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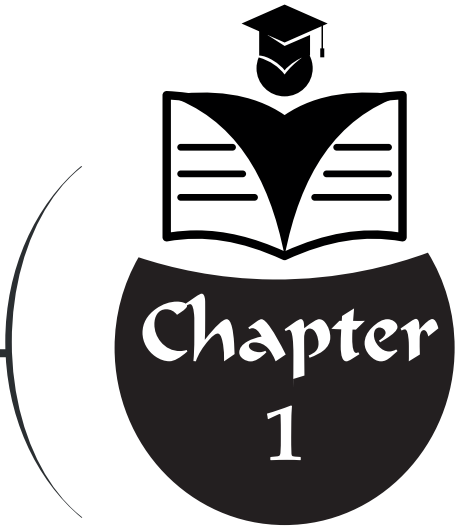

URINARY SYSTEM PROBLEMS AND REHABILITATION IN MULTIPLE SCLEROSIS

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Multiple Sclerosis (MS) is a chronic, autoimmune demyelinating disease that affects the central nervous system (CNS) (Compston & Coles, 2002). As a global health issue, the prevalence of MS is increasing worldwide, with a rate of 35.9 cases per 100,000 people (Walton et al., 2020). MS, which can progress with or without attacks, affects various systems and causes different symptoms. Sensory symptoms, paresthesia, muscle weakness, diplopia, ataxia, vertigo, fatigue, depression, anxiety, and lower urinary tract symptoms (LUTS) are common symptoms (Hauser & Cree, 2020).

Neurogenic lower urinary tract dysfunction (LUTD) is common in people with MS as a frequent result of demyelinating damage in the CNS (Ginsberg, 2013). This condition can cause LUTS involving voiding symptoms, storage symptoms, or both (Panicker et al., 2015). Urinary urgency, urinary incontinence, and nocturia are among the voiding symptoms. Storage symptoms include hesitancy, urinary retention, feeling of incomplete emptying, and increased urinary frequency (Ginsberg, 2013; Panicker et al., 2015). The prevalence of urinary incontinence has been reported to be 19-80% among people with MS (Haylen et al., 2010). LUTD seen in MS occurs due to the disruption of the connection between the micturition center in the brain stem and the sacral micturition center in the spinal cord (De Ridder et al., 2013). Lesions above the pontine micturition center are generally associated with neurogenic detrusor overactivity (NDO). NDO is characterized by uninhibited detrusor contractions (urgency, frequent urination, urge incontinence, residual urine). Medullary lesions (above the sacral micturition center and below the pons) are characterized by NDO accompanied by detrusor and external sphincter dyssynergia and are often associated with pelvic floor muscle (PFM) spasticity (Vecchio et al., 2022). Infrasacral lesions may be associated with urinary incontinence due to decreased or absent detrusor activity (urinary retention), urethral insufficiency, and/or PFM weakness (Vecchio et al., 2022).

The most common urinary dysfunctions in individuals diagnosed with MS are NDO (urge and urge-type incontinence) in approximately 60% of patients, detrusor-sphincter dyssynergia in 35%, and detrusor "underactivity" in 25% (De Ridder et al., 2013). LUTS is reported on average 8 years after the diagnosis of MS, but it can also be reported in one in ten patients with MS at the time of the first MS symptoms (De Sèze et al., 2007). Voiding dysfunction emerges at the onset of the disease and develops in 75% of patients 10 years after MS (Al Dandan et al., 2020). LUTS significantly affects the quality of life in patients with MS and can lead to increased social anxiety, isolation, and risk of falling (Hemmett et al., 2004; Sung et al., 2016). This situation highlights the importance of neurourological management in this highly complex patient population (Kaplan et al., 2022).

The management of LUTD primarily focuses on improving patients'

symptoms and quality of life and secondarily on protecting the upper urinary tract and preventing urological complications (e.g., urinary tract infections, bladder stones, and renal failure) (Tornic & Panicker, 2018). First-line treatments include fluid management, pelvic floor muscle training (PFMT), and medical treatments (e.g., antimuscarinic agents). Second-line treatments include Botulinum Toxin (BTX-A) injections, intravesical treatments, invasive and non-invasive neuromodulation, and catheterization (Sparaco & Bonavita, 2022). Surgery may be necessary in selected cases. First-line treatment can be initiated in a neurological setting, but referral to a urology service at an early stage should be considered in certain situations. Patients require comprehensive assessment prior to treatment to determine bladder function related to OAB and to identify treatment options (Aharony et al., 2017).

Evaluation of the Lower Urinary Tract in Multiple Sclerosis

History: The patient's physical characteristics, sociodemographic information, obstetric, gynecological, urological, and medical history, medications used, surgical history, primary complaint, previous treatments, and habits (smoking, alcohol, and exercise habits) are recorded. The presence of diseases that may be associated with urinary incontinence, such as chronic obstructive pulmonary disease, chronic constipation, diabetes insipidus, and/or heart failure, and the symptoms experienced in the past and present should be inquired about. In addition to urinary problems, bowel problems and sexual problems should also be evaluated (Haylen et al., 2010; Panicker et al., 2015).

Physical examination: A general neurological assessment should be performed as part of the physical examination, as neurological conditions may predispose to LUT problems. Muscle strength, mobility, reflexes, sensation, spasticity, cognition, rectal sensation, and tone are evaluated (Rogers et al., 2018). The lower abdomen, external genital organs, and perineal region are evaluated. The presence of pelvic masses, vaginal atrophy, erythema, episiotomy, pelvic organ prolapse (cystocele, rectocele), infection, and urinary leakage should be checked. Digital rectal examination can assess anal resting tone, anal sensitivity, and voluntary contraction ability (Aharony et al., 2017)..

Bladder diary (volume-frequency chart): The bladder diary is a method used to objectively and validly assess urination habits. It provides detailed information about the level of urinary incontinence, daily fluid intake, voiding volume and frequency, urgency, and nocturia (Stöhrer et al., 2009). The patient is asked to record the type and amount of fluid consumed throughout the day, the amount voided each time they use the toilet, episodes of urinary leakage, and situations where they feel urgency. Additionally, the frequency of nocturia can be calculated by noting the time the patient sleeps. The bladder diary can be kept for varying periods ranging from one day to two weeks;

however, since prolonging the duration may reduce patient compliance, short-term recording is recommended. Using a three-day bladder diary is a common practice (Kaya et al., 2015; Thüroff et al., 2011). Patients are asked not to change their urination habits, fluid intake, or sleep patterns during the period they keep the diary. Furthermore, bladder diaries completed before and after treatment allow for reliable assessment of changes in incontinence severity and treatment effectiveness. This method also contributes to patients gaining awareness of their urination habits and enables more objective monitoring of the bladder training process.

Pad Test: The pad test is widely used as an objective approach to quantify both the severity and volume of urinary leakage in individuals with urinary incontinence (Lúcio et al., 2010; Matharu et al., 2004). Different testing durations have been described, including short-duration protocols and extended assessments lasting up to 24 or 48 hours. Among these methods, the 1-hour pad test recommended by the International Continence Society is the most commonly applied in clinical practice. During the assessment, individuals are instructed to consume a predefined volume of fluid and complete a series of standardized activities designed to increase intra-abdominal pressure or provoke detrusor activity within a fixed time period. Urine loss is determined by calculating the difference between the pad's dry and post-test weight. Based on the measured leakage, results are classified into severity categories ranging from normal to very severe (JØRGENSEN et al., 1987; Matharu et al., 2004).

Q-tip test: This test measures urethral hypermobility. While the patient is in the lithotomy position, a sterile cotton swab is inserted through the urethra into the bladder. The swab is then withdrawn to the point where it first encounters resistance. The patient is asked to strain and cough. An angle of 30° or greater between the outer end of the swab and the horizontal plane indicates decreased bladder neck support and urethral hypermobility. This suggests stress urinary incontinence (Crystle et al., 1971).

Assessment of Pelvic Floor Muscles: PFM function can be assessed by observation, vaginal/anal palpation, electromyography (EMG), vaginal squeeze pressure measurement, pelvic floor dynamometry, ultrasonography, magnetic resonance imaging (MRI), myotonometry, and manometry (Frawley et al., 2021).

Patient questionnaires: These are commonly used methods in the diagnosis of urinary incontinence and in measuring the effectiveness of treatment. They help determine the type, frequency, timing, severity, and extent to which urinary incontinence affects a person's quality of life. They are also used to determine the type of incontinence. In evaluating LUTS in MS patients, the Neurogenic Bladder Symptom Score (NBSS), Bladder

Control Scale (BLCS), Urogenital Distress Inventory-Short Form (UDI-SF), the International Consultation on Incontinence Questionnaire Urinary Incontinence - Short Form (ICIQSF), the Patient Global Impression of Severity Scale (PGI-S), the Pelvic Floor Distress Inventory (PFDI), and the American Urological Association Symptom Index (AUA-SI) scales can be used (Cameron, 2022; Patel et al., 2017). In addition, quality of life related to urinary symptoms in these patients can be assessed using the Incontinence Quality of Life Scale (I-QOL), the King's Health Questionnaire (KHQ), the Qualiveen Urinary Quality of Life Questionnaire (Cameron, 2022; Patel et al., 2017), the Incontinence Impact Questionnaire (IIQ) (Cam et al., 2007), and Bristol Female Lower Urinary Tract Symptoms Index (BFLUTS) scales (Gökkaya et al., 2012).

Assessment of Sexual Function: Sexual functions are negatively affected in individuals with MS due to LUTS (Duralde & Rowen, 2017). In this context, it is important to question the presence of sexual dysfunction. Some Turkish scales with established validity and reliability that can be used in the assessment are: Female Sexual Function Index (Oksuz & Malhan, 2005), Female Sexual Distress Scale (Aydın et al., 2016), and Pelvic Organ Prolapse/ Urinary Incontinence Sexual Function Scale-12 (Cam et al., 2009).

Urodynamics: Urodynamic evaluation may be necessary in patient groups with complex LUT problems. These methods, which include tests such as uroflowmetry, cystometry, pressure-flow studies, and video urodynamics, provide the opportunity to dynamically evaluate LUT functions (Karan, 2016). Urodynamics is recommended in patients scheduled for surgical treatment, those with a history of failed urinary incontinence surgery, neurological patients, those with significant pelvic organ prolapse, and in cases where there is a discrepancy between symptoms and objective findings (Weidner et al., 2001).

Rehabilitation of Lower Urinary Tract Problems in Multiple Sclerosis

Conservative treatment methods constitute the first-line treatments for LUTS. Behavioral and lifestyle changes, bladder training, and physical therapy and rehabilitation approaches (PFMT, electrical stimulation, biofeedback, vaginal cones/tampon training, breathing exercises, taping, manual therapy, telerehabilitation) are among these methods.

Lifestyle Recommendations: Lifestyle recommendations include adequate fluid intake, dietary adjustments, weight control, prevention of constipation, limiting tobacco and alcohol use, and increasing physical activity (Arnouk et al., 2017). Adequate water consumption is necessary to flush out bladder irritants. However, restricting fluid intake due to fear of urinary incontinence can cause urine to become more concentrated, leading to irritation of the bladder mucosa and predisposing to urinary tract

infections (Beetz, 2003). In addition, reduced fluid intake can lead to decreased bladder capacity and an increased need to urinate frequently (Griffiths et al., 1993). Dietary modifications are considered an important component in the management of conditions such as obesity, constipation, and urinary incontinence (Bø & Frawley, 2015). In individuals experiencing urinary symptoms, daily nutritional habits and fluid intake patterns may significantly influence symptom severity (Dallosso et al., 2003). Accordingly, limiting the consumption of certain dietary triggers—including highly seasoned foods, caffeinated drinks, acidic food items, and artificial sweeteners—has been suggested as a supportive strategy to reduce urinary complaints (Burkhard et al., 2016).

Elevated body mass index has been associated with increased intra-abdominal pressure, which may negatively influence bladder function and contribute to detrusor muscle dysfunction. In addition, dietary patterns and beverage choices are recognized as potential factors in the exacerbation of OAB. Various studies have shown that caffeine intake has a stimulating effect on the detrusor muscle and worsens OAB symptoms (Arya et al., 2000). Similarly, alcohol has been reported to cause frequent urination due to its diuretic effect. It is also recommended to consume artificial sweeteners such as aspartame, spicy foods, citrus fruits, and tomatoes with caution (Karan, 2016).

Chronic constipation can lead to changes in the neurological functions of the pelvic floor during prolonged bowel movements. Additionally, chronic constipation and straining during bowel movements can cause pelvic organ prolapse and urinary symptoms in women (Burkhard et al., 2016). Therefore, adequate fluid intake, consumption of fiber-rich foods, regular exercise, and consideration of laxative use in cases of severe constipation are recommended to regulate bowel function (Snooks et al., 1985).

Cigarette smoking can lead to chronic cough over time, causing an increase in intraabdominal pressure. This increase, combined with coughing, creates constant pressure on the pelvic floor and contributes to the weakening of structures in this area. Anatomical damage to the urethral sphincters and vaginal support mechanisms due to pressure can result in symptoms such as urinary incontinence and frequent urination (Bump & McClish, 1994). In addition, studies have shown a relationship between nicotine and detrusor contractions (Tampakoudis et al., 1995).

Current evidence does not provide a definitive consensus on the association between physical activity and urinary incontinence. Nevertheless, from a clinical perspective, activities that substantially elevate intra-abdominal pressure are generally advised to be approached with caution in this population (Gungor & Beji, 2011).

Bladder Training: The primary goal of bladder training is to help the patient resist the urge to urinate, delay urination, and ultimately regain independent control over urination (Sparaco & Bonavita, 2022). This approach aims to control the feeling of urgency, reduce urination frequency, alleviate OAB symptoms, increase reduced bladder capacity, and prevent urinary incontinence. Thus, urination intervals are lengthened and bladder control is restored. During bladder training, if it has been less than 2 hours since the patient last urinated (this time may vary individually), they are asked not to go to the toilet; instead, they should sit down, squeeze their PFM, relax mentally, and divert their attention elsewhere. It is recommended to wait a few minutes until the urge to urinate subsides. This method is used in addition to other treatment approaches and is effective in reducing symptoms such as frequent urination, urgency, and nocturia (Fantl et al., 1991).

Physical Therapy and Rehabilitation Approaches:

Pelvic floor muscle training: This training improves the strength, endurance, relaxation, and combination of these aspects of the pelvic floor muscles. It forms the cornerstone of pelvic floor rehabilitation. In PFMT, muscle fiber hypertrophy, PFM, and connective tissue strengthening ensure more effective functioning of active motor neurons (Bø, 2004). It is also stated that training causes a decrease in the perineo-detrusor reflex and detrusor pressure, an increase in urethral pressure, and suppression of the increased voiding reflex (Amaro et al., 2005; Shafik & Shafik, 2003).

During PFMT, an exercise program is created for patients based on the 5F concept. This approach includes the stages of first noticing (Find) and feeling (Feel) the muscles, using muscle strength (Force), monitoring the exercise process (Follow), and supporting it with functional exercises (Functional training) (Berghmans, 2020; Berghmans & Seleme, 2020). Informing the patient about the structure and function of the PFM is important for the muscles to be contracted and relaxed correctly. Various teaching strategies can be used for this purpose. These include visualization, palpation of the perineal tendon, perception of movement by the patient, visual feedback using a mirror, simultaneous activation of the transversus abdominis muscle, contractions synchronized with diaphragmatic breathing, and temporary interruption of flow at the end of urination (Mateus-Vasconcelos et al., 2018).

PFMT represents a frequently applied conservative intervention for the management of LUTD in individuals with MS and may be implemented either as a standalone approach or alongside complementary therapeutic modalities (Tornic & Panicker, 2018). Evidence from clinical studies suggests that PFMT, whether administered alone or combined with interventions such as neuromuscular electrical stimulation or TTNS, can lead to improvements in urinary storage symptoms among women with MS (Lúcio et al., 2016).

Furthermore, findings from a systematic review indicate that pelvic floor–focused exercise programs contribute to enhanced health-related quality of life, a reduction in urinary incontinence severity, and improvements in sexual function in this population (Yavas et al., 2022).

Electrical Stimulation: Posterior tibial nerve stimulation (PTNS) was first described by McGuire et al. in 1983. The tibial nerve has a mixed structure containing fibers originating from the L4-S3 spinal segments, and the innervation of the bladder, rectum, and PFM occurs at the same segment level. In this treatment method, low-frequency and high-intensity electrical stimuli directly stimulate the peripheral nerve, creating a strong inhibition on the spinal spinothalamic pathways. As a result, the activation of nerves going to the external urethral sphincter and the stimulation of inhibitory afferent nerves at the spinal or supraspinal level suppress the micturition reflex (Dellis et al., 2018; Panicker & Fowler, 2015). Electrical nerve stimulation can be applied using non-invasive surface electrodes (transcutaneous), needle electrodes (percutaneous), vaginal/rectal electrodes, or implanted devices (sacral nerve modulation-SNM) (Bartley et al., 2013).

Various studies have investigated the therapeutic safety and efficacy of PTNS in patients with MS (Schneider et al., 2015). A study by Marzouk et al. investigated the therapeutic efficacy of PTNS in the treatment of neurogenic OAB in MS patients and found it to be more effective in reducing symptoms than the group that received PFMT alone (Marzouk et al., 2022). In another study, 83 MS patients suffering from LUTS underwent 12 weeks of PTNS sessions and showed significant improvement in satisfaction, bladder diary, and voiding parameters compared to pre-intervention parameters (Zecca et al., 2014).

Another method used to stimulate PFM contraction is BF therapy. BF is a method that provides visual or auditory feedback to teach the patient how to contract and relax the PFM correctly (Arnouk et al., 2017). There are also various studies in the literature on PFMT with BF in MS patients. One study investigated the effect of PFMT with PTNS and BF on OAB symptoms in female MS patients (Polat Dunya et al., 2021). The results showed that both treatments were associated with significant improvement in urgency, frequency, incontinence, nocturia, and quality of life, but no superiority of one method over the other was found.

Respiratory Exercise: The diaphragm, intercostal muscles, pelvic floor musculature, abdominal muscles, and thoracolumbar fascia form an integrated myofascial system that functions in a coordinated manner. Alterations in the performance of any single component of this system may influence the behavior and efficiency of the others due to their interconnected structure (Bø et al., 2023; Talasz et al., 2011; Talasz et al., 2022). Emerging evidence indicates

that inefficient or altered breathing patterns can disrupt effective load transfer mechanisms, potentially contributing to the development of urinary incontinence (Abidi et al., 2022; Talasz et al., 2022). In line with this concept, Toprak and colleagues proposed that targeted breathing exercises may enhance pelvic floor muscle function indirectly by optimizing diaphragmatic–pelvic floor coordination, thereby supporting continence mechanisms.

Lung function is affected even in the early stages of MS (Murrieta-Álvarez et al., 2023; Rietberg et al., 2017). Furthermore, weakness in the respiratory muscles is observed in approximately 64% of these individuals (Fry et al., 2007). Based on these findings, it is thought that lung function in individuals with MS may be related to urinary incontinence (Aguilar-Zafra et al., 2022). A study protocol examining the effectiveness of respiratory exercises applied in addition to PFMT has also been published recently.

Transcranial Stimulation: Effective PFM function in individuals with MS relies on the integrity of both central and peripheral motor pathways across different levels of the nervous system (Nardone et al., 2019). Among cortical regions, the primary motor cortex is known to undergo structural and functional alterations in this population, which may influence motor control of the pelvic floor. Interventions targeting this area have therefore been proposed as a means of enhancing PFM performance (Nardone et al., 2019). In this context, neuromodulatory approaches such as anodal transcranial direct current stimulation applied over the primary motor cortex may potentiate the effects of PFMT on pelvic floor dysfunction and urinary symptoms in patients with MS (Roy & Aziz, 2014). Transcranial direct current stimulation is generally characterized as a non-invasive, low-cost, and well-tolerated method of cortical stimulation (Benninger et al., 2011), and a growing body of evidence has demonstrated its therapeutic potential across various clinical domains in individuals with MS (Ehsani & Samai, 2019; Mortezanejad et al., 2020; Workman et al., 2019).

Vaginal Weights/Tampon Training: Vaginal devices developed by Plevnik in 1985 consist of small weights placed on the levator plate (Plevnik, 1985). The purpose of using these vaginal weights in PFM strengthening exercises is to ensure that the PFM contracts reflexively or voluntarily when the sensation of the weight falling is perceived (Herbison & Dean, 2013). As part of the exercise program, it is recommended that these cones be used twice a day for 15 minutes each (Seo et al., 2004). Training begins with the lightest cone that the person can carry for one minute while standing, and the weight is gradually increased depending on muscle strength. In the advanced stages, the goal is for the patient to reach a level where they can carry the cones for 15–20 minutes while walking (Bø, 1995; Fischer & Linde, 1997).

Telerehabilitation: Telemedicine represents a potential solution for

supporting and providing healthcare services to MS patients (Corea et al., 2021; Landi et al., 2022; Moccia et al., 2020). Telerehabilitation, on the other hand, represents one of the only possibilities for offering physical therapy interventions to people living in remote areas (Peretti et al., 2017). It has been suggested that the effectiveness of telerehabilitation protocols is comparable to face-to-face rehabilitation or better than no rehabilitation at all (Seron et al., 2021). It has also been suggested that the application of telerehabilitation for urogenital symptoms may be feasible and acceptable (Yavas et al., 2023). A study examined the effectiveness of PFMT administered via telerehabilitation on urinary symptoms and quality of life in MS patients. The findings showed that it was more effective in improving symptoms and increasing quality of life compared to the group that was only advised to make lifestyle changes (Bulbul et al., 2024).

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Chapter 2

FROM SOUND TO SPEECH: A GENERAL OVERVIEW OF AUDITORY PROCESSING IN LANGUAGE ACQUISITION

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Language is a complex cognitive system central to humans' capacity to make sense of the world, organize thoughts, and engage in social interaction. For this system to function optimally, sensory input must reach the brain accurately, completely, and in a timely manner. The quality of auditory input, which forms the foundation of language learning, influences not only the accurate perception of words but also the fidelity of phonological representations, the parsing of syntactic structures, and the establishment of semantic relationships (Salvagio et al., 2024). Consequently, auditory processing is examined as an integral component of language processing, playing a critical role at the intersection of sensory, cognitive, and neurobiological mechanisms (Thamizhmani et al., 2025).

Auditory processing begins with the encoding of sound waves by the peripheral auditory system. The tonotopic organization of the cochlea allows for the differentiation of various frequencies, serving as a fundamental mechanism for perceiving the phonemic characteristics of speech sounds (Mardin et al., 2022). Acoustic differences that enable the distinction of one phoneme from another occur over extremely brief time intervals, requiring precise peripheral analysis. If signal details are lost during this initial processing stage, the functioning of higher-level language systems is disrupted. Thus, the auditory processing chain is intimately linked to the phonological foundations of language from its very first stage (Salvagio et al., 2024). Once peripheral coding is completed, the signal progresses to central auditory structures such as the brainstem, thalamus, and auditory cortex, where it undergoes more complex analytical processing. Two fundamental mechanisms, known as auditory temporal and spectral processing, enable the resolution of the rapidly changing features of speech signals (Giroud et al., 2024). Human speech contains transient cues that can change 20–40 times per second, making temporal processing a critical factor in language perception. Auditory temporal processing refers to the auditory system's ability to detect and interpret time-varying acoustic signals, which is essential for accurate speech comprehension. This process comprises several components. Temporal resolution enables the discrimination of rapid changes in the signal and is critical for perceiving brief phonemic differences, such as the aspiration contrast between /ba/ and /pa/. Temporal ordering allows for the recognition of the temporal organization of sounds, facilitating the accurate perception of rhythm, stress, syllables, and word emphasis in speech. Temporal integration refers to the ability to combine brief or low-intensity acoustic events into a coherent percept, ensuring overall speech intelligibility. Temporal discrimination involves detecting differences in timing between sounds, supporting the perception of phoneme duration, syllable boundaries, and subtle temporal variations in speech flow.

Efficient functioning of these components allows for rapid and accurate speech comprehension, whereas deficits in temporal processing can impair phoneme and syllable discrimination, rhythm and stress perception, ultimately hindering speech understanding. Therefore, individuals with difficulties in temporal processing may struggle to accurately identify syllable

boundaries, follow rapid speech, and perceive morphological morphemes (McFarlen et al., 2024). Auditory spectral processing, on the other hand, refers to the auditory system's ability to analyze and discriminate the frequency content of a sound signal. Complex acoustic stimuli, such as human speech and music, contain not only rapidly changing temporal cues but also multiple frequency components. Spectral processing enables the auditory system to decompose, compare, and integrate these frequency elements into a coherent percept (Ankmnal et al., 2019). When an acoustic stimulus is presented, the process begins as sound waves are transmitted from the outer ear through the middle ear to the cochlea. The cochlea has a tonotopic organization, allowing different frequencies to maximally stimulate specific regions: high frequencies activate basal regions, while low frequencies activate apical regions (Moerel et al., 2012).

These mechanical vibrations are converted into electrical signals by the hair cells, with each frequency component encoded along specific nerve fibers and transmitted to the brain via the auditory nerve. In the brainstem, auditory nuclei process the incoming frequency information in a more complex manner, analyzing sound pitch, spectral content, and source location (Marin et al., 2022). The auditory cortex further evaluates these spectral cues in terms of melody, formants, and harmonic relationships. In speech perception, accurate discrimination of formant frequencies is crucial for phoneme recognition; for example, distinguishing the vowels /i/ and /e/ relies on spectral differences (Oganian et al., 2023). Finally, the brain integrates spectral information with temporal processing to construct perceptual features of the sound, enabling accurate identification and comprehension of both its source and meaning. Deficits in spectral processing can lead to difficulties in phoneme discrimination, impaired perception of speech and music, and overall challenges in auditory comprehension. These auditory spectral processes form the phonological map of language, and without accurate phonological representations, semantic, morphological, and syntactic processing cannot function optimally. Therefore, the processing of acoustic input is a primary prerequisite for an integrated and effective language processing system (Giroud et al., 2024). The impact of auditory processing on language development begins during the prenatal period and becomes particularly prominent in early childhood (Webb et al., 2015). During the fetal stage, the auditory system becomes functional around the 20th week of gestation. From this point onward, the fetus can perceive filtered acoustic features of the intrauterine environment, including maternal heartbeat, respiration, walking rhythms, vascular sounds, and, notably, the prosodic characteristics of the mother's voice (Ksilevsky et al., 2003). Due to the low-frequency transmission advantage provided by the amniotic fluid, the fetus is able to discern the basic rhythmic and melodic components of external sounds. Research has shown that newborns can recognize their mother's voice immediately after birth and show a preferential orientation to the rhythmic features of the language they were frequently exposed to in utero (Ksilevsky et al., 2003).

Early recognition of prosodic cues establishes the biological foundation for auditory pattern recognition, phonological discrimination, and rhythm perception—processes that play a critical role in postnatal language acquisition (Webb et al., 2015). Thus, auditory processing begins shaping the neuroacoustic infrastructure necessary for language even before birth (Ksilevsky et al., 2003; Webb et al., 2015). In the first months of life, infants possess the capacity to discriminate a wide range of phonemes; however, this capacity gradually becomes specialized based on environmental experience. This process reflects a neuroplasticity dynamic that aligns with the concept of critical periods in language development (Kuhl et al., 2008). If a child is exposed to insufficient, distorted, or noisy auditory input during the early years, the fine-grained aspects of the phonological system may not be learned effectively (Gervain, 2015). Even temporary conductive hearing issues, such as otitis media, have been shown to exert measurable effects on phonological awareness and verbal memory, highlighting the importance of auditory input (Tomblin et al., 2015). Similarly, congenital auditory differences or central auditory processing difficulties can directly influence the development of the language system (Kuhl et al., 2008).

Language processing does not occur solely at the phonological level; the perception of syntactic and morphological structures is also directly linked to auditory processing mechanisms. In agglutinative languages such as Turkish, a substantial portion of morphological markers consists of brief and low-intensity acoustic cues (Ketrez, 2012). Accurate perception of these affixes requires both temporal and spectral sensitivity (Tallal, 2004). Individuals with poor temporal processing skills may struggle to discriminate tense markers such as "geliyor–geliyordu–gelecek" ("is coming–was coming–will come"), leading to errors in syntactic parsing and meaning construction. Similarly, weaknesses in attentional processes can make it difficult to follow long sentences auditorily, hindering the holistic processing of syntactic structures (Steven et al., 2012). When examining the neurobiological foundations of the relationship between auditory processing and language processing, it becomes evident that these two systems are not sharply delineated. The superior temporal gyrus, Wernicke's area, Heschl's gyrus, and prefrontal cortex regions are commonly involved in both auditory analysis and language processing (Hickok & Poeppel, 2007). The network of connections among these regions facilitates complex communication that transforms auditory information into a meaningful linguistic structure. Neuroimaging studies indicate that as the quality of auditory input improves, this network becomes more organized and efficient, whereas prolonged insufficient auditory input can lead to weakened connectivity (Friederici, 2011). These findings support the notion that a portion of individual differences in language development is related to neural plasticity and auditory experience (Hickok & Poeppel, 2007; Friederici, 2011).

From a clinical perspective, auditory processing and language processing appear to be closely intertwined in many developmental disorders. Children with Developmental Language Disorder (DLD) often exhibit pronounced

weaknesses in temporal processing skills and auditory attention mechanisms (Elmahallawi et al., 2022; Hu et al., 2025). These deficits reduce the reliability of phonological representations, producing a cascading effect on syntax, morphology, and narrative abilities (Drosos et al., 2024).

Individuals with Auditory Processing Disorder (APD) demonstrate significant difficulties in distinguishing speech sounds, understanding speech in noise, and following verbal instructions (Rocha-Muniz et al., 2014; Drosos et al., 2024). The impact of these challenges on language processing frequently manifests as weakened verbal memory, impaired comprehension of complex sentence structures, and reduced academic performance (Nagaraj & Magimairaj, 2020). For example, in individuals with Autism Spectrum Disorder (ASD), auditory sensitivity and auditory attention processes exhibit distinct patterns. Some individuals demonstrate hyper-sensitivity, whereas others struggle to discriminate social auditory cues. Differences in temporal synchronization have emerged as one of the mechanisms explaining delays in social language skills. Similarly, children with a history of cleft lip and palate may experience interruptions in early auditory input, which can affect phonological memory and the accuracy of language processing (Gonçalves & Monteiro, 2023). Therefore, in addition to their intrinsic functioning, auditory and language systems can be influenced directly or indirectly by a variety of factors. Considering the complex relationship of these two systems with the cognitive system, it becomes essential that assessment and intervention processes are approached from a multidimensional and holistic perspective. This strong link between auditory and language processing necessitates an integrated approach to assessment and intervention. Combining auditory processing tests with comprehensive language evaluations provides critical clinical insights, particularly for complex profiles that fall within the so-called gray zone of overlapping auditory-language difficulties. When temporal resolution tests, frequency discrimination measures, dichotic listening protocols, and auditory attention assessments are considered alongside evaluations of phonological awareness, verbal memory, vocabulary, and syntax, a more holistic profile emerges. Such an integrated approach enhances diagnostic accuracy and informs the development of individualized intervention programs (Hu et al., 2025). The integration of auditory-based training programs with language-focused interventions is increasingly recognized as important in the rehabilitation process (Law et al., 2004). Conducting auditory discrimination exercises alongside phonological awareness training, supporting auditory memory exercises with morphological instruction, and integrating prosodic awareness activities into pragmatic language interventions have been shown to promote faster and more sustained development (Moore et al., 2005; Farag et al., 2024). Although these approaches based on the principles of neuroplasticity demonstrate more pronounced effects in early childhood, they still offer significant potential for change up to young adulthood (Schneider et al., 2023). Findings that music-based auditory training programs positively influence phonological processing and temporal resolution further support the training-sensitive nature of the auditory–language system (Kraus et

al., 2014; Nan et al., 2018). Taken together, these findings demonstrate that language processing is not merely a cognitive function; it is also shaped by the quality of auditory experience, the accuracy of signal encoding, and the effectiveness of attentional mechanisms. Auditory and language processing operate as complementary, dynamic systems. Therefore, rather than drawing sharp distinctions between the two domains, adopting a holistic perspective on their interrelationship contributes both to the development of theoretical models and to the precision of clinical applications.

In conclusion, auditory processing constitutes a fundamental component of language learning and usage. From phonological structures to syntax, from semantic interpretation to pragmatics, all levels of language are directly influenced by the fidelity of auditory input and its neural processing. Understanding, assessing, and rehabilitating language disorders can only achieve scientific validity when considered alongside auditory processing mechanisms. The reciprocal interaction between auditory and language systems provides a robust framework for understanding typical development and explaining clinical populations, serving as a guiding principle across educational and clinical contexts. In this regard, the relationship between auditory and language processing will continue to be a focal point for future research, driving the development of more precise assessment tools and more effective interventions.

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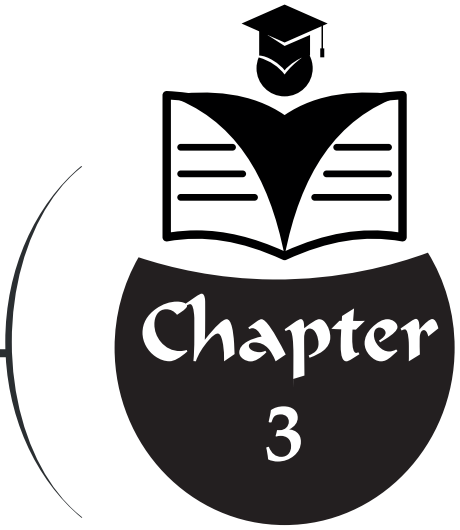

RECENT ADVANCES IN THE SPECTROPHOTOMETRIC STABILITY STUDIES OF DRUG-METAL COMPLEXES: A TEN-YEAR PERSPECTIVE

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1. INTRODUCTION TO DRUG-METAL COMPLEXES

Pharmaceuticals are chemical entities obtained from natural, semi-synthetic, or fully synthetic sources and administered to living organisms through various routes. Upon entering the body, these substances exert pharmacological effects aimed at the diagnosis, treatment, or alleviation of disease symptoms, the promotion of recovery, or the provision of prophylaxis against pathological conditions.

Historically, plant-derived drugs (herbal materials) and minerals served as primary sources of medicinal raw materials. Subsequently, active constituents isolated from these sources via appropriate extraction and purification techniques were standardized into pharmaceutical preparations and incorporated into therapeutic practice.

In recent years, metal coordination complexes formed with biologically active ligands have become the focus of intensive research owing to their critical roles in clinical therapy. Transition metals offer distinct advantages in this field due to their wide range of coordination numbers and geometries, multiple accessible oxidation states, tunable ligand-exchange kinetics, and rich redox properties. These electron-deficient metal ions, characterized by high charge-to-radius ratios, establish strong and selective interactions with pivotal biomolecules such as DNA and proteins, thereby eliciting significant therapeutic and diagnostic activities. The redox behavior of transition-metal ions plays a central role in both physiological and pathological processes. Tight regulation of cellular redox homeostasis is essential for health, as dysregulated redox cycling of these ions can trigger the generation of reactive oxygen species (ROS), culminating in oxidative stress. Conversely, uncontrolled accumulation of ROS induces damage to DNA, lipids, and proteins, thereby accelerating the progression of numerous pathologies, including cancer, cardiovascular diseases, neurodegenerative disorders, inflammation, and aging. This dual nature confers upon metal complexes the capacity to act as either pro-oxidants or antioxidants, depending on their structural features and the prevailing redox microenvironment within the cell. Such context-dependent behavior has spurred researchers to explore synergistic interactions between metal ions and natural antioxidant molecules, with the ultimate goal of designing next-generation metal-based therapeutic agents endowed with enhanced antioxidant properties.

Today, metal-containing compounds occupy an indispensable position in the treatment and diagnosis of a broad spectrum of diseases, most notably cancer. The serendipitous discovery of cisplatin (cis-diamminedichloroplatinum(II)) in 1965 by Barnett Rosenberg-during electrical stimulation experiments on Escherichia coli cultures-represents a landmark milestone in the development of metal-based chemotherapeutics. This groundbreaking finding

unequivocally demonstrated the anticancer potential of metal coordination compounds and paved the way for the clinical introduction of numerous platinum-group metal (PGM) drugs, including carboplatin, oxaliplatin, and others. The interdisciplinary convergence of coordination chemistry with biology and medicine continues to yield revolutionary advances, particularly in cancer chemotherapy and the management of diverse pathologies. It is anticipated that metal-based strategies will assume an increasingly prominent role in future drug-design paradigms (Kostova and Balkansky, 2013).

A coordination compound, or metal complex, consists of a central metal atom or ion surrounded by ligands-molecules or anions-that are bound to the metal through coordinate covalent bonds. The formation of such complexes in solution often involves proton displacement due to the interaction between the metal ion and the incoming ligand, resulting in a measurable decrease in pH. This pH drop is widely recognized as a reliable indicator of complexation. The stability and overall behavior of these complexes are governed by multiple factors, including the nature of the ligand, the strength of the metal–ligand bonds, and the physicochemical conditions of the surrounding medium. In aqueous environments, thermodynamic stability constants (log β or log K) and kinetic parameters serve as fundamental tools for characterizing metal–ligand interactions, providing insights that extend far beyond biological systems.

Complexes with very low stability constants (typically log $K < 1$, or even negative values) tend to be insoluble in water and undergo rapid dissociation, releasing free metal ions and ligands. Conversely, complexes with exceptionally high stability constants (log $K > 6$) exhibit minimal dissociation across a wide pH range, requiring substantial acid quantities to disrupt the coordination sphere; this extreme robustness can, paradoxically, reduce biological suitability by limiting ligand exchange or metal release at physiological sites. For pharmaceutical applications, stability constants in the moderate range of approximately 3–5 are generally considered optimal, as they ensure sufficient integrity of the active species while permitting controlled release or biorelevant reactivity. The kinetics and mechanisms of action of metal-based complexes differ markedly from those of conventional organic drugs. Nevertheless, the ability of transition metals to participate in redox processes confers unique advantages, enabling the modification of organic pharmacophores through coordination. Consequently, metal complexes offer integrated active entities capable of delivering tailored pharmacological outcomes that are difficult to achieve with purely organic molecules.

In recent years, the synthesis and biological evaluation of metal complexes incorporating redox-active ligands have emerged as a prominent research focus. A critical step in their development involves thorough investigation of both physicochemical properties and biological activities,

often employing in vitro enzymatic model systems that can elucidate underlying pharmacological mechanisms. Particular attention has been devoted to sterically hindered diphenolic derivatives coordinated to Ni(II), Cu(II), and Zn(II) ions. Pharmacological profiling of these compounds suggests pronounced antioxidant potential, while their antimicrobial activity is likely attributable to interference with microbial electron-transfer pathways. The concomitant manifestation of antibacterial, antifungal, and antioxidant properties positions these coordination compounds as promising broad-spectrum therapeutic candidates (Loginova et al., 2020).

The synthesis of inorganic compounds incorporating biologically relevant ligands is greatly facilitated by the pivotal roles that specific metal ions play in physiological processes. Low-molecular-weight species are particularly favored in bioinorganic design, as they effectively mimic the structural and reactivity profiles of native metallobiomolecules. The integration of metal complexes into biological systems-from catalytic roles in energy metabolism to the development of nanomaterials and bioimaging probes-necessitates a genuinely interdisciplinary approach.

One prominent strategy involves complexation of essential metal ions with biologically active molecules to enhance antimicrobial efficacy. Transition-metal coordination compounds are extensively evaluated as antibacterial and anticancer therapeutics for the management of bacterial infections and malignancies. These entities, commonly termed coordination complexes, arise from the interaction between metal ions (Lewis acids) and ligands that function as active pharmaceutical ingredients (Lewis bases). During complexation, donor ligands (e.g., H_2O, NH_3, Cl^-) provide electron pairs to acceptor metal centers (e.g., Fe^{2+}, Cu^{2+}, Zn^{2+}, Pt^{4+}). The archetypal water-soluble anticancer agent cisplatin exemplifies this principle through the coordination of Pt(II) with two chloride and two ammine ligands. Additional FDA-approved metal-based pharmaceuticals include carboplatin and oxaliplatin (platinum), silver sulfadiazine (silver), and zinc bacitracin (zinc). Although the pharmaceutical utilization of metal complexes remains relatively limited, interest is rapidly growing in the nutraceutical sector. Most such complexes are engineered primarily to improve drug stability or pharmacodynamics, with enhanced aqueous solubility regarded as a valuable secondary outcome. The electronegativity of the metal ion critically influences complex formation: higher electronegativity favors thermodynamic stability but often impairs water solubility. Consequently, judicious metal selection is essential to achieve an optimal balance between stability and solubility.

Chelates are a subclass of coordination complexes in which a single ligand (chelating agent) occupies multiple coordination sites on the metal. Agents such as EDTA are routinely employed in pharmaceutical formulations to sequester trace metal impurities. While drug–metal chelates exist, chelation is not a

prerequisite for solubility enhancement; nevertheless, certain poorly soluble drugs can form highly water-soluble coordination species. Stoichiometry, molecularity, and structural features of newly formed complexes are commonly elucidated using the continuous variation method (Job's method), which relies on UV–Vis spectrophotometric measurement of absorbance changes across varying ligand-to-metal ratios. Accumulated evidence underscores that the diverse molecular architectures and tunable physicochemical properties of drug–metal complexes provide a powerful strategy to circumvent delivery challenges associated with poorly soluble active pharmaceutical ingredients. Complexation is widely adopted in pharmaceutical sciences to augment aqueous solubility, dissolution rate, membrane permeability, chemical stability, and ultimately oral bioavailability of problematic drug candidates. A thorough understanding of pre-formulation parameters-particularly reactant stoichiometry and complex stability-is indispensable for predictable complex design. Unlike alternative approaches (e.g., taste-masking polymers), coordination complexation can intrinsically mask unpleasant flavors, although heightened solubility occasionally exacerbates bitterness.

Contemporary in silico and in vitro predictive tools efficiently forecast critical pre-formulation attributes, thereby accelerating the development of robust and reproducible drug–metal complexes. Coordination compounds are fundamentally intermolecular assemblies between ligands and acceptors; depending on the nature of the acceptor, they are classified as metal-ion complexes or organic molecular complexes. In non-metallic systems, the drug molecule associates with non-metallic atoms of neutral species or ions of ionic compounds. Inclusion complexes, by contrast, entail the physical entrapment of a guest molecule within the molecular cavity of a host. The utility of complexation strategies extends far beyond pharmaceuticals, finding widespread application in the food, cosmetic, and agrochemical industries. Overall, metal coordination compounds-particularly those incorporating essential and transition metals-offer distinct advantages over conventional organic drugs and continue to attract substantial research interest across multiple disciplines (Munnangi et al., 2023).

The evidence presented in this review clearly demonstrates that drug–metal coordination complexes represent a practical and versatile strategy to overcome numerous drug-delivery challenges associated with poorly water-soluble pharmaceutical compounds, owing to their diverse molecular architectures and tunable physicochemical properties. These complexes can significantly enhance the aqueous solubility, dissolution rate, and bioavailability of low-solubility drug molecules, thereby fulfilling the requirements for efficient delivery systems. In addition to improved solubility, the permeability of drug moieties can be substantially augmented through the formation of lipophilic coordination species. Considering the breadth

of reported studies, a comprehensive understanding of pre-formulation parameters-particularly the stoichiometric ratios of reactants and the stability constants of the resulting complexes-is crucial for predictable and reproducible complex design. Various in silico and in vitro predictive models have proven effective in forecasting these pre-formulation attributes and facilitating the development of robust, stable drug–metal complexes. The applications of the complexation approach are not limited to the pharmaceutical topics reviewed herein; they extend broadly to the food, cosmetic, and agrochemical industries, where similar enhancements in solubility, stability, and biological performance are highly desirable.

2. THE EVOLUTION OF STABILITY STUDIES ON DRUG-METAL COMPLEXES OVER THE PAST DECADE

Novel complexes of Co(II), Ni(II), Cu(II), and Zn(II) have been synthesized using a tridentate ONO-donor Schiff base ligand derived from 3-(2-aminoethylamino)quinoxalin-2(1H)-one. The structures of the obtained complexes were characterized by various analytical and spectroscopic techniques, including elemental analysis, FT-IR, UV-Vis, ^{1}H-NMR, ^{13}C-NMR, mass spectrometry, molar conductivity measurements, and magnetic susceptibility studies. DNA-binding investigations revealed that the Cu(II) complex, in particular, exhibits covalent binding to DNA with a 1:1 binding stoichiometry (metal:ligand ratio was also determined as 1:1). Cytotoxicity assays demonstrated that the synthesized mixed-ligand complexes display significantly higher cytotoxic activity compared to the reference chemotherapeutic agent cisplatin (Rajarajeswari et al., 2014).

The synthesis, structural characterization, thermal behavior, and biological evaluation of coordination complexes formed between pyridoxine hydrochloride (vitamin B6) as a drug ligand and selected rare-earth/alkali-earth metal ions-Y(III), La(III), Ce(III), and Sm(III)-were systematically investigated. The obtained complexes were characterized using a comprehensive array of analytical techniques, including elemental analysis, Fourier-transform infrared (FT-IR), UV–Visible and fluorescence spectroscopy, ^{1}H- and ^{13}C-NMR spectroscopy, scanning electron microscopy (SEM), and powder X-ray diffraction (PXRD). The thermal stability of the metal complexes followed the order Sm(III) > La(III) > Ce(III) > Y(III), as determined by thermogravimetric analysis. Furthermore, these vitamin B6–metal coordination compounds exhibited notable biocatalytic activity in the enzymatic transamination reactions of amino acids, which are critical processes in human metabolism. A direct proportional relationship was observed between the concentration of coordinated metal ions and the extent of metabolic transformation. The results suggest that pyridoxine–metal complexes are more readily assimilated by biological systems than the free pyridoxine molecule itself. Consequently, these coordination compounds are

proposed as potentially superior therapeutic agents with enhanced efficacy in both human medicine and veterinary applications (Refat et al., 2014).

Ibuprofen and paracetamol (acetaminophen) are the two most widely prescribed pharmaceutical agents characterized by their anti-inflammatory, analgesic, and antipyretic properties. Both molecules possess functional groups that can serve as donor sites for coordination to divalent transition metal ions. In the present investigation, a series of novel coordination compounds containing Cu(II), Co(II), Mn(II), and Fe(II) ions were synthesized separately using paracetamol and ibuprofen as primary organic ligands. The resulting metal complexes were extensively characterized by a number of analytical and spectroscopic methods, including molar conductance measurements, elemental (CHN) analysis, thermogravimetric analysis (TGA/DTA), Fourier transform infrared (FT-IR) spectroscopy, and UV-Visible electronic absorption spectroscopy. Interpretation of spectroscopic and analytical data revealed distinct binding modes for the two drug ligands. Stoichiometric analysis, confirmed by elemental composition and thermal decomposition patterns, consistently revealed a metal-to-ligand ratio of 2:1 (M:L = 2:1) in all synthesized complexes, regardless of the metal ion (Mn(II), Fe(II), Co(II), or Cu(II)) or drug used. These results highlight the versatility of pharmaceutical molecules commonly used as ligands in coordination chemistry and demonstrate the potential for generating metal-based derivatives of common drugs with altered solubility, stability, or biological profiles, including enhanced antimicrobial activity (Refat, El-Korashy, Hussien., 2014-1).

Coordination complexes comprising the pharmacologically active compound clioquinol (5-chloro-7-iodo-8-hydroxyquinoline) and various substituted 2,2′:6′,2″-terpyridine derivatives with copper(II) were successfully synthesized and rigorously characterized. Structural elucidation was achieved through a comprehensive suite of analytical and spectroscopic techniques, including elemental (C, H, N) analysis, molar conductivity measurements, magnetic susceptibility determinations, thermogravimetric analysis (TGA/DTG), Fourier-transform infrared (FT-IR) spectroscopy, ^{1}H-NMR spectroscopy, and fast-atom bombardment (FAB) mass spectrometry. The thermal decomposition profiles of the complexes were also systematically examined. Interpretation of the collective analytical data established a metal-to-ligand stoichiometry of 1:2 (M:L = 1:2), indicating that each Cu(II) center is coordinated by one clioquinol molecule and one substituted terpyridine ligand (or, in certain cases, two terpyridine units depending on substitution pattern and denticity). These findings underscore the ability of clioquinol to act synergistically with polypyridyl co-ligands in stabilizing copper(II) centers, yielding neutral coordination entities with potential implications for enhanced biological activity or altered pharmacokinetic behavior (Kharadi, 2014).

The interactions between the antiulcer drug omeprazole (OMZ) and Fe(III) and Co(II) ions were examined, leading to the synthesis and comprehensive characterization of the corresponding coordination complexes. The findings demonstrate that OMZ possesses a pronounced tendency to coordinate with these metal centers, forming stable complexes. A variety of analytical techniques-including X-ray powder diffraction (XRPD), elemental analysis, scanning electron microscopy (SEM), thermal analyses, magnetic susceptibility measurements, UV-Visible spectroscopy, FTIR spectroscopy, Mössbauer spectroscopy, electron paramagnetic resonance (EPR), and density functional theory (DFT) calculations-were employed to elucidate the structural and physicochemical features of the complexes. Results from elemental and thermal analyses indicate a metal-to-ligand stoichiometry of 1:2 (M:L) in both cases. Moreover, FTIR spectral data suggest that OMZ binds to the metal ions through its benzimidazole group, generating a five-membered chelate ring (Russo et al., 2014).

Thiosemicarbazides and their metal complexes have attracted considerable research interest in recent years due to their promising biological properties and potential applications in medicinal chemistry. In this context, the coordination behavior and physicochemical characteristics of binuclear cobalt(II), copper(II), and nickel(II) complexes derived from the N1-ethyl-N2-(pyridin-2-yl)hydrazine-1,2-bis(carbothioamide) ligand (H_2PET) were examined using a range of spectroscopic and analytical techniques. The stability constants of the resulting complexes were determined at different temperatures, and the values are presented below:

H_2PET: $\log K_1$ = 12.52, $\log K_2$ = 7.13 (298 K); $\log K_1$ = 12.14, $\log K_2$ = 7.04 (308 K);

$\log K_1$ = 11.74, $\log K_2$ = 6.95 (318 K)

Zn(II): $\log K_1$ = 11.99, $\log K_2$ = 8.74 (298 K); $\log K_1$ = 10.85, $\log K_2$ = 7.21 (308 K);

$\log K_1$ = 9.66, $\log K_2$ = 5.95 (318 K)

Cd(II): $\log K_1$ = 11.06, $\log K_2$ = 8.25 (298 K); $\log K_1$ = 9.04 (308 K); $\log K_1$ = 5.96 (318 K)

Hg(II): $\log K_1$ = 10.93 (298 K); $\log K_1$ = 14.27 (308 K); $\log K_1$ = 10.30 (318 K)

Furthermore, the H_2PET ligand, along with its Zn(II), Cd(II), and Hg(II) complexes, was evaluated for antitumor activity. The results revealed that the ligand and its metal complexes exhibit significant cytotoxic effects, with activity decreasing in the order: Zn(II) > Hg(II) > Cd(II) (El-Gammal et al., 2014).

Non-steroidal anti-inflammatory drugs (NSAIDs) are widely recognized

for their analgesic, anti-inflammatory, and antipyretic properties; however, they also exhibit notable anticancer activity. Complexation of these NSAIDs with copper(II), a bioessential transition metal, has been shown to significantly enhance their antitumor efficacy compared to the parent ligands. Previous studies have demonstrated that Cu(II)–NSAID complexes can interact directly with the DNA backbone, and this binding exhibits sensitivity to structural variations and hydration states of the polynucleotide. The DNA-binding affinity of these metallodrugs has been thoroughly investigated using a combination of spectroscopic methods, including UV–Visible absorption, fluorescence emission, and circular dichroism (CD) spectroscopy. Determination of the apparent binding constants (K) revealed that both the Cu(II)–piroxicam and Cu(II)–meloxicam complexes display comparable affinity toward calf thymus DNA (ctDNA) when interacting with the homopolymeric double helix poly(dG)–poly(dC). Specifically, the binding constants were found to be $K = 1.44 \times 10^5\ M^{-1}$ for $[Cu(II)(piroxicam)_2(L)_2]$ and $K = 4.3 \times 10^4\ M^{-1}$ for $[Cu(II)(meloxicam)_2(L)]$ (where L represents co-ligands, typically water or solvent molecules). Further analysis indicated that the Cu(II)–piroxicam complex exhibits relatively weaker binding to the poly(dG)–poly(dC) sequence, whereas the Cu(II)–meloxicam complex demonstrates stronger interaction with the same polynucleotide system (Chakraborty, Bose and Sarkar, 2014).

Fexofenadine is a second-generation antihistamine employed primarily for the management of seasonal allergic rhinitis and associated symptoms. Carbocisteine, in contrast, is a mucolytic agent that facilitates expectoration and alleviates respiratory symptoms in patients with chronic obstructive pulmonary disease (COPD) by reducing the viscosity of bronchial secretions. In the present study, fexofenadine and carbocisteine, along with their coordination complexes with the divalent metal ions Mn(II), Co(II), Fe(II), and Cu(II), were successfully synthesized and comprehensively characterized. Structural elucidation and physicochemical properties were investigated using a suite of analytical and spectroscopic techniques, including elemental microanalysis, electronic absorption spectroscopy, Fourier-transform infrared (FT-IR) spectroscopy, mass spectrometry, thermal analysis (TGA/DTA), molar conductivity measurements, and assessment of antimicrobial activity. Elemental microanalysis revealed distinct stoichiometric ratios in the isolated complexes. Carbocisteine formed 1:1 (ligand:metal) complexes with all examined metal ions (Mn(II), Co(II), Cu(II), and Fe(II)), whereas fexofenadine coordinated in a 2:1 (ligand:metal) ratio under the applied synthetic conditions (Refat, El-Korashy, Hussien., 2014-2).

Coordination compounds of transition and heavy metals find extensive application across diverse disciplines, including biological systems, pharmaceutical development, analytical chemistry, and separation science.

In living organisms, metal complexes perform essential functions and have emerged as promising therapeutic agents, particularly as enzyme inhibitors and as antimicrobial, antiviral, and anticancer drugs. In the present investigation, the complexation equilibria between the amino acids L-asparagine and L-serine with the divalent metal ions Cd(II) and Pb(II) were systematically examined in aqueous solution using a combination of spectrophotometric and potentiometric techniques. Stability constants (expressed as pK =-log β, where β is the overall formation constant) of the resulting 1:1 (metal:ligand) complexes were determined at two temperatures (298 K and 308 K). The calculated values are as follows:

L-Asparagine–Pb(II) : pK = 5.15 (298 K) ; pK = 7.18 (308 K)

L-Asparagine–Cd(II) : pK = 7.03 (298 K) ; pK = 7.16 (308 K)

L-Serine–Pb(II) : pK = 5.82 (298 K) ; pK = 6.19 (308 K)

L-Serine–Cd(II) : pK = 8.14 (298 K) ; pK = 6.50 (308 K)

These results provide insight into the relative binding affinities of the two amino acids toward the selected heavy metal ions and highlight the influence of temperature on complex stability (Hasan and Wahid, 2014).

The present study explores the coordination chemistry as well as the antibacterial and antifungal activities of metal complexes derived from lisinopril, a widely prescribed angiotensin-converting enzyme (ACE) inhibitor employed in the management of hypertension and congestive heart failure, with biologically essential metal ions, namely Fe(III), Mg(II), Ca(II), VO(II), and Zn(II). Lisinopril and its corresponding metal complexes were synthesized under controlled conditions and thoroughly characterized by a combination of physicochemical and spectroscopic techniques, including molar conductivity measurements, elemental microanalysis, Fourier-transform infrared (FT-IR) spectroscopy, proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy, and thermal analysis (TGA, DTG, and DTA). Spectroscopic and analytical data revealed that lisinopril acts as a monodentate ligand, coordinating to the metal centers through a single donor site. All isolated complexes exhibited a 1:1 (metal:ligand) stoichiometry, with the general formulation $[M(lisinopril)]^{n+,}$ where M = Fe(III), Mg(II), Ca(II), VO(II), or Zn(II) (Zaky et al., 2014).

In recent years, metal-based coordination compounds of pharmacologically active molecules have attracted considerable attention owing to their potential to enhance therapeutic efficacy, modulate bioavailability, and introduce novel biological properties. Within this framework, the present study reports the synthesis and comprehensive physicochemical characterization of a novel coordination complex formed between vitamin A (all-trans-retinol) and selenium(IV), a biologically essential trace element known for its critical role in selenoenzymes with antioxidant functions. The selenium(IV)-vitamin

A complex was isolated as a brown solid and systematically investigated using an array of analytical and spectroscopic techniques, including molar conductivity measurements, laser Raman spectroscopy, Fourier-transform infrared (FT-IR) spectroscopy, proton nuclear magnetic resonance (^{1}H-NMR), scanning electron microscopy (SEM), powder X-ray diffraction (XRD), and simultaneous thermogravimetric/differential thermogravimetric/differential thermal analysis (TG/DTG/DTA). Spectroscopic evidence, particularly shifts observed in the FT-IR O-H stretching and bending regions as well as characteristic ^{1}H-NMR downfield displacements of protons adjacent to the hydroxyl moiety, unequivocally demonstrates that vitamin A coordinates to the selenium(IV) center in a monodentate fashion through the oxygen atom of its terminal hydroxyl group. Thermal and structural analyses further corroborate the formation of a stable, well-defined coordination entity with distinct morphological and crystalline features compared to the free vitamin. The resulting Se(IV)–vitamin A complex is proposed as a promising antioxidant therapeutic candidate, combining the established radical-scavenging properties of selenium with the membrane-protective and gene-regulatory functions of vitamin A (Atta et al., 2014).

The essential bio-metal iron plays a pivotal role in numerous physiological processes, whereas antihistaminic agents are widely employed in the clinical management of disorders mediated by excessive histamine release. These therapeutic compounds can engage in coordination interactions with biologically relevant metal ions, thereby modulating their pharmacokinetic and pharmacodynamic profiles. In the present investigation, the coordination behavior of iron with chlorphenamine (chlorpheniramine), a first-generation H_1-receptor antagonist belonging to the alkylamine class of antihistamines, was systematically examined. Analytical and spectroscopic techniques-including elemental analysis, molar conductivity measurements, and UV-Visible spectrophotometry-were utilized to elucidate the stoichiometry and stability of the resulting complex. The data conclusively demonstrate the formation of a mononuclear [Fe–chlorphenamine] complex with a 1:1 metal-to-ligand (M:L) molar ratio. Quantitative evaluation of the equilibrium in solution yielded an overall stability constant of log β_1 = 3.6, indicative of moderate binding affinity under the experimental conditions employed (Mishra and Soni, 2014).

Thiosemicarbazide and semicarbazide derivatives, particularly their heterocyclic analogs, constitute an important class of compounds recognized for their diverse chemotherapeutic potential, encompassing antitumor, antimalarial, antibacterial, and antiviral activities. In the current study, both the free ligand and its corresponding metal complexes were thoroughly characterized using a combination of techniques, including magnetic susceptibility measurements, elemental microanalysis, spectroscopic methods

(UV–Vis, FT-IR, and NMR where applicable), and molar conductivity studies. These investigations provided comprehensive insight into the structural and electronic properties of the coordination compounds. It is well documented in the literature that platinum(II) and palladium(II) complexes of heterocyclic thiosemicarbazones display pronounced antitumor efficacy, while copper(II), platinum(II), and palladium(II) complexes derived from tetradentate bis(thiosemicarbazone) scaffolds exhibit significant anticancer activity against various cell lines. Elemental microanalysis of the cadmium(II) complex, which was isolated as a white crystalline solid, unequivocally established a 1:2 (metal:ligand) stoichiometry, consistent with the general formulation $[Cd(L)_2]$, where L represents the deprotonated thiosemicarbazone/semicarbazone ligand (Adediji, Ahmed ans Lawal, 2014).

Vitamin A (retinol) is an essential micronutrient for humans, and its deficiency represents one of the most prevalent nutritional disorders worldwide. This deficiency particularly affects young children, leading to impaired growth, xerophthalmia-with the potential progression to irreversible blindness-weakening of both innate and adaptive immune defenses, exacerbation of infectious diseases, and increased morbidity and mortality risk. Vitamin A is capable of forming coordination complexes with biologically significant metal ions, including Fe(III), Mg(II), Ca(II), Zn(II), and VO(II). A series of such metal complexes have been synthesized and thoroughly characterized using a combination of spectroscopic and analytical techniques, namely ^{1}H-NMR and ^{13}C-NMR, UV–Visible spectroscopy, molar conductivity measurements, thermogravimetric analysis (TGA), and FT-IR spectroscopy. The results indicate that vitamin A behaves as a monodentate ligand in these coordination compounds. The isolated solid complexes exhibit a metal-to-vitamin A stoichiometric ratio of 1:1 (M:Vit A) (Zaky et al., 2015).

Clioquinol (5-chloro-7-iodo-8-hydroxyquinoline, CQ) is a topical agent long employed in dermatological formulations for its well-established antifungal and antibacterial activity. Beyond its conventional use, clioquinol exhibits a broad pharmacological profile that includes antimicrobial, antineoplastic, antiprotozoal, and metal-chelating properties. The present investigation describes the synthesis and comprehensive characterization of novel coordination complexes formed between clioquinol, employed as a multifunctional ligand, and the metal ions Ag(I), Hg(II), Cr(III), and Fe(III). The isolated complexes were thoroughly analyzed by elemental microanalysis and a suite of spectroscopic techniques, including proton nuclear magnetic resonance (^{1}H-NMR), Fourier-transform infrared (FT-IR), and UV–Visible electronic absorption spectroscopy. These data, supported by molar conductivity measurements, confirmed the formation of discrete coordination compounds with distinct metal-to-ligand stoichiometries: 1:1 for the silver(I) complex, 1:2 for the mercury(II) complex, and 1:3 for both

the chromium(III) and iron(III) complexes. Nanostructural features of the clioquinol–metal complexes were further evaluated using scanning electron microscopy (SEM), powder X-ray diffraction (XRD), and energy-dispersive X-ray spectroscopy (EDX). The results revealed homogeneous morphologies with particle sizes falling within the nanoscale regime and a narrow size distribution, alongside confirmation of the expected elemental composition. In addition, the antibacterial and antifungal activities of the synthesized complexes were systematically assessed, providing insight into the influence of metal coordination on the biological performance of the parent clioquinol ligand (El-Megharbel and Refat, 2015).

Levofloxacin (LFX), a third-generation fluoroquinolone antibiotic, exhibits broad-spectrum bactericidal activity and is clinically employed in the management of diverse infections, including community-acquired pneumonia, acute bacterial sinusitis, Helicobacter pylori-associated gastritis, chronic bacterial prostatitis, complicated urinary tract infections, and selected forms of infectious gastroenteritis. The present study reports the synthesis, detailed physicochemical characterization, computational analysis, and evaluation of antimicrobial potency of novel coordination complexes formed between levofloxacin and the metal ions Fe(III), Au(III), Pd(II), and Ca(II). The complexes were prepared via a straightforward reflux procedure in which levofloxacin (1 mmol) was reacted with the respective metal chloride (1 mmol) in methanol at approximately 70 °C for 2–3 hours, yielding products with a uniform 1:1 (metal:ligand) stoichiometry. Structural elucidation of the isolated complexes was accomplished through a comprehensive suite of analytical and spectroscopic techniques, including elemental microanalysis, Fourier-transform infrared (FT-IR) spectroscopy, Raman spectroscopy, proton and carbon-13 nuclear magnetic resonance (^{1}H-NMR and ^{13}C-NMR), and UV–Visible electronic absorption spectroscopy. These data collectively confirmed coordination of the levofloxacin ligand to the metal centers, predominantly through the characteristic 3-carboxylate and 4-oxo donor sites typical of fluoroquinolone metallation. In addition to experimental characterization, density functional theory (DFT) calculations were performed to gain further insight into the geometric and electronic structures of the complexes, while in vitro antimicrobial screening was conducted to assess the influence of metal coordination on the biological activity of the parent antibiotic (Al-Khodir and Refat, 2015).

The present investigation reports the systematic synthesis and comprehensive characterization of a series of novel coordination compounds formed between diclofenac-an extensively prescribed non-steroidal anti-inflammatory drug (NSAID)-and the alkaline-earth metal ions Mg(II), Ca(II), Sr(II), and Ba(II). The complexes were isolated as crystalline hydrates and subjected to rigorous structural elucidation employing a combination of

elemental microanalysis, thermogravimetric analysis (TGA/DTG), Fourier-transform infrared (FT-IR) spectroscopy, powder X-ray diffraction (XRD), and molar conductivity measurements. The analytical and spectroscopic data converge to establish an octahedral coordination environment around each metal center, with the general molecular formula $[M(diclofenac)_2(H_2O)_2]\cdot nH_2O$ (where n=0–4 depending on the metal cation). Stoichiometric evaluation consistently confirmed a 1:2 metal-to-ligand (M:L) ratio across the entire series. Preliminary biological evaluation in relevant preclinical models has revealed that administration of these alkaline-earth metal–diclofenac complexes, in contrast to the sodium salt of the free drug, significantly attenuates diclofenac-induced renal oxidative stress and markedly improves key functional parameters, including creatinine clearance and urinary N-acetyl-β-D-glucosaminidase excretion. In this study, newly formed complexes of Mg(II), Ca(II), Sr(II) and Ba(II) with diclofenac, which are successful in improving renal function parameters and antioxidant capacities in treatment with diclofenac, were made. The general formula was found to be $[M(dic)_2(H_2O)_2]*nH_2O$ and the molar ratio was 1:2 (M:dic) (El-Megharbel, Hamza and Refat, 2015).

Metformin, a biguanide derivative widely used as a first-line oral antidiabetic agent, behaves as a moderately strong base and possesses excellent metal-binding capacity. This property arises primarily from the spatial proximity of its two imino nitrogen atoms, which favors the formation of stable chelate complexes with a broad spectrum of transition metal ions, particularly Cu(II), Ni(II), and Pt(II). In the study under consideration, researchers systematically investigated the coordination chemistry of metformin with chromium(III) and oxidovanadium(IV) ions-two metals recognized for their insulin-enhancing and glucose-lowering effects-with the aim of developing improved metallotherapeutic agents for diabetes management. The complexes $[(VO)_2(MFN)_2(SO_4)_2]\cdot 2H_2O$ and $[Cr(MFN)_3]Cl_3\cdot 6H_2O$ were synthesized and characterized using molar conductivity measurements, microanalytical techniques, scanning electron microscopy (SEM), spectroscopic methods (ESR, XRD, UV-Vis, and IR), thermal analyses (TG/DTG), and effective magnetic moment determinations. Elemental analysis indicated a 1:1 metal-to-ligand ratio for the VO(II) complex and a 1:3 ratio for the Cr(III) complex. Furthermore, the antimicrobial activities of the free metformin ligand and its Cr(III) and VO(II) complexes were evaluated against various Gram-positive and Gram-negative bacterial strains, as well as different fungal strains. The results revealed moderate antimicrobial activity for the metal complexes, suggesting potential biological relevance in addition to their coordination chemistry (Adam et al., 2015).

Copper is an essential trace element indispensable for the survival of all living organisms, playing critical roles in numerous enzymatic and redox

processes. A growing body of evidence demonstrates that various copper(II) complexes possess therapeutic potential against a wide range of pathological conditions, including gastric ulcers, rheumatoid arthritis, tuberculosis, and diverse malignancies. Ofloxacin (OFL), a second-generation fluoroquinolone antibiotic, is widely employed for the effective treatment of numerous bacterial infections due to its potent DNA-gyrase inhibitory action. The researchers have successfully synthesized and fully characterized a series of seven ternary copper(II) complexes (1-7) incorporating ofloxacin as the primary antibacterial ligand and seven structurally diverse 2,2′-bipyridine derivatives (Ln) as ancillary co-ligands. Structural and compositional elucidation was achieved through a comprehensive suite of physicochemical techniques, including Fourier-transform infrared (FT-IR) spectroscopy, electronic reflectance and solution absorption spectra, LC-mass spectrometry, magnetic susceptibility measurements at ambient temperature, and elemental microanalysis. The analytical data unequivocally confirmed a 1:1:1 (metal:ofloxacin:bipyridine derivative) stoichiometry for all complexes, consistent with the general molecular formula $[Cu(OFL)(Ln)Cl]\cdot 3H_2O$. Coordination occurs predictably through the characteristic 3-carboxylate and 4-oxo donor sites of the fluoroquinolone and the bidentate chelating mode of the bipyridine co-ligand, yielding distorted octahedral or square-pyramidal geometries around the Cu(II) center. The synthesized complexes were further subjected to extensive biological evaluation encompassing antibacterial, antifungal, and potentially other pharmacological activities, the results of which highlight the beneficial influence of ternary complexation on overall therapeutic performance (Patel et al., 2015).

Indomethacin (IndoH; systematic IUPAC name: 2-[1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indol-3-yl]acetic acid) is a widely used non-steroidal anti-inflammatory drug (NSAID) recognized for its potent analgesic and anti-inflammatory properties. The present study examines the interaction of indomethacin with Cu(II) ions in both aqueous and ethanolic solutions across a range of concentrations, employing UV-Visible spectrophotometry as the primary investigative tool to monitor complexation equilibria. Overall stability constants (log β) were obtained from combined potentiometric and spectrophotometric titration data using the SQUAD software package, and the stoichiometric molar ratios of the complexes formed between Cu(II) and indomethacin in water and ethanol were calculated using continuous variation methods and are given below:

$[Cu(Indo)(H_2O)_2]^+$; $\log \beta = 6.83 \pm 0.08$; $CuIndo^+$ (M:L) ; 1:1 (in water)

$[Cu(Indo)_2(H_2O)_2]$; $\log \beta = 16.72 \pm 0.44$; $CuIndo_2$ (M:L) ; 1:2 (in ethanol)

$[Cu_2(Indo)(H_2O)_4]^{3+}$; $\log \beta = 14.06 \pm 0.43$; Cu_2Indo^{3+} (M:L) ; 2:1 (in ethanol)

$[Cu_2(Indo)_2(H_2O)_2]^{2+}$; $\log \beta = 9.38 \pm 0.05$; $Cu_2Indo_2^{2+}$ (M:L) ; 2:2 (in ethanol)

Complementary density functional theory (DFT) calculations were performed to elucidate the geometric and electronic structures of the proposed complexes, providing theoretical validation of the experimentally observed coordination modes and supporting the preference for carboxylate-O,O′ chelation supplemented by aqua ligands. The pronounced solvent dependence of speciation-ethanol promoting both increased ligand coordination and dimerization-underscores the critical role of solvation effects in governing the solution chemistry of this metallodrug system. These findings contribute valuable thermodynamic and structural insights that may inform the design of copper-based derivatives with optimized pharmacological profiles (Rodríguez-Laguna et al., 2016).

Four novel coordination compounds of the broad-spectrum antibiotic chloramphenicol with Ca(II), Fe(III), Pd(II), and Au(III) metal ions have been successfully synthesized and thoroughly characterized by a combination of elemental microanalysis, Fourier-transform infrared (FT-IR), UV–Visible electronic absorption, and ^{1}H-NMR spectroscopy, magnetic susceptibility measurements, and thermogravimetric analysis (TGA/DTA). Infrared spectral data revealed distinct coordination modes depending on the metal center. For Ca(II), Pd(II), and Au(III), chloramphenicol acts as a neutral or monoanionic bidentate ligand, binding through its two hydroxyl groups in 1:1 or 1:2 (metal:ligand) stoichiometries. In contrast, the Fe(III) complex adopts a 1:2 ratio in which the ligand undergoes keto–enol tautomerism, enabling chelation via the amide oxygen and nitrogen atoms. The nanoscale morphology of the Fe(III) and Au(III)–chloramphenicol complexes was confirmed by powder X-ray diffraction (XRD), scanning electron microscopy (SEM), and transmission electron microscopy (TEM), demonstrating uniform particle sizes within the nanometer range. Antimicrobial screening against a panel of Gram-positive and Gram-negative bacteria as well as pathogenic fungi indicated that metal coordination generally enhances the inhibitory potency of the parent antibiotic, with the Au(III) complex exhibiting the most pronounced activity. Furthermore, the cytotoxic potential of the gold(III)–chloramphenicol complex was evaluated in vitro against human colorectal carcinoma (HCT-116) and hepatocellular carcinoma (HepG-2) cell lines, where it displayed significant antiproliferative effects, suggesting possible repurposing as a metallodrug scaffold in anticancer therapy (Al-Khodir and Refat 2016).

Doxorubicin (also known as hydroxydaunorubicin; trade name Adriamycin) is a cornerstone anthracycline chemotherapeutic agent widely employed in the clinical management of a broad spectrum of malignancies, including hematological cancers, carcinomas of diverse origin, and soft-

tissue sarcomas. Structurally related to the naturally occurring antibiotic daunorubicin, doxorubicin possesses multiple oxygen-rich coordination sites that render it highly amenable to chelation by transition metal ions, a property that has been exploited to improve its pharmacokinetic profile and facilitate encapsulation within liposomal delivery systems. In the present investigation, the coordination chemistry of doxorubicin with Cu(II) and Mn(II) ions was systematically explored, resulting in the isolation and characterization of dimeric complexes exhibiting a 1:2 metal-to-ligand (ML_2) stoichiometry. Spectroscopic and analytical data confirmed that each metal center is tightly bound by two doxorubicin molecules, yielding neutral [$M(doxorubicin)_2$] assemblies. The authors propose that these Cu(II)- and Mn(II)-doxorubicin dimers represent promising candidates for liposomal encapsulation, offering a viable alternative formulation strategy that may reduce the dose-limiting cardiotoxicity associated with free doxorubicin while preserving or potentially augmenting its antitumor efficacy. Such metallated derivatives thus open new avenues in the rational design of next-generation anthracycline-based nanomedicines (Wehbe et al., 2016).

In the present study, a series of novel zinc(II)-based coordination polymers were synthesized under mild, one-pot conditions by employing the non-steroidal anti-inflammatory drug ibuprofen as an anionic co-ligand and bis(4-pyridylthio)methane (SCS) as a neutral N-donor bridging unit. Ibuprofen, widely used for the short-term relief of pain, fever, and inflammation, was successfully incorporated into the metal–organic frameworks, thereby imparting potential therapeutic functionality to the resulting materials. The compounds were fully characterized by elemental analysis, Fourier-transform infrared (FT-IR) spectroscopy, ^{1}H-NMR, UV–Visible spectrophotometry, electrospray ionization mass spectrometry (ESI-MS), and thermogravimetric analysis (TGA). These data confirmed the formation of two distinct mixed-ligand zinc(II) complexes with stoichiometries of M:ibuprofen:SCS = 1:2:1 and 1:1:1, corresponding to the molecular formulae [$Zn(ibu)_2(SCS)$] (1) and [ZnCl(ibu)(SCS)], respectively. A particularly these coordination assemblies is their straightforward synthesis and the inherent ion-exchange capability of the carboxylate-based framework, which enables pH-dependent release of the bioactive ibuprofen payload. Kinetic studies of the drug-release process demonstrated sustained and controllable delivery profiles, underscoring the potential of these zinc–ibuprofen–SCS hybrid materials as promising platforms for localized, stimuli-responsive anti-inflammatory therapy (Lago et al., 2016).

Naringin, a flavanone-7-O-glycoside composed of the aglycone naringenin and the disaccharide neohesperidose, is a naturally occurring flavonoid responsible for the characteristic bitterness of grapefruit and other citrus fruits. The current investigation details the synthesis and exhaustive

characterization of novel coordination compounds formed between naringin (or selected phenolic acids) and vanadium(V) or platinum(II) ions. Cisplatin, the prototypical platinum-based chemotherapeutic agent widely used for the treatment of solid tumors and hematological malignancies, was included as a reference standard for comparative biological evaluation. Binary complexes were consistently obtained with a ligand-to-metal stoichiometry of 2:1 (L_2M), where L denotes either deprotonated naringin (NRG^-) or various phenolate anions and M represents Pt(II) or V(V). Additionally, a series of ternary mixed-ligand complexes incorporating 1,10-phenanthroline (phen) as an auxiliary N,N-bidentate chelator were prepared, displaying a precise 1:1:1 (NRG:M:phen) composition. Structural authentication and compositional verification were accomplished through including elemental microanalysis, Fourier-transform infrared (FT-IR) and UV–Visible spectroscopy, multinuclear NMR spectroscopy, and thermogravimetric analysis (TGA/DTG). The results underscore the exceptional ligating versatility of these multidentate oxygen-donor coordination mode of naringin and phenolic acids and establish them as promising platforms for the development of hybrid metallotherapeutics that synergistically combine pronounced antioxidant activity with potent antitumor efficacy (Fazary et al., 2017).

Paracetamol (also known as acetaminophen or 4′-hydroxyacetanilide) is a widely prescribed non-opioid analgesic and antipyretic agent employed for the symptomatic relief of fever and mild-to-moderate pain, whereas diclofenac sodium is a potent non-steroidal anti-inflammatory drug (NSAID) routinely used to alleviate pain, edema, and inflammation associated with various musculoskeletal and rheumatic disorders. A series of new coordination compounds involving thorium(IV) and cerium(III) ions with two commonly prescribed analgesic agents—diclofenac sodium (diclo) and paracetamol (para)—have been synthesized under controlled conditions and subjected to comprehensive physicochemical characterization employing a range of spectroscopic (FT-IR, UV–Vis, NMR) and analytical techniques (elemental microanalysis, thermogravimetric analysis, and molar conductivity measurements). The resulting metallodrug entities exhibited markedly enhanced stability compared to the free ligands and were subsequently screened for antimicrobial activity against selected bacterial and fungal strains. Elemental analysis and supporting spectroscopic data established the following compositions and metal-to-ligand stoichiometries:

$[Ce(diclo)_3]\cdot 2H_2O$ (M:L = 1:3)

$[Th(diclo)_2(OH)_2]\cdot 4H_2O$ (M:L = 1:2, with two hydroxo co-ligands)

$[Ce(para)_3(H_2O)_3]$ (M:L = 1:3)

$[Th(para)_4(H_2O)_2]$ (M:L = 1:4)

The distinct coordination modes observed are attributable to differences in ionic radius, charge density, and preferred coordination numbers of Th(IV) and Ce(III), as well as to the specific donor atoms available in each pharmaceutical ligand (carboxylate in diclofenac; carbonyl and phenolic oxygen in paracetamol). Preliminary biological evaluation revealed that complexation with these lanthanide and actinide ions significantly potentiates the antimicrobial properties of the parent drugs, suggesting that such metal-based derivatives may offer a viable strategy for overcoming resistance mechanisms or broadening therapeutic applications (Al-Khodir, 2018).

Sulpiride (SPR), a substituted benzamide derivative, is a well-established antipsychotic agent widely employed in the management of schizophrenia. It exerts its therapeutic effect primarily through selective blockade of central dopamine D_2 receptors, with additional contributions from other neurochemical pathways. The present investigation integrates principles of coordination chemistry and nanomedicine to develop an advanced pH-responsive polymeric nanocomposite system incorporating a ruthenium(II)–sulpiride metal complex, aimed at enhancing the oral bioavailability of this poorly water-soluble drug. Spectroscopic and analytical studies confirmed the formation of a stable Ru(II)–SPR complex with a 1:2 metal-to-ligand stoichiometry. Determination of the overall stability constant yielded a value of log K = 5.45, reflecting moderate binding strength. This relatively modest stability is strategically advantageous, as it predicts controlled dissociation of the complex under the acidic conditions of the gastric environment (pH ≈ 1.5–3.5), thereby facilitating release of the active pharmaceutical ingredient in the stomach while the nanocomposite carrier protects it during transit through the harsh gastrointestinal milieu. These findings demonstrate a promising dual-role metallodrug–nanocarrier platform that simultaneously exploits reversible metal–ligand coordination for pH-triggered delivery and leverages nanoscale encapsulation to overcome the inherent limitations of sulpiride's oral bioavailability (M'bitsi-Ibouily et al., 2019).

The present investigation reports the synthesis and detailed characterization of a series of novel azomethine metal chelates derived from the tridentate dianionic Schiff base ligand 2-[(2-hydroxyphenylimino) methyl]-6-methoxyphenol (H_2OVAP), which itself exhibits pharmacological activity. Coordination compounds with Cu(II), Pd(II), Zn(II), and Cr(III) ions were successfully prepared under mild conditions. Physicochemical studies, including elemental analysis, molar conductivity measurements, electronic and infrared spectroscopy, magnetic susceptibility, and thermal analysis, confirmed the formation of mononuclear complexes with a uniform 1:1 ligand-to-metal stoichiometry (M:L). The dianionic nature of the ligand enables O,N,O-tridentate coordination, resulting in stable five- and six-membered chelate rings around the metal centers. Stability constants of the

complexes in solution at 298 K were determined spectrophotometrically. The overall formation constants (K_f) and corresponding pK values (-log K_f) are as follows:

[Pd(OVAP)]: $K_f = 2.62 \times 10^4$; pK = 4.41

[Cr(OVAP)]: $K_f = 4.15 \times 10^4$; pK = 4.61

[Cu(OVAP)]: $K_f = 1.28 \times 10^4$; pK = 4.11

[Zn(OVAP)]: $K_f = 3.85 \times 10^4$; pK = 4.58

The observed order of stability (Cr(III) > Zn(II) > Pd(II) > Cu(II)) reflects the combined influence of metal ion charge, ionic radius, and electronic configuration on the strength of the metal–Schiff base interaction. These results underscore the robust chelating ability of the H_2OVAP scaffold and highlight the potential of such metallated derivatives as candidates for further biological evaluation, particularly in light of the inherent bioactivity of the parent azomethine ligand (Abu Dief, El Sagher and Shehata, 2019).

Thiazole-containing compounds are widely recognized for their diverse pharmacological profiles, encompassing anticancer, antiviral, antibacterial, and anti-inflammatory activities. Capitalizing on these favorable properties, the present study aimed to develop novel molybdenum-based metallodrugs as potential chemotherapeutic agents. Four new oxomolybdenum(V) and oxomolybdenum(VI) complexes incorporating a benzothiazole-derived ligand (L) were successfully synthesized and fully characterized. The isolated compounds adopt the following molecular compositions:

Mononuclear species: $MoO(X)_3L$, where X = Cl (complex 1) or Br (complex 2)

Binuclear species: $Mo_2O_4(X)_2L_2$, where X = Cl (complex 3) or Br (complex 4)

Complexes 1 and 2 were obtained through direct reaction of the ligand with the MoO^{3+} moiety in a 1:1 molar ratio, whereas complexes 3 and 4 formed under slightly modified conditions favoring dimerization. DNA-binding affinities of the free ligand and the four molybdenum complexes were quantitatively assessed via spectrophotometric titration in buffered aqueous media. The apparent binding constants (Kb, M^{-1}) were determined as follows:

Free ligand (L): 2.269×10^6

Complex 1 ($MoOCl_3L$): 2.004×10^6

Complex 2 ($MoOBr_3L$): 1.987×10^6

Complex 3 ($Mo_2O_4Cl_2L_2$): 1.436×10^6

Complex 4 ($Mo_2O_4Br_2L_2$): 1.258×10^6

These findings, combined with the established redox versatility of oxomolybdenum centers, position these complexes as promising candidates for further evaluation of their cytotoxic potential and mechanism of action in neoplastic cells (Roy et al., 2019).

Cefoperazone sodium is a third-generation cephalosporin antibiotic widely employed for the management of severe bacterial infections, including those caused by multidrug-resistant Gram-negative pathogens. In the present study, a novel coordination compound was designed and synthesized by reacting cefoperazone (CFZ) with the chromium(III) precursor $[Cr(C_2O_4)_2(H_2O)_2]^-$, with the aim of generating a metallodrug capable of retaining or potentially enhancing the antimicrobial profile of the parent antibiotic. The resulting complex was thoroughly characterized using a combination of spectroscopic and analytical techniques, including UV–Visible and Fourier-transform infrared (FT-IR) spectroscopy, elemental analysis, and thermal methods. These data unequivocally established a 3:1 metal-to-ligand stoichiometry, corresponding to the formulation $[Cr_3(CFZ)]$ (or an equivalent polynuclear assembly in which three Cr(III) centers are bridged by cefoperazone anions). In vitro antimicrobial susceptibility testing against a panel of clinically relevant bacterial strains demonstrated that the chromium(III)–cefoperazone complex exhibits inhibitory potency comparable to that of free cefoperazone sodium. This retention of bioactivity, despite extensive coordination to the metal centers, underscores the feasibility of employing bioessential metal ions as carriers or modulators in cephalosporin-based metallodrug design, offering a promising strategy for circumventing resistance mechanisms or improving pharmacokinetic properties (Behera and Behera, 2020).

Prednisolone, a synthetic glucocorticoid (chemical formula $C_{21}H_{28}O_5$), is widely prescribed for the management of a broad spectrum of acute and chronic inflammatory conditions, including allergic disorders, bronchial asthma, and rheumatoid arthritis. In the present investigation, a series of mixed-ligand coordination compounds incorporating prednisolone (L) and paracetamol (L′, $C_8H_9NO_2$) as co-ligands with copper(II) and cobalt(II) ions were successfully synthesized and rigorously characterized. Spectroscopic and analytical studies (FT-IR, UV–Vis, ESR, magnetic susceptibility, and thermal analysis) revealed two distinct stoichiometric patterns:

1:1:1 (metal:prednisolone:paracetamol) complexes of the general formula $[Cu(L)(L')(H_2O)]$ and $[Co(L)(L')(H_2O)]$

1:2:1 (metal:prednisolone:paracetamol) complexes formulated as $[Cu(L)_2(L')(H_2O)]$ and $[Co(L)_2(L')(H_2O)]$

In both series, coordination occurs primarily through the deprotonated enolate oxygen of prednisolone and the carbonyl oxygen of the paracetamol acetamido group, with an aqua ligand completing the coordination sphere.

Comparative in vitro antimicrobial screening against representative Gram-positive and Gram-negative bacterial strains as well as pathogenic fungi demonstrated that the mixed-ligand Cu(II) and Co(II) complexes exhibit significantly enhanced antifungal and antibacterial potency relative to the free prednisolone and paracetamol ligands. This synergistic improvement is attributed to increased lipophilicity, facilitated cellular uptake, and possible metal-mediated redox mechanisms, highlighting the therapeutic potential of such metallodrug constructs in combating microbial infections associated with glucocorticoid therapy (Olagboye, Adekeye and Akinwunmi, 2020).

Levofloxacin, a third-generation fluoroquinolone antibiotic, exhibits broad-spectrum bactericidal activity and is clinically indicated for the treatment of diverse infections, including community-acquired pneumonia, acute bacterial sinusitis, Helicobacter pylori eradication regimens, chronic bacterial prostatitis, complicated urinary tract infections, and selected forms of bacterial gastroenteritis. Previous studies have established that coordination of fluoroquinolones to metal ions often preserves or modestly enhances their antimicrobial potency. In the present investigation, the complexation equilibria of levofloxacin hemihydrate (LFH) with a series of transition metal ions (Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}) and alkaline-earth metal ions (Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}) were systematically examined using UV–Visible spectrophotometry, potentiometry, and conductometric titrations. Complexes with 1:1 and 1:2 (metal:ligand) stoichiometries were identified. It was observed that the binding constant (Kf) of LFH with alkaline earth metal ions increased with increasing temperature, while the opposite trend was observed in the case of transition metal ions. DFT results showed that 1:1 complexes were more stable than 1:2 complexes. In a complementary study, novel Co(II), Ni(II), Cu(II), and Zn(II) complexes of a tetradentate Schiff base derived from 3-(2-aminoethylamino) quinoxalin-2(1H)-one were synthesized and exhaustively characterized by by spectrophotometric and various methods. Stoichiometric and spectroscopic evidence confirmed mononuclear 1:1 (metal:ligand) formulations. DNA-binding studies established covalent adduct formation, while in vitro cytotoxicity assays against a panel of human cancer cell lines revealed significantly higher potency than cisplatin, underscoring the potential of these quinoxaline-based metallodrugs as next-generation anticancer agents (Uddin et al., 2021).

A novel multifunctional thiazole derivative, namely 2-amino-6-oxo-3-(piperidin-1-ylcarboximidamido)-4-(4-methoxyphenyl)-6,7-dihydro-4H-pyrano[2,3-d]thiazole (abbreviated as MPTP), has been designed and synthesized. This compound exhibits a broad spectrum of promising pharmacological activities, including antibacterial, antifungal, antioxidant, and anticancer effects. In the current investigation, coordination compounds of MPTP with Pd(II), Fe(III), and Cu(II) ions were prepared and subjected

to comprehensive structural and thermodynamic characterization using a combination of their stoichiometry and stability constants were determined using analytical and spectral techniques. Analytical and spectroscopic data consistently demonstrated the formation of mononuclear complexes with a 1:1 metal-to-ligand stoichiometry. Stability constants of the resulting species, determined in solution at ambient temperature, are reported as logarithmic values:

$[Cu(MPTP)]^{2+}$: log K = 5.83

$[Ce(MPTP)]^{3+}$: log K = 6.30

$[Pd(MPTP)]^{2+}$: log K = 6.04

The relatively high stability constants (in the range of 10^5–10^6 M^{-1}) reflect strong coordination of the thiazole-based ligand to the metal centers, primarily through its nitrogen-rich amidino and amino donor sites. These findings underscore the potential of MPTP-derived metallodrugs as therapeutically relevant agents, warranting further evaluation of their biological profiles in comparison to the free organic scaffold (Abu-Dief et al., 2021).

Ciprofloxacin (1-cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid) is a second-generation fluoroquinolone antibiotic widely employed for the treatment of infections caused by both Gram-negative and Gram-positive pathogens. The present study examines the formation, stability, and biological implications of ternary Metal(II)–ciprofloxacin–glycine complexes (M:Cf:Gly) generated in aqueous solution through initial coordination of Cu(II), Zn(II), or Mn(II) ions with ciprofloxacin and glycine (1:1:1 stoichiometry), followed by metal-mediated condensation of the primary amine of glycine with the piperazine moiety of ciprofloxacin, yielding a Schiff base (imine) linkage within the coordination sphere. Speciation studies employing potentiometric and spectrophotometric methods established overall ternary complexes formation constantsand stability constants follows:

$[Cu(cf)gly(H_2O)_3]$ H_2O k=6.75x10^4 ; K=317 (mol^2 dm^6)

$[Zn(cf)gly(H_2O)_3]$ $2H_2O$ k=5.43x10^4 ; K=1460 (mol^{-2} dm^6)

$[Mn(cf)gly(H_2O)_3]$ H_2O k=12.3x10^4 ; K=988 (mol^{-2} dm^6)

In vitro antibacterial assays against representative bacterial strains demonstrated that the ternary metallated species exhibit comparable or moderately enhanced activity relative to free ciprofloxacin. These findings suggest that such in situ-generated ternary complexes may offer advantages in terms of altered pharmacokinetic profiles, reduced resistance development, or improved cellular uptake, thereby presenting a promising strategy for enhancing the therapeutic utility of fluoroquinolone antibiotics (Panda, 2021).

Ciprofloxacin hydrochloride (CPFH), a synthetic fluoroquinolone antibiotic bearing the systematic name 1-cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid hydrochloride, is widely prescribed for the treatment of a broad range of bacterial infections, including urinary tract infections, gonorrhea, and anthrax prophylaxis. Its bactericidal efficacy is known to be modulated by coordination interactions with metal ions. In the present investigation, the coordination chemistry of CPFH with biologically relevant divalent transition metal ions-namely Zn(II), Cu(II), and Ni(II)-was systematically explored in aqueous media using a combination of spectrophotometric titration, potentiometric methods, and supporting spectroscopic techniques. The results unequivocally demonstrate the formation of mononuclear 1:1 (metal:ligand) complexes across all systems studied. Stability constants (expressed as log K_f) for the respective $[M(CPFH)]^{2+}$ species were determined as follows:

$[Ni(CPFH)]^{2+}$: log K_f = 3.71

$[Cu(CPFH)]^{2+}$: log K_f = 3.83

$[Zn(CPFH)]^{2+}$: log K_f = 3.71

These thermodynamic data provide valuable insight into the speciation of ciprofloxacin in biological environments and underscore the potential influence of metal coordination on its pharmacokinetic behavior and antibacterial performance (Uddin et al., 2022).

Cefotaxime (CFT), a third-generation parenteral cephalosporin antibiotic, exhibits potent broad-spectrum bactericidal activity against both Gram-positive and Gram-negative pathogens, including many β-lactamase-producing strains. It is clinically indicated for the management of serious infections encompassing lower respiratory tract infections, complicated urinary tract infections, meningitis, septicemia, skin and soft-tissue infections, intra-abdominal infections, bone and joint infections, pelvic inflammatory disease, and gonorrhea. Motivated by substantial evidence that coordination of cephalosporins to metal centers can favorably modulate their pharmacokinetic properties, stability, and biological efficacy, the present study reports the synthesis and characterization of novel metallodrug derivatives incorporating cefotaxime with platinum(II) and iron(II) ions. The investigations spectrophotometric measurements and other analytical technics, established a uniform 1:1 metal-to-ligand stoichiometry (M:L) for both the Pt(II)–cefotaxime and Fe(II)–cefotaxime complexes. These findings provide a solid basis for further evaluation of the antimicrobial spectrum, cytotoxic profile and mechanical properties of metallophotaxime structures. (Demir et al., 2022).

Eflornithine (α-difluoromethylornithine), an irreversible inhibitor

of ornithine decarboxylase, is a clinically established antiprotozoal agent (molecular formula $C_6H_{12}F_2N_2O_2{\cdot}HCl{\cdot}H_2O$, abbreviated as EHM). In this investigation, novel coordination compounds of eflornithine hydrochloride monohydrate with biologically relevant divalent metal ions-Cu(II), Co(II), and Ni(II)-were successfully synthesized and subjected to comprehensive physicochemical characterization. Spectrophotometric studies supported by unambiguously confirmed the formation of stable mononuclear complexes with a consistent 1-2 metal-to-ligand stoichiometry ($M:L_2$). The resulting $[M(EHM)_2]^{2+}$ species exhibit enhanced spectral features compared to the free ligand, reflecting coordination through the carboxylate and/or α-amino groups of the eflornithine scaffold. These metallated derivatives are currently under evaluation for their anti-inflammatory and antiproliferative properties relative to the parent drug (Ayipo, 2022).

Cefixime and cefuroxime are third- and second-generation cephalosporin antibiotics, respectively, widely employed in the clinical management of various bacterial infections, including those affecting the upper and lower respiratory tract, uncomplicated gonorrhea, and chronic obstructive pulmonary disease (COPD). In this study, the researchers synthesized novel coordination complexes of cefixime and cefuroxime with manganese(II) ions with the primary objective of enhancing the antimicrobial potency of the parent ligands. Using a combination of spectrophotometric techniques (UV–Vis, FT-IR, and AAS), the stoichiometry of the resulting complexes was determined to be 1:1 (metal:ligand), yielding the formulations [Mn(CFU) Cl_2] for the cefuroxime complex and [Mn(CFE)Cl_2] for the cefixime complex, where CFU and CFE represent the deprotonated forms of cefuroxime and cefixime, respectively. Antimicrobial susceptibility testing revealed that the manganese(II)–cefuroxime complex exhibited significantly greater antibacterial activity against the tested microbial strains compared to the free cefuroxime ligand, whereas the activity enhancement was less pronounced or variable in the case of the cefixime complex. These findings suggest that coordination of cephalosporins with Mn(II) may represent a promising strategy for improving the therapeutic efficacy of existing β-lactam antibiotics (Makinta et al., 2022).

2-Quinolinone (2-quinolinol/2-hydroxypyridine quinoline form) is a heterocyclic organic compound that exhibits lactam–lactim tautomerism. It serves as a privileged scaffold in pharmaceutical chemistry and constitutes the core structure of numerous pharmacologically active agents, including antipsychotic and cardiovascular drugs. The compound and its derivatives display a broad spectrum of biological activities, such as antibacterial, anticancer, antimalarial, antipyretic, antifungal, anti-inflammatory, and antiviral properties. In the present study, a di-2-quinolinone-based bis-benzylidene ligand derived from terephthalaldehyde was synthesized by

analytical technics (UV-Vis, IR) and subsequently complexed with divalent transition metal ions, namely Mn(II), Fe(II), Co(II), Ni(II), Cu(II), and Zn(II). Characterization studies revealed the formation of binuclear metal complexes with 2:2 (metal:ligand) stoichiometry. In the absence of co-ligands, complexes of the general formula $[M_2L_2]Cl_4$ were obtained, whereas in the presence of 1,10-phenanthroline as a secondary ligand, ternary complexes of the type $[M_2L_2(1,10\text{-phen})_2]Cl_4$ were formed. The structural composition and coordination behavior of both series of complexes were thoroughly elucidated using a combination of spectroscopic and analytical techniques (Alnuaimy, 2022).

Nalidixic acid (NAL), a member of the quinolone class, exhibits bactericidal or bacteriostatic activity against Gram-negative bacteria and is used in the treatment of various infections of the urinary and gastrointestinal tracts. The aim of this study was to determine the effects of Cu^{2+}, Zn^{2+}, and Mn^{2+} ions on the thermodynamic parameters, protonation constants, and structural properties of nalidixic acid complexes. In this work, potentiometric, UV spectrophotometric, MS, and MS/MS techniques were employed to elucidate the thermodynamic behavior, protonation equilibria, and structural characteristics of nalidixic acid complexes with Cu^{2+}, Zn^{2+}, and Mn^{2+}. The results indicated that the complexes formed with Zn^{2+} and Mn^{2+} follow a 1:1 metal-to-ligand (M:L) stoichiometry, whereas Cu^{2+} forms both 1:1 and 1:2 ($M:L_2$) complexes. The protonation constants determined at 25 °C were $\log\beta(Zn:L) = 3.45$, $\log\beta(Mn:L) = 4.24$, $\log\beta(Cu:L) = 5.160$, and $\log\beta(Cu:L_2) = 5.160$. (Carnamucio et al., 2023).

The benzothiazole derivative 2-(1,3-benzothiazol-2-ylimino)-2,3-dihydro-1H-imidazol-4-ol (abbreviated as BSI) is used in the treatment of various diseases as anthelmintic, antiulcerative, antiviral, anti-inflammatory, antihistamine, and antioxidant. It has attracted considerable attention owing to its versatile biological and coordination properties. In the present investigation, novel coordination complexes of the BSI ligand with Pd(II), Ni(II), and Fe(III) ions were successfully synthesized and comprehensively characterized spectroscopic and analytical. Stoichiometric studies, supported by Job's method and molar ratio plots, unequivocally confirmed a metal-to-ligand binding ratio of 1:2 (ML_2) for both the Fe(III) and Ni(II) complexes, whereas a 1:1 (ML) stoichiometry was established for the Pd(II) complex. The overall stability constants (log β) of the formed species were determined potentiometrically at constant ionic strength and 25 ± 0.1 °C, yielding the following values:

$\log \beta\ [Fe(BSI)_2]^{3+} = 7.98$

$\log \beta\ [Ni(BSI)_2]^{2+} = 7.87$

$\log \beta\ [Pd(BSI)]^{2+} = 4.95$

These results highlight the relatively high affinity of the BSI ligand toward Fe(III) and Ni(II) ions compared to Pd(II), consistent with the ligand's donor atom set and the preferred coordination geometries of the respective metal centers (Abu-Dief et al., 2023).

Moxifloxacin, a fourth-generation fluoroquinolone antibiotic, exerts its therapeutic effect acts by killing or inhibiting the growth of bacteria. In this study, six novel metal-based coordination compounds were designed and synthesized utilizing Fe(III), Cu(II), and Hg(II) ions. Three mononuclear complexes were obtained using moxifloxacin as the sole organic ligand, while the other three mixed-ligand complexes were prepared by incorporating hydrazine as a secondary co-ligand together with moxifloxacin. All complexes exhibited a metal-to-ligand stoichiometry of 1:1 (M:L) in their predominant forms. The synthesized compounds were comprehensively characterized using UV-Vis, FT-IR, elemental analysis (CHN), atomic absorption spectroscopy, TGA, scanning electron microscopy (SEM), and powder XRD. Biological screening demonstrated that the metal complexes possess significantly enhanced antifungal efficacy compared to free moxifloxacin, with several derivatives exhibiting potent activity against resistant fungal strains. Furthermore, all these compounds were screened for their antimicrobial, cytotoxic, and antidiabetic potential. These findings conclusively demonstrate that coordination of moxifloxacin with Fe(III), Cu(II), and Hg(II) ions-particularly in the presence of hydrazine as a co-ligand-markedly potentiates its antifungal and antidiabetic properties. This enhancement underscores the promising role of metallopharmaceutical in repurposing existing antibiotics and positions as valuable lead structures for the future development of dual-action antifungal and antidiabetic therapeutic agents (Ali et al., 2023).

In a similar study, three novel mixed-ligand coordination compounds have been successfully synthesized utilizing moxifloxacin (MOX) as a primary antibiotic ligand and a trisubstituted imidazole derivative (TSI) as an ancillary ligand, in the presence of Fe(III), Ni(II), and Cu(II) metal ions. Due to the increasing interest and need for innovative bioactive molecules in the healthcare field in recent years, this study was conducted on the synthesis of mixed ligands formed by the active pharmaceutical ingredients moxifloxacin (MOX) and tri-substituted imidazole (TSI) with the metals Fe(III), Ni(II), and Cu(II).The synthesized complexes were thoroughly characterized using analytical techniques, including elemental (CHN) analysis, molar conductivity measurements, UV-Visible spectroscopy, Fourier-transform infrared (FT-IR) spectroscopy, electrospray ionization mass spectrometry (ESI-MS), Mass spectrometry(MS), and thermogravimetric analysis (TGA/DTG). These investigations provided unequivocal evidence of coordination through the carbonyl oxygen and carboxylate oxygen atoms of moxifloxacin, as well as the hydroxyl oxygen and one ring nitrogen atom of the trisubstituted imidazole

moiety. Stoichiometry in solution was established as 1:1:1 (M:MOX:TSI) for all three ternary species. Biological evaluation revealed that the metal complexes exhibit significantly enhanced antimicrobial efficacy against some bacteria, fungi and standard reference agents. In addition, the ternary systems demonstrated markedly superior radical-scavenging capacity and antioxidant assays. These findings underscore the beneficial role of mixed-ligand ternary complexes thus emerge as promising multifunctional metallopharmaceutical candidates with potential applications in combating microbial resistance and oxidative stress-related disorders. (Abd El-Lateef, 2023).

Clotrimazole (CTZ) and ketoconazole (KTZ) are nitrogen-containing heterocyclic antifungal agents of the imidazole class with exceptional biological and pharmaceutical properties.For over a century, the imidazoles has remained a cornerstone of medicinal chemistry research due to its coordination chemistry and broad-spectrum bioactivity. Clinically, both compounds are widely employed in the management of diverse fungal infections, including vaginal candidiasis, oral thrush, dermatophytoses (ringworm, athlete's foot, and jock itch), and pityriasis versicolor. In the present study, a series of novel silver(I) coordination compounds derived from clotrimazole (CTZ) and ketoconazole (KTZ)-both representatives of the N-heterocyclic azole family-were systematically synthesized and characterized using by analytical and spectroscopic techniques. Reactions of CTZ and KTZ with silver(I) salts in the presence of iodide-functionalized imidazolium co-ligands afforded six distinct complexes with metal-to-ligand stoichiometries of 1:1 or 1:2 (M:L). The isolated species were formulated as: $[Ag(L^1)I]\cdot H_2O$, $[Ag(L^2)I]$, $[Ag(L^1)_2]I\cdot 4CH_2Cl_2$, $[Ag(L^2)_2]I\cdot 2CH_2Cl_2$, $[Ag(CTZ)_2]NO_3$, and $[Ag(KTZ)_2]NO_3$ (where L^1 and L^2 represent deprotonated forms or modified derivatives of the parent azole ligands under the reaction conditions).Stability constants of the complexes in solution were determined spectrophotometrically, revealing the following values:

$\log \beta\ [Ag(L^1)I]\cdot H_2O = 6.00$

$\log \beta\ [Ag(L^2)I] = 6.23$

$\log \beta\ [Ag(L^1)_2]I = 6.48$

$\log \beta\ [Ag(L^2)_2]I = 6.68$

$\log \beta\ [Ag(CTZ)_2]^+ = 6.03$

$\log \beta\ [Ag(KTZ)_2]^+ = 6.60$

The data reveal that bis-chelated and ketoconazole-derived complexes generally exhibit superior thermodynamic stability compared to their counterparts. These findings highlight the synergistic potential of combining the established antifungal efficacy of azole pharmacophores with the intrinsic

antimicrobial properties of silver(I) ions, paving the way for the development of next-generation metallodrug hybrids with enhanced potency and possibly broader therapeutic applications. (Dos Reis et al., 2023).

In another study with levofloxacin,, a novel ternary cobalt(II) complex incorporating levofloxacin as the primary anionic ligand and imidazole (ImH) as a neutral co-ligand (denoted CoLevim) was successfully synthesized and fully characterized using FT-IR, UV–Vis, Fluorescence spectra, Luminescence spectrophotometer, and thermal analyzer. When Levofloxacin (HLVX), a known quinolone drug, forms a complex with Co2+, the synergistic mechanism between the drug molecule and the metal center results in enhanced physicochemical and biological properties of the metal-drug complex. Interaction studies have shown that both free levofloxacin and the CoLevim complex bind to CT-DNA predominantly via intercalation, forming a stable complex (M:L; 1:1). The binding constant of the Co(II) complex of HLVX and ImH (CoLevim) is higher than that of HLVX (Kb = 4.8×10^3 M^{-1}) and the quinolone, enabling it to act as a broad-spectrum antibiotic that inhibits the growth of pathogenic bacteria. More importantly, the complex exhibits antitumor activity and antiproliferative potential against breast cancer cells, with a decrease observed in viable cells even at very low concentrations. CoLevim's behavior as an effective resistance-modifying agent offers promise for the development of new antimicrobial and DNA-targeted therapeutic agents as a metal-drug complex that will respond to treatment in patients developing drug resistance in cancer treatment. (Singh et al., 2023).

Acylhydrazones (HL) are important Schiff bases that can form highly stable coordination compounds with transition metal ions. Such metal complexation frequently results in significant enhancement of the inherent biological activity of the parent organic ligand. Leveraging the versatile coordination chemistry of the pyrazole moiety, a novel pyrazole-derived acylhydrazone Schiff (HL) was designed, synthesized, and fully characterized. The interaction of the free ligand (HL) and its metal complexes with bovine serum albumin (BSA) was investigated. Subsequent reactions of HL with Ni(II), Co(II), Zn(II), and Cu(II) salts in the presence of an ancillary pyrazole-based ligand (L) yielded four new mixed-ligand complexes with a 1:1:1 (M:HL:L) stoichiometry. The complexes were formulated as $[Ni(HL)L]NO_3$, $[Co(HL)L]NO_3$, $[Zn(HL)L]NO_3$, and $[Cu(HL)L]NO_3$ on the basis on the basis of fluorescence spectroscopy, UV–Vis and and microcalorimetry technics. The apparent binding constants (K_b) were determined as follows: HL: 2.0×10^5 M^{-1}

$[Co(HL)L]^+$: 4.8×10^5 M^{-1}; $[Zn(HL)L]^+$: 3.6×10^5 M^{-1}

$[Cu(HL)L]^+$: 2.3×10^6 M^{-1} ; $[Ni(HL)L]^+$: 7.6×10^6 M^{-1}

Notably, the Ni(II) and Cu(II) complexes exhibited substantially stronger

affinity toward BSA than the free ligand, suggesting that coordination markedly improves protein-binding capability. These results underscore the value of strategic metallation of pyrazole-containing acylhydrazones as a promising approach for developing new bioactive agents with optimized pharmacokinetic profiles. (Zhang et al., 2023).

A series of coordination compounds were synthesized using a tridentate Schiff base ligand derived from 2-chlorobenzaldehyde, hydrazine, and glycine hydrate-formally designated as (2-chlorobenzylidene)amino)acetohydrazide hydrate (L)-with Cu(II), Co(II), and Ni(II) ions. Physicochemical characterization employing elemental analysis, atomic absorption, FT-IR, molar ratio analysis UV–Vis spectroscopy, magnetic properties, XRD, NMR and conductance measurements of mononuclear complexes with a 1:1 metal-to-ligand stoichiometry. The complexes were formulated as $[M(L)(H_2O)_3]Cl_2$ (where M = Cu(II), Co(II), or Ni(II)), indicating coordination through the azomethine nitrogen, hydrazide carbonyl oxygen, and carboxylate oxygen of the ligand, with three water molecules completing the coordination sphere. Preliminary biological screening revealed that both the free ligand (L) and its metal complexes (Schiff base and hydrazide groups) exhibited significant antimicrobial, antioxidant, and antifungal activities, with the complexes generally exhibiting higher potency compared to the parent Schiff base-hydrazide ligand. These results highlight the beneficial role of metal coordination in enhancing the bioactivity of multifunctional hydrazone-based ligands. (Al-Azab, Mosa'd Jamil and Al-Gaadbi, 2023).

Humic acids (HA) are complex, heterogeneous mixtures of polydisperse organic macromolecules formed through the microbial decomposition of plant and animal residues via a combination of chemical and biological processes.In the present study, a refined UV–Visible spectrophotometric methodology was developed for the accurate determination of conditional stability constants of Fe(III) complexes in aqueous systems. The reliability and applicability of the method were rigorously validated using humic acid (HA) as a representative natural dissolved organic matter, alongside a series of well-characterized synthetic and pharmaceutical ligands: ethylenediaminetetraacetic acid (EDTA), nitrilotriacetic acid (NTA), oxalic acid (OA), kanamycin (Kana), and tetracycline (TTC).Beyond quantifying conditional formation constants, the approach enabled comprehensive characterization of complex speciation and stoichiometry. Stoichiometric ratios were determined by Job's method of continuous variations, while binding strengths were assessed through spectrophotometric titration and supported by numerical modeling.The resulting conditional stability constants (log K′) at the experimental pH and ionic strength are as: Fe(III)–EDTA: 7.08; Fe(III)–NTA: 4.67; Fe(III)–OA: 4.32; Fe(III)–TTC: 4.28; Fe(III)–Kana: 3.07; Fe(III)–HA: 5.02. In every instance, a 1:1 (Fe:L) binding stoichiometry was confirmed. These findings underscore

the strong complexing capacity of Fe(III) toward both anthropogenic chelators and natural humic substances, while revealing comparatively weaker interactions with the tested antibiotics. The results contribute significantly to understanding iron speciation, mobility, and bioavailability in natural waters and biologically relevant media (Zhang, Wu and Sun, 2023).

Flumequine (FLQ), a first-generation synthetic fluoroquinolone antibiotic, is widely recognized for its efficacy against urinary tract infections and broad-spectrum activity toward both Gram-positive and Gram-negative bacterial pathogens. In the present study, novel lanthanide(III) coordination compounds incorporating flumequine as the primary ligand were designed, synthesized, and comprehensively evaluated for their structural features and biological properties. The complexes, formulated as $[M(FLQ)_2Cl(H_2O)]\cdot nH_2O$ (where M = La^{3+}, Sm^{3+}, or Tb^{3+}; n = 2–4), were prepared by reacting the corresponding lanthanide(III) chlorides with flumequine under controlled conditions. The compounds were thoroughly characterized using a multifaceted analytical approach, including elemental analysis, Fourier-transform infrared (FT-IR) spectroscopy, molar conductivity measurements, UV–Visible spectroscopy, powder X-ray diffraction (PXRD), scanning electron microscopy (SEM), energy-dispersive X-ray analysis (EDX), and thermogravimetric analysis (TGA/DTG). These investigations confirmed a 1:2 (metal:ligand) stoichiometry, with flumequine acting as a bidentate anionic ligand coordinated through its carbonyl and carboxylate oxygen atoms. Spectral and thermal data further supported an octahedral coordination geometry around the lanthanide center, completed by one chloride ion and one coordinated water molecule. Biological screening revealed that the La(III), Sm(III), and Tb(III) complexes exhibit significantly enhanced antibacterial activity against selected pathogenic strains compared to free flumequine. Moreover, in vitro cytotoxicity assays against various human cancer cell lines demonstrated pronounced antitumor potential, with certain derivatives displaying superior efficacy and selectivity relative to the parent drug. Overall, the results highlight the synergistic benefits of lanthanide coordination in markedly potentiating both the antimicrobial and anticancer properties of flumequine. These findings position the reported complexes as promising dual-action metallopharmaceutical candidates worthy of further preclinical development for infectious diseases and oncological applications (El-Habeeb, 2024).

Amoxicillin, a broad-spectrum aminopenicillin antibiotic of the penicillin family, is widely used clinically for the treatment of diverse bacterial infections, including otitis media, streptococcal pharyngitis, pneumonia, skin and soft-tissue infections, odontogenic infections, and urinary tract infections. In the present investigation, a novel Schiff base derivative of amoxicillin (denoted AA) was synthesized via condensation

with acetaldehyde. This ligand was subsequently employed to prepare a mononuclear vanadyl(II) complex with a 1:2 (metal:ligand) stoichiometry. The primary aim was to elucidate the structural characteristics and therapeutic potential of these amoxicillin-derived compounds, with particular focus on their dual efficacy against breast cancer and diabetes. The Schiff base ligand (AA) and its vanadyl complex were comprehensively characterized using FT-IR, UV-Vis, and NMR spectroscopy, elemental analysis, thermal analysis, and magnetic susceptibility measurements. Biological evaluation, supported by in vitro assays and in silico molecular docking studies, revealed strong DNA-binding affinity, marked anti-inflammatory activity, potent inhibition of key diabetes-related enzymes (α-glucosidase and α-amylase), and significant antiproliferative and antimigratory effects against breast cancer cell lines. These findings clearly demonstrate that targeted chemical modification of the amoxicillin scaffold-through straightforward Schiff base formation followed by 1:2 coordination with vanadyl(II)-confers remarkable dual-action therapeutic properties. The resulting complex functions both as a DNA-intercalating anticancer agent capable of suppressing tumor growth and metastasis and as an effective enzyme inhibitor with anti-inflammatory benefits relevant to diabetes management. In conclusion, this study establishes amoxicillin-derived vanadyl complexes as highly promising multifunctional metallopharmaceutical candidates for addressing two major global health challenges: cancer and diabetes (Banbela, 2025).

3. THE RENAISSANCE OF METALLODRUGS THERAPEUTICS: PROSPECTS FOR THE COMING DECADE

Coordination complexes formed between established pharmaceutical agents (fluoroquinolones, β-lactams, azoles, macrolides, etc.) and transition-metal or lanthanide ions represent one of the most dynamic frontiers in contemporary bioinorganic chemistry. The journey that began with the approval of cisplatin in 1978 has now entered a new era, in which clinically used organic drugs are systematically repurposed and potentiated through deliberate metallation. Metal ions play indispensable roles in biological systems, mediating catalysis, electron transfer, and structural stabilization. Consequently, metal-based pharmacophores are not a novel concep-platinum drugs have long been staples in oncology. However, the strategic coordination of approved organic drug molecules with metal centres has opened dramatically broader therapeutic horizons in recent years. These hybrid complexes, thanks to their chemically and biologically tunable properties, have emerged as key components of "smart drug" design.

The inherent responsiveness of metal-ligand bonds to physiological stimuli such as pH, light, or redox potential offers unique opportunities for controlled drug release. For instance, complexes that disassemble selectively in the acidic tumour microenvironment enhance target specificity, while

photoresponsive systems enable on-demand activation for site-specific therapy. Such attributes position metal-drug conjugates as leading candidates for next-generation controlled-delivery platforms.

Metal-based therapeutic platforms are now widely seen as one of the most promising directions in modern medicine, and the reasons are straightforward. A single compound can attack disease through several independent mechanisms at the same time: it can cycle electrons to generate reactive oxygen species, become activated by light, and act as a catalyst-all while retaining the original drug's primary biological target. This multi-pronged attack makes resistance far harder to evolve, whether in cancer cells or bacteria, because any mutant would need to escape both the parent drug's effect and the additional metal-driven oxidative damage simultaneously.

Progress from idea to clinic is also dramatically faster and cheaper than conventional drug discovery. Instead of designing entirely new molecules from scratch, researchers simply coordinate metal ions to existing, clinically proven, off-patent drugs. Most of the safety, dosing, and behaviour-in-humans data already exist, so development risk and time are slashed. Moreover, swapping metals or tweaking the surrounding ligands gives exquisite control over how the drug behaves in the body - its water solubility, stability in blood, ability to cross cell membranes, and even which organelle it homes in on (the mitochondrion is a frequent and highly effective choice).

Looking ahead a decade, some organometallic complexes are ready to enter Phase 1 trials as serious non-platinum options in oncology; light-activated metal drugs for both photodynamic therapy and photoactive chemotherapy are on a realistic path toward market approval within the next 10 years, starting with localized infections and accessible tumors; and AI-guided, high-throughput screening of large metal-drug libraries suggests that the usual 8-10 year optimization phase could be reduced to just 2-4 years. In short, these systems combine scientific elegance with practical advantages that are hard to match which is why they are increasingly regarded as a cornerstone of tomorrow's therapeuticsIn conclusion, coordination complexes of approved drug molecules with metal ions occupy a pivotal boundary domain for the development of innovative therapeutic strategies. With continuing advances in molecular design, target specificity, multimodal action, and theranostic potential, these systems are poised to assume an increasingly central role in oncology, infectious diseases, and personalised medicine. Over the coming years, refinements in toxicity management, targeting strategies, and nanotechnology integration are expected to drive a substantial expansion of their clinical applications. Far from remaining academic curiosities, metal–drug hybrids are on a clear trajectory to become a mainstream therapeutic modality in 21st-century medicine.

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Chapter 4

NATURE'S COLOR PALETTE: NATURAL DYES IN FOODS

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1. INTRODUCTION TO NATURAL FOOD COLORINGS.

The food industry uses a large number of additives. Colorants, one of the most important, are used to improve the appearance of products, make them more attractive, compensate for color loss from raw materials, and establish a color standard that meets consumer expectations. Today, the food industry uses a variety of natural and artificial substances. These substances encompass a wide range of components with very different technological functions, including "preservatives, conditioners, emulsifiers, colorants, acids, bases, flavor enhancers, sweeteners, thickeners, solvents, polishing and bleaching agents, anti-caking agents, antioxidants, and flavor components" (Sengar and Sharma, 2014).

The use of food additives is regulated by international authorities to protect consumer health and ensure commercial standards, with each substance having specific purity criteria and acceptable daily intake values. Natural additives are obtained from plant, animal, or mineral sources, while artificial additives are generally produced through chemical synthesis. Although the primary aim of both types of additives is to contribute to food safety and quality, public perception, especially towards artificial additives, is often negative. Therefore, it is important to scientifically evaluate both natural and artificial food components, understand their toxicological properties, and determine their safe usage limits. Color, one of the most important sensory cues consumers use in evaluating food, is as decisive as taste and aroma in the evaluation of a food, so colorants have become one of the fundamental components of modern food technology (Branen and Haggerty, 2002).

Color is a strong guiding factor in the initial stage of purchasing behavior and directly affects the perception of basic quality elements such as the freshness, ripeness, naturalness, and reliability of the product. The color, size, and physical structure of the food play a critical role in the formation of the overall quality impression; an aesthetically appealing color appearance supports the perception that the product is fresher, of higher quality, and more delicious. Therefore, the food industry widely uses colorants to meet consumer expectations and increase the marketability of products. Food colorings are currently classified into four main categories: inorganic colors, synthetic colors, nature-identical colors, and natural colors (Singh et al., 2023).

It is thought that the application of coloring food began around 1500 BC, with ancient Egyptian inscriptions recording the coloring of medicines, while methods of wine coloring were described by the Romans in 400 BC. Although the historical development of the synthetic dye industry is generally associated with W. H. Perkin's synthesis of mauveine in 1856, the foundations of this field were laid much earlier. In the 17th century, J. R. Glauber's study of coal tar distillates was among the first systematic studies on organic dye chemistry.

In the 18th century, C. W. Scheele's work on purifying naturally occurring organic compounds made significant contributions that would later form the basis of synthetic dye production. Even before Perkin, some synthetic or semi-synthetic dyes such as picric acid, orchyl, indigo sulfate (Saxe blue), and murexides derived from uric acid had found limited applications. Rumney's production of murexides in the 19th century and F. F. Runge's description of various violet, blue, and red synthetic dyes, as well as aniline black, were significant advances in organic dye chemistry. Furthermore, the bright pigment of lichen origin, known as "French purple," developed in Lyon in the 1850s, attracted great interest in European fashion circles and influenced the color trends of the time. These early findings and technological innovations formed the scientific foundation of the modern synthetic dye industry, which gained momentum with Perkin's discovery of mauveine (Ismail, 2011).

2. CLASSIFICATION OF COLORANTS

The use of colorants in food is fundamentally based on a scientific assessment that takes into account consumer perception, potential color loss during product processing, and the physicochemical properties of the food. Since the color of a food creates the first impression for the consumer regarding freshness, quality, and flavor, manufacturers use colorants to maintain or standardize the expected appearance of the product. For example, processes such as heat treatment, oxidation, or fermentation can degrade natural pigments, causing the product's color to fade or change as it should normally be. In this case, colorants are added to replace the lost color or correct the unwanted tones caused by the process. The type of colorant to be selected is determined by the product's properties such as pH, fat content, water activity, and heat exposure level; because not every colorant remains stable in every environment. For example, fat-soluble beta-carotene can be used in margarine, while heat and light-resistant synthetic colorants are preferred in more acidic beverages (Hendry and Houghton, 1996).

This selection process also includes the decision on whether the coloring agent is natural or synthetic. Natural pigments are less stable and are easily affected by changes in light, temperature, and pH; in contrast, synthetic coloring agents provide more intense color and are more resistant to production conditions. However, the use of coloring agents must comply with food safety regulations. Which coloring agent can be used in which food and in what quantity is defined by acceptable daily intake (ADI) limits determined as a result of toxicological evaluations, and these limits are regulated by international authorities (FAO/WHO, 2011). Some coloring agents can provide not only aesthetic but also nutritional or functional effects; for example, beta-carotene functions as a precursor of vitamin A, while anthocyanins have antioxidant properties. All these scientific, technological, and legal factors together determine which coloring agent the manufacturer can use, in what

quantity, and for what purpose (Scotter, 2011). Natural coloring agents are divided into two groups, natural and synthetic, according to their production methods. This section presents nature's miraculous coloring agents and their uses.

3. NATURAL FOOD COLORANTS

Beyond adding aesthetic value to food products, natural food colorants can also play an important role in human health thanks to their biological effects such as anti-inflammatory, antioxidant, anti-rheumatic, and antimutagenic properties. These potential therapeutic properties have led to the increasing use of natural dyes in the food industry. These substances, which have limited color ranges, have poor stability and weak coloring power. They are highly affected by heat and pH depending on environmental conditions.

Today, many natural dyes are imported into the United States, while countries like Germany can produce approximately 900 tons annually, and exports of natural dyes across Europe amount to millions of dollars. India has high potential in the production and export of natural dyes thanks to its wide plant diversity and traditional knowledge (Sigurdson et al., 2017). Natural colorants are generally obtained from plants, microorganisms, animal/insect-derived pigments, and minerals. Among plant-based sources, pepper, saffron, red beet, and grape stand out, while fermented pigments also find use in industry; for example, carotene obtained from Blakeslee and Apink red pigments produced from Penicillium oxylium are examples of this. In addition, some bacterial species such as Serratia and Streptomyces are also evaluated for color production in small quantities (Khair et al., 2017).

It has long been known that natural food colorings exhibit low stability against many physical and chemical agents and therefore cause various technological problems during their use in food. However, as studies in recent years have shown that these substances can have positive effects on health, interest in natural colorings has increased significantly and the trend of their use in food products has strengthened.

4. NATURAL MICROBIAL PIGMENTS

Natural pigments are compounds obtained from plants, animals, and microorganisms, and are therefore also called "bio-colorants."

Plant-derived pigments are generally obtained from plant tissues such as flowers, fruits, vegetables, seeds, and roots. Plant pigments are often sensitive to heat, light, and pH conditions and exhibit technological disadvantages such as low solubility. Therefore, microbial pigments, which are gaining increasing interest in industry, stand out as an important alternative due to their resistance to seasonal fluctuations, their ability to be produced on a large scale at a lower cost, and their higher yield (Sancar et al., 2023).

Microbial pigments are obtained from a wide variety of microorganisms such as molds, bacteria, yeasts, protozoa, and algae, and these organisms are commonly found in different environmental settings such as water, soil, plants, insects, and animals. These naturally occurring pigments are secondary metabolites of significant commercial importance in the dairy, food, pharmaceutical, cosmetic, dyeing, and textile industries (Rajendran et al., 2023).

Microbial pigments not only exhibit coloring properties but also possess antioxidant, anticancer, antiproliferative, anti-inflammatory, antimicrobial, and immunomodulatory effects. Their biodegradability, suitability for environmentally friendly production processes, continuity of raw material supply, and rapid production in culture media make these pigments valuable from a sustainability perspective. Since microorganisms can be produced in low-cost culture media and independently of environmental conditions, this method is practical both economically and industrially.

While different color shades can be obtained with microbial pigments, red, yellow, and blue pigments are more commonly produced. Known disadvantages include causing undesirable changes in aroma, difficulty in standardization, and having a narrower color range compared to synthetic colorants. For microorganism-derived pigments to be used more effectively in industrial applications, they need to provide an acceptable level of stability, especially against UV light and oxidative stress. These pigments are used not only as food colorants but also in a wide range of applications in the cosmetics, pharmaceutical, and textile industries (Tuli et al., 2015).

1.1. ANTHOCYANINS (E163): The Miracle of Purple Foods

Anthocyanins are water-soluble phenolic compounds found in the flowers, fruits, and leaves of plants, and have wide potential for use as natural colorants in foods. These pigments create red, purple, and blue color tones, and their importance in the food industry is increasing, especially with the growing consumer demand for natural and clean-label products. The main advantage of using anthocyanins as colorants is that they possess both strong color-imparting capacity and antioxidant properties. Therefore, anthocyanins not only enhance visual appeal but also add functional value to food (Khoo et al., 2017).

One of the most important factors affecting the use of anthocyanins in foods is their pH-sensitive color change. Anthocyanins, which exhibit red tones in acidic environments, can turn purple and blue tones as the pH increases. Therefore, anthocyanins are frequently preferred as a natural coloring agent, especially in low pH fruit juices, carbonated beverages, jams, ice creams, and yogurt derivatives (Giusti and Wrolstad, 2003). However, their sensitivity to environmental factors such as light, oxygen, and high

temperatures is a limiting factor that must be considered in terms of stability in product formulation.

Recent research has focused on the use of microencapsulation, co-pigmentation, and nanotechnological carrier systems to increase the stability of anthocyanins, and these techniques have been shown to significantly improve color stability. Nevertheless, the sensitivity of anthocyanins to environmental factors such as light, oxygen, and temperature may limit their stability in some products. Therefore, research aimed at increasing color stability using microencapsulation, co-pigmentation, and nanotechnological carrier systems has accelerated in recent years (Castañeda-Ovando et al., 2009). In conclusion, anthocyanins, due to their natural origin, functional biological activity, and positive consumer perception, constitute a strong alternative to synthetic colorants in the food industry. Although stability issues persist, the reduction of these disadvantages through modern technologies indicates that anthocyanins will be used more widely in the future. Anthocyanins have wide areas of use as natural colorants in the following food categories:

· Fruit juices and fruit-based beverages

· Carbonated beverages and sports drinks

· Dairy products

· Jams, marmalades, jellies, and fruit sauces

· Confectionery and chewing gum

· Bakery and pastry products

· Dried fruit products and snacks

4.2.BETALAINS (E162): Yellow (Betaxanthins) and red-purple (Betacyanins) as Natural Pigments

In recent years, with the development of a conscious consumer society and the increasing interest in food safety and natural additives, the use of naturally sourced colorants instead of synthetic colorants in the food industry has come to the forefront, and betalains have become one of the prominent natural pigment groups in this context. Betalains are mainly found in the fruit, root, leaf, flower, and young shoot tissues of plants. They are most concentrated in the fruit peel and fruit pulp, giving plants red, purple, yellow, and orange colors. They accumulate in high amounts, especially in the roots, which serve as storage organs; the best-known example of this is red beet. In some plants, such as amaranth (leaves and seeds) and red quinoa varieties, cactus fruit, pitaya/dragon fruit (Hylocereus spp.), prickly pear, and Barbados cherry, betalains are found around the leaves and leaf veins, giving the leaves

reddish or purplish tones, while they are present in flower petals, providing bright color and playing a role in attracting pollinators. They have also been detected in the stems and young stalks of some species. At the cellular level, betalains are water-soluble pigments stored in the vacuoles of plant cells and are naturally found only in plants of the Caryophyllales order; in these plants, betalains are synthesized instead of anthocyanins (Kumorkiewicz-Jamro et al., 2021).

Betalains are water-soluble, naturally occurring pigments with strong color-imparting capacity, and are divided into two main groups: red-purple betacyanins and yellow-orange betaxanthins. These pigments are obtained from plant sources and, in addition to giving foods vibrant colors, also exhibit antioxidant, lipid oxidation-reducing, and in some cases antimicrobial effects. Studies have shown that betalains can be successfully used in different food matrices. High color stability has been observed in products such as jelly candies, ice cream, and yogurt; color loss is particularly reduced under low-temperature and light-free storage conditions. In milk and dairy products, betalain addition has been shown to delay microbial spoilage and improve sensory properties. In meat products, betalain-containing extracts have been reported to slow oxidation, extending shelf life and increasing consumer acceptance. The following are the food products in which they are used (Martins et al., 2016):

· Milk and dairy products (Milk, yogurt, cream)

· Frozen products (Ice cream)

· Confectionery and dessert products (Jelly candies, sweets)

· Beverages (Soft drinks, fruit-based drinks)

· Meat and meat products (Sausage, salami, burger)

· Other products (Jams, sauces, soups)

4.3.CAROTENOIDS (E160b): Fire colors; Yellow, orange, red

Carotenoids are lipophilic, naturally occurring pigments containing numerous conjugated double bonds in their structure, giving them a strong coloring capacity. They are also biologically active compounds that exhibit antioxidant properties, capable of scavenging reactive oxygen species and free radicals. Carotenoids are an important group of pigments found naturally in many fruits, vegetables, and flowers such as tomatoes, carrots, papayas, pineapples, marigolds, sunflowers, annatto, saffron, and green leafy vegetables. There are approximately 700 carotenoids. Synthesized by plants, some bacteria, algae, and fungi, these compounds impart yellow, orange, and red colors to foods, and are widely used as natural colorings in the food

industry due to these properties. The color characteristics of carotenoids stem from the conjugated double bonds in their molecular structure; the darker the color, the higher the number of conjugated bonds. These pigments also hold an important place in nutrition due to their role as vitamin A precursors, their antioxidant properties, and their positive health effects. Since animals cannot synthesize carotenoids, they obtain these compounds through their diet and accumulate them in their tissues. While numerous carotenoids have been identified from natural sources, the most common in human nutrition are β-carotene, α-carotene, lycopene, β-cryptoxanthin, lutein, and zeaxanthin. However, the use of carotenoids in the food industry has some limitations due to their low water solubility, chemical instability, and sensitivity to heat, light, oxygen, and oxidation (Shen et al., 2014; González-Peña et al., 2023). Carotenoids are widely used as natural coloring agents in the following food groups:

· Fruit and vegetable-based products

· Milk and dairy products

· Fats and fat-based products

· Confectionery and sweets

· Baked goods

· Spices and flavorings

· Seafood and aquaculture-based products

4.4.CARAMEL (E 150): From light yellow to amber and dark brown… The caramel dream

Defined as a coloring agent and antioxidant, caramel is used in various food products. Caramel colors, used in the production of various foods and beverages, have been on the market for over 100 years. Caramelization is one of the main reactions that cause some foods to brown during heating. This reaction begins when the process temperature reaches the melting point of the sugars contained in the food. Color and flavor changes depending on the temperature and heating time. Caramel colors are dark brown to black liquids or solids with a burnt sugar smell and a pleasant, albeit slightly, aroma. Caramel color, ranging from the lightest yellow to the darkest brown, constitutes more than 80% (by weight) of all colorings added to the foods we eat and drink (Sengar and Sharma, 2014).

The color change comes from caramel, a natural and coffee-colored pigment; Flavor changes are caused by compounds such as furfural, furanone, diacetyl, acetoin, and maltol. Caramelization is the process of browning sugar during cooking. This caramelization, which occurs depending on the process, is quite important in terms of the formation of typical color and flavor in many

foods. In addition, each type of caramel color has specific functional properties that ensure compatibility with the product and eliminate undesirable effects such as cloudiness, clumping, and separation (Millstone, 1985).

Sucrose is mostly used for caramel production. When sucrose is heated at 160°C, it first decomposes into anhydrous glucose and fructose, and caramel formation occurs when the temperature reaches 200°C. It is divided into 4 classes to meet the requirements of various food and beverage systems. However, different types of caramel are needed depending on the chemical composition of the food it will be added to. This is because caramel stability varies according to the chemical composition of the food. The International Technical Caramel Association (ITCA) and the European Technical Caramel Association (EUTECA) have standardized the properties of 4 classes and 10 types of caramel. Different caramel types are obtained by varying the environment during the heating of saccharides. According to the process applied, they are divided into 4 groups and named as follows, and their areas of use are also given (Sengar and Sharma, 2014; https://ggd.org.tr/en/karamel-ve-gida-guvenligi/):

COLOR-I: Plain Caramel (E 150a): Acid, alkali and salt can be used to accelerate caramelization, but ammonium compounds and sulfites are not used (Plain or alcoholic caramel).

· Alcoholic beverages (whiskey, rum, liqueur)

· Beer

· Bakery products

· Confectionery

· Hard candy and toffee products

COLOR-II: Caustic Sulfite Caramel (E 150b): Prepared in the presence of sulfite compound, in the absence of ammonium compound, with or without acid or alkali (Caustic sulfite caramel).

· Alcoholic beverages

· Wine and liqueurs

· Certain soft drinks

· Vinegar and vinegar-based products

COLOR-III: Ammonia Caramel (E 150c): Prepared in the presence of ammonium compounds, in the absence of sulfite compounds, and in the presence or absence of acid or alkali (ammonia or beer caramel, baker's and confectionery caramel).

· Meat and meat products

· Ready-made meals

· Soups

· Sauces and Soy sauce

COLOR-IV: Sulfite-Ammonia Caramel (E 150d): Prepared in the presence of sulfate and ammonium compounds, and in the presence or absence of acid or alkali (sulfite-ammonia, non-alcoholic beverage caramel or acid-resistant caramel).

· Cola-type carbonated drinks

· Acidic non-alcoholic beverages

· Flavored beverages

· Certain sauces and condiments

4.5.CARMINIC ACID (Carmine) (E120): The Truth Behind the Red Color!

Carmine is a naturally occurring red pigment of animal origin, belonging to the anthraquinone group, and its main coloring component is carminic acid. Carmine is a dark red extract obtained from the drying and solvent treatment of female cochineal insects; it is a solution with carminic acid as its main pigment. The ability of carminic acid to form complexes with metal ions provides a significant advantage in the production of more concentrated and stable carmine pigments. Furthermore, carminic acid has been reported to function as a free radical scavenger in aqueous and methanolic solutions and to exhibit antioxidant activity comparable to common antioxidants such as ascorbic acid or trolox (Lazova-Borisova and Adamopoulos, 2024).

Carminic acid is primarily synthesized in nature by Dactylopius coccus (American cochineal), a species that contains up to approximately 26% carminic acid by dry body weight. This ratio is quite high compared to other cochineal species, and due to this high yield, Dactylopius coccus became the main commercial source of carminic acid from the 16th century onwards. Commercially used carmine contains approximately 50% carminic acid and, thanks to its high resistance to heat and light, exhibits long-term color stability during storage, especially in foods with a pH above 3.5. The main color components are carminic acid, kermestic acid, and laccaic acid, respectively (Ferreyra-Suarez et al., 2024; Cooksey, 2019).

Carminic acid is a compound soluble in water, alcohols, esters, acid and alkali solutions, but insoluble in nonpolar solvents such as petroleum ether, benzene, and chloroform. Its color properties are extremely sensitive to pH. Cochineal dye exhibits pale orange tones below pH 4.5, changes from light red to red in the pH range of 7–7.7, and becomes magenta-red above

pH 12. Cochineal dye is notable for its high stability against light, heat, and oxidation, offering superior durability compared to many natural and synthetic colorants. Commercially, cochineal dye is available in two main forms: cochineal extract (E120(ii)) and carmine (E120(i)). Carmine, having undergone further purification processes, is widely used in the food industry in powder or liquid form, and in water-soluble or oil-soluble varieties. The absence of toxic or carcinogenic effects is an important factor supporting the preference for carmine as a natural colorant (Frick, 2003). Foods and food products in which Carmine (E120) is used are listed below:

· Milk and dairy products, flavored milks, milk-based desserts

· Confectionery and sweet products

· Jelly candies, dragees and coated products

· Turkish delight and similar traditional sweets

· Beverages, fruit-flavored beverages

· Alcoholic beverages (liqueurs)

· Flavored beverage concentrates

· Baked goods and pastry products (Biscuits, cakes)

· Meat and meat products, processed meat products

· Sauces and condiments, dessert sauces, fruit-based sauces, some salad dressings

· Other food products

4.6.CHLOROPHYLS (E140, E141, E140i, E140ii): Green; A Symbol of Peace, Security, and Abundance

Plant pigments are natural chemical compounds that give fruits and vegetables their characteristic colors and enhance their visual appeal. Chlorophylls, in particular, are widely used in the food industry as natural green colorants. Chlorophylls are natural pigments responsible for the formation of green color in plants and play a role in all photosynthetic organisms in nature (Sharma et al., 2021). Found in all photosynthetic organisms, these pigments absorb light energy, enabling the reduction of carbon dioxide into organic compounds and thus contributing to the formation of plant biomass. They are mainly found in high concentrations in the green tissues of higher plants, especially in leaves. Green leafy vegetables (spinach, lettuce, chard, broccoli), grasses and forage crops (alfalfa, meadow grasses), legumes (peas, beans), and aromatic and medicinal plants (parsley, dill, nettle) are rich sources of chlorophyll. In addition, chlorophyll is naturally found in algae and cyanobacteria (green algae, brown algae, red algae, and blue-green algae) and

enables primary production through photosynthesis in aquatic ecosystems. Chlorophyll a and chlorophyll b are natural color pigments approved as food additives worldwide. Chlorophyll is a pigment consisting of a tetrapyrrole (porphyrin) ring with a magnesium ion at its center and esterified with a phytol side chain. This structure determines chlorophyll's photosynthetic function and its usability as a natural coloring agent in foods. Its selective absorption of light at different wavelengths gives plants their characteristic green color (Ebrahimi, 2023).

Chlorophylls are mostly fat-soluble pigments. While water-soluble chlorophyllin is formed by removing the phytol chain under alkaline conditions, pheophytin is formed in acidic environments as a result of the replacement of the central magnesium ion with protons, leading to decreased color stability. Chlorophyll and its derivatives are identified as food additives with codes E140 and E141. Chlorophylls obtained by extraction from edible plants are used as E140i, while chlorophyllin obtained by saponification, which is more stable, is used as E140ii. Furthermore, copper chlorophyll (E141), obtained by replacing the magnesium ion with copper, is widely preferred in the food industry due to its high color stability (Nabi et al., 2023). Recent studies show that chlorophylls are noteworthy not only for their coloring properties but also for their bioactive properties. It is reported that chlorophylls possess high antioxidant activity and can exhibit anti-inflammatory, anti-cancer, anti-mutagenic, and wound-healing effects. These properties make chlorophylls valuable as a functional food ingredient. Below are some foods and food products in which chlorophyll is used (Silva et al., 2022; Martins et al., 2023):

· Confectionery, gelatin desserts, jams

· Milk and dairy products (yogurt, ice cream, cheese)

· Beverages (fruit juices, carbonated and non-carbonated soft drinks, energy and sports drinks)

· Sauces and soups

· Margarine and fat-based products

· Ready-made meals, canned goods, frozen foods

· Baked goods and cakes

· Spice blends and herbal flavorings

· Colorings and health additives in functional foods

· Smoothies and herbal blends

4.7.CURCUMIN (E100): Yellow; natural golden color

Natural food colorings are generally safe for health, and while some examples, such as turmeric (Curcuma longa), occasionally offer health-improving effects, it is widely used as a yellow coloring agent in the food industry thanks to its main natural pigment, curcumin, and the fact that its use does not lead to environmental waste is an important advantage. This very valuable yellow powder has been widely used for centuries in kitchens, for coloring foods, and also in various preparations in Ayurveda and Chinese medicine (Sharifi-Rad et al., 2020).

Turmeric (Curcuma longa) is a tropical plant belonging to the Zingiberaceae (ginger) family that grows in tropical and subtropical climates, and a yellow powder is obtained by drying and grinding its rhizomes; this powder is widely used in traditional medicine. Curcumin (50-60%) is the main pigment found in the rhizome of turmeric and, together with two other curcuminoids, demethoxycurcumin (20-30%) and bis-demethoxycurcumin (7-20%), forms the characteristic yellow color of turmeric. These pigments, especially curcumin, can be used in the food industry as both a natural flavoring agent and a food coloring, replacing synthetic dyes such as tartrazine. Curcumin is a naturally occurring phenolic dye and is used in the food industry as a natural yellow coloring agent identified by the code E-100. This pigment, extracted from turmeric, is evaluated for providing yellow food colors, contributing to traditional spice applications, and offering an alternative to synthetic dyes. It is reported that the turmeric rhizome contains approximately 5-6% curcumin, and this compound provides color pigments ranging from lemon-yellow to orange tones (Abdeldaiem, 2013). Curcumin is integrated into a wide variety of products not only as a natural yellow coloring agent in foods, but also as a plant-based chemical pigment. It can be used especially in foods where a yellow color is needed, such as sauces, baked goods, beverages, and dairy products. In addition, curcumin is considered a potential pH indicator in smart packaging systems due to its pH-dependent color change; its transformation to red in alkaline conditions and to yellow in acidic environments serves as a packaging color indicator. In this respect, curcumin goes beyond being a traditional spice component, and the curcumin-rich rhizomes of turmeric can be used in modern applications as both a natural food coloring agent and a functional natural compound with antioxidant and antimicrobial preservative properties. This pigment is an important natural component in improving food safety and nutritional quality as an alternative to synthetic dyes (Sarı and Sarıışık, 2025). The places where curcumin is used in foods are given below (Lan et al., 2023; Buniowska-Olejnik et al., 2024).

As a coloring agent:

· Milk and dairy products

· Baked goods

· Sauces, condiments, soups

· Fruit juices and plant-based beverages

· Desserts and confectionery

· Prepared foods

As a preservative (antioxidant and antimicrobial):

· Meat and meat products

· Bread and cereal products

· Tofu and plant-based protein products

· Oils and oil-based foods

· Fruit and vegetable products

As a coloring and preservative in food (shelf life and microbial spoilage prevention):

· Milk-based ice creams and cheeses

· Prepared food sauces

· Processed meat and poultry products

4.8.ANNATTO(E160b): Sun Yellow Annatto

Annatto is a plant-derived natural coloring agent with a long history of historical and industrial significance, obtained from the outer shells of the seeds of Bixa orellana, a tropical shrub species. Annatto seeds are known as "achiote" in Latin America and have been widely used in Central and Latin American cuisines since pre-Columbian times, during the Aztec and Mayan civilizations. These societies used annatto especially to give foods a bright red-orange color; they added it to chocolate and various foods to obtain a more intense color. This strong coloring property of annatto has paved the way for its importance as a natural dye throughout history (Picasso, 2022).

Today, annatto stands out among natural coloring additives, especially in the United Kingdom; thanks to its different pigment forms that are soluble in oil and water, it is widely used in numerous food systems. Annatto's coloring activity primarily stems from its carotenoid compounds bixin and norbixin. Treatment of the seeds with suitable organic solvents yields high-purity (80–97%) crystalline bixin, which is used as a key component in the production of commercial annatto preparations with varying solubility properties. Bixin, exhibiting lipophilic properties, is preferred in fat-based

foods, while norbixin, formed by hydrolysis of bixin's ester bonds and soluble in water, provides more effective coloring in protein-rich food matrices due to its ability to interact with proteins. To increase the stability of annatto-based colorants in food applications, these pigments are often formulated with emulsifiers and similar auxiliary substances; thus, products exhibiting higher stability against external factors such as acidic environments, metal ions, and the presence of salts are obtained. From a regulatory perspective, annatto and its derivatives are classified as food additives under code E160b in the European Union and are permitted for use in a wide range of products. Similarly, in the United States, annatto seed extracts are among the natural colorings approved by the US Food and Drug Administration (FDA) for use in food and beverages, demonstrating that annatto is a globally recognized natural dye with high application potential (Raddatz-Mota et al., 2017; Rather and Mohammad, 2016). The uses of bixin and norbixin in annatto in food are listed below (EFSA FAF Panel, 2019):

· Dairy products

· Baked goods and pastry products

· Bakery products and snacks

· Breakfast cereals

· Processed meat and fish products

· Non-alcoholic beverages and powdered beverage mixes

· Dry soup and sauce mixes

· High-protein ready-to-eat foods

· Fat-containing ready-to-eat foods and emulsion systems

· Oil-based sauces

4.9.PHYCOCYANIN (E18): The Blue of Purity and Serenity

Microalgae have long been used as food and food additives due to their high nutritional value and positive effects on health. These organisms offer rich sources of pigments and other bioactive compounds. The main pigments obtained from microalgae include chlorophyll, β-carotene, astaxanthin, xanthophyll, phycoerythrin, and phycocyanin. Phycobiliproteins are a class of water-soluble pigments in microalgae that constitute a significant portion of the cellular protein content. These proteins are formed by the combination of non-protein compounds with proteins, and their main components are phycocyanin, allophycocyanin, and phycoerythrin.

Phycocyanin (PC) is an oligomeric protein known for its blue color and is found in different microalgal species such as cyanobacteria and some red

algae. PC used in the food industry is obtained especially from cyanobacteria belonging to the genus Spirulina; The main species include Spirulina platensis, Spirulina subsalsa, and Spirulina maxima. PH-cycocyanin (PC) is structurally classified as C-phycocyanin (C-PC) and R-phycocyanin (R-PC); C-PC is predominantly found in cyanobacteria, while R-PC is concentrated in red algae. As a component of phycobilisomes, phycocyanin is responsible for both its characteristic blue color and its antioxidant and radical scavenging properties. Specifically, C-PC has been approved as "Generally Recognized as Safe" (GRAS) by the United States Food and Drug Administration (FDA) and is considered a natural blue colorant. Due to its dual function as both a pigment and a protein, phycocyanin has a versatile potential for use in the food industry. In the European Union, phycocyanin is evaluated within the scope of spirulina extract obtained from Spirulina species and is classified as a natural blue colorant with the code E18. Due to its iron-binding properties and essential amino acid content, it can be considered as a nutritional supplement and, thanks to its protein structure, can be applied to improve the organoleptic and nutritional properties of foods (Mao et al., 2024).

PCs are widely used in food products. Furthermore, potential applications are being investigated in areas such as food preservation, quality testing, and cultured meat production. These properties allow microalgae to be consumed directly as functional foods and to be used as raw materials in the production of natural products aimed at preventing various diseases. Commercially important microalgal species include Chlorella, Dunaliella, and Spirulina, which are widely used in the production of pigments and other bioactive compounds. Pigment production varies depending on the species, but can be achieved with chlorophyll at 0.5–1.5%, carotenoids at 0.1–0.2%, and phycobiliprotein at 14–20% on a dry weight basis. These pigments can be used in place of synthetic colorants in the food, pharmaceutical, cosmetic, and textile industries, and can be utilized in nutritional supplements and pharmaceutical products due to their antioxidant, anticancer, anti-inflammatory, and cholesterol-lowering effects (Arslan and Aksay, 2022). In particular, phycocyanin obtained from Spirulina platensis exhibits anti-inflammatory and immune system-supporting biological properties.

Microalgae are a rich source of polyunsaturated fatty acids, sterols, pigments, proteins, enzymes, vitamins, and other bioactive compounds. Potential health effects of microalgae include antiviral, anticancer, antidiabetic, antibiotic, antioxidant, prebiotic, probiotic, immune-boosting, cardiovascular protective, hypocholesterolemic, and antiallergic effects (Yua et al., 2023). In these respects, microalgae are among the strategic biological resources that can be consumed directly as functional foods and used as raw materials in the development of pharmaceutical and nutraceutical products. Phycocyanin is considered "GRAS" (Generally Recognized As Safe) in the US

and can be safely used in many food products (except baby foods). The uses of phycocyanin, found in microalgae, in food are listed below (Mao et al., 2005):

· Confectionery and sweets

· Ice cream and frozen desserts

· Beverages and alcoholic and non-alcoholic beverages

· Pastries and baked goods

· Functional foods and supplements

· Ready-to-eat food products

4.10.ASTAXANTINE (E161j): Nature's Red Miracle

Astaxanthin was first identified in the early 20th century as the pigment responsible for the characteristic red-pink color of shellfish and some fish species. It was later shown to be a xanthophyll belonging to the carotenoid class. Its unique chemical structure, containing hydroxyl and keto functional groups with a conjugated double bond system, gives astaxanthin both high coloring capacity and superior antioxidant activity among carotenoids. Due to these properties, astaxanthin is considered one of the most expensive and important industrial pigments used in aquaculture today (Stachowiak and Szulc, 2021).

Astaxanthin is found primarily in aquatic ecosystems and is synthesized by microorganisms, mainly Haematococcus pluvialis, as well as Xanthophyllomyces dendrorhous algae, yeasts, and microorganisms. Through the food chain, the pigment is found in the muscle tissues of fish such as salmon, red snapper, Arctic trout, and trout; Astaxanthin accumulates in the shells of crustaceans such as shrimp, lobster, and krill, as well as in the feathers of aquatic birds like red ibis and flamingo, causing red and pink hues. In aquatic environments, astaxanthin is found in algae that can synthesize this pigment and in plankton that can convert astaxanthin. The intensity of tissue color largely depends on the amount of astaxanthin consumed in the diet. In nature, astaxanthin often forms complexes with proteins or lipids. The crustacyanin–astaxanthin complex found in crustaceans is blue, while astaxanthin released as a result of protein denaturation during heat treatment produces the typical red color. Similarly, in some organisms, astaxanthin is found in the form of lipoglycoprotein complexes, causing different color tones. This is an important factor that directly affects the color behavior of the pigment during food processing and cooking (Martínez-Álvarez et al., 2020; Montero et al., 2016).

Astaxanthin's significant antioxidant capacity and potential benefits for human health have led to its consideration not only as a coloring additive but also as a component with functional properties. Accordingly, natural

astaxanthin is widely used in various product groups, primarily nutritional supplements and cosmetic formulations; however, its use in food and beverage applications is limited in some countries. Synthetic astaxanthin, being a combination of different stereoisomers not found in its natural form, exhibits lower bioavailability and stability; this is considered one of the main reasons why its use in food is not permitted under European Union legislation 1925/2006. Astaxanthin and astaxanthin-containing lipid extracts can offer a dual effect when used as food components. Firstly, thanks to their intense red pigments, they provide an attractive color to foods, making a significant technological contribution. Secondly, thanks to their antioxidant properties, they help protect foods against oxidative spoilage during production and storage, thus supporting product quality. After consumption, astaxanthin and lipid extracts containing these components can exhibit biologically active effects; this makes them a valuable component in the development of functional food products. With these properties, astaxanthin is considered not only a coloring additive but also a "functional colorant" that adds functional value to foods thanks to its strong antioxidant capacity (Martínez-Álvarez et al., 2020).

From a food technology perspective, astaxanthin offers a natural alternative to synthetic dyes; it can also contribute to the sensory and nutritional quality of products by supporting oxidative stability. However, the limited availability of natural resources and high production costs are the main factors limiting its use in food. Nevertheless, in line with the increasing interest in the literature on natural colorants, innovative approaches such as micro- and nano-encapsulation aimed at increasing the stability of astaxanthin and integrating it into food systems while preserving the color-function relationship are the focus of current research. These technological developments allow astaxanthin to be positioned as a strong alternative to synthetic colorants in functional foods and active packaging systems. The uses of astaxanthin as a coloring agent in food products are listed below (Stachowiak and Szulc, 2021):

· Functional Foods

· Beverages and Food Supplements

· Milk and Dairy Products

· Fat-Based Foods

· Confectionery and Specialty Products

· Bakery and Pastry Products (Limited Use)

4.11. VEGETABLE CARBON (VEHICLE ACTIVATED CARBON) (E153): The Science of Natural Black

Carbon-based natural food dyes are pigments that are structurally composed of carbon atoms and are obtained from natural, mostly plant-based raw materials. The most common and regulatoryly defined example in this group in the food industry is vegetable carbon, which is included in the European Union legislation with the code E153. Vegetable carbon is a coloring agent obtained as a result of the controlled carbonization of plant materials, giving an intense black color and considered chemically inert (EFSA, 2012). Within the general classification of natural food dyes, carbon-based pigments are distinguished by the fact that, unlike biomolecular structures such as anthocyanins, carotenoids, or chlorophylls, they are not molecules containing a specific chromophore; they consist of amorphous carbon particles (Scotter, 2011).

The raw materials used in the production of vegetable carbon are generally wood, tree bark, coconut shell, or similar plant-derived materials. These raw materials are carbonized by being exposed to high temperatures under conditions of limited oxygen. During the carbonization process, organic components such as cellulose, hemicellulose, and lignin found in the plant tissue are broken down, volatile fractions are removed, and a structure containing largely elemental carbon remains (Downham and Collins, 2000). The resulting carbonized material is then mechanically ground into fine particles and standardized according to the purity and particle size suitable for food applications. In some production processes, additional activation or purification steps may be applied to increase the surface area of the carbon and reduce unwanted impurities (Scotter, 2011).

One of the fundamental physical properties of carbon-based natural food dyes is their insolubility in water and oil, and their use in dispersion form. This necessitates the use of appropriate formulation techniques to ensure homogeneous distribution of the pigment within the food matrix. However, plant-based carbon exhibits very high stability to heat, light, and pH changes, making it particularly preferred in bakery and confectionery products (Martins et al., 2016). Furthermore, its taste and odor inertness allows it to provide only a visual enhancement without affecting the product's sensory profile (Downham and Collins, 2000).

In the food industry, carbon-based natural dyes are mainly used in products where a black or dark gray color is desired. One of the reasons for the increasing popularity of plant-based carbon black is its versatility. Its dark black color can enhance the visual appeal of a wide variety of foods. However, the areas of use for these pigments are limited by product categories and maximum usage levels determined by regulatory bodies (Scotter, 2011). In the

European Union, E153 has been re-evaluated by the European Food Safety Authority (EFSA) and it has been concluded that it does not pose a risk to human health under the recommended conditions of use (EFSA, 2012). EFSA reports indicate that vegetable carbon is largely inert, not absorbed from the gastrointestinal tract, and does not exhibit systemic toxicity. In contrast, the United States does not permit the use of carbon black type pigments in food, limiting their use primarily to cosmetic and industrial applications (FDA, 2019). Consequently, carbon-based natural food dyes, particularly vegetable carbon, offer significant advantages in certain food applications due to their high stability, sensory inertness, and plant-derived origin. Vegetable carbon is a dye with limited use in food under EU and Turkish legislation. High consumption may reduce nutrient absorption. Furthermore, products containing activated carbon may interact with certain medications, reducing their effectiveness. Current academic research focuses on developing forms of these pigments with more controlled particle sizes and integrating them into functional food systems (Martins et al., 2016). The uses of vegetable carbonb as a coloring agent in food products are listed below:

· Milk and Dairy Products

· Confectionery and Desserts

· Bakery and Baked Goods

· Sweet and Frozen Products

· Functional and Detox Drinks

· Ready-to-Eat and Specialty Foods

· Decorative and Special Purpose Uses

5.NATURAL COLORANTS IN THE FOOD INDUSTRY: Future Perspectives

This section comprehensively addresses natural food colorings within the framework of their sources, classifications, technological functions, areas of use, advantages, and potential health risks. In the food industry, color is one of the fundamental quality parameters that directly influences consumer perception, and the use of natural colorings is of strategic importance in this context, both technologically and in terms of marketing. Natural pigments obtained from plant, animal, and microbial sources not only enhance the visual appeal of products but also increase their added value by offering functional properties such as antioxidant activity.

Natural food colorings are increasingly preferred by consumers due to their perceived safety and health benefits compared to synthetic colorings, and are becoming a key component of the "clean label" approach. However, the sensitivity of these colorings to environmental factors such as heat, light,

pH, and oxidation presents various technological challenges in industrial applications. Therefore, approaches such as microencapsulation to increase stability, the use of suitable carrier systems, and optimization of process conditions play a critical role in the effective and sustainable use of natural colorings.

From a health perspective, while most natural food colorings are considered safe at recommended usage levels, issues such as allergic reactions, differences in bioavailability, and long-term consumption effects remain the focus of scientific research. This highlights that being of natural origin alone does not imply absolute safety, and that toxicological assessments must be conducted in light of scientific data. Establishing acceptable daily intake (ADI) levels and enforcing legal limits are fundamental elements in protecting consumer health.

From a regulatory standpoint, legislation regarding the use of natural food colorings varies between countries and regions. Regulations established by the European Union, the United States, and other international authorities serve as guidelines for manufacturers and regulatory bodies. These differences necessitate a careful approach to formulation and labeling processes for food businesses operating in the global market.

From a future perspective, natural food colorings are expected to gain even more importance in the coming years. Developments in **biotechnology and synthetic biology** will enable the production of more stable and high-yield pigments through microorganism and cell cultures; Thus, dependence on traditional agricultural resources will decrease, and seasonal fluctuations will be minimized. Furthermore, **microencapsulation and innovative carrier systems** will increase the stability of colorants, extending their shelf life and enabling a wider range of applications in industrial settings.

Increasing consumer demand for health and functional properties will encourage the integration of natural colorants with functional components that provide antioxidant, anti-inflammatory, or other biological benefits. In addition, growing awareness of sustainability and environmentally friendly production methods will contribute to reducing the environmental impact of natural colorant production processes.

In conclusion, natural food colorants are increasingly gaining importance in the food industry in line with consumer expectations, sustainability goals, and the growing interest in functional foods. However, for this potential to be effectively realized, scientific research, technological innovations, and regulatory compliance must be developed simultaneously. In the future, the development of more stable, safe, and functional natural colorants will significantly contribute to the quality, safety, and innovation goals of the food industry.

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Chapter 5

THE CHANGING FACE OF CHILDHOOD OBESITY IN TURKEY: POLICY ANALYSIS AND GLOBAL SOLUTIONS IN LIGHT OF COSI FINDINGS

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1. Introduction

Childhood nutritional problems are on the global agenda as a "double burden," encompassing undernutrition issues such as wasting and stunting, alongside increasingly prevalent overweight and obesity. According to World Health Organization (WHO) data, the prevalence of overweight among children and adolescents aged 5-19 rose from 8% in 1990 to 20% in 2022, reaching an alarming level (World Health Organization, 2022). Beyond physiological consequences, the negative psychosocial outcomes of obesity, such as stigmatization, discrimination, and bullying, profoundly affect children's quality of life and school performance.

The need for evidence-based and internationally comparable policies in combating this global epidemic mobilized the WHO European Region, leading to the inception of the WHO European Childhood Obesity Surveillance Initiative (COSI) in 2007 (Wijnhoven et al., 2014). The initiative's primary aim is to monitor growth and obesity trends among children in member states by collecting data at regular intervals under a standard protocol and to utilize this data for developing national health policies. Turkey first participated in this significant international initiative in the 2012-2013 academic year, and continued its participation in 2016 and 2022, creating a valuable national dataset regarding the course of childhood obesity (T.C. Sağlık Bakanlığı, 2014, 2017, 2024).

The strength of Turkey's COSI data lies in its extensive scope and methodological rigor. Across the three survey rounds, a total of 29,612 second-grade primary school children were reached, and 29,514 were included in the final analysis (4,958 in 2013; 11,523 in 2016; and 13,131 in 2022). The sampling strategy evolved from a rural-urban representation in 2013 to a more detailed NUTS-1 (Nomenclature of Territorial Units for Statistics) level regional representation in 2016 and 2022, ensuring that the findings reflect the diversity of the entire country. Data collection was conducted by trained health personnel using standardized forms and calibrated anthropometric tools, strictly adhering to the WHO protocol to minimize measurement errors.

This book chapter analyzes the results of the three fundamental researches in Turkey's COSI journey (2013, 2016, and 2022) from a holistic perspective. The data and evaluations presented are based on a narrative review of official reports collected in compliance with the WHO COSI protocol and representing the country (at NUTS1 level). In the analyses, children's thinness, overweight, and obesity statuses were classified according to WHO 2007 growth references using Body Mass Index (BMI) Z-Scores based on age and gender. Although there are some differences in sampling design among the researches in the analyzed periods, the general trends at the national level present a consistent

pattern. Scrutinizing Turkey's ten-year obesity trends, changing nutrition and physical activity habits, and chronic issues in parental perception, this chapter aims to evaluate the data in the context of socio-economic changes and the effects of the COVID-19 pandemic experienced between 2013 and 2022.

1.1. Policy Context: The Awakening of National Awareness

The fight against childhood obesity in Turkey gained national priority status significantly earlier than the period analyzed in this chapter. A milestone was reached in 2010 with the implementation of the "Turkey Healthy Nutrition and Active Life Program." Within the framework of this extensive program, numerous regulatory policies were developed, ranging from introducing nutritional standards for food sales in school canteens and regulating food advertising to children, to promoting physical activity within the national curriculum (T.C. Sağlık Bakanlığı, 2010). These early interventions laid the structural groundwork for the monitoring processes initiated with the first COSI round in 2013, creating a baseline to evaluate the effectiveness of these public health strategies over the subsequent decade.

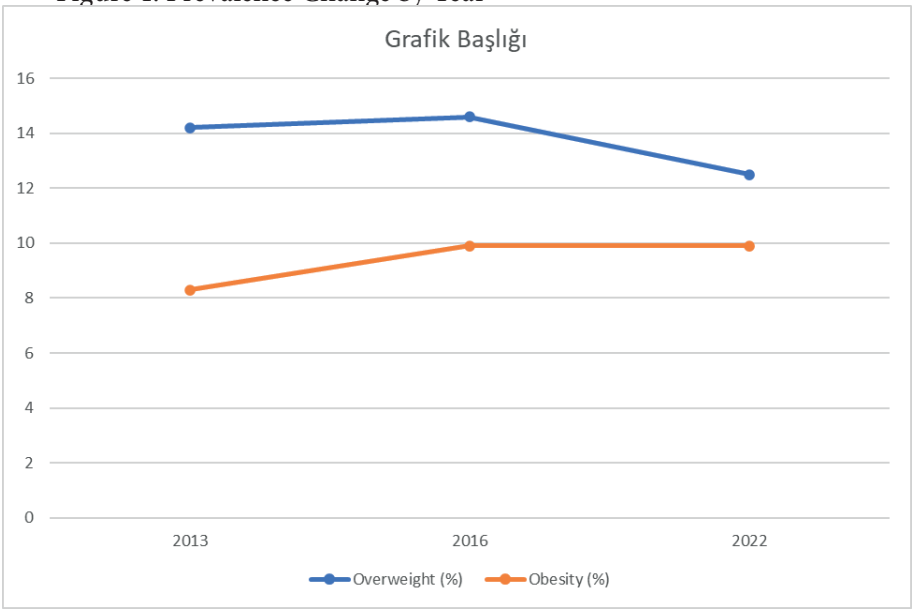
2. Turkey's Ten-Year Obesity Report Card: Risks and Trends (2013-2022)

When the ten-year course of childhood obesity in Turkey is examined, the struggle exhibits a dynamic yet complex structure. The first national COSI survey conducted in 2013 depicted the scale of the problem by revealing that 14.2% of children were overweight and 8.3% were obese (Figure 1) (T.C. Sağlık Bakanlığı, 2014). However, the real alarm bells began to ring in 2016 when the overweight rate rose to 14.6% and the obesity rate to 9.9% (T.C. Sağlık Bakanlığı, 2017). This period, where total prevalence reached 24.5%, clearly demonstrated that combating obesity must become a national priority.

The 2022 data indicates a noteworthy and partially promising break in this upward trend. According to the latest data, the overweight rate declined to 12.5%, but obesity prevalence remained constant at the 9.9% level (T.C. Sağlık Bakanlığı, 2024). Consequently, the total rate of children who are overweight or obese fell to 22.4% in 2022, returning to 2013 levels.

This picture shows that Turkey is caught between "partial success" and "structural resistance." The decline in overweight rates suggests that preventive policies, such as school canteen regulations, have been effective in slowing the transition of children in the risk group to obesity. However, the fact that the group with the highest health risk (the obesity category) has stagnated at the 9.9% level for ten years and has not regressed proves that current strategies are insufficient in combating established obesity. This stagnation suggests that although screening is performed in primary care, identified cases cannot be included in an effective referral and treatment mechanism, and the 'treatment' limb of the struggle is not as robust as the 'prevention' limb.

Figure 1: Prevalence Change by Year



3. Critical Turning Points in Nutrition and Physical Activity Habits

To understand the change in obesity prevalence, it is essential to look at the ten-year transformation in nutrition and physical activity habits that determine children's energy balance. Turkey's report card in this area harbors both "success areas" where policy interventions have been effective and "crisis areas" deepened by the impact of the pandemic.

The brightest spot in this picture is undoubtedly the steady decline in the consumption of sugar-sweetened and carbonated beverages. While 73.6% of children consumed these beverages at least once a week in 2013, this rate retreated to 62.4% in 2016 and 48.4% in 2022, recording a very serious reduction over ten years (Figure 2) (T.C. Sağlık Bakanlığı, 2014, 2017, 2024). This success is directly related to the circular regulating the sale of unhealthy foods in school canteens issued in 2016 and the decisive bans by the Ministry of National Education (T.C. Milli Eğitim Bakanlığı, 2016). However, while the success of school-based policies in this decline is undeniable, it should not be ignored that rising food prices during the same period may also be another factor limiting access to sugary products. Similarly, the increasing trend in the habit of "eating breakfast every day," which is critical for a healthy start to the day, from 2013 to 2022 (from 68.1% to 78.4%) is another positive indicator.

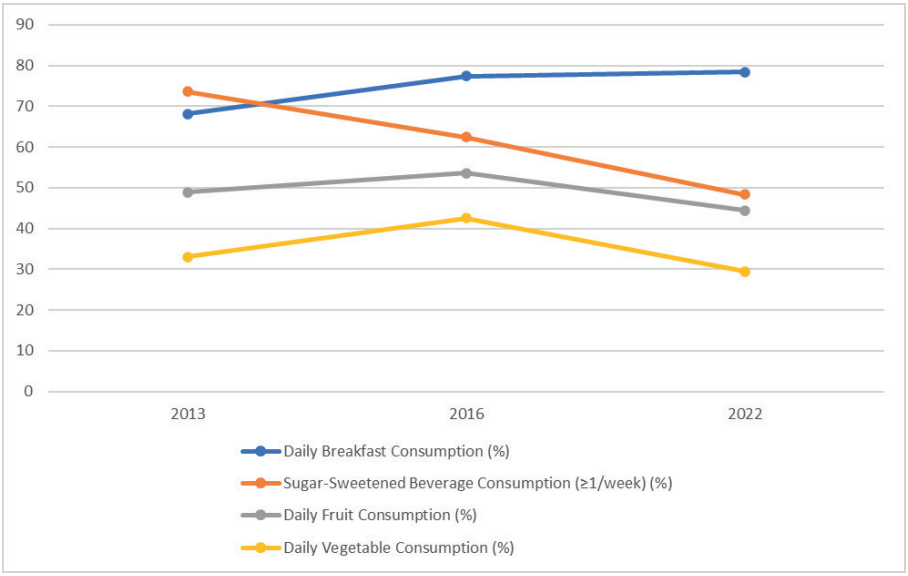
However, the other half of the picture is worrying, especially regarding fresh food consumption and physical activity. A fluctuating and recently

declining trend is observed in fruit and vegetable consumption. For instance, daily vegetable consumption, which rose to 42.6% in 2016, retreated to 29.5% in 2022 (T.C. Sağlık Bakanlığı, 2024). This decline may be associated not only with children's changing preferences but also with increasing economic barriers to accessing fresh food.

The most dramatic picture is seen in physical activity data. The rate of "regular sports participation outside of school," which reached a promising level of 40.8% in 2016, experienced a sharp drop to 25.2% in 2022 (T.C. Sağlık Bakanlığı, 2024). Although the impact of restrictions brought by the COVID-19 pandemic is undeniable in this decline, reducing the problem solely to the pandemic would be an incomplete approach. Indeed, international analyses showed that even before the pandemic, Turkish children were among the groups with the lowest sports club membership and highest screen time in Europe (Spinelli et al., 2019). The 2022 data confirms that Turkey is facing a "physical inactivity crisis," revealing that approximately three quarters of children (74.8%) do not engage in regular sports activities.

International comparative studies provide a broader context for these alarming physical activity trends. For instance, a 2021 study by Whiting et al., which utilized COSI data, revealed that Turkish children faced significant lifestyle challenges compared to their European peers. The study highlighted that Turkey had one of the highest rates of non-membership in sports clubs (83.1%) among 25 countries. Furthermore, Turkish children ranked 7th highest in spending more than two hours a day in front of screens (64.5%) and ranked 2nd lowest in sleeping less than the recommended 9-11 hours (71.3%). These figures underscore that the issue in Turkey is not solely nutritional but stems from a comprehensive lifestyle crisis encompassing sleep deprivation and excessive screen time (Whiting et al., 2021).

Figure 2: Trends in Nutritional Habits and Physical Activity (2013-2022)



4. The Invisible Barrier of the Struggle: Parental Perception

The largest and most resilient obstacle facing policy makers and health professionals in the fight against obesity is the inability of parents to correctly perceive their children's weight status. Ten-year data in Turkey shows that no improvement has been achieved in this regard; on the contrary, the problem has become chronic.

The results of the 2022 survey are quite striking: Only 0.7% of parents of children determined to be medically "obese" according to anthropometric measurements defined their child as "obese." 65.7% of the same group perceive their child's weight as "normal" (T.C. Sağlık Bakanlığı, 2024). As long as the existence of a problem is not accepted by the family, which is the most important stakeholder in the solution, no external intervention can achieve lasting success.

This "perception blindness" also differentiates Turkey negatively from other countries in Europe. A comparative analysis by Salas et al. (2021) using COSI data showed that while the rate of families viewing their obese child as 'normal weight' was 37.2% on average in Europe, this rate was 49.4% in Turkey (Salas et al., 2021). These data suggest that the problem in parental perception stems not only from a lack of knowledge but also that cultural norms tending to count a "chubby child" as healthy may be effective in this picture.

5. Global Perspective: Where Does Turkey Stand in the Mediterranean Basin?

To correctly interpret Turkey's ten-year course in the fight against obesity, it is necessary to read the data not in isolation but by comparing it with other countries sharing similar geographical and sociocultural characteristics. In this context, two Mediterranean countries that regularly participate in the COSI survey, Greece and Italy, serve as important reference points for Turkey (table-1).

When data is examined, it is seen that the Mediterranean basin is the riskiest region in Europe in terms of childhood obesity. However, countries' ways of managing this risk differ. Greece is one of the countries with the highest rates in Europe, with a total overweight and obesity prevalence approaching 40%, and has been experiencing a "stagnation" at these high levels in recent years (Kosti et al., 2021). Although Turkey's total prevalence of 22.4% (2022) paints a better picture compared to Greece, it gives similar signals in terms of the risk of "stagnation."

On the other hand, Italy follows a different course. Although Italy also has high prevalence rates, thanks to its long-running national surveillance system (OKkio alla SALUTE) and consistent public health programs, it captured a statistically significant, slight declining trend in both overweight and obesity rates in the 2008-2016 period (Spinelli et al., 2019). Turkey's complex picture of "decline in overweight, but stagnation in obesity" diverges from Italy's overall downward trend. This suggests that while interventions targeting the pre-obesity risk group (overweight children) work in Turkey, management as effective as in Italy cannot be exhibited in the group where the disease is established (obese).

Table 1: Comparative Analysis of Childhood Obesity and Related Factors in Turkey and Selected Countries

Indicator	Turkey (2022)	Greece (COSI, ~2019)	Italy (COSI, 2016)	Ireland (National Data)	Finland (National Data, ~2020)	Japan (National Data, 2019)
Obesity Trend	Complex Course: Overweight ↓, Obesity →	High Level Stagnation	Slight Declining Trend	High-Level Stagnation	Increase Halted / Stagnant	Consistently Low
Total Prevalence Overweight+ Obesity	22.4%	~40%	30.6%	~25-30%	~27%	~11%

Daily Breakfast	78.4%	~80%	~80-85%	High	>90%	>95%
Walking to School	Low	Medium	Medium	Low	High	>90%
School Meal Program	None (Limited pilots)	Yes Targeted/ Municipal	Yes Widespread Paid	Limited/ Variable	Yes Universal, Free	Yes Universal, "Shokuiku"
Physical Activity Policy	Standard Curriculum	Standard Curriculum	Standard Curriculum	Standard Curriculum	"Schools on the Move" Program	High (Walking Culture)
Sugary Drink	Distinct Decline (48.4%)	High	Declining Trend	Decline Observed (Post-Tax)	Low	Low

6. Solution Models: Good Practice Examples from the World

Turkey's success in reducing sugar-sweetened beverage consumption in school canteens has proven the power of structured policies. However, structural problems such as the physical activity crisis and the decline in fresh food consumption require more holistic solutions that go beyond simple restrictions. At this point, the Finland and Japan models, where Finland has succeeded in halting the increase in obesity and Japan keeps rates consistently low, offer a concrete roadmap for Turkey.

The Finland Model: Universal School Meals and Schools on the Move

Finland is one of the rare countries that managed to stabilize the increase in obesity in the 2000s. One of the cornerstones of this success is the "Universal and Free School Meal" program implemented since 1948 (Manninen et al., 2021). This program not only feeds children but also teaches them balanced nutrition, offers opportunities to try new tastes, and shapes the national nutrition culture. Considering the economic difficulties in accessing fresh food in Turkey, this model, which ensures equal access to at least one nutritious meal a day for children, carries importance as a vital social support tool. Additionally, Finland's "Schools on the Move" program transforms recess and the way to school into physical activity opportunities, offering a practical solution alternative to the sedentary lifestyle, which is Turkey's biggest problem (Lehto et al., 2020).

The Japan Model: "Shokuiku" and Walking to School Culture

The secret of Japan, which keeps obesity rates at the lowest level among developed countries, is the "Shokuiku" (Food and Nutrition Education) philosophy. In Japan, school lunch (Kyushoku) is part of education; children participate in meal preparation, learn the source of nutrients, and eat with their teachers (Tanaka & Shindo, 2018). This approach is a powerful source of

inspiration to overcome the "blind spot" in parents' perception of obesity in Turkey. Furthermore, the fact that more than 90% of children in Japan walk to school is the most striking proof of how physical activity can be integrated into daily life. Of course, the success of these models depends on their not being limited to schools alone, but being supported by safe city planning and social state policies. The model proposed for Turkey should be a hybrid approach adapted to the country's urban and economic realities.

7. Study Limitations

When interpreting the results of this analysis, certain limitations inherent to the study design should be considered. First, since the COSI surveys employed a cross-sectional design, the observed relationships between variables cannot be interpreted as direct causality. For instance, while strong evidence suggests that school canteen policies have contributed to the reduction in sugar-sweetened beverage consumption, this cannot be empirically proven as a sole cause-and-effect relationship within this study's scope. Second, behavioral data regarding nutrition and physical activity rely on parental declarations. This methodology may be susceptible to "social desirability bias," where parents might overreport healthy behaviors or underreport unhealthy ones. However, the strict adherence of the Turkish COSI implementation to the international WHO protocol, combined with the high national representativeness of the sample, renders the findings of this study highly valuable and reliable in reflecting the epidemiological landscape of childhood obesity in Turkey.

8. Roadmap for the Future and Policy Recommendations

Turkey's ten-year COSI data (2013-2022) indicates that a crossroads has been reached in the fight against childhood obesity. Gains such as the "victory over sugary drinks" and the "regression in overweight" achieved through school-based restrictive policies (canteen bans, etc.) are valuable; however, the stagnation of obesity and the collapse of physical activity levels indicate that current strategies have reached their limits.

For the next stage of the struggle, it is essential for Turkey to transition from "prohibitive" policies to "supportive and transformative" policies. The main steps proposed in this context are:

1. Deepening School Centered Success: Canteen inspections should continue, but the focus must shift from merely "not selling harmful food" to "providing beneficial food."

2. Physical Activity Mobilization: To break the inactivity that has become permanent post-pandemic, schoolyards and local government areas should

be transformed into "active living centers," and mobility should be spread to extracurricular times as in the Finland example.

3. Managing Parental Perception: The language of communication directed at parents should be supportive rather than accusatory; clinicians should take a more active role in raising family awareness by visualizing growth curves during routine checks.

4. Main Recommendation: "National School Meal and Active Living Program": As a holistic solution to Turkey's most stubborn problems (fresh food consumption, physical activity, parental perception), a national program inspired by Finland's principle of accessibility and Japan's philosophy of education (Shokuiku) should be implemented. Such a program should be in the nature of a structural reform involving physical activity, table culture, and nutrition education, beyond offering children one free and balanced meal a day by overcoming economic barriers to accessing fresh food.

In conclusion, Turkey's fight against childhood obesity is at a critical junction. Protecting the gains achieved and overcoming structural problems will only be possible with bold policies that are monitored by surveillance data like COSI, learn from international good examples, and invest in the health of generations.

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Chapter 6

MESENCHYMAL STEM CELL DERIVED EXTRACELLULAR VESICLES IN REGENERATIVE AND AESTHETIC MEDICINE

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1. Biological Foundations of Stem Cell-Based Regenerative Medicine

1.1. Fundamental Concepts of Stem Cell Biology and Developmental Hierarchy

Stem cells are specialized cells capable of self-renewal and differentiation into various cell types under appropriate biological conditions. These cells divide as single cells, either producing new stem cells that retain their own characteristics or entering a differentiation pathway to form specialized somatic cells (Poliwoda et al., 2022). These two fundamental properties place stem cells at the center of embryonic development, tissue regeneration, and cellular repair processes. Owing to the division and differentiation potential of stem cells, numerous cell types, such as nerve, muscle, epithelial, and blood cells, can be produced. The fate of stem cells is determined by the type of cell division they undergo. Symmetric cell division results in two stem cells with the same genetic and functional characteristics as one stem cell, which is important for maintaining or expanding the stem cell population. In asymmetric cell division, one stem cell retains its characteristics, whereas the other acquires progenitor cell characteristics (Evano et al., 2020). Progenitor cells have a limited ability to divide and enter the differentiation process by directing themselves toward specific lineages. This process results in the formation of functionally specialized somatic cell. Asymmetric division is a fundamental biological mechanism that maintains tissue homeostasis and cellular diversity. Stem cells are classified into various groups based on their differentiation capacities. Totipotent stem cells have the ability to form both embryonic and extraembryonic tissues and, theoretically, can independently generate a complete organism. In contrast, pluripotent stem cells can differentiate into cell types originating from all three germ layers but cannot form extra-embryonic tissues. Multipotent stem cells can differentiate into a limited number of cell types within a specific tissue or system, whereas in oligo-, bi-, and unipotent cells, this differentiation capacity is increasingly narrowed (Aprile et al., 2024). This hierarchical structure provides a fundamental framework for understanding processes ranging from embryonic development to adult tissue renewal.

Totipotent stem cells are early embryonic cells that emerge after fertilization. The zygote formed after fertilization and the early blastomeres that follow exhibit this totipotency (Du and Wu, 2024). During this period, the cells undergo successive mitotic divisions, forming two- and four-cell stages, respectively. As the embryo travels from the fallopian tubes to the uterus, it reaches the morula stage, which typically occurs on the third and fourth day. Following the morula stage, the blastocyst forms, consisting of the inner cell mass, outer cell mass, and blastocoel cavity. The inner cell mass contains cells that form the embryo, whereas the outer cell mass plays a role in developing placental structures. Totipotency is limited to these early stages, and cell po-

tential gradually decreases after the blastocyst stage (Li et al., 2025). Between approximately the seventh and tenth day, the embryo attaches to the uterine wall, initiating the implantation process. Pluripotent stem cells originate from the inner cell mass at the blastocyst stage and have the capacity to differentiate into all cell types of the three germ layers. Embryonic stem cells are well-known examples of pluripotent cells. In addition, epiblast-derived and primordial germ cells exhibit pluripotency (Zhu et al., 2023). Induced pluripotent stem cells (iPSCs) obtained by reprogramming somatic cells are of great interest because of their reduced ethical constraints and broad potential applications. Furthermore, cells derived from embryonic carcinomas and teratocarcinomas also possess pluripotent properties but have limited clinical applications because of the risk of tumor formation in the host. Pluripotent stem cells demonstrate the ability to differentiate into cells belonging to various organs, such as nerve and pigment cells from the ectoderm, muscle and blood cells from the mesoderm, and cells from the pancreas and lungs from the endoderm (Smith et al., 2025).

Embryonic stem cells (ESCs) are isolated from the inner cell mass at the blastocyst stage and possess high levels of pluripotency. These cells can be propagated under appropriate culture conditions and, when transferred back into the blastocyst, contribute to normal embryonic development (Ren et al., 2025). Embryonic germ cells, on the other hand, originate from primitive germ cells at a more advanced stage of embryonic development and exhibit pluripotent properties. When experimentally isolated and cultured, these cells demonstrate broad differentiation potential. Embryonic carcinoma cells are derived from germ cell tumors, particularly teratocarcinomas. Although these cells also exhibit pluripotent properties, they differ from physiological stem cells in terms of their potential for uncontrolled proliferation and tumor formation. Although these three cell types share similar characteristics in terms of pluripotency, they are distinctly different in terms of their origin and biological behaviors. Specific molecular markers are used to identify and characterize human embryonic stem cells. Among the transcription factors that play a key role in maintaining pluripotency in these cells, Oct4, Nanog, and Sox2 are prominent. These factors suppress cell entry into differentiation pathways, thereby sustaining the self-renewal capacity (Cancedda and Mastrogiacomo, 2025). Additionally, a factor known as the germ cell nuclear factor (GCNF) is involved in the developmental regulation of embryonic and germline-derived cells. The expression of these nuclear factors forms the molecular basis for the pluripotent phenotype of embryonic stem cells. Surface molecules are integral to the identification of ESCs. Surface markers, including CD30, SSEA-3, and SSEA-4, which are glycoproteins and glycolipids, are routinely employed in the identification of these cells. Additionally, surface proteins such as CD9 and CD133 serve as markers that reflect the phenotypic

characteristics of embryonic stem cells (Suresh Babu et al., 2023). These molecules facilitate the identification and purification of cells using techniques such as flow cytometry and immunohistochemistry. The high proliferative capacity of embryonic stem cells is associated with specific enzymatic activity. Telomerase activity contributes to the unlimited division potential of cells by maintaining telomere length. Moreover, alkaline phosphatase activity is regarded as a significant biochemical indicator of the undifferentiated state of embryonic stem cells. These enzymatic properties are utilized for the identification of embryonic stem cells and their monitoring under culture conditions (Jiang et al., 2025). Embryonic stem cells produce specific molecules that contribute to the definition of the pluripotent phenotype. TRA-1-60 and TRA-1-81 antigens are cell surface structures that are specific to embryonic stem cells. GCTM-2, although primarily identified in germ cell tumors, is another marker used to identify pluripotent cell populations. The combined evaluation of these molecules ensures the reliable identification of embryonic stem cells. Pluripotent stem cells contribute to the formation of three primary germ layers during gastrulation. The endoderm layer gives rise to cell types associated with internal organs, such as pancreatic, thyroid, and alveolar cells, among others. The mesoderm layer forms the heart, skeletal, and smooth muscle cells, as well as blood cells such as erythrocytes and lymphocytes. The ectoderm layer is responsible for the development of cells originating from the nervous system and skin, such as the epidermis, neurons, and melanocytes. This differentiation process is a definitive indicator of the developmental potential of pluripotent stem cells (Shahbazi and Pasque, 2024).

1.2. Mesenchymal Stem Cells with Their Phenotypic Characteristics and Paracrine Signaling

Multipotent stem cells found in adult tissues have a more limited differentiation capacity than ESCs. Mesenchymal stem cells (MSCs) are among the most well-defined cell types. These cells exhibit adhesion to plastic surfaces under in vitro conditions and highly express specific surface markers. CD105, CD73, and CD90 positivity are considered characteristic of these cells, while hematopoietic and immune cell markers such as CD45, CD34, CD14, CD11b, CD79α, CD19, and HLA-DR are expected to be negative (Sensebé, et al., 2013). Under appropriate stimuli, mesenchymal stem cells have the capacity to differentiate into mesoderm-derived cells, such as adipocytes, osteoblasts, and chondrocytes. With these properties, multipotent stem cells constitute an important cell group for tissue repair and regenerative medicine applications. The biological effects of stem cells, particularly mesenchymal stem cells, are not limited to their differentiation capacity; they also exert potent non-differentiation (paracrine) effects on surrounding tissues. Cytokines, growth factors, and extracellular vesicles secreted by these cells suppress fibrotic tissue formation and create an anti-fibrotic microenvironment (Lv et al., 2022). They

also play a regulatory role in the immune system by suppressing macrophage infiltration, microglia, and T lymphocyte activation. This immunosuppressive effect contributes to the regulation of the inflammatory response in cases of tissue damage or disease. Furthermore, they increase cellular survival by inhibiting apoptotic cell death mechanisms and stimulating neurogenesis by supporting neuronal differentiation and proliferation. In the central nervous system, the proliferation of reactive astrocytes, angiogenesis, synaptogenesis, and axonal remyelination are important outcomes of paracrine effects.

2. Extracellular Vesicles and Exosome-Mediated Therapeutic Approaches

Cellular therapy strategies are primarily classified into two categories based on the cell source: autologous and allogeneic therapies. Autologous cell therapy involves collecting cells from the patient, expanding them in the laboratory, and reintroducing them into the same patient's body. In contrast, allogeneic therapies use cells from healthy donors. These strategies include stem cell-based and non-stem cell-based therapies that employ immune or other somatic cells. The primary aim of cellular therapies is to foster a healing microenvironment through the secretion of biological factors by cells rather than through the direct incorporation of cells into tissues. In this regard, a significant portion of the therapeutic benefits of stem cells is attributed to extracellular vesicles, such as exosomes and microvesicles (Lener et al., 2015). Exosomes are derived from endosomes, whereas microvesicles are formed by budding from the plasma membrane. Both types of vesicles transport proteins, lipids, microRNAs, and other nucleic acids. Tetraspanins, such as CD9, CD63, and CD81, are typical markers of these vesicles, and the absence of HLA-DR expression is immunologically significant. Due to the biomolecules they carry, these vesicles play a role in regulating intercellular communication, modulating immune responses, and supporting tissue repair. Consequently, extracellular vesicle-based approaches are gaining increased attention, alongside cell-based therapies, in the field of regenerative medicine. Microvesicles and exosomes are extracellular vesicles secreted by numerous cell types. MSCs, neural stem cells, iPSCs, macrophages, dendritic cells, platelets, and tumor cells are the primary sources of these vesicles. Exosomes originate from early endosomes within the cell, mature into multivesicular bodies (MVBs) through late endosomes, and are released into the extracellular environment as a result of fusion with the cell membrane (Yeat and Chen, 2025). During this process, proteins, lipids, mRNA, microRNA, and other nucleic acids are selectively packaged into the vesicles. Natural exosomes carry cell-specific surface molecules, tetraspanins, and biologically active cargo, whereas modified exosomes can be enriched with targeting peptides, growth factors, microRNAs, and drug delivery systems for therapeutic purposes. After release, exosomes interact with target cells via receptor-ligand interactions, membra-

ne fusion, or endocytosis, thereby regulating intracellular signaling pathways (Mathieu et al., 2019). These mechanisms enable stem cell-derived exosomes to support cellular proliferation, angiogenesis, suppress inflammation, and promote tissue regeneration in various pathologies, including wound healing, neurological damage, ischemic stroke, osteoarthritis, myocardial infarction, liver fibrosis, and chronic kidney disease.

In stem cell-based methodologies, adult, embryonic, and iPSCs are directly employed in tissue repair, wound healing, and tissue engineering applications following cell culture and differentiation processes. Conversely, exosome-based strategies utilize the systemic or controlled delivery of extracellular vesicles isolated from MSCs (Phinney and Pittenger, 2017). Exosomes facilitate regenerative processes in various organs, including the brain, spinal cord, heart, lungs, liver, kidneys, bones, cartilage, and skin, by transporting biologically active cargo, such as proteins, lipids, and microRNAs, to target tissues, independent of the cells. The predominant techniques for exosome isolation are differential ultracentrifugation and density gradient-based separations. Recently, advanced approaches such as size exclusion chromatography, acoustic separation, nanotrapping, and flow field fractionation have gained prominence. Experimental studies have demonstrated that MSC-derived exosomes from the bone marrow and adipose tissue exhibit neuroprotective, anti-inflammatory, angiogenic, and neurogenesis-supporting effects in models of traumatic brain injury, ischemic stroke, and hemorrhagic stroke (Hade et al., 2021). It has been reported that these effects are largely mediated by specific microRNAs carried by exosomes. The clinical research continuum is structured around a stepwise evaluation of safety, optimal dosing, and therapeutic efficacy across phases 1-4, beginning with rigorous preclinical experimentation. Within this framework, exosome-based strategies have emerged as promising translational approaches to treat various diseases. Although most exosome-focused interventions are currently confined to discovery, preclinical, and early phase clinical trials, interest in these approaches continues to expand rapidly. This growing attention is largely driven by the inherent advantages of exosomes, including their low immunogenicity, favorable biosafety profile, ability to cross biological barriers, and suitability as cell-free therapeutic agents compared with conventional cell-based therapies (Wiklander et al., 2019). An overview of the current clinical trial landscape reveals that a substantial proportion of exosome-related studies are directed toward their use as diagnostic, prognostic, and predictive biomarkers. Indeed, available registry data indicate that approximately half of all ongoing clinical trials involving exosomes are primarily focused on biomarker development, whereas a comparatively smaller fraction investigates their direct therapeutic application or utilization as drug-delivery vehicles. Biomarker-oriented studies are predominantly concentrated in oncology, reflecting the critical need

for minimally invasive tools to support early detection, disease stratification, and treatment monitoring in patients with cancer. In contrast, clinical trials exploring the therapeutic potential of exosomes encompass a broader and more heterogeneous range of medical conditions than those of EVs. These include inflammatory and infectious diseases, such as SARS-CoV-2-associated pneumonia and sepsis; degenerative disorders, such as osteoarthritis; various neurological diseases; and conditions related to tissue repair and wound healing (Herrmann et al., 2021). Despite these diverse applications, the distribution of clinical phases highlights a significant developmental bottleneck in their use. Most exosome-based products remain in the early stages of clinical translation, with relatively few advancing to late-phase trials and an even smaller number achieving regulatory approval or market entry. Collectively, these observations underscore both the considerable translational promise of exosome-based technologies and the substantial scientific, technical, and regulatory challenges that must be addressed to facilitate their progression into routine clinical practice. MSCs can effectively regenerate damaged tissues through direct cellular differentiation and paracrine mechanisms. These cells accelerate tissue repair in the local microenvironment by secreting growth factors and cytokines and suppressing inflammation by modulating immune responses (Han et al., 2025). However, long-term use poses significant biosafety and efficacy concerns, including the risk of tumor formation due to genetic instability, low tissue engraftment, and limited cell survival. Furthermore, the biological heterogeneity of MSCs derived from different tissues complicates the standardization of clinical outcomes. Exosomes, on the other hand, are cell-free biological products that carry a large portion of the paracrine effects of MSCs. Since they do not contain cells, they do not carry the risk of tumor formation and have low immunogenicity. Their ability to be stored for long periods and to maintain their structural integrity during distribution provides important advantages for clinical use. However, the production, isolation, and purification of exosomes are technically complex and costly. Furthermore, the limited amount of therapeutic payload they can carry and the fact that their ability to specifically target tissues has not yet been fully optimized are among the main factors limiting their clinical applications (Chen et al., 2025).

2.1. Mechanism-Based and Translational Approaches in Wound Repair, Burns, and Flap Survival

Following tissue injury, the wound microenvironment is rapidly enriched with cytokines and chemokines that activate and mobilize cells residing in various stem cell niches, most notably the bone marrow. These mobilized stem cells enter the systemic circulation and are guided to the wound bed through homing mechanisms, where they contribute to the early phases of healing by supporting cellular proliferation and tissue repair through paracrine and autocrine signaling. At the injury site, stem cells either differentiate into

tissue-specific cell types or enhance regeneration by secreting growth factors and extracellular matrix-regulatory molecules that are essential for wound repair (Duscher et al., 2016). Current wound-healing strategies encompass both cellular and acellular approaches, including adult epidermal stem cells, embryonic stem cells, induced pluripotent stem cells, somatic cells, and exosome- or microvesicle-based applications, which can be delivered through intralesional, intradermal, or intravenous routes. In tissue and cell engineering, these approaches are often combined with biodegradable organic or synthetic scaffolds or involve genetic modification to further accelerate repair. A landmark demonstration of the clinical potential of stem cell-based therapies in skin regeneration was reported in a patient with junctional epidermolysis bullosa, in whom autologous epidermal stem cells underwent ex vivo gene correction to restore LAMB3 function, followed by the generation of cultured epidermal grafts that successfully covered more than 80% of the body surface and resulted in a stable, full-thickness functional epidermis during long-term follow-up, sustained by holoclone-derived stem cell populations (Hirsch et al., 2017). Despite these remarkable outcomes, the single-patient nature of the report, personalized and costly production process, and the need for long-term genetic safety monitoring represent important limitations. In parallel, allogeneic MSC therapies have been investigated as adjuncts to standard care in patients with severe and extensive burns, with both intravenous and local administration shown to accelerate epithelialization and significantly improve wound closure without inducing serious immunological adverse effects or pronounced hypertrophic scarring, suggesting the favorable tolerability of this treatment. These beneficial effects are thought to be mediated primarily through paracrine and immunomodulatory mechanisms, including the promotion of angiogenesis, resolution of inflammation, and regulation of fibroproliferative responses (Jeschke et al., 2020). However, the absence of large randomized controlled trials, limited sample sizes, and heterogeneity in treatment protocols currently hinder definitive conclusions regarding their clinical efficacy.

Exosome-based approaches have gained increasing attention as cell-free strategies in burn, wound, and flap surgeries, particularly because of their potential to preserve tissue viability, limit ischemia-reperfusion injury, and influence scar formation. Compared with direct cell transplantation, MSCs-derived exosomes appear to activate key regenerative processes, such as angiogenesis, mitophagy, and immune regulation, in a more controllable and biologically safe manner. Experimental evidence indicates that exosomes obtained from hypoxia-preconditioned mesenchymal stem cells can significantly improve flap survival under ischemia-reperfusion. In a rat random-pattern skin flap model, local application of hypoxic MSC-derived exosomes was associated with a reduction in necrotic tissue and expansion of viable flap areas

(Niu et al., 2022). At the molecular level, suppression of pro-inflammatory cytokines, including IL-1β, IL-6, and MCP-1, was observed, whereas inhibition of the mTOR pathway led to activation of ULK1/FUNDC1-dependent mitophagy, a mechanism reported to support angiogenesis. These changes were accompanied by improved endothelial function, decreased oxidative stress and apoptosis markers, and enhanced microcirculatory perfusion (Deng et al., 2023), suggesting that hypoxic preconditioning is an effective strategy for enhancing the biological activity of exosomes. Additionally, genetically modified exosomes have shown promise in regulating hypertrophic scar formation following burn injuries. Exosomes derived from adipose-derived MSCs and enriched with miR-29a suppressed fibroblast proliferation and activation, thereby limiting excessive scar development. In a mouse thermal burn model, miR-29a-loaded exosomes inhibited the TGF-β2/Smad3 signaling pathway, leading to reduced collagen deposition and a clear anti-fibrotic effect, with histological analyses demonstrating a more organized extracellular matrix and lower fibrotic response compared with non-modified exosomes (Yuan et al., 2021). Overall, these findings support the concept that targeted microRNA delivery via exosomes may offer a promising and flexible cell-free approach for antifibrotic and regenerative applications.

2.2. Regenerative and Aesthetic Medicine: Clinical Evidence and Emerging Applications

In fat grafting and body contouring procedures, cell-supported strategies are supported by substantial clinical evidence demonstrating improved graft survival and durability. Enrichment of autologous fat grafts with ex vivo expanded adipose tissue-derived MSCs (AD-MSCs) has been shown to significantly enhance volume retention. In a randomized, double-blind, placebo-controlled clinical trial, fat grafts supplemented with AD-MSCs exhibited superior volume preservation at 121 days compared to the control group, along with histological evidence of increased vascularization and capillary density. Importantly, the similarity in adverse event profiles between the treatment and control groups supported the clinical safety of this approach (Kølle et al., 2013). Comparable results have been reported in randomized controlled split-body studies on breast augmentation surgery, where AD-MSCs-enriched fat grafts led to improved short- and long-term volume retention and higher patient satisfaction. MRI-based evaluation at 12 months revealed low rates of fat necrosis and no serious adverse events, further confirming the favorable safety profile of this technique (Kølle et al., 2020). Together, these findings provide strong clinical support for the efficacy of the cell-assisted lipotransfer. Beyond volumetric outcomes, numerous animal studies and review articles have demonstrated that adipose-derived mesenchymal stem cells positively influence flap viability by promoting neoangiogenesis through increased capillary density, reducing inflammatory cell infiltration, and

accelerating granulation tissue formation and re-epithelialization. These regenerative effects are largely attributed to the activation of IL-6/STAT3 and VEGF signaling pathways, leading to improved microcirculation and enhanced preservation of the tissue integrity (Conese et al., 2020). In a prospective, randomized split-face study involving individuals with facial aging, the combined use of human AD-MSCs-derived exosomes and microneedling resulted in significant improvements in multiple skin aging parameters after 12 weeks of follow-up. The treated side of the face showed a reduction in wrinkle depth, along with increases in skin elasticity, hydration, and overall tissue uniformity, findings that were further supported by Visual Imaging System for Skin Analysis (VISIA) assessments. The intervention was well tolerated, and no serious adverse events were reported (Park et al., 2023). Similarly, in a double-blind, randomized split-face study of patients with atrophic acne scars, the application of an AD-MSCs-derived exosome solution following fractional CO_2 laser treatment led to more pronounced clinical improvement compared with laser treatment combined with a vehicle alone, as evidenced by lower standardized scar scores, improved skin texture and homogeneity, and a shorter recovery period, with post-laser erythema and edema resolving more rapidly on the exosome-treated side (Kwon et al., 2020). Complementing these findings, a randomized, single-blind split-face clinical study demonstrated that the addition of intradermal stromal vascular fraction to nanofat application significantly enhanced both aesthetic outcomes and tissue regeneration in patients with atrophic acne scars, with greater reductions in scar volume, area, and depth, accompanied by a significant increase in dermal and total skin thickness, indicating that SVF augments the regenerative potential of nanofat and contributes to improved skin quality (Behrangi et al., 2022).

Exosome-based strategies for the management of keloids and hypertrophic scars have garnered increasing interest owing to their pronounced anti-fibrotic effects. In a mechanistic study using human keloid fibroblast cultures, AD-MSCs-derived exosomes suppressed fibroblast proliferation, reduced collagen deposition, and limited excessive extracellular matrix accumulation through modulation of ferroptosis-related signaling pathways. It has been demonstrated that these exosomes attenuate the persistent fibrogenic activity characteristic of keloids by regulating lipid peroxidation and oxidative stress-associated pathways; however, these observations remain confined to the preclinical in vitro level (Xie et al., 2021). Consistent with these findings, a systematic review evaluating regenerative strategies for keloid and hypertrophic scar treatment reported that MSCs, exosomes, and conditioned media commonly exert their effects by suppressing the TGF-β/SMAD pathway, inhibiting fibroblast-to-myofibroblast differentiation, and improving collagen organization in the ECM. Nevertheless, the review highlighted that the existing body of evidence is largely derived from preclinical and early stage clini-

cal studies and is characterized by substantial heterogeneity with respect to product source, dosing, administration route, and treatment frequency, which currently limits the strength of the clinical conclusions (Jafarzadeh et al., 2024). Exosome-based applications for treating pigmentation disorders and melasma are supported by limited but gradually expanding clinical evidence. In a case-based prospective study, topical application of exosomes following superficial microneedling resulted in a progressive and sustained reduction in melasma-consistent hyperpigmentation, as assessed by validated scoring systems, including the Melasma Area and Severity Index, with no serious adverse events reported during a 21-month follow-up period; however, the single-patient design limits the broader generalization of the findings (Lee, 2025). Complementing this observation, a prospective, randomized, double-blind split-face clinical trial demonstrated that a topical formulation containing human AD-MSC-derived exosomes significantly reduced melanin content in individuals with hyperpigmentation compared to the placebo and produced a noticeable skin-brightening effect. The investigators noted that treatment efficacy may be further enhanced by optimizing transdermal delivery and suggested that combining exosome-based formulations with techniques such as microneedling or radiofrequency represents a rational strategy in melasma management protocols (Bonjorno et al., 2020).

3. Conclusion

An integrated synthesis of stem cell-based and cell-free regenerative approaches is presented through a combined evaluation of fundamental stem cell biology, the underlying mechanisms, and emerging translational and clinical evidence. Accumulating data indicated that the therapeutic effects of mesenchymal stem cells were mediated predominantly through paracrine signaling rather than direct cellular engraftment, which has led to an increased focus on extracellular vesicles, particularly exosomes, as key biological effectors of tissue repair. Across a broad range of experimental models and early clinical investigations, exosome-based strategies have been shown to regulate essential regenerative processes, including angiogenesis, immune modulation, oxidative stress control, mitophagy, extracellular matrix remodeling, and fibrosis. These mechanisms were associated with improved wound healing, burn injury management, flap viability, fat graft retention, scar modulation, pigmentation disorders, and aesthetic tissue regeneration. Compared with conventional cell-based therapies, exosomes offer distinct biological and practical advantages, such as reduced immunogenicity, favorable safety profiles, and feasibility of cell-free administration, thereby enhancing their translational potential. Despite these promising observations, clinical implementation remained limited by persistent challenges, including variability in source material, isolation and characterization methodologies, dosing regimens, and delivery strategies, as well as the predominance of small-

scale and early-phase clinical studies. In addition, the regulatory pathways and long-term safety considerations have not yet been fully established. Recent advances in hypoxic preconditioning, targeted cargo enrichment, and delivery-enhancing techniques suggested that the therapeutic efficacy and specificity of exosomes could be further optimized. Overall, the body of evidence supported the view that stem cell-derived exosomes represented a versatile and biologically potent therapeutic platform capable of bridging fundamental regenerative mechanisms with clinical application, while underscoring the necessity for methodological standardization, rigorous mechanistic research, and well-designed randomized clinical trials to define their definitive role in regenerative and aesthetic medicine.

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Chapter 7

FATIGUE MANAGEMENT AND POST-EXERCISE RECOVERY METHODS IN ATHLETES

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Introduction

Fatigue is defined as the disruption of internal homeostasis caused by an external stimulus, such as exercise, leading to an increase in energy production. Fatigue can generally be defined as a decrease in physical performance due to an increase in the perceived difficulty of exercise, and the inability of muscles to reach the intended strength level during exercise (Abd-elfattah et al., 2015).

Fatigue can be triggered by various mechanisms, ranging from the accumulation of metabolites within muscle fibers to the generation of inadequate motor commands in the motor cortex. The effect of fatigue on other areas, such as physical or cognitive performance, is not fully understood and is still being researched (Abd-elfattah et al., 2015; Enoka & Duchateau, 2008).

Fatigue can be classified structurally as physiological and psychological fatigue. Physiological fatigue, defined as a decrease in the ability of muscles to produce force due to a decrease in energy stores and a disturbance in acid-base balance during physical activity, is divided into central and peripheral fatigue (Polito et al., 2017). Central fatigue is the decrease in maximum force production during physical activity resulting from the gradual loss of voluntary activation or a decrease in neuromuscular excitation. Central fatigue is known to originate in the cerebral cortex (Ross et al., 2007). Peripheral fatigue, on the other hand, can be defined as a decrease in the force production capacity of skeletal muscles due to changes occurring within the muscle or at the neuromuscular junction. In peripheral fatigue, impairments may be observed in the transmission of action potentials, in the processes of converting action potentials into mechanical contractions, in the excitability of the sarcolemma, or in the cross- bridge cycle (St Clair Gibson et al., 2001).

Psychological fatigue, on the other hand, represents a psychobiological state caused by prolonged strenuous cognitive activity. Psychological fatigue can manifest subjectively, behaviorally, and physiologically. Subjectively, it can present as increased feelings of fatigue, lack of energy, and decreased motivation. Behaviorally, psychological fatigue is considered to be a decline in performance on a cognitive task (accuracy and/or reaction time, etc.). Finally, changes in brain activity are noted to be a physiological indicator of psychological fatigue (Cutsem et al., 2017).

Symptoms, formation mechanisms and origin of fatigue, a complex process that significantly affects performance and is considered a subjective experience depend on gender, type of sport, psychometric, emotional, psychological, and environmental factors. Considering the morphological and structural differences between genders, women's lower lactic anaerobic energy efficiency may lead to earlier onset of fatigue in activities requiring speed and agility. The type and form of sport is another factor affecting the etiology of fatigue. For example, endurance sports contribute more to central fatigue than shorter athletic events (Cutsem et al., 2017; Tornero-Aguilera et al., 2022). In athletes who perform explosive power and team sports, both

central and peripheral fatigue occur more frequently (Amann, 2011). Factors such as the athlete's emotional state, anxiety level, or sleep status can also cause changes in subjective fatigue perception, leading to central fatigue (Craig et al., 2006). Furthermore, external factors such as temperature and altitude can also affect fatigue (Tang, 2021). The main factors affecting fatigue, related to the internal and external factors mentioned above, can be listed as follows (Akgul, 2013).

- Accumulation of substances (increased lactic acid concentration in muscle cells and blood, etc.).
- Exhaustion of materials (glycogen, etc.).
- Changes in physiological-chemical status (new organization in structures through changes in body temperature via acidity).
- Impairment in coordination regulation through the need for the neurohormonal system.
- Limitations in stimulus transfer.

Assessment of fatigue

Fatigue is a critical factor that directly affects athletes' performance optimisation, training adaptation and planning, mental resilience, injury risk and general health. In this context, various objective and subjective techniques are used to assess training and competition-induced physiological and psychological fatigue in athletes. The methods commonly used to assess fatigue in athletes are summarised below.

-*Maximal voluntary contraction;* this technique is a frequently preferred method for assessing muscle fatigue. Fatigue is generally assessed using maximal voluntary isometric contraction strength. Maximal voluntary contraction performance can be negatively affected by peripheral and central factors, such as lack of motivation. Therefore, methods that apply maximum voluntary contraction with electrical stimulation are also preferred (Vøllestad, 1997).

-*Electromyography (EMG);* it is possible to evaluate the electrical signals produced during muscle contraction using non-invasive approaches such as electromyography, sonomyography, mechanomyography, or near-infrared spectroscopy. Many methods have been developed to characterize muscle fatigue from surface EMG signals during isometric and dynamic contractions (Shair et al., 2017).

-*Scales;* many assessment scales can be used to measure the athlete's perceptual, cognitive, and metabolic responses during exercise and to evaluate the difficulty level of the exercise performed. Scales used to measure fatigue are based on the dynamic integration of afferent inputs related to the cardiorespiratory system, metabolic processes, and thermoregulation with advanced feeding strategies. The most commonly used scale for measuring perceived fatigue is the Borg Perceived Exertion Scale (Eston, 2012). Apart

from this scale, other scales that can be preferred to assess fatigue in athletes are the "Session Rating of Perceived Exertion" (Session RPE Scale) (Foster et al., 2001) for calculating exercise load, the "Estimated Time Limit Scale" (Garcin et al., 1999) for estimating the athlete's exhaustion time, and the OMNI (Robertson et al., 2003) which uses numerical and sport-specific pictorial descriptors for resistance exercise, cycling or running.

-*Biomarkers;* different biomarkers can be used to determine fatigue during or after exercise, depending on the type of exercise (aerobic or anaerobic) performed by athletes. The concentration of biomarkers produced during exercise will vary depending on the athlete's level of fatigue, the type of exercise, and the duration of exercise. Further research on the subject is also investigating the biochemical pathways from which the biomarker originates. Since fatigue does not have a single cause, various biomarkers must be evaluated to assess fatigue. ATP metabolism biomarkers (lactate, ammonia), oxidative stress biomarkers (lipid peroxidation, protein peroxidation, antioxidant capacity), or inflammatory biomarkers (TNF-α, leukocytes, interleukin 6) can be used to assess the athlete's fatigue status (Finsterer, 2012).

-*Dynamic performance tests;* to assess both performance capacity and fatigue in athletes, isoinertial exercises involving eccentric, isometric, and concentric contractions may be preferred. Weight exercises (1 maximal squat, 1 maximal power clean) or sport-specific ballistic movements (vertical jump, sprint) can be used as dynamic performance tests (Gathercole, 2014).

-*Wearable technology;* kinematic, kinetic, and EMG changes related to fatigue can be observed in athletes. In this context, the athlete's fatigue level can be estimated by analyzing movements during exercise and monitoring changes in muscle electrical activity (Gholami et al., 2020). Furthermore, using wearable technologies and physiological signal-based methods, changes in physiological activities such as heart rate or brain activity can be measured to assess the onset or severity of fatigue (Martins et al., 2021).

Various recovery strategies are applied to athletes to both improve performance and facilitate recovery. The most commonly used techniques are summarized below.

Post-exercise recovery methods

It is known that the physiological and psychological changes that occur in athletes after training and competitions temporarily negatively affect performance. These changes can last for a few minutes, a few hours, or even a few days after training or competition. Therefore, establishing a balance between training and competition loads and the speed and quality of recovery will provide countless benefits to the athlete during repeated training and competitions. Overloading athletes before they have recovered can lead to chronic fatigue and injuries. In such cases, the athlete may have to stay away from sports for a certain period of time or even quit sports altogether. The

recovery processes following training and competition that affect athletes' recovery are complex and generally depend on the type of exercise and other external stress factors (Alemdaroğlu, 2011; Halson, 2013).

-The sport performed (type, intensity, duration, severity of fatigue caused, quality of recovery after the previous load)

-The athlete's health status (illness, inflammation, injuries, muscle damage)

-Environmental factors (temperature, humidity, altitude)

-The athlete's lifestyle (sleep quality and quantity, living conditions, relationship with teammates and technical staff)

-Athlete nutrition (carbohydrate, protein, and other nutrient intake amounts, fluid and electrolyte balance)

-Psychological stress experienced by athletes (competition-related stress and anxiety)

There are many active and passive methods used to accelerate recovery in athletes after exercise. The use of these methods may vary depending on the type of physical activity performed, the time until the next training session or competition, and the available equipment and/or personnel. Some of the most popular recovery techniques for athletes are hydrotherapy, physiotherapy, active recovery, rest, compression garments, massage, sleep, and nutrition.

Rest; adequate passive rest and sufficient sleep are very important for managing fatigue and accelerating recovery in athletes. After high-intensity exercise, simply resting will cause a significant decrease in blood lactate levels. A 15-minute rest period after exercise reduces by half blood lactate levels. After high-intensity exercise, a 90-minute rest period returns lactate levels to pre-exercise levels (Barnett Anthon, 2006).

Sleep is reported by elite and sub-elite athletes as the most effective recovery method (Erlacher et al., 2011; Tuomilehto et al., 2017). Sleep has a restorative effect on both the endocrine and immune systems. There is a relationship between sleep and recovery processes. Sleep is vital for physical recovery, particularly due to the relationship between sleep and growth hormone (Doherty et al., 2021).

Hydrotherapy; athletes commonly use contrast bath applications, hot and cold water immersion as a recovery method after exercise. The benefits of hydrotherapy on recovery arise from the effects of hydrostatic pressure and different temperatures, either alone or in combination. The mobilization of body fluids through hydrostatic pressure increases the rate at which metabolic waste products are removed from muscle tissue, thereby supporting recovery processes (Robson-ansley et al., 2009).

Cold application, the most commonly used treatment method for acute soft tissue injuries, is also used for recovery after exercise loading. The cold

water immersion method reduces muscle damage and inflammation, and the change in body core temperature leads to improved performance. In addition, the decrease in tissue temperature reduces nerve conduction and pain, leading to an improvement in the perception of fatigue (Robson-ansley et al., 2009).

Another hydrotherapy method, immersion in hot water, causes peripheral vasodilation due to the increase in superficial tissue temperature, leading to an increase in cutaneous blood flow. The increase in heart rate following immersion in hot water will also cause an increase in cardiac output. Changes in cardiac output and peripheral resistance cause an increase in subcutaneous and cutaneous blood flow, leading to increased cellular, lymphatic, and capillary permeability. The increased in permeability leads to increased metabolism and nutrient transport, and metabolic waste is removed more quickly. Furthermore, improvements in nerve conduction, proprioception, and reaction time achieved through immersion in hot water will support recovery processes. Increased muscle flexibility and joint range of motion, along with a reduction in muscle spasms, will also shorten recovery time in athletes (Wilcock et al., 2006).

The contrast bath application, used to support post-injury recovery processes, has been increasingly applied in recent years to shorten the post-exercise recovery process. Contrast baths, a hydrotherapy application that uses both hot and cold water, cause an increase in blood flow after repeated vasodilation and vasoconstriction. Both the stimulation of the central nervous system and the increase in peripheral circulation shorten post-exercise recovery processes. Reducing of post-exercise edema, inflammation, and muscle spasms, along with increased lactate excretion, can be counted among the other physiological effects of contrast baths. It is also known that contrast baths slow down metabolism and have positive effects on psychological well-being (Cochrane DJ., 2004).

Manual therapy; various manual therapy techniques are used in athletes to both improve performance and accelerate recovery. Different manual therapy applications such as massage, joint manipulations, joint and soft tissue mobilization, and fascial techniques can be used to accelerate post-exercise recovery processes. Manual therapy techniques increase the flexibility of the muscle-tendon junction by altering the viscoelastic properties of muscle tissue, thereby reducing muscle stiffness. The increase in circulation associated with the increase in nitric oxide production after application will contribute to the reduction of delayed muscle soreness and serum creatine kinase concentration. Improved lymphatic flow will also lead to a reduction in edema. Considering the neurophysiological effects of manual therapy, inhibition of the H reflex and Golgi tendon reflex will also contribute to the recovery process after exercise. Mobilization of the fascia will increase both muscle function and joint range of motion (Garc et al., 2021; Manuel et al., 2024).

Active recovery; active recovery, which involves performing aerobic and low-intensity exercise after intense exercise, is the removal of lactic acid with

the help of active muscles. Aerobic exercise performed below the anaerobic threshold will increase the utilization of lactate by the liver, kidneys, heart, and active muscles. Aerobic exercises such as running and swimming are examples of exercises that can be preferred for active recovery. For active recovery to be performed effectively, the duration and intensity of the preferred aerobic exercise must be carefully planned. A poorly planned active recovery program will cause blood lactate levels to rise and the athlete to become even more fatigued. For this reason, active recovery methods performed below the anaerobic threshold and lasting between 10 and 30 minutes should be preferred (Alemdaroğlu, 2011; Halson, 2013).

Stretching exercises; although there are conflicting results in the literature, studies show that stretching exercises support post-exercise recovery processes (Chen et al., 2011; Eguchi A, 2004; Mika et al., 2007). Stretching exercises performed after exercise will contribute to restoring joint range of motion and reducing muscle soreness and stiffness. They will also provide advantages in reducing delayed onset muscle soreness and restoring strength after exercise.

Exercise combinations consisting of voluntary contractions and stretches will reduce post- exercise edema by mechanically supporting lymph flow and venous return. Stretching exercises for recovery in athletes should be performed dynamically and painlessly. High-intensity stretching exercises can cause loss of strength and power in athletes. Uncontrolled ballistic stretching may result in increased muscle pain and stiffness (Sands et al., 2013).

Electrostimulation; interest in the use of electrostimulation is increasing due to its effects occurring at the cellular level (increased protein synthesis activity and ATP production), its painless nature, lack of side effects, low cost, and ease of application. Electrostimulation has effects on microcirculation, vascularization, and cellular energy production. The applied current stimulates tissue restoration and growth and reduces edema. Electrostimulation also has positive effects on pain and muscle spasm (Akkoc & Caliskan, 2019; Piras et al., 2021).

Compression garments; compression garments that allow external pressure application without restricting movement are used to both enhance performance and shorten post-exercise recovery time. The use of compression garments has been observed to cause physiological and biochemical changes in the venous, arterial, and lymphatic systems. Nutrient delivery and metabolite elimination processes in muscles are accelerated, and positive effects are observed on post-exercise edema, delayed muscle soreness, and muscle damage complaints (Brown et al., 2017; Kaçoğlu, 2015). It is known that compression garments worn during the recovery process reduce the concentrations of creatine kinase and lactate dehydrogenase, which are markers of muscle damage (Figueiredo & Penha-silva, 2011).

Hyperbaric oxygen therapy: hyperbaric oxygen therapy or hyperoxygenation is a treatment method involving the application of 100% oxygen in a high-pressure environment. This treatment method accelerates

cell renewal and tissue repair, thereby speeding up fatigue recovery and endurance capacity restoration. Hyperbaric oxygen therapy increases the level of dissolved oxygen in the blood and causes an increase in partial oxygen pressure in peripheral tissues. In this case, it potentially stimulates healing and recovery processes. It increases oxygenation of skeletal muscles, accelerates the removal of metabolites that cause fatigue, and speeds up ATP production. Hyperbaric oxygen therapy is also a safe and effective method that can be used to reduce local hypoxia and inflammation (Gušić et al., 2024; Moghadam et al., 2020; Sperlich et al., 2017).

Conclusion

Effectively managing fatigue and ensuring rapid recovery after training or competition provides numerous benefits for an athlete's optimal recovery and performance. There are many active and passive physical recovery methods for athletes to recover after exercise loads, including cell renewal, tissue repair, lactate removal, restoration of muscle physical properties, and reduction of pain and stiffness. We believe that future studies on this topic will contribute to the literature by determining the optimal application principles of recovery methods and investigating the effects of combined methods involving different recovery techniques on fatigue and recovery processes.

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Chapter 8

THE EFFECTS OF EXERCISE ON ATTENTION DEFICIT AND HYPERACTIVITY DISORDER

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Introduction

Attention deficit hyperactivity disorder (ADHD) is one of the most common psychiatric disorders in childhood. This disorder is characterized by symptoms of inattention, hyperactivity, and impulsivity that are inappropriate for the person's age and developmental level and begin in childhood. ADHD can cause significant academic, social, and psychiatric problems, and its negative effects can last a lifetime. It affects 5-12% of children worldwide (Özaslan & Bilaç, 2015; Rowland et., 2002).

In addition to pharmacological and non-pharmacological interventions for ADHD treatment, psycho-social and pedagogical applications with psycho-educational content also play an important role (Xiang et al., 2017). Although medication is the primary treatment method for ADHD, most guidelines recommend starting ADHD treatment with non-pharmacological interventions and switching to pharmacological treatment in cases of severe impairment (Thapar & Cooper, 2016).

In recent years, the potential effect of exercise on symptom management in individuals with ADHD has been recognized and has received increasing attention from researchers. It has been suggested that exercise can be used in conjunction with traditional pharmacological, psychological, and pedagogical intervention strategies to improve cognitive performance in individuals with ADHD. Furthermore, it is known that combining exercise with pharmacological treatment enhances the effect of pharmacological treatment (Christiansen et al., 2019). The primary focus in this area is on aerobic exercise. The effects of different exercise programs on ADHD-related functions and symptoms remain unclear, and more research is needed in this field (Li et al., 2023).

This study aims to comprehensively present different exercise programs that can be used to reduce the negative effects of ADHD and to contribute to the literature on this topic.

Overview of attention deficit hyperactivity disorder

ADHD is a disorder characterised primarily by inattention, hyperactivity and impulsivity, accompanied by a range of cognitive and behavioural symptoms (del Barrio, 2004). ADHD symptoms may begin in childhood and persist throughout life. Children with this disorder generally have weaker social skills, participate less in group games with their peers and have difficulty adapting to other children (DuPaul et., 2001). In children, hyperactivity symptoms associated with ADHD are generally more noticeable than attention deficit symptoms. For example, a child with ADHD may have difficulty sitting still during class or frequently interrupt others when they are speaking (Eiland & Gildon, 2024). Inattention in children often leads

to difficulties planning and completing lessons. Children with ADHD are consistently observed to be disinterested in classroom instruction. Bu durum akademik performansın düşmesine neden olur. Additionally, sleep problems, defiant behavior, risky behavior that leads to accidents, bedwetting, and resistance to participating in daily household chores and personal care are behavioral problems frequently seen in children with ADHD (Jangmo et., 2019; Torun et., 2009). Emotional distress in adults is more easily noticeable compared to hyperactivity symptoms. Emotional fluctuations, the reduction of ADHD hyperactivity symptoms with age, and the compensation of symptoms with cognitive overactivity make diagnosing ADHD in adults difficult. Inattention becomes a more persistent symptom of ADHD in adults. This can lead to frequent job changes, career instability, and difficulties in maintaining relationships (Martel et al., 2012; Weyandt et al., 2003; Williams et al., 2023).

The causes of ADHD are not fully understood. Biological, psychological, sociological and cultural factors are thought to contribute to ADHD. The literature discusses genetic, neurodevelopmental, and environmental factors. The consensus is that ADHD is a brain disorder that develops at birth and is due to genetic factors. Furthermore, socioeconomic status, neglect, abuse, trauma, and adverse living conditions may also play a role in its development (Çöpür & Çöpür, 2018).

Studies in neuroimaging, neuropsychology, genetics, and neurochemistry indicate that abnormalities in the frontal lobe and striatal regions are associated with ADHD. Changes in these regions constitute the fundamental neural basis of ADHD and contribute to the symptomatic features of the disorder (Bush, 2010). Knowledge regarding the genetic causes of ADHD is based on twin, adoption and family studies. It is known that the prevalence of ADHD is higher among first- and second-degree relatives or siblings of individuals diagnosed with ADHD, and that ADHD shows approximately 80% heritability, independent of gender (Faraone, et ., 1994). It is known that certain environmental factors may also be responsible for the development of ADHD. It has been reported that exposure to toxic, metabolic, and circulatory causes during the perinatal period may affect brain development, leading to the onset of ADHD. Intrauterine rubella and other infections, postnatal encephalitis, meningitis, nutritional disorders, metabolic disorders such as phenylketonuria, eclampsia, smoking during pregnancy, postmaturity, low birth weight, and antepartum haemorrhage are other environmental factors associated with ADHD (Kotimaa, et., 2003; Milberger et al., 1997). When the effects of the psychosocial environment on the etiology of ADHD are examined, it has been reported that early losses, divorce, problems in parent-child relationships and family attitudes play a role in the etiology of ADHD (Öncü & Şenol, 2002).

ADHD has three subtypes: combined type, predominantly inattentive type, and predominantly hyperactive-impulsive type. In the predominantly inattentive type, the inability to sustain focus is predominant. The main complaints of families and teachers are the child's inability to concentrate, lack of order and organizational skills, forgetfulness and being easily distracted attention. In the ADHD type characterised by hyperactivity and impulsivity, these symptoms are intense and involve excessive, uncontrolled movements and hasty actions without thinking (impulsivity) are intense. Complaints observed in this type include restlessness, disruptive behaviour in the classroom or environment, impatience and hastiness, and an inability to wait during conversations and other activities. In the combined type, symptoms from the other two subtypes are observed together, and combined type ADHD is reported to be the most common type (Willcutt, 2012).

Since the 2000s, the prevalence of ADHD reported by families and the number of children with ADHD taking medication have increased rapidly (Visser et., 2014) and the prevalence of ADHD has been reported to be approximately three times higher in boys than in girls (Polanczyk et., 2014). A study of children diagnosed with ADHD indicated that the type characterized by hyperactivity is more common in boys, while the type characterized by inattention is more common in girls (Tahiroğlu et., 2010). According to research, Turkey ranks 7th among countries where people consult a psychiatrist or psychologist with suspected ADHD (Şenol & İşeri, 2004).

Effects of attention deficit hyperactivity disorder on motor skills and physical fitness

In ADHD, in addition to the main symptoms such as inattention, hyperactivity, and impulsivity, differences can be observed in many functions such as brain volume, timing, balance and motor control, walking, and motor planning (Cundari et al., 2023; Luders et al., 2009; Seidman et al., 2005).

The cerebellum is known to play a crucial role in many tasks, including balance, motor control, and sensory-motor integration, as well as cognition, language, and emotional regulation. ADHD, like some other neuropsychiatric disorders, has been reported to be associated with differences in cerebellar function (Cundari et al., 2023). Cerebellar dysfunctions seen in ADHD lead to negative effects such as balance deficits, postural sway disorders, impulsivity, and poor inhibitory control (Goetz et al., 2017; Hove et al., 2015). Impaired inhibition due to cerebellar dysfunction in individuals diagnosed with ADHD affects postural control, gait, and balance performance. Furthermore, individuals with ADHD are known to have lower motor skill performance and more severe motor coordination problems compared to typically developing individuals (Buderath et al., 2009; Fliers et al., 2008; Kaiser et al., 2015).

Dynamic balance impairments seen in individuals with ADHD directly impact activities requiring greater balance, such as walking on uneven surface, climbing stairs, and engaging in sports activities. Furthermore, poor postural control can increase the risk of injury during daily activities (Goetz et al., 2017; Shum & Pang, 2009).

In addition to balance and postural sway disorders, low physical fitness and problems with gross and fine motor skills are observed in the majority of individuals diagnosed with ADHD (Tascioglu et al., 2024). Individuals with ADHD have been found to have deficiencies in physical fitness parameters such as strength, agility, endurance, anaerobic performance, and lower extremity muscle strength performance (Jeyanthi et al., 2019). Individuals with higher ADHD tendencies and symptoms have been found to have higher rates of abdominal fat and lower grip strength, muscular strength, and endurance (Jeoung, 2014).. A related study reported that children with ADHD have below-average VO_2 max values Harvey & Reid, 1997).

It is known that the inattention deficit symptoms seen in ADHD are associated with motor coordination disorders (Fliers et al., 2008). Individuals with ADHD experience difficulties in motor coordination and performing tasks requiring multiple motor skills. Impairments in gross motor skills such as running, climbing, and hopping on one foot, as well as in fine motor control and hand-body coordination, may also be observed. These movement-related problems also lead to an increased rate of injury due to accidents in school-aged children with ADHD (Li et al., 2023; Shilon et al., 2012).

Another problem observed in individuals with ADHD is prolonged reaction time. The inadequacy in the temporal organization of motor outputs seen in children and adults with ADHD is associated with interruptions in attention, irregularities in neuronal and temporal activity, executive dysfunction, and difficulties regulating energy levels. Changes in reaction time lead to fluctuations in both behavior and task performance (Castellanos et al., 2006; Tamm et al., 2012).

Increasing evidence in recent years suggests a link between ADHD and obesity (Holtkamp et al., 2004; Strimas et al., 2008). Within the biopsychosocial framework, cognitive-behavioural and physiological factors such as genetic factors, foetal programming, executive function impairments, psychosocial stress, positive energy balance, and sleep pattern alterations may be considered among the reasons for the relationship between ADHD and obesity (Han & Cortese, 2018). Furthermore, the use of medication for ADHD treatment, the type of medication used, its dosage and duration of use, and comorbid conditions may influence the relationship between ADHD and obesity (Dubnov-Raz et al., 2011; Fliers et al., 2013; Nigg et al., 2016).

Treatment of attention deficit hyperactivity disorder

ADHD is not only seen in childhood; it is known that if left untreated, it continues into adulthood and impairs functioning in many areas such as education, career, and interpersonal relationships Barkley, 2002). As ADHD is a multifactorial disorder involving various social factors, its treatment should also be comprehensive and holistic (Santos & Albuquerque, 2019). Both pharmacological and non-pharmacological methods are used in the treatment of ADHD. Pharmacological treatment is the first-line treatment for ADHD (Philipsen et., 2015). However, pharmacological treatment can sometimes lead to undesirable situations (side effects, poor tolerance, lack of response to treatment) Mulas et al., 2012). Most guidelines on the subject recommend a stepwise approach to treatment, starting with non-pharmacological interventions and progressing to pharmacological treatment for those severely affected (Polanczyk, et., 2014). Non-pharmacological treatment methods such as psychoeducation, social skills training, cognitive behavioural therapy, mindfulness training, neurofeedback, and dietary interventions. The guidelines state that psychoeducation is fundamental to ADHD treatment and recommend a comprehensive treatment approach that should be offered to everyone diagnosed with ADHD, their families, and caregivers(Çalışkan & Tarakçıoğlu, 2019; Seixas et., 2012.

Exercise interventions are also among the newly emerging methods used in individuals diagnosed with ADHD, and their effects on ADHD symptoms have not yet been sufficiently researched. In addition to the general health benefits of exercise, it is known to have beneficial and lasting effects on cognitive functions, increasing blood flow to the brain, neurotransmitter levels, and plasticity, and improving focus, attention, and information processing skills (Akin, et., 2017; Neudecker et al., 2019; Ng et al., 2017; Salici & Söyleyici, 2020)

The effect of exercise in the treatment of attention deficit hyperactivity disorder

Exercise is a treatment method that has been increasingly studied in recent years for ADHD, both in terms of symptoms, executive function, and motor skills, as well as its effects on the brain. Studies provide evidence that exercise therapy represents a promising alternative or additional treatment option for ADHD (Heijer et al., 2017; Neudecker et al., 2019). Compared to medication, exercise therapy is considered to be an effective method for improving areas such as attention, motor skills, and executive functions without any adverse side effects. It can be combined with all treatment approaches for ADHD (including pharmacological treatments). It is inexpensive, non-invasive, and provides additional health benefits (Christiansen et al., 2019; Heijer et al., 2017; Smith et al., 2013). Various types of exercise, such as walking, treadmill,

cycling, ball games, water exercises, fitness, team games, or combined exercise programs, are recommended for ADHD treatment. Although exercise-related motor development has been found to have positive effects on ADHD symptoms, discussions regarding the type, intensity, duration, and frequency of exercise are still ongoing (Neudecker et al., 2019).

The changes that exercise induces in central nervous system structures, neuronal network activity, and biochemical signaling have positive effects on the core symptoms of ADHD (Christiansen et al., 2019). Individuals diagnosed with ADHD may experience certain issues in the developmental processes of midbrain and cortical structures (Nagel et., 2012). Research conducted on children and adolescents has found that exercise can cause neuroplastic changes in subcortical and cortical structures (Chaddock, et., 2010; Voss et al., 2013). Exercise also helps to increase attention by contributing to the development of coordination between the brain regions necessary for attention distribution and other specific areas in individuals with ADHD (Sun et al., 2022). The core symptoms of ADHD originate from deficits in executive functions. In ADHD, reduced activation is observed in neural networks within key brain structures associated with executive functions. These deficits in executive functions negatively affect the physical and mental health, behaviour management, learning activities, and social behaviours (Kelly et al., 2012; Yang et al., 2024). According to the neurobiological hypothesis, physical activity is thought to increase catecholamine neurotransmission, neurogenesis/angiogenesis, and neuroplasticity. Exercise-induced increases in catecholamines such as dopamine and norepinephrine also support executive functions (Voss et al., 2013; Wigal et al., 2013). Exercise is also known to increase serotonin levels, which are typically reduced in individuals with ADHD. Increased serotonin levels help improve focus and attention (Heijer et al., 2017). Furthermore, levels of brain-derived neurotrophic factor, which plays a role in the aetiology of ADHD, increase with exercise. This also has positive effects on cognitive and mental functions in individuals with ADHD (Shim et al., 2008).

Individuals with ADHD have lower motor skills and physical fitness compared to their same-age peers. This situation causes ADHD symptoms to become more pronounced. These deficiencies in motor skills and physical fitness observed during childhood lead to postural sway and low static balance performance as the individual ages. Poor balance and motor performance skills are also a significant cause of physical injuries in these individuals. Furthermore, the poor motor performance observed in individuals with ADHD negatively affects social competence, academic performance, and quality of life (Muntaner-Mas et al., 2021; C. Pan et al., 2016; Ziereis & Jansen, 2015). In this context, beyond the cognitive advantages of exercise, it facilitates the management of ADHD symptoms by causing improvements in physical parameters such as balance, coordination, reaction time, and motor

skills in individuals with ADHD (Christiansen et al., 2019; Villa-gonzález et al., 2020).

Anxiety, depression, and emotional dysregulation are clinical problems observed to varying degrees in individuals with ADHD. Individuals with ADHD who experience emotional dysregulation are more prone to anxiety, depression, increased attention deficit symptoms, and aggressive and destructive behaviours compared to other individuals with ADHD (Antony et al., 2022; Marques et al., 2024; Martz et al., 2023). Exercise increases motivation for cognitive tasks and feelings of energy in individuals with ADHD, while reducing fatigue and depressive symptoms (Fritz & O'Connor, 2016). Furthermore, moderate to high-intensity exercise leads to improvements in emotional regulation and self-efficacy in individuals with ADHD (Hoza et al., 2015).

Exercise type

Exercises such as aerobic, anaerobic, running training, water exercises, football, active games with video content, bicycle ergometer, rope skipping, ball exercises, dynamic stretching, jumping on a trampoline, taekwondo, running, throwing, swimming, yoga, and table tennis have positive effects on ADHD (Cerrillo-Urbina et al., 2015; Mehren et al., 2020; Pan et al., 2016; Song et al., 2023; Wang et al., 2023).

Although there is no consensus on which exercise type is most ideal for individuals with ADHD, aerobic exercise appears to be the most frequently used. A study on the effects of acute aerobic exercise in adults with ADHD found that cognitive performance improved after 20 minutes or more of moderate-intensity aerobic exercise (Chang et al., 2012). Weng et al. (2017) reported that increased functional connectivity in networks related to executive functions and attention after aerobic exercise. Aerobic exercises such as walking, running, and ball games are also known to have positive effects on selective attention (Jeyanthi et al., 2019). Rassovsky and Alfassi (2019) indicated that aerobic exercise had positive effects on psychometric test results and reduced reaction time and omission errors in individuals with ADHD. McKune et al. (2004) found that a program consisting of various aerobic exercises resulted in improvements in attention, behavior, affect, and motor skills in individuals with ADHD.

Intermittent exercise programmes are another type of exercise applied to individuals diagnosed with ADHD. Intermittent exercises are frequently recommended due to the impulsivity, lack of motivation and impatience observed in individuals with ADHD. These exercises cause positive changes in the physical fitness, motor skills, aerobic capacity, and quality of life of individuals with ADHD (Mehren et al., 2020; Sun et al., 2022; Zhang et al., 2023). Furthermore, studies have shown that intermittent exercises also

affect neurotransmitter production and functioning processes and improve behavioural control (Lee et al., 2015). It is known that an intermittent exercise programme consisting of balance and rope skipping exercises in individuals with ADHD causes a significant increase in cardiovascular endurance, muscle strength, muscular endurance, flexibility, and epinephrine levels (Lee et al., 2015. Meßler et al. (2018) found that high-intensity interval training improved physical fitness, motor skills, quality of life, and attention levels in individuals with ADHD.

Individuals diagnosed with ADHD experience difficulties in motor skills such as force control, contraction timing, and producing appropriate movement speed. In particular, changes in force control and contraction timing negatively affect movement production. Furthermore, the time to reach maximum strength is prolonged in individuals with ADHD. In this context, strength and power exercises contribute to the development of both functional muscle strength and gross motor performance in individuals with ADHD (Pitcher et al., 2002; Steger et al., 2001). A study on this subject found that strength and power exercises improved coordination, limb strength, functional walking performance, running speed, and agility in individuals diagnosed with ADHD. The same study also noted that the effect size of strength exercises was greater than power exercises (Tascioglu et al., 2024).

In recent years, positive effects of digital games known as 'exergaming' on ADHD have been identified. These games, which combine cognitive activity with physical activity, have significant effects on cognitive functions. A combination of physical activity and cognitive stimulation improved reaction times in inhibition and switching neural efficiency (Ji et al., 2023). Exergame applications stimulate the frontal lobes of the brain, leading to improvements in attention, processing speed, cognitive control, and executive functions (Benzing et al., 2018; Ji et al., 2023). Furthermore, these games increase calorie expenditure and aerobic capacity, leading to improvements in balance, coordination, and agility, thereby enhancing physical fitness in individuals with ADHD (İnce 2023; Ji et al., 2023).. Traditional cognitive programmes may not be sufficiently engaging for individuals with ADHD due to their motivation and positive reinforcement issues (Dovis et al., 2015). In this context, exargame applications also stand out in increasing motivation and participation in individuals with ADHD (Staiano et al., 2017).

In addition to the exercise types mentioned above, stretching exercises are also known to have positive effects on ADHD. According to sensorimotor theory, physical activity is extremely important for cognitive development. Stretching exercises have positive effects on cognitive flexibility, executive functions, and processing speed. Stretching exercises increase the brain's oxygen utilization capacity, and this in turn improves individuals' learning skills (Patar et al., 2017; Sharififar et al., 2017). Choi et al. (2016)

determined increased cortical activity in individuals with ADHD after electroencephalography measurements performed after stretching exercises. In addition to isolated stretching exercise programs, yoga exercises that incorporate stretching exercises can also be preferred in the management of ADHD. Yoga exercises can be used to support hyperactivity, social anxiety, and shyness in individuals with ADHD (Jensen & Kenny, 2004). Furthermore, yoga exercises are known to cause improvement in attention levels, anger outbursts, mood swings, and neuropsychological functions in individuals with ADHD (Manjunath et al., 2024).

In addition to land-based exercises, aquatic exercise programs consisting of swimming and water exercises may also be preferred for the management of ADHD symptoms. Aquatic exercise improves physical, cognitive, and socio-emotional status in individuals with ADHD (Hosokawa et al., 2024). Chang et al. (2014) reported improvements in motor skills, coordination, and reaction time following aquatic exercise consisting of aerobic and coordination exercises. Another study found that swimming exercise significantly improved mental health, cognitive skills, and motor coordination in children with ADHD (Silva et al., 2020). A study conducted on children diagnosed with ADHD demonstrated the positive effects of aquatic games on inattention, hyperactivity, and impulsivity (Al-Ashkar, H. I., & Abo Heshima, 2019). Sabzi et al. (2021) also reported that aquatic treadmill exercise had positive effects on psychosomatic, behavioral, social, and anxiety-shyness problems in individuals with ADHD.

Duration, frequency and intensity of exercise

Exercise studies conducted with individuals diagnosed with ADHD demonstrate a lack of consensus regarding exercise duration. It is recommended that exercise should be 30-60 minutes (Jeyanthi et al., 2019) for the core symptoms of ADHD, while 70 minutes of exercise per day (Sun et al., 2022) is recommended for a positive impact on cognitive and executive functions. It has been stated that exercise should be at least 45 minutes to improve the inhibitory functions of individuals with ADHD. In a meta-analysis on the subject, it was emphasized that short-term exercises (15-30 minutes) would be effective in increasing inhibitory control (Chueh et al., 2022). Consequently, further studies are needed to determine the appropriate exercise duration for individuals with ADHD.

Studies aimed at determining exercise frequency have reported that exercising 2-3 times a week can significantly increase inhibitory functions. Reducing exercise frequency, however, may trigger physical fatigue in children with ADHD, negatively affecting cognitive functions (Cian et al., 2001; Song et al., 2023. Pan et al. (2016) reported that school-based exercise programmes conducted five days a week in children with ADHD led to improvements in

inattention, hyperactivity, and defiant behaviour.

Recent studies on the subject indicate that the effect of exercise programmes on ADHD is more related to exercise intensity than to the type of exercise. As with other parameters related to exercise, there is no consensus on the intensity of exercise to be applied to individuals with ADHD (Mehren et al., 2020; Wang et al., 2023). Studies have shown that moderate-intensity exercise contributes more to executive functions, inhibitory functions, increased attention, academic performance, working memory, motor skills, learning, and target behaviours (Montalva-Valenzuela et al., 2022; Sun et al., 2022). Some studies on the subject have also indicated that high-intensity exercise leads to greater improvement in physical fitness parameters but also enhances cognitive functions (Chan et al., 2021; Jeyanthi et al., 2019).

Conclusion

In recent years, exercise programmes have become increasingly important in the management of ADHD symptoms, the prevalence of which has been steadily rising. The changes exercise brings about in cognitive capacity, executive functions, inhibitory control, and social competence improve the quality of life for individuals with ADHD. The literature indicates that various exercise regimens, such as aerobic, resistance, balance, and aquatic, are used alone or in combination in the management of ADHD. In summary, studies demonstrate that exercise is a non-pharmacological method that can be used in the management of ADHD throughout life.

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Chapter 9

GLP-1 RECEPTOR AGONISTS AND NUTRITIONAL MONITORING IN OBESITY MANAGEMENT

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Introduction

Obesity remains a major global public health challenge, with its prevalence continuing to rise despite decades of public health campaigns and behavioural interventions. The World Health Organization defines obesity as abnormal or excessive fat accumulation that may impair health, and it is now linked to a range of comorbidities including type 2 diabetes mellitus (T2DM), cardiovascular disease, certain cancers, and reduced quality of life (Ard, Lewis, & Moore, 2024). In this context, novel pharmacotherapeutic tools have emerged that enhance the armamentarium for weight-management beyond traditional diet and exercise. Among these, the class of glucagon-like peptide-1 (GLP-1) receptor agonists has gained considerable attention for its efficacy in inducing substantial weight loss in individuals with overweight or obesity (Mozaffarian, 2024). GLP-1 receptor agonists (GLP-1 RAs) act by mimicking the endogenous incretin hormone GLP-1, thereby modulating glucose metabolism, satiety, and energy intake. Mechanistically, GLP-1 RAs both delay gastric emptying and stimulate central satiety pathways via hypothalamic and brain-stem circuits, thus reducing caloric intake (Christensen, Robinson, Thomas, & Williams, 2024). Empirical data demonstrate that use of GLP-1 RAs in clinical trials can lead to body-weight reductions in the order of 15 %–25 % of baseline weight over approximately one year of therapy (Despain & Hoffman, 2024). Moreover, the therapeutic benefits extend beyond mere weight loss: improved glycaemic control, reductions in ectopic fat deposition, amelioration of cardiometabolic risk factors, and favourable effects on inflammation have all been described (Moran & Roberto, 2024).

Despite these remarkable advances, reliance on pharmacotherapy alone is unlikely to guarantee sustainable success in obesity management. Several studies emphasise that behavioural and lifestyle factors remain critical determinants of both initial weight loss and long-term maintenance (Jhe et al., 2025). Indeed, even with GLP-1 RA therapy, individuals who do not adopt or maintain healthy eating behaviours, dietary monitoring, or physical-activity habits tend to experience suboptimal outcomes or weight regain upon medication discontinuation. It is therefore timely to consider the interaction between pharmacologic treatment and behavioural strategies in particular, nutritional monitoring (Wang et al., 2023).

Nutritional monitoring which may include self-monitoring of dietary intake, use of mobile nutrition applications, logging of food/beverage consumption, and structured feedback from dietitians has long been recognised as a key behavioural strategy in weight-loss interventions (Garvey, Mahle, Bell, & Kushner, 2024). The act of recording one's diet fosters awareness, supports self-regulation, and aligns with behavioural theories of change (Mehrtash, Dushay, & Manson, 2025). Systematic reviews indicate that greater adherence to dietary self-monitoring is associated with greater weight

loss, independent of other intervention components (Wharton et al., 2022). Indeed, a recent meta-analysis found that frequent self-monitoring (whether full diet logs or abbreviated tracking) correlates with improved outcomes (A. Brown et al., 2024). Hence, in an era where potent pharmacotherapies are increasingly used, the question arises: Does the presence or absence of structured dietary monitoring modulate the weight-loss efficacy of GLP-1 receptor agonists?

This review addresses this question by examining the interplay between GLP-1 RA therapy and nutritional monitoring, with a focus on how dietary tracking behaviours influence weight-loss magnitude, composition (fat versus lean mass), maintenance, and rebound phenomena. First, we will summarise the mechanisms of GLP-1 RAs, their clinical efficacy, and limitations. Then we will explore the literature on nutritional monitoring per se, including adherence, technological supports, and behavioural moderators. Next, we will conceptualise how nutritional monitoring might enhance or attenuate the effects of GLP-1 RAs through enhanced dietary quality, improved macronutrient distribution, maintenance of lean mass, and reduced compensatory behaviours. Finally, we will discuss implications for clinical practice and research: how integration of diet-tracking tools can optimize outcomes, and what gaps remain.

2. Mechanisms of GLP-1 Receptor Agonists

Glucagon-like peptide-1 (GLP-1) is an incretin hormone secreted by enteroendocrine L cells of the distal ileum and colon in response to nutrient ingestion, with actions that extend beyond glucose regulation to appetite suppression, delayed gastric emptying, and modulation of energy balance. GLP-1 receptor agonists (GLP-1 RAs) are synthetic analogs or mimetics that activate GLP-1 receptors (GLP-1R) across multiple organ systems to mimic and amplify the native hormone's effects. At pancreatic β-cells, GLP-1R couples to Gs to elevate cAMP and activate PKA and Epac2, enhancing closure of KATP channels, augmenting voltage-gated Ca2+ influx, mobilizing insulin granules, and potentiating glucose-dependent insulin exocytosis thereby lowering postprandial and fasting glucose with minimal hypoglycemia risk (Butsch et al., 2025). In α-cells, GLP-1 signaling suppresses glucagon in a glucose- and context-dependent manner via direct receptor effects and indirect intra-islet paracrine mechanisms, further reducing hepatic glucose output. Centrally and via gut–brain vagal pathways, GLP-1 RAs act on nucleus tractus solitarius, area postrema, and hypothalamic circuits (activating POMC/CART and inhibiting NPY/AgRP neurons) to increase satiety, reduce energy intake, and modestly influence energy expenditure; peripherally, they slow gastric emptying by increasing pyloric tone and modulating vagovagal reflexes, flattening postprandial glucose excursions and reducing glycemic variability (Popoviciu, Păduraru, Yahya, Metwally, & Cavalu, 2023). Beyond glycemia

and weight, GLP-1 RAs confer pleiotropic benefits: they improve endothelial function, reduce oxidative stress and low-grade inflammation, and may favorably modulate atherothrombosis, translating in several large outcome trials into reductions in major adverse cardiovascular events for specific agents. Renal effects include decreased albuminuria and slower decline in eGFR, likely mediated by hemodynamic, metabolic, and anti-inflammatory mechanisms. Preclinical and early clinical data suggest neuroprotective actions dampening neuroinflammation, improving synaptic plasticity, and promoting neuronal survival which are being explored in neurodegenerative diseases. Collectively, through integrated pancreatic, gastrointestinal, central nervous system, cardiovascular, and renal actions, GLP-1 RAs deliver robust glycemic control, clinically meaningful weight loss, and organ-protective benefits in people with type 2 diabetes and obesity (Crespo, Rodríguez-Duque, Iruzubieta, Morel Cerda, & Velarde-Ruiz Velasco, 2025; Kennedy, Moyer, & Herdes, 2025) (**Figure 2.**).

2.1 The GLP-1 Signaling Pathway

GLP-1 R is a G-protein-coupled receptor expressed in several tissues, notably the pancreas, gastrointestinal tract, central nervous system (CNS), heart, and kidney. Upon ligand binding, GLP-1R activates adenylate cyclase, increasing intracellular cyclic adenosine monophosphate (cAMP), which in turn modulates protein kinase A (PKA) and exchange protein directly activated by cAMP (Epac) pathways (Jalleh et al., 2024). In pancreatic β-cells, this cascade enhances glucose-dependent insulin secretion, promotes β-cell proliferation, and inhibits apoptosis. Simultaneously, in α-cells, GLP-1R activation suppresses glucagon secretion, collectively resulting in improved postprandial glucose control (Wharton et al., 2023).

2.2 Central Appetite Regulation

One of the most critical mechanisms underlying weight reduction is the central effect of GLP-1 on appetite and satiety centers. GLP-1 RAs penetrate or signal across the blood-brain barrier to influence neuronal circuits within the hypothalamus and brainstem, particularly the arcuate nucleus (ARC) and the nucleus tractus solitarius (NTS) (Grill, 2020). Within the ARC, GLP-1 stimulates pro-opiomelanocortin/cocaine- and amphetamine-regulated transcript (POMC/CART) neurons while inhibiting neuropeptide Y/agouti-related peptide (NPY/AgRP) neurons, creating an anorexigenic state. This dual modulation reduces hunger signals and increases satiety perception, leading to lower caloric intake (Shoemaker et al., 2022).

Peripheral vagal afferents further mediate these effects: GLP-1 released in the intestine activates receptors on vagal neurons projecting to the NTS, amplifying satiety signals to higher brain centers (E. Brown, Cuthbertson, & Wilding, 2018). This gut-brain axis forms the cornerstone of the appetite

suppression observed in GLP-1 RA therapy. Importantly, GLP-1 also attenuates reward-related eating behavior through dopaminergic modulation in the mesolimbic system, decreasing hedonic food cravings (Al-Najim, Raposo, BinMowyna, & le Roux, 2025).

2.3 Gastric Emptying and Gastrointestinal Effects

GLP-1 RAs delay gastric emptying by reducing gastric motility and tone. This prolongs the digestive process and blunts postprandial glucose excursions by slowing nutrient absorption. The delayed gastric emptying contributes directly to enhanced satiety and reduced meal size, forming a synergistic mechanism with central appetite suppression. However, this same mechanism explains common gastrointestinal side effects nausea, vomiting, bloating, and early satiety experienced during early phases of treatment (stachowska Stachowska, Korus, Cembrowska-Lech, & Kłoda, 2025).

2.4 Effects on Energy Expenditure and Metabolic Adaptation

While reduced caloric intake remains the dominant mechanism of weight loss with GLP-1 receptor agonists (GLP-1 RAs), converging preclinical and translational evidence suggests a complementary, modest rise in energy expenditure via central and peripheral pathways. Neuronal GLP-1R activation within brainstem–hypothalamic circuits (notably NTS→PVN/ARC) can engage sympathetic outflow to thermogenic depots, increasing norepinephrine-driven lipolysis and, in some contexts, upregulating mitochondrial uncoupling proteins in brown and beige adipocytes. In rodent models, central GLP-1 signaling has been linked to heightened brown adipose tissue thermogenesis and improved cold tolerance, consistent with a sympathetic mechanism (E. Brown, Wilding, Barber, Alam, & Cuthbertson, 2019). However, effects on non-shivering thermogenesis and UCP1 induction vary across studies, species, exposure duration, and dosing paradigms, and human data remain heterogeneous, with several trials showing little or no sustained increase in resting energy expenditure after adjusting for changes in fat-free mass (Sánchez-Garrido et al., 2017). Beyond thermogenesis, GLP-1 RAs appear to improve metabolic flexibility shifting substrate use toward greater lipid oxidation at rest and during the postprandial period reflected by lowered respiratory quotient and enhanced mobilization/oxidation of fatty acids, while concomitantly preserving lean mass when combined with adequate protein intake and resistance exercise (Arillotta et al., 2023). These adaptations may attenuate the typical decline in energy expenditure seen with hypocaloric weight loss (i.e., adaptive thermogenesis), thereby supporting weight-loss maintenance even as body mass decreases. Overall, any direct thermogenic effect is likely small relative to intake suppression, but favorable shifts in substrate partitioning, sympathetic tone to adipose tissue, and maintenance of fat-free mass collectively contribute to improved energy

balance and reduced weight-regain susceptibility (Kadouh et al., 2020).

2.5 Peripheral Organ Effects

GLP-1 receptor agonists (GLP-1RAs) exert organ-specific benefits through converging metabolic, hemodynamic, and anti-inflammatory mechanisms. In the pancreas, GLP-1R couples to Gs to raise cAMP and activate PKA and Epac2, which enhances closure of KATP channels, augments voltage-gated Ca2+ entry, mobilizes insulin granules, and potentiates glucose-stimulated insulin secretion; in parallel, GLP-1 signaling upregulates insulin gene transcription (Pdx1, MafA), improves proinsulin processing, mitigates ER stress, and promotes β-cell survival via PI3K–Akt pathways, while suppressing α-cell glucagon secretion through direct receptor effects and intra-islet paracrine signaling together delivering potent glucose-dependent insulinotropic effects with a lower hypoglycemia risk than agents like sulfonylureas (Baggio & Drucker, 2021). In the liver, GLP-1RAs indirectly reduce hepatic glucose production by lowering glucagon (improving the portal insulin:glucagon ratio) and enhancing hepatic insulin sensitivity; they also improve lipid handling by dampening de novo lipogenesis (e.g., downregulating SREBP-1c), reducing hepatic diacylglycerol content, improving mitochondrial β-oxidation, and decreasing inflammation, thereby ameliorating steatosis and potentially nonalcoholic steatohepatitis through body-weight loss–independent and -dependent components (Au et al., 2025). In the heart and vasculature, GLP-1RAs improve endothelial function by increasing nitric oxide bioavailability (eNOS activation), reducing oxidative stress and NADPH oxidase activity, attenuating vascular inflammation and adhesion molecule expression, and favorably modulating plaque biology; myocardial metabolism shifts toward more efficient substrate use (enhanced glucose uptake/oxidation), which can improve ischemia tolerance and left ventricular energetics, while clinical trials demonstrate reduced major adverse cardiovascular events with specific agents, reflecting these vascular and metabolic effects alongside weight, blood pressure, and glycemic improvements (Thota-Kammili, Tama, & Hurtado Andrade, 2024). In the kidney, GLP-1R is expressed along the nephron particularly in proximal tubule and preglomerular vasculature where receptor activation promotes natriuresis via PKA-mediated phosphorylation/inhibition of NHE3, increases tubular flow to the macula densa (supporting tubuloglomerular feedback), and may reduce afferent arteriolar hyperfiltration; systemic effects include modest blood-pressure lowering via natriuresis, weight loss, and improved endothelial function. Additionally, anti-inflammatory and antifibrotic actions (reduced macrophage infiltration, TGF-β signaling) and improved metabolic milieu contribute to decreased albuminuria and slower eGFR decline observed in outcome studies (Alluri et al., 2025). Collectively, through synchronized pancreatic, hepatic, cardiovascular, and renal mechanisms,

GLP-1RAs deliver glucose-dependent insulinotropic action, suppression of inappropriate glucagon, improvements in hepatic and vascular biology, natriuresis and hemodynamic benefits, and clinically validated reductions in cardiovascular and renal risk (Santos et al., 2025)(Figure 1).

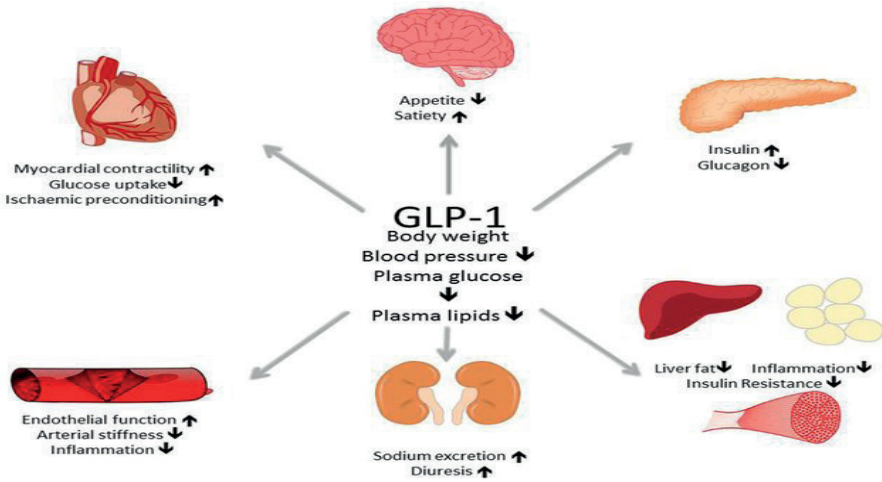

Figure 1. Visualizing Cardiovascular, Renal, and Metabolic Effects of GLP-1 (Boyle, Livingstone, & Petrie, 2018).

2.6 Pharmacokinetics and Molecular Modifications

Native GLP-1 is cleared within minutes (half-life ~2 min) due to rapid N-terminal truncation by DPP-4 and renal elimination; therapeutic analogues therefore embed stabilizing design features that extend exposure and reshape distribution. Substituting the alanine at position 2 (Ala8 in mature GLP-1 numbering) with DPP-4–resistant residues prevents enzymatic cleavage and is foundational to most agents (Min et al., 2025). Fatty-acid acylation at specific lysine residues confers reversible albumin binding, creating a circulating depot that slows renal filtration, reduces proteolysis, and supports prolonged target engagement; linker chemistry and acyl chain length also modulate receptor micropharmacology by altering tissue access and local concentration at the endothelial interface (Min et al., 2025). Liraglutide employs a C16 palmitic acid with a spacer to enable once-daily dosing, whereas semaglutide uses a longer C18 diacid with optimized linker geometry to markedly increase albumin affinity and support once-weekly administration; semaglutide's enhanced stability also derives from amino-acid substitutions that further resist DPP-4 and neutral endopeptidases. Alternative half-life extension strategies fuse the peptide to large carriers (e.g., Fc, albumin) or engineer dual- or multi-agonism. Tirzepatide, a single peptide with biased agonism at GIPR and GLP-1R plus a C20 fatty diacid for albumin binding,

exhibits prolonged pharmacokinetics and augmented efficacy by engaging complementary incretin pathways. These molecular choices not only prolong half-life but also influence volume of distribution, tissue partitioning (e.g., gut wall versus CNS access), receptor residence time, and signaling bias (PKA/Epac balance), which together can shape clinical phenotypes such as appetite suppression potency, gastrointestinal tolerability, and glycemic durability (Jakhar, Vaishnavi, Kaur, Singh, & Munshi, 2022; Sun et al., 2024).

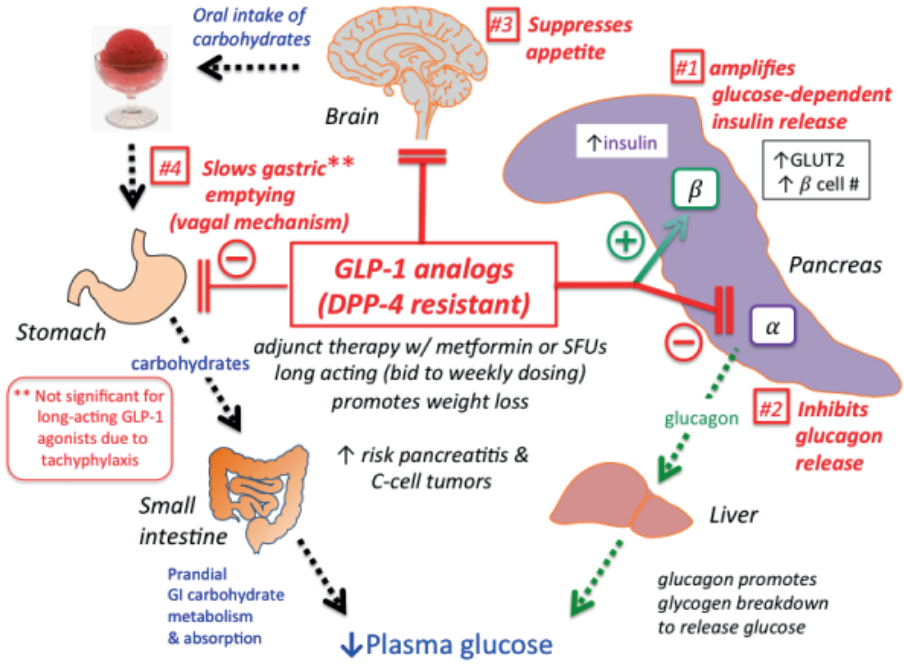

Figure 2. Potent glucagon-like peptide-1 (GLP-1) receptor agonists exert their therapeutic effects through multiple mechanisms, including delayed gastric emptying, stimulation of glucose-dependent insulin secretion, suppression of glucagon release, and central nervous system mediated appetite reduction. Short-acting GLP-1 receptor agonists, such as exenatide, primarily lower postprandial glucose levels by markedly delaying gastric emptying. In contrast, long-acting formulations such as albiglutide, dulaglutide, or extended-release exenatide exert a more pronounced effect on fasting plasma glucose, largely through sustained modulation of insulin and glucagon secretion. The impact of these long-acting agents on gastric emptying is minimal due to the development of tachyphylaxis to GLP-1–mediated gastric inhibitory effects, likely involving adaptive changes in parasympathetic tone (Camilleri, 1993; Madsbad, 2016).

2.7 Integration of Mechanisms in Weight Regulation

Weight reduction with GLP-1 pathway agonism emerges from coordinated actions across central and peripheral systems that collectively produce a sustained negative energy balance (Young et al., 2014). Within the central nervous system, pharmacologic GLP-1R activation engages brainstem–hypothalamic circuits (e.g., NTS, area postrema, ARC) and corticolimbic networks involved in reward valuation, decreasing homeostatic hunger signals, dampening cue-driven and hedonic eating, and enhancing meal satiation; this reflects a recalibration of appetite circuitry rather than a nonspecific aversive state, with learning-related changes that persist beyond acute dosing (Moiz et al., 2025b). In the gastrointestinal tract, increased pyloric tone and modulation of vagovagal reflexes slow gastric emptying and potentiate intestinal satiation signals, flattening postprandial glycemic excursions and extending inter-meal intervals. At the pancreas and liver, improvements in the insulin–glucagon axis enhance nutrient partitioning reducing hepatic glucose output and de novo lipogenesis while promoting glycogen storage thereby lowering energetic overflow into ectopic depots (Kanoski, Hayes, & Skibicka, 2016). In peripheral tissues, modest increases in lipid oxidation and improvements in mitochondrial efficiency, endothelial function, and natriuresis support small but cumulative increments in energy expenditure and cardiometabolic health (Campbell et al., 2023). The integration of these effects reduces energy intake predominately, with secondary benefits on energy utilization, leading to clinically meaningful, durable weight loss; importantly, the satiety signal is dissociable from nausea and persists at maintenance doses and in individuals with minimal gastrointestinal adverse effects, consistent with direct, circuit-level modulation of appetite control (Li et al., 2025).

2.8 Mechanistic Implications for Nutritional Monitoring

The pharmacologic suppression of appetite raises critical questions about nutrient adequacy and dietary composition. As caloric intake declines, so too may the intake of essential nutrients particularly protein, calcium, and micronutrients (Ullah & Tamanna, 2025). Without structured nutritional monitoring, individuals may experience disproportionate lean-mass loss or nutrient deficiencies despite significant weight loss. Conversely, diet tracking can ensure balanced macronutrient distribution and adequate micronutrient consumption, complementing GLP-1 pharmacodynamics. Understanding these interactions is essential for integrating pharmacotherapy into holistic weight-management paradigms (Abdalla Ahmed, Ssemmondo, Mark-Wagstaff, & Sathyapalan, 2024).

3. Discussion

The widespread use of GLP-1 receptor agonists (GLP-1 RAs) marks a major paradigm shift in obesity management, providing pharmacologic efficacy once deemed unattainable without bariatric surgery. However, their introduction also reignited fundamental questions about the *role of human behavior, diet quality, and nutritional monitoring* in sustaining pharmacologically induced weight loss. Evidence increasingly suggests that the success of GLP-1 RAs depends not only on their biological potency but also on behavioral scaffolding that reinforces dietary awareness and metabolic resilience (Azhari & Dawson, 2024).

While GLP-1 RAs suppress appetite and promote caloric deficit, this pharmacologic advantage may paradoxically weaken self-regulatory eating behaviors if not accompanied by structured dietary tracking. Individuals experiencing pharmacologically induced satiety often underreport food intake and neglect macronutrient balance, particularly protein and fiber (Veniant et al., 2024). Consequently, loss of lean mass, micronutrient deficiencies, and metabolic slowdown may emerge, offsetting the long-term benefits of weight reduction (Celik et al., 2025).

Integrating *nutritional monitoring* through food diaries, wearable sensors, or dietitian-guided follow-ups restores the behavioral dimension of treatment. These tools maintain awareness of food quality and portion control, reinforcing patient engagement even as appetite signals diminish (Mosterd, Bjornstad, & van Raalte, 2020; Yun et al., 2024). Behavioral reinforcement appears particularly critical when GLP-1 therapy is tapered or discontinued, preventing the rapid weight regain observed in clinical extensions (Abdalla Ahmed et al., 2024).

The phenomenon of *adaptive thermogenesis* a reduction in resting metabolic rate beyond what is predicted by weight loss represents a biological counterforce to sustained fat reduction (Gentinetta, Sottotetti, Manuelli, & Cena, 2024). During GLP-1 RA therapy, reduced caloric intake triggers this adaptive response, which persists even after treatment withdrawal. Without dietary tracking, patients frequently revert to pre-treatment eating patterns while metabolism remains suppressed, leading to rapid fat recovery(Chang, Ripoll, Lopez, Krishnan, & Bittner, 2024). Structured nutritional monitoring, particularly with a focus on protein distribution and resistance exercise, mitigates this adaptive slowdown by preserving muscle mass and energy expenditure. Therefore, nutritional guidance serves not merely as a behavioral adjunct but as a physiological stabilizer in long-term obesity care (He et al., 2024).

Gastrointestinal side effects of GLP-1 RAs such as nausea, vomiting, and early satiety can precipitate subclinical nutrient deficiencies (Christensen

et al., 2024; Moiz et al., 2025a). Vitamin B12, iron, and fat-soluble vitamins may be compromised due to reduced intake or altered absorption. Without systematic monitoring, such deficiencies remain undetected until functional impairments arise. Evidence from recent clinical audits shows that incorporating registered dietitians into obesity pharmacotherapy teams reduces side-effect discontinuations by up to 40% and enhances adherence. Thus, *nutritional surveillance* is not ancillary it is integral to therapeutic safety (Zakaria et al., 2024).

The growing popularity of GLP-1 RAs among non-obese populations and for cosmetic weight reduction raises ethical and practical concerns. Unsupervised use without dietary oversight can foster restrictive eating patterns, disordered body image, and rebound weight gain once treatment stops (Butsch et al., 2025). Moreover, disparities in access to medical nutrition therapy create inequities in long-term outcomes, as individuals without professional dietary support may regain weight more rapidly (Kennedy et al., 2025).

Healthcare systems must therefore frame GLP-1 RAs not as *standalone weight-loss solutions*, but as components of integrated metabolic care, combining pharmacologic, nutritional, and behavioral dimensions. In this context, *nutritional monitoring* represents the bridge that translates pharmacologic efficacy into sustainable lifestyle transformation (Wiener et al., 2025).

4. Future Perspectives

The next frontier in obesity management lies at the intersection of pharmacology, digital health, and personalized nutrition. While GLP-1 RAs have revolutionized short-term weight reduction, the future challenge is to maintain their benefits through scalable, data-driven nutritional frameworks (Ahmed et al., 2025). Advancements in artificial intelligence and mobile health technologies are transforming dietary monitoring from self-reporting to *automated, objective tracking*. Machine-learning algorithms can now estimate caloric and macronutrient intake from food images with over 90% accuracy (Fitch & Ingersoll, 2021). Integrating these tools into GLP-1 therapy offers real-time feedback loops, allowing dynamic adjustment of dosage, nutrient composition, and activity levels. Future models of obesity care may combine AI-driven nutrition analytics, continuous glucose monitoring, and metabolic biomarkers to personalize therapy in real time, optimizing outcomes while minimizing side effects. Combining GLP-1 RAs with behavioral therapy, physical activity programs, and dietary supervision represents a synergistic approach. Clinical evidence already suggests that concurrent dietitian support enhances pharmacotherapy efficacy by 15–25% (Sforzo, Gordon, Peeke, & Moore, 2025). Future

randomized controlled trials should evaluate *structured nutritional monitoring* as an independent variable influencing long-term weight maintenance. Moreover, research should explore optimal macronutrient profiles during GLP-1 therapy particularly protein-to-energy ratios that minimize lean mass loss and prevent rebound hunger after cessation. Despite growing enthusiasm, critical gaps persist (Kramer, Retnakaran, & Viana, 2024). Most clinical trials focus on weight loss magnitude rather than *diet quality* or *nutritional adequacy* during GLP-1 therapy. Few studies measure changes in nutrient intake, dietary diversity, or food choices over time. Additionally, the interaction between GLP-1 signaling and gut microbiota potentially influenced by diet composition remains largely unexplored (Mohammed & Mishra, 2025). Future studies integrating metabolomic and microbiome analyses with dietary tracking could uncover novel pathways linking pharmacologic modulation and nutritional metabolism (Elmaleh-Sachs et al., 2023).

The long-term sustainability of GLP-1 therapies extends beyond individual outcomes. Given their high cost and limited availability, public health systems must identify *who benefits most* and how to integrate pharmacotherapy with cost-effective lifestyle interventions. Nutritional monitoring tools particularly digital platforms and tele-nutrition offer scalable, low-cost strategies for sustaining behavioral change at the population level (Ali, Ghodsimaab, Rusling, & Rashid, 2024). Embedding these approaches in national obesity management programs could ensure that pharmacologic advances translate into equitable and durable public health benefits. Ultimately, the future of obesity treatment lies in synergy, not substitution. GLP-1 RAs have proven that pharmacologic modulation of appetite is possible; nutritional monitoring ensures that it becomes sustainable. The convergence of digital nutrition science, behavioral psychology, and endocrinology heralds a new model of obesity care one where weight loss is not a transient pharmacologic effect but a sustained metabolic recalibration supported by informed, adaptive nutrition (Fredrick, Camilleri, & Acosta, 2025; Valladares et al., 2025).

5. Conclusions

GLP-1 receptor agonists (GLP-1 RAs) represent a transformative advance in the pharmacologic management of obesity, offering potent appetite suppression, delayed gastric emptying, and favorable metabolic effects that together drive substantial and sustained weight reduction. Mechanistic insights reveal that these agents act via a multi-organ network, integrating central appetite regulation, gastrointestinal signaling, pancreatic hormone modulation, and peripheral energy metabolism. This comprehensive biological effect underlies the clinically observed efficacy of agents such as liraglutide, semaglutide, and tirzepatide.

However, pharmacologic potency alone is insufficient to achieve optimal and durable outcomes. Evidence consistently demonstrates that structured nutritional monitoring through dietitian-guided counseling, digital food tracking, and macronutrient optimization enhances weight loss efficacy, preserves lean body mass, and prevents micronutrient deficiencies. Behavioral and dietary adherence serves as a critical mediator of the translation from pharmacologic effect to long-term health benefit.

Clinical trials indicate that discontinuation of GLP-1 therapy often results in partial or complete weight regain, highlighting the necessity of sustainable lifestyle integration. Therefore, GLP-1 therapy should not be regarded as a stand-alone intervention; rather, it must be embedded within a multidisciplinary framework that combines pharmacotherapy, precision nutrition, and behavioral reinforcement.

Future research should prioritize the development of integrated treatment models that leverage digital nutrition tools, artificial intelligence, and individualized dietary strategies to optimize outcomes. Additionally, studies examining the interplay between GLP-1 signaling, gut microbiota, and dietary composition may reveal novel pathways to further enhance efficacy and safety.

In conclusion, GLP-1 receptor agonists offer unprecedented pharmacologic support for weight reduction, but their maximal potential is realized only when combined with systematic nutritional monitoring and behavioral support. This integrative approach ensures not only significant weight loss but also the preservation of metabolic health, lean mass, and long-term sustainability, establishing a new standard for comprehensive obesity management.

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Chapter 10

MACHINE LEARNING APPROACHES IN NUTRITION SCIENCE: MODELING GLUCOSE VARIATION USING SYNTHETIC DATA

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INTRODUCTION

Obesity and related chronic diseases are becoming increasingly prevalent worldwide. In addition, increased blood glucose levels are a significant public health problem negatively impacting the course of these diseases. The primary goal is the application of medical nutritional therapy, which addresses modifiable environmental factors in blood glucose regulation. Postprandial blood glucose levels can vary among individuals, and it has been shown that postprandial blood glucose prediction can be made by evaluating various characteristics.

In recent years, machine learning methods have attracted significant interest in the field of nutrition science. The reason for this is the diversification and growth of data. When traditional statistical methods in nutrition are insufficient to capture complex and multidimensional data structures, we can benefit from machine learning algorithms. The study by (Van et al., 2021) shows the potential to create personalized nutrition plans based on multidimensional data such as meal macronutrient content, microbiome, lifestyle, sleep, and activity. As these developments show, machine learning has begun to be preferred.

Postprandial glucose responses are traditionally assessed by calculating the incremental area under the curve of blood glucose levels over a 2-hour period based on the glycemic index values of foods (Gentilcore vd., 2006). Accordingly, while carbohydrates primarily affect postprandial blood glucose, protein and fat intake levels are also known to have a significant impact. Recently, decision tree machine learning models have begun to be used in studies aimed at predicting post-meal blood glucose based on food consumption record data. Accordingly, differences in post-meal glycemic response between individuals were evaluated using data sources such as age, gender, etc., dietary patterns, physical activity, and blood test results (Berry vd., 2022; He vd., 2021).

The aim of this chapter is to illustrate the application of machine learning methods in nutrition research by using a synthetic dataset to model postprandial glucose variation. Therefore, synthetic data with 500 subjects and the dependent variable being postprandial glucose change (mg/dL) was generated to detail the application areas of machine learning algorithms. The Random Forest algorithm, one of the machine learning algorithms, was used to predict glucose change.

Glucose Metabolism and Nutrition Relationship

The prevalence of diabetes, characterized by high blood glucose levels, is increasing and is estimated to affect 783 million individuals by 2045. Failure to maintain blood glucose homeostasis can lead to chronic diseases and

serious complications, primarily cardiovascular diseases, kidney diseases, and nervous system diseases. (IDF, 2021). Research has been conducted using machine learning and artificial intelligence methods to reduce the effects of diabetes and improve the quality of life of patients. Machine learning-based methods for predicting diabetes occurrence are divided into two groups: current condition identification and prediction approaches. While the classification of existing data samples is done using case description methods, the prediction of incidence using data from both current and previous medical records is done using advanced estimation methods (Ravaut vd., 2019).

According to the American Diabetes Association (ADA) guidelines, prediabetes is defined as fasting plasma glucose (FPG) levels between 100 and 125 mg/dL, while values ≥126 mg/dL indicate diabetes.

Precision Nutrition and Machine Learning

Precision nutrition involves the use of personal information to generate nutritional advice that, in theory, leads to superior health outcomes than generic advice (Berciano et al., 2022). Machine learning facilitates both the production of accurate nutritional outcomes and the collection of related data. Traditional methods of assessing nutrient intake are limited because they rely on estimated intakes. Machine learning-assisted moitoring of nutrient intake can make the data collection process more efficient and enable more detailed evaluation (Mezgec vd., 2019).

Monitoring changes in blood glucose levels is necessary for achieving glycemic control. Continuous Glucose Monitoring (CGM) systems play an important role because they provide real-time data for monitoring individual variations in blood glucose levels. In addition, regulating food intake in diabetes management significantly affects the management of post-meal blood glucose. The study showed that even though the content of the meal given to individuals was the same, the differences in post-meal blood glucose values highlighted the need for personalization of medical nutritional therapy (Zeevi ve ark, 2015). In addition, machine learning model predictions reported that personalized medical nutrition therapy recommendations would significantly lower post-meal glucose levels. Another study indicated that a machine learning algorithm working with real-world data (RWD) collected from a digital cohort had a high level of accuracy in predicting post-meal blood glucose (Singh ve ark, 2025).

An Overview of Machine Learning

Machine learning is an artificial intelligence field that makes predictions or classifications from data. This method can capture non-linear relationships between variables, work with high-dimensional data structures, and learn complex interactions in large samples. Thus, machine-learning methods

possess great advantages when several multivariate data types must be assessed simultaneously in the context of nutrition research.

Machine learning methods are grouped into two main categories: supervised and unsupervised learning approaches. In supervised learning methods, there is an output variable during model training, and the algorithm makes predictions or classifications by learning the relationship between the input variables and this output. It is divided into regression and classification problems.

In unsupervised learning methods, there is no output variable, and the main goal is to reveal the internal structure of the data, its patterns, and the similarities between variables. Clustering and dimension reduction techniques are commonly used.

In this study, the Random Forest algorithm, one of the supervised learning methods, was applied due to the continuous nature of the dependent variable under investigation. Regression-based machine learning algorithms enable the modeling of linear and nonlinear relationships between input variables and the output variable. This approach has begun to be preferred in multivariate and complex data structures.

Machine Learning: Practical Advantages

Machine learning (ML) has many beneficial applications in nutrition science studies due to its capability of learning from data. The aspect of algorithms learning from data without programming significantly saves time and energy while improving their adaptability to different problems. The same machine learning program can be trained on different data and applied to perform different tasks. Additionally, machine learning has the capability of processing different data structures. Machine learning techniques can process non-structured data such as image and sensor data. Machine learning techniques are also able to process and evaluate nutrition data with various data structures. Machine learning techniques can aid in reducing time and costs needed during experimental verification. The techniques develop accurate predictions on data already generated. Machine learning techniques in health studies have been able to demonstrate the reduction of unnecessary verification processes with non-invasive and low-cost variables (Sorino et al., 2020). Additionally, big data generation and increasing openness in data sharing have increased access to applications of machine learning techniques. Using basic computer infrastructure and data availability, scientists are able to perform potentially meaningful data analysis without requiring high budget laboratories. Therefore, machine learning techniques can be viewed as powerful aids in promoting advances and developments in nutrition and health sciences (Jordan & Mitchell,2015).

Machine Learning and Traditional Statistical Methods

The reporting and analysis of dietary information, using traditional approaches like questionnaire-based dietary recall, can involve errors, and the variability in data both in terms of variance and dimension can be challenging using traditional statistical analysis. Nutrition is also considered to be a multi-dimensional process, with thousands of different foods being consumed in different proportions, making the task of dietary pattern analysis rather complicated. It is here that machine learning can make in-roads, helping to understand high-dimensional data with a focus on the presence of certain non-linear relationships that might be difficult for traditional pattern analysis or linear regression approaches in understanding the linkages of dietary patterns with health. For instance, while traditional analytical approaches using linear assumptions in terms of interactions, like Principal Component Analysis, are limited in terms of understanding dietary patterns, machine-learning algorithms like XGBoost or Random Forest can help in understanding the linkages with certain non-additive, non-linear relationships (Quan et.al., press).

Machine learning and its sub-field of computer vision (CV), which employs deep learning, have greatly impacted nutritional data collection. For instance, food photographs taken using smartphones or wearable cameras can be analyzed using models such as convolutional neural networks to automatically distinguish them, thereby eliminating the need for personalized documentation of nutritional information; this helps contributions to diet tracking to be made using objective, real-time, and high-quality visual information. Wang et al. were successful in applying this technique to obtain 5% more accurate classification than other models for 233 different food categories using a CNN-based model. Likewise, photographs taken from food intake sources after extended periods using wearable cameras can be analyzed using machine learning to automatically calculate calorific and nutrient component analyses on a daily basis. These practices offer profound time- and money-saving advantages when considering normal food recording methods while also making it possible to determine nutritional quality using objective standards. Machine learning has proven to work alongside and/or better conventional methods for models describing nutrient intake and health relationships (Jordan & Mitchell, 2015).

Chin et al., in one of the studies, the data was linked across different food databases through 24-hour recall, and the quantity of lactose not present in the ASA24 output was able to be predicted using machine learning algorithms like XGBoost. It is seen in one of the examples given above that machine learning algorithms are capable of predicting what is not present in food. Overall, machine learning algorithms are more informative for nutrition research because they are able to identify the relationship between diet and

disease, which is non-linear.

Random Forest

The Random Forest model was created by Breiman (2001) and is composed of multiple independent decision trees that predict their outputs and then combine them.

The capability to model nonlinear associations and interactions among multiple dimensions is automatically incorporated within Random Forest. This model is highly useful for research involving complex behavioral and biological datasets. Additionally, the capacity to efficiently deal with noisy datasets, missing values, with minimal assumptions is highly applicable for regression analysis tasks.

Performance Measurements

To evaluate the predictive accuracy of different machine learning models, error metrics were used to compare model performance. For regression models, commonly used performance metrics, including root mean square error (RMSE), R^2, and explained variance, were employed to assess model performance on both training and test datasets. R^2 represents the proportion of the total variance in the dependent variable that is explained by the model. Its formula is given below:

$$R^2 = 1 - \frac{\sum_{i=1}^{n}(y_i - \hat{y}_i)^2}{\sum_{i=1}^{n}(y_i - \bar{y})^2}$$

In the formulas, denotes the observed value, represents the value

predicted by the model, indicates the mean of the observed values, and corresponds to the number of observations.

The MSE is the mean of the square of the difference between the predicted and actual values. Classification makes significant errors more obvious; Thus, a rigorous evaluation of the error reduction capability of the model can be performed. However, interpreting it can be challenging because its unit differs from the original variable (Alpaydın, 2011).

$$MSE = \frac{1}{N}\sum_{i=1}^{n}(y_i - \hat{y}_i)^2$$

The RMSE is obtained by taking the square root of the MSE and expressing the number of errors in the model in terms of the average of the original variables. It, therefore, facilitates the interpretation of the predicted

error. A low RMSE value indicates that the model's prediction is close to the true value (Uğuz, 2019).

$$RMSE = \sqrt{\frac{1}{N}\sum_{i=1}^{n}(y_i - \hat{y}_i)^2}$$

Synthetic Data Example

In this chapter, a synthetic dataset was employed to demonstrate the methodological workflow of machine learning applications in nutrition research rather than to represent real clinical populations. The overall aim with the synthetic data was rooted in the necessity for having a flexible and controllable structure in order to evaluate the application and efficiency of machine learning methods. Additionally, synthetic datasets allow for model construction and variable inclusion as they mimic real-world distribution.

It has 500 observations with 11 variables. Variables range from demographics and meal items to short-term physical activity, sleep duration, microbiome score, and glucose response. It provides information on the increase in blood glucose levels after meals (ranging from –16.4 to +59.1 mg/dL).

Table 1. Descriptive statistics of the synthetic dataset variables

Variables	***Mean ± SD***
Age (years)	***45.6 ± 14.8***
BMI (kg/m²)	***24.9 ± 4.06***
Carbohydrates (g)	***59.6 ± 15.1***
Protein (g)	***24.9 ± 4.93***
Fat (g)	***25.2 ± 8.17***
Fiber (g)	***7.92 ± 1.93***
Steps in last 3 hours	***370 ± 260***
Sleep duration (hours)	***6.59 ± 1.49***
Microbiome score	***0.504 ± 0.173***

For this particular task, the model was created with a Random Forest Regressor for predicting the change in postprandial glucose levels, proper

handling for categorical features was employed, and the dataset was split for training and testing with 80% and 20% split ratios. The model was implemented with the number of trees set at 500, and performance metrics (R^2 and RMSE) are reported for illustrative purposes rather than model optimization.

Table 2. Performance metrics of the Random Forest regression model

Metric	Value
R^2	0.402
RMSE (mg/dL)	8.42
% Variance Explained	39.63%

The analysis of variable importance ranking revealed that the amount of carbohydrates (%IncMSE ~55) is the most important variable influencing the change in glucose, thus confirming that the glycemic index of the meal is the dominant factor determining the metabolic response. Other important factors were behavioural variables, including sleep time, fibre intake, and activity levels in the previous three hours. This implies that the response to meals is driven by more than just differences in meals themselves, but is also influenced by the subject's physiological and lifestyle variables. BMI and fat/protein were found to be less important, as were the meal type categories.

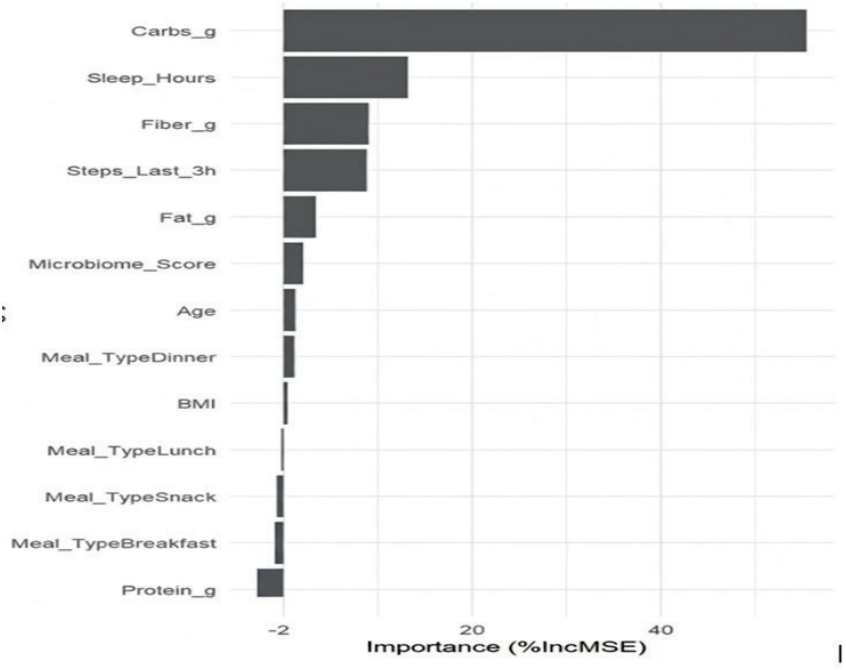

Figure 1. Random Forest variable importance plot

For instance, for a given person aged 45 years, with a BMI of 24; and having taken breakfast with 70 g carbohydrates, 22 g protein, 18 g lipid, and 4 g fiber; having taken 200 steps for the last 3 hours; and with a sleep duration of 6 hrs and a microbiome score of 0.40, the model predicted a rise of +31.8 mg/dL.

CONCLUSION AND DISCUSSION

This chapter demonstrates how artificial intelligence-based models can be used to explore individual responses to food intake within a controlled, simulation-based framework. The model under consideration emphasizes that the amount of carbohydrates, fiber content, exercise undertaken within the previous time periods, and sleep time influence the levels of glucose.

For any future research works, the model could be optimized with real datasets including broader age and metabolism criteria, implement time series-based glucose models and incorporate sleep quality and stress factors within the model. It would be very beneficial to conduct a comparison between various machine learning approaches for more reliable and applicable models for glucose levels within the context of personalized nutrition.

In conclusion, machine learning has a high rate of progress in the fields of nutrition and chronic diseases. Analyzing complex data related to this is important in terms of contributing to the scientific literature. Machine learning methods have a higher advantage over traditional and field-specific methods in terms of predictive ability, increased efficiency, and ease of use.

Rapid advances in artificial intelligence and machine learning have the potential to support and enhance medical practices in the management of diabetes and cardiovascular complications. However, significant challenges remain, including a lack of standards for evaluating model performance, limited interpretability, and the risk of bias in application processes. Addressing these challenges with a holistic approach and strengthening collaboration among all relevant stakeholders will enable the effective use of artificial intelligence in developing patient-centered care.

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Chapter 11

NON-PHARMACOLOGICAL AND COMMUNITY-BASED APPROACHES TO FEAR OF CHILDBIRTH

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1.Introduction

Fear of childbirth (FOC) is not merely an individual psychological response to labour and delivery; rather, it is a complex and multidimensional phenomenon shaped by biological, psychological, social, and cultural factors. Previous studies have consistently demonstrated that fear, anxiety, and stress experienced during childbirth influence women's decision-making processes, birth preferences, and overall birth experiences, as well as maternal and neonatal outcomes (Olza et al., 2018; Wigert et al., 2020). In this context, understanding fear of childbirth requires moving beyond a purely biomedical framework toward a culturally and socially informed perspective.

The literature indicates that women's perceptions of childbirth are deeply embedded within cultural narratives, social expectations, and collective meanings transmitted across generations. Cultural beliefs, family structures, religious interpretations, and community practices play a central role in shaping how childbirth is anticipated and experienced (Fenwick et al., 2018; Callister & Khalaf, 2010). Consequently, fear of childbirth varies considerably across societies and cultural contexts (Saisto & Halmesmäki, 2003).

This chapter examines fear of childbirth through a cultural lens and synthesizes evidence on non-pharmacological interventions shown to reduce childbirth-related fear. By integrating perspectives from midwifery, social sciences, and public health, the chapter aims to support culturally sensitive maternity care and to guide midwives and healthcare professionals in developing context-appropriate support strategies.

2. Conceptual Framework: Fear of Childbirth and Culture

Fear of childbirth can be conceptualized as a socially constructed experience that emerges at the intersection of individual psychology and collective cultural meaning. The cultural-historical framework proposed by Vygotsky emphasizes that psychological processes are inseparable from the social and cultural tools through which individuals interpret their experiences (Vygotsky, 1978; Wertsch, 1991). Applied to childbirth, this framework suggests that women's fears, coping strategies, and expectations are shaped by culturally mediated interactions, traditions, and narratives.

From this perspective, fear of childbirth is not solely the result of pain anticipation or medical risk perception, but also a reflection of societal attitudes toward women's bodies, reproductive roles, and autonomy during birth. Cultural discourse surrounding childbirth—whether it frames birth as a natural and empowering process or as a dangerous and traumatic event—significantly influences women's emotional responses (Hofberg & Ward, 2003; Wigert et al., 2020).

Understanding fear of childbirth within this conceptual framework

allows for a holistic interpretation of women's experiences and highlights the necessity of culturally informed interventions that address both psychological and social dimensions of fear.

3. Cultural Determinants of Fear of Childbirth

The determinants of fear of childbirth differ markedly across cultural settings. Studies conducted in Western societies frequently associate fear with uncertainty about the birth process, anticipated pain, loss of control, and previous traumatic birth experiences (Sheen & Slade, 2018; Wigert et al., 2020). In contrast, research from collectivist cultures, including Turkey, Iran, and Ethiopia, highlights the role of social norms, family expectations, and the intergenerational transmission of negative birth stories (Uçar & Gölbaşı, 2015; Arslantaş et al., 2020; Fentie et al., 2025).

Religious beliefs and traditional practices further shape women's interpretations of childbirth. For some women, perceiving childbirth as a sacred or spiritually meaningful event fosters resilience and reduces fear, whereas in other contexts, narratives emphasizing danger and risk intensify anxiety (Callister & Khalaf, 2010; Piccinini et al., 2021). Qualitative evidence from Ethiopia suggests that practices such as prayer, holy water use, and ritualized support may simultaneously reduce fear while posing potential health risks if not integrated with professional care (Aynalem et al., 2023).

Gender norms, power relations, and communication styles within healthcare systems also contribute to fear of childbirth. Limited autonomy, lack of respectful care, and insufficient emotional support may exacerbate anxiety and undermine women's confidence during pregnancy and labour (Mantula et al., 2023).

In many societies, fear of childbirth is transmitted intergenerationally through family narratives, social expectations, and collective memories of birth. Stories shared by mothers, sisters, and other female relatives often emphasize pain, risk, and loss of control, shaping women's anticipatory fears long before pregnancy occurs. These narratives are not merely anecdotal but represent culturally embedded forms of knowledge that influence how childbirth is perceived and emotionally processed.

In contexts where childbirth is framed as a test of endurance or moral strength, women may experience additional pressure to suppress fear or avoid expressing vulnerability. Such expectations can intensify internalized fear and reduce women's willingness to seek emotional or informational support during pregnancy. Understanding these cultural dynamics is essential for midwives, as fear of childbirth cannot be fully addressed without acknowledging the social and cultural environments in which women are embedded.

4. Non-Pharmacological Interventions from a Cultural Perspective

Non-pharmacological interventions have been widely recognized as effective strategies for reducing fear of childbirth. When implemented within a culturally sensitive framework, these interventions not only alleviate fear but also enhance women's sense of control, self-efficacy, and satisfaction with the birth experience.

4.1 Education and Psychoeducation

Prenatal education and psychoeducational programs aim to provide women with accurate information about the childbirth process, coping strategies, and available support options. Evidence indicates that psychoeducational interventions significantly reduce fear of childbirth and improve psychological well-being (Fenwick et al., 2015; Fenwick et al., 2018). In Turkey, structured prenatal education combined with breathing exercises has been shown to increase birth satisfaction and reduce fear levels (Akın et al., 2018).

Culturally tailored education programs that incorporate local beliefs, language, and social norms demonstrate higher acceptability and effectiveness. Midwife-led psychoeducation, in particular, fosters trust and enhances women's sense of preparedness and control during childbirth.

From a cultural perspective, the effectiveness of prenatal education depends not only on the content delivered but also on how information is framed and communicated. Standardized education models may fail to address culturally specific beliefs about childbirth, pain, and medical intervention. When educational approaches align with women's cultural values, language preferences, and lived experiences, they are more likely to reduce fear and enhance engagement.

Culturally adapted psychoeducation allows women to reinterpret childbirth as a meaningful and manageable life event rather than a purely medicalized or threatening experience. Such approaches also facilitate shared decision-making and promote trust between women and healthcare providers, which are critical factors in reducing fear of childbirth.

4.2 Breathing and Relaxation Techniques

Breathing and relaxation techniques, including Lamaze, deep breathing, and meditation-based practices, are widely used to manage fear and anxiety during childbirth. Randomized controlled trials and narrative reviews have demonstrated that these techniques reduce perceived pain, shorten labour duration, and enhance women's sense of control (Yüksel et al., 2017; Heim & Makuch, 2023). By regulating physiological stress responses, breathing techniques contribute to both emotional and physical comfort during labour.

Cultural acceptance of breathing and relaxation practices varies; however, when aligned with traditional beliefs or integrated into prenatal education, these methods are more readily adopted and sustained.

4.3 Mindfulness and Cognitive-Behavioral Approaches

Mindfulness-based interventions and cognitive-behavioral approaches focus on increasing awareness of childbirth-related thoughts and emotions while restructuring maladaptive beliefs. Systematic reviews and meta-analyses indicate that mindfulness-based education significantly reduces fear of childbirth and enhances self-efficacy (Abdolalipour et al., 2023; Rondung et al., 2018). Psychopedagogical counseling models, such as the BELIEF protocol, further support women in reframing fear and strengthening coping capacities (Salomonsson et al., 2013).

Cultural adaptation of these interventions is essential to ensure relevance and engagement, particularly in settings where mental health services are stigmatized or underutilized.

4.4 Continuous Midwifery and Doula Support

Continuous support during labour, provided by midwives or doulas, has been consistently associated with reduced fear, improved birth outcomes, and increased satisfaction with the childbirth experience. Evidence from systematic reviews and cohort studies demonstrates that continuous support lowers cesarean section rates, shortens labour duration, and enhances women's perception of control (Bohren et al., 2017; Kozhimannil et al., 2013).

Culturally congruent support models, in which caregivers respect women's cultural values and preferences, further strengthen the effectiveness of continuous care and contribute to respectful maternity services (Fentie et al., 2025).

Key non-pharmacological interventions and their cultural contexts are summarized in Table 1.

Table 1. Fear of Childbirth and Non-Pharmacological Interventions from a Cultural Perspective (2000–2025)

Author /Year	Country/ Cultural Context	Method / Sample	Nonpharmacological Intervention	Key Findings
Olza et al., 2018	Multicenter (meta-synthesis)	Qualitative, 20+ studies	Spontaneous vaginal birth experiences	Physiological birth enhanced the perception of control and provided a positive psychological experience.
Wigert et al., 2020	European countries	Meta-synthesis	—	Fear of birth was associated with trauma, ignorance, and negative stories.
Fenwick et al., 2015	Australia	RCT, 330 women	Midwifery-based psychoeducation	Fear and anxiety about childbirth decreased; postpartum recovery accelerated.
Akın et al., 2018	Türkiye	Cross-sectional, 320 pregnant women	Breathing exercises and education	It increased birth satisfaction and reduced fear levels.
Bohren et al., 2017	24 countries	Systematic review (26 studies)	Ongoing support, doula	Cesarean section rates decreased; birth experience satisfaction increased.
Kozhimannil et al., 2013	USA	Cohort, 2,500 births	Doula support	Lower anxiety; higher perception of control and satisfaction.
Abdolalipour et al., 2023	Iran	Meta-analysis	Mindfulness-based approaches	Significant decrease in fear of childbirth; increase in self-efficacy.
Heim & Makuch, 2023	Multicenter (narrative review)	Literature review	Breathing techniques	Effective in pain control and anxiety management; positive results in trained groups.
Fentie et al., 2025	Ethiopia	Mixed methods, 420 women	Culturally sensitive birth support	Respectful care increased satisfaction and decreased anxiety levels.
Callister & Khalaf, 2010	Middle East	Qualitative, 60 women	Spiritual rituals	Spiritual support reduced birth anxiety and strengthened the perception of control.

5. Implications for Midwifery Practice and Health Policy

The findings synthesized in this chapter underscore the importance of integrating cultural sensitivity into midwifery practice and maternity care policies. Midwives are uniquely positioned to address fear of childbirth through culturally informed communication, individualized care planning, and the promotion of non-pharmacological interventions.

At the policy level, incorporating culturally appropriate prenatal education programs, supporting midwife-led models of care, and promoting respectful maternity care are essential strategies for reducing fear of childbirth. Such approaches not only improve maternal experiences but also contribute to positive clinical outcomes and health system sustainability.

6. Limitations and Research Gaps

Despite the growing body of literature on fear of childbirth, several conceptual and methodological limitations remain that constrain a comprehensive understanding of this phenomenon. First, a substantial proportion of existing studies are quantitative in nature and focus primarily on measuring fear levels and intervention outcomes, while fewer studies explore women's lived experiences in depth. This limits insight into how cultural meanings, narratives, and social relationships shape fear of childbirth across different contexts.

Second, culturally contextualized and comparative studies remain limited, particularly in low- and middle-income countries and in societies with strong collectivist traditions. As a result, much of the available evidence reflects Western perspectives, which may not fully capture culturally specific fears, coping strategies, or expectations related to childbirth. This gap is particularly relevant for countries such as Türkiye, where family structures, religious beliefs, and social norms play a significant role in shaping childbirth experiences.

Third, although non-pharmacological interventions have demonstrated effectiveness, there is insufficient evidence regarding their long-term impact and sustainability when culturally adapted. Few studies examine how culturally sensitive interventions are implemented in routine clinical practice, how they are perceived by women and families over time, or how they influence midwifery education and service organization.

Future research should therefore prioritize qualitative and mixed-methods designs that foreground women's voices and sociocultural contexts. Cross-cultural comparative studies and community-based participatory research approaches are particularly needed to inform the development of culturally responsive and context-specific midwifery care models.

7. Conclusion

Fear of childbirth is a culturally embedded experience that requires comprehensive and context-sensitive approaches. This chapter highlights the effectiveness of non-pharmacological interventions—including psychoeducation, breathing and relaxation techniques, mindfulness-based approaches, and continuous midwifery or doula support—in reducing fear and enhancing women's sense of control and well-being.

The success of these interventions is closely linked to their cultural relevance and adaptability. By integrating cultural awareness into midwifery practice, education, and health policy, healthcare systems can foster more supportive, respectful, and empowering childbirth experiences for women across diverse cultural contexts.

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Chapter 12

THE MANAGEMENT OF NON-CYTOTOXIC AGENTS EXTRAVASATION

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INTRODUCTION

Intravenous (IV) access and medication administration are invasive procedures frequently used in patients receiving both outpatient and inpatient treatment, particularly in hospital settings. Although IV therapies are essential for diagnostic procedures, treatment, and patient recovery, they may lead to complications in certain circumstances (Duggan et al., 2024; Fernández-García et al., 2017; Tuğrul et al., 2025). The frequency and severity of these complications vary. Among IV-related complications, extravasation is particularly concerning as it may cause tissue necrosis and result in destructive and long-term consequences (Shibata et al., 2023).

Extravasation is a serious complication associated with IV therapy and is defined as the inadvertent leakage of vesicant (tissue-damaging) agents from the intended vascular pathway into the surrounding tissue (Al-Sheedi et al., 2025; Duggan et al., 2024; Sütçü Çiçek et al., 2024; Le & Patel, 2014; Simin et al., 2019). It may occur regardless of the type of venous catheter used (central or peripheral) and can cause more severe damage than other IV complications such as infiltration, phlebitis, occlusion, or venous spasm (Blazquez-Vidal et al., 2025; Saruhan, 2020).

The incidence of extravasation varies widely: 23–78% in neonates, 11–28% in children, and 16–32% in adults for all agents; 0.5–6.5% in adults receiving cytotoxic agents (Duggan et al., 2024; Shibata et al., 2023); and approximately 0.09% in patients receiving blood transfusions (Sütçü Çiçek et al., 2024). Extravasation may occur due to a wide range of IV-administered agents used for diagnostic and therapeutic purposes, including antineoplastic drugs, radiographic contrast media, electrolytes, parenteral nutrition solutions, blood, and blood products that possess tissue-irritating properties (Blazquez-Vidal et al., 2025; Sütçü Çiçek et al., 2024).

When extravasation occurs, symptoms such as pain, tingling, stinging or burning sensation, warmth, redness (erythema), and edema develop at the IV injection site. These symptoms serve as early warning signs and are often first noticed by the patient (Duminuco et al., 2025; Kim et al., 2020; Şen et al., 2019). Delayed recognition and improper management may increase tissue erosion, leading to blister formation, damage to underlying muscles, tendons, and nerves, necrosis, skin discoloration, contractures, and neuropathies. These complications may result in severe outcomes ranging from soft tissue loss requiring skin grafting to amputation and permanent disability (Kim et al., 2020; Shibata et al., 2023; Şen et al., 2019).

The extent of tissue damage caused by extravasation is influenced by several factors, including the concentration of the drug or solution, the volume of fluid leaked into the tissue, the duration of tissue exposure, and the anatomical site of extravasation. In addition to these factors, patient-

related characteristics and various clinical process-related factors also play a significant role (Arslan et al., 2018; Saruhan, 2020; Shibata et al., 2023; Tuğrul et al., 2025).

Severe extravasation may result in prolonged treatment, reduced functional capacity, decreased quality of life, extended hospital stays, increased care requirements and costs, and higher morbidity and mortality rates (Durmuş et al., 2025; Shibata et al., 2023; Tuğrul et al., 2025). Since nurses are primarily responsible for administering IV therapies, they must be aware of the factors contributing to extravasation, recognize its signs and symptoms, critically evaluate preventive interventions, and apply systematic problem-solving approaches in the management of extravasation (Atay et al., 2015; David et al., 2020; Yıldırım, 2010; Yıldırım & Koç, 2013).

RISK FACTORS FOR EXTRAVASATION

Extravasation occurs when vesicant medications leak out of the vascular system into surrounding tissues. Several factors influence the development of extravasation in patients. These factors may be related to the patient, the catheter used, the administered therapy, the anatomical site selected for treatment, and the institutional procedures followed during IV therapy administration (Blazquez-Vidal et al., 2025; Duggan et al., 2024; Eren, 2022; Shibata et al., 2023).

Patient-Related Factors

Patient-related risk factors include neonates, children (≤10 years), older adults (≥60 years), and individuals with chronic conditions such as type 1 diabetes mellitus, peripheral neuropathy, diabetes, Raynaud's syndrome, a history of radiotherapy, lymphedema, and obesity. Patients with communication difficulties—such as young children, foreign patients, individuals with cognitive impairment, and intubated or sedated patients—are also at increased risk (Blazquez-Vidal et al., 2025; Duggan et al., 2024; Durmuş, 2015; Fernández-García et al., 2017; Genç, 2025).

Factors Related to the Administration Site and Venous Structure

Extravasation risk increases when the infusion site is close to joints, when venous access is difficult (e.g., in obese patients or in those with fragile, narrow, or mobile veins), and in the presence of circulatory or coagulation disorders such as diabetes, Raynaud's syndrome, or cerebrovascular disease. Limited use of the extremity (e.g., due to dialysis fistula, axillary lymph node dissection, or amputation) and repeated IV access attempts in the same anatomical region further increase the risk (Arslan et al., 2018; Durmuş, 2015; Genç, 2025; Kim et al., 2020).

Catheter-Related Factors

Extravasation most commonly occurs with peripheral intravenous catheters (PIVCs). Although extravasation is less frequent with central venous catheters (CVCs) and implanted venous access devices, it may be more easily overlooked, potentially delaying diagnosis (Blazquez-Vidal et al., 2025; Shibata et al., 2023). Risk factors include the use of an oversized PIVC relative to vein diameter, butterfly needle use for infusion therapy, improper catheter placement, the quality of fixation materials (tapes and dressings), excessively loose or tight catheter stabilization, and moisture exposure of fixation dressings (Arslan et al., 2018; Durmuş, 2015; Genç, 2025; Fernández-García et al., 2017). For CVCs, contributing factors include catheter kinking or dislodgement, incomplete or incorrect needle placement in implanted ports, excessive back pressure around the needle, flushing with small-volume syringes, fibrin sheath formation, or catheter-related thrombosis (Avdal & Aydınoğlu, 2012).

Therapy-Related Factors

The physicochemical properties of the administered medication or solution, including pH, osmolarity, concentration, volume, and vasoactive characteristics, play a critical role in the development of extravasation. Hyperosmolar solutions, blood, and blood products may damage the vascular endothelium, increasing the risk of extravasation and associated skin injury. Tissue injury commonly occurs following extravasation of vasoconstrictive agents, hyperosmolar solutions, acidic or alkaline substances (pH <5.5 or >8.5), and irritant medications, as well as antineoplastic agents, radiographic contrast media, total parenteral nutrition (TPN), blood, and blood products. Commonly used agents that cause extravasation in patients are given in Table 1 (Blazquez-Vidal et al., 2025; Durmuş, 2015; Le & Patel, 2014; Shibata et al., 2023). Other treatment-related factors include bolus infusion administration and the duration of the infusion (Fernández-García et al., 2017). In addition, the amount of fluid leaking into the tissue and the duration of tissue exposure to the drug affect the severity of extravasation (Saruhan, 2020).

Institutional Procedure-Related Factors

Institutional factors include the presence and implementation of standardized procedures for the administration of both cytotoxic and non-cytotoxic agents, the education level and clinical experience of nurses or healthcare professionals, years of professional practice, catheter insertion expertise, and multiple venipuncture attempts during catheter placement (Eren, 2022; Genç, 2025; Kim et al., 2020).

Table 1. Non-Cytotoxic Vesicants

Anti-infective Agents	Vasopressors	Hyperosmolar Solutions (>600 mOsm)	Concentrated Electrolytes	Other Agents (Acidic/ Alkaline)
Acyclovir	Epinephrine	Dextrose (≥10%)	Calcium chloride	Amiodarone
Amphotericin	Norepinephrine	Mannitol (10–20%)	Calcium gluconate	Phenytoin
Ampicillin	Dopamine hydrochloride	Radiographic contrast media	Potassium chloride	Furosemide
Cloxacillin	Dobutamine hydrochloride	Total parenteral nutrition (TPN)	Sodium bicarbonate	Lansoprazole
Gentamicin	Phenylephrine hydrochloride	Human albumin	3% Sodium chloride	Thiopental sodium
Metronidazole	Methylene blue	Lipids (20%)		Arginine
Oxacillin	Angiotensin II	Immunoglobulin		Lorazepam
Penicillin				Morphine
Tetracycline				Promethazine
Vancomycin				Propofol
				Digoxin
				Iron preparations

SIGNS AND SYMPTOMS OF EXTRAVASATION

When vesicant medications or solutions leak into surrounding tissues during IV administration, a wide range of symptoms may occur. Some symptoms develop during infusion, whereas others may appear 2–3 days later (Avdal & Aydınoğlu, 2012). The most common early signs include pain at the injection site, tingling, swelling, pallor, redness (erythema), venous discoloration, and numbness. Within 24 hours following extravasation, patients may experience persistent tingling, sensory changes, tissue erosion, induration, and skin roughening (Duminuco et al., 2025; Kim et al., 2020; Shibata et al., 2023; Şen et al., 2019).

Leakage from the insertion site may be observed. Depending on the type of extravasated agent, ulceration, necrosis, and cyanosis may develop within 48–96 hours or up to 1–2 weeks after the event (Duminuco et al., 2025; İşeri et al., 2019).

In cases of extravasation, the flow rate of IV fluids may slow or stop altogether (Avdal & Aydınoğlu, 2012; Kim et al., 2020), and increased resistance during infusion may be noted. However, it is essential to first rule out other causes such as patient positioning (e.g., wrist or elbow flexion) or issues related to cannula support, including tight bandaging (Duminuco et al., 2025).

Another possible sign of extravasation is the absence of venous blood return; however, this finding alone is not definitive. Conversely, the presence of blood return does not exclude extravasation. In cases where the cannula has migrated, blood aspiration may reposition the cannula into the vessel lumen, damage the vessel wall, and falsely restore blood return (Duminuco et al., 2025). Drug leakage may also be observed around the needle insertion site. These symptoms may occur with both peripheral and centrally placed catheters (Blazquez-Vidal et al., 2025; Kim et al., 2020).

Extravasation symptoms associated with central venous catheters (CVCs) or implanted port devices exhibit specific characteristics. In such cases, edema may develop around the port access site or chest area, drug leakage may be observed around the catheter entry site, and redness may appear in the chest, clavicular region, or neck where the CVC is inserted. A stinging-type pain is commonly reported. These signs may present either early or late in the course of extravasation (Kim et al., 2020).

PREVENTION OF EXTRAVASATION

To prevent extravasation, it is essential to understand the characteristics (vesicant or irritant) and risk factors associated with non-cytotoxic agents (David et al., 2020; Shibata et al., 2023). Non-cytotoxic agents are classified into three categories: vesicants, irritants, and non–tissue-damaging agents. To ensure patient safety, all IV administered medications should be clearly labeled as "irritant" or "vesicant," thereby increasing awareness among nurses and other healthcare professionals during IV therapy administration (Duggan et al., 2024).

When inserting a PIVC, veins in the forearm should be prioritized. Joint areas should be avoided due to mobility and the risk of catheter displacement. The dorsum of the hand, the volar aspect of the wrist, the antecubital fossa, and lower extremities should generally not be preferred for IV therapy. Extravasation is particularly difficult to detect in the antecubital fossa, and damage to nerves and tendons may occur; therefore, this site should be avoided. Veins on the dorsum of the hand may be used in certain cases, as observation is easier; however, extreme caution is required, as this area is more susceptible to severe injury following extravasation. Catheters should not be placed in areas with impaired circulation, sclerosis, or prior IV access (Atay et al., 2015; David et al., 2020; Kim et al., 2020; Uçar & Arıkan, 2019). The catheter size should be appropriate for the patient's vein structure and diameter and inserted using correct technique. Special care should be taken with older adults, obese patients, individuals with limited use of the contralateral extremity, or those with fragile or mobile veins. In such cases, catheter insertion should be performed by experienced nurses. A single nurse should not attempt venipuncture more than twice, and no more than four

total attempts should be made on the same patient (Arslan et al., 2018; David et al., 2020; Eren, 2022).

The catheter should be securely stabilized to prevent movement while avoiding circulatory compromise. Transparent dressings should be used for catheter fixation. The dressing must be changed if it becomes moist, loose, disrupted, or visibly contaminated (İşeri et al., 2019).

Before administering medications or solutions via bolus or infusion, blood return should be aspirated to confirm patency. The catheter should be flushed with 5 mL of isotonic saline before, after, and between medication administrations (İşeri et al., 2019; Kim et al., 2020). Whenever possible, vesicant medications should be administered before non-vesicant agents (Atay et al., 2015). Patients who are unable to communicate or who are agitated should be closely monitored for signs of extravasation. Patients should be instructed to immediately inform a nurse or healthcare professional if they experience burning, tingling, pain, or any sudden change at the injection site (David et al., 2020; Duminuco et al., 2025; Kim et al., 2020).

Healthcare institutions should establish a care protocol for managing IV therapy-related extravasation that is readily accessible and easy to implement. Such protocols can help identify key surveillance points and clinical indicators of extravasation while increasing healthcare professionals' awareness of potential sequelae (David et al., 2020).

MANAGEMENT OF EXTRAVASATION

General Principles

Extravasation of non-cytotoxic agents requires immediate intervention to prevent potential complications, including progression to necrosis and the possible need for amputation (Blazquez-Vidal et al., 2025). Once extravasation is identified, the infusion must be stopped immediately, and any residual drug should be aspirated through the cannula before catheter removal. If available, an agent-specific antidote should be administered, followed by catheter removal. Pressure should not be applied to the affected area, as this may increase the spread of the extravasated agent. The affected area should be marked with a pen and photographed to monitor progression. The estimated volume of extravasated fluid should be documented. To minimize inflammation, the affected extremity should be elevated. The use of warm or cold compresses remains controversial and depends on the physicochemical properties of the extravasated agent (Blazquez-Vidal et al., 2025; İşeri et al., 2019; Tuğrul et al., 2025).

In some cases, pharmacological (e.g., hyaluronidase, corticosteroids, phentolamine) and surgical interventions may be required depending on the severity and extent of extravasation (Atay et al., 2015; Blazquez-Vidal et al.,

2025; Tuğrul et al., 2025).

The choice of management strategy should consider both patient-specific and event-specific factors, including age, body weight, comorbidities, communication barriers, skin integrity, extravasation site, anatomical abnormalities, care setting, response to current treatment, time to diagnosis, and the type, volume, and concentration of the vesicant agent involved. Pharmacological and non-pharmacological supportive interventions used in extravasation management are presented in Table 2 (Ong et al., 2020).

Table 2. Stages of Extravasation and Management

Stage	Assessment	Management
1	Pain at the infusion site No redness Localized swelling (1–10% of the affected area, above or below the site)	Removal of the cannula Elevation of the affected extremity Warm or cold compresses
2	Pain at the infusion site Mild edema (up to 25% of the affected area, above or below the site) Mild erythema (fluid leakage in the affected area) Palpable distal pulse Rapid capillary refill (1–2 seconds)	Removal of the cannula Elevation of the affected extremity Warm or cold compresses Consideration of antidote administration
3	Pain at the infusion site Moderate edema (25–50% of the area, above or below the site) Marked erythema extending beyond the central area of extravasation Pallor (vasopressor extravasation only) Palpable distal pulse Rapid capillary refill (1–2 seconds) Cool sensation upon palpation of the skin	Leave the cannula in place; aspirate the drug using a syringe Remove the cannula unless antidote administration is required Elevation of the affected extremity Warm or cold compresses Consideration of antidote administration
4	Pain at the infusion site Severe edema (>50% of the area, above or below the site) Intense erythema extending beyond the edematous borders Pallor (non-vasopressor extravasation) Decreased or absent distal pulse Prolonged capillary refill (>4 seconds) Cool skin temperature Skin damage including blistering or necrosis	Leave the cannula in place; aspirate the drug using a syringe Remove the cannula unless antidote administration is required Elevation of the affected extremity Warm or cold compresses Consideration of antidote administration Surgical consultation if edema, tension, and pallor are present

NON-PHARMACOLOGICAL SUPPORTIVE METHODS

Providing appropriate general care and interventions following extravasation is essential to limiting tissue damage. Depending on the causative agent, additional agent-specific treatments may be required (David et al., 2020). While extravasation-related tissue injury and small wounds often heal spontaneously, supportive interventions can accelerate tissue recovery (Kim et al., 2020; Ong et al., 2020).

Non-pharmacological supportive methods used in extravasation management include cessation of infusion, aspiration of residual fluid from the catheter, elevation of the affected extremity, application of warm or cold compresses, saline irrigation, and physical massage or compression therapy (Ong et al., 2020; Stefanos et al., 2023; Şen et al., 2019).

Discontinuation of Infusion and Aspiration

If a patient reports burning or pain, or if swelling, redness, or skin discoloration is observed at the infusion site, IV injection or infusion must be stopped immediately. The infusion set should be disconnected from the catheter (PIV or CVC), and attempts should be made to aspirate the drug through the catheter—particularly in Stage 3 and Stage 4 extravasation. Aspiration may reduce tissue damage associated with extravasation, although the patient may experience discomfort during the procedure. The maximum possible amount of the extravasated agent should be aspirated (Atay et al., 2015; Kim et al., 2020; Shibata et al., 2023; Stefanos et al., 2023).

Elevation of the Extremity

The affected extremity may be elevated to enhance lymphatic absorption of the extravasated agent and reduce edema. Elevation lowers hydrostatic pressure in capillaries and facilitates fluid redistribution. Gentle movement of the extremity should be encouraged to prevent involvement of deeper tissues. Elevation should be initiated within the first 48 hours following extravasation (Atay et al., 2015; İşeri et al., 2019; Kim et al., 2020; Stefanos et al., 2023). However, some studies report that elevating the extremity by approximately 10.16 cm (4 inches) or positioning the limb at heart level does not significantly reduce pain, tissue induration, or extravasated fluid volume (Ong et al., 2020).

Cold Application

Cold application induces vasoconstriction, thereby localizing the vesicant agent, limiting its spread, and reducing tissue damage (Blazquez-Vidal et al., 2025; Ong et al., 2020). However, it may also prevent drug degradation and potentially worsen tissue injury in some cases (Blazquez-Vidal et al., 2025). Cold therapy should be applied as a dry cold compress. Cold packs should be frozen for at least 2 hours, wrapped in a clean, dry cloth, and applied to

the affected area. Applications may be performed four times daily for 15–20 minutes over a 48-hour period. Except for vasopressor extravasation, cold application is appropriate for hyper- or hypo-osmolar solutions, acidic or alkaline agents, and valproate extravasation (Atay et al., 2015; David et al., 2020; Ong et al., 2020).

Warm Application

Warm application promotes vasodilation, increasing blood perfusion, absorption, and dispersion of the extravasated agent, thereby reducing local drug concentration (Avdal & Aydınoğlu, 2012; Ong et al., 2020; Blazquez-Vidal et al., 2025). Dry warm compresses should be applied by immersing the compress in boiling water for 5 minutes or heating it in a microwave for 20 seconds, then wrapping it in a clean, dry cloth. Warm compresses should be applied four times daily for 20-minute sessions over 24–48 hours (Avdal & Aydınoğlu, 2012; Atay et al., 2015; David et al., 2020; Durmuş et al., 2025).

Warm compresses should be used in conjunction with vasodilatory antidotes (e.g., phentolamine, terbutaline, nitroglycerin) for vasoconstrictive vesicant extravasation. When combined with hyaluronidase, warm application enhances blood flow and facilitates dispersion of the extravasated drug, producing a synergistic effect (Ong et al., 2020; David et al., 2020).

Saline Irrigation

Saline irrigation is a technique used for the physical removal of vesicant agents. It is reported to promote local edema formation by diluting the extravasated drug within the affected tissue, thereby facilitating systemic absorption of the agent (Avdal & Aydınoğlu, 2012). This procedure is performed by creating multiple small surgical incisions and inserting a catheter to allow irrigation with sterile saline solution, combined with aspiration in a manner similar to liposuction. Saline irrigation is routinely used in some clinical centers (Ong et al., 2020).

Physical Massage or Compression Therapy

Physical massage may assist in the manual distribution of extravasated fluid and reduction of tissue pressure. It has been suggested that massage may facilitate physical dispersion of the extravasated agent; however, the current literature provides insufficient evidence to support routine use of this intervention (Ong et al., 2020).

PHARMACOLOGICAL METHODS

Pharmacological agents used in the management of extravasation include antidotes and supportive medications such as hyaluronidase, dimethyl sulfoxide (DMSO) 70–99% solution, sodium thiosulfate, dexrazoxane, phentolamine, terbutaline, topical anesthetics (e.g., lidocaine and prilocaine

creams), topical antimicrobials (e.g., silver sulfadiazine and chlorhexidine), topical debridement agents (e.g., collagenase ointment), topical steroids, topical vasodilators (e.g., nitroglycerin), and growth factors (Durmuş et al., 2025; Le & Patel, 2014; Stefanos et al., 2023). Management of extravasation should be performed by a clinical specialist with expertise in venous access, pharmacological properties of the administered drug, and extravasation management (Kim et al., 2020).

Hyaluronidase

Hyaluronidase is an enzyme that hydrolyzes hyaluronic acid in connective tissue, increasing tissue permeability and facilitating the dispersion, absorption, and breakdown of extravasated drugs and fluids (David et al., 2020; Ong et al., 2020; Tuğrul et al., 2025). Its effect is rapid and pronounced, leading to fading of erythema and significant reduction of edema (Ong et al., 2020).

Hyaluronidase is administered subcutaneously around the extravasation site. It may be used alone or in combination with saline irrigation (Tuğrul et al., 2025). A dose of 150 units facilitates absorption of 1 liter or more of subcutaneously infiltrated fluid. In adults and children, a typical dose is 15 units (maximum 150 units), administered as five divided subcutaneous injections of 0.2 mL each around the swelling. Doses may be repeated every 30–60 minutes until the desired effect is achieved. Total doses of up to 450 units have been reported without adverse effects (Ong et al., 2020; Stefanos et al., 2023).

For optimal effectiveness, hyaluronidase should be administered within the first 6 hours, preferably within 1 hour of extravasation (Duminuco et al., 2025; Durmuş et al., 2025). Its benefits have been reported in extravasations involving volumes exceeding 50 mL, low-osmolar radiographic contrast media, and erythrocyte suspension transfusions (Blazquez-Vidal et al., 2025).

Dimethyl Sulfoxide (DMSO) 99% Solution

DMSO should be initiated as soon as possible following extravasation, ideally within 10–25 minutes. It is applied as a thin layer to the affected skin surface (the skin must be dry) and allowed to air-dry without covering with a dressing. Application should be repeated every 6–8 hours for 7 days (Alikan & Şeref, 2025; Uçar & Arıkan, 2024).

Dexrazoxane

Dexrazoxane should be administered intravenously within 6 hours after extravasation, infused over 1–2 hours through a vein in an unaffected extremity (Alikan & Şeref, 2025; Uçar & Arıkan, 2024).

Sodium Thiosulfate

In adults, sodium thiosulfate is administered intravenously at a dose of 12.5 g over 30 minutes, which may be gradually increased to 25 g up to three times weekly (David et al., 2020; Ong et al., 2020). Patients should be monitored for anion gap metabolic acidosis, hypocalcemia, and nausea. A 25% gel-based topical sodium thiosulfate formulation may be a safe alternative. Topical use may cause mild erythema and scaling but does not require discontinuation of therapy (Stefanos et al., 2023).

Phentolamine (α_1-Antagonist)

Phentolamine is generally used in extravasation injuries caused by vasoconstrictive agents. By inducing vasodilation, it restores blood flow and reduces ischemic tissue damage. Phentolamine should be administered immediately or within 12 hours following extravasation (Atay et al., 2015; Stefanos et al., 2023). In adults and children, 5–10 mg of phentolamine diluted in 10–20 mL of 0.9% sodium chloride is administered subcutaneously in five divided doses around the blanched area. The procedure may be repeated every 30–60 minutes until the desired effect is achieved or until the patient experiences intolerable hypotension (Ong et al., 2020; Stefanos et al., 2023).

Terbutaline (β_2-Agonist)

Terbutaline is a β_2-agonist that induces vasodilation and reverses vasoconstrictive effects following vasopressor extravasation. It may be used as an alternative when phentolamine is unavailable or inaccessible (Stefanos et al., 2023). In adults and children, terbutaline 1 mg diluted in 10 mL of normal saline is administered intradermally in five divided doses around the blanched area. The procedure may be repeated every 30–60 minutes until the desired effect is achieved or until intolerable systemic effects such as tachycardia or blood pressure instability occur (Ong et al., 2020; Stefanos et al., 2023).

Topical Nitroglycerin

Topical nitroglycerin exerts a peripheral vasodilatory effect when applied to the extravasation site and has been reported to reduce extravasation and phlebitis (Avdal & Aydınoğlu, 2012; Ong et al., 2020). In adults, a 1-inch (2.54 cm) strip is applied to the affected area; in neonates, a dose of 4 mm/kg is recommended. Applications may be repeated every 8 hours as needed until symptoms resolve (Ong et al., 2020).

Localized Steroid Therapy

The outcomes of topically or locally injected steroids are mixed. Steroids are used for their anti-inflammatory effects; however, their use remains controversial because inflammatory cell infiltration is typically minimal in extravasation-injured tissue (Avdal & Aydınoğlu, 2012; Ong et al., 2020).

Growth Factors

Growth factors have been reported to prevent the development of necrosis in extravasated tissue; however, evidence is limited. Agents such as sargramostim and filgrastim have been used (Avdal & Aydınoğlu, 2012).

Antimicrobial Therapy

Because extravasation injuries are considered chemical burns, topical silver sulfadiazine may be used. Secondary bacterial infections of damaged tissue should be treated with appropriate topical or systemic antimicrobial agents (Ong et al., 2020).

Surgical Management

If extravasation-related skin necrosis is smaller than 20 mm, conservative wound care and minimal debridement are recommended (Durmuş et al., 2025). Surgical intervention should be considered if the affected area is extensive, if pain does not decrease after 2–3 weeks, or if no visible improvement in tissue damage is observed. Surgical options include liposuction, fasciotomy, incision and drainage, skin grafting, or flap reconstruction for non-healing extravasation wounds (Atay et al., 2015; Blazquez-Vidal et al., 2025; Durmuş et al., 2025; Stefanos et al., 2023; Şen et al., 2019; Tuğrul et al., 2025).

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Chapter 13

EVIDENCE-BASED PRACTICES IN BREASTFEEDING

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Introduction

Breastfeeding is the most important practice necessary for a baby to start life with the strongest possible foundation from the moment they open their eyes for the first time. Breast milk provides infants with the complete range of nutrients required during the first six months of life. The advantages of breastfeeding include that breast milk is suitable for the baby's digestion, inexpensive, and easily accessible. It is also considered the first vaccine for the newborn, regulating the baby's immunity (Başer et al., 2018; Durmazoğlu and Okumuş, 2019).

Although the benefits of breastfeeding are widely recognized both globally and nationally, breastfeeding rates remain below the desired level. It is recommended that babies be fed only breast milk from the first minutes of life for 6 months and that children continue to receive breast milk with complementary foods until the age of two and beyond (WHO, 2017; UNICEF, 2024).

According to the Global Breastfeeding Report Card 2023, the rate of breastfeeding initiation within the first hour after birth is reported as 46%, exclusive breastfeeding during the first six months as 48%, breastfeeding continuation up to one year is 71%, and breastfeeding continuation up to two years is 45% (Global Breastfeeding Report Card, 2023).

Evidence-based practices ensure that care is provided according to a single standard. When practices such as initiating and maintaining breastfeeding, breastfeeding frequency and duration, timing of complementary feeding introduction, pacifier/bottle use, milk expression and storage, and finally weaning are implemented based on evidence, the care provided by each clinic will be consistent. Breastfeeding practices, grouped under the title "Successful Breastfeeding in Ten Steps," aim to promote, protect, and support breastfeeding and are intended to be implemented to the same standard in all baby-friendly hospitals (Ministry of Health, 2020a).

Nurses play an active role in ensuring the continuous provision of breastfeeding support and counseling services to mothers during the prenatal, delivery, and postnatal stages. Effective breastfeeding education has been shown to rapidly increase breastfeeding rates and duration among mothers (Yadav et al., 2022). The aim of this review is to evaluate recent evidence-based breastfeeding practices carried out by nurses.

Breastfeeding

Breast milk is a unique food that provides all essential nutrients, immune-supporting components, growth factors, enzymes, and hormones required to support optimal growth and development in early infancy (Brown et al., 2019).

Breastfeeding is unique in that it is the most ideal food for the baby's health and development, is available to the baby whenever needed, is fresh, sterile, easily digestible, protects against disease, is inexpensive, and is culturally valued (Ministry of Health, 2024). Many organizations, such as the World Health Organization (WHO) and the United Nations Children's Emergency Fund (UNICEF), support breastfeeding. According to recommendations from the WHO and UNICEF, infants should be exclusively breastfed during the first six months, with breastfeeding maintained together with complementary feeding for a minimum of two years (WHO, 2017; UNICEF, 2024).

According to the 2023 Global Breastfeeding Report Card, the rate of breastfeeding initiation within the first hour after birth is reported as 46%, exclusive breastfeeding during the first six months as 48%, breastfeeding continuation up to one year is at 71%, and breastfeeding continuation up to two years is at 45% (Global Breastfeeding Report Card, 2023). Accordingly, the rate of exclusive breastfeeding for the first 6 months has reached 48%, approaching the 50% target set by the World Health Assembly for 2025. The Global Breastfeeding Collective aims to raise the global rate of exclusive breastfeeding during the first six months to 70% by 2030 (Global Breastfeeding Report Card, 2023).

According to data published by the Turkey Population and Health Survey (TNSA) in 2018, the proportion of newborns who are breastfed within the first hour of life is currently 71%, the proportion of infants aged 0–6 months who are exclusively breastfed is 41%, breastfeeding continuing for one year is 66%, and breastfeeding continuing until the age of two is 34% (TNSA, 2018).

It is reported that ensuring optimal breastfeeding, as advocated by the WHO and UNICEF, could save the lives of over 800,000 children under the age of five annually. In addition, it is reported that 45% of child deaths are due to malnutrition (WHO, 2017; UNICEF, 2024; Victora et al., 2016).

Evidence-Based Breastfeeding Practices

In order to protect, promote, and support the health of infants and children, WHO and UNICEF recommend initiating breastfeeding within the first hour after birth and providing only breast milk for the first six months, and that breastfeeding continue until the age of two, alongside appropriate, clean, and safe complementary foods (WHO, 2017; UNICEF, 2024). Breast milk, a food that is easy to digest, inexpensive, and equipped to meet all the needs of a newborn during the first six months, is the most ideal food a baby can receive (Brown et al., 2019).

Skin-to-skin contact

Placing the newborn on the mother's breast in a naked prone position with the head turned to the side, either at birth or within the first hour, is defined as skin-to-skin contact (SSC) (Yerlikaya and İldan Çalım, 2021). The

baby should be given the opportunity to initiate breastfeeding by orienting toward the nipple with smell, hand-mouth movements, touching the nipple, grasping, and positioning appropriately at the breast (Widström, 2011).

The Society of Obstetricians and Gynecologists of Canada (SOGC), the WHO, and the American Women's Health, Obstetric and Neonatal Nurses Association (AWONN) the importance of immediate skin-to-skin contact and early initiation of breastfeeding within the first hour after birth is strongly emphasized (Ludington-Hoe, 2015). The fourth step of the "Ten Steps to Successful Breastfeeding," developed as a result of the Baby-Friendly Hospital Initiative, aims to initiate skin-to-skin contact immediately after birth (Ministry of Health, 2020a).

There are many evidence-based studies on the positive effects of SSC on the baby and mother. Early SSC plays an important role in improving the newborn's vital signs, weight gain in a short time, reducing crying, early discharge from the hospital, and reducing morbidity (Köse et al., 2013; Coşkun Erçelik et al., 2023). A systematic review of 2,177 women in 2012 indicated that SSC reduces infant crying and improves breastfeeding outcomes. Studies have also shown that early breastfeeding stimulates uterine contractions, thereby facilitating placental expulsion after delivery (Moore et al., 2012). Safari et al. (2018) found that SSC shortened the third stage of labor (Safari et al., 2018).

It has been reported that initiating skin-to-skin contact during the immediate postpartum period enables mothers to breastfeed their babies more quickly and for longer periods, and also protects against postpartum depression by facilitating mother-infant bonding (Eroğlu and Aslan, 2018). Early skin-to-skin contact is necessary for effective bonding, without waiting for breastfeeding (Çetinkaya and Ertem, 2017). Skin-to-skin contact can be made not only with the mother but also with other caregivers in the family, such as the father (Höbek Akarsu et al., 2017). Recent evidence-based studies recommend that newborn care procedures (taking anthropometric measurements, dressing the newborn, etc.) performed immediately after birth should be done while in the SSC position or postponed until after breastfeeding (Evcili et al., 2014). Sharma's randomized controlled trial found that early SSC increased breastfeeding rates in the first six weeks and reduced pain experienced by mothers during episiotomy (Sharma, 2016).

Start time, duration, and frequency of breastfeeding

All healthy newborns should begin initiation of breastfeeding within the first 30 minutes of life or at the latest within one hour after birth. The WHO states that breastfeeding should be initiated within the first hour after birth (WHO, 2017). According to the Global Breastfeeding Scorecard (2023) data, the proportion of infants breastfed within the first hour following birth is

46%, while in our country, according to TNSA (2018) data, this rate is 71% (Global Breastfeeding Scorecard, 2023; TNSA, 2018). A study examining the link between the time of initiation of breastfeeding after birth and the risk of neonatal death found that when breastfeeding was initiated within 2-23 hours, the risk of neonatal death was 1.3 times higher than when breastfeeding was initiated within the first hour, and the risk of neonatal death was twice as high among infants who were breastfed 24 hours or later after birth (Smith et al., 2017).

A review of the literature reveals many factors that prevent the initiation and continuation of breastfeeding. The main ones are the mother's lack of knowledge about breastfeeding techniques, lack of information, lack of milk, the baby being in an incubator, flat nipples, and pain (Işık et al., 2016). Studies examining the timing of breastfeeding initiation based on the mode of delivery report that cesarean deliveries negatively affect the timing of breastfeeding initiation due to pain and delayed milk production (Akkuş and Çoban, 2023).

Newborns should be breastfed every two hours, with a frequency of 10-12 feedings per day. To ensure the newborn can feed efficiently, they should remain at the breast for at least ten to fifteen minutes. If the baby has been breastfed on one breast, the next feeding should be on the other breast. However, if both breasts have been fed, breastfeeding should continue on the last breast used (Çökelek, 2017). The frequency of breastfeeding should be adjusted according to the baby's movements indicating that they want to feed (making sucking movements with their mouth, bringing their hand to their mouth, etc.) rather than according to the hours. The baby's restlessness and crying are the latest signs that they want to be breastfed (Akkuş and Çoban, 2023).

Breastfeeding positions

Effective and successful breastfeeding is possible when the baby is positioned correctly. To ensure effective breastfeeding, it is recommended that the baby be positioned with their face toward the breast, their nose, chin, and cheeks touching the breast, most of the areola below the nipple in the baby's mouth, the lower half of the baby's face buried in the breast, and no visible gap between the breast and the baby's mouth (Figure 1). (Figure 2) (Douglas and Geddes, 2018; Ministry of Health, 2020b).

For effective breastfeeding, both the mother and baby should be in a comfortable position. The mother should support the breast tissue with her hand, holding the breast in a C-shape. In the C-shaped breast hold, the thumb should be on top, the other four fingers should grasp the bottom of the breast, and the mother's fingers should be away from the baby's mouth (Kültürsay et al., 2018).

Different positions can be recommended to support effective breastfeeding. These include the reclining position, cradle hold, cross-cradle hold, and underarm hold (Figure 3) (Ministry of Health, 2020b).

Reclining position: This position is frequently used by mothers who have delivered by cesarean section. It can also be comfortably used by women who have undergone an episiotomy following vaginal birth. In this position, it is essential that the infant's face is fully oriented toward the mother's breast (Ministry of Health, 2020b).

Cradle hold: This is the most commonly used breastfeeding position. The mother sits upright, holds the infant in her arms, supports the infant's hips and back with her arm, and positions the infant so that the head is turned toward the breast. This position is considered ideal for infants with low birth weight (Ministry of Health, 2020b).

Cross-cradle hold (reverse cradle hold): This position is particularly useful for breastfeeding preterm newborns and infants with clavicular fractures. In this position, the infant's head and shoulders are supported by the mother's hand, ensuring stable positioning during breastfeeding (Ministry of Health, 2020b).

Underarm hold (football hold): This position is ideal for breastfeeding twins, preterm or weak infants, mothers with large breasts, and women who have delivered by cesarean section. It is also an effective technique for facilitating the emptying of both breasts. In this position, the infant's head is supported with a pillow and positioned under the mother's arm (Kültürsay et al., 2018).

When to start complementary foods

The WHO and UNICEF recommend introducing complementary foods after the sixth month of life and continuing breastfeeding in combination with complementary foods until two years of age (WHO, 2017; UNICEF, 2020). A review of the literature indicates that bottle/pacifier use and formula consumption are associated with mothers starting complementary foods early, eading to decreased rates of exclusive breastfeeding during the first six months and a shorter duration of continued breastfeeding (Uçar and Öztürk Şahin, 2021).

Pacifier and bottle use

The WHO advises against the use of bottles and pacifiers, as they may increase the risk of illness in infants (WHO, 2017). Bottle feeding is easier than breastfeeding, so nipple rejection is common. Many studies show that bottle use leads to early cessation of breastfeeding (Çalık et al., 2017).

Pacifiers are not recommended because their use causes an increase in

otitis, tooth decay, malocclusion, and diarrhea in infants and children (Çamurdan et al., 2008). The American Academy of Pediatrics (2005) reported that pacifier use during sleep in infants reduces the risk of sudden infant death and can be used at the family's discretion (Jenik and Vain, 2009).

In a study by Yahşi and Saylı (2022), the rate of pacifier use among mothers was reported to be 41.8%, and the main reason for giving pacifiers was to calm the baby. In the same study, the use of pacifiers was found to shorten the duration of breastfeeding and increase the risk of weaning before six months by 5.1 times (Yahşi and Saylı, 2022).

Milk expression and storage

After birth, every mother should be taught methods for expressing and storing breast milk (WHO, 2017; Ministry of Health, 2020b). For preterm infants, it is recommended that expressed breast milk be fed with a spoon, cup, or syringe (WHO, 2017; Ministry of Health, 2020b). Expressing breast milk both ensures the continuity of the mother's milk production and helps relieve the breast in cases such as blockages (Kültürsay et al., 2018). Breast milk expression can be performed either by hand or with the use of a breast pump.

Hand expression: Hands should be washed, and pressure should be applied to the breast tissue with the thumb and index finger. The milk sinuses in the breast should be felt by hand and pressed and released several times. Manual expression can take 20-30 minutes. During expression, pressure on the nipple can block milk flow and cause nipple irritation (Figure 4). If the mother feels pain/discomfort during manual expression, it indicates that the technique is being performed incorrectly (Kültürsay et al., 2018).

Expressing with an electric pump: These battery- or electric-powered pumps can have a pressure range of 30 mmHg-300 mmHg. Expressing should begin at a low pressure to stimulate the breast. The pressure is then increased to support vigorous milk expression. Pump sets should be changed daily and sterilized (Eker and Aslan, 2018; Kültürsay et al., 2018).

Storage conditions for breast milk

To prevent breast milk from spoiling, it must be stored in glass jars with tight-fitting lids or in sterile breast milk storage bags. Expressed breast milk should be stored in 100 ml portions. The date and time must be written on the storage container. It is important for the circadian rhythm that milk expressed during the day is given to the baby during the day, and milk expressed at night is given at night (Kültürsay et al., 2018). Another important condition for storing breast milk is the storage conditions in the refrigerator or freezer. The Academy of Breastfeeding Medicine (ABM) and the Turkish Ministry of Health have established a standard for these conditions (Table 1) (Eglash and Simon, 2017; Ministry of Health, 2024).

Breast milk taken out of the freezer should first be thawed in the refrigerator and then warmed using the bain-marie method. Heating milk in the microwave or on the stove can cause the breakdown of immune-supporting substances and protein structure in its content (Rodrigo et al., 2018). Breast milk heated to the appropriate temperature can be safely stored for about one hour at room temperature or up to 24 hours when kept in a refrigerator (Kültürsay et al., 2018; Eglash and Simon, 2017).

Weaning

Weaning is defined as the gradual replacement of breast milk with complementary foods in children's nutrition (Abu Hamad and Sammour; 2013). Weaning is an experience of separation from the mother for the baby. Although the decision to start breastfeeding is made before birth, the decision to wean may be unplanned (Nuzrina, Roshita, and Basuki, 2016). Mothers' decision to wean may be due to health, illness, cultural, environmental, and child-related factors (Dinç and Dombaz, 2015; Nuzrina et al., 2016).

Today, gradual weaning, abrupt weaning, and traditional methods are used in the weaning process. The gradual weaning method is applied by skipping the baby's breastfeeding session and replacing the sucking behavior with a bottle or other food. The abrupt weaning method involves abruptly stopping breastfeeding within a short period due to the baby refusing the breast or the development of a situation that prevents breastfeeding (illness, surgery, etc.). Traditional methods of weaning include sending the child to another home for a few days or using techniques to alter the taste and appearance of the breast (such as applying tomato paste, hair, or black paint to the breast) to discourage the child from breastfeeding (Abu Hamad and Sammour; 2013; Dinç and Dombaz, 2015; Gürarslan Baş et al., 2018). Abruptly ending breastfeeding can cause the child to feel punished and exhibit aggressive and angry behavior. In terms of mother-infant separation, breastfeeding should be discontinued gradually, and mothers should be supported by nurses during this period (Alsaç and Polat, 2018). The support and counseling provided enable mothers to make appropriate decisions concerning how long to continue breastfeeding and when to begin weaning. (Altunel and Özaydın, 2022).

Evidence-based breastfeeding practices: a nursing approach

Health professionals trained in breastfeeding are the first point of contact for effectively initiating, continuing, and supporting breastfeeding. Health professionals working in Family Health Centers, Community Health Centers, maternity schools, delivery rooms, and postpartum care clinics regularly participate in training and certification programs to increase their knowledge and skills in breastfeeding. Nurses promote the spread and successful continuation of breastfeeding by using evidence-based breastfeeding practices in their work. Breastfeeding counseling should not be limited to the mother

alone but should also include her partner, family, and close circle. The role of nurses is of vital importance in terms of improving public health and ensuring the healthy development of infants (Ministry of Health, 2020b; Ministry of Health, 2024).

Awareness among health professionals regarding baby-friendly hospital initiatives and their active support for implementing these initiatives within their institutions are essential. Breastfeeding counseling, which begins during pregnancy, should cover the importance of breast milk, the benefits of breastfeeding, the risks of not breastfeeding, the timing of initiating breastfeeding, the importance of mother and baby sharing the same room, breastfeeding positions, and breast care. In particular, birth practices that support breastfeeding should be promoted, and the importance of skin-to-skin contact should be emphasized. Additionally, breastfeeding should be supported after cesarean delivery, and the risks of artificial feeding alternatives should be discussed (Işık and Arça, 2019).

Healthcare professionals have an essential role in promoting and facilitating the early initiation of breastfeeding after birth. Identifying problems related to the mother's first breastfeeding experience and providing appropriate support will encourage her to breastfeed. Healthcare workers should not rush, but rather focus on alleviating the mother's concerns about breastfeeding and being sufficient for her baby without causing her anxiety (Uzun et al., 2018).

Providing a comfortable and safe environment before beginning to assess breastfeeding will enable the mother to breastfeed her baby with more confidence. During breastfeeding, the mother's behavior, the appropriateness of her position, the baby's latch, and whether effective breastfeeding is occurring should be observed. Mothers should be informed that they should breastfeed their babies whenever they want (Ministry of Health, 2024).

Mothers should be informed about increasing breast milk production, expressing milk by hand or with an electric pump when necessary, storing it under appropriate conditions, and reusing it. Breastfeeding mothers should be informed about common breast problems encountered during this period, and support and counseling should be provided, especially if negative factors affecting breastfeeding are identified. Health professionals should be supportive and encourage breastfeeding in response to negative feelings and behaviors that may arise during the breastfeeding process (Uzun et al., 2018).

Health professionals working in family health centers or antenatal clinics should advise mothers that they should wait at least two years before becoming pregnant again after giving birth, that the lactational amenorrhea method is valid when appropriate conditions are met, and that they should recommend an appropriate family planning method based on the mother's medical history (RCOG, 2015). Health professionals providing family plan-

ning counseling should be adequately equipped with the latest evidence-based studies and play an active role in ensuring these practices are implemented (Ortaç and Koruk, 2023).

Becoming pregnant during the breastfeeding period causes women to stop breastfeeding. If mothers wish to continue breastfeeding during pregnancy, they should be carefully monitored and given appropriate support and counseling. A review of the literature reveals recent studies indicating that breastfeeding during pregnancy does not harm the health of the mother, baby, or fetus, and that the mother's milk will be sufficient for both children after delivery (İn and Koruk, 2024).

If the mother must take medication during breastfeeding, she should be educated about the medication with the support of specialists using a multidisciplinary approach, the transfer of the medication into breast milk should be evaluated, and if it will negatively affect the baby, she should be taught methods of expressing and storing breast milk. The importance of mothers informing healthcare professionals that they are continuing to breastfeed in case of any illness should be emphasized, and if breastfeeding is to be discontinued, support and counseling should be provided to the mother to prevent possible breast problems (Yurtsal, 2018).

Health professionals should support the mother in her decision to stop breastfeeding by gradually reducing breastfeeding in accordance with the baby's age and development and replacing it with solid foods to make this process easier. Gradual reduction of breastfeeding will prevent the mother from experiencing problems such as breast engorgement, as milk production will also decrease (Yurtsal, 2018).

In conclusion, breastfeeding counseling covers pregnancy, childbirth, and the postpartum period. Healthcare professionals providing breastfeeding counseling should support and encourage evidence-based practices in light of current guidelines and research.

Conclusions and recommendations

Breastfeeding is crucial for improving public health. The widespread adoption of evidence-based practices will increase science-based care. Evidence-based breastfeeding practices are methods based on scientific evidence that support the health benefits of breast milk and breastfeeding. These practices emphasize that breast milk is the ideal food for babies, strengthens the immune system, reduces the risk of infection, and contributes to the baby's physical and mental development.

Evidence-based breastfeeding practices have ensured the promotion and support of breastfeeding in society. Breastfeeding rates are increasing with the implementation of baby-friendly policies in hospitals and health institutions.

Information, education, and counseling services provided to expectant and new mothers boost confidence and success in breastfeeding. Furthermore, the creation of breastfeeding support programs in workplaces and communities will make it easier for mothers to continue breastfeeding and contribute to strengthening the culture of breastfeeding in society.

In conclusion, the widespread adoption of evidence-based breastfeeding practices will contribute to improved health outcomes for mothers and infants and strengthen public health. Supporting and promoting evidence-based breastfeeding practices contributes to raising a healthy generation and strengthens the culture of breastfeeding in society.

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Figure 1: *Incorrect positioning of the baby at the breast*

Figure 2: *Correct positioning of the baby at the breast*

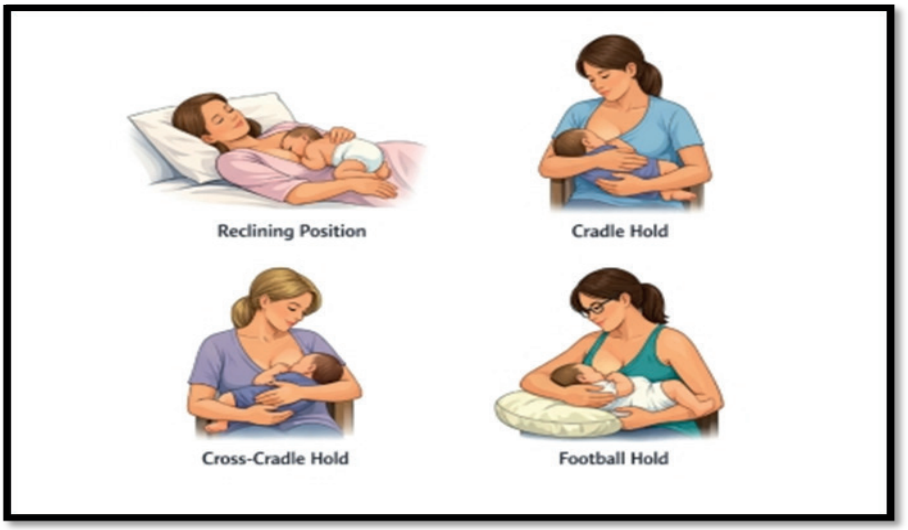

Figure 3: *Breastfeeding positions*

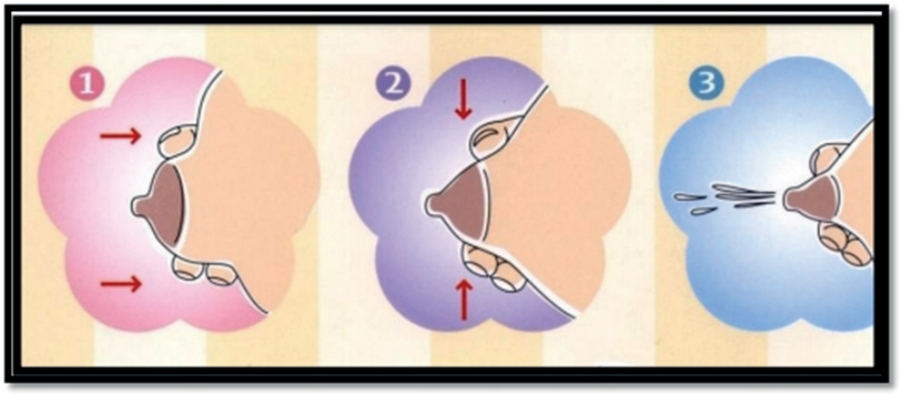

Figure 4: *Steps for expressing milk by hand*

Table 1. *Turkish Ministry of Health and ABM Protocol Committee recommendations for storing breast milk*

	ABM Protocol Committee	Ministry of Health
Room Temperature (16-29 °C)	**4 hours**	**4 hours**
Refrigerator shelf (+4 °C)	**4 days**	**4 days**
In the freezer (-18°C or below)	**6 months**	**6 months**

Chapter 14

EVIDENCE-BASED PRACTICES IN POSTNATAL RISKY SITUATIONS: A TRADITIONAL REVIEW

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Introduction

The postpartum period covers the 6-8-week period starting with the removal of the placenta in labor and lasting until all organs return to their pre-pregnancy state. This postpartum period is called puerperum, postpartum, fourth trimester (Ekşi, 2019).

Risky conditions occurring in the postpartum period are a major determinant of morbidity and mortality internationally. The global maternal mortality rate for 2020 is estimated to be 223. The World Health Organization (WHO) targets to reduce the maternal mortality rate to 70 and below by 2030 (WHO, 2023). According to the Turkey Maternal Mortality Report, between 2015 and 2019, the maternal mortality rate was 13.1 per 100,000 and the majority of maternal deaths were due to preventable causes (Ministry of Health, 2021).

The most frequent causes of maternal deaths globally are hemorrhage (27.1%), hypertensive disorders (14.0%) and sepsis (10.7%), while in Turkey, the most common causes are cardiovascular (23.9%), toxemia of pregnancy (18.3%), hemorrhage (15.7%) and embolism (14.9%) (Say et al, 2014; Ministry of Health, 2021).

Postpartum risk conditions include postpartum hemorrhage, infection of reproductive organs, urinary tract infection, thromboembolic conditions, breast problems and psychological problems. It is very important that current evidence-based practices for the prevention and treatment of postpartum risky conditions are known by nurses and used effectively in clinics to reduce maternal mortality rates. Evidence-based nursing (EBN) practices are very important in increasing both the quality of care and the patient's recovery outcomes by performing care and interventions in a certain standard (Abu-Baker et al., 2021). The evidence levels and recommendation grades of the practices obtained are presented in Table 1. The aim of this review is to examine evidence-based practices in risky postpartum situations in the light of current literature.

Evidence-Based Practices in Postpartum Risk Conditions

Postpartum bleeding

Postpartum hemorrhage (PPH) is defined as blood loss of 500 mL or more after vaginal birth or 1,000 mL or more following caesarean section. This represents an approximate 10% drop in hematocrit level. Postpartum hemorrhage is called 'early PPH' if it occurs within the first 24 hours and 'late PPH' if it occurs between 6-12 weeks. Postpartum hemorrhage may occur due to reasons such as uterine overstretching or contraction failure, trauma, blood clotting disorders and requires urgent intervention (Hamlacı, Y., Hediye Bekmezci, H. & Özerdoğan, N, 2017).

Evidence-based practices in postpartum hemorrhage are listed in line with WHO recommendations (WHO, 2012);

- The use of an effective uterotonic (Oxytocin, Carbetocin, Misoprostol, Ergometrine/methylergometrine) is recommended to prevent bleeding in the third stage of labor (all deliveries) (moderate quality, strong recommendation).

- Oxytocin (10 IU, IM/IV) is recommended for prevention of bleeding in all deliveries (moderate quality, strong recommendation).

- When oxytocin cannot be used, ergometrine/ methylergometrine, oxytocin + ergometrine fixed dose combination or misoprostol (600 μg PO) is recommended (moderate quality, strong recommendation).

- Controlled Cord Traction (CCT) by experienced birth attendants is recommended for vaginal and cesarean deliveries requiring manual removal of the placenta (moderate quality, strong recommendation).

- It is recommended that uterine tone be routinely evaluated every 15 minutes in the first two hours after birth (very low quality, strong recommendation).

-Uterine massage is recommended as an intervention in the management of postpartum hemorrhage (very low quality, strong recommendation).

- Single dose antibiotics are recommended in cases where manual placental removal is necessary (very low quality, weak recommendation)

- If the use of uterotonics for postpartum uterine atony-induced bleeding has failed, intrauterine balloon tampons, uterine artery embolization, external aortic compression, bimanual uterine compression, uterine packing and non-pneumatic anti-shock garments are recommended. If postpartum hemorrhage cannot be prevented despite available preventive measures, surgical intervention is advised when necessary (very low quality, weak recommendation) (WHO, 2012; WHO, 2015).

Infection of the reproductive tract

Postpartum infection is defined as "the occurrence of fever of 38 degrees and above for 10 postpartum days after the first 24 hours following delivery and lasting for at least two days". Postpartum reproductive tract infections include endometritis, abdominal wound infection and perineal area/episiotomy infection, salpingitis and peritonitis (Boushra & Rahman, 2020).

In the period before delivery, the mother may engage in practices to reduce the risk of infection. Women are advised to avoid routine perineal shaving before vaginal deliveries (conditional recommendation based on very low evidence) (WHO, 2015). In patients scheduled for cesarean delivery, showering

before delivery is recommended (Boushra & Rahman, 2020; Berríos-Torres et al., 2017). Vaginal preparation with chlorhexidine gluconate (CHX) or povidone-iodine should be performed immediately before cesarean delivery (Conditional recommendation based on moderate quality evidence) (WHO, 2021). There is a study showing that vaginal preparation with 4% chlorhexidine gluconate reduces the risk of endometritis in cesarean deliveries (Shea & Soper, 2019). It is recommended to use an alcohol-based agent for skin preparation for the surgical incision site (ACOG, 2019).

In surgical deliveries, a single dose of routine prophylactic antibiotic administration 60 min before the incision, not after umbilical cord clamping, is recommended (Moderate quality evidence, strong recommendation). For emergency and elective cesarean section, a single dose of cephalosporin or penicillin should be administered (WHO, 2015). In antibiotic administration, the dose should be adjusted according to the weight of the mothers (1 g if kg <80, 2 g if kg >80) (ACOG, 2018). The routine use of antibiotics at caesarean section significantly decreases the risk of wound infection and endometritis by 60–70% (evidence of moderate quality) (Smaill & Grivell, 2014).

Routine measurement of fever after delivery is not recommended unless the mother has any signs of infection [D (GPP)]. If infection is suspected in the mother, the temperature should be measured and if it is 38 degrees Celsius or above, the temperature should be repeated in 4-6 hours. Women with high fever (2 temperature measurements above 38 degrees Celsius) should be evaluated for signs of sepsis (emergency action) (NICE, 2015).

At each postpartum consultation, he/she should offer to assess the woman's perineal area [D(GPP)]. The perineal examination should assess signs of infection (redness, increased temperature, presence of exudate), perineal pain, foul-smelling discharge, the condition of the sutures, and address the woman's concerns about the wound healing process [D(GPP)]. The importance of perineal hygiene and toilet hygiene should be explained to women, and women should be encouraged to take daily standing showers (NICE, 2021).

Prophylactic antibiotics are not routinely indicated for all women who have had a vaginal delivery or episiotomy without intervention (WHO, 2015). However, the use of prophylactic antibiotics following operative vaginal delivery has been shown to lower the risk of serious infectious complications (Evidence high quality) (WHO, 2021). In mothers with premature rupture of membranes before delivery, antibiotics are recommended until delivery (moderate quality evidence, strong recommendation) (WHO, 2015; ACOG, 2018).

In a Chohrane review of 42 studies for postpartum endometritis, clindamycin + gentamicin combination therapy was considered the most appropriate treatment for endometritis (Mackeen et al., 2015). Irrigation showed no significant effect when compared (washing the uterus and surgical site with

saline solution containing antibiotics) and intravenous antibiotic prophylaxis in reducing the risk of endometritis after cesarean section. High-quality studies are needed to find the safest-effective way to give preventive antibiotics (Nabhan, Allam & Hamed Abdel, 2016).

Limiting the number of vaginal examinations and avoiding fetal monitoring during labor reduces the risk of postpartum infection (Boushra & Rahman, 2020). Manual removal of the placenta should be avoided whenever possible (Shea & Soper, 2019). In case of manual removal of the placenta, women should receive routine antibiotic prophylaxis (WHO, 2015). Adherence to aseptic techniques and rigorous handwashing procedures during labor and delivery help to reduce the risk of postpartum infection (ACOG, 2018).

Urinary tract infections

Urinary tract infection is a common postpartum infection seen in 2-4% of all deliveries (Kaya Senol, 2023). Risk factors associated with postpartum bacteriuria and urinary tract infection include bladder catheterization, epidural anesthesia, cesarean delivery, intervention delivery, and high body mass index of the mother (Dalton & Castillo, 2014). While it facilitates the spread of microorganisms due to the close proximity of the meatus, vagina and rectum in women, bleeding in the postpartum period increases the susceptibility to infections (Kaya Şenol, 2023).

Acute cystitis is the most common postpartum urinary infection. It manifests itself with fever and chills in the early postpartum period. However, suprapubic pain, feeling of urgent urination, dysuria and frequent urination, hematuria, cloudy urine color and foul odor are other important symptoms. With the development of pyelonephritis as a result of the infectious agent reaching the kidneys, symptoms of low back pain, nausea, vomiting, chills and fever occur (Kaya Şenol, 2023).

The diagnosis is confirmed by detection of the infectious agent in the urine sample. The first option for non-acute cystitis is nitrofurantoin 100 mg pro BID x 5 days or Trimethoprim-Sulfamethoxazole (TMP-SMX) 160/800 mg BID x 3 days. Acute pyelonephritis is treated orally with fluoroquinolones for 7 days or TMP-SMX twice daily for 14 days. During treatment with fluoroquinolone, it has been reported that breastfeeding of the mother should be restricted and her milk should be expressed and discarded at least 2 hours after taking the drug (Dalton & Castillo, 2014).

After delivery, the bladder catheter should be removed at the earliest appropriate time (postoperative 8th hour), and in cases where the catheter cannot be removed, intake and output should be monitored. In each postpartum follow-up, it should be questioned whether the woman experiences burning, pain, and incontinence during urination (Postpartum Care Management Gu-

ide, 2018). In women who experience postpartum urinary retention, it is recommended to apply warm water to the perineum, pour warm water, listen to the sound of water, ensure adequate fluid intake (2500-3000 ml) and empty the bladder every 2-3 hours (Postpartum Nursing Care Pathway, 2019).

Thromboembolic conditions

Postpartum is a period of increased risk of thromboembolic disease. In this period, it is aimed both to increase peripheral circulation and to prevent thrombophlebitis. In order to prevent thromboembolic diseases in cesarean delivery, wearing elastic stockings/bandages is supported and women are asked to mobilize as soon as possible after delivery (Postpartum Care Management Guide, 2018). Women in the postpartum period are advised to avoid remaining in the same sitting position for prolonged periods. A pedal pulse comparison should be made in both legs of the woman and the level of edema in the legs should be checked. The patient should be closely monitored for signs of tachycardia, blood pressure alterations, and shock (Çetinkaya Ak, 2023).

Petechiae and bruising should be checked twice a day in women who start anticoagulant treatment for thrombophlebitis. Nosebleeds, blood in urine and feces, bleeding gums and vaginal bright red lochia should be evaluated carefully and the patient should receive appropriate information regarding the management plan (Çetinkaya Ak, 2023).

It is important to mobilize women as soon as possible immediately after delivery. Women should be evaluated for deep vein thrombosis (DVT) when symptoms of unilateral leg pain, swelling and redness are observed, and for pulmonary embolism if shortness of breath or chest pain begins (emergency action). Thromboembolism is more common in obese women and individualized care is required (NICE, 2015).

Breast problems

Exclusive breastfeeding is recommended for infants during the first six months of life, starting from the first minutes of life, with continued breastfeeding alongside complementary foods beyond the second year of life (WHO, 2017; UNICEF, 2020). Skin-to-skin contact is recommended immediately after birth (WHO, 2017; Ministry of Health, 2020). The newborn should be breastfed every two hours and the frequency of breastfeeding should be 10-12 meals a day. The newborn should be kept at the breast for at least ten to fifteen minutes to receive milk efficiently. If the baby is breastfed with one breast, the next breastfeeding should be switched to the other breast. However, if both breasts are breastfed, breastfeeding should be continued from the last breast. Breastfeeding frequency should be tailored to the infant's sucking cues (such as making sucking movements with the mouth or bringing the hand to the mouth) rather than being scheduled by time intervals (Akkuş & Çoban, 2023).

Providing a comfortable and reliable environment before starting to evaluate breastfeeding will allow the mother to breastfeed her baby more confidently. The mother's behavior during breastfeeding, the appropriateness of the position, the way the baby grasps the breast and whether effective breastfeeding occurs should be observed. Incorrect breastfeeding position can cause breast problems. Early identification and management of breast-related problems during postpartum breastfeeding are of critical importance. If left untreated, such problems may result in complications including nipple fissures, nipple pain, nipple bleeding, insufficient milk production, breast engorgement, mastitis, and breast abscess (Durmuş & Can Gürkan, 2020).

Maternal breastfeeding should be assessed at each postpartum follow-up, with attention given to the identification of symptoms such as breast engorgement and mastitis. If there is engorgement, the mother is recommended to breastfeed frequently and empty her breasts with warm application. Analgesic use is recommended when necessary. The use of a properly fitted bra that does not compress the breasts is recommended during breastfeeding [D(GPP)] (WHO, 2022).

In the Cochrane (2020) review examining breast engorgement; it was reported that using cabbage leaves for pain may be more effective than using cold gel packs. It is also reported that cold cabbage leaf application may be more effective than hot water bags for breast stiffness (Zakarija- Grkovic & Stewart, 2020). In the Cochrane (2020) review examining mastitis and breast pain; acupoint massage may be more effective than breast massage and low-frequency pulse therapy (Crepinsek, Taylor, Michener & Stewart, 2020).

A study examining descriptive and supportive care practices in the treatment of breast problems (healing traumatic nipple pain and nipple cracks) according to evidence levels recommends olive oil (evidence level I-II), Lanolin (evidence level II), Lanolin breast shields (evidence level I), mint juice (evidence level II), aloe vera (evidence level I-II) and tea compress (evidence level II) (Durmuş, Can Gürkan, 2020).

Psychological problems

Physiological changes that occur during pregnancy-birth-postpartum processes in the woman's life cycle and changes in the situation such as adaptation to parenthood cause various mental problems. This condition, which starts to show its symptoms with maternal blues, can progress to postpartum depression and psychosis if necessary precautions and treatments are not taken. WHO defines "major depression episode occurring up to 1 year after pregnancy or delivery" as postpartum depression (WHO, 2022).

In the guidelines published by WHO, routine screening for depression and anxiety using a validated scale is recommended for all postpartum wo-

men. Early psychological interventions are recommended for women with postpartum psychological problems (WHO, 2022).

In a study evaluating the level of evidence for interventions implemented during the postpartum period; Antidepressant use (quality A), telephone-based peer support (evidence A), interpersonal psychotherapy (evidence B), individual therapy (evidence A), social support (evidence A) and home visits by nurses/midwives (evidence A) were found effective in preventing postpartum psychological problems (Yıldırım & Büyükkayacı Duman, 2018).

Conclusion and Recommendations

Postpartum infections remain a significant contributor to maternal morbidity and mortality. The implementation of evidence-based approaches in both the prevention and management of these infections plays a crucial role in reducing infection-related risks and serious complications through the provision of standardized care. The reviewed literature indicates that the existing evidence largely consists of studies with low to moderate levels of evidence, highlighting the need for further support through well-designed and up-to-date research.

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Table 1: Levels of Evidence and Degrees of Recommedation (Dişli & Kaydırak, 2021)			
Level of Evidence	**Diagnostics**	**Evidence Type**	**Recommendation Rating**
High	Further research is unlikely to change the confidence in the predicted outcome.	Meta-analyses, systematic reviews of RCTs or RCTs	A
Middle	It can have a significant impact on reliability, which is the result of further research.	Systematic reviews of case-control or cohort studies, well-done case-control or cohort studies	B
Low	Further research is likely to have a significant impact on the predicted outcome and change the result.	Analytical/non-experimental studies (case reports, case series, correlation)	C
Very Low	The prediction of any outcome is uncertain	Expert opinion, official consensus	D
	Basically, the consensus within the group that developed the guide was used		GDG D(GPP)
GDG (Strong/Weak) D (GPP): Good Practice Point			

Chapter 15

GENERAL INFORMATION AND PATHOLOGIES OF THE ENDOCRINE SYSTEM

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General Information and Pathologies of the Endocrine System

General Information About the Endocrine System

The maintenance of internal balance (homeostasis) in the human body and the regular functioning of vital activities such as growth, development, and reproduction are achieved through intercellular communication. This communication occurs through the nervous system and the endocrine system. While the nervous system responds very quickly to stimuli, the endocrine system's responses develop more slowly but have a longer-lasting effect. While the nervous system affects organs through nerves, the endocrine system regulates organ function through hormones. This keeps fundamental processes such as growth, development, energy production, reproduction, and adaptation to environmental conditions in balance (1-3).

Hormones are produced in the endocrine glands in the body and are transported to target organs via the bloodstream. Once they reach these organs, they regulate their functions. Endocrine glands do not have ducts; therefore, they release their secretions directly into the bloodstream. Although they are located in different parts of the body, they all together form the endocrine system. The word "hormone" means "to set in motion" or "to stimulate." Hormones are chemical substances released from endocrine glands into the bloodstream that direct the functioning of various organs. These substances can have a stimulating (exciting) effect on organs in some cases and a slowing (inhibiting) effect in others. Hormones are divided into three groups based on their chemical structure: steroid hormones, peptide hormones, and amino acid hormones. Steroid hormones are generally soluble in fat, while peptide and amino acid hormones are soluble in water (2-4).

Hypophysis (Pituitary gland)

The pituitary gland works with the hypothalamus to control the activity of many endocrine glands in the body. Therefore, it acts as a conductor in the hormone system. The pituitary gland is located in a cavity called the fossa hypophysialis in the skull. It is reddish in color and oval in shape. On average, it is 12 mm wide, 8 mm long, and weighs approximately 0.5–0.6 grams (2, 3).

The pituitary gland is connected to the tuber cinereum part of the hypothalamus by a thin stalk called the infundibulum. The upper part of the gland is covered by a membrane called the diaphragma sellae, formed by the dura mater. The hypothalamus directly controls the posterior part of the pituitary gland (neurohypophysis) and indirectly controls the anterior part (adenohypophysis) (2, 3).

Neighbors of the Hypophysis

The anterior-inferior portion of the pituitary gland contains the sphenoid

sinus, while the superior portion contains the optic chiasm and hypothalamus. On its lateral sides are the cavernous sinus, internal carotid artery, cavernous sinus veins, and cranial nerves III, IV, V1, V2, and VI (2, 3).

The pituitary gland begins to form during infancy (the 6th to 8th weeks of fetal life). Because its development originates from different tissues, it possesses characteristics of both the nervous system and the endocrine system (2).

It is examined in two parts:

Adenohypophysis (Anterior Lobe)

The adenohypophysis constitutes the largest part of the pituitary gland and has a rich vascular structure. The function of this section is controlled by releasing hormones secreted by neurons in the hypothalamus. These hormones regulate the blood levels of other hormones secreted by the adenohypophysis. This communication occurs via the tuberohypophysial tract and the hypothalamo-hypophysial portal system (1, 2, 5).

The adenohypophysis is divided into three main sections:

1. Distal lobe: It constitutes approximately three-quarters of the gland.

2. Pars tuberalis (pars infundibularis): This is the section rich in blood vessels and located around the infundibulum.

3. Pars intermedia: The part located at the rear of the adenohypophysis, adjacent to the neurohypophysis.

Adenohypophysis Hormones

· **TSH (Thyroid-Stimulating Hormone):** Regulates hormone secretion in the thyroid gland.

· **ACTH (Adrenocorticotropic Hormone):** Stimulates the cortex of the adrenal gland, increasing hormone secretion.

· **PRL (Prolactin):** Stimulates the mammary glands to initiate milk production.

· **LH (Luteinizing Hormone):** Stimulates testosterone in men and ovulation in women.

· **STH (Somatotropin / Growth Hormone):** Promotes the growth and development of body cells.

· **MSH (Melanocyte-Stimulating Hormone):** Plays a role in skin color formation (pigmentation).

FSH (Follicle-Stimulating Hormone): Supports the development of sperm and egg cells in the testes and ovaries (2).

Posterior pituitary gland (Lobus Posterior)

The neurohypophysis, which forms the posterior portion of the pituitary gland, is derived from neural ectoderm and comprises approximately one-fourth of the pituitary gland. This section lacks secretory cells and the blood-brain barrier. The neurohypophysis functions in conjunction with the brain via the median eminence (1, 2, 5).

Neurohypophysis Hormones

Vasopressin (ADH–Antidiuretic Hormone): This hormone controls water reabsorption in the kidneys. When insufficiently secreted, urine output increases. It also raises blood pressure through its vasoconstrictive effect (2, 5).

Oxytocin: Causes the uterine muscles to contract during childbirth and stimulates milk secretion from the mammary glands after childbirth (2, 5).

The Vessels of the Pituitary Gland

The pituitary gland has a rich network of blood vessels. It is supplied by the superior hypophysial artery and inferior hypophysial artery branches of the internal carotid artery (2, 3).

Pineal Gland

The pineal gland is a small, pinecone-shaped gland located between the two hemispheres of the brain, at the rear of the corpus callosum. It measures approximately 8 × 5 × 4 mm and weighs about 100–200 mg. It is connected to the diencephalon region via a stalk. The gland has a rich blood supply and consists of glial cells and cells called pinealocytes. These cells contain melanin and serotonin (1, 2, 5).

The pineal gland is active in the dark and inactive in the light. The substances it secretes reach many organs, such as the pituitary gland, adrenal glands, ovaries, testes, pancreas, and parathyroid glands, via the blood or cerebrospinal fluid (CSF) (2, 5).

Vascular and Nervous Structure

The pineal gland has a dense network of blood vessels and does not contain a blood-brain barrier. Its blood supply is provided by the posterior choroidal artery, a branch of the posterior cerebral artery. The veins drain into the systemic circulation via the vena magna cerebri (Galen vein). Nervous control is provided by the pineal nerve (nervus pinealis), which originates from the superior cervical ganglion. This nerve transmits sympathetic signals to the gland (2, 5).

Structural Features and Development

The pineal gland is located within a capsule formed by an extension of the pia mater. It reaches its full size between the ages of 7 and 14. Calcium deposition in the gland begins at around 16–17 years of age. This deposition is called acervulus cerebri (brain sand). This structure is visible on X-ray films and is an important indicator for determining age or locating the center of the brain (2, 6).

Duties and Salary

The pineal gland secretes a hormone called melatonin. Melatonin regulates the sleep-wake cycle (circadian rhythm) and the activity of certain reproductive organs. Production of this hormone increases at night and decreases during the day. Therefore, the pineal gland plays an important role in setting the body's biological clock (2, 3, 6).

Thyroid Gland Development

The thyroid gland begins to develop in the fourth week of the embryonic period. Its development takes the form of a small protrusion (diverticulum) originating from the endoderm layer located in the lower part of the pharynx, between the foramen caecum and the copula. This structure extends downward and forward, growing into a tube shape. The lower end of the tube divides into two branches, and the cell clusters formed here will eventually develop into the isthmus and lateral lobes. Over time, the ductus thyroglossus closes, leaving only the foramen caecum at the base of the tongue. In some cases, small pieces of thyroid tissue may remain along this canal (2, 6).

General Features

The thyroid gland is located in the front of the neck, between the 5th cervical (C5) and 1st thoracic (T1) vertebrae. It is reddish-brown in color and has a rich vascular structure. The thyroid gland, the largest of the endocrine glands, typically weighs around 25–30 grams, although this varies from person to person. It is slightly larger in women and may enlarge during menstruation and pregnancy. The gland consists of two lobes, right and left, and an isthmus connecting them in the middle. Sometimes a third protrusion called the lobus pyramidalis may also be present. This protrusion usually extends upward from the upper part of the isthmus (2, 6, 7).

Location and Neighborhoods

The thyroid gland is located in front of the trachea. The isthmus is usually at the level of the 2nd and 3rd tracheal rings. Each lobe is approximately 5 cm long and 2 cm wide. The gland is covered on the front by the sternohyoideus muscle, sternothyroideus muscle, and omohyoideus muscle. The fascia pretrachealis, which externally envelops the thyroid gland, can be easily separated

from the internal capsule (capsula fibrosa). The gland is closely related to the trachea, cricoid and thyroid cartilages, esophagus, and n. laryngeus recurrens nerve. In addition, parathyroid glands and vascular-nerve structures are located at the posterior edges of the gland (2, 6).

The isthmus is a narrow section connecting the two lobes. It is approximately 1.5 cm long and its location may vary from person to person. Sometimes there may be no isthmus. The anterior jugular vein passes through its front surface. In some individuals, there is a small protrusion extending upward from the upper part of the isthmus. This protrusion is called the lobus pyramidalis. It may sometimes contain muscle bundles, which are called the m. levator glandulae thyroideae (5, 6).

Connectors and Capsules

The thyroid gland is attached to the thyroid cartilage, cricoid cartilage, and trachea by ligaments. These ligaments are called the suspensory ligaments of the thyroid gland (Berry ligaments) (1, 2, 8).

The thyroid gland has two capsules:

Inner capsule (capsula fibrosa): It tightly envelops the gland and enables it to divide into small lobules (lobuli) (2, 6).

Outer capsule: It is an extension of the deep fascia of the neck and is loosely connected to the inner capsule (2, 6).

Between the two capsules are the parathyroid glands, the inferior thyroid artery, and the recurrent laryngeal nerve (6).

Thyroid Hormones

The thyroid gland regulates the body's metabolic rate and growth process by secreting the hormones T3 (triiodothyronine) and T4 (thyroxine). The production of these hormones is controlled by TSH (Thyroid-Stimulating Hormone) (2, 5).

Blood Vessels and Nerves of the Thyroid Gland

The thyroid gland is supplied with blood by the superior thyroid artery, a branch of the external carotid artery, and the inferior thyroid artery, which arises from the thyrocervical trunk (2, 6).

The veins in the upper part drain into the internal jugular vein via the superior thyroid vein and the middle thyroid vein (6).

The veins in the lower regions drain into the left brachiocephalic vein via the inferior thyroid vein (6).

Nerves

Sympathetic nerves reach the upper, middle, and lower cervical ganglia via the sympathetic trunk (6).

The parasympathetic nerves originate from the vagus nerve and reach the gland via the superior laryngeal nerve and inferior laryngeal nerve (3, 6).

Parathyroid Glands

Development: The parathyroid glands originate from the endoderm layer of the third and fourth pharyngeal pouches during the embryonic period. These glands are yellowish-brown, pea-sized endocrine organs. They are usually located between the two capsules of the thyroid gland, close to the posterior surface of the lateral lobes. Although their size varies from person to person, they are on average 6 mm long, 3–4 mm wide, and 1–2 mm thick. They weigh approximately 0.5 grams. Humans generally have 4 parathyroid glands. Those located in the upper part are called the superior parathyroid glands, while those located in the lower part are called the inferior parathyroid glands. The superior parathyroid glands are usually two in number and are located on the sides of the beginning of the esophagus, at the level of the lower edge of the cricoid cartilage. There is little variation in the location of these glands (2, 3, 6).

However, the inferior parathyroid glands can often be found in different locations. For example, they may be located between the capsules of the thyroid gland, near the lower ends of the lobes, under the inferior thyroid artery, or even in front of the jugular vein or within the thyroid tissue. Although humans generally have four parathyroid glands, it has been reported that some individuals may have fewer than four (1%) or more than four (2, 6).

Arteries, Veins, Nerves, and Lymphatics

The parathyroid glands are supplied by rich blood vessels originating from the anastomosis between the superior and inferior thyroid arteries. Venous blood drains into the veins of the thyroid gland. The glands contain a dense network of lymphatic vessels, which join the lymphatic system of the thyroid gland. The neural innervation of the parathyroid glands originates from the sympathetic ganglia in the neck region (2, 6).

Adrenal Gland

The adrenal glands are two small glands located above the kidneys, yellowish in color and weighing approximately 5 grams each. These glands are located in the retroperitoneal region and function independently of each other. While they are larger relative to body weight at birth, their size decreases with age (2).

The right adrenal gland (G. suprarenalis dextra) is generally triangular or

pyramidal in shape; it is located in the upper part of the right kidney, adjacent to the inferior vena cava and the liver (2).

The left adrenal gland (G. suprarenalis sinistra) is crescent-shaped; its anterior surface is related to the stomach, pancreas, and left kidney (2).

Structure and Sections

Each adrenal gland develops from two different embryonic origins (2, 6):

· The cortex (outer layer) originates from the mesoderm.

· The medulla (inner layer) is composed of neural crest cells.

The cortex consists of three layers:

Zona glomerulosa: Produces mineralocorticoid hormones such as aldosterone; regulates water and electrolyte balance (2, 6).

Zona fasciculata: Secretes cortisol (glucocorticoid hormone); affects carbohydrate, protein, and fat metabolism (2, 6).

Zona reticularis: Produces sex hormones such as androgens, estrogens, and testosterone (2, 6).

The medulla develops from the neural crest and is associated with the sympathetic nervous system. In this section:

Adrenaline (epinephrine) and noradrenaline (norepinephrine) hormones are synthesized (2).

These hormones are released during times of stress, fear, or excitement, preparing the body for a "fight or flight" response (2).

Increases heart rate, increases blood flow to muscles, and dilates the pupils (2).

Arteries, Veins, and Nerves

The sponge is nourished by numerous small blood vessels (6):

· Superior adrenal artery (originates from the inferior phrenic artery)

· Middle suprarenal artery (originates from the abdominal aorta)

· Inferior adrenal artery (originates from the renal artery)

Venous flow (6):

· On the right side, the right adrenal vein opens directly into the inferior vena cava.

· The left adrenal vein drains into the left renal vein.

Nervous impulses come from the sympathetic system (6).

The cortex is controlled by the ACTH hormone secreted by the pituitary gland (2, 6).

The medulla is stimulated by sympathetic fibers arriving via the greater and lesser splanchnic nerves and acts as a kind of sympathetic ganglion (2, 6).

Development (Embryology)

The development of the cortex begins with the thickening of the intermediate mesoderm. Mesothelial cells proliferate toward the mesenchyme, forming primary cortical cells; these later form the fetal cortex. This layer is quite large during the fetal period but shrinks after birth. The second layer that develops later becomes the permanent cortex and differentiates into the zona glomerulosa, zona fasciculata, and zona reticularis layers (2, 5, 6).

The medulla develops from neural crest cells. As these cells migrate to the sympathetic ganglia, some settle within the adrenal cortex and transform into chromaffin cells. These cells are responsible for the synthesis of adrenaline and noradrenaline (1, 5).

After birth, the gland shrinks and reaches its normal size in adulthood. Occasionally, accessory (ectopic) adrenal cortex tissue may be found around the kidneys or near the gonads (1, 6).

Pathologies

Many endocrine glands are prone to neoplastic growth. However, the effects of these growths on health vary depending on the tissue. Neoplasms occurring in the pituitary gland are quite common, but most are benign. However, this is not true for all types. When pituitary adenomas grow upward, they press on the optic chiasm, causing bitemporal hemianopsia. Therefore, even though patients can see straight ahead, they may collide with objects coming from the sides (1, 9).

Excessive secretion of the ACTH hormone leads to Cushing's syndrome, which is characterized by increased fat deposits in the face and body. This condition typically presents with skin changes such as facial redness, easy bruising, and purple streaks, alongside metabolic symptoms like hyperglycemia and hypertension (1, 10).

Growth hormone (STH/GH) deficiency in children causes dwarfism, while excess growth hormone causes gigantism. If excess hormone occurs after the epiphyseal plates have closed, this condition manifests as acromegaly (1, 5, 11).

If there is excess prolactin in a pituitary tumor, this condition is called prolactinoma (1, 5). Prolactinomas account for approximately 50% of pituitary adenomas requiring medical intervention. In addition, they are a significant cause of hypogonadism and infertility. Prolactinomas smaller than 10

mm or larger than 4 cm may occur (12).

Increased function of the pineal gland may reduce the activity of certain glands by increasing melatonin secretion. If the suppression of melatonin on the hypothalamus is eliminated, early puberty (pubertas precox) may develop. In addition, evaluation of the pineal gland in MR imaging provides important information about intracranial lesions (1, 5, 13).

Acromegaly is characterized by transverse enlargement of bones, especially in adults, growth of hands and feet, coarsening of facial features, enlargement of the tongue, and deepening of the voice. Headaches and visual disturbances may occur due to the effect of the adenoma mass. Hypertension and diabetes are common in patients (2, 3, 6, 11).

It is known that the thyroid gland is the only endocrine gland that stores its secretion outside the cell. Preservation of the parathyroid glands is essential during surgical removal. Enlargement of the gland tissue beyond normal size is called goiter. Usually due to iodine deficiency, the gland works excessively to increase thyroid hormone production and its volume increases (1, 5, 14).

Thyroid hormones affect metabolic rate, body temperature, heart rate, muscle strength, and bone and reproductive system functions. Therefore, changes in hormone levels cause numerous clinical symptoms (1, 5).

Goiter and Thyroid Nodules

The most common thyroid disorder in society is referred to as simple (diffuse) physiological goiter. According to studies, the highest prevalence is seen in premenopausal women, with a female-to-male ratio of 4:1 (14).

Thyroid Cancer

The clinical presentation of this cancer is generally a single nodule or a gradually enlarging goiter. Thyroid nodules are common, but cancers are rare. However, it is the most common malignant tumor within the endocrine system and accounts for 90% of endocrine gland cancers. A study reported the incidence in the United Kingdom as 3.5 per 100,000 in women and 1.3 per 100,000 in men (14).

Hyperthyroidism

It occurs due to excessive secretion of thyroid hormones (T3-T4); TSH levels are usually low. Imaging and scintigraphic examinations aid in diagnosis

Key findings:

- Weight loss, muscle weakness, fatigue
- Trembling hands, excessive sweating, intolerance to heat

- Tachycardia, hypertension
- Anxiety, insomnia, irritability
- Hair loss, menstrual irregularities
- Exophthalmos may be observed (15).

Congenital Hypothyroidism

Congenital hypothyroidism, which affects 3,500-4,000 newborns, is considered the most treatable cause of intellectual disability (16). Approximately 85% of cases occurring in iodine-rich regions are caused by sporadic developmental disorders of the gland, such as arrest of embryonic thyroid migration or complete absence of thyroid tissue. The remaining small portion involves thyroid dyshormonogenesis disorders transmitted through autosomal recessive inheritance. If rapid diagnosis and treatment do not occur, most affected children may gradually develop growth disorders, irreversible intellectual disability, and neuropsychological disorders (14).

Hypothyroidism

It develops as a result of insufficient secretion of thyroid hormones; in this case, TSH is found to be high

Clinical features:

- Metabolism slows down, chills and fatigue become more pronounced
- Constipation, tendency to gain weight, loss of appetite
- Excessive sleepiness, mental sluggishness
- In children, growth and developmental delays and cretinism may develop
- The most common cause is an autoimmune process called Hashimoto's thyroiditis (14).

Thyroid Scintigraphy

Functional assessment of the thyroid gland is performed using a radioactive substance administered intravenously (2, 3).

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Chapter 16

THE CULTURAL DIMENSION OF HOLISTIC CARE: THE PLACE OF CUPPING THERAPY IN NURSING

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Introduction

Holistic care is a nursing approach that considers individuals in terms of their physical, social, psychological and cultural needs (Ambushe et al., 2023). It considers the individual's living conditions, mental state, beliefs and culture (Nursing and Midwifery Council [NMC], 2024). The holistic approach enables the provision of patient-centred care, increases patient satisfaction and improves the quality of care (Öntürk-Akyüz et al., 2024). Nursing care is founded on the principle that nurses must be prepared to provide care in all these areas to help individuals maintain, sustain and achieve integrity in all dimensions of their existence. Advocates of holistic care believe that the body, mind, and spirit are interconnected and must be considered as a whole (Korkut-Bayındır and Biçer, 2019).

Culture influences individuals' perceptions of health, their coping mechanisms with illness, treatment preferences and adherence to treatment (Ediz et al., 2024; Güngör & Kaya, 2023). The duty and responsibility of healthcare professionals is to collaborate with individuals/patients and their families during the most sensitive, crucial and vulnerable moments of their lives, defend them and empower them. At this stage, nurses should be aware not only of the patient's strategies for coping with illness, but also of their inclination towards complementary methods that support physical and mental balance. Nurses should be aware of the strategies that healthy or sick individuals use to cope with illness and their inclination towards complementary methods that support physical and mental balance (Ediz et al., 2024).

Cupping therapy, acupuncture, herbal treatments, aromatherapy, and spiritual methods are techniques used in many cultures to support physical and mental health. Cupping therapy is one of the oldest complementary treatment methods widely used in Chinese, Middle Eastern, East Asian, and Anatolian cultural areas (Choi et al., 2021). This method is used to increase blood circulation, eliminate toxins, and regulate physical energy balance by applying negative pressure to the skin (Wang et al., 2023). Cupping therapy is associated with potential physiological effects such as reducing inflammation symptoms (Abdelfattah et al., 2024), improving blood circulation (Al-bedah et al., 2018), and increasing nitric oxide production (Furhad et al., 2023). Recent studies have shown that cupping therapy can provide symptomatic improvement in many conditions, including migraine, hypertension, musculoskeletal pain, rheumatic diseases, asthma, and even fatigue and depression (Kalaycı et al., 2025; Nasb et al., 2020; Syahruramdhani et al., 2021; Toprak et al., 2025; Yiying et al., 2025). Cup therapy not only alleviates physical discomfort, but also aims to improve a person's overall well-being through mental relaxation and energy balancing (Abdulah et al., 2024; Uçun, 2022). In this respect, cupping therapy shows a strong parallelism with the holistic care approach adopted in nursing. In this sense, cupping therapy, which is a culturally rooted practice,

can be considered an element that adds cultural sensitivity to nursing care.

Nurses practicing holistic care utilize their nursing knowledge, theories, experience, and intuition to provide individualized care services throughout the patient's treatment process (Korkut-Bayındır and Biçer, 2019). In this context, cupping therapy is a noteworthy practice both in terms of reflecting individuals' culture and traditional healing understandings and expanding the scope of holistic nursing practice. Developing a nursing perspective on cupping therapy will significantly contribute to strengthening cultural sensitivity and evidence-based treatment. The aim of this section is to support a safe and evidence-based approach to practice by relating the scientific aspect of cupping therapy to the holistic and culturally sensitive approach in nursing.

What Does Science Say About Cupping Therapy?

Cupping therapy is an important practice recognized in the fields of traditional, complementary, and alternative medicine (World Health Organization [WHO], 2019). This therapy, whose historical origins date back to ancient times, was used in Ancient Egypt around 1550 BC, as mentioned in the Ebers Papyrus (Qureshi et al., 2017). Records of cupping therapy in Europe date back to the 14th to 17th centuries, parallel to the Renaissance period (Aboushanab & AlSanad, 2018). In Turkey, in line with global developments, legal regulations have been established for Traditional and Complementary Medicine practices. Furthermore, the legal basis for traditional and complementary medicine practices is Decree Law No. 663, the "Ministry of Health Approved Education Regulation" published in the Official Gazette dated 04.02.2014 and numbered 28903 and the Regulation on Traditional and Complementary Medicine Practices published in the Official Gazette dated October 27, 2014, and numbered 29158 (Bayrak, 2025). Cupping therapy has been shaped by the influence of different civilizations throughout history and has acquired a unique character over time by blending with local cultural elements (Al-Bedah et al., 2018).

Cupping therapy is divided into two main types: dry and wet cupping therapy (Shen et al., 2022). Dry cupping therapy, widely used in East Asia, aims to increase circulation by creating negative pressure on the skin using vacuum-forming cups. Wet cupping therapy, frequently used in the Middle East, is relatively invasive and involves making small incisions or punctures in the skin before applying the cups to draw blood into them (Wang et al., 2023).

Cupping therapy is one of the long-known and used methods. Especially after the pandemic period, claims that it alleviates the possible side effects of the vaccine have increased interest in this practice and led to cupping being preferred by wider audiences (Seçer, 2025). However, thanks to its promotion on social media platforms and the support of well-known athletes and celeb-

rities, it has gained widespread popularity (Alanazi, 2025).

Studies indicate that cupping therapy is the most widely known traditional and complementary medicine practice in society (Kıskaç et al., 2024). In a study by Özen and Balcıoğlu (2024) examining the use, knowledge level, and attitudes toward traditional and complementary medicine practices among individuals aged 18 and over who visited a family medicine clinic, it was determined that the practices participants were most knowledgeable about were, in order, leech therapy, cupping therapy, phytotherapy, and acupuncture.

Research conducted in Turkey shows that approximately 17.8% of participants have benefited from cupping therapy (Samancı et al., 2023). Similarly, in Indonesia, where the majority of the population is Muslim, cupping therapy is widely accepted not only as a complementary treatment method but also as a spiritual and cultural practice (Baharudin et al., 2025; Idwar et al., 2019; Siregar, 2021).

Hajamat, also known as hijama or prophetic wet cupping therapy, is considered one of the sunnah practices directly linked to the teachings of the Prophet Muhammad and, in this respect, is seen as a holistic treatment method that combines many religious, cultural, and health-related meanings (El-Sayed, 2023; Aboonq, 2019).

Cup therapy is a complementary treatment method that has been studied in numerous scientific studies and has gained attention for its potential benefits in treating various diseases and symptoms. In the literature, these applications have been shown to be effective in controlling back and neck pain (Nasb et al., 2020; Yiying et al., 2025; Zhang et al., 2024), treating migraines, managing obesity (Kang et al., 2023), and reducing asthma symptoms (Toprak et al., 2025). Furthermore, cupping therapy has been shown to be effective in the recovery process of stroke patients (Kim and Han, 2021), alleviating the symptoms of rheumatic diseases (Toprak et al., 2025), and lowering blood pressure, cholesterol (Syahruramdhani et al., 2021), and triglyceride (Toprak et al., 2025) levels. Furthermore, it is stated that it improves the person's quality of life by reducing fatigue and depression symptoms (Kalaycı et al., 2025; Yiying et al., 2025). Randomized controlled trials have shown that cupping therapy also yields positive results in the treatment of acne vulgaris (Tabatabaei et al., 2021) and fibromyalgia (Karacaoglu et al., 2024). A systematic review and meta-analysis conducted by Zhang and colleagues (2024) revealed that the effectiveness of cupping therapy in treating back pain varies depending on the duration of application, method, and type of pain. Furthermore, it has been reported to be effective in increasing ankle range of motion in individuals with limited mobility (Schaub et al., 2024) and in contributing to functional improvement by alleviating pain and stiffness in patients with knee osteoarthritis. A meta-analysis revealed that cupping therapy may play

an important complementary role in improving physical function and alleviating symptoms (Wang et al., 2023). In addition, a systematic review conducted by Uçun (2022) found that wet cupping therapy led to a significant decrease in stress, depression, and anxiety levels and a marked increase in mental well-being in many studies. Abdulah et al. (2024) also noted in their study with migraine patients that after cupping therapy, not only physical pain but also mental tension and psychological distress decreased.

Cupping therapy is generally considered a safe complementary treatment with rare and mild side effects. Side effects such as scarring, burns, headache, itching, dizziness, anemia, and panniculitis may occur after cupping; however, these side effects are usually mild and resolve spontaneously (Kaya, 2020). However, especially in wet cupping (hijama) applications, the disruption of the skin barrier increases the risk of skin infections and abscess formation; various infectious complications associated with this practice have been reported in the literature (Wang et al., 2023; Kaya, 2020). In a case reported by Emiral et al. (2025), sudden cardiac death occurred after wet cupping therapy performed by an unlicensed physician on a patient diagnosed with ischemic stroke and bladder tumor. This situation demonstrates that wet cupping therapy, when performed under improper conditions, can lead to serious and even fatal complications.

How Well Do Nurses Know Cupping Therapy?

Since cupping therapy is a culturally accepted practice in Turkey, it is important for nurses to evaluate this method within a scientific, ethical, and safe framework (Cevahir, Kaya & Altındiş, 2025, Seçer, 2025). Providing accurate information to individuals seeking cupping therapy, explaining sterilization and complications risks, and monitoring physiological outcomes after application are part of nursing care and education roles. This approach enables nurses to guide individuals while respecting cultural values and upholding scientific safety principles. Furthermore, nurses can contribute to the development of evidence-based practices by organizing awareness campaigns and research to increase the community's knowledge on this subject. In this regard, cupping therapy is an important example that combines cultural sensitivity, ethical principles, and holistic care elements in nursing science (Kaya et al., 2020).

The WHO took the first step toward establishing a legal framework for complementary treatment methods with its 1998 study titled "Regulation of Herbal Medicine Practices." In 2001, it published relevant legal regulations from more than 150 countries, and in 2013, it defined its strategies for the 2014-2023 period. These strategies emphasized that countries should develop national laws, regulations, and guidelines, taking into account personal health choices. Furthermore, the WHO's 2002-2005 strategies included nurses and

midwives among complementary therapy practitioners, and the 2014-2023 Traditional Medicine Strategies report highlighted that these approaches vary from country to country. The report also emphasized the importance of integrating complementary medicine practices into public health and stated that this process should be carried out within the framework of evidence-based practices, trained personnel, ethical principles, and patient safety standards (WHO, 2013). In this context, the education, certification, and practice-related authorities of nurses regarding complementary therapies have been legally defined in many countries (WHO, 2019; Kaya et al., 2020; Syahruramdhani et al., 2020). Similarly, Turkey's Regulation on Traditional and Complementary Medicine Practices (2014) clearly specifies the areas of application, such as acupuncture, apitherapy, phytotherapy, hypnosis, leech therapy, homeopathy, chiropractic, cupping therapy, reflexology, and music therapy, and who can perform them. However, this regulation only allows nurses to practice certain methods. Nevertheless, the Regulation on Traditional and Complementary Medicine Practices (2014) in Turkey clearly specifies the scope of practices such as acupuncture, apitherapy, phytotherapy, hypnosis, leech therapy, homeopathy, chiropractic, cupping therapy, reflexology, and music therapy, and who can perform them. However, according to the regulation, nurses can only perform certain methods (e.g., cupping therapy, reflexology, music therapy) under the supervision of a physician, while in other areas they can only take on an advisory role (Can and Türker, 2024). This situation limits nurses' participation in complementary methods that support their patient care roles.

According to international sources, nurses lack knowledge and training regarding complementary practices. According to a study conducted by Türker and Can (2024), the vast majority of nurses have not received training on complementary practices, lack sufficient knowledge on the subject, and do not adequately inform patients about these topics. The practices that the participants in the study were most knowledgeable about included massage, music therapy, prayer, herbal applications, reflexology, ozone therapy, and cupping therapy. The nurses who participated in the study stated that they had personally experienced these practices, wanted to receive training on the subject, and that complementary therapies should be added to the nursing education program. Similarly, a study examining the public's attitudes, perceptions, and knowledge levels regarding cupping therapy emphasized the importance of healthcare professionals receiving training on this subject and creating an open and respectful environment when communicating with patients (Al-Yousef, Wajid, & Sales, 2018). Accordingly, it is recommended that cupping therapy training be included in the health sciences curriculum to develop students' knowledge, skills, and confidence and to ensure that they can effectively apply these therapy methods in their future practice settings (Alanazi, 2025).

Conclusion

In conclusion, a holistic care approach in nursing requires considering the physical, mental, and cultural integrity of the individual, rather than focusing solely on the symptoms of illness. Cupping therapy, as a practice that reflects individuals' cultural values and traditional understandings of healing and supports both physical and mental balance, strengthens the holistic dimension of this approach. The literature shows that nurses experience a lack of knowledge regarding complementary therapies, but that training and curriculum integration for these practices increase both knowledge and confidence. Accordingly, it is recommended that training on cupping therapy and other complementary practices be systematically included in nursing education programs. In clinical practice, it is important to develop guidelines that support cultural sensitivity and open communication with patients. Furthermore, including nurses as practitioners in health regulations regarding cupping therapy practices will make significant contributions to ensuring both safe and evidence-based holistic care.

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Chapter 17

AMYGDALIN AND CANCER FROM THE CONTEXT OF NATURAL COMPOUNDS

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INTRODUCTION

Cancer is an intricate, multifaceted and heterogeneous collection of diseases characterized by unregulated growth of cells that invade nearby tissues and spread to other parts of the body (metastasis). Genomic changes, epigenetic aberrations, deregulation of cell cycle and checkpoints, capacities to escape immune response as well as abilities for neoangiogenesis and remodelling of the microenvironment contribute in association with one another to cancer onset and evolution. Despite considerable advances in the early diagnosis and therapeutic strategy, cancer is still the second leading cause of death globally following cardiovascular diseases, and its global burden is exacerbated by demographic shifts. The importance of this aspect is paramount and it highlights the pressing demand for new, efficient and safe therapeutic approaches to be used in conjunction with currently available therapies.

Traditional cancer treatments such as chemotherapy, radiotherapy and targeted therapies are the cornerstones of contemporary clinical practice, yet their limited effectiveness and serious side effects along with development of resistance to them have led scientists to explore alternatives. In this scenario, phytochemicals have been the subject of much interest in the last decades for their antioxidant, anti-inflammatory and antiproliferative effects. Phytochemicals, including polyphenols, flavonoids and phenolic acids, have also been shown to employ various anticancer activities such as induction of apoptosis, cell cycle arrest regulation, angiogenesis inhibition and immune modulatory responses via genetic and epigenetic pathways.

Among these bioactive natural compounds, amygdalin is a cyanogenic glycoside which has been used for hundreds of years in traditional and modern medicine systems to treat different diseases; it mainly occurs in seeds or kernels of plants belonging to the family Rosaceae. Despite the controversy over its use as a cancer treatment in previous decades, increasing evidence from in vitro and in vivo studies suggested that apricot kernel amygdalin showed anti-proliferative, apoptotic, and autophagic effects and influences the pathways involved with oxidative stress and inflammation on cancers of various origins. Natural sources of amygdalin, its tumor apoptotic effects in mouse tumors and anticancer effect in humans are described and critically analyzed in the light of the results prevailing at present experimental and clinical findings.

1. Carcinogenesis

Cancer is a collective term for an extremely varied group of diseases resulting from unregulated proliferation of cells, their capacity to invade

local tissues and spread by the process of metastasis to distant parts of the body (Brown et al., 2023). The development and progression of cancer are the outcome of various biological processes that act through genetic changes, abnormal epigenetic regulation, disruption in cell cycle control, immunological escape mechanisms, angiogenesis mechanisms and incessant migration/interaction within a tumor microenvironment (Vlierberghe et al., 2024). Carcinogenesis is an incremental process in which genetic and/or epigenetic alterations in otherwise normal cells accumulate over time. This transformation is characterized by conversion of proto-oncogenes into oncogenes or functional inactivation of tumor suppressor genes, which causes the complete loss regulation of cellular homeostasis (Bayramova et al., 2024; Vlierberghe, 2024). In particular, mutation and inappropriate expression of major regulatory genes such as TP53, PTEN, APC, KRAS have been well associated with elevated proliferative potential and genomic instability (Yan et al., 2024). In addition to cytotoxic damage, the levels of chromatin reorganization and gene expression landscapes are substantially altered by epigenetic regulatory processes (DNA methylation patterns, histone modifications and non-coding RNA activity). These epigenetic changes are related not only to tumor initiation but also to tumor progression, metastasis and the development of resistance against anticancer therapies (Capp et al., 2025). An additional characteristic of malignant cells is their ability to evade immune surveillance. Under homeostatic conditions, the immune system guards against abnormal cells through multiple mechanisms, however most cancers will develop ways to escape immune detection or destruction; frequently by modifying immunosurveillance checkpoint molecules (including PD1 ligand, PD-L1) orchestrating the pathway of antigen presentation or by promoting a pro-tumourigenic inflamed microenvironment mediated by cytokines such as IL-10 and TGF-b (Ghorani et al., 2023). These adaptive maneuvers enable the continued survival of a tumor and are closely related with treatment resistance and recurrence of disease. Tumor growth and metastasis are also angiogenesis-dependent, because growing cancer cell population in a tumor mass cannot get their metabolic requirments met without newly formed vascular network. This mechanism is mainly controlled by growth factors and, especially, VEGF that is produced by the cancer cells as well as stromal constituents in the tumor microenvironment (Vlierberghe, 2024). Together, changes in cell–cell and cell–matrix crosstalk as well as enzymatic remodeling of the extracellular matrix and reprogramming of intracellular signaling cascades cooperate to increase invasiveness and metastatic capacity (Biray Avci et al., 2024). From an epidemiologic perspective, cancer remains a substantial global health burden. In 2022, over 19.96 million new cancer cases and nearly 9.74 million cancer-related deaths were recorded globally, making cancer the second major cause of death following cardiovascular diseases (Zhang et al., 2024).

The situation is anticipated to worsen, given the ongoing demographic transitions especially population aging and urbanization that further increase such burden, as the estimated global cancer incidence will press on almost 35 million new cases per year by 2050. In 2022, the most frequent cancers diagnosed were lung, breast, colorectal, prostate and stomach cancer with around 2.48 million, 2.29 million, 1.93 million,1.46 million and 0.97 million cases (Bizuayehu et al., 2024). These cancers also cause the most cancer-associated deaths globally (Zhang et al., 2024). Of interest, there are large disparities in cancer and its outcomes across the globe: Early diagnosis and availability of more advanced treatment options typically favor high-income nations with regard to higher detection rates (and proportionally fewer deaths), while low and middle-income regions experience relatively double the mortality rate due to lack of available healthcare infrastructure and screening programs as well as heavier burden of infection-related cancers (Zhang et al., 2024).

From the standpoint of cancer prevention and therapy, natural compounds have received increasing scientific interest in addition to established therapeutic strategies including chemotherapy and radiotherapy. Both experimental and clinical researches further demonstrate that many natural plant bioactive components, such as polyphenols, flavonoids and phenolic acids contain significant biological properties which may directly contribute to cancer prevention or treatment. These compounds are particularly attractive because of their ability to inhibit tumor cell proliferation, accompanied by antioxidant and anti-inflammatory activity. Increased literature has demonstrated that natural compound-induced the cross-talking of genetic and epigenetic regulation they affected a series pathway about carcinogenesis. By these means, they can lead to apoptosis, cell cycle arrest, altered angiogenesis and altered immune responses in the tumor microenvironment. Among these bioactive natural compounds, amygdalin has gained attention in recent years due to its anticancer effects in various experimental models. Therefore, rational ASC-based strategies to prevent and treat cancer are needed, and a comprehensive understanding of the molecular mechanisms by which natural compounds exert their biologic effects is essential in this regard. Such observations might not solely contribute to the identification of additional anticancer drugs, but may also help in the optimization of adjunctive measures as part of integrated cancer care.

2. Natural Sources of Amygdalin

Amygdalin (d(-)-mandelonitrile-B-D-gentiobioside) is one of the most prevalent cyanogenic di-glucosides and in nature it is usually obtained from the plants belonging to Prunus species of Rosaceae family. It occurs in fruit stones e.g. apricot, almond, cherry, apple, plum, pear or peach (Barceloux,

2009). Amygdalin was extracted from bitter almonds in 1830 by French biochemists Pierre-Jean Robiquet and Antoine Boutron-Charlard (Wahab et al., 2015).

3. Uses of Amygdalin in Medicine

Amygdalin is a natural cyanogenic glycoside, which is present in many plant materials including bitter almonds, apricot kernels and peach kernels. Amygdalin preparations have been used for millennia in different parts of the planet and within ancient medical practices, such as those of Egypt, China, and India to treat diverse diseases (Song et al., 2014).

Amygdalin was previously reported to be an active ingredient in alternative/ complementary medicine and has been utilized for a variety of diseases such as anemia, asthma, hypertension, atherosclerosis, diabetes, migraine and tumors (Yan et al., 2006; Wang et al., 2015; Chen et al., 2016). Development of Laetrile, a semisynthetic compound related to amygdalin, was initiated in the U.S. and patented in the 1950s by Ernst T. Krebs as an anticancer agent (Greenberg et al., 1975). Old records suggest that the use of amygdalin as an anticancer agent was established in Russia as far back as 1845 (Moss et al. 1996). However in 1953 studies initiated by the California Medical Association determined that no sound scientific evidence supported amygdalin's claim to having anti-cancer properties. These discoveries led to the outlawing of amygdalin in California in 1959. Notwithstanding its prohibition and the restrictions published by various regulatory authorities in many countries, such as US Food and Drug Administration, amygdalin is still used within alternative medicine (Greenberg et al., 1975; Blaheta et al., 2016).

Amygdalin gained attention in the field of alternative medicine in the 1970s and it has been estimated that approximately seventy thousand cancer patients in the United States were treated with amygdalin by 1980 (Kolesár et al., 2015; Moss et al., 2005). It has been shown in the experimental study that amygdalin can induce apoptosis of human cancer prostatic cells, like DU145 and LNCaP cell lines (Chang et al., 2006). In addition, Park et al. in Center reported amygdalin to have anticancer activity via modulating DNA damage which is associated with cell cycle in human colon cancer cells, implying that it may be a potential candidate of the antitumor agent (Park et al., 2005). In agreement with these results, Makarevic et al. demonstrated that amygdalin elicited significant antitumor activity against prostate cancer cells and the potential for its application clinically warranted to be confirmed through elaborate experimental and clinical research (Makarevic et al., 2016).

4. Antitumor Potential of Amygdalin

Potential Role of Amygdalin in Nervous System Tumors: An aqueous extract from apricot kernel exhibited the ability to induced apoptotic neuronal cell death in rat N2a neuroblastoma cells. Consistent with this, treatment of the extract led to expression increase in the pro-apoptotic protein Bax and inhibition on next caspase-3 enzyme activity, together with a decrease in anti-apoptotic protein Bcl-2 (Kim et al., 2005). Consequently, these results indicated that bioactive agents isolated from apricot kernels could be inducing the endogenous apoptosis pathways in cells (Mazzio et al., 2010). The antiproliferative effect of apricot pulp treated mechanically (freeze, can and dry) has also been assayed in rat C6 glioma cells as an experimental model. The results showed a significant, dose-dependent inhibition of cell proliferation with the greatest inhibitory effect elicited by methanolic extract. Of the processing methods investigated, canned apricot pulp was most inhibitory to cell growth, followed by dried and frozen pulp. It has been suggested that the canning process may favor the release or increased accessibility of bioactive compounds involved in antiproliferative activities. Rather, drying and freezing seemed to be having less potential impact on the overall phytochemical composition but their partial degradation of carotenoids and phenolic compounds might have generated new antiproliferative agents (Wani et al., 2020).

Potential Role of Amygdalin in Hepatocellular Tumors: Aqueous methanolic extract of apricot kernel and its amygdalin-enriched fraction have been demonstrated to exert potent anticancer actions via various mechanisms such as apoptosis and autophagy induction, cell proliferation suppression, increase in oxidative defense systems antioxidant enzymes and decrease in expression of pro-inflammatory cytokine TNF-α together with the bureau formation VEGF. The Aqueous-methanolic extract and amygdalin-containing fractions of the P. amarus Lower concentration susceptible to have ability the cytotoxic effect toward HepG2 Cancer Cells After 24 h (25.26μg/mL), while, after 48 hours at a concentration of Lower (6.20μg/mL) were observed in (Chen et al., 2020). Both aqueous-methanolic extract and amygdalin-rich fraction exhibited cytotoxic activity as proved by the trypan blue exclusion test towards Ehrlich ascites carcinoma (EAC) cells with IC50 of 20.2 μg/ml. The results showed partial purification of cytotoxic molecules. A hydroethanolic extract of apricot kernels (with octasiloxane-hexadecamethyl as a major constituent) also had cytotoxic activity in HepG2 cells at 22.8 μg/mL.

The in vivo antitumor activity of the extract was also studied on EAC cells bearing mice that received 100 mg/kg for six days exhibited a significant tumor volume and cell count reduction compared to control mice. Furthermore, administration of the extract mediated improvements

in liver and kidney functions, as indicated by changes in enzymatic markers such as AST, ALT, urea, creatinine, malondialdehyde (MDA) level and SOD/CAT activities (Sireesha et al., 2019). Correspondingly, aqueous, methanolic and ethanolic extracts of apricot kernels were proved to have significant inhibitory effects on the proliferation of hepatocellular carcinoma cells in a concentration-dependent manner with IC50 values 17.5 μg/ml, 19.2 μg/ml and 14.5 μg/ml respectively (Gomaa, 2013).

Extract from bitter Bulgarian apricot kernels, 20% ethanol-based, comprising amygdalin, deidaklin, linamarin and prulaurasin (cyanogenic glycosides), exerted antigenotoxic, antirecombinogenic, antimutagenic and anticarcinogenic activities in yeast test-systems. Another extract also exerted a significant antiproliferative influence on HepG2 cells at 2.5 and 5 μg/mL (Dimitrov et al., 2021). Moreover, the antiproliferative activity of extracts of kernels from several varieties (19 different) of apricot against HepG2 cells was evaluated and Waflu chuli showed the highest antiproliferative activity in HepG2 cells (Chen et al., 2020). In addition, an ethanolic extract of apricot pulp exhibited strong cytotoxic activity with 91.9% inhibition of Hep3B human hepatocellular carcinoma cells at a concentration of 4 mg/mL (Yoo et al., 2007).

Apricot kernel extract and its amygdalin fraction also expressed an in vivo antitumor activity on a DMBA-induced carcinogenesis model in mice. The effects were explained by a reduction in lipid peroxidation and an improvement of antioxidant defence mechanisms, which was demonstrated by the regulation of superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH) and malondialdehyde (MDA). The anti-cancer activity of the ADRF was proposed to be mediated through the hydrolysis of amygdalin into hydrogen cyanide (HCN) within tumour cells and accumulation of reactive oxygen species-triggered apoptotic cell death. Conversely, treatment also upregulated caspase-3 and Beclin-1 mRNA levels with concurrent inhibition of Bcl2 gene expression (Hosny et al., 2021).

More recently, the effect of dietary apricot supplementation was found to be protective against radiation and 7,12-dimethylbenz[a]anthracene (DMBA) induced oxidative stress in rat liver. In DMBA plus radiotherapy-exposed animals, treatment with 20% APR resulted in lowering of ALT, AST, 5′-nucleotidase, MDA and nitric oxide levels and expression of Bcl-2, AP-1, cAMP response element–binding protein, NF-κB as well as increase in caspase-3 activity and Bax expression GT levels. Histopathological studies also demonstrated that DMBA treatment considerably increased the mitotic activity, pericentral necrosis and cellular pleomorphism, which were partially recovered by apricot supplementation and/or radiotherapy (Karabulut et al., 2014).

It has also been reported that, ethanolic extracts of apricot kernel (70% and 99.9%) have been similarly found to possess both preventive and therapeutic effects in rats subjected to N-nitrosodiethylamine-induced hepatocellular carcinogenesis. Histopathological examination supports that administration of these extracts (labdane diterpenoids) at a dose of 200 mg/mL for eight weeks caused notable suppression in AST, ALT, ALP, total bilirubin and direct bilirubin TSF content, and an increase in albumin/total protein ratio and alpha-fetoprotein expressions as well as MDA levels accompanied by enhancing the reduced glutathion in liver tissue. Similar results were noticed in animals treated with amygdalin and silymarin at 50 mg/kg (Ramadan et al., 2020). Finally, a strong inhibition of tumor growth with both raw and heat-treated apricot kernels was reported in EAC cell transplanted mice with heat-treated kernels further extending the survival time of animals when compared to untreated controls (Yamshanov et al., 2016).

Potential Role of Amygdalin in Colon Tumors: Apricot kernel aqueous, methanol and ethanol extracts have been reported to cause the inhibition of cell proliferation in HCT-116 human colon cancer cells by a concentration-dependent manner. The IC_{50} of the crude extract was 33.6 and 36.3 μg/mL (Gomaa et al., 2013). On the other hand, growth of HCT-116 cells treated with 100 μg/mL fermented methanolic A kernelextract increased; cell proliferation rates ranging from 79.0 ± 1.5% reached to 90.6 ± 4.6% (Sohn et al., 2010). The effect of 80% ethanolic extracts of apricot kernels from South Africa and China on cell proliferation, apoptosis and cell cycle regulation in HT-29 colon cancer cells is also described. At 24 hours, the South African extract showed a biphasic behavior that support cell proliferation in low (100 mg/ml) and higher dosages (1000 mg/ml), but it was inhibitory at an intermediate dosage (500 mg/ml). Nevertheless, extracts of Chinese apricot kernels constantly inhibited cell proliferation in a dose-dependent manner after 24 and 48 h incubation. In addition, morphological changes were observed in 24-h Chinese seed extracts-treated cells and those treated with South African extract at 1000 μg/mL for 72 h including irregularly shaped cell and cellular shrinkage (Cassiem et al., 2019). A fruit-based beverage including apricots, oranges and grapes subjected to in vitro digestion by gastric and duodenal juices, was found to possess powerful antiproliferative effect against human colon cancer cells (CaCO-2). Resistant cells were arrested at the S-phase of cell cycle upon prolonged incubation with reduced cyclin B1 and cyclin D1 expression levels (Cilla et al., 2009). In another study, zinc and milk supplemented digested beverage resulted in 35% and 29% inhibition of Caco-2 and HT-29 cell proliferation after a 24 h exposure. This suppression was accompanied by an increase in the percentage of S-phase cells and decrease in those in G0/G1 phase, with no remarkable change at G2/M phase comparing to vehicle treated cells (Cilla et al., 2010).

In yeast test systems, as most cytotoxic active extract 20% ethanol on apricot kernels of Bulgarian origin containing cyanogenic glycosides-amygdalin, deidaklin, linamarin and prulaurasin previously demonstrated for antigenotoxic, antirecombinogenic antimutagenic and anticarcinogenic properties against HT-29 cells. Cells decreased viability to 32% and 41% at concentrations of 2.5 μg/mL and 5 μg/mL, respectively, and mild antiproliferative activity was detected at a concentration of 5 μg/mL (Dimitrov et al.,2021). Furthermore, apricot extracts have also been proposed to inhibit p-glycoprotein mediated transport in caco-2 cells, modulating drug efflux activity (Deferme et al., 2002).

Diet driven studies have additionally shown that 20% sun-dried or sulfur fume-treated apricots in the diet significantly decreases oxidative stress and telomerase activity, in rats with azoxymethane-induced colon carcinogenesis. Telomerase activity which is vital to end maintenance and cellular immortality was significantly reduced and dropped from 54.25 to 23.54 RTA/g protein in SF-treated group; and 3.42 RTA/g protein for sun-dried apricot group compared with control animals. Although the sun-dried apricots had a higher inhibitory effect on telomerase activity, SO2 fumes application apricots exerted greater antioxidant capacity as demonstrated by higher levels of glutathione and lower nitric oxide and malondialdehyde content (Karabulut et al., 2014).

Potential Role of Amygdalin in Pancreatic Tumors: Ethanolic extracts both from bitter and sweet almonds and pure amygdalin were also demonstrated to inhibit cell proliferation of human pancreatic cancer PANC-1 cells in a time- and dosedependent manner. By contrast, these treatments did not have an appreciable cytotoxic activity on untransformed 293/KDR epithelial cells suggesting some degree of selectivity for cancer cells. Interestingly, bitter apricot kernel extract displayed a stronger antiproliferative activity than that prepared from sweet kernels.

Characteristic apoptotic features of chromatin condensation and nuclear fragmentation were seen in treated PANC-1 cells by DAPI stain for the nucleus. Concomitantly, flow cytometric analysis revealed a significant elevation in the percentage of early and late apoptotic cell populations. Apoptotic induction was also verified at the molecular level as a significant increase in Bax and caspase-3 mRNA levels with a concomitant decrease in anti-apoptotic gene Bcl-2 expression by real-time PCR. Therefore, as a group, the present results suggest that apricot kernels and amygdalin induce apoptosis of pancreatic cancer cells by a mitochondria-dependent mechanism (Aamazadeh et al., 2020; Aamazadeh et al., 2021).

Potential Role of Amygdalin in Breast Tumors: A compound extracted from apricot was found to significantly suppress cell growth on human breast cancer cell lines (MCF-7, HDF and MDA-MB-231) in a concentration dependent manner and with incubation periods up to 24, 48 and 72 h. Moreover, time-related decreased in Bax and c-FLIP promoter gene expressions were detected in the total RNA extracted of MCF-7 and MDA-MB-231 cells treated by extract (Mahmoudi et al., 2019). Similarly, apricot kernel aqueous, methanic and ethanoic extracts formulations were proven to inhibit cell growth in MCF-7 cells dose-dependently (Gomaa et al., 2013). Likewise, hydroethanolic apricot kernel extract has manifested potent antiproliferative activity against MCF-7 cells which was found to be an octasiloxane-hexadecamethyl from GC–MS analysis of this extract (Sireesha et al., 2019).

Other studies have reported that the breast cancer cell lines MCF-7, MDA-MB-231 and T47D are also sensitive to aqueous, ethyl acetate and hydromethanolic extracts isolated from apricot kernels. The hydromethanolic extract was particularly active in all cell lines, followed by the acetone and methanol extracts. This extract was found to increase the percentage of cells in G0/G1 and decrease the number of cells at G2/M phase. Induction of apoptosis was accompanied by increase in the pro-apoptotic proteins such as Bax and caspase-3, levels coupled with decrease in anti-apoptotic one Bcl-2 (Mosadegh Manshadi et al., 2021). Soltani et al., in a research on for hydroethanolic extracts from apricot kernels of four Iranian cultivates including Jahangiri, Palmia, Jafari and N585 confirmed inhibitory function of the extractives toward MCF-7 cell proliferation. The extracts were examined at concentrations of 25, 100, 400 and 1200 μg/mL with the most noticeable inhibitory effect being observed in N585 type at the concentration of 1200 μg/ml after 24 and 72 hours exposure (Soltani et al.,2021). Similarly an ethanolic extract of apricot pulp, was also observed to produce 72.8% cytotoxicity on MCF-7cells at a dose of 4 mg/ml (Yoo et al., 2007).

The cytotoxic potential of a polyphenol-rich mixture of fruits including apricot, peach, aronia, raspberry, wild strawberry and blueberry and cranberry has been also studied in T47D human breast ductal carcinoma cells, MCF7 breast adenocarcinoma cells and normal breast epithelial cell line (MCF-12A). They showed a dose-dependent cytotoxicity against MCF-7 and T47D cancer cells. Cytopathogenic alterations of the three cell lines were observed microscopically; the preparation exerted less cytotoxicity to cancer cells than to normal cells. This effect was hypothesized to be associated with fruit polyphenol-estrogen receptor interactions which may result in changes in paracrine growth factor generation (Sołtys et al., 2021).

Apricot leaves derived ethyl acetate extract have been also shown to reduce cell viability, increase apoptosis and reactive oxygen species levels in MCF-7 cells. This extract was demonstrated to induce bax, suppress bcl-2 expression and decrease the levels of cdk4, cyclin E and cyclin D1; additionally caspase-3 activity was elevated (Kitic et al., 2022). Salarbashi et al. also investigated in other research. Compared cytotoxicity between curcumin loaded with apricot gum extract and free curcumin. Both unencapsulated and encapsulated formulations resulted in cytotoxicity concentration-dependently against 4T1 breast cancer cells, though the latter showed higher antitumor activity by a possible synergistic effect with apricot gum extract (Salarbashi et al., 2021).

Potential Role of Amygdalin in Lung Tumors: An ethanolic extract from apricot pulp was found to exhibit a potent cytotoxic effect on A549 human lung carcinoma cells, causing about 88.2% of death at a concentration of 4 mg/mL (Yoo et al., 2007). Furthermore, Fei-Liu-Ping (FLP), an oral preparation in traditional Chinese medicine for lung cancer treatment and contains several medicinal herbs including apricot has been reported to suppress A549 cell growth. This effect has been linked to the modulation of NF-κB signaling pathways, epithelial-mesenchymal transition markers such as E-cadherin and N-cadherin and MMP2/MMP9 expression.

In vivo, FLP treatment in Lewis lung derived xenografts mice models inhibited the growth of these tumors by ~40%, and combined with cyclophosphamide to 83.23%. This anti-tumor effect was associated with reduced serum Pro-inflammatory cytokine IL-6, TNF-alpha and IL-1 beta. Simultaneously, E-cadherin was increased, N-cadherin was inhibited and MMP-9 expression became partially normalized that indicated the inhibition in process of tumor invasion and metastasis (Li et al., 2014).

Recently, a clinical study demonstrated that Bufei Huayu, a traditional Chinese herbal decoction comprising bitter apricot kernels among other herbs exhibited an excellent clinical efficacy when combined with gefitinib to treat advanced non-small cell lung cancer. Such a dual therapy proved to be well tolerated by patients, while improved the response rate and diminished the thrombosis danger. Most importantly, the herbal preparation was also observed to substantially suppress specific side effects associated with gefitinib treatment such as skin rash and increased serum alanine aminotransferase (ALT) level (Yuan et al., 2020).

Potential Role of Amygdalin in Leukemia: The observed results demonstrated that the aqueous, ethyl acetate and hydromethanolic extracts derived from apricot kernels presented significant antiproliferation against NALM-6 and KG-1 acute leukemia cell lines without showing cytotoxicity to normal control cells. Of all the preparations, the ethyl acetate fraction with

approximately 0.67% amygdalin exhibited the most potent antiproliferative activity. Meanwhile, a notable increase in the gene expression of caspase-3 was detected in NALM-6 and KG-1 cells pointing towards the activation of apoptotic pathways (Manshadi et al., 2019).

Moreover, one in vivo study showed that mice treated with apricot kernel (at doses of 2 g/day for 1 month before and two days after transplantation with LYO-1 lymphosarcoma cells) had significantly less tumor growth compared to untreated control animals. These observations may indicate a possible protective and antitumor effect of the consumption of apricot kernel in hematological malignancy models (Lee et al., 2007).

Potential Role of Amygdalin in Skin Tumors: We have reported that essential oil from apricot estimated to stifle growth of HaCaT cells. Mechanisms of action studies revealed that exposure of cells to this oil caused G0/G1 phase cell cycle arrest which was easyly accompained by increased early and late apoptotic populations. Key apoptotic markers, such as caspase-3, 8 and 9, Bax and poly(ADP-ribose) polymerase (PARP), were also found to be activated. At the same time there was significant decrease in levels of anti-apoptotic Bcl-2 and Rel/NF-κB. These results imply that the apoptosis induced is death receptor-dependent, mitochondrial signal-based, and NF-κB regulatory (Li et al., 2016).

Subsequently, a 95% ethanolic extract of apricot fruit has been shown to strongly inhibit activation of Epstein–Barr virus early antigen (EBV-EA) by 12-O-tetradecanoylphorbol-13-acetate under in vitro conditions. Suppression of skin carcinogenesis was strongly correlated with this inhibitory activity in in vivo mouse models, suggesting that apricot constituents may play a cancer preventive role (Kapadia et al., 1997).

Potential Role of Amygdalin in Oral Tumors: An apricot kernel extract was previously found to exert significant cytotoxic action against mouth cancer cell lines, with the maximum inhibitory activity estimated around 82% at 100 μg/mL (Sireesha et al., 2019). These data suggest that anti-tumoral compounds present in the apricot kernels could effectively be used as inhibitors for cellular proliferation of oral cancer. In addition, in an another study extracts made from apricot with carrot and taro was found to effectively suppress the generation of 8-OH-dG, a biomarker involved in oxidative DNA damage such as that derived from lipid peroxidation among in vitro condition. Of the tested extracts and fractions, chlorogenic acid-rich samples showed more potent inhibition of 8-OH-dG formation. The same study also showed that these extracts were capable of inhibiting the generation of 8-OH-dG in rat tongue tissue treated with 4-nitroquinoline-1-oxide during carcinogenesis, indicating its protective effect against oral cancer development (Kasai et al.,

2000).

Potential Role of Amygdalin in Other Tumors: Apricot kernel extract and its solvent-partitioned fractions hexane, ethyl acetate, and aqueous were found to inhibit 12-O-tetradecanoylphorbol-13-acetate–induced ornithine decarboxylase activity in T24 human bladder carcinoma cells suggesting an inhibitory effect on tumor promotion-related enzyme activity (Kim et al., 1996). Furthermore, an aqueous extract of bitter apricot kernel has been reported to induce apoptosis in DU145 human prostate cancer cells. This pro-apoptotic effect was accompanied by increased caspase-3 activity, upregulation of the pro-apoptotic protein Bax, and downregulation of the anti-apoptotic protein Bcl-2 (Lee et al., 2016). Similarly, an ethanolic extract obtained from apricot pulp has exhibited strong cytotoxic effects on human cervical adenocarcinoma (HeLa) cells with 89.4% cell viability loss at 4 mg/mL concentration (Yoo et al., 2007). In addition, the study of Salarbashi et al. evaluated the anticancer effect of curcumin packaged in apricot gum extract and free curcumin. Results showed that curcumin aerodynamic spray-dried nanocomposite nanoparticles significantly inhibited proliferation of A2780 human ovarian cancer cells in a dose-dependent manner and had superior antiproliferative activity compared to free curcumin, which could be explained by enhanced solubility, stability or synergism between the curcumin and apricot gum extract (Salarbashi et al., 2021).

CONCLUSION

Cancer is a diverse and complex collection of diseases resulting from the crosstalk between genetic and epigenetic aberrations that accumulate during carcinogenesis, microenvironmental influences, immune surveillance processes, and cellular signaling networks. Because of this heterogeneity, the therapeutic or preventive approaches directed toward a unique molecular target are rarely effective, emphasizing the importance of broader and more integrative strategies. In this context, natural products extracted from plants have received increasing interest as an adjuvant of the traditional therapies such as chemotherapy and radiotherapy. Some bioactive molecules polyphenols, flavonoids and phenolic acids have been shown to influence key anticancer mechanisms such as restriction of cell proliferation, induction of apoptotic cell death, attenuation of the cell cycle, inhibition of angiogenesis and regulation of immune responses. The realization that a number of these effects are orchestrated by genetic and epigenetic regulatory networks highlights the significance of natural compounds in cancer biology.

Of these, amygdalin has drawn attention continuously because its biological activity in experimental cancer systems have been catalogued. Preclinical studies indicate that amygdalin might affect mitochondrial

apoptosis signaling, caspase activation pathways, and cell cycle control mechanisms. However, the knowledge about its anticancer activity, selectivity and safety is based far more upon in vitro and in vivo experimental studies than clinical interpretation should show. As a result, subsequent studies should focus on more rigorous characterization of the molecular targets of amygdalin and also natural compounds. Particular attention will need to be dedicated to a thorough assessment of their implication in all aspects of carcinogenesis, using integrative multi-omics analyses including genomic and transcriptomic as well as proteomic and epigenomic datasets. Identifying selective responsiveness to amygdalin in diverse cancer types and within specific subpopulations of cells would provide better precision toward potential intent for therapy.

Furthermore, combining it with other strategies such as nano-formulations to increase bioavailability, targeted drug delivery carriers and rational combination therapies could significantly improve its therapeutic outcome. Different combinations might yield synergistic interactions with known chemotherapeutics or immunotherapies to enhance treatment response and limit toxic side effects. However, well-designed randomised controlled clinical trials are needed to define the safety and efficacy of amygdalin in human beings. Such evidence will be essential for the science-based and evidence-based incorporation of naturopathic compounds into the oncology clinic. In this regard, amygdalin and other botanicals offer potential candidates for integrative and personalized cancer care regimens.

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