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gece kitaplığı

International Research and Evaluations in the Field of Health Sciences

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INTERNATIONAL RESEARCH AND EVALUATIONS IN THE FIELD OF HEALTH SCIENCES

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Chapter 1

LC-MS/MS METHODS FOR THE DETERMINATION OF PACLITAXEL IN BIOLOGICAL FLUIDS: A REVIEW*

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INTRODUCTION

Oncology stands out as a dynamic field of science, driven by continuous development and innovative research leading to advances in treatment methods. In this context, paclitaxel is a chemotherapy drug that is effectively used in the treatment of a wide variety of cancer types. The drug's areas of application include breast, ovarian, bladder, lung, prostate, melanoma, oesophageal cancers, Kaposi's sarcoma, and various other solid tumours. This review details paclitaxel's therapeutic indications, its mechanism of action at the cellular level, its role in suppressing tumour cell proliferation, as well as dosage adjustments, infusion protocols, routes of administration, and possible contraindications; it also emphasises the clinical importance of monitoring patients undergoing treatment. Paclitaxel, initially known as "taxol" when it was first discovered, is a natural diterpenoid compound derived from the Pacific yew tree (Taxus brevifolia). The drug's discovery was the result of natural product screening programmes conducted by the National Cancer Institute (NCI) in the United States in the late 1960s and early 1970s. During this process, researchers examined various plant extracts in an effort to identify compounds with anticancer potential, and paclitaxel's pronounced cytotoxic effects attracted attention. In particular, its capacity to halt the cell cycle and induce apoptosis by inhibiting microtubule depolymerisation has made paclitaxel a priority agent in anticancer research (1).

Liquid chromatography tandem spectrometry (LC-MS/MS) a technique combining liquid chromatography with tandem mass spectrometry, is an effective analytical method that integrates chromatographic systems providing high resolution in the separation of analytes with mass spectrometric detection offering high selectivity and sensitivity. Thanks to its mass spectrometry component in particular, this technique offers advanced sensitivity and separation capacity, thereby gaining significant momentum in analytical applications. Today, it is recognised as one of the most effective and reliable techniques in the field of pharmaceutical analysis (2).

In this review, the pharmacological properties of paclitaxel are examined, and LC-MS/MS analyses of paclitaxel in biological fluids are provided.

*GENERAL SECTIONS

1. Taxane Class Compounds and Paclitaxel

Taxanes constitute a group of chemotherapeutic agents widely employed in cancer management. Chemotherapy involves the systemic administration of cytotoxic drugs designed to destroy malignant cells and suppress tumour proliferation. Each category of chemotherapeutic agents acts through distinct biological mechanisms to accomplish this goal. Taxanes exhibit their antineoplastic properties by interfering with the cellular machinery responsible for cell division and replication in cancer cells. Since uncontrolled proliferation is essential for tumour expansion, the inhibition of this process by taxanes effectively contributes to limiting cancer progression. These compounds specifically interrupt mitosis — the stage of the cell cycle in which cells divide — and therefore are classified as mitotic inhibitors. Taxanes are naturally sourced from yew trees. Paclitaxel, the first compound identified within this class, was originally isolated from the bark of the Pacific yew (Taxus brevifolia). As a plant-derived anticancer drug, paclitaxel represents one of the two major plant-based chemotherapy classes, the other being the vinca alkaloids, which are obtained from periwinkle plants. (3).

Paclitaxel is an anticancer agent with mitotic inhibitor activity and hydrophobic properties that inhibits tumour growth by stopping cell division. This compound, together with docetaxel, belongs to a group of cytotoxic diterpene compounds derived from yew trees called taxanes. It is particularly noteworthy for its high efficacy against certain malignant tumour types known to be resistant to conventional chemotherapy agents. Paclitaxel was first identified in 1962 as part of the National Cancer Institute's research into naturally occurring anticancer compounds. During these studies, thousands of plant extracts were screened to identify substances with therapeutic potential, and it was determined that the crude extract obtained from the bark of the slow-growing, rare Pacific yew tree (Taxus brevifolia) exhibited significant antitumour activity in various tumour models (4).

2. Therapeutic Indications of Taxane Class Compounds

In ovarian cancer treatment, paclitaxel is used in combination with a platinum-derived agent as first-line therapy in advanced or metastatic cases; it is also recommended as a second- line treatment option in the same patient group. In breast cancer cases, it is included in treatment following anthracycline and cyclophosphamide administration for adjuvant thera-

py in early-stage, node-positive patients. In metastatic or advanced breast cancer cases, it is combined with anthracycline if its use is appropriate; if not appropriate, it can be used alone or in combination with trastuzumab in patients with immunohistochemically detected HER2 positivity. It is used as second-line therapy in cases where combination chemotherapy has failed. Nevertheless, in the absence of clinical contraindications, anthracyclines are generally incorporated into first-line chemotherapy protocols. For patients with non-small cell lung cancer (NSCLC) who are unsuitable for surgical intervention or radiotherapy, paclitaxel is typically administered as part of a first-line regimen in combination with a platinum-based compound. Moreover, in the management of AIDS-related Kaposi's sarcoma, paclitaxel serves as an effective chemotherapeutic option, particularly when used as a second-line treatment. (5).

3. Side Effects of Taxanes

Taxanes, like other chemotherapy drugs, target cancer cells with rapid division characteristics, inhibiting their proliferation and thus exhibiting anticancer effects. However, they can also damage rapidly dividing healthy cells and cause side effects. The group of rapidly dividing cells includes skin cells, hair follicle cells, epithelial cells in the gastrointestinal system, and haematopoietic blood cells. The interaction of taxanes with rapidly dividing cells during chemotherapy often results in the development of certain adverse effects. Among these, neutropenia is one of the most frequently observed toxicities, characterized by a reduction in white blood cell count that increases the patient's vulnerability to infections. Peripheral neuropathy is another notable complication, manifesting as a "glove-and-stocking" pattern of numbness, tingling, or weakness in the hands and feet. Additional adverse reactions commonly associated with taxane-based regimens include myalgia, arthralgia, injection-site rash, nausea, vomiting, fluctuations in blood pressure, thrombocytopenia, anaemia, and alopecia. The intensity and incidence of these side effects can vary depending on the specific taxane used and the dosage administered. (3).

4. FDA-Proven Effect of Taxanes

Paclitaxel was approved by the FDA as a chemotherapeutic agent for the treatment of ovarian cancer in December 1992. In order to meet the growing demand for the drug, a research group led by Robert Holton developed a method for the semi-synthetic production of paclitaxel. In sub-

sequent studies, the drug's efficacy against metastatic breast cancer was evaluated, and clinical trials yielded positive results. Following these findings, in 1994, the FDA also approved paclitaxel for use in the treatment of breast cancer. Paclitaxel is used effectively in combination therapy (6).

5. Chemical Formula and Molecular Information of Paclitaxel

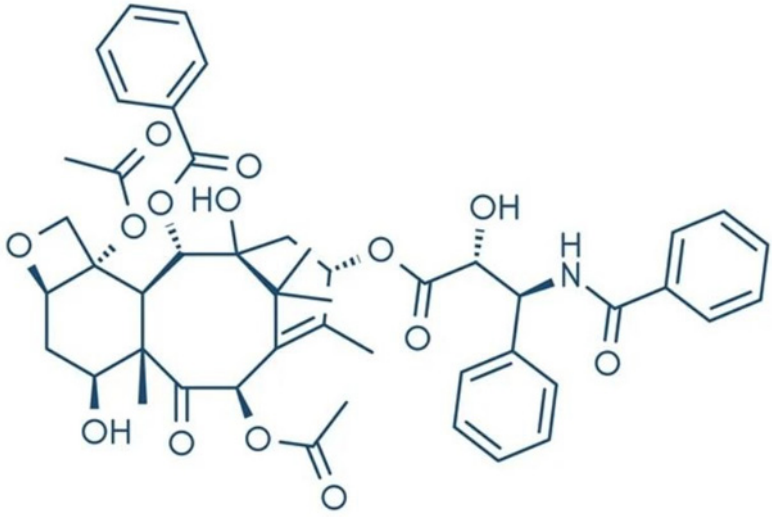

Figure 1. *Chemical formula of paclitaxel (4)*

Molecular formula: C47H51NO14, molecular weight: 853.9 g/mol, melting point: 216–217 °C, solubility: 5.56×10^{-3} g/L (7).

6. Pharmacological Aspects of Paclitaxel

Paclitaxel possesses a narrow therapeutic margin and exhibits strong lipophilic properties, rendering it almost insoluble in aqueous environments. Currently, cancer chemotherapy with paclitaxel often causes hypersensitivity reactions. One of the main challenges in achieving clinical success with paclitaxel is the availability of the active ingredient and limitations in its pharmaceutical delivery. Various problems encountered in the treatment process today clearly demonstrate the need for a more effective and safer delivery system for this drug. Therefore, current approaches have primarily focused on: developing formulations without Cremophor® EL, the possibility of large-scale preparation, and stability over longer periods. Identifying new molecules with more effective therapeutic activity will continue to be an integral part of healthcare systems (8).

It has rapid distribution, exhibits distribution in large volumes, and binds significantly to plasma proteins. Paclitaxel is metabolised in the

body primarily via the cytochrome P450 enzymes CYP2C8 and CYP3A isoenzymes. When administered intravenously, the most common toxic effects include neutropenia, hypersensitivity reactions, peripheral neurotoxicity, and hair loss (alopecia). Several new formulations are in development; albumin-bound paclitaxel (Abraxane) has recently been approved. Development studies are being conducted on oral formulations of taxane group drugs, and most of these formulations are still in Phase I clinical trials. These new dosage forms are expected to increase both treatment efficacy and safety, as well as provide ease of administration (9).

Paclitaxel and its semi-synthetic analogue docetaxel exert their antitumour effects by promoting the polymerisation of microtubule structures and inhibiting the disassembly of these structures. Both agents halt cell division by increasing intracellular microtubule stability. Although these compounds bind similarly to the β-tubulin subunit, the microtubule structures resulting from their effects differ. For example, the microtubules formed by docetaxel contain an average of 13.4 tubulin subunits, while those formed by paclitaxel are limited to approximately 12 subunits. Docetaxel penetrates cells more rapidly than paclitaxel and remains active in the cell for a longer period. In vitro and in vivo studies conducted in line with these pharmacokinetic advantages have demonstrated that docetaxel exhibits 2 to 4 times higher antitumour efficacy than paclitaxel. Furthermore, numerous studies have shown that prolonged exposure to paclitaxel significantly increases treatment efficacy (10). The antitumour efficacy of paclitaxel is largely dependent on the presence of the C13 side chain, A ring, oxetane ring, and the benzoil group at the C2 position. Paclitaxel is highly lipophilic in its chemical structure and therefore exists as a white crystalline powder with low solubility in water (11).

7. Mechanism of Action of Paclitaxel

Unlike traditional cancer drugs, paclitaxel does not affect DNA and RNA synthesis in cancer cells or damage DNA molecules. Its mechanism of action is primarily based on microtubules, which form spindle fibres during the prophase stage to transport chromosomes towards the cell poles. In the subsequent stages of cell division, these structures disintegrate and dissolve. Under normal conditions, microtubules lose their structure through depolymerisation when exposed to cold environments or calcium ions. However, paclitaxel interferes with this process; it binds to microtubules, increasing their stability. Thanks to this binding, microtubules maintain their structure without degrading despite cold or calcium effects and gain resistance to depolymerisation. Therefore, paclitaxel

treatment supports tubulin polymerisation and inhibits the progression of mitosis (12).

8. Discovery and Development of Paclitaxel

The discovery of paclitaxel and its journey to clinical use is based on a long and systematic research history. In 1964, as part of a joint project between the US National Cancer Institute (NCI) and the Department of Agriculture (USDA), Taxus brevifolia (Pacific yew) samples were found to possess cytotoxic properties. Following this discovery, in 1967, the Wall laboratory identified the active compound isolated from the plant and named it "taxol". In 1971, the chemical structure of the compound was published scientifically, and in 1978, its antitumour effects were observed in mouse tumour models. In 1979, studies conducted by the Horwitz laboratory revealed paclitaxel's effect on microtubule stabilisation. Following these scientific developments, the drug was included in phase I clinical trials in 1984 and phase II clinical trials in 1985. In 1991, the NCI transferred the commercial rights to taxol to Bristol-Myers Squibb (BMS), and this transfer of was debated in the US Congress. In 1992, BMS registered the name "Taxol," and in the same year, the FDA approved the drug for use in the treatment of ovarian cancer. Additionally, the Pacific Yew Act was enacted to protect the Taxus brevifolia species. In 1993, a second congressional hearing was held regarding this transfer process and in 1994, the FDA also approved Taxol for use in the treatment of breast cancer. In the same year, a semi-synthetic production method for the drug was also approved by the FDA. By 1995, the legal protection period for T. brevifolia had expired. Finally, in 1999, the United States Food and Drug Administration (FDA) officially approved the use of paclitaxel for the treatment of non-small cell lung cancer (13).

REVIEW OF ANALYTICAL METHODS

MA Fernández-Peralbo and colleagues conducted a quantitative investigation of paclitaxel and its principal metabolites in the serum, plasma, and tissues of ovarian cancer patients following intraperitoneal chemotherapy, employing an LC–MS/MS approach. They proposed an analytical protocol capable of detecting paclitaxel, 6α-hydroxypaclitaxel, and p-3′ -hydroxypaclitaxel at sub-nanogram per millilitre concentrations. The sample preparation involved a liquid–liquid extraction step to purify and concentrate the analytes before chromatographic analysis. Quantification was achieved using LC–MS/MS in selected reaction monitoring

(SRM) mode, which allowed for highly selective identification and precise quantitation in biological matrices. Detection limits were established at 0.03–0.15 ng/mL for serum and 0.07–0.62 ng/g for tissue samples, while relative standard deviation values between 0.5% and 2.7% confirmed the intra-day precision of the method. The analytical approach was successfully applied to samples from 13 women diagnosed with ovarian peritoneal carcinomatosis undergoing hyperthermic intraperitoneal chemotherapy (HIPEC). The results demonstrated that this method is suitable for post-treatment monitoring of therapeutic effectiveness and potential toxicity.

Similarly, Bianna Posocco et al. developed a novel LC–MS/MS method for determining paclitaxel and its main metabolite, 6α-hydroxypaclitaxel, in human plasma, which was subsequently utilized in a clinical pharmacokinetic study. The method enables sensitive and specific quantification across all clinically relevant paclitaxel dosage ranges, relying on rapid protein precipitation using minimal plasma volumes. Chromatographic separation was achieved on a SunFire™ C18 column (2.1 × 150 mm, 3.5 µm particle size, 92 Å pore size) with a mobile phase composed of bidistilled water and acetonitrile, both containing 0.1% formic acid. Detection was carried out via electrospray ionization (ESI) in positive ion mode using SRM acquisition, providing a robust and reproducible approach for pharmacokinetic evaluation. The developed bioanalytical method was successfully validated in accordance with the bioanalytical method validation guidelines published by the FDA and EMA. Calibration curves yielded linear results ($R^2 \geq 0.9948$) in the concentration ranges of 1–10,000 ng/mL for paclitaxel and 1–1,000 ng/mL for 6α-hydroxy-paclitaxel, demonstrating high accuracy and precision of the method. Intra- and inter-day precision and accuracy assessments were carried out at three concentration levels for paclitaxel and its major metabolite, 6α-hydroxy-paclitaxel. The obtained values remained within acceptable analytical ranges, with precision below 9.9% and accuracy between approximately 91% and 115%. The validated method demonstrated limits of detection within 0.03–0.15 ng/mL for serum and 0.07–0.62 ng/g for tissue matrices. Repeatability, expressed as relative standard deviation (RSD), was maintained between 0.5% and 2.7%. These findings confirm that the LC–MS/MS approach provides reliable quantification of paclitaxel and its metabolite in plasma samples, supporting its application in optimizing dosage regimens and monitoring therapy-related toxicities in pharmacokinetic investigations.

Tarek Baati and co-workers developed an ultra-sensitive LC–MS/MS method to quantify paclitaxel released from nanocarriers in vitro, using a liquid-phase extraction step for sample purification. The technique involved methanol and a mild acid (acetic acid) during sample preparation

to prevent drug degradation and to achieve recoveries exceeding 80% using an internal standard system with both paclitaxel and docetaxel. This rapid and selective approach enabled quantification of paclitaxel in cell culture media and lysates over linear ranges of approximately 1–250 nM and 5–250 nM, respectively, with limits of quantification at sub-picomole levels. The method was effectively applied to A549 human non-small cell lung carcinoma cells to evaluate the amount of paclitaxel remaining in the medium and intracellular compartment following incubation at various concentrations. Owing to its sensitivity, the method offers a valuable tool for studying the kinetic behaviour of paclitaxel release from nanocarrier systems, a crucial factor in validating drug delivery platforms for cancer therapy.

In another investigation, Pavan K. Yadav et al. examined the combined pharmacokinetic behaviour of paclitaxel (PTX) and bortezomib (BTZ) for potential co-therapy in breast cancer treatment, utilizing a validated LC–MS/MS method. Both analytes were separated using a C18 reversed-phase column under an isocratic mobile phase containing formic acid in methanol and ammonium acetate buffer. The analytical method achieved a lower limit of quantification of 1 ng/mL with a total run time of six minutes per analyte, and demonstrated linearity between 1 and 600 ng/mL. The established LC–MS/MS protocol was applied to evaluate the oral pharmacokinetic profile of a nanoformulation co-loaded with PTX and BTZ in female Sprague-Dawley rats. The method exhibited excellent reproducibility, sensitivity, and accuracy, fulfilling U.S. FDA bioanalytical validation criteria for parameters such as linearity, stability, and precision.(17).

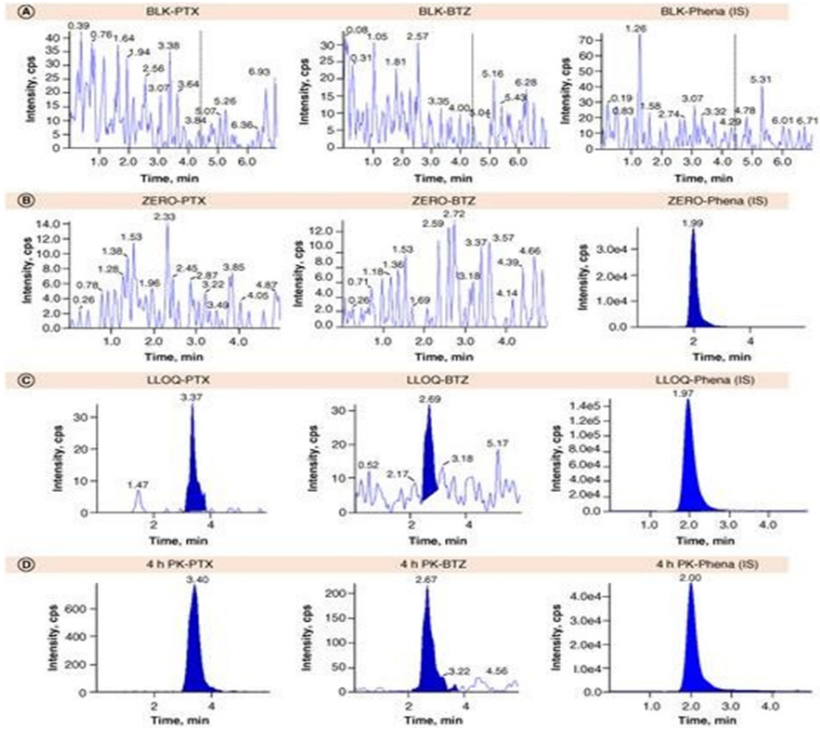

Figure 2. *(A) female blank plasma, (B) female SD-rat plasma (zero sample), (C) female SD rat plasma spiked with PTX and BTZ at LLOQ, and (D) MS/MS chromatograms of the PTX- BTZ pharmacokinetic sample at 4 hours. Chromatograms of phenacetin (IS) were used with each BTZ and PTX plasma sample (17).*

Susan M. Christner et al. quantitatively evaluated paclitaxel and its 6-alpha-OH and 3-para-OH metabolites in human plasma using LC-MS/MS (Figure 3). A high-performance liquid chromatography-mass spectrometry method was developed to quantitatively determine paclitaxel and its 6-alpha-OH and 3-para-OH metabolites in 0.1 mL of human plasma. Following liquid-liquid extraction with MTBE, chromatographic separation was achieved using a Phenomenex Synergy Polar Reverse Phase column (4 µm, 2 mm × 50 mm) and a gradient of acetonitrile and 0.1% formic acid in water over an 8-minute run time. Mass spectrometric analysis was performed on an ABI SCIEX 4000Q instrument operating in positive mode using an electrospray ionisation (ESI) source. The method yielded linear results for paclitaxel in the range of 10–10,000 ng/mL and for both major metabolites in the range of 1–1,000 ng/mL; accuracy values ranged from 94.3% to 110.4%, and the coefficient of variation was measured to be below 11.3%, demonstrating that the method is both accurate and precise. The recovery rate from plasma samples was found to

be between 59.3% and 91.3%, while the matrix effect was determined to be between -3.5% and +6.2% and negligible. In sample stability tests, the freeze- thaw stability was 90.2%–107.0%, the stability of samples stored at -80°C for 37 months was 89.4–112.6%, and the stability of samples kept at room temperature for 4 hours was 87.7– 100.0%, all of which were within acceptable limits. This method allows for detailed examination of the pharmacokinetic and pharmacodynamic behaviour of paclitaxel and its metabolites in a clinical setting; it also has potential for use in therapeutic drug monitoring and in the phenotypic assessment of patients' CYP2C8 and CYP3A4 enzyme activities (18).

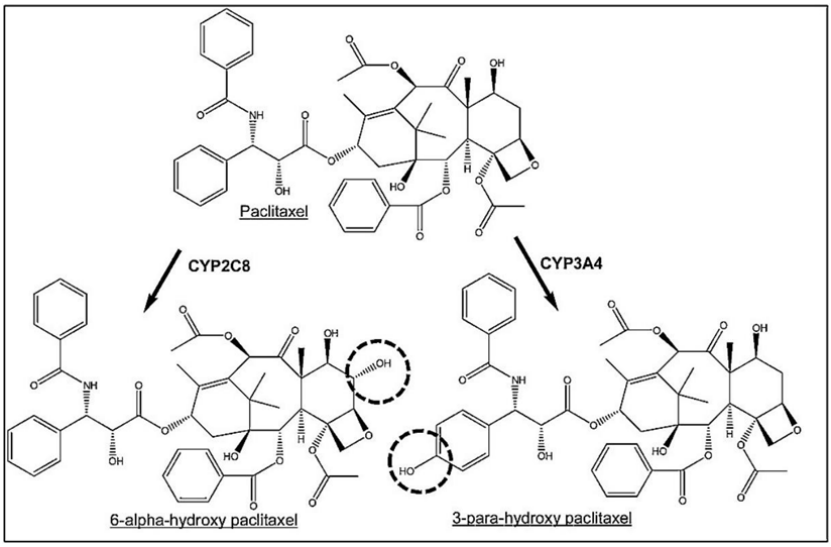

Figure 3. *Paclitaxel and its metabolites, 6-alpha-hydroxy paclitaxel and 3-para-hydroxy paclitaxel (18).*

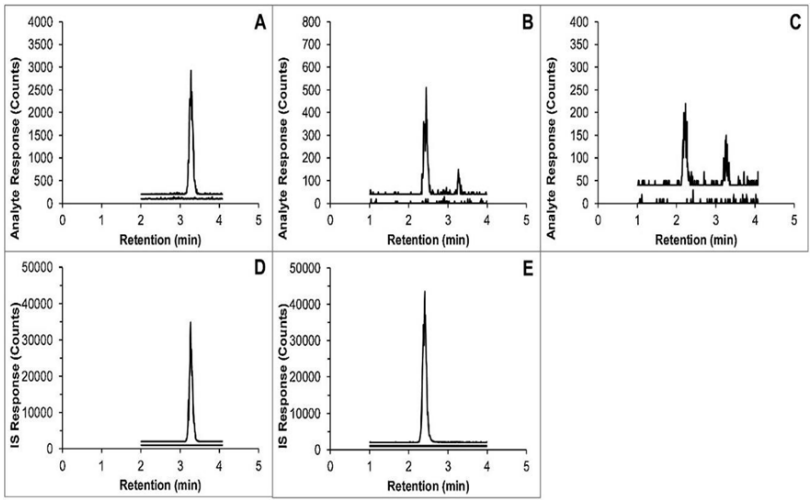

Figure 4. *The ion signals related to the detection of paclitaxel (PTX) and its metabolites at different concentrations in human plasma and control plasma (18).*

In Figure 4, Figure A shows the data obtained by adding PTX (m/z 854.5 → 286.0; 3.3 min) at a concentration of 10 ng/mL LLOQ to control plasma with an offset of 200 counts and to human plasma with an offset of 100 counts. Similarly, Figures B and C present the detection of 6-α–OH-PTX (m/z 870.5 → 286.0; 2.5 min) and 3-p–OH-PTX (m/z 870.5 → 569.5; 2.3 min) at the 1 ng/mL LLOQ level are presented. The internal standards [13C6]-PTX (m/z 860.5 → 292.0; 3.3 min) and [D5]-6-α–OH- PTX (m/z 875.5 → 291.0; 2.5 min) were analysed at a concentration of 500 ng/mL with different offset values. These methods enable the quantitative analysis of PTX and its metabolites (18).

Xinran Chen and co-workers established a validated LC–MS/MS protocol capable of simultaneously quantifying vancomycin, norvancomycin, methotrexate, paclitaxel, and imatinib in human plasma. The method was developed in accordance with international bioanalytical validation guidelines and successfully implemented in a clinical setting. The calibration ranges for these analytes were 0.5–100 μg/mL for vancomycin and norvancomycin, 5–1000 ng/mL for methotrexate, 10–2000 ng/mL for paclitaxel, and 5–500 ng/mL for imatinib. Method validation demonstrated that both accuracy (bias) and precision values for all compounds were within ±15%, fulfilling regulatory requirements. The recovery efficiency, normalized against an internal standard, was approximately 45% for vancomycin and norvancomycin, whereas near-complete recovery (≈100%) was achieved for methotrexate, paclitaxel, and imatinib. No significant carry-over was detected in the analytical sequence. Sample stability was

confirmed for at least 24 hours in the autosampler, up to 72 hours under refrigerated storage (4°C), and for one week at −80°C. Comparable drug concentrations were observed between plasma and serum matrices.

Correlation analyses indicated that methotrexate and vancomycin plasma levels were positively associated with serum creatinine concentrations, and imatinib exposure increased with patient age. For paclitaxel, systemic exposure was positively correlated with both therapeutic response and adverse event frequency. Considerable interindividual and intraindividual variability in plasma drug concentrations was reported. Nonlinear pharmacokinetic evaluation suggested that maintaining paclitaxel plasma levels above approximately 0.05 μmol/L could serve as a predictive marker for both clinical efficacy and potential toxicity. (19).

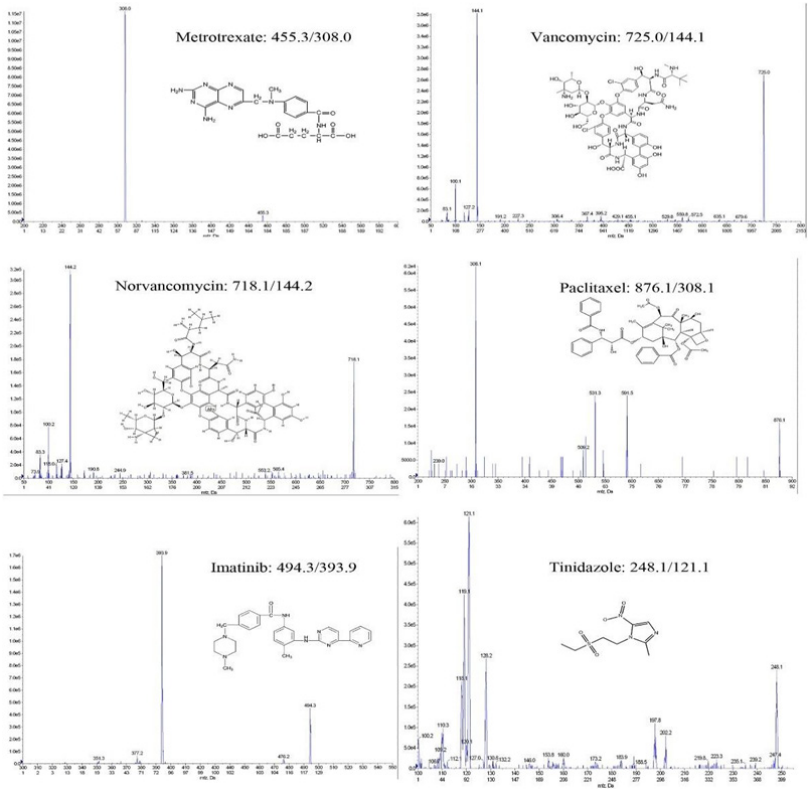

Figure 5. *MS/MS conditions for vancomycin, norvancomycin, methotrexate, paclitaxel, imatinib, and tinidazole (19).*

Lin Hou and colleagues reported, for the first time, the development and validation of an LC–MS/MS method enabling the simultaneous quantification of curcumin (CUR) and paclitaxel (PTX). Methanol was employed for analyte extraction, with docetaxel serving as the internal stan-

dard. Chromatographic separation was achieved using a C18 column (4.6 × 50 mm, 3.5 µm) under an isocratic mobile phase composed of methanol and an aqueous solution containing 0.1% formic acid (80:20, v/v), at a flow rate of 0.5 mL/min. The validated procedure was subsequently applied to assess the pharmacokinetic behaviour of CUR and PTX in rat plasma. Calibration curves exhibited strong linearity across concentration ranges of 2–1000 ng/mL for paclitaxel and 5–500 ng/mL for curcumin. Extraction recovery and matrix effect values for both compounds, as well as the internal standard, were within acceptable analytical limits. The method demonstrated high sensitivity, reliability, and ease of operation for simultaneous analysis. Interestingly, when paclitaxel was co-administered with curcumin, a notable increase in its apparent volume of distribution was observed, indicating that curcumin may modulate the pharmacokinetic profile of paclitaxel. (20).

CONCLUSION

Paclitaxel is used as a chemotherapeutic agent in oncology. Paclitaxel has a unique mechanism of action that causes microtubule stabilisation, thereby arresting cell division and inducing apoptosis in cancer cells. It treats cancer by its ability to inhibit microtubule depolymerisation. This agent, which binds to microtubules and inhibits cell division, is particularly effective in breast and ovarian cancer. LC-MS/MS is a powerful analytical technique that combines high-resolution chromatographic separation with extremely sensitive and specific mass spectrometric detection. It offers advantages such as high sensitivity, high selectivity, the ability to analyse complex matrices, short analysis time, provision of quantitative (numerical) and qualitative (descriptive) information, high resolution, and accuracy. It has a wide range of applications in many fields such as pharmaceutical analysis, clinical diagnosis, toxicology, food safety, and environmental analysis. It was considered that simple, accurate, selective, and sensitive results could be obtained using the liquid chromatography method for paclitaxel determination. Research in the literature has shown that LC-MS/MS method provides high reliability and accuracy in paclitaxel analysis. Understanding the pharmacokinetic profile of paclitaxel and monitoring the therapeutic dose range are critical for effective and safe treatment. At this point, the LC-MS/MS method stands out as one of the most widely used and reliable analytical techniques today, thanks to both its analytical performance and biological compatibility. This method is effectively used in pharmaceutical analysis and clinical applications due to its advantages, such as low limit of quantification (LLOQ), high selectivity, repeatability, and short analysis time. Studies conducted using

LC-MS/MS have enabled the accurate identification of paclitaxel and its metabolites in plasma, thereby contributing significantly to the generation of pharmacokinetic data for the individualisation of treatment.

Consequently, the LC-MS/MS technique is a highly suitable method for analysing drugs such as paclitaxel, which have a narrow therapeutic range and exhibit complex pharmacokinetic behaviour. It is anticipated that this method will continue to be widely used not only in pharmaceutical analysis but also in clinical biochemistry, toxicology, and pharmacogenetics studies. These methods developed for the analysis of paclitaxel will be further optimised in the future and will play a pioneering role in areas such as monitoring treatment response, implementing personalised treatment approaches, and evaluating the efficacy of new carrier systems. The combined use of advanced analytical techniques such as LC-MS/MS with nanotechnology-based drug delivery systems has the potential to further enhance the therapeutic efficacy and safety of paclitaxel. In this context, new studies will pave the way for the more effective and safer use not only of paclitaxel but also of other anticancer agents with similar pharmacological properties.

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Chapter 2

THE CIRCRNA/MIR-7 AXIS IN HUMAN CANCERS: ONCOGENIC SPONGES, REGULATORY MECHANISMS, AND THERAPEUTIC TARGETS

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1. Introduction

Cancer is a leading cause of death worldwide, driven by the accumulation of genetic and epigenetic changes that disrupt normal cellular balance. Recently, the non-coding genome has been identified as an important regulator of gene expression in both healthy and pathological situations, adding a complex layer of post-transcriptional control to our understanding of oncogenesis (Hansen et al., 2013). The competitive endogenous RNA (ceRNA) hypothesis has revealed an intricate layer of post-transcriptional regulation, whereby non-coding RNAs including circular RNAs (circRNAs) can sequester microRNAs (miRNAs) and affect the regulation of gene expression (Hansen et al., 2013). CircRNAs have emerged as a unique and intriguing type of non-coding RNA. CircRNAs have a covalently closed continuous loop structure due to back-splicing, and they lack 5' caps and 3' poly(A) tails, giving them extraordinary stability and resistance to exonuclease degradation (Kristensen et al., 2019). CircRNAs, previously neglected as splicing artifacts, are now recognized for their numerous regulatory activities, including transcriptional regulation, protein decoys, and, most importantly, effective miRNA sponges (Li et al., 2018). The ceRNA hypothesis proposes that RNA transcripts with similar miRNA response elements may interact and regulate one another by competing for a limited number of miRNAs. CircRNAs are ideal ceRNAs due to their abundance, stability, and high concentration of miRNA binding sites. This chapter summarizes recent data on the elevated expression of specific microRNA-7 (miR-7) sponge circRNAs, including CDR1as/ciRS-7, circHIPK3, and circERBB2, in various human cancers (Zhang et al., 2018; Zeng et al., 2018; Gao et al., 2021). A significant and functionally relevant ceRNA interaction in cancer biology involves specific circRNAs and the tumor-suppressive miR-7. miR-7 is a well-studied tumor suppressor miRNA that targets a complex network of oncogenes involved in critical signaling pathways, including EGFR/PI3K/AKT and RAF/MEK/ERK (Hansen et al., 2013). MiR-7 significantly suppresses fundamental cancer features such as uncontrolled cell proliferation, migration, invasion, and epithelial-mesenchymal transition (EMT). Thus, the potent tumor-suppressive efficiency of miR-7 is important in cancer. A main mechanism for this inactivation is molecular sponging by particular, frequently overexpressed circRNAs. These circRNAs sequester miR-7, inhibiting its interaction with target mRNAs and derepressing a number of oncogenic proteins (Gajda et al.,2021). This chapter aims to summarize available research on the circRNA/miR-7 axis in cancer. We discusses the principal circRNAs involved, the precise oncogenic pathways triggered by the sequestration of miR-7, the resultant effects on tumor behavior and therapeutic response, and the growing clinical implications of this axis

for diagnosis and treatment.

2. The Broader Landscape of MicroRNA Dysregulation in Cancer

The widespread dysregulation miRNAs during cancer pathogenesis is evident from the key role played by miR-7 in tumor suppression, a compelling piece of evidence from a much broader and more complex phenomenon. Defining the circRNA/miR-7 axis within the larger and more complex picture of miRNA biology in oncology is valuable in providing insight into its full significance. miRNAs are known to interact with the 3'-untranslated region of target mRNAs to regulate translational repression or degradation (Hussen et al., 2021; Jansson and Lund, 2012). The ability to fine-tune gene expression to a precise level makes miRNAs a key regulator of cellular physiology, and this prediction suggests that they regulate most of the human protein-coding genes (Jansson and Lund, 2012). This regulatory role is significantly disrupted during malignancy. Essentially, miRNAs can be studied in cancers in two categories: onco-miRs and onco-suppressor miRs. Therefore, miRNAs have the capacity to function as both potent oncogenes and tumor suppressors (Ali Syeda et al., 2020; Jansson and Lund, 2012). The patterns of these miRNAs are not collateral but precisely correlate with the detection, staging, development, and treatment response of cancers, providing effective diagnostic and prognostic indicators (Hussen et al., 2021). A confluence of mechanistic alterations is implicated in the dysregulation of miRNA expression during malignancy. Genomic instability predominantly affects miRNA loci, where deletions or loss of heterozygosity most frequently follow loss of function among tumor suppressor miRNAs, while overduplication among genes results from the overexpression of oncomiRs (Ali Syeda et al., 2020; Hussen et al., 2021). This genetic background is obscured by misregulation of DNA methylation of promoter regions and histones, which can silence the activity of tumor suppressor miRNAs, while oncomiRs can be activated by hypomethylation, allowing a degree of reversible regulatory control (Ali Syeda et al., 2020). Furthermore, transcriptional regulation is impaired for the host miRNA transcript; key transcription factors such as MYC and p53, oncogenes or tumor suppressors, are capable of repressing/activating miRNA clusters, directly remodeling the transcriptome (Ali Syeda et al., 2020; Jansson and Lund, 2012). Finally, the miRNA biogenesis machinery, consisting of key enzymes such as Drosha and Dicer, can introduce errors during the processing of new miRNAs and the subsequent upregulation of oncogenes (Ali Syeda et al., 2020). As a functional consequence of this multilayered misregulation, miRNAs contribute to the establishment and development of all hallmarks of cancer, including invasion and metastasis,

maintaining cell proliferative signaling and resisting cell death (Hussen et al., 2021; Jansson and Lund, 2012). Consequently, the circRNA/miR-7 interaction is not an exception, but rather a crucial node in a generally disrupted, highly interconnected circuitry. The multilayered mechanism of posttranscriptional regulation of malignancy by cancer cells is represented by the sequestration by an oncogenic circRNA of the activity of the tumor suppressor miR-7. Given that the coupling between circRNAs and miR-7 may play a critical role in various pathways involved in the cancer process, this coupling clearly holds significant therapeutic potential.

3. Mechanisms of Circular RNA Biogenesis and Regulation

The presence of circRNAs, formerly thought to be splicing artifacts, is now acknowledged to be the result of a complex and regulated biological mechanism. Unlike canonical linear splicing, which joins exons in a 5'-to-3' order, circRNAs are created using a different method known as back-splicing (Kristensen et al., 2019; Li et al., 2018). In back-splicing, a downstream 5' splice site (splice donor) chemically connects to an upstream 3' splice site (splice acceptor), forming a closed circular molecule with no free ends. CirRNAs are resistant to exonucleases due to their unusual structure, which gives them extraordinary stability and, in many cases, a longer half-life than linear mRNAs (Chen & Yang 2015). CircRNA synthesis is not an accident, but rather a competitive process that happens co-transcriptionally and competes for the same substrate with the standard pre-mRNA splicing machinery (Ashwal-Fluss et al., 2014). The efficiency of back-splicing is determined by a mixture of cis-acting regulatory components inside the pre-mRNA and trans-acting factors that interact with them. The fundamental problem in back-splicing is the physical proximity of distant splice sites. This is frequently aided by complementary sequences within the introns that flank the eventual circular exon(s). Intronic Complementary Sequences (ICSs): Short, reverse-complementary sequences, such as Alu repeats in humans, can base-pair with each other to form an RNA duplex that loops the intervening exon and brings the upstream acceptor and downstream donor splice sites close together (Chen & Yang, 2015; Kristensen et al., 2019). This "ribonucleoprotein bridge" significantly improves the likelihood of the spliceosome performing back-splicing rather than linear splicing. Specific RNA-binding proteins (RBPs) are essential for stabilizing circularization structures and recruiting splicing machinery. Ashwal-Fluss et al. (2014) reported that the RBP Muscleblind binds to specific locations in both the introns and exons of its own pre-mRNA. MBL molecules dimerize to effectively bridge the circularizing exon, boosting the synthesis of circMbl. This established an

important principle: proteins can operate as trans-acting agents, specifically promoting circRNA synthesis. Another RBP, QKI, which is activated during epithelial-mesenchymal transition (EMT), can bind to intronic sequences and enhance circRNA production by bringing splice sites together, similar to how ICSs work (Conn et al., 2015). The ADAR enzyme, which catalyzes A-to-I RNA editing, can prevent the production of circular RNA. It accomplishes this by melting the double-stranded RNA structures generated by ICSs, destroying the bridge that enables back-splicing (Ivanov et al., 2015). Back-splicing and linear splicing are processes that compete for the same pre-mRNA transcript (Ashwal-Fluss et al., 2014). The transcription rate can influence each process's relative efficiency. Slower transcription may allow the intronic complementary sequences or RBPs to generate the stabilizing structures needed for back-splicing. This competition explains why the biogenesis of many circRNAs is tissue-specific, developmentally regulated, and can be altered in diseases such as cancer, where the expression of specific trans-acting factors or gene transcription kinetics are disrupted (Li et al., 2018; Yang et al., 2022). To summarize, circRNA biogenesis is a complex, multi-layered process coordinated by the interaction of certain genomic regions with RNA-binding proteins. Understanding these methods is more than just academic; it is critical for creating treatment strategies that selectively suppress carcinogenic circRNAs by targeting their specific biogenesis pathways.

4. The circRNA/miR-7 Axis Across Human Cancers

The dysregulation of the circRNA/miR-7 axis in different human cancers underscores its integral function across the span of the axis (Figure 1). A quick reference of relevant circRNAs, different cancer types, and functions with respect to Table 1. CDR1as (ciRS-7) was the first recognized circRNA on this axis and has over 70 conserved miR-7 binding sites, thus functioning as a prolific sponge (Hansen et al. 2013). Documented in over ten cancer types, CDR1as has oncogenic potential in NSCLC, glioblastoma, hepatocellular carcinoma (HCC), and osteosarcoma, among others. In those types of cancers, it is reported to aid in the proliferation and metastasis and contribute to treatment resistance (Zhang et al., 2018; Pei et al., 2022; Xu et al., 2018). In addition to CDR1as, several additional circRNAs employ the same strategy, proving the regulatory module's universality. CircHIPK3, for example, enhances colorectal cancer development and metastasis by sponging miR-7 and activating a network of oncogenes including FAK, IGF1R, and EGFR (Zeng et al., 2018). Multiple circRNAs, including circERBB2, circWHSC1, circATXN7, and circSCAP, have been identified as tumor-promoting drivers in lung cancer by se-

questering miR-7 and activating critical pathways such as FOXM1, TAB2, PFN2, and SMAD2 (Gao et al., 2021; Guan et al., 2021; Li et al., 2022; Zhang et al., 2023). Similarly, in breast cancer, circ_0006528 sponges miR-7-5p to activate the RAF1/MAPK/ERK pathway (Gao et al., 2019), whereas circXPO1 promotes malignancy in glioblastoma by suppressing RAF1 through miR-7-5p sponging (Wang et al., 2023). This continuous pattern across multiple tumors lends evidence to the circRNA/miR-7 axis as a fundamental carcinogenic mechanism.

5. Downstream Oncogenic Pathways and Therapy Resistance

The primary molecular consequence of circRNA sponging is the alleviation of miR-7-mediated repression of target mRNAs. This results in the activation of a wide variety of oncogenic proteins, which collectively drive cancer hallmarks. Cell proliferation, development, and motility depend on these targeted pathways. MiR-7 targets key participants in growth factor signaling, including EGFR/RAF/ERK and PI3K/AKT. For example, Epidermal Growth Factor Receptor (EGFR) is a traditional miR-7 target that is regularly derepressed by circRNAs such as CDR1ases in PDAC, PTC, and osteosarcoma (Liu et al., 2019; Han et al., 2020; Xu et al., 2018). Similarly, RAF1 has been a common target of circRNA reactivation, such as circXPO1 in GBM and circ_0006528 in breast cancer, leading to activation of the pro-proliferative MEK/ERK cascade (Wang et al., 2023; Gao et al., 2019). Furthermore, miR-7 directly targets PI3K/AKT components, including PIK3CD, and induces their upregulation via circRNA sponging in laryngeal squamous cell carcinoma and non-small cell lung cancer. MiR-7 regulates cell cycle progression and stem cell proliferation by targeting key regulators. CCNE1 (Cyclin E1), which induces the G1/S phase transition, is frequently upregulated by circRNAs like CDR1as in LSCC and OS, allowing uncontrolled proliferation (Zhang et al., 2018; Xu et al., 2018). MiR-7-5p also directly targets KLF4, a transcription factor associated with cancer stem cell proliferation. In hepatoblastoma, CDR1as spongiforms miR-7-5p, causing it to upregulate KLF4 and promote tumor growth and stem cell proliferation. It is known that chemotherapy resistance significantly contributes to treatment failure. In breast cancer, CDR1as confers resistance to 5-fluorouracil (5-FU) by sponging miR-7 and upregulating CCNE1 (Yang et al., 2019). Also in prostate cancer, hsa_circ_0000735 increases docetaxel resistance by suppressing miR-7, increasing cell proliferation, and suppressing apoptosis (Gao et al., 2020). This evidence suggests that the circRNA/miR-7 axis plays a critical role in many cancer processes.

6. Therapeutic Implications and Future Perspectives

The critical role of the circRNA/miR-7 axis in accelerating tumor progression and conferring resistance to therapy makes it a promising target for therapy. Various methods can be developed to suppress oncogenic circRNAs or reactivate tumor suppressor miRNAs, which fits the general concept of targeting noncoding RNAs in cancer therapy (Mollaei et al., 2019; Jansson and Lund, 2012). The simplest strategy is to suppress oncogenic circRNA via RNA interference technology or antisense oligonucleotides (ASOs). Preclinical studies validate this approach. For example, CDR1as silencing has been shown to increase the sensitivity of breast cancer cells to 5-fluorouracil (5-FU), which suppresses miR-7 (Yang et al., 2019). Furthermore, suppression of the circ_0013912 gene in pancreatic ductal adenocarcinoma cells suppressed growth, migration, and invasion; these effects were partially mediated by the release of miR-7-5p (Guo et al., 2020). Targeting circRNAs with unique back-splice splicing specificity offers a potential avenue to avoid off-target effects against their linear mRNA counterparts. The idea of microRNA replacement therapy involves delivering tumor suppressor miRNAs, such as miR-7, to cancer cells to restore their function and inhibit tumor growth (Mollaei et al., 2019). The potential of tumor suppressor miRNAs to regulate entire signaling networks within cells makes them a promising candidate for the development of anticancer therapeutics (Mollaei et al., 2019). This strategy has a unique appeal because a single miRNA mimetic can simultaneously target multiple oncogenic pathways, potentially offering a better outcome than single-target agents. For the past several decades, researchers have attempted to design effective and safe targeting schemes to reconstitute these suppressive miRNAs in malignant cells (Mollaei et al., 2019). The ability to modulate this axis through pharmacological agents is a new and promising avenue. For example, the natural product curcumin has been identified as an epigenetic modulator that suppresses breast cancer tumorigenesis via downregulation of CDR1as and subsequent upregulation of miR-7-5p, thereby inhibiting downstream oncogenic targets (Abuaisha et al., 2025). This highlights the potential for repurposing existing compounds or finding novel small molecule agents that can specifically disrupt the circRNA/miR-7 interaction. Furthermore, the anesthetic agent propofol has been shown to have antitumor activity against lung cancer by suppressing the circ-ERBB2/miR-7-5p/FOXM1 axis (Gao et al., 2021), and therefore, it may be possible that some of the activity of commonly prescribed clinical drugs may be mediated through this axis. Since miRNAs are already used clinically as diagnostic markers and therapeutics (Jansson and Lund, 2012), the next logical step towards a more comprehensive understanding of human diseases and treatment lies in incorpo-

rating the level of regulation provided by circRNAs into this therapeutic paradigm. However, significant challenges remain. Future research must address the technical challenges of in vivo application, stability, and specificity for RNA therapies (Mollaei et al., 2019). Uncovering the deeper regulatory mechanisms of miRNAs and circRNAs in cancer will facilitate understanding the impact of tumor dysfunction and also provide insights into safe and effective therapeutic approaches (Ali Syeda et al., 2020). Furthermore, understanding the context-dependent behavior of this axis and exploring its impact on the tumor microenvironment will be crucial for designing successful combination therapy schemes alongside existing methods such as chemotherapy and immunotherapy. Consequently, the successful implementation of these methods in the clinic will require robust in vivo validation and carefully designed clinical trials to demonstrate their efficacy and safety for cancer patients.

7. Conclusion

Studies clearly demonstrate the oncogenic potential of the circRNA/miR-7 pathway. This pathway forms part of a larger deregulated miRNA network. It is encouraging to explore the mechanism(s) of circRNA biogenesis in cancers investigated in studies suggesting loss of control of miRNA biogenesis, as circRNA biogenesis is also deregulated, and this is an open focus for future research. CDR1as, in particular, circHIPK3, and many other circRNAs act as important molecular sponges by suppressing the tumor suppressor activity of miR-7, thereby activating numerous oncogenes that promote cancer processes such as proliferation, metastasis, and treatment resistance. However, to achieve clinical applicability, new studies are needed to improve the in vivo delivery of RNA therapeutics and to overcome challenges such as the multifunctionality of circRNAs and miRNAs, which must be differentiated for application.

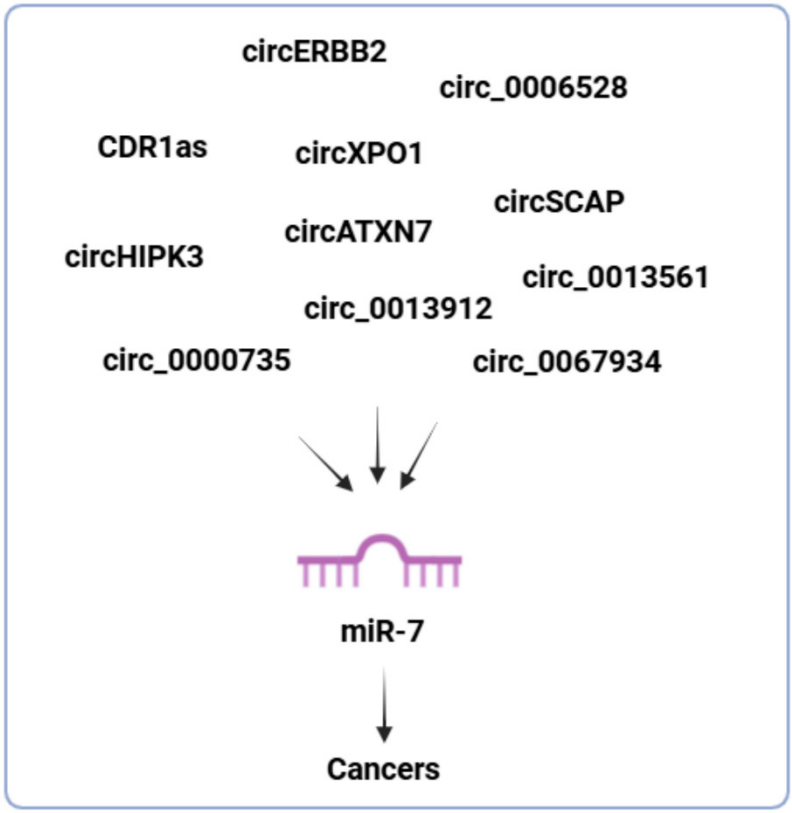

Figure 1. *circRNA/miR-7/cancer relation*

Table 1: *The circRNA/miR-7 Axis in Human Cancers*

circRNA Name	Cancer Type	Expression	miRNA	Key Target(s) / Pathway	Functional Outcome	Key Reference(s)
CDR1as	NSCLC	Up	miR-7	EGFR, CCNE1, PIK3CD	Promotes progression & proliferation	Zhang et al., 2018
CDR1as	Glioblastoma	Up	miR-7	Wnt/β-catenin pathway	Promotes tumor development	Pei et al., 2022
CDR1as	Osteosarcoma	Up	miR-7	EGFR, CCNE1, RAF1	Promotes growth & migration	Xu et al., 2018
CDR1as	ESCC	Up	miR-7	HOXB13	Promotes growth & metastasis	Li et al., 2018
CDR1as	PDAC	Up	miR-7	EGFR/ STAT3 pathway	Promotes proliferation & invasion	Liu et al., 2019

CDR1as	PTC	Up	miR-7	EGFR	Promotes proliferation & migration	Han et al., 2020
CDR1as	Gastric Cancer	Up	miR-7	PTEN/ PI3K/AKT pathway	Inhibits tumor suppression	Pan et al., 2018
CDR1as	NSCLC	Up	miR-7	CCNE1, PIK3CD	Enhances proliferation & invasion	Zhang et al., 2018
CDR1as	Breast Cancer	Up	miR-7	CCNE1	Induces chemoresis-tance	Yang et al., 2019
CDR1as	Nasopharyn-geal Carcinoma	Up	miR-7-5p	E2F3	Promotes growth & metabolism	Zhong et al., 2019
CDR1as	Hepatoblas-toma	Up	miR-7-5p	KLF4	Promotes proliferation & stemness	Chen et al., 2020
circHIPK3	Colorectal Cancer	Up	miR-7	FAK, IGF1R, EGFR, YY1	Promotes growth & metastasis	Zeng et al., 2018
circERBB2	Lung Cancer	Up	miR-7-5p	FOXM1	Facilitates tumori-genesis & invasion	Gao et al., 2021
circ_ 0006528	Breast Cancer	Up	miR-7-5p	RAF1 (MAPK/ ERK pathway)	Promotes proliferation & invasion	Gao et al., 2019
circXPO1	Glioblas-toma	Up	miR-7-5p	RAF1	Promotes malignancy	Wang et al., 2023
circATXN7	NSCLC	Up	miR-7-5p	PFN2	Promotes proliferation & metastasis	Li et al., 2022
circSCAP	NSCLC	Up	miR-7	SMAD2	Facilitates tumorigenesis	Zhang et al., 2023
hsa-circ-0013561	HNSCC	Up	miR-7-5p	PDK3	Regulates development & metastasis	Tian et al., 2024
circ_ 0013912	PDAC	Up	miR-7-5p	-	Promotes growth & invasion	Guo et al., 2020
hsa_circ_ 0000735	Prostate Cancer	Up	miR-7	-	Induces chemoresistance	Gao et al., 2020
circRNA_ 0067934	Glioma	Up	miR-7	Wnt/β-catenin pathway	Promotes development	Pei et al., 2022

Note: ESCC: Esophageal Squamous Cell Carcinoma; PDAC: Pancreatic Ductal Adenocarcinoma; PTC: Papillary Thyroid Cancer; LSCC: Laryngeal Squamous Cell Carcinoma; HNSCC: Head and Neck Squamous Cell Carcinoma.

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Chapter 3

HERBAL APPROACHES IN HEALTH: PHYTOCHEMICAL AND BIOLOGICAL EFFECTS OF *DIPLOTAENIA TURCICA*

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Introduction

In recent years, herbal remedies and their constituents have gained increasing significance as complementary or alternative strategies in the prevention and management of various health conditions. For centuries, plants have been employed in diverse cultures for therapeutic purposes, and modern scientific research has provided a rational basis for this traditional knowledge. Contemporary investigations in the field of phytotherapy demonstrate that plant-derived compounds may exert beneficial influences on health through antioxidant, anti-inflammatory, antimicrobial, and multiple biological properties. Within this context, the phytochemicals present in medicinal plants also serve as an important source of inspiration for pharmaceutical innovation and drug discovery studies.

Diplotaenia turcica is recognized as one of the endemic plant species of Turkey and has long been applied in traditional medicine for various purposes. Preliminary investigations focusing on the biological activities of its root and leaf extracts have revealed promising outcomes, including considerable antioxidant potential, possible anti-inflammatory effects, and the capacity to mitigate oxidative stress at the cellular level. A comprehensive evaluation of the phytochemical composition and biological properties of *D. turcica* is crucial not only for substantiating its traditional applications with scientific evidence but also for contributing to the development of novel therapeutic strategies.

Historical and cultural perspectives on the use of medicinal plants

Archaeological evidence indicates that the use of medicinal plants dates back nearly 60,000 years, reaching as far as the Paleolithic era. Records concerning the therapeutic application of plants can be traced to ancient Chinese inscriptions and Egyptian papyri from around 3000 BC. While certain societies, such as African and Native American communities, incorporated plants into healing rituals, others—such as those practicing Ayurveda or Traditional Chinese Medicine—developed more systematic frameworks for herbal treatments. Research further demonstrates that across different regions of the world, comparable or even identical plants have been employed for similar therapeutic purposes (Mat, 2010; Yeşilada, 2012).

In mythology, plants have often been regarded as the most precious gifts bestowed upon humanity by the gods. Since the very beginning of human existence, vegetation has played a central role in supporting life, and the interaction between people and plants has persisted across his-

tory (Gezgin, 2007). Archaeological findings from ancient times further suggest that early humans primarily relied on plants both as a source of nourishment and as remedies for health problems (Koçyiğit, 2005).

Throughout human history, numerous diseases have been managed using plants. The World Health Organization reports that approximately four billion people worldwide initially rely on herbal remedies to address their health concerns. Moreover, in developed countries, nearly 25% of prescription drugs are derived from plant-based active compounds (Farnsworth et al., 1985).

Phytotherapy and contemporary applications of medicinal and aromatic plants

The concept of "phytotherapy," referring to the use of medicinal plants for treatment, was first introduced in 1870 by the French physician Henri Leclerc. Natural compounds synthesized by plants, including primary and secondary metabolites, form the basis of various industrial products both directly and indirectly. Through their metabolic processes, plants transform water, minerals, and other soil-derived elements into bioavailable compounds that the human body can easily assimilate. As a result, these compounds enhance the body's defense mechanisms, support the functionality of organs, and accelerate the healing process (Farnsworth et al., 1985).

Today, medicinal and aromatic plants are utilized across a wide range of sectors, including food, pharmaceuticals, cosmetics, textiles, dyes, and agriculture. The extent of their therapeutic application varies depending on a country's level of development. In developing nations, approximately 80% of the population relies on plant-based treatments. In certain regions of the Middle East, Asia, and Africa, this figure can rise to 95%, whereas it is comparatively lower in developed countries. For instance, usage rates are reported at around 40–50% in Germany, 42% in the United States, 48% in Australia, and 49% in France (Acıbuca & Budak, 2018).

Turkey's role in the global medicinal and aromatic plant trade

Turkey ranks among the leading countries in the trade of medicinal and aromatic plants due to its geographic location, climatic diversity, rich flora, agricultural potential, and extensive land area. This significance is largely attributed to the presence of many plant species within the Turkish flora that serve as sources for herbal medicines, plant-derived chem-

icals, food additives, and products in the cosmetics and perfumery sectors commonly used in developed countries. Consequently, these plants are often harvested from the wild and brought to the market (Bayram et al., 2010). Of approximately 11,000 plant species found in Turkey, nearly 500 are utilized for medicinal purposes (Türkan et al., 2006). Furthermore, the production and utilization of medicinal and aromatic plants are steadily increasing worldwide (Toker et al., 2015).

Turkey exports medicinal and aromatic plants to approximately 100 countries worldwide, including regions such as North America, the European Union, Latin America, the Far East, and North Africa. Leading destinations among these are the United States, Germany, Vietnam, the Netherlands, Poland, Brazil, Canada, Italy, Belgium, Greece, France, and Japan (Dagmar, 2002). The main medicinal and spice plants exported by Turkey include thyme, bay leaves, cumin, anise, fennel seeds, juniper bark, mahaleb, fenugreek, rosemary, licorice root, mint, sumac, sage, and linden flowers (Bayram et al., 2010).

Safe use and standardization requirements of herbal products

Effective utilization of herbal drugs and products for maintaining health, preventing diseases, alleviating everyday ailments, and supporting therapeutic protocols necessitates adherence to specific standards from production to consumption. Firstly, the plant employed must be correctly identified, and its biologically active compounds should be present at adequate levels, as species that appear morphologically similar may differ significantly in their biochemical composition. For instance, medicinal chamomile (*Matricaria recutita* = *Matricaria chamomilla*) contains compounds with anti-inflammatory properties, whereas visually similar species such as *Tanacetum parthenium* carry sesquiterpene lactones like parthenolide, which are utilized in managing migraine attacks.

During cultivation, medicinal plants should preferably follow organic farming or good agricultural practices, be properly dried under suitable conditions, and be free from biological contaminants. From these raw materials, standardized extracts should be prepared in accordance with good laboratory practices, or fixed and essential oils should be obtained using appropriate techniques. The production of fixed oils rich in unsaturated fatty acids via cold pressing or supercritical carbon dioxide extraction is particularly critical for preserving therapeutic efficacy, as oils extracted using heat or chemical solvents often experience significant reductions in pharmaceutical quality.

Standardized extracts and herbal oils must then be processed following good manufacturing practices aligned with pharmaceutical technology principles and converted into pharmacy-grade phytopharmaceuticals, such as pastilles, capsules, tablets, dragees, or syrups. Throughout this process, rigorous control of raw materials, monitoring of production stages, and thorough analysis of finished products are essential. Moreover, herbal products should only be dispensed through pharmacies, under the guidance of a pharmacist and with awareness of the prescribing physician; uncontrolled use may result in reduced efficacy, unexpected adverse effects, or even mortality, while simultaneously compromising the effectiveness of ongoing medical treatment protocols (Houghton et al., 2009).

Diplotaenia turcica

Diplotaenia turcica is a member of the Umbelliferae (Apiaceae) family. According to the GENOM (Generic NOMenclator) database, the Umbelliferae family comprises approximately 464 genera and is particularly widespread across temperate regions, especially in Central Asia. In Turkey, the Umbelliferae family is represented by 109 genera and 450 species, ranking second in terms of overall plant diversity within the country. This indicates that Turkey is a highly rich region for Umbelliferae species diversity in Asia, and this richness may also be significant at a global level. Experimental studies conducted on some species within the Umbelliferae family have revealed notable biological activities, including antihyperglycemic, hypolipidemic, and antioxidant effects (Farkhad et al., 2012; Ahmed et al., 2011; Razavi et al., 2010; Duran et al., 2015).

Until 2011, *Diplotaenia turcica* was known as *Diplotaenia cachrydifolia*. However, research conducted by Pimenov and colleagues in the Bitlis-Van-Hakkâri region led to its identification as a new species within the Umbelliferae (Apiaceae) family (Pimenov et al., 2011). The plant's significance is linked to its use in traditional regional products, such as herb-infused cheese, and its widespread application in folk medicine. Moreover, it has been reported as an antidote for venomous bites from snakes and scorpions, while its root is traditionally employed to manage diabetes, hypertension, and rheumatic disorders (Kaval et al., 2014; Uce & Tunçtürk, 2014).

Preparation of *Diplotaenia turcica* root extract

Diplotaenia turcica is typically harvested between May and June and

subsequently identified taxonomically. The roots collected from the wild are washed with distilled water and cut into small pieces. They are then dried in a shaded, dry environment and ground into a fine powder. A total of 100 grams of the powdered root is soaked in 1000 ml of 96% pure ethanol for 24 hours, followed by filtration. The remaining material is re-extracted with 70% ethanol for an additional 24 hours and filtered again. Both filtrates are combined and dried in an evaporator at 50 °C with a rotation speed of 70 rpm (Farkhad et al., 2012).

Studies on *Diplotaenia turcica*

In a 28-day study conducted by Özdek et al. (2020), the effects of hydroalcoholic root extract of *Diplotaenia turcica* were investigated on lipid peroxidation, antioxidant levels, and immunohistochemical changes in the pancreatic tissue of streptozotocin-induced diabetic rats. The research was carried out on a total of 78 rats, and a notable reduction in glucose levels was observed with increasing doses of *Diplotaenia turcica*. Additionally, pancreatic antioxidant enzyme activities, including GSH, CAT, SOD, GSH-Px, and GSH-R, were significantly reduced in diabetic rats compared to the control group. Oral administration of *Diplotaenia turcica* and glibenclamide markedly improved pancreatic antioxidant activities. Particularly, the group receiving 200 mg/kg of *Diplotaenia turcica* showed more pronounced improvement than the oral glibenclamide group.

Malondialdehyde (MDA) levels in the pancreatic tissue of diabetic rats were significantly elevated compared to controls, whereas treatment with *Diplotaenia turcica* significantly reduced these levels. The researchers reported severe damage to pancreatic β-cells in streptozotocin-induced diabetic rats; however, after treatment with *Diplotaenia turcica* and glibenclamide, atrophy of the Langerhans islets, β-cell degeneration, and necrosis were markedly reduced, and pancreatic architecture was partially restored. The study also demonstrated that increasing doses of *Diplotaenia turcica* significantly improved glucose and insulin levels. Although triglyceride (TG) and LDL cholesterol levels were elevated in the diabetic group, total cholesterol and HDL levels did not change significantly. In groups treated with *Diplotaenia turcica* and glibenclamide, TG and LDL levels decreased; however, these changes were not statistically significant. Based on these findings, Özdek et al. (2020) concluded that the antihyperglycemic effect of *Diplotaenia turcica* is primarily associated with its restorative action on β-cells within the pancreatic Langerhans islets.

Özdek (2020) aimed to investigate the total phenolic and flavonoid contents of the hydroalcoholic extract of *Diplotaenia turcica* and to evaluate its antioxidant and anti-Alzheimer activities. The study revealed that the extract's total phenolic content was higher than its flavonoid content, exhibiting moderate antioxidant properties alongside strong anti-butyrylcholinesterase activity. LC-MS analysis identified malic acid and quinic acid as the most dominant compounds in the extract, while the primary flavonoids were hesperidin and rutin. Furthermore, LC-MS analysis confirmed the presence of various other compounds, including p-coumaric acid, gallic acid, caffeic acid, 4-hydroxybenzoic acid, trans-ferulic acid, chlorogenic acid, and protocatechuic acid.

In a study investigating the antimicrobial effects of *Diplotaenia turcica*, extracts obtained from the aerial parts of the plant exhibited the highest inhibitory activity against *Escherichia coli* and were also effective against *Enterococcus faecalis*. Extracts from the root showed the greatest inhibition against *Bacillus subtilis* and were additionally active against *Pseudomonas aeruginosa* and *Escherichia coli*. The researchers emphasized that *Diplotaenia turcica* holds potential for applications in health-related fields and that further comprehensive analyses could provide significant contributions to the scientific community (Seçkin & Meydan, 2021).

Seçkin (2021) investigated the antimicrobial, antioxidant, and DNA-protective effects of copper nanoparticles (Cu NPs) synthesized via a green method from *Diplotaenia turcica*. The study examined the potential antioxidant capacity and protective properties of these nanoparticles against pBR322 plasmid DNA. Additionally, their antimicrobial activity was evaluated against *Staphylococcus aureus* ATCC 29213, *Pseudomonas aeruginosa* ATCC 27853, *Enterococcus faecalis* ATCC 29212, *Bacillus subtilis* ATCC 6633, *Bacillus cereus* ATCC 10876, *Escherichia coli* ATCC 25952, and *Candida albicans* ATCC 25952. The Cu NPs/Dt were characterized using ultraviolet-visible absorption spectroscopy, Fourier-transform infrared spectroscopy, scanning electron microscopy, X-ray diffraction, and energy-dispersive X-ray analysis. Antioxidant activity analyses revealed that the structure possesses strong antioxidant properties, while Cu NPs/Dt exhibited significant antimicrobial effects. Moreover, the nanoparticles were more effective than the positive control antibiotic against some pathogens and demonstrated concentration-dependent potential to prevent DNA strand breakage.

Colcimen et al. (2019) investigated the effects of *Diplotaenia turcica* root extract on rat ovarian tissue using histological and stereological methods. Four groups, each consisting of seven rats, were established. Excluding the control group, the remaining three groups received daily oral

administration of *Diplotaenia turcica* root extract at doses of 250 mg/kg, 550 mg/kg, and 1000 mg/kg for 28 consecutive days. At the end of the study, rats were decapitated, and right ovarian tissues were collected for histological evaluation. In the treated groups, an increase in the ovarian medulla was observed, along with elevated numbers of corpora lutea and Graafian follicles in the cortex. Comparison of total tissue volume revealed no significant differences among groups. Analysis of follicle counts showed a significant decrease in primordial follicles in the treated groups compared to controls. No notable changes were observed in primary follicle numbers, while a significant increase was detected in Graafian follicles. The researchers concluded that *Diplotaenia turcica* may influence the follicular maturation process, leading to the observed increase in Graafian follicles.

Potential problems arising from the uncontrolled use of herbal products

Herbal products have been a fundamental component of traditional therapies for centuries and continue to be widely used in complementary or alternative medicine today. However, the pharmacological effects of plant-based preparations are not always thoroughly investigated scientifically, rendering the reliability of these products questionable. Uncontrolled use may lead to serious health issues due to incorrect dosages, selection of inappropriate plant species, improper preparation methods, or interactions with other medications. Toxic effects on the liver and kidneys, allergic reactions, and drug–food interactions are among the most frequently reported adverse outcomes.

Another aspect of uncontrolled herbal product use involves applications based on hearsay or anecdotal information. Such approaches, which lack scientific evidence, may lead patients to delay or refuse conventional medical treatments, potentially exacerbating existing conditions. Additionally, the presence of herbal products on the market that are produced under non-standard conditions, with uncertain active ingredient content or possible contamination, poses significant public health risks. Therefore, the use of herbal products should be guided by scientific evidence, healthcare professional recommendations should be followed, and public awareness of responsible usage must be promoted.

In phytotherapy, undesirable outcomes often arise from incorrect taxonomic identification and naming of plants. Diagnosing plants solely based on external morphology can lead to serious errors due to structural similarities. For instance, the highly toxic *Colchicum autumnale* closely

resembles parsley. Even if the plant collected from the wild is correctly identified, mislabeling during packaging may occur. Furthermore, subspecies of a single plant may possess different biochemical compositions, making accurate identification critical not only at the species level but also at the subspecies level.

Herbal products can be harmful rather than beneficial if not administered to the appropriate individual, at the correct time, in the proper manner, and at the right dose; they may also interact with conventional medications. For instance, individuals with hypertension should avoid consuming more than 2 grams of licorice daily for complaints such as hoarseness or cough, as licorice can disrupt the sodium–potassium balance, leading to elevated blood pressure. Herbal prescriptions given on television programs can also be dangerous, as the viewer's age, current medication use, and overall health status are unknown. For example, a person taking a statin for cholesterol who follows a program recommending St. John's Wort tea for depression may experience severe side effects. Similarly, someone using Ginkgo leaves to enhance memory or prevent forgetfulness while on anticoagulant therapy faces a bleeding risk. Moreover, the growing interest in herbal products has led to the discovery of synthetic compounds in certain products marketed for weight loss or performance enhancement, which may cause serious health issues, including heart attack and death (Aranson, 2008).

Conclusion

The potential health effects of herbal products represent a significant area of research, particularly for species whose traditional uses are supported by modern scientific evidence. In this context, *Diplotaenia turcica*, an endemic plant of the Turkish flora, stands out due to its bioactive compounds and merits consideration from a phytotherapeutic perspective. Initial findings indicate that this plant exhibits antioxidant and anti-inflammatory properties, as well as cellular protective effects. However, to fully elucidate these activities, advanced toxicological, pharmacological, and clinical studies are required. Such investigations would not only validate the traditional uses of *Diplotaenia turcica* scientifically but also contribute to the development of natural and reliable therapeutic alternatives.

Diplotaenia turcica is an endemic plant that has been traditionally used in medicine and is only recently gaining attention through modern scientific research. Its high content of phenolic compounds, flavonoids, and essential oils confers significant bioactive potential, enhancing its pharmacological value. The antioxidant, antimicrobial, anti-inflammato-

ry, and potential anticancer properties of these phytochemicals provide a foundation for considering this plant as a complementary or alternative therapeutic agent in healthcare.

Experimental and laboratory-based studies have demonstrated that *Diplotaenia turcica* extracts can effectively neutralize free radicals, thereby reducing oxidative stress. This property may contribute significantly to the prevention or deceleration of oxidative stress-related disorders, such as diabetes, cardiovascular diseases, and neurodegenerative conditions. Furthermore, the plant's broad-spectrum antimicrobial activity offers opportunities to explore its potential as a natural protective agent, particularly in the context of increasing antibiotic resistance.

This plant also holds potential applications in veterinary medicine, showing promise in reproductive biotechnology, tissue repair, and animal health-supporting supplements. Histological and biochemical analyses, in particular, indicate that *Diplotaenia turcica* may exert protective and restorative effects at the cellular level. However, it should be noted that most of the current evidence is limited to laboratory or animal studies, highlighting the need for human-based research.

In conclusion, *Diplotaenia turcica* holds a notable position among herbal approaches in health due to its rich phytochemical composition and multifaceted biological activities. However, translating this potential into clinical applications requires comprehensive toxicological evaluations, precise dosage determinations, and long-term safety studies. Such efforts would contribute to the development of more reliable, effective, and natural therapeutic options for both human and animal health.

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Chapter 4

PHARMACOLOGİCAL SİGNİFİCANCE OF PLANTS AND POTENTİAL HEALTH BENEFİTS OF *DİPLOTAENİA TURCİCA*

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Introduction

Throughout history, plants have served as primary resources in both traditional and modern medicine for preventing and treating diseases. Today, the pharmacological effects of naturally derived plant compounds have become a central focus of numerous scientific studies. These compounds, known as phytochemicals, exhibit diverse biological activities such as antioxidant, antimicrobial, anti-inflammatory, hepatoprotective, and anticancer effects, demonstrating considerable pharmaceutical potential. Their generally low toxicity and ability to influence multiple metabolic pathways make natural products valuable in drug development processes.

In this context, Diplotaenia turcica, a plant endemic to Turkey, has emerged as an important research target because of its bioactive constituents. Although studies on this species remain scarce, researchers have begun examining its ability to counteract oxidative stress-induced cellular damage, its antioxidant potential, and possible anti-inflammatory actions. Thorough evaluation of the pharmacological characteristics of Diplotaenia turcica will not only provide a scientific basis for its traditional applications but may also facilitate the identification of novel phytotherapeutic compounds for natural healthcare solutions.

Herbal medicines: Historical and contemporary perspectives

The earliest medicines used by humans were derived from fruits, seeds, flowers, leaves, stems, or roots of surrounding plants. Through trial and error, people identified which plant parts were effective for specific health issues and passed this knowledge across generations, forming traditional healing practices (Schulz et al., 2004). Archaeological evidence indicates that humans primarily relied on plants both for nutrition and to address health problems. This long-standing relationship between humans and plants has ultimately led to the emergence of ethnobotany, a scientific field now widely recognized and studied worldwide (Koçyiğit, 2005).

Throughout human history, numerous health conditions, including diabetes, jaundice, and shortness of breath, have been treated using plants. The World Health Organization reports that approximately four billion people worldwide primarily rely on herbal drugs to address health problems. Moreover, it is known that nearly one-quarter of prescription medicines in developed countries contain active ingredients derived from plants (Farnsworth et al., 1985).

From prehistoric times through the periods of Mesopotamia, Ancient Egypt, the Hittites, Greece, Rome, the Seljuks, and the Ottomans, herbal medicines have been employed. During the Republican era, research on traditional medicine was also conducted. It is known that people in Anatolia have used plants for therapeutic purposes since the Paleolithic period and have benefited from them for various applications for approximately 50,000 years (Özbek, 2005).

Medicinal and aromatic plants: Production, sustainability, and global trade perspectives

Medicinal and aromatic plants are primarily sourced from the Aegean, Marmara, Mediterranean, Eastern Black Sea, and Southeastern Anatolia regions. To utilize their sustainable production and market potential effectively, it is crucial that these plants are available in sufficient quantities and high quality. Developing improved varieties, determining suitable ecological conditions, harvesting wild plants without harming nature, and applying post-harvest handling and processing technologies are necessary to obtain standardized and high-quality products that meet consumer and industrial demands. These measures are expected to enhance both the production capacity and market value of medicinal and aromatic plants (Bayram et al., 2010).

Examining the production and use of medicinal and aromatic plants, socio-political events and technological advancements at the beginning of the 20th century led to a decline in herbal medicine use. The increase in synthetic drug production during the 1930s and 1940s reduced interest in plant-based treatments, particularly in Western countries. However, from the 1980s onward, growing health awareness, efforts to avoid potential chemical side effects, and rising demand for natural and organic products have restored the importance of medicinal and aromatic plants. In response, developed countries re-evaluated herbal therapies and initiated scientific studies. During this period, medicinal and aromatic plants were safely cultivated, production was increased, and they were made available for public use. Additionally, these plants began to be used in the cosmetic industry for applications such as weight management and anti-aging products. Today, medicinal and aromatic plants remain highly significant and are actively utilized in various fields (Bayram et al., 2010).

Due to the large number of medicinal and aromatic plants in trade and the considerable variation in their active compound content, a single classification system cannot be applied in trade statistics. The most reliable data on the global trade volume and value of these plants are available

from the International Trade Centre database in Geneva. Over the past five years, the international trade of herbal drugs has averaged 16.8 billion USD in exports and 18.6 billion USD in imports. The most important plant types in production include bulbs and tubers, tea and coffee, spices, condiments, roots, and other plant groups. Among the countries importing medicinal and aromatic plants, the United States, United Kingdom, Germany, France, the Netherlands, China, and India also rank among the leading exporters of many plant species (Binici, 2002).

Quality and standardization in medicinal and aromatic plants

Medicinal and aromatic plants serve as raw materials in various industries, including pharmaceuticals, food, cosmetics, and perfumery. The reliability and consistency of their effects largely depend on proper quality control and standardization procedures. Inadequate quality management can lead to variations in active compound content, incorrect plant species identification, contamination, and improper storage conditions. Such issues may not only reduce the therapeutic efficacy of the products but also pose significant health risks.

Accurate identification of the correct plant species represents a fundamental step in quality assessment. Errors in taxonomic classification, the use of incorrect species, or the mixing of visually similar toxic plants can lead to undesired pharmacological effects and, in some cases, poisoning. Therefore, verification through botanical examination, pharmacognostic analyses, and, when necessary, modern techniques such as DNA barcoding is essential.

Another aspect of standardization involves the qualitative and quantitative determination of active components. The content of these compounds in herbal products can be influenced by multiple factors, including cultivation conditions, harvest timing, drying, and storage methods. To manage such variations, active ingredient analyses are performed using chromatographic and spectroscopic techniques, and the compliance of products with the quality standards defined in pharmacopoeias is carefully evaluated.

Additionally, impurities such as heavy metals, pesticide residues, microbial contamination, and toxic metabolites must be thoroughly examined during quality control procedures. Organizations such as the World Health Organization, the European Pharmacopoeia, and similar authorities have established quality parameters and limit values to ensure the safe consumption of herbal products.

In medicinal and aromatic plants with diverse applications, it is essential to conduct quality assessments and establish standardized criteria. The importance of quality standards has been increasingly recognized in recent years. In addition to basic tests, specific investigations are also carried out (Phillipson, 1993). The Turkish Standards Institute has conducted studies on certain medicinal and aromatic plants; however, these efforts are limited and cover only selected species. Therefore, these standards should be expanded and updated to meet current requirements (Bayram et al., 2010).

In recent years, organizations such as the European Pharmacopoeia, the World Health Organization, and the European Scientific Cooperative on Phytotherapy have begun publishing monographs for the standardization of herbal raw materials intended for pharmaceutical production. In Germany, the Federal Health Agency established an expert committee called "Commission E" to evaluate the safety and reliability of herbal medicines (Başer, 1998).

In conclusion, quality and standardization in medicinal and aromatic plants are crucial for protecting public health, ensuring product reliability, and enhancing competitiveness in international trade. Without scientifically based production, effective quality control, and appropriate standardization practices, the safe and efficient use of herbal products cannot be guaranteed.

Quality, safety, and traditional use of *Diplotaenia turcica* in medicinal plants

Plants intended for therapeutic use may be contaminated with microorganisms, pesticides, or heavy metals. In processed herbal products, additional risks may include various toxins, foreign toxic plants, and residues of synthetic drugs. Such issues are particularly common when quality control is lacking. Variations in plant collection methods, harvest timing, post-harvest transport and storage conditions, and processing procedures can lead to both qualitative and quantitative differences in active compound concentrations, making accurate dosage determination challenging (Van Breemen et al., 2008; Fong, 2002; Chan, 2003).

In recent years, another factor increasing the value of medicinal plants is the emergence of resistant strains due to pathogen resistance development. Preparations derived from medicinal plants exhibit multi-target effects, making them effective against new strains. Consequently, there has been a renewed interest in plant-based remedies. A notable example is

malaria. Although synthetic drugs, such as Atebrin, are used to treat malaria, quinine obtained from the chinchona tree, which grows in tropical regions, remains highly significant (Ceylan, 1995).

In recent years, serious side effects caused by synthetic drugs, along with the resulting medical and economic problems, have contributed to the renewed popularity of plant-based therapies (Özbek, 2005).

Diplotaenia turcica is an endemic, long-lived plant with a woody root system that can reach approximately 1.5–2 meters in height. This species blooms white flowers in August and is locally known as "siyabo" (Özdek et al., 2020). The local population uses *Diplotaenia turcica* both in culinary applications and for therapeutic purposes; it is also incorporated into the production of herb-flavored cheese. Traditionally, the plant is preferred for managing diabetes, hypertension, and rheumatic disorders (Uce & Tunçtürk, 2014).

Experimental and pharmacological studies on *Diplotaenia turcica*

In an experimental study conducted on 78 rats, diabetes was induced by streptozotocin, and the effects of the hydroalcoholic extract of *Diplotaenia turcica* root on lipid peroxidation, antioxidant levels, and immunohistochemical alterations in pancreatic tissue were investigated. The researchers observed that increasing doses of *D. turcica* resulted in a marked reduction in glucose levels. Moreover, the activities of pancreatic antioxidant enzymes (GSH, CAT, SOD, GSH-Px, and GSH-R) were significantly lower in diabetic rats compared to the control group. Notably, treatment with 200 mg/kg *D. turcica* extract produced greater improvement than oral glibenclamide administration. The investigators also found that higher doses of *D. turcica* significantly improved glucose and insulin levels. Following streptozotocin exposure, HbA1c and blood glucose levels increased, whereas insulin concentrations decreased. These findings suggest that streptozotocin-induced pancreatic beta-cell damage reduces insulin synthesis, leading to elevated blood glucose levels (Özdek et al., 2020).

Özdek (2020) conducted another study aiming to evaluate the total phenolic and flavonoid contents of the hydroalcoholic extract of *Diplotaenia turcica* and to determine its antioxidant and anti-Alzheimer activities. The researcher reported that the extract inhibited the BChE enzyme by 76.57%, whereas the standard drug galantamine showed an inhibition rate of 84.3%. No inhibitory effect of the extract was detected on the AChE enzyme. The author emphasized that the extract exhibited strong

anti-butyrylcholinesterase activity. Furthermore, malic acid and quinic acid were identified as the major compounds, along with several other bioactive molecules, including p-coumaric acid, gallic acid, caffeic acid, 4-hydroxybenzoic acid, trans-ferulic acid, chlorogenic acid, and protocatechuic acid.

Seçkin & Meydan (2021) investigated the antimicrobial potential of *Diplotaenia turcica* against certain pathogenic microorganisms. Their findings revealed that the extract obtained from the aerial parts of the plant exhibited activity against the Gram-negative bacterium *Escherichia coli*, whereas the root-derived extract showed notable inhibitory effects on the Gram-positive bacterium *Bacillus subtilis*.

Seçkin (2021) examined the antioxidant and DNA-protective effects of copper nanoparticles synthesized from *Diplotaenia turcica* using the green synthesis method, and reported that the plant exhibited strong antioxidant activity. The study also evaluated the antimicrobial activity of these nanoparticles against *Staphylococcus aureus* ATCC 29213, *Pseudomonas aeruginosa* ATCC 27853, *Enterococcus faecalis* ATCC 29212, *Bacillus subtilis* ATCC 6633, *Bacillus cereus* ATCC 10876, *Escherichia coli* ATCC 25952, and *Candida albicans* ATCC 25952. The results demonstrated a significant antimicrobial effect of the nanoparticles. Furthermore, they were shown to be more effective than the positive control antibiotic against certain pathogens and exhibited the potential to prevent DNA strand breaks in a concentration-dependent manner.

In another study, the effects of the root extract of *Diplotaenia turcica* on primordial, primary, and Graafian follicles in rat ovarian tissue were examined using histological and stereological methods. In the extract-treated groups, an increase in the ovarian medulla was observed, along with a higher number of corpus luteum and Graafian follicles in the cortex. Compared with the control group, a significant reduction in the number of primordial follicles was reported in the treated groups. While no notable difference was detected in primary follicle counts, the number of Graafian follicles increased significantly. The authors concluded that *D. turcica* promotes follicular maturation, thereby enhancing Graafian follicle development. Based on these findings, they suggested that *D. turcica* exerts biological effects on ovarian tissue (Colcimen et al., 2019).

Adverse effects and drug ınteractions of medicinal plants: Safety considerations

The adverse effects of plants and their potential interactions with conventional drugs are not fully understood. When plants or herbal products are used together with prescription medications for treatment or disease prevention, careful attention is required regarding possible drug interactions and side effects (Cupp, 2000).

Particularly, the use of unlicensed herbal medicines with unproven efficacy and reliability, lacking proper labeling and standardization, and sold in uncontrolled markets, has increased. This trend has led to a noticeable rise in scientific publications worldwide, especially in the United States and Europe, drawing attention to the potential adverse effects of such products.

Without medical guidance, many medicinal plants, such as chamomile, sage, mint, and lemon grass, are used at home almost like conventional drugs to relieve minor ailments. However, uncontrolled or incorrect use can lead to adverse health outcomes. Therefore, for the treatment of serious illnesses, medical advice and supervision are essential to avoid mismanagement (Özer et al., 2001).

The main concern in herbal medicine is the use of plants that have not undergone sufficient clinical research as if they were conventional drugs. Furthermore, interactions between these products and pharmaceuticals, other herbal products, or foods can lead to serious health issues. Studies indicate that a large proportion of herbal product users do not inform healthcare professionals, such as physicians, pharmacists, dentists, or nurses, about the products they consume. The use of herbal products obtained from non-specialist herbalists, unregulated media sources, or online platforms may result in severe interactions involving herbal product–drug, herbal product–disease, or herbal product–organ relationships (Erdem & Ata Eren, 2009; Ernst, 2011; Ersöz, 2011; Koçak, 2013).

Panax ginseng is classified in the alternative medicine literature as an "adaptogen" that enhances resistance to physical, chemical, and biological stress, while also improving overall health by boosting both physical and mental capacities. Due to these properties, assessing its side effects and potential drug interactions is challenging, as available ginseng formulations vary widely and the exact content of ginseng in these products cannot be reliably determined. The use of Panax ginseng is considered inadvisable in individuals with high blood pressure, acute asthma, acute infections, nosebleeds, or excessive menstruation. These effects are particularly pronounced with high doses or prolonged use (Kiefer & Pantuso,

2003).

The mixing of visually similar plants can also lead to undesirable outcomes. For instance, medicinal chamomile (*Matricaria chamomilla*), commonly used as tea for upper respiratory tract infections, can be easily confused with various other species. While substitution with certain species may result in a lack of the intended therapeutic effect, contamination with plants such as Dalmatian chrysanthemum (*Tanacetum cinerariaefolium*), which has insecticidal properties, Canary weed (*Senecio* species), which contains hepatotoxic compounds, or dog chamomile (*Anthemis cotula*), which may trigger allergic reactions, can lead to severe poisoning (Ersöz, 2011).

In conclusion, although herbal products can provide supportive benefits when used appropriately, their misuse poses significant health risks. Therefore, it is essential that these products are not consumed arbitrarily and are used under the guidance of healthcare professionals, such as physicians and pharmacists, with careful attention to dosage, potential interactions, and duration of use.

Conclusion

Plant-based therapeutic approaches are gaining increasing importance in modern medicine, and the phytochemical potential of endemic species offers promising prospects for the development of new pharmaceutical agents. *Diplotaenia turcica*, naturally present in the flora of Turkey, is among the notable species due to its bioactive compounds. Detailed investigation of its pharmacological effects can support traditional knowledge with scientific evidence while contributing to the development of natural and safe therapeutic alternatives. Future in vitro, in vivo, and clinical studies are expected to clarify the positive health effects of *Diplotaenia turcica* and provide a new perspective on plant-based therapies.

Plants have served for centuries as one of the primary natural resources for maintaining human health and treating diseases. Modern pharmacology derives the raw materials of many drugs directly from plants or their bioactive compounds. These natural components, known as phytochemicals, exhibit diverse biological effects, including antioxidant, antimicrobial, anti-inflammatory, and anticancer activities, playing important roles in both preventive and therapeutic strategies. Today, the rising prevalence of chronic diseases and the side effects of synthetic drugs have further increased interest in plant-based treatment approaches.

Diplotaenia turcica, an endemic species primarily found in Central Anatolia, possesses high pharmacological potential due to its phenolic compounds, flavonoids, and essential oils. These constituents have been shown to reduce oxidative stress, prevent cellular damage, and strengthen the immune system. Moreover, studies indicate that the plant's antimicrobial effects may be effective against a broad range of microorganisms. This property is particularly significant today, as it supports the development of natural and reliable alternative therapeutic agents in the context of increasing antibiotic resistance.

In terms of antioxidant activity, *Diplotaenia turcica* may contribute to the protection of cell membranes, DNA structures, and enzyme functions by neutralizing free radicals. This mechanism can play an important role in reducing the risk of cardiovascular diseases, diabetes, neurodegenerative disorders, and even certain types of cancer. Additionally, its anti-inflammatory properties may serve as a supportive agent in managing chronic inflammation-based conditions.

Diplotaenia turcica also holds notable potential in veterinary medicine, where it can be utilized in the development of phytotherapeutic products supporting reproductive biotechnology, tissue repair, and overall animal health. Histological and biochemical analyses indicate that this plant may exert protective effects at the cellular level, particularly maintaining structural integrity in reproductive organs and tissues.

In summary, *Diplotaenia turcica* is a valuable plant species with natural, effective, and versatile pharmacological properties suitable for both human and animal health. However, most existing data are at the experimental level, and more comprehensive human studies, toxicity tests, and long-term safety evaluations are required before clinical application. Multidisciplinary research conducted in this context will help establish *Diplotaenia turcica* as a reliable phytotherapeutic agent in modern medicine.

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Chapter 5

PULMONARY REHABILITATION IN LUNG CANCER PATIENTS

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SUMMARY

The clinical features of lung cancer include the side effects of tumor invasion, as well as those caused by radiotherapy, chemotherapy, and targeted treatments—such as decreased physical capacity, decline in lung function, fatigue, and psychological effects. Pulmonary rehabilitation (PR) is a multidisciplinary, patient-specific, planned, effective, and non-invasive intervention for people with chronic respiratory disease and impaired lung function. PR aims to increase exercise capacity, functional status, and quality of life by addressing all aspects of the disease, promoting an active lifestyle, and encouraging positive behavior changes.

This article examines lung issues faced by cancer patients and explores pulmonary rehabilitation methods for these problems.

INTRODUCTION

The clinical features of lung cancer include the side effects of tumor invasion, as well as those caused by radiotherapy, chemotherapy, and targeted treatments—such as decreased physical capacity, decline in lung function, fatigue, and psychological effects. Although surgical resection remains the most effective treatment with curative intent, many cases are inoperable at diagnosis due to metastasis. Many patients also have chronic obstructive pulmonary disease (COPD), which often makes surgery more difficult because of the high risk of complications. COPD further complicates treatment, leading to a multidisciplinary and more complex process (1-7).

Pulmonary rehabilitation (PR) is a multidisciplinary, patient-specific, planned, effective, and non-invasive intervention for people with chronic respiratory disease and impaired lung function. PR aims to increase exercise capacity, functional status, and quality of life by addressing all aspects of the disease, promoting an active lifestyle, and encouraging positive behavior changes. These programs incorporate essential components of exercise training, such as strengthening respiratory muscles, providing nutrition counseling, offering psychosocial support, facilitating behavioral changes like smoking cessation, and delivering patient education. Today, pulmonary rehabilitation has become a fundamental part of comprehensive care for lung cancer patients (2-6).

A detailed history should be taken, symptoms evaluated, physical examination performed, and necessary laboratory tests conducted before pulmonary rehabilitation (PR). Daily life activities (GYA), basic daily activities such as changing clothes and bathing, housework, leisure activities, employment activities, and sexual behaviors should be assessed

by evaluating the daily life activities (GYA). Past and current smoking status should be determined to assess dependence. Awareness of the disease should be evaluated to see if the patient accurately recognizes their current condition and can follow their doctor's instructions. The level of training can also indicate whether the patient understands the instructions and cooperates with medical staff to complete the necessary treatment steps.

Scales such as the Beck Anxiety Inventory and the Beck Depression Scale are recommended for assessing mood. Additionally, factors like exercise capacity, nutritional status, and social and mental functioning should be evaluated. Height, weight, body mass index (BMI), fat mass to fat-free mass ratio, and muscle mass should also be measured. At the beginning and conclusion of pulmonary rehabilitation (PR), hand grip strength and quadriceps power are assessed to indicate skeletal muscle performance.

The respiratory function test alone does not fully reflect the cardiopulmonary condition; therefore, it is recommended to evaluate it together with the 6 Minute Walk Test (6MWT), staircase climbing test, and Cardiopulmonary Exercise Test (CPET). Oxygen saturation and heart rate are monitored throughout the test, and respiratory and leg fatigue after exercise are assessed using the modified Borg scale. Patients' respiratory functions are tested with spirometry, including forced vital capacity (FVC), forced expiratory volume in 1 second (FEV1), the ratio of FEV1 to FVC (FEV1%), and the forced vital capacity to FVC ratio. Therefore, a CPET is recommended if preoperative FEV1 and/or carbon monoxide diffusion capacity (DLCO) are below 80% of predicted values. Minute ventilation to carbon dioxide output ratio (VE/VCO2) predicts the slope, surgical complications, and mortality. Peak oxygen uptake (VO2peak) has also been shown to be a good predictor of surgical complications, higher VE/VCO2 slope, and increased mortality in patients undergoing lung resection.

The COPD assessment includes a BODE index that considers body mass index (BMI), airway obstruction (FEV1), dyspnea scale (mMRC), and exercise capacity. Additionally, a comprehensive evaluation of COPD severity, categorized into groups A / B / C / D, involves spirometry results, COPD ignition history, mMRC, and the COPD evaluation test (CAT).

It is recommended to begin before the operation and continue PR for an extended period afterward. Preoperative PR can help improve cardiopulmonary function, reduce surgical complications, shorten post-operative hospital stays, and lower medical costs. Patients are advised to undergo PR about two weeks prior to surgery (5 days a week) to achieve optimal

results.

Postoperative patients are advised to engage in appropriate breathing and aerobic exercises, such as deep breathing, running, swimming, and climbing stairs, early after surgery. Additionally, lung function tests should be performed regularly. The exercise intensity should be adjusted to each individual's tolerance to ensure it matches their capabilities. This level of intensity can be maintained for 3-6 months, with gradual increases based on improvements in lung function and respiratory symptoms. It has also been reported that postoperative rehabilitation exercises should ideally last about 1 year to enhance lung function. Pulmonary rehabilitation offers significant improvements in VO2 max, helping suitable candidates for surgery to undergo successful resection. It has been noted that planning personalized pulmonary rehabilitation, especially before surgery in lung cancer cases, positively influences the recovery process, increases functional capacity, and improves quality of life. Pulmonary rehabilitation programs also benefit clinical outcomes in lung cancer patients with COPD by reducing surgical complications. Poor exercise capacity (peak oxygen consumption <15 mL/kg/min) is a key factor in postoperative morbidity and mortality after pneumonectomy. Effective pulmonary rehabilitation can improve cardiopulmonary function and exercise tolerance and is valuable for improving long-term survival. A notable increase in FEV1 was observed at 1 month after surgery in patients who received intra-hospital pulmonary rehabilitation compared to those who did not. Exercise training is classified as endurance, intermittent, resistance, respiratory muscle, and balance training depending on its purpose (1-9).

Most lung cancer cases are non-small cell lung cancer (CWC). When possible, surgical resection is the main treatment option. However, there is no universal agreement in the literature on post-surgical rehabilitation methods, duration, and timing. Respiratory muscle strengthening exercises included in current pulmonary rehabilitation programs can enhance respiratory function, increase exercise capacity, and reduce dyspnea—especially by targeting inspiratory muscles. Postoperative respiratory pattern training, positive pressure breathing, resistance exercises, and abdominal muscle exercises all contribute positively to lung function, quality of life, and exercise tolerance during the 1-6 month period (8-13).

Although the effect of inspiratory muscle training (IMT) on maintaining respiratory muscle strength is limited, it can improve oxygenation in high-risk patients. A six-week combination of IMT and aerobic exercise can increase respiratory muscle strength and exercise capacity after video-assisted thoracoscopic surgery (VATS). Therefore, inspiratory muscle strengthening training is seen as a practical way to prevent complications

such as postoperative dyspnea and cough. Pulmonary rehabilitation enhances maximum inspiratory pressure (MIP), effort intolerance, quality of life, and tidal volume in CWC patients. MIP is a measure that directly assesses the strength of muscles involved in inspiration, especially the diaphragm, and relates to COPD severity. Techniques like diaphragm breathing are specifically designed to strengthen these muscles.

In addition, techniques such as deep breathing, shrunk lip breathing, and active coughing exercises have the potential to reduce airway sensitivity in CHAKRA patients (8-13).

Postoperative PR alleviates dyspnea, exercise intolerance, and wound pain caused by surgery, and also enhances disease-related anxiety and depression. Short- or long-term programs improve quality of life along with lung function. Although it typically takes 1-2 months, 20-week protocols have been established. The application frequency is 3-5 days per week.

Types of exercises included in PR are as follows:

- Endurance training involves cycling or walking; it enhances cardiopulmonary function, increases physical activity, and reduces dyspnea and fatigue. It can be adapted for low-performance patients using low-intensity or intermittent methods.
- Interval training: Alternating high-intensity exercises with brief rest periods lowers symptom scores in COPD patients.
- Resistance and strength training: Builds muscle strength and mass through repeated heavy lifting, preserves bone density, and decreases shortness of breath.
- Upper extremity exercises: These are aerobic or resistance workouts that aid daily life activities.
- Flexibility exercises: Enhance respiratory capacity by improving posture and chest mobility. Focus on the main muscle groups and the range of motion of the joints.
- Inspiratory muscle training: Enhances exercise capacity and alleviates dyspnea, especially in individuals with weakened respiratory muscles. The most common method is inspiratory pressure threshold loading (≥ 30% of MIP).
- Neuromuscular electrical stimulation: It reduces ventilation and cardiac load by inducing muscle contractions, particularly beneficial for patients with severe functional limitations.

All exercises should be planned according to FITT principles (frequency, intensity, duration, type). In individuals with COPD, according to ACSM:

- Frequency: 3 to 5 days per week
- Density: 50-80% of peak power output, 4-6 on Borg CR-10, 12-14 on RPE
- Duration: 20-60 minutes at medium-high intensity; short low-intensity intervals may be added if incomplete.
- Type: Aerobic activities like walking, stationary bike, upper body handcycle

Airway Secretion Clearance Techniques

- **Effective coughing:** Voluntary removal of secretions
- **Postural drainage**: Transport of secretions to the trachea with the help of gravity
- **Mechanical vibration:** Mobilization of airway secretions by vibrating the chest wall (7)

They are good predictors for demanding expiratory volume in one second (FEV1), vital capacity (FVC), DLCO, cardiopulmonary exercise test (CPET), and pulmonary resection. Pulmonary rehabilitation has been shown to improve cardiopulmonary function, exercise tolerance, anxiety, and depression. It has been stated that preoperative pulmonary rehabilitation can enhance FEV1, FVC, and the 6-minute walk test (6MWT). Besides lung function, it has also been indicated that cardiac function improves after preoperative exercise training. Additionally, pulmonary rehabilitation may reduce cytokine and inflammation factor levels. PR has been demonstrated to boost exercise tolerance and effectively combat dyspnea and fatigue (10-15).

In patients with lung cancer, it may be more beneficial to enhance exercise capacity and quality of life. PR involves a combination of practices, including accurate diagnosis, therapy, emotional support, and training.

The progressive course of lung cancer can cause patients to experience serious impairments in respiratory system functions. Conditions such as obstruction caused by the tumor in lung tissue, deterioration of alveolar gas exchange, weakening of respiratory muscles, and dyspnea due to physical activity limit patients' daily life activities. Additionally, as a result of chemotherapeutic treatments, radiotherapy protocols, and invasive

surgical procedures, multiple complications like systemic fatigue, loss of muscle mass, and exercise intolerance can develop. These factors lead to not only physical challenges but also emotional and cognitive ones.

The primary reason for PR is that it offers a comprehensive approach to this complex clinical situation. Key goals of PR include retraining respiratory muscles, improving oxygen utilization efficiency, enhancing exercise endurance, and increasing individual adherence to therapy. Emphasizing that planning PR to be patient-centered and personalized can boost treatment success is important. This highlights the value of rehabilitation tailored to the patient's specific clinical characteristics, rather than generic physical activity advice.

However, PR not only aids in physical recovery but also helps patients strengthen their coping mechanisms for the disease and boosts their psychological resilience. Studies in the literature have shown that PR can also improve symptoms such as depression, anxiety, and sleep disorders. Therefore, PR practices should be conducted by a multidisciplinary team: respiratory therapists, physiotherapists, psychologists, nutritionists, and oncology clinical staff are essential components that enhance the effectiveness of rehabilitation (4,8-15).

PR applications in the process of preparing for surgery play an important role in increasing patients' suitability for surgery and improving the postoperative period. The literature often reports the effects of PR on reducing surgical risks, shortening hospital stays, and preventing complications. Benzo meat. (2011) reported in two separate randomized controlled trials that postoperative complication rates were significantly lower for patients who underwent PR before surgery, along with a reduced hospital length of stay. These findings indicate that PR is a vital intervention tool before surgery.

Morano et al. (2013) showed that four weeks of pulmonary rehabilitation before surgery in patients with respiratory comorbidities such as COPD lead to significant improvements in postoperative pulmonary functions and reduced respiratory morbidity. The study found that postoperative respiratory complications were fewer, and hospital stay and chest tube duration were shorter. The content of PR included many modules such as aerobic exercises, respiratory technique training, muscle-based resistance exercises, postural drainage techniques, and patient education.

Goldsmith et al. (2021) reported that some patients previously considered "inoperable" for surgery became suitable after PR. This significant finding shows that PR offers more than symptom relief; it also provides prognostic value regarding surgical eligibility. The fact that patients with

low FEV_1 values and limited exercise tolerance can be physically prepared for surgery with PR indicates that this approach directly influences medical decision-making. This method helps to improve long-term survival rates for lung cancer patients by increasing resection rates (17).

However, there are shortcomings in standardization regarding the rehabilitation period and implementation protocol in the literature. These differences make it difficult to determine how effective PR is for different patient groups before surgery. Additionally, studies on the psychological effects of PR on preoperative stress and surgical fear are limited. In this context, both physical and psychosocial components should be taken into account.

The primary aim of pulmonary rehabilitation (PR) in patients with advanced lung cancer is symptom management, enhancing quality of life, and preserving independence. It is recognized that PR helps control symptoms such as dyspnea, muscle weakness, and fatigue, and also supports psychosocial recovery (16). It has also been suggested that during palliative care, PR reduces symptoms like shortness of breath, anxiety, and sleep disturbances, while also aiding the psychosocial adaptation process. In this context, PR is not just a way to increase physical capacity but also a comprehensive therapy that provides mental relief, helping patients find effective support during their final stages of life (16). (2022) highlighted that home PR increases physical endurance and improves the patient's independence in daily activities (18).

RESULTS

Pulmonary rehabilitation is a multidisciplinary treatment that offers various benefits, including preparing patients for lung cancer surgery, reducing postoperative complications during recovery, shortening hospital stays, increasing functional capacity, and providing palliative support.

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