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Aims, Scope, and Audience
Pharmata aims to contribute to the scientific literature by publishing manuscripts of the highest caliber. The journal accepts research articles, reviews, and short communications that adhere to ethical guidelines.

The scope of the journal encompasses various topics, including but not limited to:

1. Pharmaceutical analysis of complex systems
2. Quality control and methods for biotech drugs
3. Action mechanisms and metabolism of drugs in the body
4. Quantitative and qualitative analysis in the drug screening process
5. Molecular pharmacology
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14. New biochemistry methods for pharmaceutical analysis
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16. New analytical techniques and methods
17. Pharmaceutical chemistry
18. Synthesis and analysis of new drug molecules
19. Other areas: pharmaceutical solid materials (including biomaterials, polymers, and nanoparticles), biotechnology products (including genes, peptides, proteins, and vaccines), engineered cells.

The target audience of the journal includes researchers and specialists who have an interest in or are working in any of the fields covered by the journal's scope.

You can find the current version of the Instructions to Authors at https://pharmata-ataunipress.org/.

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Pharmata

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REVIEW

ATATURK UNIVERSITY PUBLICATIONS

Research Article

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Neuroprotective Effects of Pregnenolone Against Oxidative Damage in a 6-Hydroxydopamine-Induced Parkinson's Disease Cell Model Using SH-SY5Y Cell Line

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ABSTRACT

Objective: Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by the degeneration of dopaminergic neurons, leading to motor impairments and cognitive deficits. Despite advances in understanding PD's pathophysiology, effective therapeutic interventions are limited. Oxidative stress, resulting from the overproduction of reactive oxygen and nitrogen species, plays a crucial role in PD progression. Neurosteroids, naturally occurring brain steroids, have been implicated in dopamine signaling regulation and are disrupted in PD. Pregnenolone (Prgn), a neurosteroid, has shown potential neuroprotective properties and antioxidant effects.

Methods: In this study, we investigated the neuroprotective potential of Prgn in an in vitro PD model using SH-SY5Y cells treated with 6-hydroxydopamine (6-OHDA). Pregnenolone pretreatment significantly improved cell viability and reduced oxidative stress induced by 6-OHDA exposure. Moreover, Prgn treatment led to the modulation of antioxidative biomarkers, restoring cellular redox balance.

Results: Our findings suggest that Prgn exerts neuroprotective effects against 6-OHDA-induced neurotoxicity in SH-SY5Y cells by reducing oxidative stress and enhancing cell survival. These results support the exploration of Prgn as a potential therapeutic agent for mitigating PD progression.

Conclusion: In conclusion, our study demonstrates that Prgn, a neurosteroid, exhibits promising neuroprotective effects in an in vitro model of PD by attenuating 6-OHDA-induced oxidative damage and preserving the viability and functional integrity of SH-SY5Y cells.

Keywords: 6-OHDA, in vitro, oxidative stress, Parkinson's disease, pregnenolone

INTRODUCTION

Parkinson's disease (PD) is a gradually worsening neurodegenerative condition marked by the degeneration of dopaminergic neurons in the substantia nigra, resulting in motor difficulties and cognitive impairments.[1-3] Despite significant advances in understanding the pathophysiology of PD, the available therapeutic interventions remain limited and often fail to halt or reverse the relentless progression of the disease.[4] An essential characteristic of PD is the overproduction of reactive oxygen species (ROS) and reactive nitrogen species (RNS), which leads to oxidative stress. The accumulation of ROS and RNS triggers a cascade of detrimental events, including lipid peroxidation, protein misfolding, and DNA damage, culminating in neuronal dysfunction and death. Consequently, there is a growing interest in exploring novel neuroprotective agents that can effectively mitigate oxidative damage and ameliorate the course of PD.[5]

Various research studies have found that neurosteroids, which are naturally occurring steroids produced and active in the brain, can influence the communication of dopamine within a brain region called the striatum.[6] It is important to note that these natural substances are disrupted or imbalanced in the brains, cerebrospinal fluid, and blood of individuals with PD and in animal models of PD.[7]

Pregnenolone (Prgn), a neurosteroid synthesized in the brain, has attracted considerable attention due to its potential neuroprotective properties.[8] Pregnenolone has been involved in several cellular

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processes, such as neurogenesis, synaptic plasticity, and anti-inflammatory mechanisms.[9] Moreover, several studies have suggested that Prgn exerts antioxidant effects by scavenging free radicals and modulating antioxidant enzyme activities, thereby reducing oxidative stress-induced cellular injury.[10] To examine the neuroprotective potential of Prgn in a PD context, we employed a well-established in vitro model of PD using the SH-SY5Y cell line treated with 6-OHDA. This model mimics the key features of PD, enabling us to assess the efficacy of Prgn in attenuating 6-OHDA-induced oxidative damage and its subsequent neurotoxic effects. In this study, we aimed to elucidate the impact of Prgn on the viability and functional integrity of SH-SY5Y cells following 6-OHDA exposure. Despite numerous studies investigating the effects of neurosteroids in PD, this study represents the first investigation of Prgn's antioxidative effects on SH-SY5Y cells in an in vitro setting. The findings of this study hold significant implications for developing novel therapeutic strategies that target oxidative stress and its deleterious consequences in PD.

METHODS

Cell Culture

We used a type of human neuroblastoma cells called SH-SY5Y (ATCC Cat. CRL-2266) for our cellular model. These cells were grown in a special liquid called Dulbecco's Modified Eagle Medium (DMEM), mixed with 10% fetal bovine serum and 1% penicillin/streptomycin solution. SH-SY5Y cells are commonly used in studies related to PD because they share many characteristics with dopaminergic neurons.[4] Moreover, growing and differentiating SH-SY5Y cells are costeffective. Since these cells are derived from humans, they express specific proteins and variations not naturally present in primary cultures from rodents.[4,11] After letting the cells adhere for 24 hours, we exposed them to a substance called 6-OHDA at a concentration of 200 µM for 24 hours to induce stress. Prior to this stress, we pre-treated the cells with different concentrations (25 µM, 50 µM, and 100 µM) of Prgn for half an hour. After 24 hours, we measured cell viability.[4]

Cell Viability Test

To measure cell viability, we used a test called the 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) assay. We added MTT solution (Sigma-Aldrich, MO, USA) to each well, following the instructions provided in the kit. After a specific incubation period, we dissolved the formazan precipitate in 150 µL of a chemical called Dimethyl sulfoxide (DMSO). Then, we used a spectrophotometer (BioTek Instruments, Winooski, USA) to read the absorbance values at 540 nm.[4] This allowed us to assess the viability of the cells.

Measuring Oxidative Stress Markers

To evaluate oxidative stress levels, we used the enzyme-linked immunosorbent assay (ELISA) kits (Elabscience, Texas, USA) as per the kit's instructions.[12] These kits allowed us to measure the levels of 2 important markers: TAC (total antioxidant capacity) and TOS (total oxidant status). The measurement was performed by reading the absorbance of the samples at 450 nm using a spectrophotometer.[13]

Statistical Analyses

For data analysis, we utilized a statistical method called 1-way analysis of variance along with post hoc Tukey's test using IBM Statistical Package for the Social Sciences (IBM SPSS Corp., Armonk, NY, USA) version 22.0 software.[14,15] In this study, we considered P values less than .05 ($P < .05$) as statistically significant, indicating that the observed results were unlikely due to chance. The data are presented as mean ± SD, which allowed us to show the average value for each group along with the variation or spread of the data around the mean.

RESULTS

Cell Viability

We observed that when the SH-SY5Y cells were exposed to 6-OHDA, their cell viability significantly decreased, indicating a harmful effect of 6-OHDA on the cells (Figure 1). The pre-treatment with Prgn noticeably increased cell viability compared to the group exposed to 6-OHDA alone, suggesting that Prgn has the ability to promote cell proliferation under the influence of 6-OHDA ($P < .05$). This result indicates that Prgn could potentially counteract the damaging effects of 6-OHDA and promote the survival and growth of SH-SY5Y cells.

Oxidative Stress Results

Considering that the production of harmful oxygen radicals and products of lipid peroxidation are associated with the disease

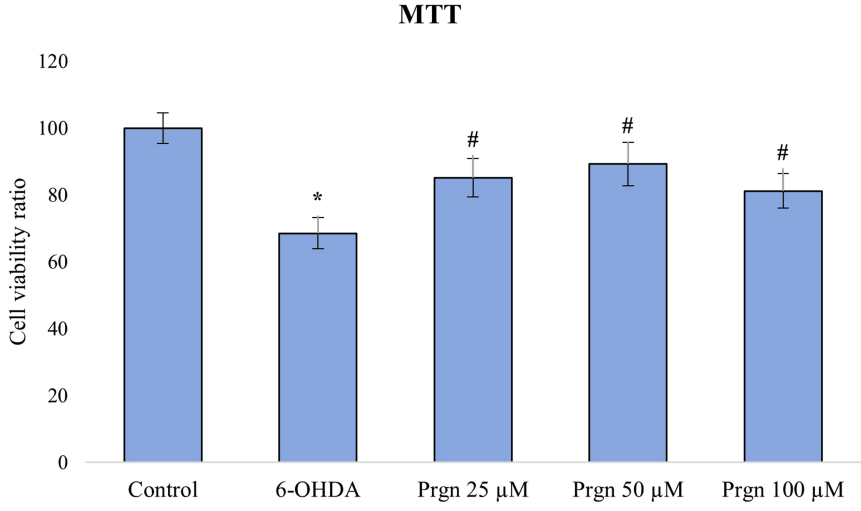

Figure 1. Effects of pregnenolone on the cell viability ratio.
Data are expressed as the means ± SD. 6-OHDA, 6-hydroxydopamine; Prgn, pregnenolone. *$P < .05$ vs. control group, #$P < .05$ vs. 6-hydroxydopamine group.

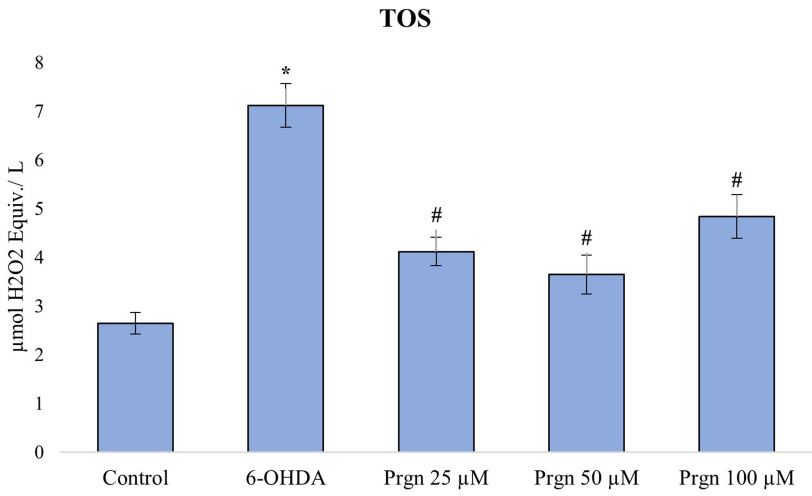

Figure 2. Effects of pregnenolone on the total oxidant status levels.
Data are expressed as the means ± SD. 6-OHDA, 6-hydroxydopamine; Prgn, pregnenolone; TOS, total oxidant status. *$P < .05$ vs. control group, #$P < .05$ vs. 6-hydroxydopamine group.

process of PD, we investigated specific biomarkers related to oxidative stress and antioxidant defense. When the SH-SY5Y cells were exposed to 200 µM 6-OHDA, the levels of TOS markedly increased in comparison with the control group ($P < .05$) (Figure 2). This indicated that 6-OHDA triggered oxidative stress in the cells. Additionally, 6-OHDA caused a significant reduction in the activity of TAC within the SH-SY5Y cells. Interestingly, when we pre-treated the cells with Prgn before exposing them to 6-OHDA, the oxidative burden induced by 6-OHDA was remarkably alleviated ($P < .05$), as depicted in Figure 3. This suggests that Prgn has the potential to counteract the oxidative damage caused by 6-OHDA, thereby protecting the cells from oxidative stress. Moreover, Prgn treatment increased the activity of TAC in the SH-SY5Y cells exposed to 6-OHDA ($P < .05$), indicating an enhancement of the cellular antioxidant defense. Taken together, these results imply that Prgn might play a beneficial role in mitigating the harmful effects of oxidative stress in PD.

DISCUSSION

Parkinson's disease is a disabling neurodegenerative condition characterized by the gradual degeneration of dopaminergic neurons in the substantia nigra, resulting in motor deficits and cognitive dysfunction.[16] Pregnenolone, a neurosteroid synthesized in the brain, has been the subject of research regarding its potential relationship with PD. Parkinson's disease is a neurodegenerative disorder characterized by the loss of dopaminergic neurons in the substantia nigra region of the brain, leading to motor dysfunction.[7] Recent studies have explored the role of Prgn in modulating neuroinflammatory responses, oxidative stress, and neuronal survival mechanisms.[10] It is proposed that Prgn might exert neuroprotective effects by influencing various pathways implicated in PD pathology. However, the exact mechanisms through which Prgn could impact PD progression remain a topic of ongoing investigation.

The quest for effective neuroprotective strategies to ameliorate PD pathology remains a significant challenge in current research. In this study, we utilized a well-established in vitro PD model, utilizing the SH-SY5Y cell line exposed to 6-OHDA, to investigate the potential neuroprotective effects of Prgn. The choice of SH-SY5Y cells as a cellular model is grounded in their relevance and widely accepted use in PD studies, as they exhibit numerous features characteristic of dopaminergic neurons and present a cost-effective approach for investigation.[4,17-19]

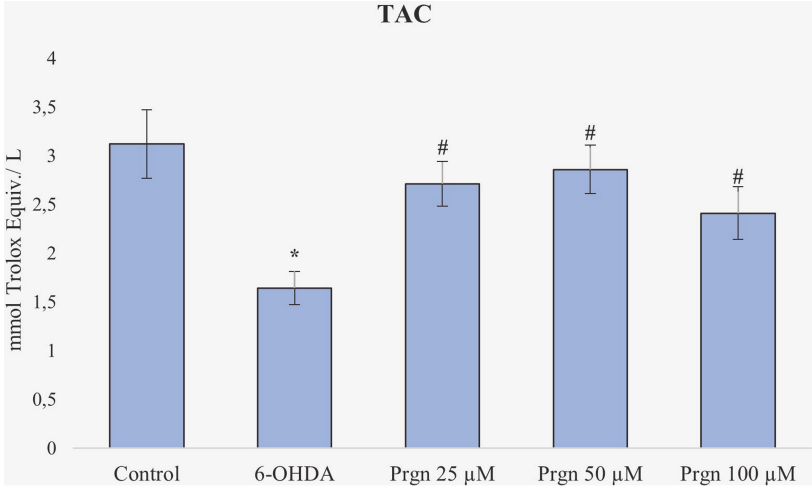

Figure 3. Effects of pregnenolone on the total antioxidant capacity levels.
Data are expressed as the means ± SD. 6-OHDA, 6-hydroxydopamine; Prgn, pregnenolone; TAC, total antioxidant capacity. *$P < .05$ vs. control group, #$P < .05$ vs. 6-hydroxydopamine group.

Importantly, the human origin of the differentiated SH-SY5Y cell culture allowed us to investigate human-specific proteins and isoforms that are not naturally present in rodent primary cultures, enhancing the translational relevance of our findings.[20] The MTT assay, an established method to assess cell viability, was employed to evaluate the impact of Prgn on cellular survival following 6-OHDA-induced stress. Our results unequivocally demonstrated that Prgn pre-treatment significantly increased cell viability in the presence of 6-OHDA, signifying a protective effect against 6-OHDA-induced cytotoxicity. This observation suggests that Prgn's administration may promote cell proliferation and counteract the deleterious consequences of 6-OHDA, potentially exerting a beneficial influence in mitigating the neurodegenerative processes associated with PD. Oxidative stress is a crucial contributor to PD pathogenesis, characterized by an imbalance between ROS production and the cellular antioxidant defense mechanisms.[21] To elucidate the mechanism underlying Prgn's protective effects, we examined oxidative stress-related biomarkers, including TAC and TOS. Exposure to 6-OHDA led to a marked increase in TOS levels, indicative of heightened oxidative stress within the SH-SY5Y cells. In contrast, pre-treatment with Prgn effectively attenuated the 6-OHDA-induced oxidative burden, restoring cellular redox balance and enhancing the activity of TAC. These results demonstrate that Prgn exerts a significant antioxidative effect, conferring cellular protection against oxidative damage in the context of our PD model. The collective findings of this study provide compelling evidence for the neuroprotective potential of Prgn in an in vitro PD model induced by 6-OHDA. By positively influencing cell viability and mitigating oxidative stress, Prgn emerges as a promising candidate for further investigation as a therapeutic intervention in PD. The identification of Prgn's beneficial effects on crucial cellular processes implicated in PD pathology opens new avenues for the development of innovative treatment strategies aimed at attenuating the relentless progression of this devastating neurodegenerative disorder. Nevertheless, the translation of these findings to in vivo and clinical studies is warranted to corroborate the full therapeutic potential of Prgn and its underlying mechanisms of action. Overall, our study provides valuable insights into the field of neuroprotection and brings us closer to the prospect of developing novel therapeutic interventions for PD.

Ethics Committee Approval: Ethical approval was not required as this study was conducted using a commercially acquired cell line.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – U.O., I.F.O.; Design – U.O., I.F.O.; Supervision – U.O., I.F.O.; Resources – I.F.O., F.Y., U.O.; Materials – I.F.O., U.O.; Data Collection and/or Processing – I.F.O., F.Y., U.O.; Analysis and/or Interpretation – U.O., F.Y., I.F.O.; Literature Search – I.F.O., U.O.; Writing Manuscript – U.O., I.F.O.; Critical Review – I.F.O.

Declaration of Interests: The authors declare that they have no competing interest.

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ATATURK UNIVERSITY PUBLICATIONS

Research Article

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Anatomy of *Paliurus spina-christi* Mill. (Blackthorn) (Rhamnaceae)

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ABSTRACT

Objective: This investigation aimed to explore the anatomical structures of the stem, leaf, petiole, petal, and fruit of *Paliurus spina-christi*.

Methods: Plant specimens were collected from Uzundere/Erzurum (Turkey) in June 2016. Subsequently, standard herbarium techniques were employed to dry and preserve the samples stored at the Herbarium of Atatürk University, Biodiversity Application and Research Center. For anatomical analysis, the materials were preserved in 70% alcohol. Characteristic elements of these plant parts were identified through sectioning, and their structures were visually documented with photographs.

Results: The leaf is bifacial, with stomata located in the lower epidermis. Abundant cluster crystals of calcium oxalate, very dense, and unicellular trichome (only stem, leaf, petiole) were shown in stem, leaf, petiole, and petal. Lignified structures were observed in the samara fruits pericarp.

Conclusion: This research thoroughly delineates the anatomical characteristics of *P. spina-christi*. The findings derived from this investigation imply that the observed anatomical variations could have valuable implications for taxonomic classification.

Keywords: Anatomy, *Paliurus spina-christi*, Rhamnaceae

INTRODUCTION

Paliurus spina-christi Mill. belongs to the Rhamnaceae family and is represented by a single species in Turkey. *Paliurus spina-christi* Mill. is known as “karaçalı” or “çaltı dikeni” in our country. It is grown naturally in the south of Europe, Turkey, Crimea, Caucasus, Syria, Iran, and Iraq.[1,2] The plant is a spiky shrub that grows to a height of 2-4 meters. The fruits of the plant are used in Turkish folk medicine as antidiabetic, diuretic, constipating, and stone-lowering agents. Moreover, it is known that it has been used in the past against eye diseases.[3,4] Flavonoids, tannins, amino acids, alkaloids, and sterol content, in addition to the antibacterial and antioxidant activities of this species, have been reported in previous studies.[4-7]

Paliurus spina-christi is used among people as an antidiarrheal, diuretic, and remedy against rheumatism. In addition, the samara-type fruits of the plant are used as an anti-inflammatory against kidney stones, chest infections, and eye infections, and the leaves are used externally for boil inflammations. In the Kastamonu region, it is known that the decoction prepared from ripe fruits is used in cases of respiratory failure. The fruits have a constipating and diuretic effect. They are thorny shrubs with yellow, small flowers in May–July, 2-4 m tall, deciduous in winter, and rarely up to 5 m tall. Young branches, axillary buds, petioles, leaf main veins, and pedicels ± dense brown short soft hairs, mature branches ± bare, often ± strongly curved. The branches are thin and weak. The stipules took the form of spines, and the spines were 2 in each nodule; the long one is straight and about 2 cm long, and the short one is curved like a hook. Petioles are 3-13 mm long. The leaves are symmetrical to asymmetrical, ovate to epileptic, 2-4 cm long, 1.5-3.5 cm wide, leaf base obtus to cordate variable, apex obtuse, thin, and paper-like structure. Flowers glabrous, 3-6 mm in diameter, pedicels 4-8 mm long, receptaculum disc-shaped. Fruits are circular, bulging in the middle, winged, 3-seeded, and a dry samara. It is an invasive, cheeky plant that often forms impenetrable woodlands on alluvial soils. This species can be found in a wide range of habitats, including sparse oak forests, shrublands, heathlands, scrubby forests, valley slopes, gorges, river valleys, cleared forest areas, degraded forested areas, and even vacant lands. It can be found at altitudes ranging from 1500 to 2300 m above sea level. [6] Since the plant develops well in arid soils, it is very effective in combating erosion. There are 5 known species, and only *P. spina-christi* is found in the flora of Turkey. *Paliurus spina-christi* is a plant that spreads in Turkey, southern Europe, the Balkans, and the Caucasus.[8]

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In this study, anatomical structures of the stem, leaf, petiole, petal, and fruit of *P. spina-christi* were investigated. Characteristic elements of stem, leaf, petiole, petal, and fruit were identified by taking the sections. Their structures are illustrated with photographs. The leaf is bifacial with its stomata located in the lower epidermis. Abundant cluster crystals of calcium oxalate and very dense and unicellular trichome (only stem, leaf, and petiole) were shown in stem, leaf, petiole, and petal. Lignified structures were observed in the samara fruits pericarp.

METHODS

Plant Material

The widely distributed *P. spina-christi*, registered in the Flora of Turkey, was collected from Erzurum/Uzundere. According to the Grid map, it was collected from Erzurum-Uzundere located in square A8. A8: Erzurum-Uzundere-Altincanak Mah. wooded areas within its borders (40°33′49″ N, 41°35′47″ E). It is at an altitude of 1060 m. The flowering time of the plant was observed on June 8, 2016. It was diagnosed by forest engineer Mehmet Önal from the Eastern Anatolia Forestry Research Institute. The herbarium specimens have been preserved at the Biodiversity Application and Research Center of Atatürk University with the AUEF 1348. Plant specimens for anatomy studies were taken into 70% ethanol during the collection of the plant (Figure 1).

Abbreviations for Figures

d → Druse, e → Epidermis, es → Ergastic substances, g → Glandular trichome, mc → Mucilage cell, ph → Phloem, s → Stomata, sc → Sclerenchyma, st → Starch grains, t → Trichome, xy → Xylem, v → Vessel

Anatomical Studies

To determine the anatomical characteristics of this species, the below-mentioned aerial part and fruit of *P. spina-christi* were collected from Erzurum, East Anatolia, Turkey, and the samples were put on 70% ethanol. Transverse and superficial sections were taken from the plant specimens manually. These sections were examined with various reagents (e.g., Sartur Reagent, Chloralhydrate). The anatomical features of the stem, leaf, petiole, petal, and fruit were determined, and photographs of the specimens and the characteristic elements were determined with the aid of ZEISS Primostar 415500, Germany. Preparations were prepared from the stem, leaf, petiole, petal, and fruit, respectively, from the samples contained in alcohol. After the specimens were placed in styrofoam, manual sections were taken with a razor blade and put on a slide with reagent dripped on it. Then, after being covered with a coverslip and heated, tests were made.

RESULTS

In this study, anatomical structures of the stem, leaf, petiole, petal, and fruit of *P. spina-christi* were investigated. Characteristic elements of stem, leaf, petiole, petal, and fruit were identified by taking the sections. Their structures were illustrated with photographs.

The leaf is bifacial, with stomata located in the lower epidermis. Abundant cluster crystals of calcium oxalate and very dense and unicellular trichome (only stem, leaf, and petiole) were shown in stem, leaf, petiole, and petal. Lignified structures were observed in the samara fruits pericarp. The findings gained in this study propose that this anatomical diversity may be beneficial in taxonomical classification.

The stem has a cylindrical shape, and vascular bundles are regular. Mucilage cells are large and found on both vascular bundles and the central cylinder. The cuticle is thin and the central cylinder is diminished. Tracheitis, druse, endodermis, and unicellular trichome are on the stem (Figure 2).

The leaf is bifacial. Mucilage cells, druse, tracheitis, and parenchyma with starch grains are on mid vein. The cuticle is thin and the mid-vein is glabrous (Figure 3).

The corners of the petiole are round and rectangular. Its cuticle is thin and mucilage cells are on the petiole. One of the edges has a concave and there are trichomes on concave. Collenchyma is more noticeable on the petiole than on the stem. There is no central cylinder or tracheitis, while there is druse. The vascular bundles are intensified in the middle (Figure 4).

The leaf is bifacial. There is no stoma on the upper surface. The stoma type is anomositic on the under surface (Figure 5).

Druses are on all surfaces, and vessels are noticeable on petal anatomy (Figure 6).

Figure 1. The general appearance of (A) *Paliurus spina-christi*. (B) Herbarium specimen. (C) Flowers and fruits.

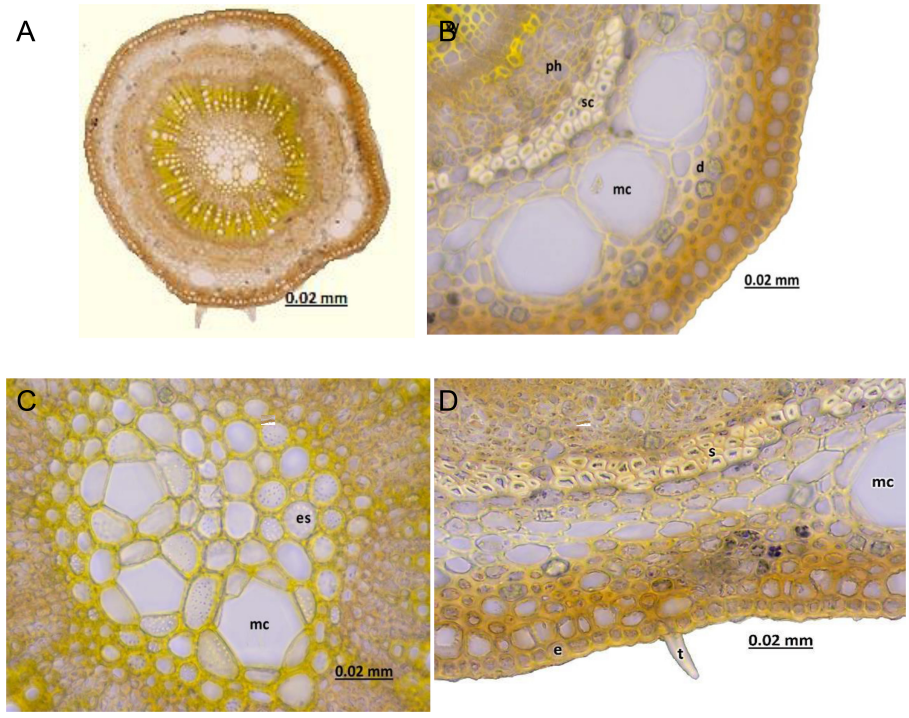

Figure 2. (A) Anatomy of stem. The stem is cylindric; vascular bundles are regular. (B-D) Mucilage cells are large and found on both vascular bundles and the central cylinder. The cuticle is thin and the central cylinder is diminished. Tracheitis, druse, endodermis, and unicellular trichome are on stem.

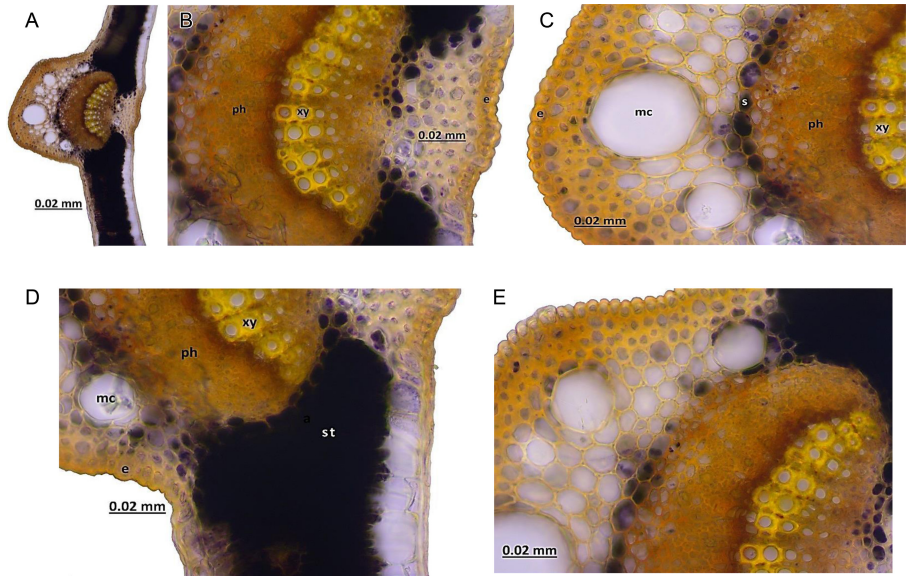

Figure 3. (A) Mid-vein anatomy. The leaf is bifacial. (B-E) Mucilage cells, druse, tracheitis, and parenchyma with starch grains are on mid-vein. The cuticle is thin and the mid-vein is glabrous.

The pedicel is cylindrical and the cuticle is thick. There are mucilage cells, phloem parenchyma, glandular trichome, trachea, and tracheitis. The sclerenchyma is arranged as cylindric. The vascular bundles are regular, and the central cylinder is too narrow (Figure 7).

The fruit is samara. The exocarp is thick and the mesocarp is small on the wings of the fruit (Figure 8).

DISCUSSION

According to our literature research, this study represents the first examination of the anatomy of *P. spina-christi*.

Ziziphus paliurus Willd., *Z. spina-christi* Georgi, and *Z. spina-christi* var. *microphylla* Hochst. ex A.Rich. are the synonyms of *P. spina-christi*.[9] It has been reported that the epidermis of *Z. spina-christi* var. *spina-christi* is glabrous and consists of 1 layer of isodiametric cells with a thick wall. Stomata are submerged and the mesophyll is isobilateral. Vascular bundles are collateral and surrounded by bundle sheaths with thin cell walls. Also, it has been found that the abaxial surface of *Z. spina-christi* var. *aucheri* is pubescent and the cuticle is thick on both surfaces. The epidermis contains 1 layer of cells with a thick wall adaxially. Papilla with high frequency is available abaxially. The mesophyll is isobilateral. Vascular bundles are

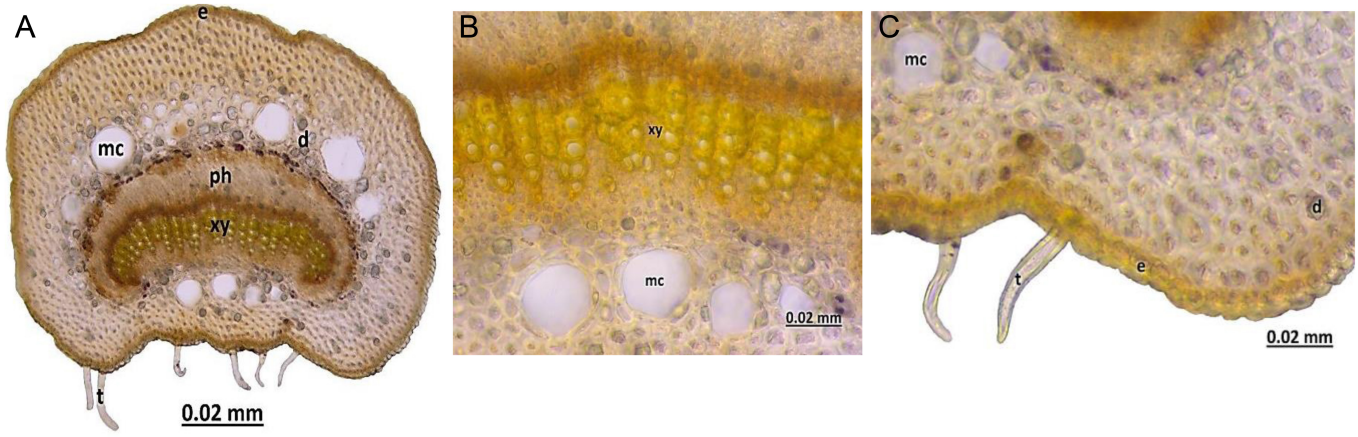

Figure 4. (A) Petiole anatomy. The corners are round and rectangular. (B-C) The cuticle is thin and mucilage cells are on the petiole. One of the edges has a concave and there are trichomes on concave. Collenchyma is more noticeable on the petiole than on the stem. There is no central cylinder and tracheitis while there is druse. The vascular bundles are intensified in the middle.

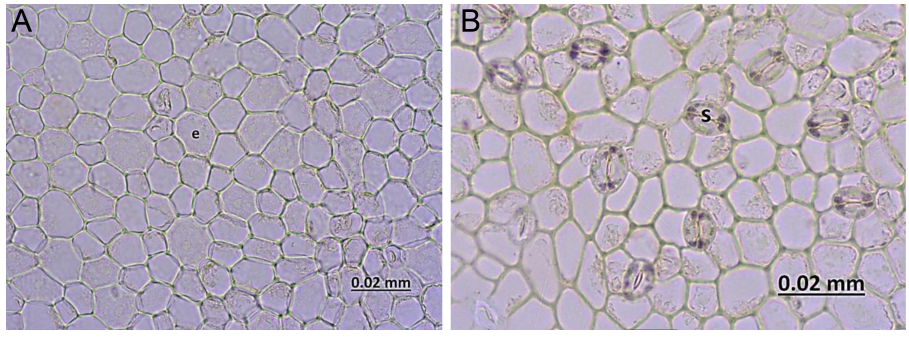

Figure 5. (A) Upper surface and (B) under surface anatomies. The leaf is bifacial. There is no stoma on the upper surface. The stoma type is anomositic on the under surface.

collateral and surrounded by a bundle sheath. The stem is glabrous and the single epidermis includes isodiametric cells with thick outer walls. The cortex includes 3-4 layers of sub-epidermal collenchyma and a large canal. In the process of vascular cylindrical secondary growth, it's worth noting that the stem develops a cylindrical shape with additional layers of vascular tissue. The innermost part of the stem, known as the pith, contains parenchymatous cells.[10] An anatomical examination of *Z. spina-christi* wood revealed certain characteristics. The wood of this species is diffuse-porous, showing distinct growth rings, simple preformation plates, polygonal openings, and parenchyma arranged either in bands or dispersed aggregates.[11]

In another study, it was mentioned that *P. spina-christi* Mill., with 2 distinct varieties, namely *P. spina-christi* L. var. *spina-christi* and *P. spina-christi* var. *macrocarpa* Beck, is naturally found in the mountainous regions of Kurdistan, occasionally extending to the upper plains of northern Iraq. Sampling from diverse locations within the Kurdistan region yielded a collection of 15 plants, serving as the basis for comparison. Precise measurements of various attributes, including leaf, inflorescence, flowers, fruit, seeds, and leaf anatomical characteristics, were obtained—30 measurements per trait—enabling a comprehensive evaluation of the 2 varieties. Remarkably, the fruit diameter of var. *macrocarpa* outshines that of *var. spina-christi*, while larger dimensions are also

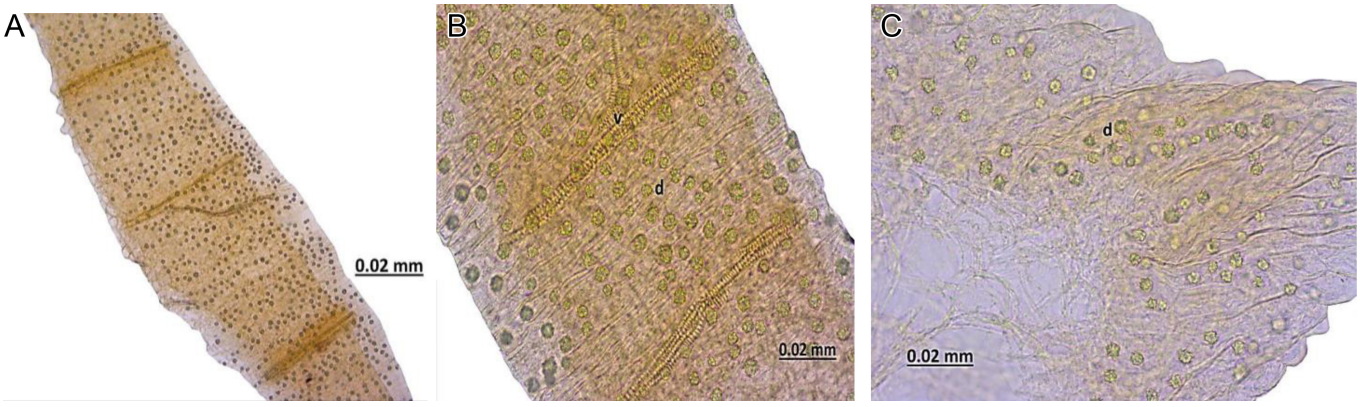

Figure 6. (A-C) Petal anatomy. Druses are on the all surfaces. Vessels are noticeable.

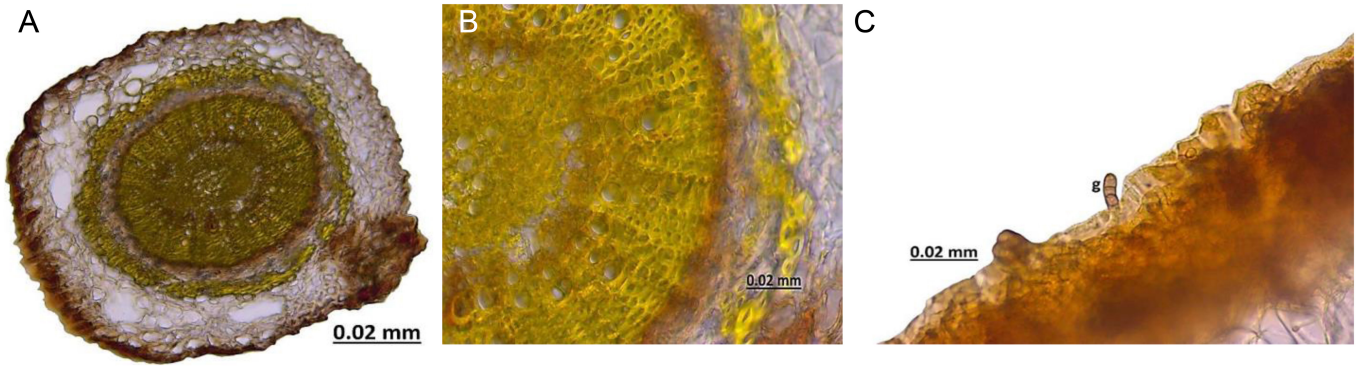

Figure 7. (A) Pedicel anatomy. The pedicel is cylindric and the cuticle is thick. (B-C) There are mucilage cells, phloem parenchyma, glandular trichome, trachea, and tracheitis. The sclerenchyma is arranged as cylindric. The vascular bundles are regular, and the central cylinder is too narrow.

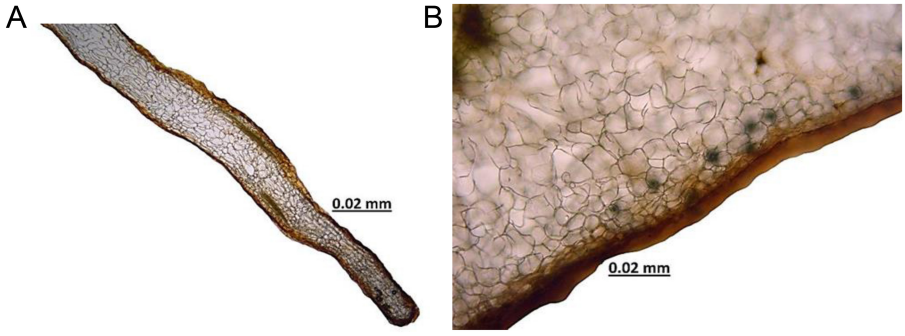

Figure 8. (A) Fruit anatomy. The fruit is samara. (B) The exocarp is thick and the mesocarp is small on the wings of the fruit. Also, exocarp is thick interior of fruit.

evident in the ovary and seed size of the former. The upper epidermal layer consistently exhibits greater thickness compared to the lower epidermal layer within the same blade. A balanced mesophyll structure emerges, featuring 2 layers of elongated palisade cells on the upper surface and 2-3 layers of shorter cells on the lower surface. Furthermore, the mesophyll houses well-distributed vascular bundles. With the exception of simple hairs exclusively present on the adaxial side of the petiole in var. *spina-christi*, absent in var. *macrocarpa*, other anatomical features appear non-taxonomically significant. Notably, the stomatal density on the adaxial leaf side reaches up to 19 stomata per mm^2 in var. *spina-christi*, contrasting the denser 38 stomata per mm^2 found in var. *macrocarpa*. Conclusively, both the fruit diameter and the stomatal density on the adaxial leaf side serve as key diagnostic indicators, facilitating the differentiation between the 2 varieties of *P. spina-christi* Mill.

The characteristics of the epidermis, stomata, and vascular bundles were similar to these studies. However, fruit, lower-upper leaf epidermis, petiole, and pedicel anatomy were not examined in these studies. While mucilage, starch cells, and glandular trichomes were seen in our study, they were not mentioned in these studies.

"Plant anatomy," which examines the internal structure of organs, is also called "internal morphology." "Plant systematics" studies plant diversity, "plant biochemistry" studies plant chemistry, "pharmacognosy" studies the drug properties of plants, and "plant ecology" studies the relationships of plants with their environment and each other. The correct diagnosis of medicinal plants is very important for their use in treatment. One of the characteristics used in the diagnosis of plants is their anatomical features.[12]

Ethics Committee Approval: The Ethics Committee Approval is not required for this study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – H.Y., S.K., Z.G.; Design – H.Y., S.K.; Supervision – Z.G.; Resources – H.Y., S.K., Z.G.; Materials – H.Y., S.K., Z.G.; Data Collection and/or Processing – H.Y., S.K.; Analysis and/or Interpretation – H.Y., S.K.; Literature Search – H.Y., S.K.; Writing Manuscript – H.Y., S.K., Z.G.; Critical Review – S.K., Z.G; Other – H.Y., S.K., Z.G..

Declaration of Interests: The authors declare that they have no competing interest.

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ATATURK UNIVERSITY PUBLICATIONS

Research Article

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Body Mass Index and Hemoglobin A1c Levels in Diabetic Adults

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ABSTRACT

Objective: Studies have shown that an increase in body mass index (BMI) increases the risk of developing diabetes and that there exist a strong relationship between hemoglobin A1c (HbA1c) and BMI. The aim of this studywas to interpret the relationship between BMI and HbA1c levels in adults.

Methods: Three hundred seven adult individuals in the patient group aged 18 years and older who presented to the internal medicine outpatient clinic and were diagnosed with type 2 diabetes and 99 healthy volunteers in the control group were evaluated within the scope of our study. Body mass index values and biochemical blood test results of diabetic and healthy individuals participating in our study were obtained prospectively from hospital records.

Results: The mean BMI of the patients was 31.2 ± 6.0 kg/m^2, glucose values were 164.7 ± 91.4 mg/dL, and HbA1c values were 8.3 ± 2.0%. The mean BMI value of all individuals with an HbA1c value of 6.5% and above was found to be above normal.

Conclusion: Diabetes and obesity are chronic and progressive diseases that are among the important public health problems today. The results of this research show the importance of HbA1c, BMI measurements, and cholesterol level measurements in health institutions applied for the improvement of public health.

Keywords: Body mass index, glucose, hemoglobin A1c, type 2 diabetes

INTRODUCTION

Obesity, according to the definition of the World Health Organization (WHO), is a chronic disease that carries the risk of increased morbidity and mortality, resulting from the interaction of genetic and environmental factors as well as factors affecting lifestyle. Although obesity is a common disease, it was determined that 1.9 billion adults aged 18 and over were overweight in 2016, and 650 million of these adults were obese.[1] The WHO recommends the use of body mass index (BMI) in adult obesity classification.[2]

Body weight gain has been shown to be associated with an increased risk of type 2 diabetes.[3] Diabetes is a metabolic disorder characterized by high blood glucose levels. Uncontrolled diabetes can lead to many adverse conditions, including renal failure, retinopathies that may even result in blindness, cardiovascular diseases, and diabetic wounds in the future.[4]

The hemoglobin A1c (HbA1c) test, which is used in the diagnosis and follow-up of diabetes, is a marker that shows the average blood glucose levels over a 3-month period. According to the American Diabetes Association, the plasma HbA1c level must be above 6.5% in order to be diagnosed with diabetes.[5]

The aim of this study was to evaluate the relationship between BMI and HbA1c levels in patients with type 2 diabetes and healthy controls without type 2 diabetes.

METHODS

Our research was carried out in two research hospitals (Erzurum Regional Training and Research Hospital and Erzurum Mareşal Çakmak State Hospital) between September 2022 and February 2023. This prospective study was carried out with the data of 307 patients aged 18 and over and 99 healthy individuals whose HbA1c measurements were made between September 2022 and February 2023. Patients and healthy individuals who accepted to participate in the study read the consent form of the study and accepted the research conditions and were included in the study. Pregnant women were not included in the study. The study was approved by the Clinical Resarch Ethics Committee of Erzurum Regional Training and Research Hospital (November 24, 2022, 2022/18-178).

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Biochemical data were obtained from the hospital automation system. After obtaining the consent of all participants, the age, gender, body weight, height, and biochemical analysis results of all individuals were obtained from the records of the automation system. As biochemical parameters, serum glucose, triglyceride, high-density lipoprotein (HDL) cholesterol (HDL-Chol), low-density lipoprotein (LDL) cholesterol (LDL-Chol), total cholesterol, uric acid, and plasma HbA1c values were evaluated.

Body mass index levels of all individuals participating in the study were calculated using the formula body weight (kg)/height^2 (m^2). Those with a BMI between 18.50 and 24.99 kg/m^2 were in normal weight; those between 25.00 and 29.99 kg/m^2 were considered overweight; and those over ≥30.0 kg/m^2 were considered obese.[2] The HbA1c value of 260 of the patients was 6.5% and above, and the HbA1c value of 47 of the patients was 6.5% and below. The entire patient group had a known diagnosis of type 2 diabetes.

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) for Windows 25 package program (SPPS Inc., Chicago, Ill, USA). The normality of the data was evaluated with the Kolmogorov–Smirnov test. Descriptive statistical methods (mean, SD) were used. The *t*-test was used to compare independent groups. *P* values less than .05 at the 95% CI were considered significant.

RESULTS

Comparisons of patient and control groups with demographic and biochemical parameters are presented in Table 1.

Patients were divided into 2 groups: those with an HbA1c value below 6.5 and those with a HbA1c value above 6.5. The mean HbA1c value (%) of patients with an HbA1c value below 6.5 was found to be 5.8 ± 0.7, and the mean HbA1c value (%) of patients with an HbA1c value above 6.5 was 8.7 ± 1.8. There was a statistically significant difference in HgA1c levels between the 2 groups (*P* < .01).

The biochemical parameters and subgroup comparisons of the 2 subgroups according to this subclassification in the patient groups are presented in Table 2.

Comparisons of the demographic characteristics and biochemical values of male and female patients are presented in Table 3.

As a result of comparing the demographic characteristics and biochemical values of male and female patients with the *t*-test in independent groups, it was determined that age, glucose and HgA1c, triglyceride, LDL-Chol, and total cholesterol values did not show a statistically significant difference between male and female patients (*P* > .05 for all parameters) (Table 2). Body mass index, HDL-Chol, and uric acid values were found to differ statistically between male and female patients (*P* < .01, *P* < .01, and *P* = .002, respectively).

Table 1. Comparisons of Patient and Control Groups with Demographic and Biochemical Parameters

	Patient Group (n=307)	Control Group (n=99)	*P*
Age (years)	59.5 ± 10.9	46.1 ± 15.4	<.01**
BMI (kg/m^2)	31.2 ± 6.0	26.9 ± 5.0	<.01**
Glucose (mg/dL)	164.7 ± 91.4	90.6 ± 11.2	<.01**
HbA1c (%)	8.3 ± 2.0	5.0 ± 0.5	<.01**
Triglyceride (mg/dL)	195.7 ± 98.9	135.9 ± 28.1	< .01**
HDL-Chol (mg/dL)	42.2 ± 11.4	49.1 ± 6.5	<**
LDL-Chol (mg/dL)	132.1 ± 36.3	138.8 ± 14.1	.074
T cholesterol (mg/dL)	179.9 ± 46.1	180.8 ± 18.3	.849
Uric acid (mg/dL)	4.8 ± 1.5	4.0 ± 1.4	<.01**

Results are presented as mean ± SD. BMI, body mass index; HbA1c, hemoglobin A1c; HDL-Chol, high-density lipoprotein cholesterol; LDL-Chol, low-density lipoprotein cholesterol; T-cholesterol, Total Cholesterol; *P*, test statistics *P* value of independent samples *t*-test; T cholesterol, total cholesterol.**Significance at *P* < .01 level.

Table 2. Demographic, Biochemical Parameters, and Comparisons of Patients with HbA1c < 6.5 and HbA1c ≥ 6.5

	Patients with HbA1c < 6.5 (n=47)	Patients with HbA1c ≥ 6.5 (n=260)	*P*
Age (years)	58.4 ± 8.7	59.6 ± 11.3	.486
BMI (kg/m^2)	29.6 ± 4.2	31.5 ± 6.3	.048*
Glucose (mg/dL)	127.2 ± 133.9	171.5 ± 79.9	.002**
HbA1c (%)	5.8 ± 0.7	8.7 ± 1.8	< .01**
Triglyceride (mg/dL)	169.7 ± 74.2	200.4 ± 102.1	.050*
HDL-Chol (mg/dL)	42.6 ± 8.9	42.2 ± 11.8	.799
LDL-Chol (mg/dL)	126.0 ± 31.2	133.2 ± 37.1	.214
T cholesterol (mg/dL)	177.7 ± 35.0	180.3 ± 47.9	.722
Uric acid (mg/dL)	5.3 ± 1.4	4.7 ± 1.5	.005**

Results are presented as mean ± SD. BMI, body mass index; HbA1c, hemoglobin A1c; HDL-Chol, high-density lipoprotein cholesterol; LDL-Chol, low-density lipoprotein cholesterol; T-cholesterol, Total Cholesterol; *P*, test statistics *P* value of independent samples *t*-test; T cholesterol, total cholesterol.**Significance at *P*< .01 level.

Table 3. Comparisons of the Demographic Characteristics and Biochemical Values of Male and Female Patients

	Female (N=180, 58.6%)	Male (N=127, 41.4%)	*P*
Age (years)	59.3 ± 10.9	59.7 ± 10.9	.766
BMI (kg/m^2)	32.4 ± 5.9	29.4 ± 5.8	<.01**
Glucose (mg/dL)	163.5 ± 94.3	166.5 ± 87.5	.778
HbA1c (%)	8.2 ± 1.9	8.4 ± 2.1	.338
Triglyceride (mg/dL)	190.3 ± 91.6	203.4 ± 108.2	.252
HDL-Chol (mg/dL)	44.0 ± 12.1	39.7 ± 9.8	< .01**
LDL-Chol (mg/dL)	131.9 ± 33.5	132.4 ± 40.1	.911
T cholesterol (mg/dL)	179.9 ± 48.0	179.9 ± 43.5	.997
Uric acid (mg/dL)	4.5 ± 1.4	5.1 ± 1.5	.002**

Results are presented as mean ± SD.BMI, body mass index; HbA1c, hemoglobin A1c; HDL-Chol, high-density lipoprotein cholesterol; LDL-Chol, low-density lipoprotein cholesterol; T-cholesterol, Total Cholesterol; *P*, test statistics *P* value of independent samples *t*-test; T Cholesterol, total cholesterol.**Significance at *P* < .01 level.

DISCUSSION

Obesity and diabetes, which are common diseases today, are related to each other. The WHO recommends the calculation of BMI for defining obesity in adults.[2] In addition to fasting glucose measurement, HbA1c measurement is also commonly preferred for the diagnosis of diabetes.[6] Since obesity and diabetes are important public health problems, measurements of BMI, glucose, and HbA1c values are important for individuals (7). The aim of this study to examine the relationship between random glucose and HbA1c measurements and BMI levels of individuals who presented to the internal medicine outpatient clinics of 2 state hospitals (Erzurum Regional Training and Research Hospital and Erzurum Mareşal Çakmak State Hospital).

The mean age of the patients participating in the study was 59.5 ± 10.9 years, the mean BMI values were 31.2 ± 6.0 kg/m^2, serum glucose values were 164.7 ± 91.4 mg/dL, and the HbA1c values were 8.3 ± 2.0%. In a meta-analysis examining the prevalence of obesity in Turkey, the mean BMI value of individuals was found to be 27.4 kg/m^2.[8] It is seen that the data of our research are higher than the BMI data across the country.

The UK Prospective Diabetes Study described plasma HbA1c measurement as the most valuable data in assessing the risk of developing diabetic complications as well as providing glycemic control.[7]

Hyperglycemia causes body weight gain due to lipid biosynthesis formation.[9] In studies examining the relationship between BMI

and fasting blood glucose levels, a positive and significant relationship was found.[9] The fact that individuals have high BMI and glucose values is due to the fact that hyperglycemia increases the body fat ratio. The best way to prevent possible complications for diabetic patients is to lose weight.[6]

In conclusion, BMI values, serum glucose values, plasma HbA1c levels, and plasma cholesterol levels were found to be higher in the patient group compared to the control group. As energy consumption increases, BMI levels increase, and so blood glucose and HbA1c levels also increase. Since obesity and diabetes are chronic diseases with increasing importance, it is very important to detect obesity, if any, and to monitor the HbA1c values of diabetes patients in health institutions applied to reduce its prevalence. In addition, diabetic patients with obesity should be provided with professional help to change their lifestyle and eating habits.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Clinical Resarch Ethics Committee of Erzurum Regional Training and Research Hospital (Date: November 24, 2022, Number: 2022/18-178).

Informed Consent: Written informed consent was obtained from patients who participated in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – A.F.K.; Design – A.F.K.; Materials – A.F.K., M.B., K.Ç.; Data Collection and/or Processing – A.F.K., M.B., K.Ç.; Analysis and/or Interpretation – A.F.K., M.B., K.Ç.; Literature Search – A.F.K., M.B., K.Ç.; Writing Manuscript – A.F.K., M.B., K.Ç.; Critical Review – A.F.K.

Declaration of Interests: The authors declare that they have no competing interest.

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ATATURK UNIVERSITY PUBLICATIONS

Research Article

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The Role of Myocardial Performance Index in Evaluating Effect of Hypothyroidism on Systolic and Diastolic Functions of Heart

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ABSTRACT

Objective: Hypothyroidism causes pathological changes in the heart. Systolic and diastolic functions of the left ventricle of the heart can be evaluated with the myocardial performance index (MPI). We compared MPI in overt hypothyroidism patients vs. healthy group.

Methods: The study included 50 healthy control subjects and 50 overt hypothyroid patients. Echocardiographic procedure was performed in all patients. The following parameters, like ejection fraction (EF), diastolic transmitral peak velocity (E and A waves), E/A ratio, and MPI, were measured.

Results: The E wave (0.79 ± 0.26 m/s) and E/A ratio (0.95 ± 0.30), which are echocardiographic parameters that reflect cardiac diastolic dysfunction, were dramatically lower in the case group ($P < .05$). Myocardial performance index (MPI) (0.42 ± 0.04), which reflects systolic and diastolic functions of the heart, was significantly higher in the case group ($P < .05$). Ejection fraction value (62.5 ± 4.0%) was not statistically different between the case and healthy subjects ($P > .05$).

Conclusion: Significant decrease in E and E/A values in the hypothyroid patients indicated the development of diastolic dysfunction. Although no difference could be detected between the groups in EF value, which reflects systolic functions of the heart, a significant increase in MPI value in the hypothyroid group indicated that systolic and diastolic functions of the heart started to deteriorate. In our study, the elevation of the MPI in hypothyroid patients without EF impairment suggests that MPI should be practiced more widely in the evaluation of cardiac functions in hypothyroid patients.

Keywords: Diastolic dysfunction, hypothyroidism, myocardial performance index, systolic dysfunction

INTRODUCTION

Hypothyroidism affects the functions of all organs and systems. Typically, there is a slowing in physical and mental activity and the functioning of many organs. Basic clinical symptoms include weakness, coarse, dry and cold skin, lethargy, slow speech, facial edema, constipation, weight gain, dyspnea, peripheral edema, hair thickening, menorrhagia, and bradycardia.[1,2]

Major cardiovascular changes due to hypothyroidism include decrements in cardiac output, myocardial contractility, and heart rate, and an increment in peripheral vascular resistance.[3,4] Because of decreased cardiac output, all values related to left ventricular performance deteriorate in overt hypothyroidism.[5] Ventricular diastolic relaxation rate is also decreased due to impairment in compliance and diastolic filling.[6]

In 1995, Tei Chuwa described the myocardial performance index (MPI).[7] It reflects the systolic and diastolic functions of the heart. It has found to be related to morbidity and mortality in cardiovascular diseases. It is easily calculated and has a narrow range in normal healthy individuals. This index can be measured by Doppler tracings obtained from mitral and aortic flows; MPI is not affected by heart rate, ventricular structure, and afterload.[8] MPI value can be determined using pulsed wave Doppler (PWD) echocardiography by dividing the sum of isovolumetric relaxation time (IVRT) and isovolumetric contraction time (IVCT) by ejection time (ET) (Figure 1).

In this study, we tried to investigate the role of MPI in the evaluation how hypothyroidism affects the systolic and diastolic functions of the heart.

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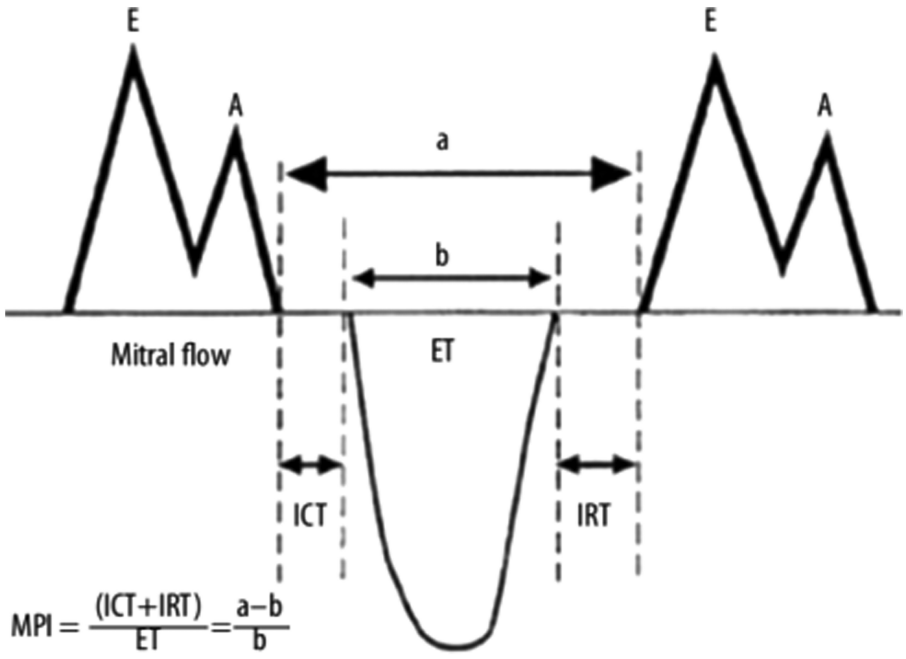

Figure 1. Schematic display of MPI calculation. a, the time between late diastolic and early diastolic waves; A, late diastolic wave; b and ET, systolic ejection time; E, early diastolic wave; ICT, isovolumetric contraction time; IRT, isovolumetric relaxation time; MPI, myocardial performance index..

METHODS

Data were obtained from 100 subjects who were admitted to our internal medicine clinic in Prof. Dr. Cemil Taşçıoğlu City Hospital. Informed consent forms from the study participants and approval from the ethics committee of our hospital were obtained. Ethics committee approval number is E-48670771-903.99-2190425 63. Subjects with diseases that may impair cardiac systolic and diastolic functions, such as chronic renal disease (creatinine > 1.4 mg/dL), patients with EF < 50, hypertension (> 140/90 mmHg), coronary heart disease, non-ischemic dilated cardiomyopathy, hypertropic cardiomyopathy, and diabetes, were excluded. Blood samples from the patient and the control groups were obtained at 08.00-08.30 AM in the morning after 10-12 hours of fasting. Creatinine, total cholesterol, triglyceride, and low-density lipoprotein values were measured from everyone participating in the study.

All echocardiographies were performed transthoracically using a 2.5 MHz transducer and Vivid 3 pro (Cingmed Technology, USA, 2006) echocardiography device. The patients were examined at left lateral decubitus position; measurements were made using two-dimensional, M-mode, continuous wave Doppler and pulsed wave Doppler in the presence of parasternal long, short, apical, and four-chamber images. In accordance with the recommendations of the American Echocardiography Association, all echocardiographies were performed by the same person at midday to eliminate the effects of circadian changes on cardiac functions. Appropriate M-mode images were attained in the parasternal long axis between the mitral valve and papillary muscle. Ultrasound beam was adjusted to be perpendicular to the interventricular septum and the posterior wall of the left ventricle. By this way, end-diastolic and end-systolic internal diameters of the left ventricle were measured from the extreme endocardial points, and the left ventricular ejection fraction was calculated using the Teichholz method. In terms of diastolic dysfunction, the E wave, which shows early diastolic peak transmitral filling rate, the A wave, which shows late diastolic peak transmitral atrial filling rate, and the E/A ratio were measured.

For MPI calculation, records were obtained with pulse wave Doppler from the sample volume placed under the aortic valve for mitral valve diastolic flow and from the sample volumes placed at mitral valve tips at the apical 4 chambers using the apical long axis image of the left ventricular outflow tract. For every cycle, typical diastolic early filling (the E wave), diastasis period, and atrial contraction (the A wave) periods were obtained. Isovolumetric contraction time (IVCT), isovolumetric relaxation time (IVRT), and ejection time were recorded. Myocardial performance index was figured out by dividing the summation of isovolumetric times by ejection time (ET). The normal value for MPI was accepted as 0.39 ± 0.05.[7]

For descriptive statistics of the data obtained in this study, mean, standard deviation ratio, and frequency were taken into consideration. The distribution of data was analyzed with the Kolmogorov–Smirnov test. Mann–Whitney *U*-test and independent samples *t*-test were applied for the evaluation of quantitative data, and the chi-square test was used for qualitative data. The Statistical Package for the Social Sciences Statistics, version 22.0, (IBM SPSS Corp., Armonk, NY, USA) program was utilized for the analyses.

RESULTS

The hypothyroidism group included a total of 50 patients, 24 females and 26 males between 30 and 70 years of age, and the control group was made up of 50 subjects, 30 females and 20 males. No statistically significant difference was found among the case and control groups in terms of age and gender distribution ($P = .904$, and $P = .229$, respectively) (Table 1).

In the hypothyroidism group, low density lipoprotein (LDL) and total cholesterol levels were higher than those in the control group ($P < .05$). And there was no significant difference in

Table 1. Demographic Parameters of Hypothyroid and Control Groups

	Hypothyroid Group		Control Group		
	Avg. ± SS/n %	Med.	Avg. ± SS/n %	Med.	*P*
Age (years)	49.4 ± 11.6	49 (30-74)	49.1 ± 8.0	48 (36-64)	.904
Gender					
Female	24 (48%)		30 (60%)		.229
Male	26 (52%)		20 (40%)		

Table 2. Biochemical Data for Hypothyroid and Control Groups

	Hypothyroid Group		Control Group		
	Avg.	Med.	Avg.	Med.	*P*
Creatinine (mg/dl)	0.77 ± 0.17	0.74 (0.51-1.18)	0.77 ± 0.14	0.74 (0.5-1.18)	.772
LDL (mg/dL)	131.8 ± 35	126 (57-205)	88.5 ± 19.4	87 (56-156)	<.001
TG (mg/dL)	146.4 ± 63.2	160 (43-325)	127.1 ± 35.1	119 (67-198)	.122
T-Cholesterol (mg/dL)	203.2 ± 35.3	205 (108-293)	155.8 ± 28.3	156 (108-210)	<.001

LDL, low density lipoprotein; T-Cholesterol, total cholesterol; TG, triglycerides.

Table 3. Echocardiographic Data for Hypothyroid and Control Groups

	Hypothyroid Group		Control Group		
	Avg.	Med.	Avg.	Med.	*P*
E'' (m/s)	0.79 ± 0.26	0.70 (0.42-1.80)	1.02 ± 0.23	1.05 (0.60-1.80)	<.001
A' (m/s)	0.87 ± 0.25	0.86 (0.49-1.80)	0.83 ± 0.25	0.80 (0.20-1.80)	.397
E''/A''	0.95 ± 0.30	0.84 (0.50-1.60)	1.35 ± 0.55	1.24 (0.60-4.0)	<.001
MPI	0.42 ± 0.04	0.41 (0.33-0.51)	0.40 ± 0.04	0.41 (0.31-0.49)	.033
EF (%)	62.5 ± 4.0	63 (52-71)	62.3 ± 4.0	62 (52-71)	0.594

A, late diastolic wave; E, early diastolic wave; EF, ejection fraction; MPI, myocardial performance index.

triglyceride (TG) and creatinine levels between hypothyroid and control subjects ($P > .05$) (Table-2).

The MPI value in the hypothyroidism group was (0.42 ± 0.04) significantly higher than the control group (0.40 ± 0.04) ($P = .033$) (Table 1). The E value (0.79 ± 0.26) and E/A ratio (0.95 ± 0.30) in the hypothyroidism group were significantly lower than the control (1.02 ± 0.23)-(1.35 ± 0.55) group ($P < .05$) (Table 1). No significant difference in A value was detected between the hypothyroid (0.87 ± 0.25) and the control groups (0.83 ± 0.25) ($P > .05$) (Table 1). No significant difference in EF value was detected between the hypothyroid (62.5 ± 4.0) and the control (62.3 ± 4.0) groups ($P > .05$) (Table 3).

DISCUSSION

It is known that the most characteristic and frequently observed symptoms and findings of thyroid diseases are caused by the impacts of the thyroid hormone on the cardiovascular system.[9] Hypothyroidism is a disease characterized by a decrease in the use of oxygen by all the major organs in the body. This reduction in oxygen demand reduces cardiac output. By the way, hypothyroidism deteriorates cardiac function by changing the expression of the myocyte-specific gene.[10] Major changes in hypothyroidism are decreased cardiac contractility and heart rate and increased peripheral vascular resistance.[11-13]

In hypothyroidism, the left ventricular performance of the heart is impaired and cardiac output is reduced. Diastolic filling and compliance are impaired due to decreased ventricular diastolic relaxation. Decreased ventricular performance is probably multifactorial.[14] Possible mechanisms include increases in heart rate and changes in the gene expression of proteins that regulate myocardial calcium. Various enzymes regulating calcium entry are controlled by thyroid hormones, and impaired activity of these enzymes due to hypothyroidism disrupts systolic performance and diastolic relaxation.[12]

Changes in cardiac gene expression in hypothyroidism lead to decreased cardiac contractility.[11] Decreased sarcoplasmic reticulum calcium adenosine triphosphatase (Ca-ATPase) expression and increased phospholamban expression (Ca-ATPase inhibitor) are the main reasons for cardiac diastolic dysfunction. All these proteins regulate the intracellular calcium cycle and diastolic function. These genomic changes explain the slowed isovolumetric relaxation phase, which is a characteristic diastolic marker of hypothyroidism.[12,15] Indeed, a study conducted in 2013[16] demonstrated significant prolongation of both the systolic pre-ejection period and the isovolumetric relaxation period in hypothyroid patients.

A study performed in 2013 in Turkey[17] included 25 patients with subclinical hypothyroidism, 21 patients with overt hypothyroidism, and 28 healthy controls and detected a decreased E/A ratio, which is among cardiac diastolic echocardiographic parameters both in subclinical and overt hypothyroidism patients when they are compared with the control subjects. Consistent with the findings of this paper, we also found a low E/A ratio in our study and detected diastolic dysfunction in hypothyroidism patients. In another study,[18] a decrease in both E and E/A values was found in hypothyroidism patients. Again, consistent with this literature, we found an important decrease in E and E/A values in our study.

In our study, we found MPI value was high, and EF value was normal between the control subjects. Several factors, like left ventricular volume and cardiac geometry, play a role in the calculation of cardiac ejection fraction.[19] We think this may be the reason that we found a high MPI and a normal EF value. A study performed in Brazil in 2004[20] demonstrated a significantly high MPI value in untreated central hypothyroidism patients. We also detected a statistically higher MPI value than the control group.
In conclusion, overt hypothyroidism causes pathological changes in the cardiovascular system and impairs both systolic and diastolic functions of the heart. Calculation of MPI values with echocardiography is a suitable method to determine these functional disturbances. It may give better information than EF measurements to predict future cardiac failure. For this reason, we think that its wider use in clinical practice would be beneficial.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Prof. Dr. Cemil Taşçıoğlu University (Number: E-48670771-903.99-219042563).

Informed Consent: Written informed consent was obtained from all patients participating in this study.

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Research Article

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1,1-Diphenyl-2-picrylhydrazyRadical-Scavenging Capabilities of 13 Essential Oils and Analysis of Volatile Components of the Most Effective Clove Essential Oil

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ABSTRACT

Objective: In this study,it is aimed to research the antioxidant activity of 13 essential oils and 4 synthetic standards, which are frequently used in aromatherapy.

Methods: The essential oils were determined by their 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging abilities and the volatile components of the most effective essential oil were analyzed by GC-MS.

Results: Among the essential oils, the one with highest DPPH radical-scavenging activity was clove essential oil (Eugenia Caryophyllus Flower Oil) (IC_{50} 2.31 µg/mL). A low IC_{50} (half-maximal inhibitory concentration) value indicates high radical-scavenging ability. The IC_{50} values of DPPH scavenging of essential oils and standard antioxidants decreased in the following order: ylang-ylang, frankincense, thyme, cedarwood (23.11 µg/mL), ylang-ylang, eucalyptus, bergamot, rosemary, wintergreen, lemon, ginger, immortelle, myrrh, grapefruit (34.66 µg/mL), and patchouli (69.32 µg/mL). help_outlineThe analysis of clove essential oil with the highest DPPH activity was performed by gas chromatography–mass spectrometry, and it was determined that it was mainly composed of eugenol (81.80%) and caryophyllene (13.90%).

Conclusion: Clove can be used for medicinal and food preservation purposes as a natural antioxidant.

Keywords: Clove, DPPH, Essential oil, GC-MS

INTRODUCTION

Essential oils are named after the plant from which they are derived. These are aromatic, volatile liquids obtained by steam distillation from the leaves, fruit, bark, or root parts of plants. Essential oils are chemically pure volatile compounds, usually colorless or light yellow, that can easily crystallize. Essential oil components vary greatly, sometimes due to genetic reasons but also due to climate, precipitation, or geographic origin.[1,2]

Essential oils are composed of hydrocarbons (monoterpenes, sesquiterpenes, and diterpenes), oxygenated derivatives of terpenes (esters, aldehydes, ketones, alcohols, phenols, and oxides), phenylpropanoids, and nonterpenic substances according to their chemical components.[3-5]

Thanks to the secondary metabolites contained in essential oils, they show antispasmodic, irritating, antiseptic, antifungal, antiviral, antimicrobial, and antioxidant properties. In addition, since essential oils have free radical-scavenging activity, it is reported that they have a preventive effect against many types of cancer, including liver, breast, and colon cancer cells caused by cellular damage caused by these radicals.[4-7]

Essential oils are widely used in hygiene products such as soaps and detergents, in perfumery and dermatological cosmetics, and in insecticide products due to their aromatic scents. Medicinal and aromatic plants are used as antioxidants, for antimicrobial activities, and as flavoring components in herbal tea, food supplements, and additives.[8,9] It has also been experimentally shown that essential oils used in aromatherapy are effective for memory, mental balance, and emotion and increase work efficiency.[7]

Essential oils contain natural antioxidants and help reduce oxidative stress. Antioxidants neutralize the effects of harmful molecules known as free radicals and prevent cell damage .[10]

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Clove essential oil (Eugenia Caryophyllus Flower Oil) has been used for years as a topical anesthetic and sweetener. It is known to have antimicrobial, anti-inflammatory, and antioxidant activity, mostly in relation to the content of eugenol and other polyphenolic compounds. Other uses of clove have also emerged, such as an insect repellent or growth-promoting agent .[11]

Oxidative stress is a condition that occurs when cells produce more free radicals than normal or when the cells' antioxidant defense mechanisms are insufficient. Free radicals are reactive molecules that can damage cells and cause oxidative damage because oxygen molecules carry more electrons than normal.[12]

Normal physiological processes and environmental factors (cigarette smoke, air pollution, radiation, etc.) are among the causes of oxidative stress. Oxidative stress can damage cell functions by damaging cell components such as deoxyribonucleic acid, proteins, and lipids. As a result, it may be associated with the development of various diseases; for example, conditions such as cancer, cardiovascular diseases, neurodegenerative diseases, and aging may be associated with oxidative stress.[13,14]

Free radicals, known to have harmful effects on human metabolism, cause deterioration during the processing and storage of fatty foods. To prevent this situation, natural antioxidants are preferred over synthetic antioxidants due to their side effects.

Today, there are many bioanalytical methods that measure the antioxidant effect. One of them, 1,1-diphenyl-2-picrylhydrazyl (DPPH) removal test, is the most preferred, popular, and widely used method to determine antioxidant ability.[15]

1,1-Diphenyl-2-picrylhydrazyl, is a compound used to measure antioxidant activity and can react with free radicals. This compound is widely used in the DPPH radical-scavenging test, which is a test used to detect substances with antioxidant activity that have significant benefits for human health. DPPH has a purple crystal structure and can accept electrons because it is a free radical. Antioxidants neutralize DPPH radicals to form a colorless compound, and by measuring this change, their antioxidant activity can be determined.[16,17]

In our study, it was aimed to analyze the DPPH radical-scavenging activity of 13 of the essential oils known for their antioxidant properties and the most used in aromatherapy, and the essential oil analysis of the essential oil with the highest antioxidant effect by gas chromatography–mass spectroscopy (GC-MS).

METHODS

Plant Materials

Clove (Eugenia Caryophyllus Flower Oil), ylang-ylang (Cananga Odorata Flower Oil), eucalyptus (Eucalyptus Radiata Leaf Oil), bergamot (Citrus Aurantium Bergamia Fruit Oil), rosemary (Rosmarinus Officinalis Leaf Oil), wintergreen (Gaultheria Procumbens Leaf Oil), lemon (Citrus Limon Peel Oil), ginger (Zingiber Officinale Root Oil), immortelle (Helichrysum Italicum Flower Oil), myrrh (Commiphora Myrrha Oil), grapefruit (Citrus Paradisi Peel Oil), pelargonium (Pelargonium Graveolens Oil), and patchouli (Pogostemon Cablin Leaf Oil) essential oils were obtained from Elantra Pharmaceuticals Health Cosmetics Ltd. Co.

1,1-Diphenyl-2-picrylhydrazyl Radical–Scavenging Assay

Using the DPPH-scavenging technique, the free radical-scavenging capacity of the 13 essential oils and 4 standards was assessed.[18] The technique relies on antioxidants to remove DPPH free radicals. Standards and extracts were generated with concentrations ranging from 20 to 60 µg/mL. For each sample, 500 µL of DPPH (0.1 mM) was added to tubes. For 30 minutes, these tubes were kept in the dark at 25°C. At 517 nm, the measurements were taken. Samples of DPPH potentials were calculated and compared to standards. Finally, the half maximal inhibitory concentration (IC_{50}) values for each sample were determined. The decrease in absorbance demonstrates the sample's capacity to scavenge DPPH free radicals.[18]

Gas Chromatography–Mass Spectrometry

In the GC-MS study, a Agilent 6890N Network GC system, a 5977 mass spectrometer detector, an Agilent 7693 series autosampler, and an HP-5 MS column (30 m × 0.250 mm ID, film thickness 0.25 µm) were used. Helium was used as the carrier gas (0.8 mL/min). Chromatographic analysis was performed with a flow rate of 0.8 mL/min and an injection volume of 1 µL split mode (40:1). The GC oven temperature was kept at 60°C for 10 minutes, increased by 4°C, and fixed at 220°C for 10 minutes. It was incubated at 240°C for 1 minute at a rise of 1.0°C per minute. Injector and detector temperatures were chosen as 250°C and 300°C, respectively. Mass spectra were recorded at 70 eV scanning mode (35-450 m/z) and analyzed according to the peak area.

RESULTS

The DPPH free radical-scavenging activities of essential oils and positive antioxidants, such as α-tocopherol (1.14 µg/mL; $r^2 = 0.9377$), Trolox (1.14 µg/mL; $r^2 = 0.9377$), BHA (butylated hydroxyanisole) (1.33 µg/mL $r^2 = 0.9526$), clove (2.31 µg/mL; $r^2 = 0.9136$), BHT (butylated hydroxytoluene) (3.85 µg/mL; $r^2 = 0.9535$), ylang-ylang (34.66 µg/mL; $r^2 = 0.9281$), eucalyptus (34.66 µg/mL; $r^2 = 0.9722$), bergamot (34.66 µg/mL; $r^2 = 0.9889$), rosemary (34.66 µg/mL; $r^2 = 0.9019$), wintergreen (34.66 µg/mL; $r^2 = 0.9453$), lemon (34.66 µg/mL; $r^2 = 0.9777$), ginger (34.66 µg/mL; $r^2 = 0.8340$), immortelle (34.66 µg/mL; $r^2 = 0.8770$), myrrh (34.66 µg/mL; $r^2 = 0.9049$), grapefruit (34.66 µg/mL; $r^2 = 0.9452$), pelargonium (34.66 µg/mL; $r^2 = 0.9551$), patchouli (69.32 µg/mL; $r^2 = 0.9889$) (Table 1, Figures 1 and 2).

Table 1. The IC_{50} (µg/mL) Values for DPPH˙ Scavenging of Essential Oils and Standard Antioxidants

	DPPH˙ Scavenging	
Antioxidants	IC_{50}	r^2
α-Tocopherol	1.14	0.9377
Trolox	1.14	0.9377
BHA	1.33	0.9526
Clove	2.31	0.9136
BHT	3.85	0.9535
Ylang-ylang	34.66	0.9281
Eucalyptus	34.66	0.9722
Bergamot	34.66	0.9889
Rosemary	34.66	0.9019
Wintergreen	34.66	0.9453
Lemon	34.66	0.9777
Ginger	34.66	0.8340
Immortelle	34.66	0.8770
Myrrh	34.66	0.9049
Grapefruit	34.66	0.9452
Pelargonium	34.66	0.9551
Patchouli	69.32	0.9889

BHA, butylated hydroxyanisole; BHT, butylated hydroxytoluene; DPPH˙, 1,1-diphenyl-2-picrylhydrazyl radical.

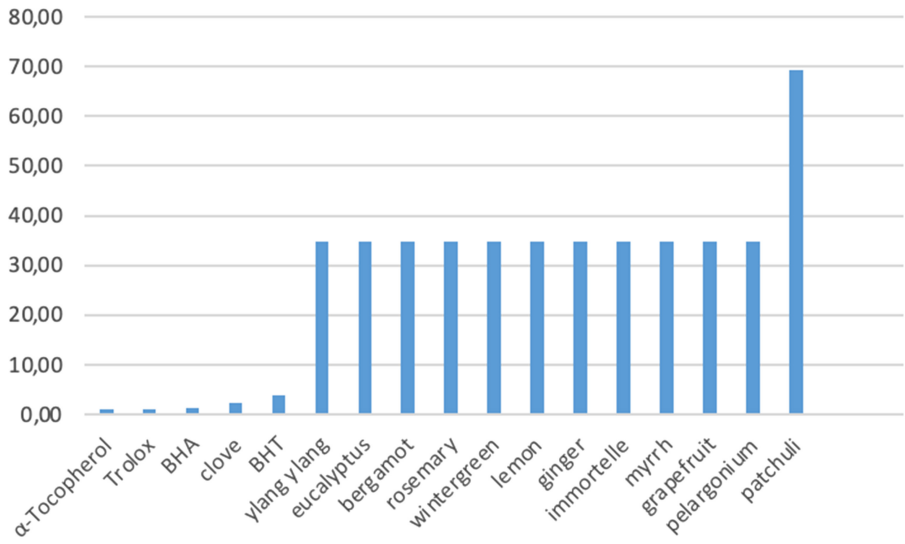

Figure 1. Comparison of DPPH•-scavenging abilities of essential oils and positive controls. DPPH•, 1,1-diphenyl-2-picrylhydrazyl radical.

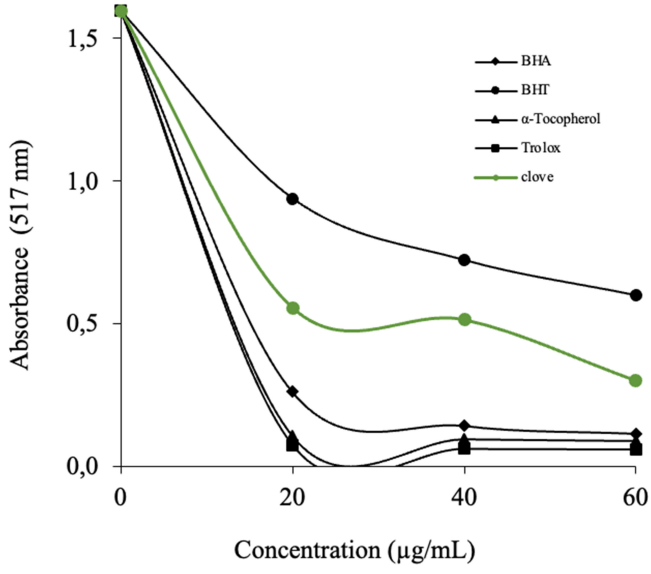

Figure 2. DPPH•-scavenging ability of clove and positive controls. DPPH•, 1,1-diphenyl-2-picrylhydrazyl radical.

The analysis of the clove essential oil was carried out by GC-MS. In the resulting chromatogram, the representative and characteristic components are defined. The ratios of these components specified by the integrator are shown in Table 2. This creates the chromatographic profile of the essential oil. According to the results of the analysis, eugenol was determined to be 81.80% and caryophyllene was determined to be 13.90%. It is thought that eugenol may be responsible for antioxidant and other biochemical and pharmacological activities.

Table 2. GC-MS Analysis of Clove Essential Oil

Peak	Compounds	RT (Minutes)	Area%
1	Chavicol	23.685	0.51
2	Eugenol	30.375	81.80
3	Caryophyllene	34.287	13.90
4	*α*-Humulene	36.496	3.60
5	Cadinene *δ*	40.541	0.19
	Total identified		100

RT, retention time.

DISCUSSION

In this study, the DPPH radical-scavenging abilities of the 3 most commonly used oils in aromatherapy treatments were determined and compared. In our study, clove oil had a very high radicalscavenging effect compared to BHT and other oils used as positive controls. Half maximal inhibitory concentration was used to indicate the practicality of antioxidant testing using DPPH radicals. The lower the IC_{50} values, the higher the DPPH radical-removing ability of the antioxidants. In this context, the IC_{50} value is widely used in biochemistry to compare the radical-scavenging capacities of different antioxidants. When the previous studies are examined, the IC_{50} values of the DPPH radical-scavenging activity of the 13 essential oils we studied are as follows: clove 1.8 µL/mL DPPH IC_{50},[19] ylang-ylang 1.30 ± 0.03 µL/mL,[20] eucalyptus > 10 000 mg/L,[21] bergamot 1040 ± 0.9 µg/mL,[22] rosemary 77.6 µL/mL,[23] wintergreen 34.16 mg/mL,[24] lemon 16 mg/mL,[25] ginger 675 µg/mL,[26] immortelle 438.9 ± 15.6 µg/mL,[27] myrrh 11.33 mg/mL,[28] grapefruit 22.06 ± 0.92 mg/mL,[29] pelargonium 83.26 ± 0.01 µg/mL,[30] patchouli IC_{50} = 1.31 µg/mL.[31]

Clove essential oil is popularly used for sore throat and toothache, due to its beneficial effects on the oral–dental mucosa. It is generally used in mouthwash as a mouthwash between 1% and 5%.[32,33] In our study, the clove essential oil contained 81% eugenol, and the oil's effect is thought to be due to this active ingredient. The effects of essential oils can vary depending on the amount and type of phenolic compounds in their content. In addition, the content of essential oils can vary according to environmental factors such as soil, climate, and temperature in the environment where the plants grow.[2]

With a general perspective, the DPPH radical-scavenging activity of 13 essential oils and synthetic antioxidants (BHT, BHA, α-tocopherol, and Trolox) most commonly used in aromatherapy was determined in our study. Essential oil components were determined by GC-MS analysis of the most active clove. It is possible that clove essential oil can be used as a radical scavenger in the food or pharmaceutical industries. For further studies, the antioxidant mechanisms of clove and major eugenol should be explained by different methods.

Ethics Committee Approval: Ethics committee approval is not required for this study.

Informed Consent: There is no need to obtain patient consent.

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ATATURK UNIVERSITY PUBLICATIONS

Research Article

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Anatomy of *Paeonia mascula* (L.) Mill. (Paeoniaceae)

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ABSTRACT

Objective: The primary aim of this investigation was to explore the anatomical structures of the stem, leaf, petiole, petal, and fruit of *Paeonia mascula*.

Methods: Plant specimens were collected from Aşkale/Erzurum (Turkey) in July 2017. Subsequently, standard herbarium techniques were employed to dry and preserve the samples, which were stored at the Herbarium of Atatürk University, Biodiversity Application and Research Center. For anatomical analysis, the materials were preserved in 70% alcohol. Characteristic elements of these plant parts were identified through sectioning, and their structures were visually documented with photographs.

Results: The leaf exhibited a bifacial arrangement, and stomata were observed on the lower leaf epidermis. Furthermore, unicellular trichomes and druses were observed to prominent in both the leaf and stem of *P. mascula*. Additionally, starch-bearing parenchyma was identified in the pericarp anatomy, and druses were also present in the seed.

Conclusion: Comprehensive characterization of the anatomical properties of *P. mascula* was demonstrated in this study. The findings obtained in this study suggest that the observed anatomical diversity may have potential benefits in taxonomical classification.

Keywords: *Paeonia mascula*, Paeoniaceae, plant anatomy

INTRODUCTION

The name *Paeonia* is known to come from the Greek mythology, referring to the supreme god who was the physician of the gods or the one who healed the gods, known as "Paean" (Paeeon, Paeon). In ancient Greece, the origin of the name signifies "healing."[1]

The genus *Paeonia* has attracted significant attention from the scientific community due to its potential as a source of bioactive compounds. *Paeonia* belongs to the Paeoniaceae family and comprises 179 scientifically named plant species. Among these, 36 species have been accepted according to The Plant List database. The genus has a rich traditional use in treating various conditions such as amenorrhea, hematemesis, dysmenorrhea, epilepsy, spasms, and gastritis, with 21 species, 2 subspecies, and 7 varieties reported in traditional treatments. The root and root bark are the most commonly used parts of *Paeonia* plants. 451 compounds from these plants have isolated, including monoterpenoid glucosides, flavonoids, tannins, stilbenes, triterpenoids and steroids, and phenols. Additionally, *Paeonia* has demonstrated various biological activities such as antioxidant, anti-inflammatory, antitumor, antimicrobial, cardioprotective, and neuroprotective effects .[2]

Paeonia mascula (L.) Mill, which is relatively widespread from Spain to Iraq via France, Italy, the Balkans, Cyprus, and Turkey, holds particular significance. An infusion of young aerial parts of *P. mascula* subsp. *arietina* is used on an empty stomach to lower blood glucose in folk medicine *P. mascula* (L.) Miller is employed in managing jaundice and urinary system disorders. Furthermore, *P. mascula* roots have been used as a sedative for epilepsy and cough management, as well as a respiratory regulator. In Anatolia, *P. mascula* roots and flowers are utilized to deal with ulcer, cough, and epilepsy.[2]

P. mascula (L.) Mill. Paeoniaceae) is known as "Gülorç, Gülhorç, Ayı gülü, Eşek gülü in Turkey[3]. It is grown in Amasya, Gümüşhane, Bayburt, Rize, Balikesir, Yozgat, Tunceli, Elazığ, Bitlis and Bingöl cities in our country.[4] The aerial part of *P. mascula* contains tannins, flavonoids, essential oils, and alkaloids.[5,6] The leaves of the plant are used in Turkish folk medicine as antidiabetic, sedative, and antitussive.[3,6] In this study, anatomical structures of the stem, leaf, petiole, petal, and fruit of *P. mascula* were investigated. The characteristic elements of stem, leaf, petiole, petal, and fruit were identified with taking the sections. Theirs structures were illustrated with photographs.

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METHODS

Plant Material

The sample of *Paeonia mascula* was collected on July 2017, at an elevation of 2020 meters, from the foothills of Aşkale Mountain, Erzurum, Turkey. The plant specimen is stored at the Herbarium of Atatürk University, Biodiversity Application and Research Center (AUEF 1351). The general appearance of *P. mascula*,fruits, and a herbarium specimen with flower, are shown in Figure 1.

Anatomical Studies

The specimens for anatomical analysis were kept in a 70% alcohol solution. This study aimed to examine the anatomical features of the stem, leaf, petiole, petal, and fruit of *P. mascula*. Sections were made to distinguish and identify the specific components of these plant parts. The structures were visually represented through the use of photographs. The imaging process involved a Zeiss 51425 camera, attached to a light microscope (Zeiss 415500-1800-000, Carl Zeiss Microscopy).

RESULTS

Anatomy of Stem

The stem cross section appears like a bear head, approximately 7 vascular bundles are regular. Cuticle is thin and striated. The central cylinder is large. Starch grains and druses can be found within the central cylinder of parenchyma. The stem has also trichomes, which are long and short. The anatomy of stem is shown in Figure 2.

Anatomy of Leaf

Anatomy of the Leaf Midrib

The leaf is bifacial. Druses and parenchyma containing starch grains are located in the mid-vein. Cuticle is thin and the leaf midrib vein has trichomes which are long and short. The anatomy of the leaf midrib is shown in Figure 3.

Figure 1. (From left to right) The general appearance of *Paeonia mascula*, fruits, and a herbarium specimen with flower.

Anatomy of the Leaf Upper and Under Surfaces

The leaf is bifacial. There isn't stoma on the upper surface. Stoma type is anisocytic on the under surface. The anatomy of the leaf upper and under surfaces is shown in Figure 4.

Anatomy of Petiole

There are 5 vascular bundles and it is regular. One of them is central and is the biggest, others are laterals. The size of the others is gradually diminishing. Cuticle is thin and there are druses everywhere especially on central vascular bundle. The anatomy of petiole is shown in Figure 5.

Anatomy of Fruit

Both exocarp and mesocarp are thick. Pericarp is bright yellow. There are parenchyma with starch grains and stone cell. The anatomy of fruit is shown in Figure 6.

Anatomy of Seed

There is parenchyma with starch grains. There are druses on central parenchyma cells. The anatomy of seed is shown in Figure 7.

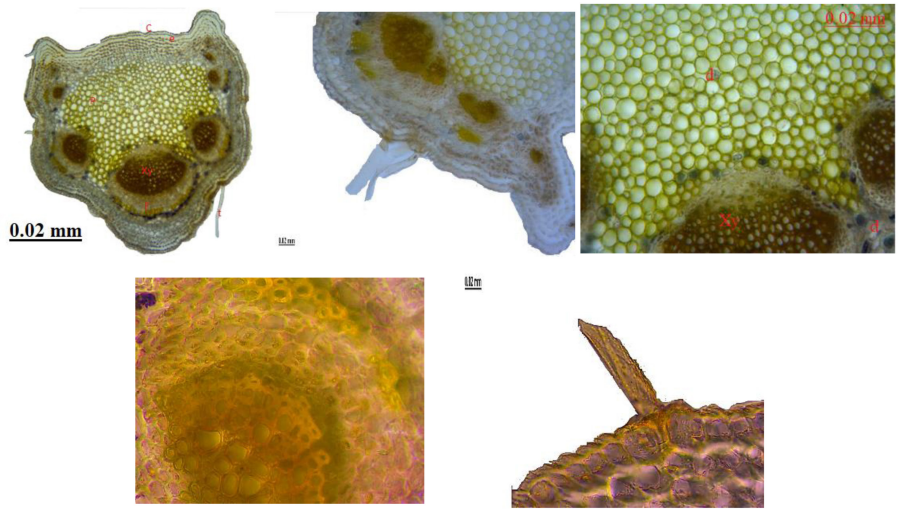

Figure 2. The anatomy of stem. c, cuticle; d, druse; e, epidermis; P, parenchyma; ph, phloem; t, trichome; xy, xylem.

Figure 3. The anatomy of mid-vein.

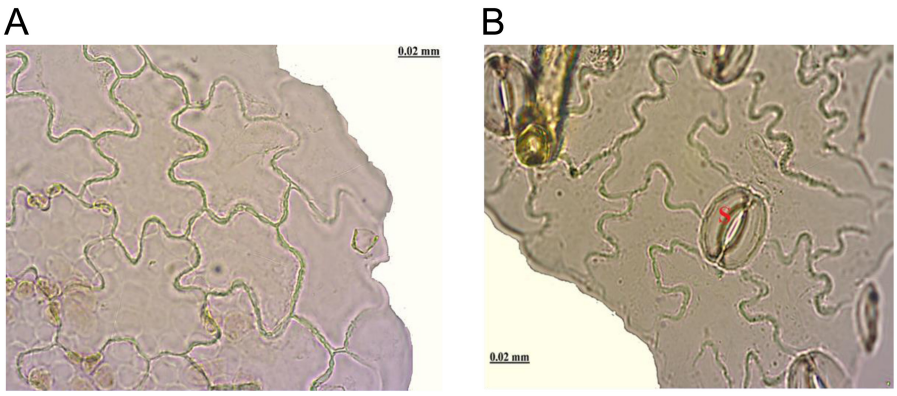

Figure 4. The anatomy of upper (A) and under (B) surfaces. S, stoma.

DISCUSSION

This study focused on examining the anatomical characteristics of the stem, leaf, petiole, seed, and fruit of *P. mascula*. The anatomical attributes described in this investigation offer a comprehensive portrayal of *P. mascula*. The results obtained from this study suggest that the observed anatomical variations could hold significance in the taxonomical categorization of the species.

The diverse array of plant species found on Earth serves as the primary source of raw materials for crude drugs. In the pharmacopoeial texts of crude drugs, a comprehensive description

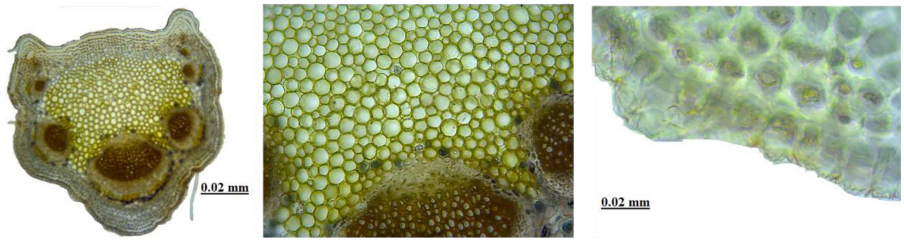

Figure 5. The anatomy of petiole.

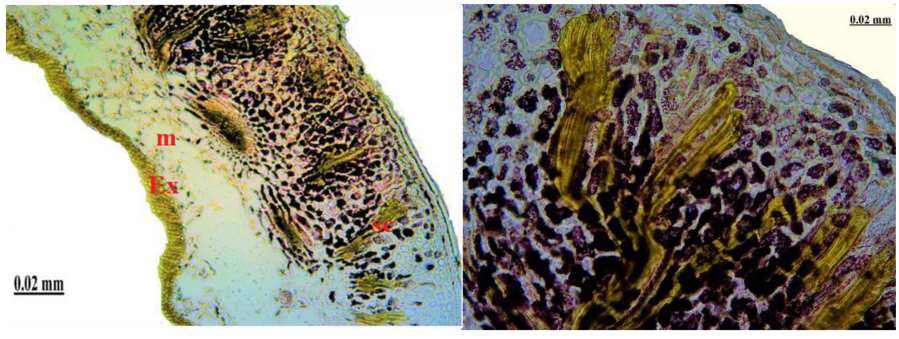

Figure 6. The anatomy of fruit. Ex, exocarp; M, mesocarp; Sc, stone cell.

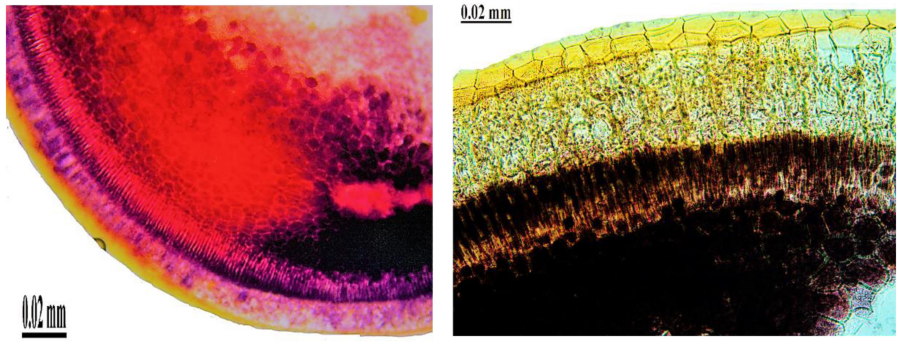

Figure 7. The anatomy of seed.

of a drug plant at both the macroscopic (morphological) and microscopic (anatomical) levels is crucial for establishing its botanical identity, ensuring the quality of herbal preparations, and establishing pharmacognostic standards. Higher plants possess various morphological organs, including roots, stems, leaves, flowers, and fruits. Some of these organs may undergo modifications to fulfill diverse functions. During analysis, factors such as the shape, size, and form, along with the color, texture, fracture characteristics, and features of the cut surface of these major organs and associated minor structures are carefully considered to establish the plant's identity and quality.[7]

The comparative examination of plant structure and anatomy has always been fundamental to plant systematics, aiming to unravel the intricacies of plant diversity, phylogeny, and evolution. The latter half of the 20th century witnessed an intriguing era during which systematics and structural studies greatly benefited from the emergence of novel techniques and methodologies.[8]

Anatomical studies in plants are of significant importance in understanding their structure, function, and overall biology. In summary, anatomical studies in plants are fundamental to numerous scientific disciplines, ranging from taxonomy and evolution to ecology, medicine, and agriculture. This comprehensive knowledge provides valuable insights into the complexity and diversity of the plant kingdom and informs various aspects of human life and the environment.

This study provides a detailed description of the anatomical properties of *P. mascula*. The findings suggest that the observed anatomical diversity could be beneficial in taxonomical classification. Our study provided a comprehensive anatomical characterization of various plant parts of *P. mascula*. We identified distinctive features such as bifacial leaf arrangement, stomata on the lower leaf epidermis, unicellular trichomes, druses in the leaf and stem, starch-bearing parenchyma in the pericarp, and druses in the seeds. These findings contribute to a better understanding of *P. mascula*'s anatomical diversity and may prove valuable in its taxonomical classification.

Ethics Committee Approval: Ethical approval was not required as there were no animal or human studies conducted.

Peer-review: Externally peer reviewed.

Author Contributions: Concept – S.G., S.K.; Design – S.G., S.K.; Supervision – S.K., Z.G.; Resources – S.G., S.K., Z.G.; Materials – S.G., S.K., Z.G.; Data Collection and/or Processing – S.G., S.K.; Analysis and/or Interpretation – S.G., S.K.; Literature Search – S.G., S.K.; Writing Manuscript – S.G., S.K.; Other – S.G., S.K., Z.G.

Declaration of Interests: The authors declare that they have no competing interest.

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ATATURK UNIVERSITY PUBLICATIONS

Research Article

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Simultaneous Spectrophotometric Determination of Dexketoprofen Trometamol and Thiocolchicoside by Using Principal Component Regression Multivariate Calibration Model in Combined Pharmaceutical Formulation

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ABSTRACT

Objective: The combination of the non-steroidal anti-inflammatory drug dexketoprofen trometamol (DXT) and the centrally acting muscle relaxant thiocolchicoside (TH) is used for symptomatic relief in various conditions, with the aim of alleviating associated symptoms. The study involves the development, validation, and application of the chemometrics-based spectrophotometric method.

Methods: An integral aspect of the investigation is the strategic application of experimental design techniques for calibration and validation mixtures to facilitate the assessment of factor effects within complex matrices. In this study, a factorial design was used to prepare calibration (25 samples) and validation (8 samples) sets comprising mixtures of DXT and TH within their linear ranges (2.5-25 µg/mL for DXT and 2-16 µg/mL for TH).

Results: Spectra of the acquired mixtures and samples were recorded at wavelengths between 220nm and 460 nm at $\Delta\lambda = 1$ nm intervals. Using regression models based on the principal component regression algorithm, the results obtained showed satisfactory performance, with a recovery rate of ≤98.54% for DXT and ≤98.88% for TH.

Conclusion: These models offer the potential for accurate identification and quantification of DXT and TH in pharmaceutical preparations

Keywords: Dexketoprofen trometamol, experimental design, PCR, thiocolchicoside

INTRODUCTION

Pain is often associated with potential tissue damage or described as a similarly unpleasant emotional experience. It is a major reason why people seek medical attention. Pain can be acute or chronic.[1] Physiologically, there are 3 types of pain: nociceptive (protective), inflammatory (tissue damage), and pathological (nervous system origin).[2] Today, one of the most preferred groups of analgesic drugs for the prevention of pain is the non-steroidal anti-inflammatory drugs (NSAIDs). This group of drugs works by inhibiting the enzyme cyclooxygenase. In this way, they prevent the synthesis of prostaglandins, which leads to peripheral sensitization, reduction of pain, and inflammation.[3,4]

Dexketoprofen is a ketoprofen (S+) enantiomer of NSAIDs. The chemical composition includes 2-amino-2-(hydroxymethyl) propane-1,3-diol and 2-(3-benzoylphenyl)propanoic acid.[5] Dexketoprofen trometamol (DXT) has analgesic, anti-inflammatory, and antipyretic properties and is more potent than ketoprofen.[6] Thiocolchicoside (TH) is used as a muscle relaxant. The chemical composition consists of N-[(7S)-3-(beta-D-glucopyranoxyloxy)-1,2-dimethoxy-10-(methylsulfanyl)-9-oxo-5,6,7,9-tetrahydrobenzo[a]heptalen-7-yl]acetamide and is a semi-synthetic colchicoside derivative.[7] Thiocolchicoside acts as a selective agonist by binding to the GABA-A receptor and the citrickinin-sensitive glycine receptor. It also has analgesic and anti-inflammatory properties.[8] There are many preparations of dexketoprofen that combine trometamol and TH. The combination of DXT and TH has a higher analgesic effect than DXT alone.[9] Dexketoprofen trometamol and TH, a centrally acting muscle relaxant, are used together in the symptomatic treatment of osteoarthritis, painful syndromes of the spine,

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extra-articular rheumatism, painful muscle contractions, and the treatment of pain following trauma and surgery.[10,11] In order to carry out quality control studies in combination drug preparations, it is necessary to determine the concentrations of the drugs. Both DXT quantification methods, including spectrophotometric[12] and chromatographic[13,14] methods, and TH measurements in various combined preparations, utilizing spectrophotometric[15-17] and chromatographic[18,19] methods, have been employed in previous studies. In addition, it was found that the determination in the combined pharmaceutical preparations of DXT and TH was performed using Ultra Violet (UV) spectrophotometer,[20-23] High-Performance Liquid Chromatography (HPLC),[24] Reverse Phase High-Performance Liquid Chromatography with Photodiode Array Detection (RP-HPLC-PDA),[25,26] and High-Performance Thin-Layer Chromatography (HPTLC)[27,28] methods in the literature review. The spectrophotometric method is a simple and cost-effective method used in quality control studies of drugs. In recent times, a chemometric approach has emerged as a promising solution for tackling the issue of overlapping spectra in various media within mixtures. This approach involves the development of chemometric calibration models based on sample spectra, offering advantages such as reduced chemical consumption and sample preparation, elimination of time-consuming processes, and enhanced accuracy and precision values without the need for chromatographic elution. This technique has been explored in studies[17,29] to effectively address the challenges posed by spectral overlaps in complex samples. The most popular multivariate calibration algorithms in chemometrics are the classical least squares method or K-matrix method, the inverse least squares method or P-matrix method, the principal component regression (PCR) method, and the partial least squares regression (PLS) method, which is recognized as a straightforward and efficient calibration approach.[30]

METHODS

Instrument

The UV-visible measurements were performed using a Thermo Scientific Multiscan GO51119300 model instrument. In this study, spectrophotometric measurements were performed using a microplate reader with the following settings: data mode: absorbance, start wavelength (nm): 220, stop wavelength (nm): 460, scan speed: fast, bandwidth: 1 nm, and microplate temperature (°C): 25.

Materials

Dexketoprofen trometamol and TH were acquired from Sigma-Aldrich (Darmstadt, Germany). Methanol (Merck, Darmstadt, Germany) and double deionized water (Milli-Q water) were utilized as chemical reagents and were of analytical grade. Stock solutions of both analytes were prepared by dissolving the analytes in methanol at a concentration of 250 µg/mL for DXT and 40 µg/mL for TH. The commercial pharmaceutical preparation named "Dexplus effervescent tablet" was purchased from a pharmacy in Erzurum, Turkey.

Chemometric Method and Data Preprocessing

In this study, PCR, which is one of the chemometric calibration methods, was employed. Principal component regression is based on the decomposition of the measured absorbance[31] data of the concentration set into mutually orthogonal (orthogonal) axes. These obtained axes serve as the coordinate system for constructing the calibration.

Construction of the Training Set

Different working solutions of DXT (2.5-25 µg/mL) and TH (2-16 µg/mL) were prepared in methanol by using their stock solutions. A calibration batch of 25 mixtures was meticulously prepared following a well-established full factorial design with 5 levels in 2 categories. The UV absorption spectra of these resulting mixtures were meticulously recorded in the wavelength range of 220-460 nm at increasing intervals of 1 nm. Detailed concentration details can be found in Figure 1A.

Construction of the Validation Set

In order to assess the predictive capability of the PCR method, a validation set was generated. This set included 8 different laboratory-prepared mixtures, each containing different concentrations of DXT and TH, following a full factorial design with 3 levels in 2 categories (Figure 1B). Following the formulation process, absorbance measurements were taken within the 220-460 nm spectrum.

For data analysis, the PCR model was constructed using MATLAB R2019b with PLS-Toolbox software version 8.5.1. The calibration and validation sets were created using Design-Expert 12.0 software (Stat-Ease Inc., Minneapolis, Minn, USA). Additionally, statistical analyses were performed utilizing Microsoft Excel 2018.

Preparation of Pharmaceutical Formulations

In this study, the pharmaceutical formulation containing DXT and TH, namely Dexplus effervescent tablets, was meticulously carried out through the following steps:

Powdering of the commercial product: The commercial product containing DXT and TH was finely ground into a powder using a mortar and pestle.

Sample extraction: Approximately 1 tablet of the powdered commercial product, equivalent to 25 mg DXT and 8 mg TH, was accurately weighed and placed in a volumetric flask.

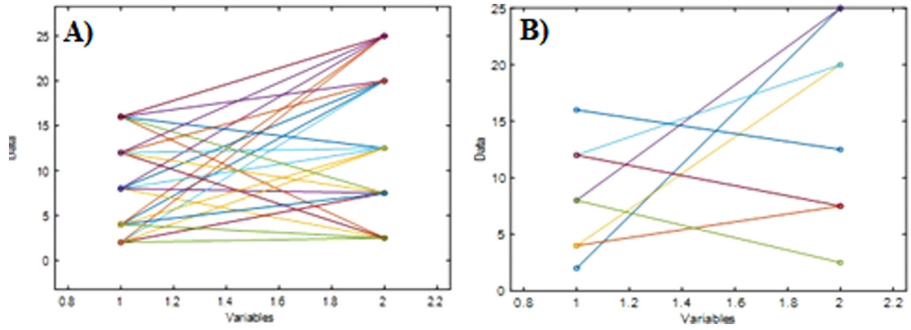

Figure 1. (A) Calibration and (B) Validation sets with varied dexketoprofen trometamol and thiocolchicoside concentrations.

Dissolution and solution preparation: The weighed tablet powder was dissolved in a methanol solution, and the volume was adjusted to 100 mL to ensure thorough mixing.

Ultrasonication and cooling: The prepared solution was ultrasonicated for 15 minutes to enhance dissolution. After this step, the solution was allowed to cool to room temperature.

Dilution and spectral recording: To ensure accurate measurements, the resulting solution was diluted accordingly. Spectra in the UV-visible region of the diluted solutions were recorded for further analysis.

RESULTS AND DISCUSSION

Optimization of Spectrophotometric Method

According to previous studies, it has been reported that compounds DXT and TH are insoluble in organic solvents, while they exhibit good solubility in water and methanol.[16] Based on this information, it was observed that compounds DXT and TH dissolved effectively in a methanol solution. After determining the optimal spectroscopic conditions, individual spectra of compounds DXT and TH were obtained within the wavelength range of 220-460 nm in the methanol solution. Regarding standard DXT and TH, the respective maximum absorbances are observed to occur at 258 nm and 370 nm, respectively (Figures 2A and 2B). It was noted that both compounds exhibited a linear wavelength range. Examination of the absorption spectra of DXT and TC (Figure 2C) revealed a significant overlap between the 2 compounds, which posed a challenge to their direct quantification. Therefore, the primary objective of this study was to implement highly sensitive and accurate analytical techniques to enable the simultaneous quantification of DXT and TH in their individual forms, laboratory-prepared mixtures, and pharmaceutical dosage forms without the need for prior separation.

Principal Component Regression Multivariate Calibration Model

Spectral Data Processing and Calibration Model Development

Recently, spectrophotometric analysis with chemometric calibration models has proven to be a robust method for the quantification of binary drug-active substances with overlapping spectra. This approach has gained popularity due to its effectiveness in solving the challenges posed by the spectral overlap of these compounds. Chemometric calibration models have been used to obtain accurate quantitation. In particular, the PCR model was developed to provide simultaneous and precise quantitative analysis of each drug in the binary mixture. But, careful preparation of calibration and validation data matrices is necessary for a comprehensive chemometric analysis. In this context, this study involved the pooling of several samples containing different concentrations of DXT- and TH-active pharmaceutical ingredients (2.5-25 µg/mL for DXT and 2-16 µg/mL for TH) for the preparation of a calibration set. By including a wide range of concentrations, the calibration model effectively captured spectral variations across the entire concentration spectrum, resulting in a more robust and reliable prediction model. This set included absorbance values measured at 241 points with $\Delta\lambda = 1$ nm intervals in the wavelength range of 220-460 nm. For each sample, the measured absorbance values were associated with the corresponding compound concentrations. In this context, a pair was created for each sample: the measured absorbance value and the known compound concentration. The prepared calibration sets were subjected to PCR algorithm-based modeling. The variance–covariance matrices of absorbance and concentration values within the calibration set were calculated, thereby forming a fundamental basis for the PCR calibration models. By processing absorbance data through a composition process in the variance–covariance matrix, a mathematical relationship was established between absorbances and concentrations, laying the foundation for robust calibration.

Optimal Factor Determination and Cross-Validation

To ascertain the optimal factor numbers for the PCR algorithm, a cross-validation procedure was conducted, employing 25 calibration spectra. During each iteration of this process, a single calibration solution was treated as a sample. The procedure was repeated 25 times in total, and using 24 of the calibration spectra, PCR calibrations were conducted. The estimated concentrations of analytes were cross-validated by comparing them with the actual concentrations. The root- mean square error of cross-validation (RMSECV) was computed as an evaluation metric using the following formula:

$$\mathrm{RMSECV} = \sqrt{1/N}\left(\sum^{N_{i=1}}\left(C_{\mathrm{estimated}} - C_{\mathrm{actual}}\right)^2\right)$$

Optimal Factor Selection, Model Performance and Model Evaluation, and Performance

The selection of the optimal number of factors is crucial for ensuring accurate quantitation in PCR calibration. The chosen factor numbers are guided by criteria such as minimizing the prediction error sum of squares, RMSECV, or *F*-statistic. Figure 3 illustrates the variation of RMSECV as a function of the number of factors for DXT and TH. For both compounds, an optimal factor number of 3 was determined for accurate quantitation in mixtures containing DXT and TH. Among the selected models, the one with the lowest RMSECV value was preferred. The 5-factor PCR calibration yielded RMSECV values of 0.486 and 0.295 for DXT and TH, respectively.

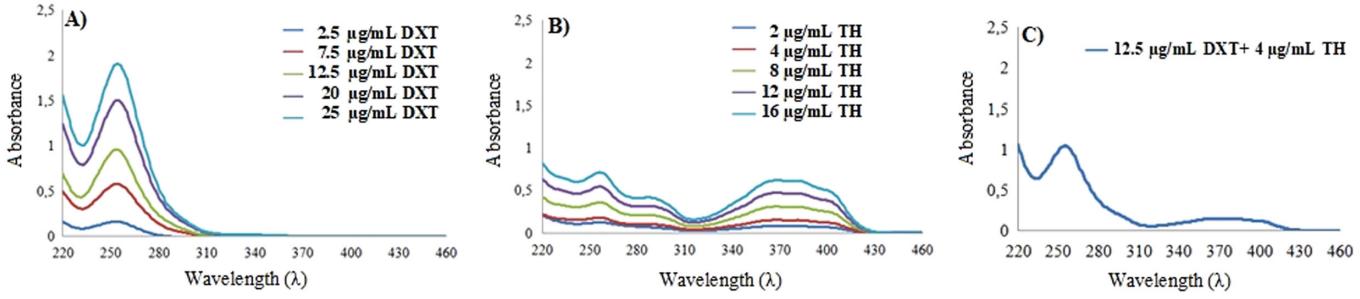

Figure 2. UV absorption spectra: (A) Ddexketoprofen trometamol (DXT) (2.5-25 µg/mL), (B) thiocolchicoside (TH) (2-16 µg/mL), and (C) spectral overlay of DXT (12.5 µg/mL) and TH (4 µg/mL).

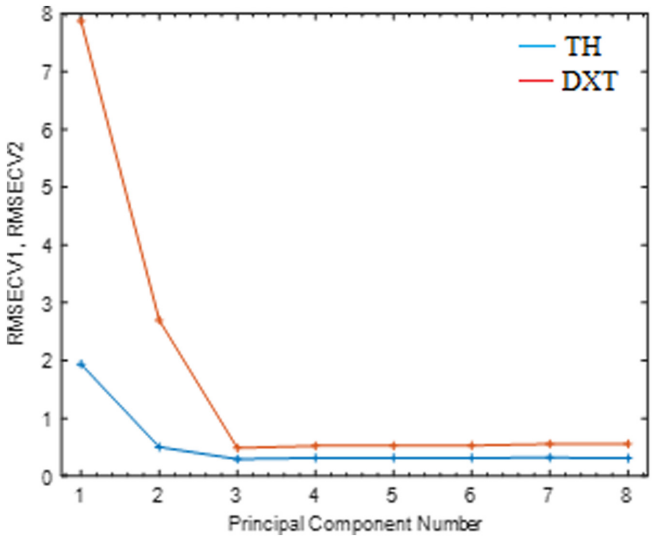

Figure 3. Principal component number curves versus root mean square error of cross-validation by Principal Component Regression multivariate calibration for DXT and TH."

The predictive capabilities of the models were assessed by graphing the predicted concentration against the known true concentration. The statistical results are presented in Table 1.

Validation of Chemometric Calibration

The validation of the developed chemometric model was conducted using the validation set depicted in Figure 1B. The validation set underwent the same analysis procedures as the calibration data set. The accuracy assessment was conducted by determining recovery values, which were found to be ≤99.71% for DXT and ≤98.73% for TH. To evaluate precision, the relative standard deviation values were in the range of 0.08%-3.63% for TH and 0.62%-2.21% for DXT. The statistical outcomes of this validation process are summarized in Table 2. Prior to the application of the PCR calibration method to actual commercial pharmaceutical preparation, recovery studies were conducted using the standard addition method. The pharmaceutical formulation, Dexplus effervescent tablets (8 mg TH and 25 mg DXT), as outlined in Section 2.3, was dissolved in methanol. Standard solutions were prepared at concentrations of 2-16 µg/mL for TH and 2.5-25 µg/mL for DXT. UV-visible spectra were obtained for each standard addition.

The PCR calibration method was applied, with formulation contributions subtracted. Percentage recovery values for both compounds within the added standards were calculated. The recovery percentages obtained from the PCR model were greater than 98.54% for DXT and 98.88% for TH, indicating a remarkable level of accuracy (Table 2).

Table 1. Summary of Statistical Parameters for Simultaneous Quantification of DXT and TH via PCR Multivariate Calibration Model

PCR Model/Cross-Validation Results	DXT	TH
Concentration (µg/mL)	2.5-25	2-16
Spectral region (nm)	220-460	220-460
Optimum number of factors	3	3
Calibration curves	1.0141x+0.0321	1.0039x+0.0068
R^2	0.9999	0.9999
RMSECV	0.486	0.295
RMSEC	0.420	0.254
Kalibrasyon bias	$-1.77.10^{-15}$	0

DXT, dexketoprofen trometamol; PCR, principal component regression; R, correlation coefficient; RMSEC, Root Mean Square Error of Calibration; RMSECV, root mean square error of cross-validation; TH, thiocolchicoside.

Table 2. The Prediction Results Obtained with Validation Set for the PCR Calibration Model

Drug Mixture (µg/mL)		Found (µg/mL)∓SD		Recovery %		RSD%	
TH	DXT	TH	DXT	TH	DXT	TH	DXT
4	7.5	4.06 ± 0.01	7.81 ± 0.05	101.44	104.17	0.08	0.62
4	20	4.13 ± 0.07	20.26 ± 0.28	103.20	101.31	1.76	1.40
8	25	8.15 ± 0.09	25.87 ± 0.17	101.85	103.48	1.12	0.65
8	2.5	8.09 ± 0.01	2.51 ± 0.03	101.09	100.41	0.18	1.18
12	20	11.93 ± 0.12	19.94 ± 0.24	99.42	99.71	1.03	1.21
12	7.5	11.96 ± 0.20	7.53 ± 0.08	99.69	100.35	1.70	1.09
2	12.5	2.01 ± 0.07	12.51 ± 0.20	100.69	100.06	3.63	1.64
16	12.5	15.80 ± 0.16	12.53 ± 0.28	98.73	100.21	1.00	2.21

DXT, dexketoprofen trometamol; PCR, principal component regression; RSD, relative standard deviation; SD, Standard Deviation, TH, thiocolchicoside.

Application to Commercial Pharmaceutical Preparation

In order to demonstrate the applicability of the developed and validated PCR method to pharmaceutical formulations, quantification analyses of active substances in pharmaceutical preparations containing DXT and TH were performed. The pharmaceutical preparation containing DXT and TH, namely Dexplus effervescent tablets, was dissolved in methanol as described in "Preparation of Pharmaceutical Formulations"Section. Subsequent dilutions were performed to prepare solutions with concentrations of DXT and TH ranging from 4 to 8 µg/mL and 12.5 to 25 µg/mL, respectively. The absorbance values of the prepared solutions, measured in the wavelength range 220-460 nm with $\Delta\lambda = 1.0$ nm intervals, were recorded (Figure 4).

The PCR algorithm described above was then applied to calculate the quantities of DXT and TH within the tablet content. The results presented in Table 3 underline the reliability and effectiveness of the PCR method for quantitative analysis of DXT and TH in complex pharmaceutical matric. This successful application further validates the practical value of the developed PCR method in pharmaceutical analysis, providing a robust and accurate approach to the quantification of active substances in commercial pharmaceutical formulations.

In this study, we developed, validated, and applied a chemometrics-based spectrophotometric method for the simultaneous quantification of DXT and TH in pharmaceutical formulation. An important aspect was our use of experimental design techniques for calibration and validation mixtures. This approach allowed us to study the effects of factors in the presence of varying levels of other factors, thereby increasing the efficiency of the method

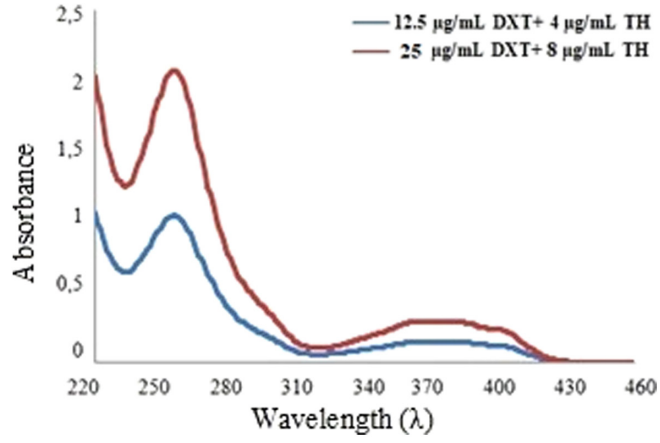

Figure 4. The UV absorption spectra of the drug (Dexplus effervescent tablets) solutions containing dexketoprofen trometamol (DXT) and thiocolchicoside (TH).

Table 3. Simultaneous Determination of DXT and TH in the Commercial Pharmaceutical Preparation

Commercial Pharmaceutical	Active Substance	Number of Analyses	Mean ± SD	Recovery %	RSD %
Dexplus effervescent tablets	TH (8 mg)	30	7.94 ± 0.015	99.43	0.195
	DXT (25 mg)	30	25.14 ± 0.54	100.54	2.15

DXT, dexketoprofen trometamol; RSD, relative standard deviation; SD,Standard Deviation, TH, thiocolchicoside.

in complex matrix analysis. The successful development of our chemometric calibration model using UV-visible spectra demonstrated the potential of the PCR method for accurate DXT and TH quantification. The model demonstrated remarkable precision and accuracy, confirming its effectiveness for complex matrix analysis. Validation, including accuracy, precision, and recovery studies, further confirmed the robustness of the method. Consistently achieved recovery percentages within the predefined range underscored the method's ability to recover true concentrations in the midst of complex matrices. Application of our method to a pharmaceutical formulation demonstrated its practical utility by accurately determining DXT and TH concentrations. This confirmed the applicability of the method for real-world pharmaceutical analysis.

Ethics Committee Approval: There was no need for ethics committee approval for this study. The research conducted did not involve human subjects, animal experimentation, or sensitive personal data. Therefore, ethical approval was not required in accordance with the established regulations and guidelines governing research ethics.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – F.D.M., N.K.; Design – F.D.M.; Supervision – F.D.M., N.G.; Resources – F.D.M., N.K.; Materials – F.D.M., N.K.; Data Collection and/or Processing – F.D.M., N.K.; Analysis and/or Interpretation – F.D.M., N.K.; Literature Search – F.D.M., N.K.; Writing Manuscript – F.D.M., N.K.; Critical Review – F.D.M., N.K.

Declaration of Interests: The authors declare that they have no competing interest.

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ATATURK UNIVERSITY PUBLICATIONS

Review

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The Relationship Between Euthyroid, Hyperthyroid, Hypothyroid, and Type 2 Diabetes

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ABSTRACT

Diabetes mellitus (DM) and thyroid dysfunction, which have a significant incidence worldwide, are the most common endocrine system disorders that occur together in patients. Our aim is to explain these 2 diseases with high incidence and the relationship between these 2 diseases. Thyroid hormones (TH) are essential hormones that govern body metabolism. Thyroid hormone changes are thought to be effective on the pathogenesis of DM. Diabetes mellitus treatments can be beneficial, as TH changes may contribute to the pathogenesis of DM. However, more research needs to be done. This lack of information limits potential biomarkers and targets for diagnosis, prognosis, and the development of new DM treatments. The limitations of the use of natural THs have led to the development of synthetic hormones called thyromimetics. However, most of the thyromimetics tested so far have been ineffective or toxic.

Keywords: Diabetes, euthyroid, hyperthyroid, hypothyroid

INTRODUCTION

Diabetes mellitus (DM) is a significant global health issue that has a profound impact on the quality of life of affected individuals. The prevalence of diabetes is increasing worldwide, and it is projected to continue to rise in the coming years. According to the International Diabetes Federation (IDF), the number of adults with diabetes was estimated to be 1 in 11 in 2015, and this number is expected to reach 642 million by 2040. Furthermore, diabetes-related mortality is a major concern. The IDF reported that in 2019, approximately 4.2 million deaths were attributed to diabetes. Without effective intervention and management, this number is projected to increase to 700 million by 2045. Diabetes is among the top 10 causes of death globally, highlighting the severity of the disease and its associated complications.[1]

Diabetes mellitus is indeed a rapidly growing disease worldwide, characterized by chronic elevated blood sugar levels. It is an endocrine system disorder that disrupts the metabolism of carbohydrates, fats, and proteins due to insufficient insulin secretion or reduced tissue sensitivity to insulin. Insulin, which is produced by the beta cells in the pancreas, plays a vital role in regulating blood sugar levels. In individuals with DM, there is a loss of functional beta-cell mass, leading to an imbalance in insulin production and utilization. This results in an inability to effectively regulate blood glucose levels.[2]

Types of Diabetes Mellitus

Today, diabetes is classified in four different ways, and it now includes 4 main categories

- Type 1 diabetes (T1D): This type of diabetes is characterized by autoimmune destruction of the beta cells in the pancreas, resulting in an absolute deficiency of insulin. It often develops early in life, and individuals with T1D require lifelong insulin therapy to manage their blood sugar levels.
- Type 2 diabetes (T2D): Type 2 diabetes is the most common form of diabetes and is usually associated with insulin resistance, where the body's cells become less responsive to insulin. Over time, the beta cells in the pancreas may also progressively lose their ability to produce sufficient insulin. Type 2 diabetes is often associated with lifestyle factors such as obesity, sedentary behavior, and poor dietary habits. It can be managed through lifestyle modifications, oral medications, or insulin therapy.
- Gestational diabetes: Gestational diabetes occurs during pregnancy and is diagnosed in the second or third trimester. It is characterized by elevated blood sugar levels that were not present before pregnancy. Gestational diabetes usually resolves after delivery, but women who have had gestational diabetes have an increased risk of developing T2D later in life.
- Specific types due to other causes: This category includes various forms of diabetes that have specific underlying causes. It encompasses monogenic diabetes syndromes, which are genetic disorders

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that affect insulin production or utilization. Exocrine pancreatic diseases, such as pancreatitis or cystic fibrosis, can also lead to diabetes. Additionally, certain medications or chemicals can induce diabetes as a side effect.[3]

The classification of DM into these 4 categories helps guide appropriate treatment strategies and management approaches. It allows healthcare professionals to tailor treatment plans based on the underlying causes and mechanisms of the disease in each individual case.[4]

Epidemiology and Prevalence

Incidence in Children and Adolescents

According to the IDF in 2017, more than 96 000 new cases of T1D are diagnosed each year in children and adolescents under the age of 15. This highlights the significant impact of T1D on the younger population.

Prevalence

Type1 diabetes accounts for approximately 10% of all diabetes cases, as reported by the Diabetes Association of America. It affects around 20 million people worldwide, indicating its global prevalence and impact on individuals living with the condition.

Age of Diagnosis

Although T1D can occur at any age, it is most commonly diagnosed in early childhood (around ages 4 to 5) or during adolescence. This aligns with the observation that T1D is usually diagnosed during critical periods of growth and development.

Increasing Incidence

The incidence of T1D in children under 15 years of age has been observed to increase across Europe. The EURODIAB ACE study group reported an average annual increase of 3.4% in this age group, with the highest increase seen in children under 5 years of age. This suggests a concerning trend of rising T1D cases in younger children.

These statistics emphasize the significant burden of T1D on individuals, families, and healthcare systems globally. Further research and efforts are needed to understand the underlying causes and develop effective strategies for prevention, early diagnosis, and management of T1D.[5]

Pathophysiology

Type 1 diabetes is characterized by an autoimmune response that leads to the selective destruction of insulin-producing pancreatic β-cells. This autoimmune process results in a deficiency of insulin production, leading to high blood sugar levels. The onset of T1D occurs when a significant number of β-cells are destroyed. Type 2 diabetes is primarily characterized by insulin resistance, where the body's cells become less responsive to the effects of insulin. This reduced sensitivity to insulin leads to impaired glucose uptake by cells and elevated blood sugar levels. In addition to insulin resistance, T2D also involves impaired insulin secretion by pancreatic β-cells, contributing to elevated blood glucose levels.

Interaction Between Insulin and the Liver

In normal physiological conditions, insulin plays a crucial role in maintaining appropriate plasma glucose concentrations. One of its functions is to interact with the liver, regulating glucose production and storage. Disruption of this interaction, as seen in T2D, can lead to dysregulated glucose metabolism, impaired insulin secretion, and insulin resistance.

Pathological Defects in T2D

Type 2 diabetes is characterized by a combination of insulin resistance, impaired insulin secretion, and abnormal glucose metabolism. These pathological defects contribute to the development of persistent hyperglycemia and the long-term complications associated with T2D.

Understanding these underlying mechanisms is crucial for developing targeted interventions and treatments for both T1D and T2D. It highlights the importance of insulin production, secretion, and sensitivity in maintaining glucose homeostasis and the need to address these dysfunctions in diabetes management.[5]

Type 1 diabetes is characterized by the autoimmune destruction of pancreatic β-cells, resulting in insulin deficiency. Insulin gene mutations can lead to insulinopathies, causing abnormal insulin synthesis and secretion. Hyperinsulinemia is commonly observed in patients with insulinopathies. In T1D, abnormalities are seen in beta, alpha, and delta cells in pancreatic islets. Insulin secretion defects in T1D include a relative decrease in basal secretion, impaired response to glucose and amino acids, and reduced insulin sensitivity. Type 1 diabetes is also associated with an increase in alpha-cell mass, leading to hyperglucagonemia. Islet amyloid deposits containing islet amyloid polypeptide may play a role in T2D pathogenesis, but its exact role is not fully understood. Type 2 diabetes involves insulin resistance, impaired insulin secretion, and abnormal glucose metabolism. Insulin resistance may result from decreased insulin receptor number, hyperinsulinemia, hyperglycemia, or abnormalities in glucose transporter proteins. Genetic factors, including mutations in the insulin receptor gene and certain glucose transporter genes (GLUT2 and GLUT4), contribute to T2D susceptibility. Environmental factors, such as obesity, sedentary lifestyle, dietary factors, and age, also play a role in insulin resistance. The risk of T2D is influenced by genetic factors, but the individual effects of genetic variants have limited predictive value. Impaired glucose tolerance is characterized by hyperglycemia despite high insulin levels, indicating insulin resistance. As the condition progresses to diabetes, insulin secretion decreases. It is important to note that the information you provided is a general overview, and the development and progression of T1D and T2D involve complex interactions between genetic, environmental, and physiological factors. Further research is needed to fully understand the mechanisms underlying these diseases.[3-5]

Thyroid

The thyroid gland is an endocrine gland responsible for producing and releasing hormones into the bloodstream. The main hormones produced by the thyroid gland are triiodothyronine (T3) and thyroxine (T4). These hormones play a crucial role in regulating metabolism, growth, development, and the functioning of various organs and tissues in the body. The thyroid gland is located in the front of the neck, below the Adam's apple (in men) or the thyroid cartilage. It has a butterfly-shaped structure with 2 lobes connected by a narrow band of tissue called the isthmus. The gland is richly supplied with blood vessels and receives signals from the brain, specifically the hypothalamus and pituitary gland, which help regulate the production and release of thyroid hormones (THs).[6]

Thyroid Physiology

The body needs THs for the metabolism to work correctly and functionally. Thyroid hormone production is a tightly regulated process controlled by a classical negative feedback loop involving

the hypothalamus, pituitary, and thyroid, giving rise to the common name hypothalamus–pituitary–thyroid axis. Thyrotropin-releasing hormone (TRH) is produced in the hypothalamus. After TRH is released, it reaches the pituitary gland and binds to the TRH receptor, stimulating the production and secretion of thyroid-stimulating hormone (TSH), also known as thyrotropin.[6]

In the thyroid, TSH binds to the TSH receptor and induces TH production. Triiodothyronine and T4 are released into the circulation when needed. In the hypothalamus and pituitary, THs act through the nuclear TH receptor β (THRβ) to inhibit TRH and TSH production and secretion, completing a negative feedback loop that maintains physiological levels of TRH, TSH, and THs. In target cells, deiodinases (DIO2 and DIO3) produce T3 from T4 by removing iodine at the 5' position of T4. The expression of different deiodinases is cell type and tissue specific, providing a mechanism to control TH movements independent of circulating TH levels.[7]

Thyroid hormones are essential for the development and maturation of various tissues and for general well-being. In areas of adequate iodine exposure, the prevalence of TH changes in the general population is estimated to be ~0.5%-4%. There are different types of TH changes (hyperthyroidism, subclinical hyperthyroidism, subclinical hypothyroidism, and hypothyroidism) that cause different clinical symptoms. Recent epidemiological meta-analyses have identified a clear association between TH changes and risk of death in the general population.[8]

Thyroid Types

There are thyroid diseases that occur when the homeostasis of the thyroid and thyroid signaling is impaired. These are hypothyroidism, characterized by decreased production and/or circulation of TH; hyperthyroidism, characterized by increased production and/or circulation of TH; and euthyroidism, which is any thyroid enlargement characterized by selective (limited to one or several sites) thyroid tissue enlargement. The onset of these diseases is caused by genetic and environmental factors. Dietary iodine consumption is one of the determinants of thyroid disease risk, but other factors such as aging, gender, genetic predisposition, ethnicity, smoking, endocrine disruptors, and immune system inhibitors also affect the epidemiology of the disease. Between 3% and 10% of adults, especially women, are affected by hypothyroidism. This condition is associated with iodine deficiency, and about a third of the world's population lives in areas with low iodine diets. This is the main cause of hypothyroidism and endemic goiter. In fact, 80% of people living in areas with severe iodine deficiency suffer from goiter. In areas where iodine deficiency is not present, Hashimoto's thyroiditis is the most common type of autoimmune hypothyroidism, occurring in 1% to 2% of cases. The prevalence of hyperthyroidism is 0.8% in Europe and 1.3% in the United States. Marked hyperthyroidism is a disease characterized by low serum TSH concentrations and high levels of serum T4 (tetraiodothyronine), T3, or both. On the other hand, subclinical hyperthyroidism is characterized by low serum TSH but normal serum T4 and T3 levels. As a result of overproduction of T4, diseases such as Graves' disease (GD), thyroid nodules, and thyroiditis occur.[9]

Hashimoto's thyroiditis (HT) is one of the leading autoimmune thyroid diseases (AITD) of hypothyroidism. Approximately 20%-30% of thyroid patients suffer from HT. The cause of the disease is thought to be a combination of genetic predisposition and environmental factors that cause the loss of immunological tolerance. This causes an autoimmune attack on the thyroid tissue and the emergence of the disease. Hashimoto's thyroiditis, which causes chronic inflammation in the thyroid tissue, is the most common of the autoimmune thyroid disorders.[10]

Graves' disease, which occurs as a result of impaired immune tolerance to thyroid antigens, is the most common cause of hyperthyroidism in developed countries. The annual incidence of GD is 20 cases/100 000 people. It is generally seen between the ages of 30 and 60 and is 5 to 10 times more common in women than in men. Genetic predisposition explains 79% of GD risk, and environmental factors explain 21%. Among the endogenous factors of GD, estrogens, X-chromosome inactivation, and microchimerism occupy an important place. Environmental risk factors include smoking, iodine excess, selenium and vitamin D deficiency, and occupational exposure.[11]

Effects of Thyroid Hormones on Glucose and Lipid Metabolism

Thyroid hormones induce catabolism of all types of energy sources by increasing oxygen consumption. Thyroid hormones are effective modulators of lipid and glucose metabolism. Thyroid hormones specifically reduce circulating triglycerides and cholesterol-containing lipoproteins. Thyroid hormones stimulate the expression of sterol response element-binding protein 2 (Srebp-2). Increased Srebp-2 levels contribute to increasing low-density lipoprotein (LDL) receptor expression, which enhances hepatic cholesterol uptake. Moreover, THs are known to increase lipolysis and liponeogenesis simultaneously. In fact, THs are known to increase the expression of carnitine palmitoyltransferase (mitochondrial fatty acid uptake) and acetyl-coenzyme A carboxylase (lipogenic).[12] A comprehensive analysis of these processes has shown that liponeogenesis is enhanced to maintain lipid levels under conditions of elevated lipolysis. Under these conditions, lipolysis is enhanced to provide substrates for thermogenesis. Carbohydrate metabolism is also affected by THs. Gluconeogenesis and glycogenolysis are known to be enhanced by THs in a process that supports tissues with fuel to maintain their energy requirements. In this sense, hepatic insulin resistance has been shown to increase gluconeogenesis and subsequently hepatic glucose output in hyperthyroid individuals.[13] Increased rates of gluconeogenesis are supported by increased Cori cycle activity, which involves muscle tissue in providing substrates (lactate and certain amino acids such as alanine and glutamine) for hepatic gluconeogenesis. This process represents a dynamic glucose buffer that allows it to be used by other tissues under their glucose requirements as needed. It is known that THs in the liver increase the expression of phosphoenolpyruvate carboxykinase, which is the rate-limiting step in gluconeogenesis, and supports the direct role of THs in the regulation of these processes. Studies in mice exposed to T4 mimicking hyperthyroidism have shown that insulin signaling is active in insulin-target tissues even under fasting conditions due to dysregulated function of the endocrine pancreas (for example, increased insulin secretion followed by circulating levels). Overall, compelling data in the literature suggest that THs produce effects in a few, if not all, tissues involved in glucose and lipid homeostasis.[14]

Changes in Thyroid Hormones in Diabetes Mellitus

The relationship between changes in thyroid function and the development of different types of DM has been the focus of intense research. The prevalence of hyperthyroidism in DM individuals is higher than in nondiabetic subjects, and a study has determined that patients suffering from hyperthyroidism are at higher risk of developing DM. Among adult patients with T2D, ~4.4% have overt hyperthyroidism and 2%-4% have subclinical

hyperthyroidism. Interestingly, improved diabetic control in T2D patients normalizes TSH levels in patients with subclinical hyperthyroidism; this suggests that treatments that improve T2D may contribute to normalizing thyroid function. However, a recent study has shown that nondiabetic patients diagnosed with hyperthyroidism have an increased risk of developing T2D later in life, suggesting that thyroid dysfunction may precede diabetogenic processes.[15] Accordingly, hyperthyroid patients showed increased basal hepatic glucose production and increased fasting insulin levels compared to healthy individuals, while hyperthyroid patients treated with methimazole became euthyroid, exhibiting significantly reduced levels in the same parameters, reaching the levels of the healthy control group. One study showed that patients with overt or subclinical hyperthyroidism undergoing a glucose tolerance test had higher circulating glucose and insulin levels. Glucose intolerance in these patients is due to enhanced hepatic gluconeogenesis.[16] These effects may be related to the control-exerting THs in the expression of genes involved in glucose and lipid metabolism, suggesting that several physiological abnormalities that contribute to the loss of metabolic homeostasis are common in hyperthyroidism and T2D. Another study showed that hypothyroidism is associated with insulin resistance and dyslipidemia. Other evidence also points to an increased risk of DM in patients with hypothyroidism and has reported an increased prevalence of subclinical hypothyroidism in patients with T2D. Other studies have failed to link hypothyroidism with the development of T2D, in contrast to compelling research showing the relationship between DM and thyroid dysfunction, supported by the well-defined role of THs on glucose metabolism and insulin secretion. Increasing evidence links changes in thyroid function with other types of DM, such as T1D and gestational DM (GDM). Various studies have shown that patients with T1DM, an autoimmune disease, are prone to exhibit AITD such as HT and GD. Available data show that up to 30% of adults with T1D have thyroid diseases of autoimmune origin.[17]

Gestational DM is a common complication affecting ~10% of all pregnancies associated with adverse pregnancy outcomes such as preeclampsia, macrosomia, and cesarean delivery. Gestational DM disappears after birth, but in many cases different types of DM (GDM in a subsequent pregnancy or T2DM) can occur later in life. [18] Among the changes that occur during pregnancy, the placenta is known to increase the secretion of pro-inflammatory cytokines that induce insulin resistance to support nutrient availability for the fetus.[46] Under these conditions (e.g., transient insulin resistance during pregnancy), GDM is the result of the impaired capacity of pancreatic β-cells to increase insulin secretion to compensate for insulin resistance in insulin-target tissues. [19] Several reports have identified that maternal hypothyroidism predisposes the offspring to exhibit limited insulin secretion and develop glucose intolerance, increasing the risk of T2D in the offspring.[20] In addition, separate reports have identified hypothyroidism as being associated with GDM. In this regard, we found several mutations in PAX8. It leads to hypothyroidism associated with the development of GDM, suggesting that human GDM may have a genetic component. Remarkably, this study revealed that PAX8 expression in pancreatic islets modulates cellular pathways involved in cellular survival.[21]

The Physiological and Pathophysiological Role of Thyroid Hormones in the Endocrine Pancreas

One of the main organs involved in the control of circulating glucose levels is the endocrine pancreas. Extensive research has demonstrated the role of THs in the differentiation, maturation, and functionality of metabolic tissues.[22] In vivo research has determined that circulating T3 levels increase during postnatal development and induce the expression of MAF bZIP transcription factor A and THRs in pancreatic β-cells to facilitate their maturation.[23] A study on experimental animals showed that TH supplementation had severe effects on β-cells as well as increased β-cell proliferation and apoptosis. Strikingly, β-cells of T4-treated mice have been reported to exhibit increased glucokinase expression. At the organism level, T4-treated mice exhibit increased insulin expression and secretion in pancreatic islets under fasting conditions. This indicates that the insulin secretory mechanism is constitutively active to facilitate nutrient uptake by insulin-target tissues.[14]

Separate investigation in mildly hypothyroid PAX8 heterozygous knockout mice, which exhibit several hallmarks of T2D, demonstrated that pancreatic islets exhibited a transcriptional profile associated with increased metabolic activity and impaired antioxidant capacity.[24] Pancreatic β-cells are particularly vulnerable to oxidative stress due to very limited expression of antioxidant genes such as catalase and glutathione peroxidase (e.g., <5% of hepatic levels). More importantly, under typical cellular stress situations such as high glucose, high oxygen, or heat shock, pancreatic islets have virtually no capacity to increase the expression of antioxidant enzymes. Increased metabolic activity states with limited antioxidant defenses can generate oxidative stress, which can lead to accumulation of oxidative damage. In these cases, pancreatic endocrine function may be compromised and apoptotic processes may be initiated if cellular stress is not resolved.[25]

Thyroid Hormone-Related Changes in Insulin-Target Tissues

Changes in TH function have tremendous effects on liver tissue. Thyroid hormones cause increases in intracellular glucose production and insulin resistance.[26] Thyroid hormone-mediated insulin resistance can be produced by increased levels of cytokines produced in peripheral tissues such as adipose tissue[27] Given the central role of insulin action in the regulation of hepatic gluconeogenesis and glycogenolysis, the compromised insulin sensitivity produced by THs may have important implications of its own for glucose homeostasis.[28] Interestingly, effects on endogenous glucose production in the liver have been shown, in part, to mediate the effects of THs in the paraventricular nucleus of the hypothalamus, which mediates effects via sympathetic projections to the liver.[26] In this regard, Klieverik et al showed that increases in endogenous glucose production mediated by the paraventricular nucleus are independent of circulating levels of glucoregulatory hormone.[29] Furthermore, these studies showed that hepatic sympathetic denervation completely blunted the paraventricular TH-induced increase in endogenous glucose production. Nonalcoholic fatty liver disease (NAFLD) is the hepatic manifestation of metabolic syndrome. Various changes have been found in the pathogenesis of NAFLD in hypothyroid patients, including the development of insulin resistance, dyslipidemia, and increased adiposity.[30] Epidemiological studies have identified the existence of an inverse correlation between circulating TH levels and the incidence of NAFLD.[31] A study also showed that patients with NAFLD exhibit higher serum TSH levels and lower free T4 levels.[32] Accordingly, hypothyroidism is more common in patients with NAFLD when compared to healthy individuals matched for ethnicity, age, sex, and body mass index. Patients with nonalcoholic steatohepatitis (NASH), a more serious form of fatty liver disease, had a higher incidence of hypothyroidism compared to patients

suffering from NAFLD without NASH. Individuals diagnosed with hypothyroidism were 2.1 times more likely to experience NAFLD and NASH, respectively.[33] In addition, NASH and advanced fibrosis are more common in patients with overt and subclinical hypothyroidism.[34] Interestingly, overt and subclinical hypothyroidism, as well as even upper TSH levels in the euthyroid range, have been associated with NAFLD regardless of established metabolic risk factors.[35] In addition, hypothyroid patients exhibit increased esterification of hepatic fatty acids with restricted lipoprotein lipase activity and decreased hepatic uptake of high-density lipoprotein, indicating an inappropriate cholesterol metabolism.[36]

Thyroid hormones also play an important role in other insulin-targeted tissues such as adipose tissue and skeletal muscle. The association between hypothyroidism and impaired insulin-stimulated glucose uptake in muscle and adipose tissue has been documented in animals and humans.[33,37] However, conflicting results were obtained when hyperthyroid patients were analyzed for positron emission tomography with 2-deoxy-2-[fluorine-18]fluoro-D-glucose integrated with computed tomography (18 F-A fluorodeoxyglucose [FDG] -positron emission tomography [PET]/CT). One report showed that hyperthyroid patients exhibit increased uptake of radioactive glucose in brown adipose tissue compared to euthyroid patients.[38] A second study achieved similar results (e.g., increased radioactive glucose uptake) in hypothyroid patients with thyroid carcinoma when these patients became mildly hyperthyroid upon TSH suppression. However, other studies have shown no difference in glucose uptake in patients with hyperthyroidism and in hypothyroid patients with thyrotoxicized thyroid cancer.[39]

Thyroid hormones exert significant effects on energy metabolism, which predisposes tissues and organs with high metabolic demand, such as skeletal muscle, to severe effects in patients suffering from hypothyroidism and hyperthyroidism. In skeletal muscle, glucose uptake represents the limiting step in glucose metabolism and is mediated by plasma membrane glucose transporter 4 (Glut4). Thyroid hormones induce Glut4 expression through a positive TRE (DR+4) region in its promoter, which represents a direct link with carbohydrate metabolism. Studies focusing on determining the physiological and pathophysiological role of THs in skeletal muscle have shown that up to 57% of patients with hypothyroidism show muscle damage manifested by elevated creatine kinase levels.[40]

Remarkably, TH treatment in hypothyroid patients reduced creatine kinase levels and improved muscle complications. Individuals with hyperthyroidism also show muscle weakness, and normalization of TH function in these patients increases muscle strength and muscle cross-sectional area and improves insulin response upon administration of TH in patients and experimental models of hypothyroidism.[24] At the tissue level, THs increase insulin sensitivity in skeletal muscle; this effect is due to the proper conversion of T4 to T3 by DIO2. Accordingly, cell cultures of myotubes developed from DIO2 knockout mice and DIO2 knockout mice exhibit insulin resistance.[41] In vivo research mice treated with T4-treated hyperthyroid have shown that insulin signaling is chronically activated in skeletal muscle lysates, which may be detrimental in the long run.[24]

Treatment Approaches Based on Thyroid Hormones or Thyromimetics for Diabetes Mellitus

As mentioned earlier, hypothyroidism is associated with metabolic disorders that increase the tendency to develop T2DM. Thyroid hormone supplementation reduces the risk of developing this disease by normalizing DM-related parameters such as lipid and lipoprotein levels. Studies in mice have also supported the potential of THs to improve metabolic health. Thyroid hormone supplementation has been shown to increase glucose tolerance in wild-type mice and reduce hyperglycemia[42] in leptin receptor-deficient mice. Remarkably, research in mice has also identified the potential of THs to improve metabolic health and survival in experimental models of T1DM.[43] Our results showed that levothyroxine supplementation blunted the onset of experimental T1DM using the RIP-B7.1 model, which summarizes the β-cell-specific autoimmune attack in patients with T1D.[24] Interventional studies in humans using levothyroxine and the thyroxine enantiomer dextrothyroxine have shown increased serum levels of LDL cholesterol after treatment. However, treatments were discontinued as participants developed serious adverse events, confirming the narrow therapeutic window of interventions based on the use of THs.[44] However, the beneficial effects of interventions based on the use of THs in certain metabolic parameters have prompted the development of thyromimetics as promising agents to improve metabolic health. Newly produced thyromimetics could in principle provide therapeutic benefits in certain cell types or organs, resulting in improved metabolic homeostasis while avoiding side effects.[45] Therefore, fatty liver can be treated with thyromimetics designed to specifically target hepatic tissue. One study determined that the liver can be directly targeted using a glucagon-T3 mixed agonist that mediates selective delivery of T3 to the liver.[24,39] Studies have shown beneficial effects on hepatic steatosis, cholesterol, and triglyceride levels in rodents.[46] However, in some cases, thyromimetics (e.g., eprotirome and 3,5-d iiodothyropropionic acid [DIPTA]) have failed due to ineffectiveness or toxicity in human or preclinical studies.[47] Selective THRβ agonists MGL-3196 and VK2809 have recently been investigated in phase 2 clinical trials as lipid-lowering agents and for the treatment of NASH. The most recent results using MGL-3196 in humans indicate a significant reduction in hepatosteatosis after 12 and 36 weeks of treatment.[48] In addition, VK2809 has been shown to reduce hepatic lipid content and circulating LDL levels. However, given the potential side effects of thyromimetics, a better understanding of their selective metabolic effects and experimental and clinical studies evaluating the long-term effects of these interventions are needed.

CONCLUSION

Thyroid hormones are essential hormones that govern all body metabolism, acting on every cell of the body. Changes in THs leading to different forms of hyperthyroidism and hypothyroidism have been associated with various diseases such as DM. This suggests that TH changes share specific pathogenesis mechanisms in which THs contribute at different stages of DM pathogenesis. Therefore, treatments or interventions designed to treat DM may also have beneficial consequences on DM. Further research is needed to increase knowledge about the changes that occur in the early stages of DM and in diseases associated with THs; this currently limits potential biomarkers and targets to facilitate diagnosis, prognosis, and development of new treatments for DM. The pleiotropic effects of TH changes in different tissues indicate that personalized and precision medicine must be applied to provide the most appropriate treatment for each patient. The narrow therapeutic window of interventions based on the use of natural THs has led to the development of thyromimetics. However, most of the thyromimetics tested to date have failed due to ineffectiveness or toxicity.

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