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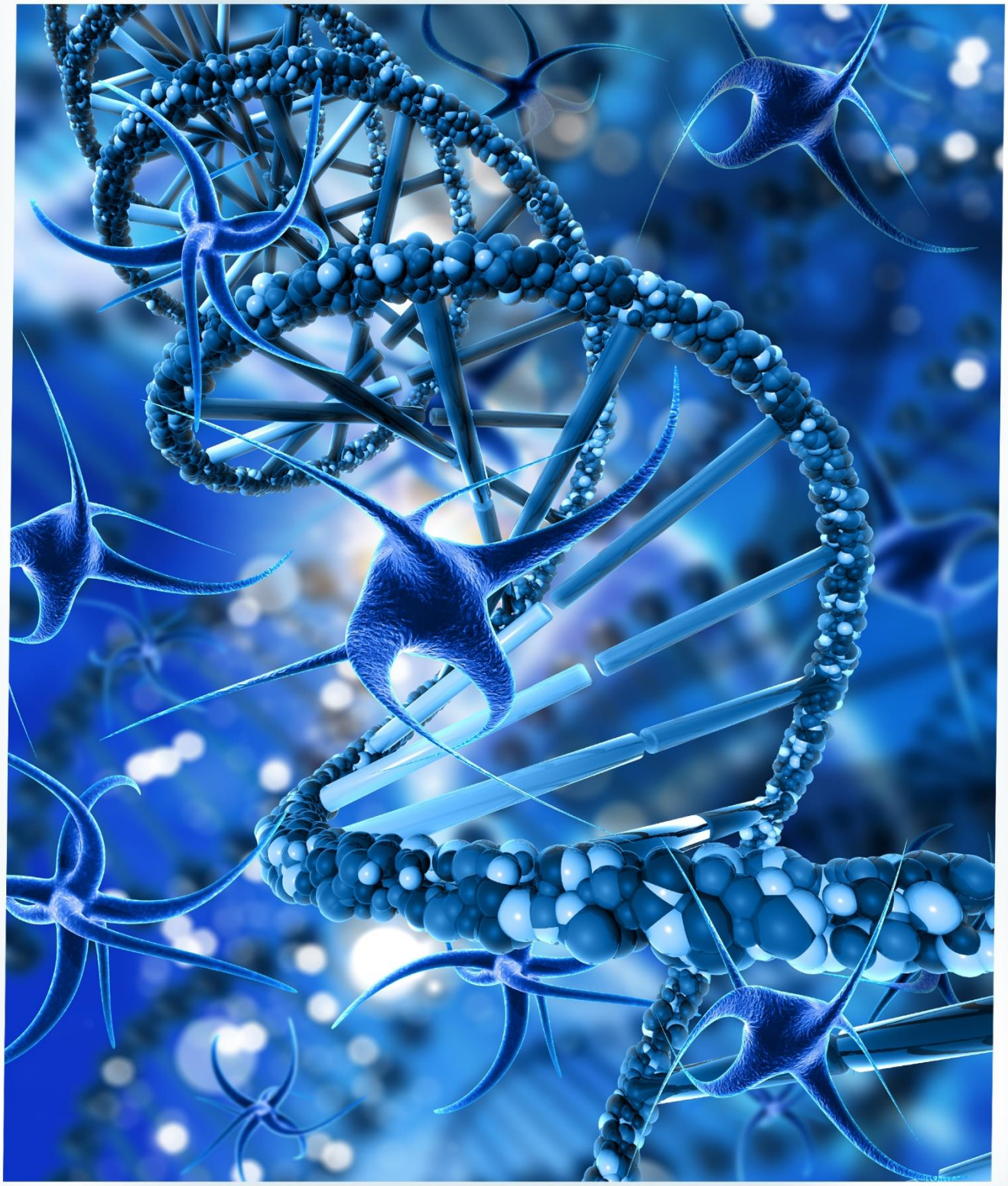

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Issue Content / *Sayı İçeriği*

Research Articles / ***Araştırma Makaleleri***

Selim Demir, Nihal Turkmen-Alemdar, Elif Ayazoglu-Demir, Ahmet Mentese, Yuksel Aliyazicioglu

Gallic Acid Alleviates Methotrexate-Induced Oxidative Ovarian Damage in Rats

*(Gallik Asit Sıçanlarda Metotreksatın Neden Olduğu Oksidatif Yumurtalık Hasarını Azaltır)*__***119-125***

Gokçe Seyhan, Ceren Boguslu, Can Ozgur Yalcın, Zekeriya Bıyıklıoglu, Burak Barut

DNA Binding, Nuclease/Photonuclease, and Phototoxicty Properties of Water Soluble Silicon (IV) Phthalocyanine

*(Suda Çözünür Silisyum (IV) Ftalosiyaninin DNA Bağlanma, Nükleaz/Fotonükleaz ve Fototoksik Özellikleri)*___***126-133***

Zekeriya Duzgun, Birgül Kural, Asim Orem, Ilkay Yildiz

Molecular Docking Analysis of the Affinities of Lipid-Lowering Drugs to Paraoxonase-1 Enzyme and Its Polymorphic Structures

*(Lipid Düşürücü İlaçların Paraoksonaz-1 Enzimine ve Polimorfik Yapılarına Afinitelerinin Moleküler Docking Analizi)*___***134-144***

Case Reports / ***Olgu Sunumları***

Ezgi Selcuk-Ozmen, Asli Enzel-Koc

Persistent Genital Arousal Syndrome; A Case Report

*(Persistan Genital Uyarılma Sendromu; Bir Olgu Sunumu)*___***145-148***

Review / ***Derleme***

Meltem Çelik

Antik Çağlarda Bir Kraniyotomi Uygulama Yöntemi: Trepanasyon

*(A Method of Performing Craniotomy in Ancient Times: Trepanation)*___***149-154***

Bunyamin Sevim, Hasan Basri Savas, Salim Bilici

Basic Principles in the Application of Problem-Based Learning in Medical Biochemistry Education

*(Tıbbi Biyokimya Eğitiminde Probleme Dayalı Öğrenim Uygulamasında Temel Esaslar)*___***155-158***

Issue Reviewers / *Sayı Hakemleri*

Farabi Tıp Dergisi 3(4):119-125,2024
Farabi Medical Journal 3(4):119-125,2024
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Gallic Acid Alleviates Methotrexate-Induced Oxidative Ovarian Damage in Rats

Gallik Asit Sıçanlarda Metotreksatın Neden Olduğu Oksidatif Yumurtalık Hasarını Azaltır

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ABSTRACT

Although methotrexate (MTX) is an effective chemotherapeutic agent in the treatment of cancer, its use is limited due to the occurrence of systemic tissue toxicity, including those affecting the reproductive system. Gallic acid (GAL) is a phenolic compound that has been demonstrated to exert beneficial effects in a number of pathological conditions associated with oxidative stress (OS) in recent years. This study was designed to investigate the potential therapeutic benefits of GAL in the treatment of MTX-induced ovarian damage, for the first time. Adult female rats (n=30) were randomly allocated to five groups: control, MTX, MTX+GAL (2.5 and 5 mg/kg) and high-dose GAL only (5 mg/kg). A single intraperitoneal injection of MTX (20 mg/kg) was administered to induce ovarian toxicity. The treatment groups were administered 2.5 and 5 mg/kg of GAL intraperitoneally for a period of three consecutive days. The levels of OS, inflammation and apoptosis were determined in ovarian tissue samples collected on the fifth day of the study using spectrophotometric methods. The results showed that GAL treatment reduced the level of ovarian lipid peroxidation, inflammation, and apoptosis and promoted the ovarian antioxidant system in rats subjected to MTX. The results of this study indicate that GAL may have the potential to ameliorate MTX-associated oxidative and inflammatory ovarian damage. The ovarian protective effect of GAL requires further confirmation through more extensive preclinical studies.

Keywords: Apoptosis, Gallic acid, Inflammation, Methotrexate, Ovarian damage, Oxidative stress, Rat model

ÖZET

Metotreksat (MTX) kanser tedavisinde etkili bir kemoterapötik ajan olmasına rağmen, üreme sistemi de dahil olmak üzere sistemik doku toksisitesinin ortaya çıkması nedeniyle kullanımı sınırlanmaktadır. Gallik asit (GAL), son yıllarda oksidatif stres (OS) ile ilişkili çeşitli patolojik durumlara karşı faydalı etkiler gösterdiği kanıtlanmış bir fenolik bileşiktir. Bu çalışma, MTX'in neden olduğu yumurtalık hasarının tedavisinde GAL'ın terapötik potansiyelini ilk kez araştırmak için tasarlandı. Yetişkin dişi sıçanlar (n=30) rastgele beş gruba ayrıldı: kontrol, MTX, MTX+GAL (2,5 ve 5 mg/kg) ve yalnızca yüksek doz GAL (5 mg/kg). Yumurtalık toksisitesi tek doz intraperitoneal MTX (20 mg/kg) enjeksiyonu ile oluşturuldu. Tedavi gruplarına ise 2,5 ve 5 mg/kg dozundaki GAL ardışık üç gün boyunca intraperitoneal yoldan uygulandı. Çalışmanın beşinci gününde toplanan yumurtalık doku örneklerinde OS, inflamasyon ve apoptoz seviyeleri spektrofotometrik yöntemler kullanılarak belirlendi. Bulgular, GAL tedavisinin, MTX'e maruz bırakılan sıçanlarda yumurtalık lipid peroksidasyon, inflamasyon ve apoptoz seviyesini azalttığını ve yumurtalık antioksidan sistemini desteklediğini gösterdi. Bu çalışmanın sonuçları, GAL'ın MTX ile ilişkili oksidatif ve inflamatuar yumurtalık hasarını iyileştirme potansiyeline sahip olabileceğini göstermektedir. GAL'ın bu yumurtalık koruyucu etkisinin daha kapsamlı klinik öncesi çalışmalarla doğrulanması gerekmektedir.

Anahtar Kelimeler: Apoptoz, Gallik asit, İnflamasyon, Metotreksat, Oksidatif stres, Rat modeli, Yumurtalık hasarı

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INTRODUCTION

Methotrexate (MTX) is an anti-folic acid compound employed for the treatment of ectopic pregnancy, autoimmune and malignant diseases, due to its inhibitory activity against dihydrofolate reductase.[1,2] This inhibitory feature affects one-carbon metabolism, which in turn disrupts nucleotide acid synthesis and amino acid metabolism, thereby exerting an anticancer effect.[3,4] MTX is employed in high doses in the treatment of cancer, and has the capacity to negatively affect rapidly dividing cells, including trophoblast and gonadal cells.[5] The toxicity of systemic MTX administration to healthy tissues, including ovaries, represents a significant limitation to its use and a source of concern in young female patients.[3,6] MTX has been documented to exert gonadotoxic effects on ovarian tissue, resulting in a reduction in the number of ovarian follicles.[7-9] It is well documented that high doses of MTX used in cancer treatment can cause early menopause[10], and one study of breast cancer patients undergoing chemotherapy even found that 68% of those treated with MTX experienced menopause.[11] It is proposed that the elevation in reactive oxygen species (ROS) and pro-inflammatory molecules may be a significant contributing factor in the development of MTX-induced tissue toxicity.[4,12-15] Nicotinamide adenine dinucleotide phosphate (NADPH), produced by the pentose phosphate pathway (PPP) and malic enzyme, plays a pivotal role in anabolic reactions and the regeneration of oxidized glutathione (GSSG) back to reduced glutathione (GSH).[16] It is well established that MTX inhibits glucose-6-phosphate dehydrogenase (G6PD), the control enzyme of the PPP. This results in a reduction in the quantity of NADPH, which possesses reducing power, and thus a depletion of GSH, the most significant endogenous antioxidant molecule.[7,17,18] MTX induces oxidative stress (OS) in cells by two mechanisms: firstly by depleting the GSH pool, and secondly by directly increasing the amount of ROS.[19] Consequently, OS results in the damage of lipids, proteins and DNA.[20] In general, tumor necrosis factor-alpha (TNF-α) is considered to be one of the pro-inflammatory cytokines that regulate inflammatory responses.[21] MTX is a known cause of inflammation, with a particular effect of increasing TNF-α concentration.[14,15] Chronic inflammation has been shown to further exacerbate OS and apoptosis over time.[22] Consequently, it is a reasonable approach to assess the efficacy of antioxidant molecules in counteracting the toxicity of MTX, which is exacerbated by elevated OS and inflammation.[6,14]

Gallic acid (GAL) is a trihydroxybenzoic acid with hydroxy groups in the 3, 4 and 5 positions. It is currently employed to a significant extent in the cosmetic, food and dyeing industries.[23] It can be found in a multitude of commonly consumed plants and fruits.[24] A series of experimental and clinical investigations have revealed that GAL can exert advantageous biological effects on a number of disparate physiological systems, including the cardiovascular, central nervous, gastrointestinal and reproductive systems.[23,24] Previous research has shown that GAL has therapeutic and/or protective effects against ovarian damage caused by exposure to cisplatin, letrozole and doxorubicin.[20,25,26] However, to date, there have been no reports that have evaluated the potential beneficial effects of GAL in the context of MTX-associated ovarian damage. This experimental study aimed to elucidate the effects of GAL treatment on MTX-induced ovarian OS, inflammation and apoptosis, for the first time.

METHODS

Animals

A total of 30 healthy adult female Sprague-Dawley rats (weighing approximately 195±5 g) were utilised in this study. The animals were housed under standard laboratory conditions (12 h light/dark cycle and 22±2°C) with *ad libitum* access to pellet feed and tap water.

Experimental protocol

The protocol was approved by the Local Animal Ethics Committee of Karadeniz Technical University (Protocol Number: 2023/07). Vaginal smears were conducted on a daily basis, and following three consecutive cycles, rats exhibiting normal estrous cycles of 4-5 days were included in the experiments.[7] The 30 animals were divided into five groups (six subjects in each group): Control, MTX, MTX+GAL (2.5 and 5 mg/kg) and GAL only (5 mg/kg). The control group was administered an intraperitoneal injection of physiological saline solution for a period of four days. The MTX group was administered an intraperitoneal MTX (20 mg/kg) injection on the first day, followed by an intraperitoneal physiological saline solution injection for the subsequent three days. The GAL treatment groups were administered an intraperitoneal MTX injection at a dose of 20 mg/kg on the first day. This was followed by two different doses of GAL (2.5 and 5 mg/kg)

intraperitoneal injection over the subsequent three days. The GAL *per se* group was administered an intraperitoneal physiological saline solution injection on the first day, followed by an intraperitoneal high-dose GAL (5 mg/kg) injection for the subsequent three days. GAL (Cat no: G7384, purity ≥98.5%) and MTX (Cat no: 454126, purity ≥98%) were purchased from Sigma-Aldrich Chemical Company (St Louis, MO, USA) and dissolved in physiological saline solution and administered to rats. The doses of GAL[20,27] and MTX[3,6,15] were determined based on previous experimental studies. On the morning of the fifth day of the study, cervical dislocation and subsequent oophorectomy were performed on the rats under general anesthesia. The excised tissues were subsequently stored at -80ºC for subsequent biochemical analyses.

Biochemical analysis

The ovary samples were homogenised in phosphate buffer solution (1:10 w/v, pH 7.4) using a homogeniser (IKA, T25 Ultra-Turrax, Staufen, Germany) with cooled tubes within ice. The resulting homogenates were then subjected to centrifugation at 1800xg for a period of 15 min at a temperature of 4°C, after which the supernatants were separated for further analysis. The protein content of supernatants was quantified using the bicinchoninic acid assay[28], and the supernatants were subsequently employed in subsequent analyses. The degree of tissue lipid peroxidation (LPO) was determined by the previously described spectrophotometric measurement of malondialdehyde (MDA), a LPO end product.[29] The absorbance of the pink complex formed by MDA and thiobarbituric acid, which is indicative of LPO, was measured at 532 nm. The standard employed was 1,1,3,3-tetramethoxypropane, with the results expressed in nanomolar per milligram of protein.[30]

The total antioxidant status (TAS) and the total oxidant status in ovarian tissue were quantified using commercially available kits (Rel Assay Kit Diagnostics, Gaziantep, Turkey) and the OS index (OSI) was calculated.[31] In the supernatants, the levels of superoxide dismutase (SOD) as an antioxidant enzyme[21], TNF-α as an inflammatory cytokine[13] and caspase-3 (CASP3) as an apoptosis marker[20] were determined using commercial assay kits (BT LAB, Zhejiang, China). The measurements were conducted in accordance with the manufacturer's instructions, and the intra-assay CV% values were found to be less than 8% in all three analyses.

Statistical analysis

The data are presented as the arithmetic mean ± standard error of the mean (SEM). The statistical analysis of the data was conducted using ANOVA, with Tukey's test employed for comparisons between groups. A p-value of less than 0.05 was considered statistically significant.

RESULTS

Effects of GAL treatments on ovarian OS levels induced by MTX

As illustrated in Table 1, treatment with MTX resulted in a significant elevation of MDA, TOS and OSI levels and a reduction of SOD and TAS levels in comparison to the control group. Nevertheless, three-day treatments with GAL following MTX demonstrated a dose-dependent improvement in OS parameters, with a concomitant strengthening of the antioxidant system.

Table 1. Effects of GAL on OS biomarkers in MTX-induced ovarian injury

	Control	MTX (20 mg/kg)	MTX+GAL (2.5 mg/kg)	MTX+GAL (5 mg/kg)	GAL (5 mg/kg)
MDA (nmol/mg protein)	47.50±7.63	95.04±6.28***	64.11±6.29$^{\#}$	50.28±2.45$^{\#\#\#}$	54.73±7.15
TOS (μM H_2O_2 equivalent/L)	16.60±3.47	30.99±2.04**	25.00±2.83	19.54±1.68$^{\#}$	14.32±1.47
TAS (mM trolox equivalent/L)	2.52±0.09	0.71±0.12***	1.69±0.16$^{**,\#\#\#}$	2.04±0.17$^{\#\#\#}$	2.43±0.14
OSI (arbitrary unit)	0.66±0.13	5.75±1.81**	1.59±0.28$^{\#}$	1.00±0.15$^{\#\#}$	0.61±0.08
SOD (ng/mg protein)	1.82±0.38	0.84±0.05*	1.20±0.16	1.51±0.11$^{\#}$	2.11±0.31

MTX: methotrexate, GAL: gallic acid, MDA: malondialdehyde, TOS: total oxidant status, TAS: total antioxidant status, OSI: oxidative stress index, SOD: superoxide dismutase.
P-values according to one-way ANOVA test, post-hoc Tukey test. Data were expressed as mean±SEM.
Compared with control group *p<0.05, **p<0.01 and ***p<0.001.
Compared with MTX group $^{\#}$p<0.05, $^{\#\#}$p<0.01 and $^{\#\#\#}$p<0.001.

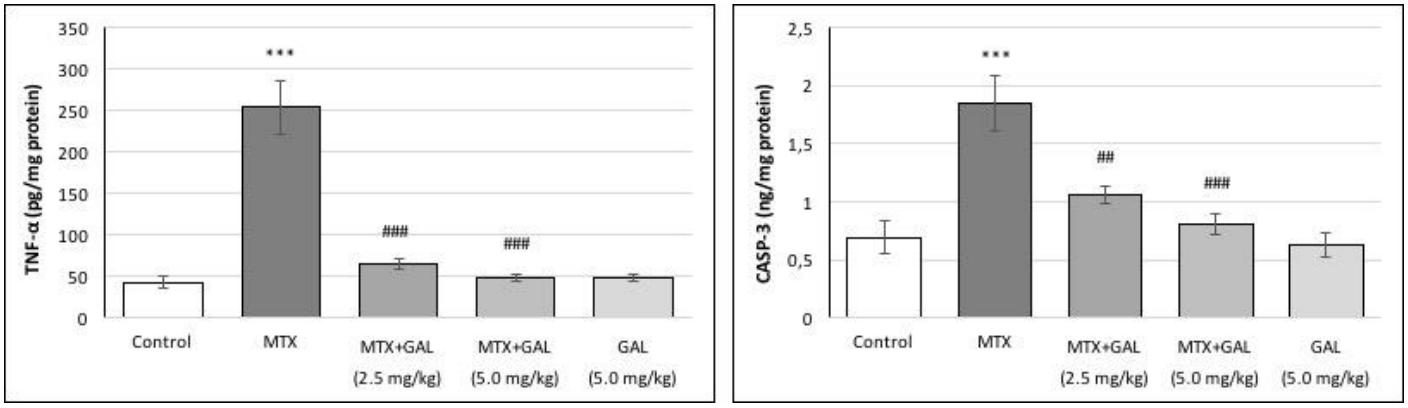

Figure 1. Effects of GAL on inflammatory and apoptosis biomarkers in MTX-induced ovarian injury
MTX: methotrexate, GAL: gallic acid, TNF-α: tumor necrosis factor-alpha, CASP3: caspase-3.
P-values according to one-way ANOVA test, post-hoc Tukey test. Data were expressed as mean±SEM.
Compared with control group ***p<0.001,
Compared with MTX group ##p<0.01 and ###p<0.001.

Effects of GAL treatments on ovarian inflammation and apoptosis levels induced by MTX

As illustrated in Figure 1, MTX treatment led to a notable elevation in TNF-α and CASP3 levels in ovarian tissue in comparison to the control group. Nevertheless, the administration of GAL following MTX administration resulted in a significant reduction in TNF-α and CASP3 levels in a dose-dependent manner.

DISCUSSION

Although MTX is one of several chemotherapeutics that can be employed alone or in combination with other drugs in clinical practice, the primary concern of young female patients undergoing MTX therapy is the potential impact of treatment on future fertility.[6] Although low-dose MTX is not considered to be relatively gonadotoxic in the context of autoimmune disorders[32,33], experimental studies have demonstrated that high-dose MTX used in chemotherapy harms ovarian tissue.[4,7-9] This study was therefore conducted to investigate the therapeutic effects of GAL on MTX-induced ovarian damage for the first time. In order to fulfill these objectives, a model was devised for the study of ovarian toxicity. In it, MTX was administered in a dose of 20 mg/kg[3,6,15], and the levels of biochemical biomarkers were subsequently determined in tissue samples taken three days after the commencement of the GAL treatments.[20] The results of the study demonstrated that the established hypothesis was indeed correct. Specifically, the findings disclosed that MTX administration led to an increase in OS, inflammation and apoptosis levels. Furthermore, the findings also disclosed that GAL treatments significantly alleviated MTX toxicity.

Although the precise molecular mechanism of MTX-induced toxicity remains unclear, OS and chronic inflammation resulting from the depletion of the antioxidant system are identified as the primary drivers of tissue damage.[4,12,14,15] The OS is defined as an imbalance between the amount of oxidants and the antioxidant system capacity.[15] Increased ROS attacks membrane lipids, resulting in LPO. Consequently, the level of reactive aldehyde derivatives, such as MDA, is elevated as a consequence of the chain reactions of LPO.[34] MDA is a highly reactive molecule that not only inhibits enzymes but also damages membrane integrity.[35] The TOS, TAS and OSI have been employed with considerable frequency in recent years as straightforward and useful parameters for evaluating the overall OS degree in a biological sample.[14] It has been demonstrated that elevated OS can precipitate infertility by inducing aberrations in oocyte development.[8,14] Our study, which was in accordance with previous experimental literature, demonstrated that MTX application led to an increase in OS, which was attributed to a depletion of the antioxidants.[4,6,12,14,15,36] It is established that MTX reduces the intracellular concentration of NADPH by inhibiting G6PD and malic enzyme.[37] As a consequence of the reduction in intracellular NADPH, the regeneration of GSH is impaired, thereby increasing the levels of OS within the cells. It is highly probable that the elevated MDA, TOS and OSI levels and the reduced TAS level observed in the MTX-treated group were a consequence of GSH depletion in the tissue.[14,18] Nevertheless, the administration of GAL treatments subsequent to MTX was found to alleviate ovarian OS by supporting the levels of TAS and SOD. The reduction in OS levels following the administration of MTX and concurrent treatment with GAL may be attributed to the *in vivo* antioxidant activity of GAL. In accordance with our findings, it has been demonstrated that GAL can exert protective and/or therapeutic effects by supporting the

antioxidant system and quenching LPO in a range of experimental models.[38-41]

In addition to the previously discussed mechanisms, inflammation and apoptosis have been proposed as other potential mechanisms for MTX-induced tissue damage.[14,15,18] MTX stimulates neutrophils, resulting in an increase in the amount of hydrogen peroxide, which in turn leads to an elevation in the level of ROS. This process ultimately culminates in the induction of cellular damage.[42] Furthermore, MTX also increases the concentration of pro-inflammatory cytokines, including TNF-α, by activating the nuclear factor kappa B (NF-κB) pathway.[12] TNF-α is a crucial pro-inflammatory cytokine that plays a pivotal role in numerous inflammatory processes.[14] Although inflammation is an acute adaptive response of the body to an invading attack, in the process of chronic inflammation, the sustained production of TNF-α increases tissue destruction, resulting in the activation of CASP3.[43] In accordance with previous experimental literature, our findings demonstrated that MTX application resulted in elevated levels of inflammation and apoptosis in ovarian tissue.[6,14,36,44] Conversely, the administrations of GAL following MTX has been observed to suppress the inflammation and apoptosis levels dose-dependently. In accordance with our findings, it has been demonstrated that GAL can exert protective and/or therapeutic effects by inhibiting inflammation and apoptosis in a range of experimental models.[20,45-47] The reduction in levels of inflammation and inflammation-induced apoptosis observed following the administration of MTX in conjunction with GAL treatment may be attributed to the *in vivo* anti-inflammatory activity of GAL. In support of this hypothesis, the anti-inflammatory activity of GAL is attributed to its capacity to inhibit the synthesis of pro-inflammatory mediators and activation of mitogen-activated protein kinase (MAPK) and NF-κB signaling pathways.[24]

It should be noted that the present study is not without limitations. Firstly, no histopathological analysis was conducted on the ovarian tissues. In future studies, the therapeutic effect of GAL on MTX-induced ovarian damage should be confirmed through histological analysis. Secondly, the impact of three consecutive days of GAL administration was assessed in an experimental acute ovotoxicity model induced by a single dose of MTX. In future studies, the efficacy of GAL can be evaluated in a chronic toxicity model induced by repeated doses of MTX. Thirdly, the therapeutic efficacy of GAL was demonstrated using basic OS, inflammation, and apoptosis biomarkers. In future studies, the ovoprotective efficacy of GAL should also be evaluated in terms of cell signalling.

CONCLUSION

The findings of this study corroborate previous reports indicating that MTX can induce OS, inflammation, and apoptosis in ovarian tissue. GAL treatments (in particular at a dose of 5 mg/kg) administered after MTX were found to be highly effective in eliminating the damage caused by MTX, due to their antioxidant and anti-inflammatory properties. These findings suggest that GAL application may be a promising approach to alleviating chemotherapy-induced reproductive toxicity. Nevertheless, before clinical use, the ovoprotective activity of GAL should be supported by more comprehensive preclinical studies that elucidate the underlying molecular mechanisms.

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Authorship contribution statement

Consept and desing: AM and SD.

Acquisition of data: SD, NTA, EAD, AM and YA.

Analysis and interpretation of data: SD, NTA, EAD, AM and YA.

Drafting of the manuscript: SD.

Critical revision of the manuscript for important intellectual content: AM and YA.

Statistical analysis: AM.

Declaration of competing interest

None of the authors have potential conflicts of interest to be disclosed.

Ethical approval

This study was approved by the Local Animal Research Ethics Committee of Karadeniz Technical University (Protocol no: 2023/07) and performed according to the animal research reporting of in vivo experiments (ARRIVE) guidelines.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

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REFERENCES

1. Valerio V, Kwok M, Loewen H, et al. Systematic review of recommendations on the use of methotrexate in rheumatoid arthritis. Clin Rheumatol. 2021;40(4):1259-1271. doi:10.1007/s10067-020-05363-2
2. Hamed KM, Dighriri IM, Baomar AF, et al. Overview of methotrexate toxicity: A comprehensive literature review. Cureus. 2022;14(9):e29518. doi:10.7759/cureus.29518
3. Hortu I, Ozceltik G, Ergenoglu AM, Yigitturk G, Atasoy O, Erbas O. Protective effect of oxytocin on a methotrexate-induced ovarian toxicity model. Arch Gynecol Obstet. 2020;301(5):1317-1324. doi:10.1007/s00404-020-05534-1
4. Fu J, Liu Y, Wang C, et al. Persistent follicular granulosa cell senescence and apoptosis induced by methotrexate leading to oocyte dysfunction and aberrant embryo development. Clin Transl Sci. 2021;14(5):2043-2054. doi:10.1111/cts.13068
5. Benian A, Guralp O, Uzun DD, Okyar A, Sahmay S. The effect of repeated administration of methotrexate (MTX) on rat ovary: measurement of serum antimullerian hormone (AMH) levels. Gynecol Endocrinol. 2013;29(3):226-229. doi:10.3109/09513590.2012.738725
6. Turkler C, Onat T, Yildirim E, et al. An experimental study on the use of lycopene to prevent infertility due to acute oxidative ovarian damage caused by a single high dose of methotrexate. Adv Clin Exp Med. 2020;29(1):5-11. doi:10.17219/acem/111809
7. Karri S, Vanithakumari G. Effect of methotrexate and leucovorin on female reproductive tract of albino rats. Cell Biochem Funct. 2011;29(1):1-21. doi:10.1002/cbf.1711
8. Gol M, Saygili U, Koyuncuoglu M, Uslu T. Influence of high-dose methotrexate therapy on the primordial follicles of the mouse ovary. J Obstet Gynaecol Res. 2009;35(3):429-433. doi:10.1111/j.1447-0756.2008.00945.x
9. Ulug P, Oner G. Evaluation of the effects of single or multiple dose methotrexate administration, salpingectomy on ovarian reserve of rat with the measurement of anti-mullerian hormone (AMH) levels and histological analysis. Eur J Obstet Gynecol Reprod Biol. 2014;181:205-209. doi:10.1016/j.ejogrb.2014.07.011
10. Kyaw MT, Sakthiswary R, Ani Amelia Z, Rahana AR, Munirah MM. Effects of methotrexate therapy on the levels of gonadotropic hormones in rheumatoid arthritis patients of reproductive age. Cureus. 2020;12(4):e7632. doi:10.7759/cureus.7632
11. Murthy V, Chamberlain RS. Menopausal symptoms in young survivors of breast cancer: A growing problem without an ideal solution. Cancer Control. 2012;19(4):317-329. doi:10.1177/107327481201900408
12. El-Sheikh AA, Morsy MA, Abdalla AM, Hamouda AH, Alhaider IA. Mechanisms of thymoquinone hepatorenal protection in methotrexate-induced toxicity in rats. Mediators Inflamm. 2015;2015:859383. doi:10.1155/2015/859383
13. Yucel Y, Oguz E, Kocarslan S, et al. The effects of lycopene on methotrexate-induced liver injury in rats. Bratisl Lek Listy. 2017;118(4):212-216. doi:10.4149/BLL_2017_042
14. Soylu Karapinar O, Pinar N, Özcan O, Özgür T, Dolapçıoğlu K. Protective effect of alpha-lipoic acid in methotrexate-induced ovarian oxidative injury and decreased ovarian reserve in rats. Gynecol Endocrinol. 2017;33(8):653-659. doi:10.1080/09513590.2017.1306847
15. Gunyeli I, Saygin M, Ozmen O. Methotrexate-induced toxic effects and the ameliorating effects of astaxanthin on genitourinary tissues in a female rat model. Arch Gynecol Obstet. 2021;304(4):985-997. doi:10.1007/s00404-021-06000-2
16. Eigenbrodt E, Glossmann H. Glycolysis-one of the keys to cancer? Trends Pharmacol Sci. 1980;89:359-362. doi:10.1016/0165-6147(80)90009-7
17. Babiak RM, Campello AP, Carnieri EG, Oliveira MB. Methotrexate: pentose cycle and oxidative stress. Cell Biochem Funct. 1998;16(4):283-293. doi:10.1002/(SICI)1099-0844(1998120)16:4<283::AID-CBF801>3.0.CO;2-E
18. Uraz S, Tahan V, Aygun C, et al. Role of ursodeoxycholic acid in prevention of methotrexate-induced liver toxicity. Dig Dis Sci. 2008;53(4):1071-1077. doi:10.1007/s10620-007-9949-3
19. Jahovic N, Çevik H, Şehirli AÖ, Yeğen BÇ, Şener G. Melatonin prevents methotrexate-induced hepatorenal oxidative injury in rats. J Pineal Res. 2003;34(4): 282-287. doi:10.1034/j.1600-079X.2003.00043.x
20. Ayazoglu Demir E, Mentese A, Livaoglu A, Alemdar NT, Demir S. Ameliorative effect of gallic acid on cisplatin-induced ovarian toxicity in rats. Drug Chem Toxicol. 2023;46(1):97-103. doi:10.1080/01480545.2021.2011312
21. Demir S, Kazaz IO, Kerimoglu G, et al. Gallic acid attenuates torsion/detorsion-induced testicular injury in rats through suppressing of HMGB1/NF-κB axis and endoplasmic reticulum stress. Rev Int Androl. 2024;22(1):1-7. doi:10.22514/j.androl.2024.001
22. Mittal M, Siddiqui MR, Tran K, Reddy SP, Malik AB. Reactive oxygen species in inflammation and tissue injury. Antioxid Redox Signal. 2014;20(7):1126-1167. doi:10.1089/ars.2012.5149
23. Gao J, Hu J, Hu D, Yang X. A role of gallic acid in oxidative damage diseases: A comprehensive review. Nat Prod Commun. 2019;14(8):1-9. doi:10.1177/1934578X19874174
24. Bai J, Zhang Y, Tang C, et al. Gallic acid: Pharmacological activities and molecular mechanisms involved in inflammation-related diseases. Biomed Pharmacother. 2021;133:110985. doi:10.1016/j.biopha.2020.110985
25. Ul-Haq Shah MZ, Soni M, Shrivastava VK, Mir MA, Muzamil S. Gallic acid reverses ovarian disturbances in mice with letrozole-induced PCOS via modulating Adipo R1 expression. Toxicol Rep. 2022;9:1938-1949. doi:10.1016/j.toxrep.2022.10.009
26. Silva RLDS, Lins TLBG, Monte APOD, et al. Protective effect of gallic acid on doxorubicin-induced ovarian toxicity in mouse. Reprod Toxicol. 2023;115:147-156. doi:10.1016/j.reprotox.2022.12.008
27. Ramkumar KM, Vijayakumar RS, Vanitha P, et al. Protective effect of gallic acid on alloxan-induced oxidative stress and osmotic fragility in rats. Hum Exp

Toxicol. 2014;33(6):638-649. doi:10.1177/0960327113504792
28. Smith PK, Krohn RI, Hermanson GT, et al. Measurement of protein using bicinchoninic acid. Anal Biochem. 1985;150(1):76-85. doi:10.1016/0003-2697(85)90442-7
29. Mihara M, Uchiyama M. Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. Anal Biochem. 1978;86(1):271-278. doi:10.1016/0003-2697(78)90342-1
30. Demir S, Kazaz IO, Kerimoglu G, et al. Propolis ameliorates ischemia/reperfusion-induced testicular damage by reducing oxidative stress. Rev Int Androl. 2023;21(3):100364. doi:10.1016/j.androl.2023.100364
31. Demir S, Mentese A, Livaoglu A, Demir EA, Alemdar NT, Aliyazicioglu Y. Therapeutic effect of sinapic acid against 5-fluorouracil-induced oxidative stress and inflammation in rat ovarium: An experimental approach. Farabi Med J. 2023;2(2):1-7. doi:10.59518/farabimedj.1221397
32. Boots CE, Gustofson RL, Feinberg EC. Does methotrexate administration for ectopic pregnancy after *in vitro* fertilization impact ovarian reserve or ovarian responsiveness? Fertil Steril. 2013;100(6):1590-1593. doi:10.1016/j.fertnstert.2013.08.007
33. Mahajan N. Fertility preservation in female cancer patients: An overview. J Hum Reprod Sci. 2015;8(1):3-13. doi:10.4103/0974-1208.153119
34. Ayazoglu Demir E, Mentese A, Livaoglu A, Alemdar NT, Aliyazicioglu Y, Demir S. Chlorogenic acid attenuates cisplatin-induced ovarian injury in rats. Drug Chem Toxicol. 2024;47(2):213-217. doi:10.1080/01480545.2023.2172181
35. Selimoğlu Şen H, Şen V, Bozkurt M, et al. Carvacrol and pomegranate extract in treating methotrexate-induced lung oxidative injury in rats. Med Sci Monit. 2014;20:1983-1990. doi:10.12659/MSM.890972
36. Yuksel Y, Yuksel R, Yagmurca M, et al. Effects of quercetin on methotrexate-induced nephrotoxicity in rats. Hum Exp Toxicol. 2017;36(1):51-61. doi:10.1177/0960327116637414
37. Vardi N, Parlakpinar H, Cetin A, Erdogan A, Cetin Ozturk I. Protective effect of beta-carotene on methotrexate-induced oxidative liver damage. Toxicol Pathol. 2010;38(4):592-597. doi:10.1177/0192623310367806
38. Kulkarni JM, Swamy AHMV. Cardioprotective effect of gallic acid against doxorubicin-induced myocardial toxicity in albino rats. Indian J Health Sci. 2015;8(1):28-35. doi:10.4103/2349-5006.158219
39. Olayinka ET, Ore A, Ola OS, Adeyemo OA. Ameliorative effect of gallic acid on cyclophosphamide-induced oxidative injury and hepatic dysfunction in rats. Med Sci. 2015;3:78-92. doi:10.3390/medsci3030078
40. Kilic K, Sakat MS, Akdemir FNE, Yildirim S, Saglam YS, Askin S. Protective effect of gallic acid against cisplatin-induced ototoxicity in rats. Braz J Otorhinolaryngol. 2019;85(3):267-274. doi:10.1016/j.bjorl.2018.03.001
41. Abarikwu SO, Mgbudom-Okah CJ, Njoku RCC, Okonkwo CJ, Onuoha CC, Wokoma AFS. Gallic acid ameliorates busulfan-induced testicular toxicity and damage in mature rats. Drug Chem Toxicol. 2022;45(4):1881-1890. doi:10.1080/01480545.2021.1892949
42. Phillips DC, Woollard KJ, Griffiths HR. The anti-inflammatory actions of methotrexate are critically dependent upon the production of reactive oxygen species. Br J Pharmacol. 2003;138(3):501-511. doi:10.1038/sj.bjp.0705054
43. Althagafy HS, Sharawi ZW, Batawi AH, et al. Buspirone attenuated methotrexate-induced hippocampal toxicity in rats by regulating Nrf2/HO-1, PPAR-γ, NF-κB/nNOS, and ROS/NLRP3/caspase-1 signaling pathways. J Biochem Mol Toxicol. 2023;37(9):e23414. doi:10.1002/jbt.23414
44. Famurewa AC, Folawiyo AM, Enohnyaket EB, et al. Beneficial role of virgin coconut oil supplementation against acute methotrexate chemotherapy-induced oxidative toxicity and inflammation in rats. Integr Med Res. 2018;7(3):257-263. doi:10.1016/j.imr.2018.05.001
45. Nikbakht J, Hemmati AA, Arzi A, Mansouri MT, Rezaie A, Ghafourian M. Protective effect of gallic acid against bleomycin-induced pulmonary fibrosis in rats. Pharmacol Rep. 2015;67(6):1061-1067. doi:10.1016/j.pharep.2015.03.012
46. Dehghani MA, Maram NS, Moghimipour E, Khorsandi L, Khah MA, Mahdavinia M. Protective effect of gallic acid and gallic acid-loaded Eudragit-RS 100 nanoparticles on cisplatin-induced mitochondrial dysfunction and inflammation in rat kidney. Biochim Biophys Acta Mol Basis Dis. 2020;1866(12):165911. doi:10.1016/j.bbadis.2020.165911
47. Altındağ F, Meydan İ. Evaluation of protective effects of gallic acid on cisplatin-induced testicular and epididymal damage. Andrologia. 2021;53(10):e14189. doi:10.1111/and.14189

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DNA Binding, Nuclease/Photonuclease, and Phototoxicty Properties of Water Soluble Silicon (IV) Phthalocyanine

Suda Çözünür Silisyum (IV) Ftalosiyaninin DNA Bağlanma, Nükleaz/Fotonükleaz ve Fototoksik Özellikleri

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ABSTRACT

Photodynamic therapy (PDT) is known as a method in which photosensitizers produce reactive oxygen species in the presence of light and oxygen, leading to cell death. In this paper, DNA interaction properties of bis[4-({8)-[3-(trimethylamino)phenoxy]octyl}oxy)] substituted silicon (IV) phthalocyanine (**GsB-SiPc**) were examined using a UV-Vis spectrophometer and agarose gel electrophoresis techniques. Afterwards, cytotoxic/phototoxic effects of GsB-SiPc were examined using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays on A549 cells. The results showed that GsB-SiPc bound to ct-DNA via a groove binding mode. In nuclease/photonuclease experiments, **GsB-SiPc** had low nuclease activity in the dark but it showed high photonuclease activity in the presence of light, depending on compound concentration and light dose. In addition, **GsB-SiPc** demonstrated remarkable phototoxicity toward human lung adenocarcinoma (A549) cell line at 50 and 100 µM in the presence of light. The *in vitro* data revealed the potential of **GsB-SiPc** as a photodynamic therapy agent for the treatment of lung cancer. These findings need to be supported by further studies.

Keywords: DNA Binding, DNA Nuclease, Lung Cancer, Photodynamic Therapy, Silicon (IV) Phthalocyanine

ÖZET

Fotodinamik terapi (PDT), fotosensitizörlerin ışık ve oksijen varlığında reaktif oksijen türleri ürettiği ve hücre ölümüne yol açtığı bilinen bir yöntemdir. Bu makalede, bis[4-({8)-[3-(trimetilamino)fenoksi]oktil}oksi)] yan grubu içeren silisyum (IV) ftalosiyaninin (GsB-SiPc) DNA etkileşim özellikleri bir UV-Vis spektrofotometresi ve agaroz jel elektroforezi teknikleri kullanılarak incelenmiştir. Daha sonra, GsB-SiPc'nin sitotoksik/fototoksik etkileri A549 hücreleri üzerinde 3-(4,5-dimetiltiazol-2-il)-2,5-difeniltetrazolium bromür (MTT) deneyleri kullanılarak incelenmiştir. Sonuçlar, **GsB-SiPc**'nin ct-DNA'ya bir oluk bağlama modu aracılığıyla bağlandığını göstermiştir. Nükleaz/fotonükleaz deneylerinde, **GsB-SiPc** karanlıkta düşük nükleaz aktivitesine sahipti ancak bileşik konsantrasyonuna ve ışık dozuna bağlı olarak ışık varlığında yüksek fotonükleaz aktivitesi gösterdi. Ek olarak, **GsB-SiPc** ışık varlığında 50 ve 100 µM'de insan akciğer adenokarsinomu (A549) hücre hattına karşı dikkate değer fototoksisite gösterdi. *İn vitro* veriler, **GsB-SiPc**'nin akciğer kanserinin tedavisi için bir fotodinamik terapi ajanı olarak potansiyelini ortaya koydu. Bu bulguların daha fazla çalışmayla desteklenmesi gerekiyor.

Anahtar Kelimeler: Akciğer Kanseri, DNA Bağlama, DNA Nükleaz, Fotodinamik Terapi, Silisyum (IV) Ftalosiyanin

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INTRODUCTION

According to the latest Global Cancer Observatory (GLOBOCAN) (2022) data, 9.7 million women and 10.3 million men were diagnosed with cancer. 4.3 million women and 5.4 million men died from the disease. In particular, lung cancer is the most common type of cancer after breast cancer and ranks at the top when the death/incidence rate is examined.[1] Although conventional therapeutic methods such as chemotherapy, radiotherapy, and surgery are used to treat cancer and reduce mortality, they are inadequate due to their undesirable side effects (e.g., morbidity, damage to healthy cells, and drug resistance) and the complex, progressively worsening nature of cancer. Therefore, new treatment methods with high therapeutic efficacy and low side effects are needed.[2,3]

In recent years, photodynamic therapy (PDT), a light-activated photosensitizer-based treatment method, has attracted considerable attention in the treatment of various types of cancers, including breast, brain, lung, head and neck, and cervical cancers.[4,5] Compared to other methods, PDT has no long-term side effects when used correctly, is non-invasive, targets the vasculature along with the tumor, can be applied repeatedly to the same area, does not leave scars after application, and is cost-effective.[6] As mentioned, the method has many advantages but requires photosensitizers that accumulate in cancerous tissues, a wavelength of light that activates the photosensitizer, and the presence of molecular oxygen for the formation of reactive oxygen species. These three factors are essential for the effectiveness of PDT.[7]

The PDT process begins with the administration of a photosensitizer to the patient. The photosensitizers that accumulate in the target tissue are then excited by light of an appropriate wavelength. A series of energy transfer events occur, whereby the ground state photosensitizer passes into the excited state and then into the excited triplet state. At this point, a reaction occurs, which is generally referred to as the Type II mechanism. This mechanism involves energy transfer from the excited triplet state photosensitizer directly to molecular oxygen, producing singlet oxygen. The singlet oxygen formed is highly reactive and causes death in cancer cells.[8,9] Cell death after PDT is caused by apoptosis, necrosis, and autophagy.[10,11]

Photosensitizers are molecules activated by light to induce cancer cell death in PDT. A photosensitizer suitable for clinical use should be non-toxic until activated by light, be mobile in body fluids, have high water solubility, selectively accumulate in the tumor, be rapidly excreted, have high absorption in the red and near-infrared (NIR) spectral ranges, and not cause pain during treatment.[12,13] Second-generation photosensitizers, namely phthalocyanines, have garnered significant interest in PDT due to their numerous advantageous properties. These include low *in vitro* toxicity, minimal or no absorption at 400-600 nm, high absorption within the therapeutic window (650-800 nm), high extinction coefficients, rapid excretion from the body, and minimal fluorescence following topical application. Additionally, their chemical structures can be readily modified by introducing central metals and substituent groups such as silicon (IV) phthalocyanines.[14,15]

In line with this information, this study aimed to reveal the potential of bis[4-({8-[3-(trimethylamino)phenoxy]octyl}oxy)] substituted silicon (IV) phthalocyanine (GsB-SiPc) as a PDT agent by examining its *in vitro* DNA interactions and phototoxicity using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays on human lung adenocarcinoma (A549) cells.

METHODS

Chemicals

Acetic acid (Sigma-Aldrich, A6283), agarose (Sigma-Aldrich, A9539), bromophenol blue (Sigma-Aldrich, B0126), calf thymus-DNA (ct-DNA) (Sigma-Aldrich, D1501), Dulbecco's modified eagle medium (DMEM) (Gibco, 41966029), dimethyl sulfoxide (DMSO) (Sigma-Aldrich, 472301), (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Serva, 2039502), ethidium bromide (EB) (Sigma-Aldrich, E7637), ethylenediaminetetraacetate (EDTA) (Sigma-Aldrich, E5134), glycerol (Sigma-Aldrich, G5516), hydrogen peroxide (H2O2) (Sigma-Aldrich, 216763), methylene blue (MB) (Sigma-Aldrich, M9140), supercoiled pBR322 plasmid DNA (Thermo Scientific, SD0041), sodium dodecyl sulphate (SDS) (Sigma-Aldrich, L3771), streptomycin/penicillin (Multicell, 420-201-EL), trizma-base (Tris) (Sigma-Aldrich, 93362), trypsin/EDTA (Multicell, 3R5-542-EL), and xylene cyanol (Sigma-Aldrich, X4126) were purchased from commercial companies. Bis[4-({8-[3-(trimethylamino)phenoxy]octyl}oxy)] substituted silicon (IV) phthalocyanine (**GsB-SiPc**) were synthesized by our research group (Figure 1).[16]

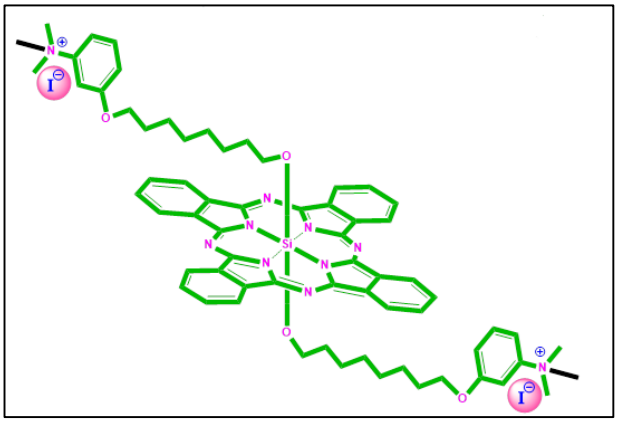

Figure 1. The molecular structure of **GsB-SiPc**

DNA binding experiments

Firstly, **GsB-SiPc** were dissolved in distilled water and then diluted in a buffer containing 5 mM Tris-HCl and 50 mM NaCl (pH 7.2) (TBS). A solution of ct-DNA was prepared in TBS, stirred for three days, and stored at 4 °C for up to a week. To assess the percentage hypochromicity of **GsB-SiPc**, experiments were conducted with fixed concentrations of the compound (50 µM) while varying the concentrations of ct-DNA (0-25 µM). The mixtures, containing increasing amounts of ct-DNA and the compounds, were incubated for 10 minutes at room temperature, and the changes in absorption spectra were recorded. The hypochromicity percentage of **GsB-SiPc** was calculated using formula: Hypochromicity (%)= ((A0-A1)/(A0))×100 A0: Maximum absorbance of the compound; A1: Absorbance of the compound:ct-DNA complex.[17]

Competitive binding experiments of **GsB-SiPc** with ethidium bromide (EB) were conducted using UV-Vis spectroscopy. The EB-ct-DNA complex was formed by mixing EB and ct-DNA at concentrations of 40 µM each. The concentrations of **GsB-SiPc** were gradually varied (5, 10, and 20 µM), and the changes in absorption spectra were measured in the range of 425-550 nm.[18]

To confirm the interaction of the compounds with ct-DNA, agarose gel electrophoresis was performed. A fixed concentration of ct-DNA (100 µM) was used, while the concentrations of **GsB-SiPc** were varied (0-100 µM) and incubated at 37 ºC for 60 min. The mixtures were then loaded with buffer onto a 0.8% agarose gel (1 mg/mL in TAE buffer: Tris-acetate-EDTA), and electrophoresis was carried out for 30 min at 100 V. The resulting gel was visualized using the BioRad Gel Doc XR system.[19]

DNA nuclease/photonuclease experiments

The DNA nuclease/photonuclease properties of **GsB-SiPc** were analyzed by agarose gel electrophoresis using supercoiled pBR322 plasmid DNA, both with and without irradiation. For the DNA-photonuclease studies, the samples were exposed to light irradiation (white light, 17.5 mW/cm²) for 15, 30, and 60 min. Methylene blue (MB) was used as a positive control. Supercoiled pBR322 plasmid DNA was treated with increasing concentrations of **GsB-SiPc** (10 and 50 µM) in a buffer containing 50 mM Tris-HCl (pH 7.0). All samples were incubated at 37 ºC for 60 min. After incubation, loading buffer (containing bromophenol blue, xylene cyanol, glycerol, EDTA, and SDS) was added, and the mixtures were loaded onto a 0.8% agarose gel with ethidium bromide staining in TAE buffer (Tris-acetic acid-EDTA). Electrophoresis was conducted at 100 V for 90 min, and the results were visualized using the BioRad Gel Doc XR system and analyzed with Image Lab Version 4.0.1 software.[20]

Cell culture studies

Human lung adenocarcinoma (A549) cells were cultured in high-glucose DMEM supplemented with 10% fetal bovine serum, 1% penicillin (100 U/mL), and 1% streptomycin (100 µg/mL), and maintained at 37°C in a 5% CO_2 incubator. When the cells reached 90% confluence, they were treated with 0.25% trypsin-EDTA and sub-cultured into 96-well plates. Cells were seeded at a density of 1×10^4 cells per well and incubated for 24 h. A stock solution of GsB-SiPc (10 mM) was prepared in water, and cells were treated with medium containing **GsB-SiPc** (5-100 µM). MB and 0.1% water were used as positive and negative controls, respectively. After treatment, the medium was replaced with 100 µL of serum-free medium containing 0.5 mg/mL MTT, and the cells were incubated at 37°C for 4 h. The MTT-containing medium was then removed, and 150 µL of DMSO was added to each well to dissolve the formazan crystals. The plates were shaken for 10 min, and absorbance was measured at 570 and 690 nm using a microplate reader. For the dark group, the plates were incubated at 37°C in 5% CO_2 without light exposure.

For the irradiation group, after a 2-h incubation, the plates were exposed to light irradiation (17.5 mW/cm²) for 60 min, and then re-incubated under the same conditions as the dark group.[21]

Statistical analysis

In this study, all data were analyzed using Microsoft Excel for Windows and GraphPad Prism 5.0, and results were expressed as mean±standard deviation (n=6). Statistical analyses were conducted using two-way ANOVA, followed by Bonferroni post-tests for multiple comparisons ($p<0.05$).

RESULTS

DNA binding studies of GsB-SiPc

The binding interactions of **GsB-SiPc** with DNA were first investigated by titration methods using a UV-Vis spectrophotometer. The results are presented in Table 1. In this study, different concentrations of ct-DNA were added to the fixed concentration of **GsB-SiPc** and the change in the spectrum was observed. The maximum absorbance value of **GsB-SiPc** was recorded at 622 nm. Then, with the addition of different concentrations of ct-DNA, a decrease in absorbance was observed. The hypochromism rate was calculated as 16.45±2.20%. In addition, no shift in the wavelength of maximum absorbance was observed when ct-DNA was added.

Table 1. DNA binding parameters of **GsB-SiPc**

Compound	λ (nm)	Change in Absorbance	Shift (nm)	H%
GsB-SiPc	622	Hypochromism	0	16.45 ± 2.20

Secondly, the competitive ethidium bromide (EB) experiment of **GsB-SiPc** was also carried out using a UV-Vis spectrophotometer. The results are presented in Figure 2. In this study, after first recording the spectrum of EB, ct-DNA is added to the mixture at a ratio of 1:1. The absorbance value of the complex formed dramatically decreased. Then, the change of low absorbance was observed with the addition of different concentrations of compounds. Despite the addition of **GsB-SiPc** (5 and 10 µM), no significant changes in absorbance change were observed.

The results of another binding experiment, agarose gel electrophoresis DNA binding studies, are presented in Figure 3. In this study, different concentrations of **GsB-SiPc** were added to the fixed ct-DNA concentration. When the intensity of the lanes in Figure 3 was analyzed, it was observed that the intensities did not change significantly in lanes 2-6.

DNA nuclease/photonuclease studies of GsB-SiPc

Plasmid DNA is found in three forms on agarose gel electrophoresis. Form I is defined as the supercoiled form and migrates the fastest on the gel. Form II is formed when one strand of the supercoiled form is damaged and is the slowest moving form on the gel. Form III is formed by the breakage of two strands and is seen between Form I and Form II. The DNA nuclease/photonuclease activities of **GsB-SiPc** were investigated using agarose gel electrophoresis method. The results are presented in Figure 4. MB was used as a positive control in this study. In this study, light dose was 17.5 mW/cm2, white light was used for 15, 30, and 60 min. The results showed that **GsB-SiPc** did not show any nuclease activity compared to negative control in the dark (Figure 4a). In the presence of light, it was determined that nuclease activities enhanced with the decrease in Form I percentage at the concentrations used depending on the increase in light dose. While the Form I percentage was found to be 79.60% after 15 min of light stimulation in the presence of **GsB-SiPc** (50 µM) (Figure 4b), it decreased to 59.00% and 43.50% after 30 and 60 min of stimulation (Figure 4c and 4d).

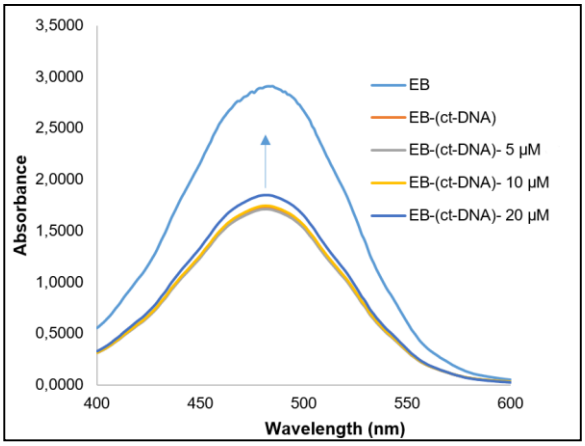

Figure 2. Competitive EB binding assay studies of **GsB-SiPc**. EB:40 µM; ct-DNA: 40 µM

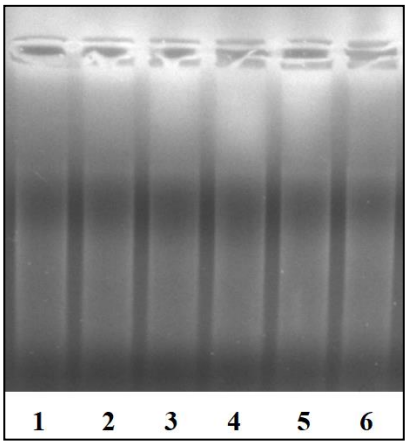

Figure 3. Electrophoresis binding study of **GsB-SiPc** (lane 1: 100 µM ct-DNA; lane 2: 100 µM ct-DNA + 10 µM **GsB-SiPc**; lane 3: 100 µM ct-DNA + 20 µM **GsB-SiPc**; lane 4: 100 µM ct-DNA + 50 µM **GsB-SiPc**; lane 5: 100 µM ct-DNA + 100 µM **GsB-SiPc**; lane 6: 100 µM ct-DNA + 200 µM **GsB-SiPc**

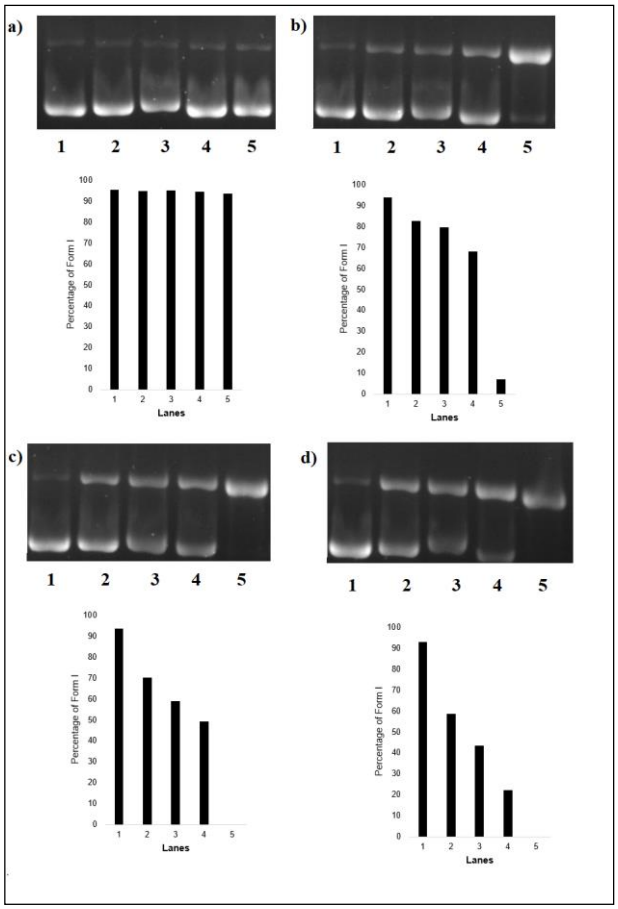

Figure 4. DNA nuclease/photonuclease effects of **GsB-SiPc** on plasmid pBR322 DNA. Lane 1: DNA control; lane 2: DNA+**GsB-SiPc** (10 µM); lane 3: DNA+**GsB-SiPc** 1 (50 µM); lane 4: DNA+MB (10 µM); lane 5: DNA+MB (50 µM) a) dark; b) 15 min light; c) 30 min light; d) 60 min light (Light: white light, 17.5 mW/cm²)

Cytotoxic/phototoxic effects of GsB-SiPc

The cytotoxic and phototoxic activities of **GsB-SiPc** were investigated using MTT cell viability test on A549 cells. MB was used as a positive control. In this study, 5, 25, 50, and 100 µM were used to investigate the toxicity profiles of the compounds. The results are showed in Figure 5. When the cytotoxic activity of **GsB-SiPc** was examined, 99.00±3.00% and 90.00±4.20% cell viability was observed at 5 and 10 µM of **GsB-SiPc**, while these values were found to be 83.02±3.33% and 54.12±4.46% at 50 and 100 µM. As a result of 60 min of light stimulation, no statistically significant change was observed at 5 and 10 µM, while cell viability was determined as 57.00±2.70% and 20.22±2.05% at 50 and 100 µM on A549 cells. This situation revealed a statistically significant difference between darkness and light ($p<0.001$). In the presence of MB, the cell viabilities were 4.44 ± 0.88% (light) and 35.04 ± 2.09% (dark) at 100 µM on A549 cells.

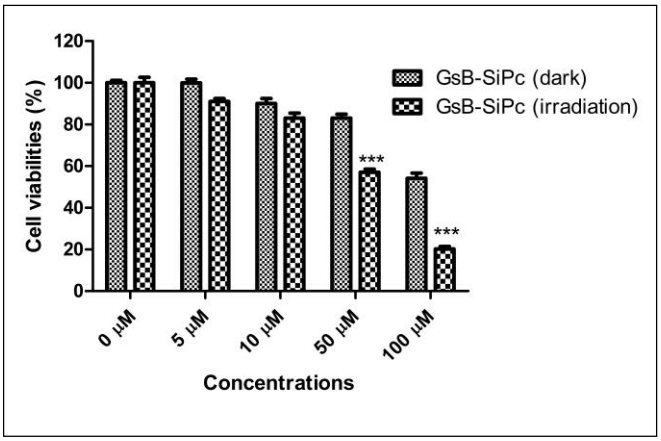

Figure 5. Cell viabilities of A549 in the presence of **GsB-SiPc** w/o light irradiation (17.5 mW/cm², 60 min, white). ***$p < 0.001$ irradiation vs dark at same concentrations on A549 cells. Two-way analysis of variance Bonferroni posttests. Positive control: Methylene blue (4.44 ± 0.88% (light) and 35.04 ± 2.09% (dark) at 100 µM).

DISCUSSION

Since most anticancer drugs target DNA, which plays a crucial role in uncontrolled cell growth, the study of compound interactions with DNA has become a topic of great interest in recent years.[22] In this study, UV-Vis spectrophotometric methods and agarose gel electrophoresis were used to study the DNA binding of Compound 1 and to provide insight into the binding mode of the compound with DNA. Hypochromism percentages and changes in wavelengths showing maximum absorbance play an important role in determining the binding modes of compounds with DNA.[23] High hypochromic values and shifts in wavelengths reveal an intercalative interaction between the compound and DNA. Otherwise, it is stated that the compounds interact in the direction of binding to the groove. In this work, when ct-DNA was added to **Compound 1** at different concentrations, the hypochromism rate was determined as 16.45±2.20%, while no shift in wavelength was observed. This situation suggested that the compound interacted with DNA by binding to the groove.

A competitive EB experiment was performed to support this data. In this experiment, when EB and ct-DNA were treated at a 1:1 ratio, a high hypochromism in absorbance was observed due to intercalative interaction. If the compounds show an intercalative interaction, a rapid increase in absorbance value is expected when they are added.[24] In the study, no effective increase was observed when different concentrations of the compound were added to the

EB: ct-DNA complex. This situation can be accepted as supporting data for the groove binding interaction. In the binding experiment with the agarose gel method, which is one of the most preferred methods in recent years, it is stated that there is a correlation between the decrease in the band intensity of DNA and the binding of the compound to DNA when increasing concentrations of compounds are added to DNA at constant concentrations.[25] In other words, it was revealed that the compound binds strongly to DNA at concentrations where the band intensity of DNA decreases. The decrease in band intensity at low concentrations is presented as a sign that the compound is a strong intercalator. However, in this study, it was observed that the band intensity did not change in all well contents. Thus, it reveals that the compound interacts by binding to the groove of DNA in the three experiments performed. The reason for this interaction is thought to be the long chain side group of **GsB-SiPc**.

In the DNA nuclease/photonuclease activities of the compounds, it is realized with the assumption that the damage caused by the compounds on DNA may cause cell death.[26] It is known that the properties of ideal photosensitizers are low toxicity in the dark and high toxicity in the presence of light.[14] In this area, the damage activities of the compounds on DNA are examined by agarose gel electrophoresis method to obtain preliminary data before examining the compounds on cell lines. In this study, it was examined whether **GsB-SiPc** causes damage on DNA both in the dark and using different light doses. The results showed that **GsB-SiPc** did not cause any DNA damage in the dark, whereas in the presence of light, it caused DNA damage depending on the compound concentration and light dose. This is expected considering that phthalocyanines generate reactive oxygen species in the presence of light. Although many studies examining the DNA binding and nuclease activities of silicon (IV) phthalocyanines containing different side groups are available in the literature, there has been no DNA interaction study of **GsB-SiPc** synthesized by our research group before.

PDT is known as a method in which photosensitizers produce reactive oxygen species in the presence of light and oxygen, leading to cell death.[27] In recent years, it has been applied against many types of cancer such as breast, brain, lung, head and neck, and cervical.[4,5] In particular, lung cancer is the most common type of cancer after breast cancer and ranks at the top when the death/incidence rate is examined.[1] Considering the side effects of the current drugs used in treatment, the search for many alternative treatments for the treatment of this disease continues. The MTT cell viability test is a common method that measures the metabolic activity of cells and evaluates their viability. The test is based on the conversion of a tetrazolium salt into insoluble formazan crystals by mitochondrial enzymes of living cells. As a result of the reduction, insoluble purple formazan crystals are formed. This reaction only occurs in metabolically active (living) cells; formazan is not formed in dead or metabolically reduced cells. Purple formazan crystals accumulated in the cells are dissolved with a solvent that can dissolve the cell membrane (e.g. DMSO, isopropanol). The solution turns purple. In this way, cell viability is determined.[28] In the present study, the cytotoxic and phototoxic activities of GsB-SiPc against A549 cell line were investigated using MTT cell viability assay. The results of the study revealed that GsB-SiPc at 50 and 100 μM showed effective cell death in the presence of light. There are studies in the literature examining the effects of phthalocyanines against lung cancer cell lines. Ma et al. investigated phototoxic and photothermal effects of zinc(II) phthalocyanine encapsulated with boronate-linked polydopamine polydopamine-poloxamers on A549 cells. These results claimed it shows synergistic effects with PDT and photothermal activity.[29] In another study, Önal et al. investigated the PDT efficacy on A549 cells using metal free phthalocyanine containing triphenylphosphine groups The results claimed that the IC50 values of the compound were approximately 3.3 μM and 2.4 μM with light irradiation on A549 cells for 24 and 48 h.[30] The results reveal that different metals, ligands, and concentrations affect the PDT results.

CONCLUSION

In this paper, DNA binding, nuclease/photonuclease and cytotoxic/phototoxic properties of **GsB-SiPc** were investigated using UV-Vis spectrophotometer and agarose gel electrophoresis methods. The UV-Vis titration, competitive EB, and electrophoresis binding studies of **GsB-SiPc** revealed that it interacted with ct-DNA via groove binding. **GsB-SiPc** was observed to have low nuclease activity in the dark and high photonuclease activity in the presence of light, depending on compound concentration and light dose. In addition, **GsB-SiPc** showed high phototoxic activity against A549 cell line at 50 and 100 μM in the presence

of light. All findings in the study revealed the potential of **GsB-SiPc** as a therapeutic agent for PDT.

Authorship contribution statement

Consept and desing: GS, COY, ZB and BB.
Acquisition of data: GS, COY, ZB and BB.
Analysis and interpretation of data: GS, COY and BB.
Drafting of the manuscript: GS, CB, COY and BB.
Critical revision of the manuscript for important intellectual content: GS, CB and BB.
Statistical analysis: BB.

Declaration of competing interest

None of the authors have potential conflicts of interest to be disclosed.

Ethical approval

Since we did not use human/animal or human/animal data in this study, our study does not require ethics committee approval.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

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REFERENCES

1. Bray F, Laversanne M, Sung H, et al. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2024;74(3):229-263. doi:10.3322/caac.21834
2. Debela DT, Muzazu SG, Heraro KD, et al. New approaches and procedures for cancer treatment: Current perspectives. SAGE Open Med. 2021;9:20503121211034366. doi:10.1177/20503121211034366
3. Nguyen VN, Pham HL, Nguyen XT. Recent progress in organic carbon dot-based photosensitizers for photodynamic cancer therapy. Dyes and Pigments. 2024;230:112359. doi: 10.1016/j.dyepig.2024.112359
4. Baskaran R, Lee J, Yang SG. Clinical development of photodynamic agents and therapeutic applications. Biomater Res. 2018;22:25. doi:10.1186/s40824-018-0140-z
5. Li X, Lovell JF, Yoon J, Chen X. Clinical development and potential of photothermal and photodynamic therapies for cancer. Nat Rev Clin Oncol. 2020;17(11):657-674. doi:10.1038/s41571-020-0410-2
6. Karbasi M, Varzandeh M, Karbasi M, Mobarakeh AI, Falahati M, Hamblin MR. Photodynamic therapy based on metal-organic framework in cancer treatment: A comprehensive review of integration strategies for synergistic combination therapies. Nano-Structures & Nano-Objects. 2024;40:101315. doi:10.1016/j.nanoso.2024.101315
7. Kim TE, Chang JE. Recent Studies in Photodynamic Therapy for Cancer Treatment: From Basic Research to Clinical Trials. Pharmaceutics. 2023;15(9):2257. doi:10.3390/pharmaceutics15092257
8. Kwiatkowski S, Knap B, Przystupski D, et al. Photodynamic therapy - mechanisms, photosensitizers and combinations. Biomed Pharmacother. 2018;106:1098-1107. doi:10.1016/j.biopha.2018.07.049
9. Oluwajembola AM, Cleanclay WD, Onyia AF, et al. Photosensitizers in photodynamic therapy: An advancement in cancer treatment. Results in Chemistry. 2024;10:101715. doi:10.1016/j.rechem.2024.101715
10. Kessel D. Death pathways associated with photodynamic therapy. Photochemistry and Photobiology. 2021;97(5):1101-1103. doi:10.1111/php.13436
11. Nowak-Perlak M, Ziółkowski P, Woźniak M. A promising natural anthraquinones mediated by photodynamic therapy for anti-cancer therapy. Phytomedicine. 2023;119:155035. doi:10.1016/j.phymed.2023.155035
12. Brilkina AA, Dubasova LV, Sergeeva EA, et al. Photobiological properties of phthalocyanine photosensitizers Photosens, Holosens and Phthalosens: A comparative *in vitro* analysis. J Photochem Photobiol B. 2019;191:128-134. doi:10.1016/j.jphotobiol.2018.12.020
13. Adnane F, El-Zayat E, Fahmy HM. The combinational application of photodynamic therapy and nanotechnology in skin cancer treatment: A review. Tissue Cell. 2022;77:101856. doi:10.1016/j.tice.2022.101856
14. Li X, Zheng BD, Peng XH, et al. Phthalocyanines as medicinal photosensitizers: Developments in the last five years. Coordination Chemistry Reviews. 2019;379:147-160. doi:10.1016/j.ccr.2017.08.003
15. Santos KLM, Barros RM, da Silva Lima DP, et al. Prospective application of phthalocyanines in the photodynamic therapy against microorganisms and tumor cells: A mini-review. Photodiagnosis and Photodynamic Therapy. 2020;32:102032. doi:10.1016/j.pdpdt.2020.102032
16. Barut B, Barut EN, Yalçın CÖ, et al. The synthesis and therapeutic effect of silicon(IV) phthalocyanines for colorectal cancer cells in photodynamic therapy by altering Wnt/β-catenin and apoptotic signaling. Journal of Photochemistry and Photobiology A: Chemistry. 2024;453:115663. doi:10.1016/j.jphotochem.2024.115663
17. Barut B, Çoban Ö, Yalçın CÖ, et al. Synthesis, DNA interaction, *in vitro/in silico* topoisomerase II inhibition and photodynamic therapy activities of two cationic BODIPY derivatives. Dyes and Pigments. 2020;174:108072. doi:10.1016/j.dyepig.2019.108072
18. Kocak A, Yilmaz H, Faiz O, Andac O. Experimental and theoretical studies on Cu (II) complex of N, N′-disalicylidene-2, 3-diaminopyridine ligand reveal indirect evidence for DNA intercalation. Polyhedron. 2016;104:106-115. doi:10.1016/j.poly.2015.11.037
19. Yabaş E, Bağda E, Bağda E. The water soluble ball-type phthalocyanine as new potential anticancer drugs. Dyes and Pigments. 2015;120:220-227. doi:10.1016/j.dyepig.2015.03.038
20. Baş H, Biyiklioglu Z, Barut B, Yalçın CÖ, Özel A. Highly water soluble axial disubstituted silicon (IV) phthalocyanine, naphthalocyanine: Synthesis, DNA interaction and anticancer effects against human lung (A549), liver (SNU-398), melanoma (SK-MEL128), prostate (DU-145), breast (BT-20) cell lines. Inorganic

Chemistry Communications. 2023;156:111139. doi:10.1016/j.inoche.2023.111139
21. Barut B, Yalçın CÖ, Demirbaş Ü, Akçay HT, Kantekin H, Özel A. The novel Zn (II) phthalocyanines: Synthesis, characterization, photochemical, DNA interaction and cytotoxic/phototoxic properties. Journal of Molecular Structure. 2020;1218:128502. doi:10.1016/j.molstruc.2020.128502
22. Torres-Martinez Z, Delgado Y, Ferrer-Acosta Y, et al. Key genes and drug delivery systems to improve the efficiency of chemotherapy. Cancer Drug Resistance. 2021;4(1): 163. doi:10.20517/cdr.2020.64
23. Sirajuddin M, Ali S, Badshah A. Drug–DNA interactions and their study by UV–Visible, fluorescence spectroscopies and cyclic voltametry. Journal of Photochemistry and Photobiology B: Biology. 2013;124:1-19. doi:10.1016/j.jphotobiol.2013.03.013
24. Phadte AA, Banerjee S, Mate NA, Banerjee A. Spectroscopic and viscometric determination of DNA-binding modes of some bioactive dibenzodioxins and phenazines. Biochemistry and Biophysics Reports. 2019;18:100629. doi:10.1016/j.bbrep.2019.100629
25. Barut B, Seyhan G, Keleş T, Kulein B, Biyiklioglu Z. Nonperipherally and peripherally substituted water-soluble magnesium (II) phthalocyanines and their DNA binding, nuclease activities. Applied Organometallic Chemistry. 2024;38(5):e7421. doi:10.1002/aoc.7421
26. Borges HL, Linden R, Wang JY. DNA damage-induced cell death: lessons from the central nervous system. Cell Research. 2008;18(1):17-26. doi:10.1038/cr.2007.110
27. Alvarez N, Sevilla A. Current advances in photodynamic therapy (PDT) and the future potential of PDT-combinatorial cancer therapies. International Journal of Molecular Sciences. 2024;25(2):1023. doi:10.3390/ijms25021023
28. Ghasemi M, Turnbull T, Sebastian S, Kempson I. The MTT assay: utility, limitations, pitfalls, and interpretation in bulk and single-cell analysis. International Journal of Molecular Sciences. 2021;22(23):12827. doi:10.3390/ijms222312827
29. Ma D, Zhang H, Zhao M, et al. A novel boronate-linked polydopamine-poloxamer 407 loaded zinc phthalocyanine nanoparticles for photothermal and photodynamic synergy therapy. Journal of Drug Delivery Science and Technology. 2023;87:104870. doi:10.1016/j.jddst.2023.104870
30. Onal E, Tuncel O, Erdoğan Vatansever I, et al. Development of AB3-type novel phthalocyanine and porphyrin photosensitizers conjugated with triphenylphosphonium for higher photodynamic efficacy. ACS Omega. 2022;7(43):39404-39416. doi:10.1021/acsomega.2c05814

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Molecular Docking Analysis of the Affinities of Lipid-Lowering Drugs to Paraoxonase-1 Enzyme and Its Polymorphic Structures

Lipid Düşürücü İlaçların Paraoksonaz-1 Enzimine ve Polimorfik Yapılarına Afinitelerinin Moleküler Docking Analizi

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ABSTRACT

Paraoxonase-1 (PON1) is a high-density lipoprotein (HDL)-associated enzyme that exhibits paraoxonase, arylesterase, and lactonase activities. This multifunctional enzyme plays a crucial role in preventing atherosclerosis by inhibiting low-density lipoprotein (LDL) oxidation and reducing oxidized lipid levels. The present study aimed to investigate the affinities of various lipid-lowering drugs to PON1 and its polymorphic structures [(M/L)55 and (Q/R)192] using advanced molecular docking methods. The research utilized a comprehensive computational approach, including homology modeling, molecular dynamics simulation, and AutoDock 4 software to analyze the interactions between PON1 and several classes of lipid-lowering agents. These included statins (simvastatin, atorvastatin, lovastatin, mevastatin, fluvastatin, rosuvastatin, pravastatin), fibrates (fenofibrate, gemfibrozil, bezafibrate, ciprofibrate), niacin, ezetimibe, orlistat, sibutramine, probucol, and phytosterols (brassicasterol, campesterol, β-sitosterol, stigmasterol). The study revealed varying affinities of these drugs to PON1 and its polymorphic structures. Notably, brassicasterol showed the highest affinity for the normal PON1 structure, while sibutramine and stigmasterol demonstrated the highest affinities for the Q/R 192 and M/L 55 polymorphic structures, respectively. Conversely, orlistat exhibited the lowest affinity for both normal PON1 and the M/L 55 polymorphic structure, while atorvastatin showed the lowest affinity for the Q/R 192 polymorphic structure. These findings provide valuable insights into the potential interactions between lipid-lowering drugs and PON1, suggesting that consideration of PON1 affinity might be important in the selection of lipid-lowering therapies, particularly in individuals with different PON1 polymorphisms. However, further *in vitro* and *in vivo* studies are necessary to validate these computational results and establish their clinical relevance.

Keywords: Cardiovascular disease, Lipid-lowering drugs, Molecular docking, Paraoxonase-1 (PON1), PON1 polymorphisms

ÖZET

Paraoksonaz-1 (PON1), paraoksonaz, arilesteraz ve laktonaz aktiviteleri gösteren, yüksek yoğunluklu lipoprotein (HDL) ile ilişkili bir enzimdir. Bu çok fonksiyonlu enzim, düşük yoğunluklu lipoprotein (LDL) oksidasyonunu önleyerek ve oksitlenmiş lipid seviyelerini azaltarak aterosklerozun önlenmesinde önemli bir rol oynamaktadır. Bu çalışma, çeşitli lipid düşürücü ilaçların PON1 ve polimorfik yapılarına [(M/L)55 ve (Q/R)192] olan afinitelerini gelişmiş moleküler doking yöntemleri kullanarak araştırmayı amaçlamıştır. Araştırma, PON1 ile çeşitli lipid düşürücü ajanlar arasındaki etkileşimleri analiz etmek için homoloji modellemesi, moleküler dinamik simülasyonu ve AutoDock 4 yazılımını içeren kapsamlı bir hesaplamalı yaklaşım kullanmıştır. Bu ajanlar arasında statinler (simvastatin, atorvastatin, lovastatin, mevastatin, fluvastatin, rosuvastatin, pravastatin), fibratlar (fenofibrat, gemfibrozil, bezafibrat, siprofibrat), niasin, ezetimib, orlistat, sibutramin, probukol ve fitosteroller (brasikasterol, kampesterol, β-sitosterol, stigmasterol) yeralmaktadır. Çalışma, builaçların PON1 ve polimorfik yapılarına değişen afiniteler gösterdiğini ortaya koymuştur. Özellikle, brasikasterol normal PON1 yapısına en yüksek afiniteyi gösterirken, sibutramin ve stigmasterol sırasıyla Q/R 192 ve M/L 55 polimorfik yapılarına en yüksek afiniteleri göstermiştir. Buna karşılık, orlistat hem normal PON1 hem de M/L 55 polimorfik yapısına en düşük afiniteyi gösterirken, atorvastatin Q/R 192 polimorfik yapısına en düşük afiniteyi göstermiştir. Bu bulgular, lipid düşürücü ilaçlar ile PON1 arasındaki potansiyel etkileşimler hakkında değerli bilgiler sağlamakta ve PON1 afinitesinin, özellikle farklı PON1 polimorfizmleri olan bireylerde lipid düşürücü tedavilerin seçiminde önemli olabileceğini göstermektedir. Bununla birlikte, bu hesaplamalı sonuçları doğrulamak ve klinik önemini belirlemek için daha fazla *in vitro* ve in vivo çalışma gereklidir.

Anahtar Kelimeler: Kardiyovasküler hastalık, Lipid düşürücü ilaçlar, Moleküler doking, Paraoksonaz-1 (PON1), PON1 polimorfizmleri

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INTRODUCTION

Paraoxonase-1 (PON1) has emerged as a subject of intense research interest in recent years, primarily due to its pivotal role in lipid metabolism and cardiovascular health. As an enzyme associated with high-density lipoprotein (HDL), PON1 exhibits a remarkable ability to hydrolyze a wide range of substrates, including oxidized lipids, homocysteine thiolactone, and various toxic organophosphate compounds.[1,2] The enzyme's capacity to prevent low-density lipoprotein (LDL) oxidation and reduce oxidized lipid levels has positioned it as a key player in the prevention of atherosclerosis and, by extension, cardiovascular diseases.[1]

The PON1 gene, located on chromosome 7 in humans, is known to have several polymorphisms that can affect the enzyme's activity and concentration in the serum. Two of the most studied polymorphisms are the (M/L)55 and (Q/R)192 variants, which have been associated with varying levels of enzymatic activity and different susceptibilities to cardiovascular diseases.[3] These genetic variations add a layer of complexity to the study of PON1 and its interactions with various compounds, including lipid-lowering drugs.

Lipid-lowering drugs represent a cornerstone in the management of dyslipidemia and the prevention of cardiovascular diseases. This diverse group of pharmaceuticals includes several classes of compounds, each with unique mechanisms of action.[4] While the primary mechanisms of these drugs in lipid lowering are well established, their potential interactions with other physiological systems, including enzymes like PON1, are not fully understood. The relationship between lipid-lowering drugs and PON1 has been a subject of investigation, with some studies reporting conflicting results regarding the effects of these drugs on PON1 activity.[5,6]

The advent of computational methods in drug discovery and molecular biology has opened new avenues for investigating such complex interactions. Molecular docking, in particular, has emerged as a powerful tool for predicting the binding affinities and orientations of small molecules to their target proteins. This in silico approach allows for the rapid screening of multiple compounds and can provide valuable insights into potential drug-enzyme interactions, guiding further experimental studies and potentially informing clinical decision-making.[7]

The present study aims to leverage these computational techniques to examine the affinities of various lipid-lowering drugs to PON1 and its polymorphic forms by utilizing molecular docking methods. This comprehensive in silico analysis aims to contribute to our understanding of the complex interplay between lipid-lowering drugs and PON1, potentially shedding light on the broader implications of these interactions in the context of cardiovascular health and personalized medicine.

METHODS

Ligands and paraoxonase protein

Lipid-lowering drugs such as ezetimibe, ciprofibrate, clofibrate, fenofibrate, gemfibrozil, beta-sitosterol, brassicasterol, campesterol, stigmasterol, bezafibrate, niacin, orlistat, probucol, sibutramine, atorvastatin, fluvastatin, lovastatin, mevastatin, pitavastatin, pravastatin, rosuvastatin, and simvastatin have been used. All ligands had hydrogen atoms added using Marvin Sketch software before processing (Marvin 5.6.0.5, 2011, (ChemAxon). Each ligand underwent a total of 100,000 steps of minimization using the mmff94 (Merck Molecular Force Field) force field and the "steepest descent" optimization algorithm.[8] Ligands were charged using the ADT (Autodock Tools) program with Gasteiger, and all bonds except amide bonds were set to be freely adjustable. All ligands were obtained from the PubChem ligand database.[9,10] The modeling, simulation, and docking methodology of Paraoxonase Protein was established based on the previous work conducted by Duzgun et al.[11]

Hardware

All docking and molecular dynamics simulations were conducted on TÜBİTAK's high-performance computing clusters, Trgrid TRUBA. Each of the computer clusters used in Trgrid consists of nodes with two 12-core "Opteron 6174" processors, making a total of 24 cores. Molecular dynamics simulations were run in parallel across multiple clusters using GROMACS 4.5.5 software.[12] Docking processes were performed on a computer with a 4-core Intel Core i3 processor using "Autodock 4" and "Autodock Vina" software.[13,14]

Modeling of paraoxonase protein

The protein structure modeling process began with the PON1 protein structure (PDB code: 3SRE) obtained from the Protein Data Bank.[15] The structure was preprocessed using Chimera software to remove existing ligands, preparing it for subsequent homology modeling.[16] Homology modeling was performed using

MODELLER software, with the human serum paraoxonase enzyme sequence from Uniprot (accession code: P27169) serving as the template.[17,18] The sequence alignment showed 85% identity between the template and target sequences, indicating a high probability of accurate model generation. To study the polymorphic variants, specific amino acid modifications were made at positions 55 and 192 to create the Q/R192 and M/L55 variants. For each variant (wild-type, Q/R192, and M/L55), MODELLER generated 20 distinct models. The modeling process included the retention of Ca^{2+} cofactors by enabling the 'include HETATM residues other than water' option. The calcium ions were maintained in their crystallographic positions due to their critical role in structural stability and catalytic function. The final model selection for each variant was based on quality assessment using the Molprobity server, with the highest-scoring model chosen for further analysis.[19]

Molecular dynamics simulation

All molecular dynamics calculations were conducted on the computer clusters on Trgrid, each with 24 cores (AMD Opteron) using GROMACS 4.5.5 software.[20] The parallel computing setup allowed for efficient handling of the computationally intensive simulations. The structures obtained in PDB file format from the previously used "MODELLER" homology modeling software were subjected to a series of stages according to the following diagram. The simulation box was constructed with periodic boundary conditions using a dodecahedron geometry, with a minimum distance of 1.2 nm between the protein and box edges. The "forcefield" used was AMBER99SB-ILDN, and SPC (simple point charge) was chosen as the water model.[21,22] Na^+ and Cl^- ions were added for neutralization of the system. The ionic strength was adjusted to 0.15 M to mimic physiological conditions. A total of 5000 steps of the "steepest descent" minimization algorithm were performed. The minimization was continued until the maximum force was less than 1000 kJ/mol/nm. The equilibrium phase occurred in two phases, NVT (for temperature and volume stability) and NPT (for pressure and density stability). Initially, a 100 ps NVT phase was initiated. The other phase was a two-step NPT phase. The first step was a 100 ps phase using position restraining algorithms, while the second step was a 1 ns final equilibrium phase without position restraining algorithms. A 10 ns simulation was applied in the production phase. To ensure that the simulation was successfully conducted, several data analyses were performed. One of these was structural stability, which was evaluated using RMSD calculations. RMSF analysis was conducted to show the mobility of each residue in the protein. The radius of gyration (Rg) is a measure of the compactness of a protein; if a protein is stably folded, it will show a certain stable Rg value in the corresponding graph.

Molecular docking

Docking was performed using two different computational algorithms: Autodock4 and Autodock Vina.[13,14] Both programs were chosen for their complementary strengths in binding prediction and scoring functions. The human PON1 model, obtained from the rabbit PON1 enzyme through homology modeling, underwent MD simulation with GROMACS software to gain appropriate structure and behavior under in vivo conditions. The final structure for docking was selected from the MD trajectory based on clustering analysis of conformations. The flexible missing residues in the range of 72-81 in the 3SRE model were added in this process. The Y71 and R292 residues were treated as flexible in both docking processes. The docking procedure used for AutoDock 4.2 was based on the protocol established by Ben-David et al. In AutoDock 4.2, the active site of the PON1 enzyme was targeted with an average grid volume of 39 Å (Angstrom) for the docking process. Grid maps were generated with 0.375 Å spacing. In AutoDock Vina, the docking process was similarly based on the active site of the PON1 enzyme, with an average grid volume of 17 Å (Angstrom). Exhaustiveness was set to 4. Vina and AutoDock 4.2 considered all ligands as flexible except for the Y71 and R292 residues. The electrostatic field was calculated on a 1 Å grid, and all other settings related to Vina were left as default. The docking results were analyzed based on binding energy scores and clustering of binding poses. AutoDock 4 was preferred due to its ability to calculate the Ca^{2+} cofactor and electrostatic charge of the protein. Since AutoDock Vina could not perform these calculations, resulting in poor correlation and incorrect conformations, only AutoDock 4 was used for affinity calculations of the drugs. The final binding poses were selected based on both energy scores and visual inspection of the protein-ligand interactions.

RESULTS

Molecular dynamics simulation

A molecular dynamics study was conducted using Gromacs software on PON1 protein and its polymorphic

structures. The protein systems, with an average molecular weight of 39.75 kDa, each contained two calcium atoms as cofactors. The analysis focused primarily on RMSD, RMSF and Radius of gyration (Rg) measurements. RMSD, measured using alpha carbon positions, serves as an indicator of system stability and structural integrity. A stable RMSD value suggests system equilibration. The MD simulations revealed distinct conformational patterns. All systems showed an initial RMSD increase from 0.05 nm during the first 2 ns. The wild-type system stabilized between 0.15-0.17 nm after 6 ns. The M/L 55 variant showed lower RMSD values (0.13-0.15 nm), indicating increased structural rigidity. The Q/R 192 variant displayed the highest RMSD values (0.16-0.19 nm) with greater fluctuations, suggesting enhanced flexibility. All systems reached equilibrium within 6-7 ns, with M/L 55 showing the most stable trajectory. Protein flexibility was analyzed using RMSF calculations, which measure residue-specific mobility throughout the simulation. The analysis identified key structural regions: loops (L1, L2, L3) and helices (H1, H2). The N-terminal H1 region showed maximum flexibility, followed by the L1 region near the active site. Rg measurements provided insights into protein compactness. The wild-type maintained an Rg around 1.91 nm, while both variants showed slightly higher values around 1.93 nm. The M/L 55 and Q/R 192 variants demonstrated similar Rg values but with different fluctuation patterns. All systems maintained stable Rg values within ±0.01 nm after equilibration, indicating that while mutations caused subtle structural changes, they did not induce major conformational alterations. The higher Rg values in mutant systems suggest slightly less compact structures compared to the wild type, potentially affecting their functional dynamics.

Molecular docking

After 10 ns of molecular dynamics simulation, docking was performed using Autodock 4.2 on a total of 22 lipid-lowering drugs for each polymorphic structure of the proteins at 0.1 ns intervals starting from the 9th ns. As seen in figure 1, the best interaction was determined to be brassicasterol with the Normal PON1 protein structure, sibutramine with the Q/R 192 PON1 polymorphic structure, and stigmasterol with the M/L 55 PON1 polymorphic structure. When examining the interaction of brassicasterol with the Normal PON1 structure, no hydrogen bond formation was observed, while electrostatic interactions were observed with His285 and Leu267 (Figure 2A). When examining the interaction of sibutramine with Q/R 192 PON1, only Phe77 was observed to engage in electrostatic interaction (Figure 2B). When examining the interaction of stigmasterol with M/L 55 PON1, it was observed that the hydroxyl group at the end of the compound's steroid structure formed electrostatic interactions with Asn168 and Asn224. Additionally, His115, Glu53, and Asp269 were also observed to participate in electrostatic interactions (Figure 2C).

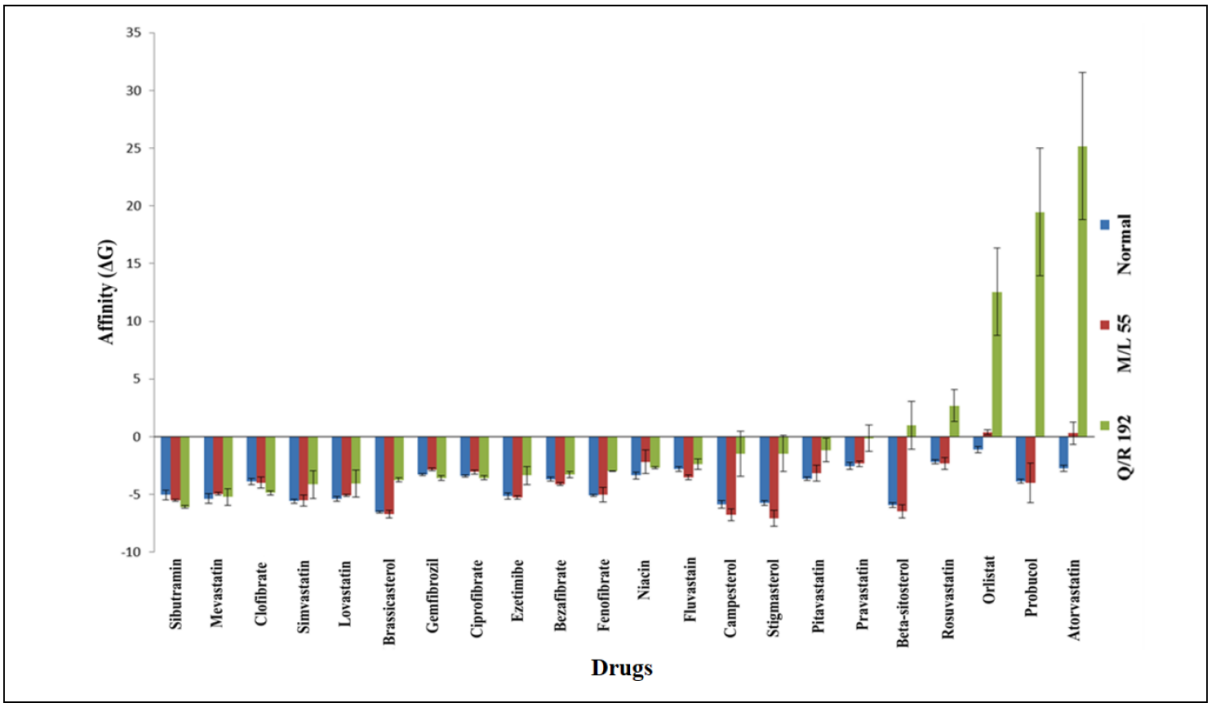

Figure 1. Affinity values of lipid-lowering drugs to PON1 and its polymorphic structures

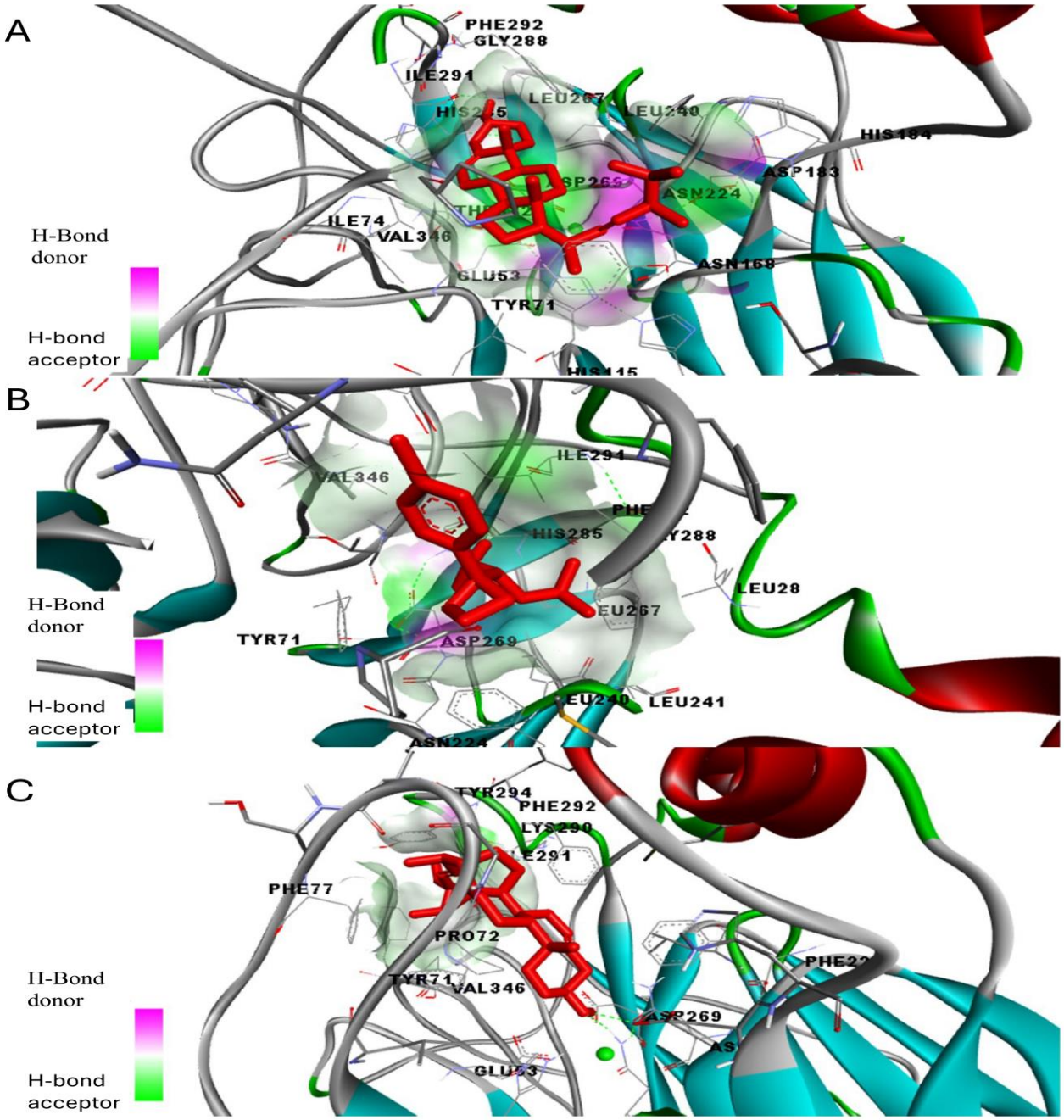

Figure 2. A- Conformation of brassicasterol shown in red structure in the active center of Normal PON1
B- Conformation of sibutramine shown in red structure in the active center of Q/R 192 PON1
C- Conformation of stigmasterol shown in red structure in the active center of M/L 55 PON1

DISCUSSION

In this study, the affinities of lipid-lowering drugs to PON1 enzyme were evaluated by applying *in silico* (computer-aided) approaches, which have been widely used in recent years to guide research before experimental studies and to save time and money. The affinities of PON1 enzyme substrates and lipid-lowering drugs were compared. Before proceeding to the molecular docking method used in affinity determination, the three-dimensional molecular structure of the PON1 enzyme was created by considering its polymorphic structures. Since the X-

RAY crystallographic structure of human PON1 enzyme was not revealed, 100% human PON1, M/L 55 and Q/R 192 polymorphic structures were obtained by homology modeling from a human-rabbit hybrid X-RAY crystal structure (PDB code: 3SRE) with 83.66% similarity at the amino acid level with the same enzymatic activity. These structures were subjected to 10 ns molecular dynamics simulation to give them their natural conformation and behavior under laboratory conditions. The characteristics of serum PON1 enzyme and (M/L) 55, (Q/R) 192 polymorphic structures were analyzed by molecular dynamics simulation.

Homology Modeling

In this study, 100% human serum PON1 protein structure and (M/L) 55, (Q/R) 192 polymorphic structures were obtained from rePON1-G2E6, a recombinant PON1 variant with identical enzymatic activity (83.66% similar to human serum paraoxonase) using homology modeling.[15] The normal and polymorphic structures generated by MODELLER, a homology modeling tool, and the experimentally obtained rePON1-G2E6 variant were aligned using the "matchmaker" tool with Chimera software. The alignment resulted in RMSD values of 0.133 with the PON1 normal construct, 0.155 RMSD with PON1 (M/L) 55 and 0.141 RMSD with PON1 (Q/R) 192. Very low RMSD values indicate that the construct produced has very high structural similarity with the experimental G2E6 variant. A value close to 0 increases the significance considerably.[23] The most deviation occurred in the L1 knot region of the PON1 enzyme close to the catalytic site. Since the knot in this region of the protein is very flexible, it could not be shown in the experimentally obtained rePON1-G2E6 variant and remained as a missing residue.[15] The MODELS tool provides a great advantage by not only producing 100% human serum PON protein and polymorphic structures, but also complementing these missing residues. MODELLER is one of the most widely used, reliable and fully automated comparative homology modeling programs. Its speed compared to other homology modeling software has made it useful for whole genome modeling studies.

In genetic, cell and molecular biology studies, experimental methods are applied to reveal protein structures. However, due to the lack of information on the atomic structure of these proteins, their molecular functions and mechanisms cannot be fully elucidated. Current methods to obtain biomolecules at atomic resolution (X-ray crystallography and NMR spectroscopy) require the preparation of high concentrations of pure proteins under physiological conditions. NMR spectroscopy can be applied for proteins with a maximum size of 15 kDa. However, many biologically important proteins have larger structures. Homology modeling can reveal the structures of proteins with different polymorphic and mutated structures.[24]

Molecular Dynamics Simulation

After homology modeling of the PON1 enzyme and its polymorphic structures were established, molecular dynamics simulations were performed. Molecular dynamics simulations were performed for 10 ns each on the normal, M/L 55 and Q/R 192 polymorphic structures of PON enzyme. With these simulations, conformational changes were examined by giving the protein its unique dynamic character in the system and molecular docking was performed with its substrates and lipid-lowering drugs on protein conformations at certain time intervals.

In this study, Parinello-Rahman' method was used to adjust the temperature, pressure and density values in molecular dynamics simulation. One of the reliability criteria of molecular dynamics simulation is the density value of the system. The density of a system containing water, ions and protein should be close to 1000 kg/m^3 in accordance with laboratory conditions.[25] In our study, the density of the system containing all three protein structures was very close to the laboratory conditions. Density differences of less than 1% between the systems were predicted to be related to the different numbers and types of atoms in the systems. In addition, small temperature and pressure differences between the systems also have an effect on the density.[25]

Over a period of 10 ns, the positional changes of each amino acid in the polymorphic structures are shown. Amino acids in the range of 70-80% are very flexible and located very close to the active site of the enzyme. This raises the question of whether the position of this region is effective in enzyme-substrate interactions. As a matter of fact, a detailed study on the enzyme PON suggested that this region may have a substrate-selective character and especially the 71st tyrosine residue may act as a cap in enzyme-substrate complexes.[26]

The choice of an appropriate energy function to describe intramolecular and intermolecular interactions is critical for a successful molecular dynamics simulation. Energy functions are usually composed of many parametric

terms. These parameters are mainly obtained from experimental and quantum mechanical studies of small molecules or fragments. Groups of functions associated with parameter settings are expressed by the term force field.[27] The force field parameters, which are vital for molecular dynamics simulations, are now being developed with the help of quantum mechanical calculations and continue to be improved with higher accuracy. AMBER (Energy Simplification Assisted Model Building) is a family of force fields for molecular dynamics simulations of biomolecules developed by the Peter Kollman group at the University of California, San Francisco. The correlation of various force fields of the AMBER family with experimental data was shown by Hornak *et al*.[28] In this study, a high correlation between the experimental NMR parameters and the parameters generated by the ff99SB force field was shown with 0.83 for lysozyme and 0.95 for ubiquitin.[28]

Molecular Docking

The most widely used Autodock program was used to determine the affinity of different polymorphic structures of PON1 with drugs and its natural substrates. While the interactions of PON1 with various substrates have been studied in depth with the docking method, there is no docking study on its affinity or interaction with lipid-lowering drugs in the literature. Xin Hu *et al*. used molecular docking, MD simulation and free energy calculation methods to investigate the interactions between the PON1 enzyme and its various substrates such as esters, lactones and phosphotrioester.[29] In their study, they showed that tyrosine 71 residue may have an important role in the binding of substrates and suggested that it may have a gate function that facilitates substrate identification. In our study, it was observed that tyrosine 71 residue has a gating function but has no direct catalytic effect in the interaction with substrates. Ben-David *et al*. and Harel *et.al*. showed that in the structure at pH 4.5 (PDB:1V04), residue 71 was close to the catalytic site, while at pH 6.5 (PDB:3SRE, 3SRG), residue 71 was outside the active site.[15,30] In other words, it has been shown that the 71st residue shows open or closed conformation at different pH. Since we used 3SRE-derived structures (operating at neutral pH) in our study, it is possible that residue 71 showed mostly open conformation.[15] A study on the structure and activity of PON1 revealed that calcium is a vital co-factor in catalytic activity.[31] As a matter of fact, in the conformations of the substrates obtained by molecular docking method on different polymorphic structures of PON1, calcium has an important effect on the formation of the enzyme-substrate complex by attracting the oxygen in the lactone structure towards itself. The Q/R 192 polymorphic structure had a narrower active center and the oxygens of its substrates were located closer to the catalytic calcium than the other polymorphic structures.

Lipid Lowering Drugs and Paraoxonase

The affinity of lipid-lowering drugs for PON1 itself and its polymorphic structures was shown in figure 1. When the comparison of the drugs between the PON structures was made, it was observed that the affinity of the drugs in the Q/R 192 polymorphism was generally lower than the other PON structures. The Y71 residue, which is located in the lid position of the active site, is open in other structures of paraoxonase, while it is closed in the Q/R 192 polymorphic structure. This may have resulted in lower affinity of the drugs. In contrast to other drugs, atorvastatin, probucol, orlistat, rosuvastatin and betasterol showed positive ΔG in the Q/R 192 polymorphic structure. In other structures of paraoxonase, atorvastatin, probucol, orlistat, rosuvastatin and pravastatin were found to have low affinity for PON1. Therefore, these drugs may not be very effective on paraoxonase activity. This suggests that drugs with high affinity may decrease paraoxonase activity, whereas drugs without any affinity or with low affinity may not affect paraoxonase activity much.

In this study, it was observed that atorvastatin could not affect paraoxonase activity, while no study was found that atorvastatin decreased or did not affect paraoxonase. In fact, it has been reported to increase PON1 activity in many studies. Kural *et al.* found that atorvastatin significantly increased serum paraoxonase activity and HDL levels in a study with dyslipidemic patients.[32,33] Similarly, Harangi *et al.* observed that atorvastatin treatment increased paraoxonase activity.[34] According to Oranje *et al.* atorvastatin decreased LDL oxidation in type 2 diabetic patients.[35] These studies pointed that atorvastatin has an important effect in preventing atherosclerotic diseases. However, Bergheanu *et al* investigated the effect of rosuvastain and atorvastatin on PON1 activity in men with cardiovascular disease and showed that both drugs increased PON1 activity, but rosuvastatin, unlike atorvastatin, increased PON1 activity in a dose-dependent manner.[36] In our study, it was observed that rosuvastatin has a weak affinity for PON1, so it may not be effective. We could not find any studies showing that

rosuvastatin reduces PON1 activity. Orlistat, another drug used in this study, showed very little affinity for PON1 in normal structure but not in polymorphic structures. Audikovszky *et al.* expressed that orlistat increased paraoxonase activity.[37]

Among the statins, simvastatin, lovastatin and mevastatin were found to have the highest affinity values. The fact that these drugs have lactone structures is the probable reason for this result. Due to this similarity, it is expected that PON activities would decrease by competitive inhibition. Consistent with our study, Billecke *et al.* reported that these three statin group drugs showed affinity for PON1 and were metabolized by PON1.[38] Another study also identified that statins such as pravastatin, fluvastatin and simvastatin reduced PON1 activity.[39] On the other hand, Tomas *et al.* stated that simvastatin increased paraoxonase activity and therefore may have actioxidant properties.[40] In a meta-analysis study conducted by Farretti *et al*., it was shown that statin therapy provides cardiovascular benefits by increasing PON1 paraoxonase and arylesterase activities, and this could be among the lipid-independent pleiotropic effects.The fact that this effect is independent of statin dose, treatment duration, or changes in LDL cholesterol levels indicates additional mechanisms underlying the cardiovascular protective effects of statins.[41] In our study, while mevastatin and simvastatin were calculated to have stronger interactions with PON1, atorvastatin was observed to be unable to interact with PON1, consistent with the study conducted by Farretti *et al.*[41]

Among all drug groups, the highest affinity was found in brassicasterol with PON1-normal structure, stigmasterol with M/L 55 polymorphic structure and sibutramine with Q/R 192 polymorphic structure. Therefore, these drugs may be effective in decreasing PON1 activity. Phytosterols showed high affinity for PON1 and M/L 55 polymorphic structure unlike fibrate type drugs. In the Q/R 192 polymorphic structure, the Y71 residue and the narrow structure of the active center together with the relatively large molecules of phytosterols may have caused them to show low activity. No study was found on the effects of phytosterols, which are similar to cholesterol in chemical structure, on paraoxonase enzyme activity. However, there are conflicting studies showing the relationship between cholesterol and PON1 and studies on HDL in which paraoxonase is involved. Consistent with our study, Yi *et al.* showed the decreased serum PON1 activity in mice on a high cholesterol diet.[42] On the other hand, Kim *et al.* expressed that cholesterol increased PON1 activity.[43] There are also studies on the effect of phytosterol-rich foods on PON1 activity.While Sutherland *et al.* found a positive correlation between plasma phytosterol and HDL cholesterol levels, Zak *et al.* showed that phytosterol consumption increased the cholesterol level in HDL.[44,45]

Clofibrate, gemfibrozil, ciprofibrate and bezafibrate showed low affinity values. Fenofibrate showed above average affinity in normal and M/L 55 structures and below average affinity in Q/R 192 polymorphic structures. Yesilbursa *et al.* observed that fenofibrate increased PON1 activity.[46] This result contradicts our study in normal and M/L 55 constructs with above average affinity values. Macan *et al.* found that gemfibrozil significantly decreased PON1 activity.[47] Increased PON1 activity by using bezafibrate was reported by Durrington *et al.*[48] As mentioned above, the fact that fibrate-type drugs generally increase PON1 activity is consistent with the low affinity values found in our study.

Ezetimibe was in the group of drugs with high affinity for PON1. Niacin was found to have low affinity. A study identified that niacin did not affect paraoxonase and arylesterase activity, but ezetimibe decreased paraoxonase and arylesterase activity.[49] This result support our view that ezetimibe with high affinity value may have a negative effect on PON1 activity.

Sibutramine was measured as the compound with the highest affinity in the Q/R 192 polymorphic structure. As far as we have researched, there is no study showing a direct effect of sibutramine on PON1 activity. However, James et al. observed that sibutramine significantly increased plasma HDL levels. Since there were no studies showing a direct effect of sibutramine on PON1, a comparison with experimental studies could not be made.[50]

Our study has several limitations. Molecular docking analyses were performed on static protein structures and may not fully reflect dynamic interactions under physiological conditions. Additionally, computational results need to be validated in the *in vivo* environment.

These *in silico* results found in this study need to be supported by experimental studies. Although *in silico* molecular docking method saves time and money, its reliability is still a matter of debate. When the correlation between the Autodock 4 software used in this study and the affinity of PON1 with its substrates

and other experimental studies was evaluated, it was observed that the Autodock software worked well for lactone structures, but the error rate increased for compounds such as phenylacetate and very high affinity compounds. Although Autodock is one of the most widely used programs, more reliable software such as CDOCKER may be preferred in the future. Nowadays, more reliable results can be obtained by conducting molecular dynamics studies together.

In light of the findings of this study, it is evident that paraoxonase-1 (PON1) polymorphisms play a critical role in determining the interaction dynamics with lipid-lowering drugs. This highlights the importance of integrating genetic profiling into clinical practice to tailor treatment strategies effectively. Personalized medicine approaches could help optimize drug efficacy and minimize adverse effects by selecting treatments based on an individual's genetic predispositions.

Future studies should focus on validating these computational findings through in vitro and in vivo experiments to confirm their clinical relevance. Additionally, investigating the molecular mechanisms underlying PON1 interactions with a broader range of therapeutic agents could provide deeper insights into its role in personalized treatment approaches. Expanding on these findings, the development of advanced molecular dynamics simulations and large-scale genotype-phenotype correlation studies will be instrumental in bridging the gap between computational predictions and practical applications in clinical settings.

CONCLUSION

This computational study investigated the interactions between various lipid-lowering drugs and PON1, including its polymorphic forms. Our findings revealed significant variations in binding affinities, with brassicasterol, sibutramine, and stigmasterol showing the highest affinities for normal PON1, Q/R 192, and M/L 55 polymorphic structures, respectively. These results suggest that the efficacy of lipid-lowering drugs may be influenced by their interactions with PON1 and its polymorphisms, potentially impacting personalized treatment approaches. However, further *in vitro* and *in vivo* studies are necessary to validate these computational findings and establish their clinical relevance. This research demonstrates the value of *in silico* methods in exploring drug-enzyme interactions and opens new avenues for personalized medicine in cardiovascular health management.

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Note

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Authorship contribution statement

Consept and desing: ZD and BK.
Acquisition of data: ZD.
Analysis and interpretation of data: ZD, BK, AO and IY.
Drafting of the manuscript: ZD and BK.
Critical revision of the manuscript for important intellectual content: IY, AO and BK.
Statistical analysis: ZD.
Supervision: BK.

Declaration of competing interest

None of the authors have potential conflicts of interest to be disclosed.

Ethical approval

Ethical approval was not required for this study.

Availability of data and materials

Data and materials are available from the authors upon reasonable request.

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REFERENCES

1. Durrington PN, Bashir B, Soran H. Paraoxonase 1 and atherosclerosis. Front Cardiovasc Med. 2023;10:1065967.
2. Dornas W, Silva M. Modulation of the antioxidant enzyme paraoxonase-1 for protection against cardiovascular diseases. Nutr Metab Cardiovasc Dis. 2024;34(12):2611-2622. doi:10.1016/j.numecd.2024.04.005
3. Nasreen FJ, Balasubramaniam G. Paraoxonase gene polymorphisms: Understanding the biochemical and genetic basis of coronary artery disease. J Taibah Univ Med Sci. 2023;18(2):257-264.
4. Hsu HY, Lin CJ, Lee YS, Wu TH, Chien KL. Efficacy of more intensive lipid-lowering therapy on cardiovascular diseases: a systematic review and meta-analysis. BMC Cardiovasc Disord. 2020;20:1-12.
5. Godbole C, Thaker S, Salagre S, Shivane V, Gogtay N, Thatte U. A prospective study to assess the role of paraoxonase 1 genotype and phenotype on the lipid-lowering and antioxidant activity of statins. Indian J Pharmacol. 2023;55(3):179-184.
6. Zaragoza-García O, Guzmán-Guzmán IP, Moreno-Godínez ME, et al. PON-1 haplotype (-108C> T, L55M, and Q192R) modulates the serum levels and activity PONase promoting an atherogenic lipid profile in rheumatoid arthritis patients. Clin Rheumatol.

2021;40:741-752.
7. Muhammed MT, Aki-Yalcin E. Molecular docking: principles, advances, and its applications in drug discovery. Lett Drug Des Discov. 2024;21(3):480-495.
8. Halgren TA. Merck molecular force field. I. Basis, form, scope, parameterization, and performance of MMFF94. J Comput Chem. 1996;17(5-6):490-519.
9. Gasteiger J, Marsili M. Iterative partial equalization of orbital electronegativity—a rapid access to atomic charges. Tetrahedron. 1980;36(22):3219-3228. doi:10.1016/0040-4020(80)80168-2
10. Morris GM, Huey R, Olson AJ. Using autodock for ligand-receptor docking. Curr Protoc Bioinforma. 2008;24(1):8-14.
11. Duzgun Z, Kural BV, Orem A, Yildiz I. In silico investigation of the interactions of certain drugs proposed for the treatment of Covid-19 with the paraoxonase-1. J Biomol Struct Dyn. 2023;41(3):884-896.
12. Pronk S, Páll S, Schulz R, et al. GROMACS 4.5: a high-throughput and highly parallel open source molecular simulation toolkit. Bioinformatics. 2013;29(7):845-854.
13. Morris GM, Huey R, Lindstrom W, et al. AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. J Comput Chem. 2009;30(16):2785-2791.
14. Trott O, Olson AJ. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J Comput Chem. 2010;31(2):455-461.
15. Ben-David M, Elias M, Filippi JJ, et al. Catalytic versatility and backups in enzyme active sites: The case of serum paraoxonase 1. J Mol Biol. 2012;418(3-4):181-196. doi:10.1016/j.jmb.2012.02.042
16. Pettersen EF, Goddard TD, Huang CC, et al. UCSF Chimera--A visualization system for exploratory research and analysis. J Comput Chem. 2004;25(13):1605-1612. doi:10.1002/jcc.20084
17. Consortium U. UniProt: a worldwide hub of protein knowledge. Nucleic Acids Res. 2019;47(D1):D506-D515.
18. Webb B, Sali A. Comparative protein structure modeling using MODELLER. Curr Protoc Bioinforma. 2016;54(1):5-6.
19. Chen VB, Arendall WB, Headd JJ, et al. MolProbity: all-atom structure validation for macromolecular crystallography. Acta Crystallogr Sect D Biol Crystallogr. 2010;66(1):12-21. doi:10.1107/S0907444909042073
20. Pronk S, Pall S, Schulz R, et al. GROMACS 4.5: a high-throughput and highly parallel open source molecular simulation toolkit. Bioinformatics. 2013;29(7):845-854. doi:10.1093/bioinformatics/btt055
21. Lindorff-Larsen K, Piana S, Palmo K, et al. Improved side-chain torsion potentials for the Amber ff99SB protein force field. Proteins Struct Funct Bioinforma. 2010;78(8):1950-1958.
22. Zielkiewicz J. Structural properties of water: Comparison of the SPC, SPCE, TIP4P, and TIP5P models of water. J Chem Phys. 2005;123(10).
23. Damm KL, Carlson HA. Gaussian-weighted RMSD superposition of proteins: a structural comparison for flexible proteins and predicted protein structures. Biophys J. 2006;90(12):4558-4573.
24. Ramachandran S, Dokholyan N V. Homology modeling: generating structural models to understand protein function and mechanism. In: Computational Modeling of Biological Systems: From Molecules to Pathways. Springer; 2012:97-116.
25. Levitt M, Sharon R. Accurate simulation of protein dynamics in solution. Proc Natl Acad Sci. 1988;85(20):7557-7561.
26. Hu X, Jiang X, Lenz DE, Cerasoli DM, Wallqvist A. In silico analyses of substrate interactions with human serum paraoxonase 1. Proteins Struct Funct Bioinforma. 2009;75(2):486-498.
27. Adcock SA, McCammon JA. Molecular dynamics: survey of methods for simulating the activity of proteins. Chem Rev. 2006;106(5):1589-1615.
28. Hornak V, Abel R, Okur A, Strockbine B, Roitberg A, Simmerling C. Comparison of multiple Amber force fields and development of improved protein backbone parameters. Proteins Struct Funct Bioinforma. 2006;65(3):712-725.
29. Hu X, Jiang X, Lenz DE, Cerasoli DM, Wallqvist A. In silico analyses of substrate interactions with human serum paraoxonase 1. Proteins Struct Funct Bioinforma. 2009;75(2):486-498. doi:10.1002/prot.22264
30. Harel M, Aharoni A, Gaidukov L, et al. Structure and evolution of the serum paraoxonase family of detoxifying and anti-atherosclerotic enzymes. Nat Struct Mol Biol. 2004;11(5):412-419.
31. Yeung DT, Josse D, Nicholson JD, et al. Structure/function analyses of human serum paraoxonase (HuPON1) mutants designed from a DFPase-like homology model. Biochim Biophys Acta (BBA)-Proteins Proteomics. 2004;1702(1):67-77.
32. Kural BV, Örem C, Uydu HA, Alver A, Örem A. The effects of lipid-lowering therapy on paraoxonase activities and their relationships with the oxidant–antioxidant system in patients with dyslipidemia. Coron Artery Dis. 2004;15(5):277-283.
33. Abdin AA, Hassanien MA, Ibrahim EA, Abou El SEDA. Modulating effect of atorvastatin on paraoxonase 1 activity in type 2 diabetic Egyptian patients with or without nephropathy. J Diabetes Complications. 2010;24(5):325-333.
34. Harangi M, Seres I, Varga Z, et al. Atorvastatin effect on high-density lipoprotein-associated paraoxonase activity and oxidative DNA damage. Eur J Clin Pharmacol. 2004;60:685-691.
35. Oranje WA, Sels JPJE, Rondas-Colbers GJWM, Lemmens PJMR, Wolffenbuttel BHR. Effect of atorvastatin on LDL oxidation and antioxidants in normocholesterolemic type 2 diabetic patients. Clin Chim acta. 2001;311(2):91-94.
36. Bergheanu SC, Van Tol A, Dallinga-Thie GM, et al. Effect of rosuvastatin versus atorvastatin treatment on paraoxonase-1 activity in men with established cardiovascular disease and a low HDL-cholesterol. Curr Med Res Opin. 2007;23(9):2235-2240.
37. Audikovszky M, Pados G, Seres I, et al. Orlistat increases serum paraoxonase activity in obese patients. Nutr Metab Cardiovasc Dis. 2007;17(4):268-273.
38. Billecke S, Draganov D, Counsell R, et al. Human serum paraoxonase (PON1) isozymes Q and R hydrolyze

lactones and cyclic carbonate esters. Drug Metab Dispos. 2000;28(11):1335-1342.
39. Gouédard C, Koum-Besson N, Barouki R, Morel Y. Opposite regulation of the human paraoxonase-1 gene PON-1 by fenofibrate and statins. Mol Pharmacol. 2003;63(4):945-956.
40. Tomás M, Sentí M, García-Faria F, et al. Effect of simvastatin therapy on paraoxonase activity and related lipoproteins in familial hypercholesterolemic patients. Arterioscler Thromb Vasc Biol. 2000;20(9):2113-2119.
41. Ferretti G, Bacchetti T, Sahebkar A. Effect of statin therapy on paraoxonase-1 status: a systematic review and meta-analysis of 25 clinical trials. Prog Lipid Res. 2015;60:50-73.
42. Yi GH, Mo ZC, Ye YP, et al. Effects of probucol on paraoxonase 1 expression and oxidative stress in hyperlipidemic mice. Cell Biol Int. 2008;32(3):S19-S20.
43. Kim DS, Burt AA, Ranchalis JE, et al. Dietary cholesterol increases paraoxonase 1 enzyme activity. J Lipid Res. 2012;53(11):2450-2458.
44. Sutherland WHF, Robertson MC, Williamson SA, Nye ER. Plasma noncholesterol sterols in male distance runners and sedentary men. Eur J Appl Physiol Occup Physiol. 1991;63:119-123.
45. Zak A, Zeman M, Vitkova D, Hrabak P, Tvrzicka E. Beta-sitosterol in the treatment of hypercholesterolemia. Cas Lek Cesk. 1990;129(42):1320-1323.
46. Yesilbursa D, Serdar A, Saltan Y, et al. The effect of fenofibrate on serum paraoxonase activity and inflammatory markers in patients with combined hyperlipidemia. Polish Hear J (Kardiologia Pol. 2005;62(6):530.
47. Macan M, Vrkić N, Lucić Vrdoljak A, Radić B, Bradamante V. Effects of high sucrose diet, gemfibrozil, and their combination on plasma paraoxonase 1 activity and lipid levels in rats. Acta Biochim Pol. 2010;57(3):321-326.
48. Durrington PN, Mackness MI, Bhatnagar D, et al. Effects of two different fibric acid derivatives on lipoproteins, cholesteryl ester transfer, fibrinogen, plasminogen activator inhibitor and paraoxonase activity in type IIb hyperlipoproteinaemia. Atherosclerosis. 1998;138(1):217-225.
49. Tang WH, Villines T, Hazen S, et al. Effect of niacin and ezetimibe on serum paraoxonase and arylesterase activities of HDL cholesterol. J Am Coll Cardiol. 2012;59(13S):E1497-E1497.
50. James WPT, Astrup A, Finer N, et al. Effect of sibutramine on weight maintenance after weight loss: a randomised trial. Lancet. 2000;356(9248):2119-2125.

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Olgu Sunumu
Case Report

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Persistent Genital Arousal Syndrome; A Case Report

Persistan Genital Uyarılma Sendromu; Bir Olgu Sunumu

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ABSTRACT

The purpose of this case report is to present a case involving the etiology, diagnosis, and treatment of Persistent Genital Arousal Disorder (PGAD). The described patient meets the criteria for PGAD. In this case, organic lesions in the nervous and urogenital systems were excluded, and a psychogenic background of the syndrome was assumed. Due to the patient's refusal of pharmacological treatment, psychoeducation regarding PGAD and sexuality was provided within the framework of cognitive-behavioral therapy, resulting in a moderate effect.

Keywords: Cognitive behavioral therapy, Persistent genital arousal disorder, Sexual dysfunction

ÖZET

Bu olgu sunumunun amacı, Kalıcı Genital Uyarılma Bozukluğu'nun (PGAD) etiyolojisi, tanısı ve tedavisini içeren bir vakayı sunmaktır. Tanımlanan hasta PGAD kriterlerini karşılamaktadır. Bu vakada, sinir ve ürogenital sistemlerdeki organik lezyonlar dışlanmış ve sendromun psikojenik bir temele dayandığı varsayılmıştır. Farmakolojik tedaviyi reddetmesi nedeniyle, hastaya bilişsel davranışçı terapi çerçevesinde PGAD ve cinsellik konusunda psikoeğitim verilmiş ve orta düzeyde bir etki elde edilmiştir.

Anahtar Kelimeler: Bilişsel davranışçı terapi, Cinsel işlev bozukluğu, Kalıcı genital uyarılma bozukluğu

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INTRODUCTION

Persistent Genital Arousal Disorder (PGAD) is a diagnosis that has gained increased awareness in recent years and is gradually being introduced into the literature. It has not yet been included in the ICD-10 or DSM-5 classifications. Since it was first defined by Leiblum and Nathan in 2001, numerous case reports describing individual cases or small groups of patients with this disorder have been published.[1] In 2019, the International Society for the Study of Women's Sexual Health (ISSWSH) published the first expert consensus on the diagnosis and treatment of PGAD.[2] These criteria include:

1. Unwanted or intrusive genital arousal sensations that persist for at least 3 months or longer, which may be disturbing,
2. It may also include other types of genito-pelvic dysesthesia, such as buzzing, tingling, burning, throbbing, itching, and pain,
3. Additionally, frequent occurrences in the clitoris and other genito-pelvic areas such as mons pubis, vulva, vestibule, vagina, urethra, perineal area, bladder, and/or rectum,
4. It may involve being on the brink of orgasm, experiencing uncontrollable orgasms, and/or an excessive number of orgasms,
5. Not associated with accompanying sexual desire, thoughts, or fantasies.

Patients with PGAD commonly use terms like itching, tightening, pain, or swelling to describe their symptoms. These sensations can last for hours or throughout the day, causing extreme discomfort for the patient. Due to feelings of shame and embarrassment, patients tend to conceal their symptoms. Another challenging factor for patients is the lack of experience among many physicians regarding these symptoms and the absence of a clear consensus on treatment.

The pathophysiology and etiology of PGAD are not clearly understood. Central neurological changes (e.g., post-injury, specific brain lesion/anomaly), peripheral neurological changes (e.g., pelvic nerve hypersensitivity or compression), vascular changes (e.g., pelvic obstruction), mechanical pressure on genital structures, medication-related changes (such as the use or discontinuation of antidepressants and other mood stabilizers), psychological changes (stress), onset of menopause, physical inactivity, and overactive bladder are implicated in the etiology.[1]

The frequency of the disorder has not been determined to date. Until recently, this disorder was only identified in women; however, a few cases of PGAD have also been reported in men.[3] Symptoms frequently occur in women aged 25-51 and men aged 38-74.[4]

In more than 60% of PGAD cases, an overactive bladder syndrome has been found, strongly associated with Restless Leg Syndrome (RLS).[1] The relationship between PGAD and RLS is uncertain. RLS is believed to arise from iron deficiency in the central nervous system, dysfunction of the nigrostriatal system, and imbalances in dopaminergic and glutamatergic neurotransmission involving opioids and hypocretin.[5] The neuropathogenesis of RGS proposed by Waldinger is presented in Figure 1.[6] In this case report, a female patient diagnosed with Persistent Genital Arousal Syndrome is being discussed in line with the literature.

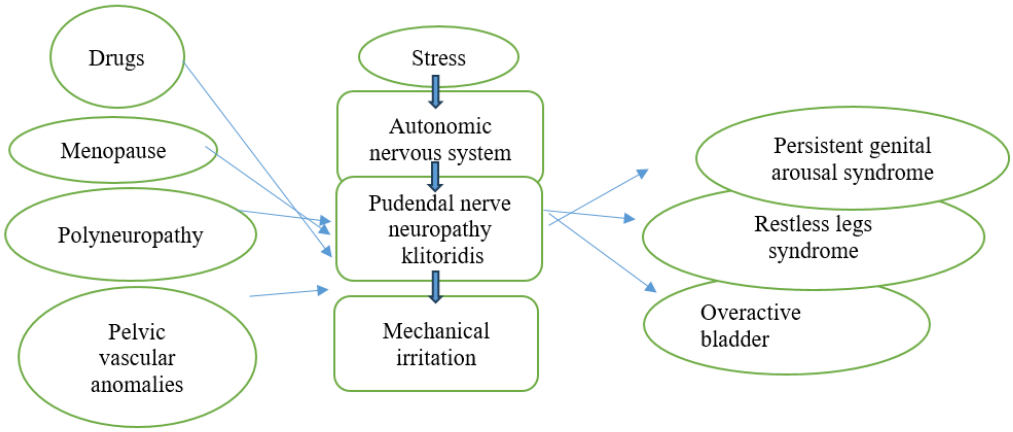

Figure 1. Diagram of the hypothetical neuropathogenesis of restless genital syndrome

CASE REPORT

A 24-year-old single woman applied to the hospital. She complained of experiencing spontaneous orgasms during daily activities lasting less than 20 minutes, occurring 4-5 times a day, without feeling sexual desire for the past 2 years. The patient mentioned feeling pain in her right groin, followed by a pulsating sensation in her vagina, along with vaginal discharge. She stated that due to the need to shower after each orgasm experience during the day, she had become unable to leave home, experiencing significant social and academic difficulties. There were no organic disorders in the patient's medical history, and she did not have a history of chronic illness, continuous medication use, alcohol, or substance abuse.

Due to her symptoms, she first consulted a gynecologist. During the urogynecological examination, it was observed that her external genital structure was in line with sex norms, her hormone levels were normal, and there were no abnormalities in the thyroid function test or biochemistry values. Her neurological examination was normal. No pathology was detected in the computerized brain tomography and Electroencephalography (EEG).

In the mental status examination, her overall appearance and physical development were in line with her socioeconomic status. It was determined that not feeling sexual desire and still being able to have an orgasm caused psychological tension and dissatisfaction.

The psychological evaluation, based on the Minnesota Multiphasic Personality Inventory (MMPI), indicated that her responses were defensive, with no clinical pathology detected.

During the period of sexual development, the patient re membered that she had not received any information about menstrual bleeding from her elders or peers before, and did not react when it first occurred. The patient stated that she had never been in a flirtatious situation, engaged in sexual activity, or masturbation. She recalled an incident at the age of 9 when she played a sexually themed game with her 19-year-old cousin's son, during which he exposed his genitalia to her. When she attempted to touch him, she felt extreme anxiety and ran away upon hearing her sister calling them from another room. The patient mentioned missing school for a month after the incident due to daily abdominal pain, and she had never shared this incident with anyone.

A followup interview was carried out with the patient a bout her illness. Showing limited knowledge about sexuality, she received 2 sessions of psychoeducation. Paroxetine 10 mg (often preferred for treating anxiety and depression, regulating serotonin levels, managing sexual desire, and controlling involuntary arousal with sexual side effects) was prescribed to manage her anxiety symptoms, such as fear, restlessness, irritability, palpitations, sweating, muscle tension, and abdominal discomfort due to genital stimulation at unwanted times. A follow-up appointment was scheduled for 15 days later.

During followup interviews, the patient expressed that she did not want to use the recommended medical trea tment and confirmed that she did not use it. Following psychoeducation, the patient reported a decrease in the frequency of pain in her right groin and expressed relief, feeling that there was no reason to blame herself for her complaint. The patient was advised to seek further help if symptoms increased or if she experienced additional complaints. Written informed consent was obtained from the patient who participated in this case.

RESULTS

Persistent Genital Arousal Disorder (PGAD) is a highly distressing and disgraceful condition for patients. Since its first report in 2001, clinical treatment strategies for PGAD have significantly expanded, providing a rational basis for managing this condition in many patients.[7] However, it should be emphasized that the care process for PGAD/PGD is somewhat limited to expert opinion due to a lack of awareness about the condition and its impact, insufficient research, the absence of large-scale studies on effective treatments, and inadequate research support. The numerous and varied etiologies also hinder the development of a single treatment strategy, necessitating a personalized, biopsychosocial approach. Increasing awareness of this condition, combined with expanding clinical experience and efforts to improve patient outcomes, may lead to affected individuals achieving a better quality of life.

Benzodiazepines such as clonazepam and oxazepam are often prescribed to alleviate PGAD symptoms. The medication's mechanism of action is likely based on the 'gating' phenomenon of the Central Nervous System.[6] Tramadol, a medication that activates μ-opioid receptors and has a limited effect on serotonin and noradrenaline reuptake, has also been documented as beneficial for some patients.[1] The tricyclic antidepressant amitriptyline has proven effective in treating patients with depressive or compulsive disorders. While researchers have explored the potential

use of dopamine receptor agonists (such as pramipexole) as a treatment for Restless Leg Syndrome (RLS) and Restless Genital Syndrome (RGS), the results have been inconsistent. The effectiveness of Botulinum toxin injections in treating pudendal neuropathy has also been demonstrated.[8]

Family therapy and couples therapy may benefit patients experiencing long-term stress due to family conflicts.[9]

In our case, the patient's psychological symptoms of stress related to preparing for a central exam, along with relief from non-pharmacological methods after receiving psychoeducation, suggest that the complaints could be psychologically driven. Some PGAD cases have shown that psychotherapy, particularly cognitive-behavioral therapy used in the treatment of genital pain syndrome, may be effective.

CONCLUSION

As there is currently no standard treatment algorithm for PGAD, a thorough psychiatric and physical medical history, along with comprehensive examinations and accompanying diagnoses, is essential in guiding treatment selection. In our case, psychotherapy was effective in reducing tension and symptoms, supporting the psychosomatic nature of the disorder. However, the etiology and treatment options for PGAD remain under-researched. Detailed assessment for PGAD symptoms is crucial, and structured research is necessary to establish effective long-term treatment approaches for this condition.

Authorship contribution statement

Consept and desing: ESO and AEK.

Acquisition of data: ESO and AEK.

Analysis and interpretation of data: ESD and AEK.

Drafting of the manuscript: ESO.

Critical revision of the manuscript for important intellectual content: ESO and AEK.

Declaration of competing interest

None of the authors have potential conflicts of interest to be disclosed.

Ethical approval

No ethical approval was required for this study. Informed consent was obtained from the patient who participated in this case.

Availability of data and materials

All data generated or analyzed during this study are included in this published case.

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REFERENCES

1. Waldinger MD, Schweitzer DH. Persistent genital arousal disorder in 18 Dutch women: Part II. A syndrome clustered with restless legs and overactive bladder. J Sex Med. 2009;6(2):482-497. doi:10.1111/j.1743-6109.2008.01114.x
2. Goldstein I, Komisaruk BR, Pukall CF, et al. International Society for the Study of Women's Sexual Health (ISSWSH) review of epidemiology and pathophysiology, and a consensus nomenclature and process of care for the management of persistent genital arousal disorder/genito-pelvic dysesthesia (PGAD/GPD). J Sex Med. 2021;18(4):665-697. doi:10.1016/j.jsxm.2021.01.172
3. Kruger TH, Hartmann U. A case of comorbid persistent genital arousal disorder and premature ejaculation: Killing two birds with one stone. J Sex Marital Ther. 2016;42(1):1-3. doi:10.1080/0092623X.2015.1053022
4. Pink L, Rancourt V, Gordon A. Persistent genital arousal in women with pelvic and genital pain. J Obstet Gynaecol Can. 2014;36(4):324-330. doi:10.1016/S1701-2163(15)30608-3
5. Allen RP, Barker PB, Horská A, Earley CJ. Thalamic glutamate/glutamine in restless legs syndrome: increased and related to disturbed sleep. Neurology. 2013;80(22):2028-2034. doi:10.1212/WNL.0b013e318294b3f6
6. Waldinger MD, Venema PL, van Gils AP, Schweitzer DH. New insights into restless genital syndrome: static mechanical hyperesthesia and neuropathy of the nervus dorsalis clitoridis. J Sex Med. 2009;6(10):2778-2787. doi:10.1111/j.1743-6109.2009.01435.x
7. Leiblum SR, Nathan SG. Persistent sexual arousal syndrome: a newly discovered pattern of female sexuality. J Sex Marital Ther. 2001;27(4):365-380. doi:10.1080/009262301317081115
8. Nazik H, Api M, Aytan H, Narin R. A new medical treatment with botulinum toxin in persistent genital arousal disorder: successful treatment of two cases. J Sex Marital Ther. 2014;40(3):170-174. doi:10.1080/0092623X.2013.788109
9. Lofrisco BM. Female sexual pain disorders and cognitive behavioral therapy. J Sex Res. 2011;48(6):573-579. doi:10.1080/00224499.2010.540682

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Antik Çağlarda Bir Kraniyotomi Uygulama Yöntemi: Trepanasyon

A Method of Performing Craniotomy in Ancient Times: Trepanation

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ÖZET

Trepanasyon veya baş delgi operasyonu, tarih boyunca kullanılan ve kraniumun cerrahi olarak delinmesini içeren eski bir tıbbi prosedürdür. MÖ 6500 yıllarına kadar dünyanın çeşitli bölgelerinde, özellikle Güney Amerika, Avrupa ve Afrika'da yaygın olarak kullanılan bu yöntem genellikle hastaların kafatasında delikler açarak beyin üzerindeki baskıyı azaltmayı hedeflemiştir. Bugün, modern tıpta yerini daha gelişmiş cerrahi tekniklere bırakan bu tedavi yöntemi sonucunda arkeolojik kalıntılardan elde edilen yorumlara dayanarak hastaların yaşamına devam ettiğini gösteren kanıtlar mevcuttur. Bazı arkeolojik incelemelerde, daha önce trepanasyon cerrahisinin uygulanmadığı düşünülen coğrafi bölgelerden ve dönemlerden olası trepanasyon örnekleri keşfedilmeye devam edilmektedir. Bu çalışmanın amacı, trepanasyonun tarihsel uygulama yöntemleri ve arkeolojik buluntuların sistematik bir özetini sunmaktır. Bu amaçla, geçmişten günümüze trepanasyon operasyonlarına ilişkin bilgiler Pubmed, Google Akademik gibi çeşitli veri tabanlarından sistematik olarak derlenmiştir. Gerek bulunduğu coğrafi bölgeye gerek tespit edilen tarihi döneme ait böyle operasyonların yapıldığına dair kanıt bulunamayan arkeolojik çalışmalarda, elde edilen kafatasındaki olası bir trepanasyonu değerlendirirken, kapsamlı bir ayırıcı tanı teşhisi kolaylaştırabilmektedir.

Anahtar Kelimeler: Kranium, Tedavi, Trepanasyon

ABSTRACT

Trepanation or head puncture operation, is an ancient medical procedure that has been used throughout history and involves the surgical puncture of the cranium. This method, which was widely used in various parts of the world, especially in South America, Europe and Africa, until 6500 BC, generally aimed to reduce the pressure on the brain by opening holes in the skull of patients. Today, as a result of this treatment method, which has been replaced by more advanced surgical techniques in modern medicine, there is evidence that patients continue their lives based on the interpretations obtained from archaeological remains. In some archaeological investigations, possible examples of trepanation continue to be discovered from geographical regions and periods previously thought to be incurred for trepanation surgery. The aim of this study is to present a systematic summary of the historical application methods of trepanation and archaeological finds. For this purpose, information regarding trepanation procedures from the past to the present has been systematically collected from various databases such as PubMed and Google Scholar. In archaeological studies where there is no evidence that such operations were performed both in the geographical region and in the historical period identified, a comprehensive differential diagnosis can facilitate the diagnosis when evaluating a possible trepanation in the skull obtained.

Keywords: Cranium, Treatment, Trepanation

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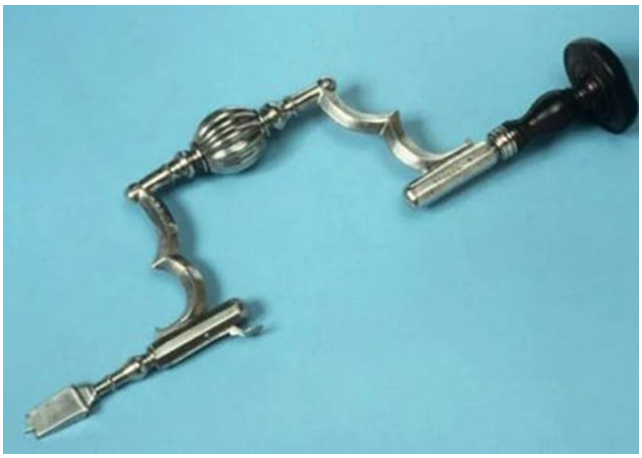
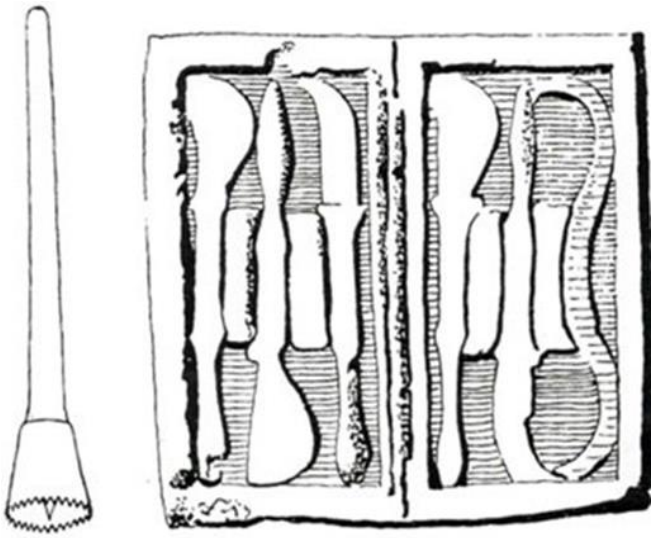
GİRİŞ

Trepanasyon, beyne ulaşmak amacıyla kranium üzerine bir veya birden fazla delik açmak için delme, kazıma veya testereyle kesme işleminden oluşan primitif bir nöroşirürji prosedürüdür. Trepanasyon işleminde kafatası altında bulunan yapıları açığa çıkarmak için kranium üzerinden bir parça kemiğin beyin ile dura mater encephali'ye zarar vermeden çıkarılması gereklidir.[1,2] Yunanca "delici" anlamına gelen terebra ve trepanon terimlerinden üretilmiştir ve zamanla bu terim trepanasyon olarak kullanılmaya başlamıştır. 16.yy'da kranium üzerine açılacak delik için kullanılan Fransız aleti tres fines olarak isimlendirilmiştir (Şekil 1) ve bu Latince'de "üç uç" anlamına gelen bu terim trepanasyon'un oluşumuna katkıda bulunmuştur.[3] Beyinde yaralanma, kanama ve enfeksiyon gibi çeşitli riskler barındırmasına rağmen, arkeologlar Avrupa, Afrika, Balkanlar ve Güney Amerika'daki antik uygarlıklarda trepanasyonun yaygın ölçüde uygulandığına dair kanıtlar bulmuşmuş olup Romalılar, Yunanlılar, Mısırlılar ve İnkalar gibi antik kültürlere ait olduğu düşünülen ve Neolitik döneme ait binlerce trepanasyon yapılmış kraniumu ortaya çıkarmış ve incelemişlerdir.[2,4] Bu derlemenin amacı, trepanasyonun tarihsel gelişimi ve yöntemleri üzerine edinilen bilgiler ile trepanasyon vakaları olarak yanlış teşhis edilebilecek olası durumların sistematik bir özetini sunmaktır.



Şekil 1. 18. yy'a ait trepanasyon aleti[5]

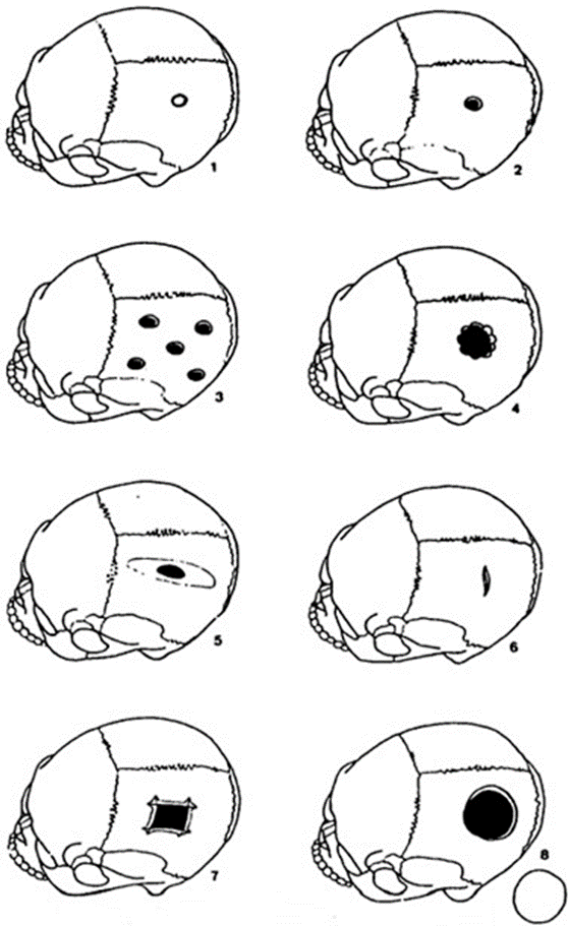
Trepanasyon uygulanan kişilerde norma superior üzerinde kemik dokularda izlenen iyileşme belirtileri, hastaların ameliyattan sonra hayatta kaldıklarını düşündürmektedir. Antik çağlarda trepanasyonun uygulanmasının amaçları kültürden kültüre farklılık göstermekle birlikte sıklıkla terapötik ve dini inanışlar gereği kötü ruhlardan arınma ayini yer almaktadır.



Şekil 2. Antik Yunan'da cerrahların kullandığı farklı trepanasyon aletleri[10]

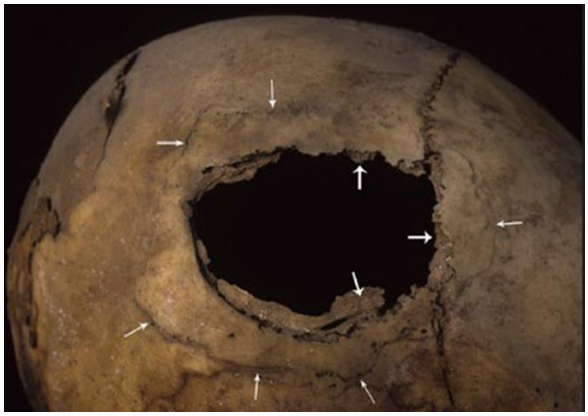
Terapötik nedenlerden doğumsal ve gelişimsel anomalliler, kafa travmaları, enfeksiyon ve tümöral dokuların varlığı üzerine tedavi amaçlı uygulandığı söylenmektedir.[2,6,7] Edinilen bulgulara göre travmatik lezyonların genellikle sağ elini kullanan bir rakip tarafından verildiği düşünülerek birçok ameliyat kranium sol yarısında yapılmış olması dikkat çekmektedir. Antik Çin döneminden kalan tıbbi ve tarihi eserlerde, ünlü hekim Hua Tuo gibi cerrahların çalışmaları ve beyin patolojilerinin tedavisi için kullanılan cerrahi prosedürler de dahil olmak üzere trepanasyon uygulamasına ilişkin kanıtlar yer almaktadır.[6] Trepanasyon, farklı çalışma aletleri (Şekil 2) ve tekniklerle yapılmakla birlikte kullanımı en yaygın olduğu düşünülen yöntemler sıralanmıştır: (i) Scalp katmanları ve diploe'yi nazikce inceltme işlemiyle iç katmanda yer alan beyin zarlarının diseksiyonu ile beyne ulaşılan kazıma yöntemi, (ii) Kafa derisi ile altta yer alan yumuşak doku diseke edildikten sonra kranium üzerinde özel aletlerle delik açarak ortada kalan parçanın kesilerek çıkarılması yöntemi, (iii) Kesişen doğrusal çizgilerden oluşan bir dikdörtgen şekilli kemik bölgenin çıkarılması yöntemi, (iv) Küçük dairesel oluklardan bir oval sınır oluşturarak çoklu delme işlemiyle bir büyük kemik parçanın çıkarılması yönteminden oluştuğu bilinmektedir (Şekil 3).[2,8] Diğer yöntemlerden biri olarak küçük delik grupları ile oluşturulan "burr deliği" yer almaktadır.[8] Kazıma, tarih öncesi kafataslarında en yaygın görülen yöntemdir ancak doğrusal kesme, delme ve kesme ve matkapla delme daha nadir uygulamalardır.

Paleopatolojik ve arkeolojik çalışmalarda yeni vakalar sunulmakta olup, özellikle daha önce bu tür uygulamalara dair kanıt olmadığı düşünülen coğrafi bölgelerde uygulandığı tespit edilen trepanasyon çalışmaları ve olgular dikkat çekmektedir.[9]



Şekil 3. Çeşitli trepanasyon teknikleri[2]

Kenarlarında kemik reaksiyonu gösteren trepanasyonlar, ameliyatın yaşayan bir hastada yapıldığını belirtmektedir. Kemik reaksiyonu belirtisi olmayan açıklıklar için hastanın ameliyat sırasında yada ameliyattan kısa bir süre sonra yaşamını yitirdiğini veya alternatif olarak açıklığın hastanın ölümünden sonra teşhis ve çalışmalar adına yapılmış olduğu varsayılmaktadır. Kemik dokuda iyileşme belirtileri, hayat kalım süresini tahmin etmede önemli bir kanıttır. Erken iyileşme belirtilerinden kemik doku etrafında hiperemi ve osteoklastik aktivite varlığı, trepanasyon sonrası sağkalım süresi hakkında bilgi sunmaktadır.[4] İncelenen olgularda sıklıkla trepanasyon etrafında, periosteum'un çıkarılmasıyla oluşan kanamalara bağlı nekrotik kemik dokulara rastlanılmaktadır. Nekrotik alanın dış kenarları osteoklastik çukurlaşmayı belirtmek üzere "sınırlama hattı" olarak tanımlanan bir yöntemle işaretlenmiştir (Şekil 4).[8,9,11] Küçük delme işlemlerinden oluşan burr deliği açıklıkların kapanması oldukça nadir olmakla birlikte diğer yöntemlerden oluşan trepanasyon açıklığının tamamen kapandığı olguların varlığı üzerine kanıtlar bulunmamaktadır.[7,8]

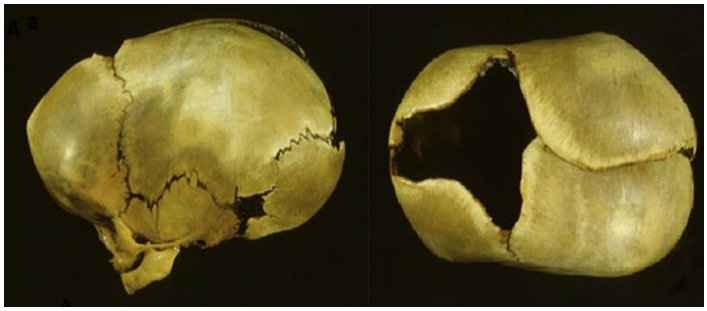


Şekil 4. Sağ os parietale trepanasyonu yapılan kraniumda ortadaki büyük oklar açıklığın kenarlarındaki osteoklastik aktiviteyi ve alttaki ok trepanasyondan kalan kazıntı izlerinin bir kısmını belirtmektedir. Etraftaki daha küçük oklar nekrotik kemiğin dış kenarlarında "sınırlama hattı"nı göstermektedir.[8]

Trepanasyon uygulama yöntemleri çeşitli olgularda ve ayırıcı tanıda farklılık arz ettiğine dair kalıntılar ile açıklığın yapılış yöntemleri de değişiklik göstermektedir.[12] Bu nedenle kalıntılarda trepanasyon uygulaması ve tekniğinin doğru teşhisini yapabilmenin önemi adına, uygulamanın ayırt edici özellikleri ile vakalarda karşılaşılabilecek diğer deformasyonlar aşağıda özetlenmeye çalışılmıştır.

1.1. Doğumsal ve Gelişimsel Anomaliler

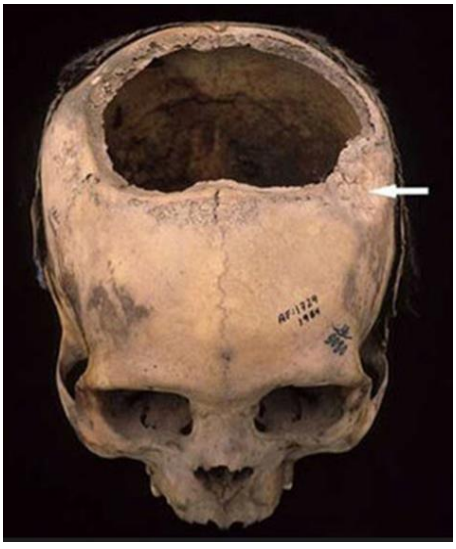
Bebek ve çocukluk çağında kraniumda var olan fontanellerin trepanasyon açıklığı ile karıştırılması pek mümkün olmamakla birlikte bunlar alışıklığın dışında büyükse veya beklenenden daha uzun süre açık kaldığı düşünüldüğünde karışıklık yaşanabilir. Bu dönemde görülen kranium anomalileri büyüme ve gelişme sırasında normal kemikleşmenin meydana gelmemesinden oluşabilir. Cleidocranial dysostosis yada Hidrosefali olgularında olduğu gibi, suturalarda birleşmenin kısmi ya da tam olmadığı durumlarda meydana gelen gelişim anomalileri buna örnek olabilir (Şekil 5). Bu tür anomaliler için yaygın bir konum, sutura sagittalis ve sutura coronalis'in birleştiği yerde, bregma'nın hemen posteriorunda veya sutura sagittalis boyunca uzanır. Stewart (1975), trepanasyon olarak yanlış teşhis edilen tarihsel kalıntı olduğu bilinen kraniumda meningosel vakasını bildirmiştir.[8,13]



Şekil 5. Norma lateralis ve norma superior'dan görünen fontanel açıklıkları[8]

1.2. Kafa Travmaları

Travma ile kraniumda oluşan depresyon kırıkları iyileşmiş bir trepanasyon defekti olarak teşhis edilebilmektedir. Bunun sebebi genellikle trepanasyon defektlerinin yeni kemik oluşumu ile açıklığın kapanarak iyileştiğine dair yanlış inanıştır. Kraniumda oluşan parçalı kırıklarda, izole parçaların kan akımı yok olur, bu parçalar yara tarafından emilimi olmayacak büyüklükte ise parçaların çıkarılarak bölgenin temizlenmesi söz konusudur. Birey hayatta kalırsa, travma alanında farklı büyüklükte ve şekillerde defektler oluşumu gözlenebilir. Bu durumda sağkalımı gerçekleşen vakalarda, kırık tedavisi için herhangi bir trepanasyon müdahalesinde bulunulduğunu belirlemek güçtür.[14] Savaşlar esnasında kafatasına alınan darbeler nedeniyle oluşan açıklıklar da yanlışlıkla trepanasyon olarak değerlendirilebilmektedir. Bu tür vakalarda döneme ait boyutları ve şekilleri, mızrak uçları veya yıldız başlı topuzlar gibi silahlarla eşleştirilerek, cerrahi müdahalenin varlığı ya da yokluğu belirlenebilmektedir (Şekil 6).[8,15]



Şekil 6. Peru'da MÖ 400-200 yılları arasında geçirilen kafa travması (beyaz ok) sonrası uygulanan trepanasyon operasyonu[16]

1.3. Enfeksiyon

Kraniumda os parietale üzerinde gelişmeye müsait Streptococcus pyogenes, Corynebacterium pyogenes ve Pseudomonas aeruginosa gibi piyojenik irin yapıcı bakterilerin üremesi, tüberküloz veya osteomyelit gibi kemik iltihabına neden olan vakalarda oluşan defektlerin varlığı trepanasyon olarak yanlış teşhis edilebilecek açıklıklar oluşturabilir.[17,18] Ölüm esnasında enfeksiyon aktifse, bu defektlerin sınırları gözenekli ve düzensizdir, ancak iyileşme durumunda yeniden şekillenerek iyileşmiş trepanasyon olarak değerlendirilebilir. Trepanasyon yapılan bir olguda osteomyelit de eşlik edebilir. Travma kraniumun belirli bir bölgesinde oluşan kırık veya kafa derisinde oluşan açık yarayı takiben meydana gelen enfeksiyon, trepanasyona zemin hazırlayabilir ya da enfeksiyon başlı başına ameliyatın bir komplikasyonu olabilir. Kazılarda edinilen olgularda enflamasyona bağlı olduğu düşünülen birçok trepanasyon örnekleri mevcuttur ancak defektin enfeksiyon ya da trepanasyon kökenli olup olmadığı net değildir.[7]

1.4. Neoplasm

Neoplasm, tek bir hücrenin normal formundan sıyrılarak normalin dışında çoğalarak oluşturduğu hücre kümesi olarak bilinir ve tümör olarak adlandırılır. Metastatik karsinom ve multipl myelom gibi malign neoplasmatik hücre formasyonu nörokranyumda yıkıcı lezyonlar üretebilir. Bunlar, vücudun diğer bölgelerinden metastaz yaparak hızla gelişen tümör hücre grubu oluşturarak yıkıcı lezyonları kısa vadede gösteren neoplazm grubudur. Karekteristik olarak düzensiz, "delinmiş" kenarlar ve çok az veya hiç yeni kemik oluşumu göstermeyen defekt görünümü yaratırlar.[18] Malign neoplazmların bir ürünü olmaları nedeniyle bu vakalarda iyileşme beklenemez ve bu durumdaki açıklıkların trepanasyon olarak yanlış değerlendirilme olasılığını daha düşük kılar.[8]

1.5. Postmortem Tafonomik Hasarlar

Tafonomi, Efremov'un 1940 yılında tanımladığı gibi, canlıların gömülme süreçlerini araştıran bir bilim dalı olup bu terim Yunanca "taphos" (gömülme) ve "nomos" (kanunlar) kelimelerinin birleşiminden türetilmiştir. Paleontoloji biliminin bir alt dalı olarak bilinen tafonomi, organizmanın postmorem süreçte vücudunda değişime uğrayan alanların nedenleri üzerinde çalışmaktadır.[19] Postmortem süreçte çeşitli etmenlere maruz kalma sonucu kraniumda erozyon ve aşınma nedenli kırılma, hava koşullarına maruz kalma,

kemirgen veya yırtıcı hayvanlar tarafından verilen zarar gibi çeşitli nedenlerle oluşabilir. Kırılma veya erozyon genellikle kemikleşme reaksiyonu görülememesi ve kırılan bölgenin sınırlarında renk farklılığı sebebiyle ayırt edilebilir. Ancak, güneş ışınlarına maruz kalma nedeniyle kemik yüzeyde beyazlama ve rüzgarla savrulan kumun aşındırması, bazen trepanasyon defektlerini taklit edecek düzeyde kemik dokuda hasarlar oluşturabilir.[8,20,21]

1.6. Suprainion Lezyonlar

Inion (protuberentia occipitalis eksterna) üzerindeki lezyonlar, ilk olarak Perulu beyin cerrahı Fernando Cabieses tarafından bebeklerde yapılan olağandışı bir profilaktik trepanasyon şekli olarak bilinmektedir. Suprainion lezyonları, squama occipitalis'de incelme ve aralıklı kemik perforasyonu gösteren sığ çöküntülerdir. Cabieses ve Weiss, bunları os occipitale'de kazıma yöntemiyle trepanasyon uygulaması olarak sınıflandirmıştır ancak T. Dale Stewart ve diğer bazı araştırmacılar, bu lezyonların bebeklerin beşik tahtalara sırtüstü yatırılmasına veya ilkel kafa şekillendirme aparatlarının kullanımına oluşabileceğini ileri sürmüşlerdir. Stewart ve Diane Holliday gibi araştırmacılar, lezyonlarda kesik veya kazıma izlerine rastlayamamaları nedeniyle bu vakaları trepanasyon olarak değerlendirmemişlerdir. Suprainion lezyonlar üzerine tartışmalar sürmektedir.[8]

SONUÇ

Trepanasyon, tıp tarihinin en ilkel cerrahi prosedürlerinden biri olarak, sağlık ve hastalıkla mücadelenin en belirgin kanıtlarından biri olduğu bilinmektedir. Antik çağlardan itibaren birçok farklı kültürde uygulanan bu prosedür, dönemin tıbbi bilgisi ve teknolojisinin kısıtlığına rağmen dikkat çekici ölçüde başarılı olduğu düşünülmektedir. Genellikle kafatası travmaları, beyin üzerindeki baskıyı azaltma ve ruhsal hastalıkları tedavi etme amacıyla kullanılan trepanasyon, arkeolojik bulgulara göre başarılı bir şekilde uygulanmış ve edinilen bulgular ışığında hastaların bu işlemden sonra yaşamaya devam ettiğine dair kanıtlara rastlanılmıştır. Günümüz modern tıpta daha teknolojik cerrahi yöntemler yer alsada, trepanasyonun incelenmesi tıp biliminin gelişimini kavramak açısından önemlidir. Bu ilkel prosedür, sadece tıbbi bir uygulama olarak değil, aynı zamanda halk sağlığını koruma adına verilen mücadelenin bir sembolü olarak görülmektedir. Trepanasyonun, tıp tarihinin gelişiminde önemli bir perspektif olduğu düşünülür ve geçmişten günümüze cerrahi tekniklere bulunduğu katkıları anlamanın önemi büyüktür. Yapılan araştırmalarda trepanasyon müdahalesi olduğu düşünülen vakalarda eşlik edebilecek olası klinik durumlar ve tafonomik deformasyonlar nedeniyle teşhis edilmesi zor olabilir. İyileşmiş defektlerde cerrahi müdahale izlerin yok olması ya da iyileşmemiş deliklerin premortem veya postmortem olup olmadığının belirlenmesi gibi zorluklar söz konusudur. Trepanasyon çeşitli bölgelerde keşfedilmesi durumlarında bölgenin tarihte bilinmeyen izole vakaları tanımlamak teşhisi zorlaştırabilmektedir. Literatürde de belirtildiği gibi, aynı coğrafi alanda trepanasyonun doğru belirlenmesi, bilinmeyen vakaları yorumlamak açısından önemlidir ve tanıda trepanasyonların diğer defektlerden ayırt edilmesi tıp bilimi ve tarihe önemli katkılar sağlayacağı düşünülmektedir.

Yazarlık katkı beyanı

Bu çalışmanın tasarımını, veri toplamasını ve analizini Meltem Çelik gerçekleştirmiştir. Tek yazarlı bir derleme makalesi olduğu için literatür taraması, makalenin yazımı, revizyon ve son revizyonlar aynı yazar tarafından yapılmıştır.

Yazar çıkar çatışması

Yazarın açıklanması gereken potansiyel çıkar çatışması yoktur.

Veri ve materyallerin mevcudiyeti

Çalışmada kullanılan tüm makaleler, üretilen veya analiz edilen tüm veriler bu derlemede yer almaktadır.

Destek

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KAYNAKLAR

1. Hobert L, Binello E. Trepanation in Ancient China. World Neurosurg. 2017;101:451-456. doi:10.1016/j.wneu.2016.10.051
2. Özbek M. Çayönü'nde kafatası delgi operasyonu. Hacettepe Üniversitesi Edebiyat Fakültesi Dergisi. 1998; 15:109-126.
3. Ganz JC. Trepanation technique. Prog Brain Res. 2024;284:11-17. doi:10.1016/bs.pbr.2024.02.002
4. Özbek M. Geç Bizans Devrinde Trepanasyon (Kafatası Delgi Ameliyatı). TTK BELLETEN. Aralık 1988;52(205):1567-1574. doi:10.37879/ttkbelleten.971198
5. https://www.sciencephoto.com/media/637955/view
6. Tsoucalas G, Kousoulis AA, Mariolis-Sapsakos T, Sgantzos M. Trepanation Practices in Asclepieia: Systematizing a Neurosurgical Innovation. World Neurosurg. 2017;103:501-503. doi:10.1016/j.wneu.2017.04.022

7. Toyne JM. Possible cases of scalping from prehispanic highland Peru. Int. J.Osteoarchaeol. 2011;21 (2):227–242.
8. Verano JW. Reprint of-Differential diagnosis: Trepanation. Int J Paleopathol. 2017;19:111-118. doi:10.1016/j.ijpp.2017.03.004
9. Katsevman GA, Brandmeir NJ. Trepanation of the Outer Table as a Treatment for Scalping Injuries: Historical Perspective and Modern Applications. World Neurosurg. 2021;145:301-305. doi:10.1016/j.wneu.2020.09.147
10. https://educalingo.com/en/dic-en/trephine
11. Partiot C, Lepetit A, Dodré E, et al. Cranial trepanation and healing process in modern patients-Bioarchaeological and anthropological implications. J Anat. 2020;237(6):1049-1061. doi:10.1111/joa.13266
12. Bynum B, Bynum H. Trepanned cranium. Lancet. 2018;392(10142):112. doi:10.1016/S0140-6736(18)31509-5
13. Stewart TD. Cranial dysraphism mistaken for trephination. Am. J. Phys.Anthropol. 1975;42(3): 435–437.
14. Pavlovic T, Djonic D, Byard RW. Trepanation in archaic human remains - characteristic features and diagnostic difficulties. Forensic Sci Med Pathol. 2020;16(1):195-200. doi:10.1007/s12024-019- 00158-7
15. Andrushko VA, Verano JW. Prehistoric trepanation in the Cuzco region of Peru: a view into an ancient Andean practice. Am Phys Anthropol. 2008;137(1):4-13. doi:10.1002/ajpa.20836
16. Verano JW, Asencios BI, Kushner D, et al. Holes in the Head: The Art and Archaeology of Trepanation in Ancient Peru. Studies in Pre-Columbian Art and Archaeology. 2016; *38*:126.
17. Kaufman MH, Witaker D, McTavish J. Differential diagnosis of holes inthe calvarium: application of modern clinical data to palaeopathology. J.Archaeolog. Sci. 1997;24:193–218.
18. Ortner DJ. Identification of Pathological Conditions in Human SkeletalRemains, 2nd ed. Academic Press, Amsterdam 2003; Boston.
19. Gümrükçü M. Tafonominin tarihçesi ve tafonomik araştırmaların paleoantropolojideki önemi. Antropoloji. 2018;35:83-92. doi:10.1501/antro_0000000355
20. Dinçarslan İ. Adli Antropolojide Tafonomi. Turkiye Klinikleri J Foren Med-Special Topics. 2017;3(1):81-7.
21. Wilson-Taylor RJ, Dautartas AM. Time since death estimation and bone weathering, the post mortem interval. Forensic anthropology: a. 2017.

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Basic Principles in the Application of Problem-Based Learning in Medical Biochemistry Education

Tıbbi Biyokimya Eğitiminde Probleme Dayalı Öğrenim Uygulamasında Temel Esaslar

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ABSTRACT

Although the methods of applied education vary, it is well-known that medical faculties are among the faculties with the most intensive curriculum across all universities. In the medical education system applied throughout our country, the aim is to build a strong medical foundation by starting with an intensive theoretical curriculum, with basic science courses predominantly taught during the first three years. Following this, the objective is to provide clinical training through practical rotations in clinics, building on the acquired foundational knowledge. However, some challenges are encountered in the traditional lecture-based approach, particularly in delivering theoretical courses during the first three terms. As an alternative to the traditional teaching method, the problem-based learning (PBL) model, which was first introduced in the 1960s at McMaster University Faculty of Medicine in Canada, has been developed. Today, this model is widely used in medical education globally, either as a standalone method or in combination with traditional approaches. Medical biochemistry, which evolved in the 19th and 20th centuries and has become a significant field that bridges basic and clinical medical knowledge in the 21st century, has a detailed and comprehensive curriculum. Given its importance in medical education, as well as its broad and detailed scope, different teaching methods are required for the effective and long-lasting retention of medical biochemistry knowledge. The problem-based learning method, which promotes interactive learning, encourages students to research and access new information, and motivates them to work collaboratively in a student-centered, brainstorming-based group setting, offers a strong alternative to classical lecture-based teaching in medical biochemistry. Research has also highlighted the numerous benefits of using problem-based learning in medical biochemistry education. Incorporating problem-based learning into the curriculum of faculties offering medical biochemistry education could enhance the quality of education. In this study, it is aimed to discuss the effects of problem-based learning method on medical biochemistry education with the current literature.

Keywords: Education, Medical biochemistry, Medical faculty, Problem-based learning

ÖZET

Uygulanan eğitim metotları değişkenlik gösterse de bütün üniversitelerde en yoğun müfredatın verildiği fakülteler arasında tıp fakültelerinin ilk sıralarda yerini aldığı bilinmektedir. Ülkemiz genelindeki tıp fakültelerinde uygulanan eğitimde de yoğun teorik müfredatla başlanarak, ilk 3 yıl boyunca temel bilimler derslerinin ağırlıklı olarak verilerek tıbbi alt yapının oluşturulması amaçlanmaktadır. Ardından edinilen temel alt yapı üzerine kliniklerde rotasyona girilerek pratikler eşliğinde klinik yaklaşım verilmesi hedeflenmektedir. İlk üç dönemde verilen teorik derslerin aktarımında geleneksel ders öğretim uygulamasında bazı problemlerle karşılaşılmaktadır. Kanada'daki McMaster Üniversitesi Tıp Fakültesinde 1960'lı yıllarda ilk olarak tıbbi alanda uygulanan PDÖ modeli, geleneksel ders metoduna alternatif olarak geliştirilmiştir. Günümüzde birçok ülkenin tıp eğitiminde salt veya karma kullanımı söz konusu olacak şekilde yaygın kazanmıştır. 19 ve 20. yüzyıllarda gelişen ve 21. yüzyılda tıp biliminin temel ve klinik bilgilerini birleştiren çok önemli bir bilim haline gelen tıbbı biyokimya eğitimi detaylı ve geniş kapsamlı bir müfredata sahiptir. Hem tıp eğitimindeki önemi hem de geniş kapsamı ve detayı sebepleriyle tıbbi biyokimyanın kalıcı ve etkili öğretilmesi için farklı eğitim metotları kullanılmasına ihtiyaç bulunmaktadır. İnteraktif öğrenme imkânı veren, araştırmaya ve yeni bilgiye ulaşmaya yönlendiren, öğrenci merkezli ve beyin fırtınasına dayalı grup çalışmasına motive eden probleme dayalı öğrenim metodu tıbbi biyokimya eğitiminde klasik teorik derslere iyi bir alternatif olarak görünmektedir. Yapılan araştırmalar da probleme dayalı öğrenim metodunun tıbbi biyokimya eğitimindeki çok sayıda faydasını detaylı olarak ortaya koymuştur. Tıbbi biyokimya eğitimi veren fakültelerin müfredatlarının kapsamına probleme dayalı öğrenim metodunu almaları daha üstün bir eğitim verilmesini sağlayabilir. Bu çalışmada, probleme dayalı öğrenim metodunun tıbbi biyokimya eğitimine etkilerinin güncel literatür eşliğinde tartışılması amaçlanmıştır.

Anahtar Kelimeler: Eğitim, Probleme dayalı öğrenim, Tıbbi biyokimya, Tıp fakültesi

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INTRODUCTION

Medical education has a deep-rooted and institutionalized systematic structure that has persisted from ancient times to the present day. Although the methods of education vary, medical faculties are recognized as having some of the most intensive curriculum among all university faculties. In the medical education system throughout our country, the goal is to build a solid medical foundation by beginning with an intensive theoretical curriculum, primarily consisting of basic science courses in the first three years. After this foundation is established, clinical training is introduced through rotations in clinics, allowing students to apply their acquired knowledge in practice. However, challenges arise in the traditional lecture-based teaching method, particularly in transferring the heavy theoretical load presented during the first three terms. Due to the one-sided, instructor-centered, and monotonous nature of traditional teaching, it becomes difficult to maintain students' attention and interest in the courses. While the integrated board or committee system remains a prevalent model in medical faculties across the country, various new approaches—such as "Outcome-Based Education", "Case-Based Learning," and "Problem-Based Learning" (PBL)—are being integrated into existing systems.[1]

The sources related to the subject in the current literature were examined and the subject was explained by considering the articles that would be useful for a better expression of the subject. In this study, it is aimed to discuss the effects of problem-based learning method on medical biochemistry education with the current literature.

The rapid developments in medical sciences, driven by intensive research, have led to an exponential increase in theoretical knowledge. This situation necessitates more effective methods for knowledge transfer in medical education. Several educational models have been developed and documented in the literature to address this need, and among these, problem-based learning (PBL) stands out as one of the most commonly used approaches.

PBL is a teaching method similar to, yet distinct from, the previously developed Case-Based Learning (CBL) model. Although the two methods are sometimes used interchangeably in the literature, leading to confusion, they are indeed different. CBL was first introduced in 1870 by Professor Christopher Columbus Langdell, Dean of Harvard University Law School at the time. In contrast, PBL was first implemented at McMaster University in the late 1960s, almost a century later. Studies have explored how these methods influence one another, highlighting their similarities and differences.[2]

Problem-based learning: History and application principles

The PBL model, first implemented in the medical field in the late 1960s at McMaster University Medical School in Canada, was developed as an alternative to the traditional lecture-based approach.[3,4] Today, it has become widespread in medical education worldwide, either as a standalone method or in combination with other models. In Turkey, PBL was first introduced at Dokuz Eylül University Medical School during the 1997-1998 academic year. Since then, it has spread to other universities, such as Pamukkale University, Ondokuz Mayıs University, and Istanbul University. Currently, there are many medical faculties where PBL is at the forefront, as well as universities like Akdeniz University and Mardin Artuklu University, where PBL is blended with the integrated model in a hybrid approach.[5-7]

Although PBL can be applied in various forms, its core structure is shaped by a few common principles:[4,5]

1. Problems that align with the teaching objectives are prepared in advance, either as fictional cases or real-life patient cases.
2. 5-10 students participate in a group, supervised by an instructor.
3. Learning is student-initiated, making it an active learning model.
4. The facilitator asks guiding questions to encourage comprehensive discussion of the case.
5. Students independently research the case and perform analyses both individually and as a group.
6. A key distinction between PBL and CBL is that students are not informed about the case beforehand.

An important factor in the success of PBL is ensuring that the prepared case is aligned with the learning objectives and the students' prior knowledge. Some studies have highlighted the need for standardized case preparation guides to ensure consistency in case design.[8]

Application of problem-based learning in medical biochemistry education

As in many parts of the world, the first three terms of medical education in our country consist of an intensive

curriculum covering basic science disciplines. Following the foundational knowledge gained through basic sciences, clinical education, which includes surgical and internal medicine disciplines, is carried out practically in the final three terms through internships. Among the basic sciences, Medical Biochemistry is one of the courses with the most extensive lecture hours during the first three years. Medical Biochemistry is a rapidly evolving field that is open to new developments, with much of its theoretical content centered around metabolic cycles and processes. Because the course covers a vast array of proteins, enzymes, molecules, pathways, cycles, steps, and reactions related to metabolic processes, its content can become quite complex. This complexity can make Medical Biochemistry—a course that requires extensive memorization due to its dense and intricate content—less engaging, more challenging, and harder to learn compared to other courses from the students' perspective.[9]

When the traditional classroom method is applied, the monotonous lecture environment created by the instructor further diminishes students' interest and focus, making it harder to sustain attention and enthusiasm in the subject. Therefore, Medical Biochemistry is one of the courses where alternatives to the passive, traditional education methods commonly used in medical education can have the greatest impact and significance. Indeed, a review of the literature reveals numerous studies from various institutions where problem-based learning (PBL) methods have been applied to the Medical Biochemistry course with similar concerns, often comparing PBL to traditional teaching methods.[9-14]

In these studies, feedback from both students and faculty members, collected through surveys, shows that PBL is generally considered more engaging. Furthermore, many studies report that when comparing test results between student groups that received traditional lectures and those that experienced PBL or hybrid PBL models, the latter demonstrated higher success rates in learning.[10,12,14,15] Given the inherent complexity of Medical Biochemistry, it has been suggested that exposing students to well-designed and thoughtfully planned cases during the learning process, with a clear scope and purpose, can make learning more interesting and the course easier to understand.[10,11]

As a result of the positive feedback and improved learning outcomes achieved through the PBL method, some researchers have concluded that this method should be considered a necessity and integrated as a required component of the medical curriculum.[12]

Nearly all of these studies focus on the application of the PBL model in the theoretical aspects of Medical Biochemistry, sharing comparisons and results. However, researchers at Dokuz Eylül University Faculty of Medicine took the PBL method a step further by applying it to biochemistry laboratory practices using a "dry laboratory" model, aiming to increase the efficiency of experimental practices. This approach was adopted to address the lack of relevance of certain experiments conducted in what they referred to as the "flowing laboratory." In medical education, which aims to train medical doctors, the use of PBL in dry laboratory practices, designed with clinical relevance, was found to be much more effective compared to flowing laboratory experiments that lack direct clinical connection.[13]

As demonstrated by this application, the PBL model not only facilitates the teaching of the theoretical aspects of Medical Biochemistry but also stands out as an effective teaching method in practical settings, such as in the dry laboratory model. PBL includes group work. It is interactive. However, not every individual in the group may actively participate to the same extent. For this reason, it may not be equally instructive for every student. The aim is to learn the subject through a concrete problem. However, learning the subject may be limited to the problem. Important details of the subject may not be emphasized enough. Using the PBL method by synthesizing it with other classical and modern learning methods may be beneficial for better learning.[16,17]

CONCLUSION

Medical biochemistry, which developed in the 19th and 20th centuries and has become a critical science combining basic and clinical knowledge in the 21st century, possesses a detailed and comprehensive curriculum. Given its importance in medical education, as well as its broad and complex content, there is a need for diverse educational methods to ensure the permanent and effective teaching of medical biochemistry. The problem-based learning (PBL) method, which fosters interactive learning, encourages students to conduct research and acquire new information, and motivates them to engage in group work based on brainstorming in a student-centered environment, appears to be an excellent alternative to traditional lecture-based courses

in medical biochemistry education. Numerous studies have highlighted the many benefits of the PBL method in medical biochemistry instruction. Incorporating PBL into the curriculum of medical biochemistry programs can significantly enhance the quality of education. Further detailed scientific research is needed to continue exploring and assessing the impact of the problem-based learning method on medical biochemistry education.

Authorship contribution statement

Designed the study: HBS.

Performed study and collected data: HBS and BS.

Discussed the results and strategy: HBS, BS, SB.

Supervised, directed and managed the study: HBS.

Final approved of the version to be published: HBS, BS, SB.

Declaration of competing interest

None of the authors have potential conflicts of interest to be disclosed.

Ethical approval:

This study wasn't need approved by the Local Research Ethics Committee because this paper is a review study.

Availability of data and materials

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REFERENCES

1. Turan-özdemir S. Tıp Eğitimi ve Standartlar. Uludağ Tıp Derg. 2005;31(2):133-137.
2. Servant VFC. The Harvard Connection: How the Case Method Spawned Problem-Based Learning at McMaster University, Health Professions Education. 2019;5(3): 163-171. doi:10.1016/j.hpe.2018.07.004
3. Neufeld VR, Barrows HS. The "McMaster Philosophy": an approach to medical education. J Med Educ. 1974;49(11):1040-1050.
4. Mishra S, Padmanaban P, Sarka G. PBL-An approach in medical biochemistry. BCAIJ. 2012;6(6):205-208.
5. Al-Azri H, Ratnapalan S. Problem-based learning in continuing medical education: review of randomized controlled trials. Can Fam Physician. 2014;60(2):157-165.
6. Musal B, Akalın E, Kılıç O, Esen A, Alıcı E. Dokuz Eylül Üniversitesi Tıp Fakültesi Probleme Dayalı Öğretim Programı, Süreçleri ve Eğitim Yönlendiricilerinin Rolü. TED. 2002;9(9):39-49.
7. Gürpınar E, Akyıldız F, Özbudak Ö, Şenol Y. Akdeniz Üniversitesi Tıp Fakültesinde Uygulanan Probleme Dayalı Öğrenimin Eğitim Yönlendiricileri Tarafından Değerlendirilmesi. TED. 2005;20(20):46-52.
8. Williams B. Case based learning—a review of the literature: is there scope for this educational paradigm in prehospital education? Emergency Medicine Journal. 2005:22(8):577-581.
9. Tarhan L, Ayyıldız Y. The views of undergraduates about problem-based learning applications in a biochemistry course. Journal of Biological Education. 2015:49(2):116-126.
10. Tailor P, Patel S. Comparison of problem-based learning with traditional lecture-based learning for first MBBS students in biochemistry using pre and post MCQ test of the students. Int.J.Med.Sci.Educ. 2019;6(3):83-86.
11. Smith HC. A course director's perspectives on problem-based learning curricula in biochemistry. Acad Med. 2002;77(12 Pt 1):1189-1198. doi:10.1097/00001888-200212000-00006
12. Narad SG, Chari S, Gupta M. Implementing problem based learning for first MBBS students in biochemistry. J Educ Technol Health Sci. 2016;3(3):115-121.
13. İşlekel H, Güner G. Tıp Fakültesi Probleme Dayalı Öğrenim Programında Biyokimya "Kuru Laboratuvar Uygulama" Modeli: Öğrenci Geri Bildirimleri. TED. 2006;21(21):16-21.
14. Yan Q, Ma L, Zhu L, Zhang W. Learning effectiveness and satisfaction of international medical students: Introducing a Hybrid–PBL curriculum in biochemistry. Biochemistry Molecular Biology Education. 2017;45(4):336-342.
15. Yennita Y, Zukmadini AY. Problem-based learning (PBL) and blended learning in improving critical thinking skills and student learning activities in biochemistry courses. J. Phys. Conf. Ser. 2021;1731:012007. doi: 10.1088/1742-6596/1731/1/012007
16. Ibrahim ME, Al- Shahrani AM, Abdalla ME, Abubaker IM, Mohamed ME. The effectiveness of problem-based learning in acquisition of knowledge, soft skills during basic and preclinical sciences: Medical students' points of view. Acta Inform Med, 2018;26(2):119-24.
17. Maçin S, Samadzade R, Aydoğan S. Selçuk Üniversitesi Tıp Fakültesi Eğitim Yönlendiricilerinin ve Öğrencilerinin Probleme Dayalı Öğrenme Uygulamasına İlişkin Geri Bildirimlerinin Değerlendirilmesi. STED. 2023;32(3):208-19.

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