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On behalf of the Medical Faculty of Gaziantep Islam Science and Technology University
Gaziantep İslam Bilim ve Teknoloji Üniversitesi Tıp Fakültesi adına

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Chief Editor/Baş Editör

İbrahim Halil Türkbeyler, Prof.

Clerk of Editorial Office/Sorumlu Yazı İşleri Müdürü

Mehmet Göl, Asst. Prof.

Aim

Experimental and Applied Medical Science aims at being a current and easily accessible academic publication in which striking research results that will improve the quality of life and are unique from every field of medical sciences.

Amaç

Experimental and Applied Medical Science, yaşam kalitesini arttıracak çarpıcı araştırma sonuçlarının sunulduğu, tıp bilimlerinin her alanında benzersiz, güncel ve kolay erişilebilir bir akademik yayın olmayı hedeflemektedir.

Scope

Experimental and Applied Medical Science is an open-access, internationally double-blind peer reviewed academic medical journal which is published in English four times a year, under the auspices of Medical Faculty of Gaziantep Islam Science and Technology University. The journal receives manuscripts for consideration to be publishing in the form of research articles, reviews, letter to editor, brief notification, summary notification etc. which could have been presented from within the country or abroad and including experimental animal studies related to the pathogenesis of diseases, pharmacological, clinical, epidemiological and deontological studies, also studies in the fields of improving public health, health services or health insurance.

During evaluation or publication no charge is demanded from authors. The journal is published every 3 months (March, July, September and December) with 4 issues per year. The literary language of the journal is English. Abstract part of the manuscript only should also be submitted in Turkish.

Kapsam

Experimental and Applied Medical Science, Gaziantep İslam Bilim ve Teknoloji Üniversitesi Tıp Fakültesi himayesinde yılda dört kez İngilizce olarak yayınlanan açık erişimli, uluslararası çift kör hakemli bir akademik tıp dergisidir. Dergi, yurt içinden veya yurt dışından, hastalık patogenezleri ile ilişkili deneysel hayvan çalışmaları, klinik, farmakolojik, epidemiyolojik, deontolojik çalışmalar ile beraber halk sağlığının geliştirilmesi amacı taşıyan ve sağlık hizmetleri veya sağlık sigortaları konularında araştırma makaleleri, derlemeler, vaka sunumları, kısa bildirimleri, özet bildirimleri vs. yayınlamak için değerlendirmeye kabul etmektedir. Değerlendirme veya yayın sırasında yazarlardan herhangi bir ücret talep edilmez.

Dergi 3 ayda bir (Mart, Temmuz, Eylül ve Aralık) yılda 4 sayı olarak yayımlanır. Derginin yazı dili İngilizcedir. Makalenin sadece özet kısmı Türkçe olarak da gönderilmelidir.

Ethical Principles and Publication Policy

Manuscripts are only considered for publication provided that they are original, not under consideration simultaneously by another journal, or have not been previously published. Direct quotations, tables, or illustrations that have extracted from any copyrighted material must be accompanied by written authority for their use from the copyright owners. All manuscripts are subject to review by the editors and referees. Deserving to be publishing is based on significance, and originality of the material. If any manuscript is considered to deserve publishing, it may be subject to editorial revisions to aid clarity and understanding without changing the data presented.

Experimental and Applied Medical Science strictly adheres to the principles set forth by "Helsinki Declaration" whose web address is below.

https://www.gibtu.edu.tr/Medya/Birim/Dosya/20210525133548_b192cec0.pdf

Editorial Board declares that all reported or submitted studies conducted with "human beings" should be in accordance with those principles.

Manuscripts presenting data obtained from a study design conducted with human participants must contain affirmation statements in the *Material and Methods* section indicating approval of the study by the institutional ethical review committee and "informed consent" was obtained from each participant. Also all manuscripts reporting experiments in which laboratory animals have been used should include an affirmation statement in the *Material and*

Etik İlkeler ve Yayın Politikası

Makaleler, orijinal/özgün olmaları, eş zamanlı olarak başka bir dergi tarafından incelenmemeleri veya daha önce yayınlanmamış olmaları koşuluyla yayına kabul edilir. Telif hakkıyla korunan herhangi bir materyalden alınan doğrudan alıntılar, tablolar veya resimler, kullanımları için telif hakkı sahiplerinden alınan yazılı izinle birlikte sunulmalıdır. Tüm yazılar editörler ve hakemler tarafından incelemeye tabidir. Yayınlanmaya hak kazanılması, materyalin önemine ve özgünlüğüne bağlıdır. Herhangi bir makalenin yayınlanmayı hak ettiği düşünülürse, sunulan veriler değiştirilmeden netlik ve anlayışa yardımcı olmak için editör revizyonlarına tabi tutulabilir.

Experimental and Applied Medical Science, internet adresi aşağıda yer alan "Helsinki Deklarasyonu" ile belirlenen ilkelere sıkı sıkıya bağlıdır.

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Editör Kurulu, "insan" ile yapılan tüm raporlanan veya sunulan çalışmaların bu ilkelere uygun olması gerektiğini beyan eder. İnsan katılımcılarla yürütülen bir çalışma tasarımından elde edilen verileri sunan makaleler, *Gereç ve Yöntemler* bölümünde çalışmanın kurumsal etik inceleme komitesi tarafından onaylandığını ve her katılımcıdan "bilgilendirilmiş onam" alındığını belirten onay ifadeleri kullanmalıdır. Ayrıca laboratuvar hayvanlarının kullanıldığı deneyleri bildiren tüm yazılar, *Gereç ve Yöntemler* bölümünde, internet adresi aşağıda

Methods section validating that all animals have received human care in compliance with the "Guide for the Care and Use of Laboratory Animals" whose web address is below and reveal approval by the institutional ethical review board. https://www.gibtu.edu.tr/Medya/Birim/Dosya/20210818130308_dca61056.pdf

If there is a commercial relation that contributes to the study process or there is an institution that provides financial support for the study; the authors must declare that they have no commercial relationship with the commercial product, drug, company used, or what kind of relationship (consultant or any other agreement) they have, if any.

Processing and publication are free of charge with the journal. No fees are requested from the authors at any point throughout the evaluation and publication process. All manuscripts must be submitted via the online submission system, which is available at https://dergipark.org.tr/tr/pub/eams. The journal guidelines, technical information, and the required forms are available on the journal's web page.

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belirtilmiş olan "Laboratuvar Hayvanlarının Bakımı ve Kullanımı Kılavuzu"na uygun olarak tüm hayvanların insanî bir bakım aldığını doğrulayan bir beyan ile kurumsal etik inceleme kurulunun onayını içermelidir. https://www.gibtu.edu.tr/Medya/Birim/Dosya/20210818130308_dca61056.pdf

Çalışma sürecine katkı sağlayan ticari bir ilişki veya çalışmaya maddi destek sağlayan bir kurum varsa; yazarlar ticari ürün, ilaç, aracılık eden şirket ile ticari bir ilişkilerinin olmadığını veya varsa ne tür bir ilişkisi (danışmanlık veya başka bir anlaşma) olduğunu beyan etmelidir.

Değerlendirme ve yayınlama süreçleri ücretsizdir. Değerlendirme ve yayın sürecinin hiçbir aşamasında yazarlardan ücret talep edilmez. Tüm yazılar https://dergipark.org.tr/tr/pub/eams adresinde bulunan çevrimiçi başvuru sistemi üzerinden gönderilmelidir. Dergi ile ilgili kullanım kılavuzları, teknik bilgiler ve gerekli formlar derginin internet sayfasında yer almaktadır.

Derginin tüm masrafları Gaziantep İslam Bilim ve Teknoloji Üniversitesi Tıp Fakültesi tarafından karşılanmaktadır. Reklam vermeyi düşüne kişi veya kurumlar yayın ofisi ile iletişime geçmelidir. Reklam görselleri sadece Baş Editör'ün onayı ile yayınlanabilir.

Tüm araştırmacılar, makaleye doğrudan akademik veya bilimsel olarak katkıda bulunmuş olmalıdır. Yazarlar, makalenin planlanması, uygulanması, yazılması veya gözden geçirilmesi aşamalarından birine veya birkaçına katkıda bulunmuş olmalıdır. Tüm yazarlar nihai versiyonu onaylamalıdır. Bilimsel kriterlere uygun bir makale hazırlamak yazarların sorumluluğundadır.

the final version. It is the authors' responsibility to prepare a manuscript that meets scientific criterias.

Statements or opinions expressed in the manuscripts published in the journal reflect the views of the author(s) and not the opinions of the Medical Faculty of Gaziantep Islam Science and Technology University, editors, editorial board, and/or publisher; the editors, editorial board, and publisher disclaim any responsibility or liability for such materials.

All manuscripts involving a research study must be evaluated in terms of biostatistics and it must be presented altogether with appropriate study design, analysis and results. ***p*** values must be given clearly in the manuscripts. Other than research articles, reviews, case reports, letters to the editor, etc. should also be original and up to date, and the references and, if any, their biostatistical parts should be clear, understandable and satisfactory.

The publication language of the journal is English. In addition, the abstract part of the article must be uploaded in both Turkish and English. Manuscripts should be evaluated by a linguist before being sent to the journal.

All manuscripts and editorial correspondence must be submitted online to the editorial office, https://dergipark.org.tr/tr/pub/eams.

According to the Law on Intellectual and Artistic Works, which was first published in the Official Gazette with the law number 5846 on 13/12/1951, whose web address is below, and on which subsequently various changes have been made or novel parts have been added in time, all kinds of publication rights of the articles accepted

Dergide yayınlanan yazılarda ifade edilenler veya görüşler, Gaziantep İslam Bilim ve Teknoloji Üniversitesi Tıp Fakültesi, editörler, yayın kurulu ve/veya yayıncının görüşlerini değil, yazar(lar)ın görüşlerini yansıtır; editörler, yayın kurulu ve yayıncı bu tür materyaller için herhangi bir sorumluluk veya yükümlülük kabul etmez.

Araştırma çalışması içeren tüm yazılar biyoistatistiksel açıdan değerlendirilmeli ve uygun çalışma düzeni, verilerin analizi ve sonuçları ile birlikte sunulmalıdır. ***p*** değerleri yazılarda açık olarak verilmelidir. Araştırma makaleleri dışında derlemeler, olgu sunumları, editöre mektuplar vb. de orijinal/özgün ve güncel olmalı, kaynaklar ve varsa biyoistatistiksel kısımlar açık, anlaşılır ve tatmin edici olmalıdır.

Derginin yayın dili İngilizce'dir. Ayrıca makalenin özet kısmı hem Türkçe hem de İngilizce olarak yüklenmelidir. Yazılar dergiye gönderilmeden önce bir dilbilimci/konunun uzmanı tarafından değerlendirilmelidir.

Bütün çalışmalar ve editör kurulu ile yazışmalar çevrimiçi olarak, https://dergipark.org.tr/tr/pub/eams adresi üzerinde yayın ofisine gönderilmelidir.

İnternet adresi aşağıda belirtilmiş olan, ilk olarak 13/12/1951 tarih ve 5846 sayılı Kanun ile Resmi Gazete'de yayımlanan, sonraları üzerinde değişiklikler yapılmış veya yeni kısımlar eklenmiş olan Fikir ve Sanat Eserleri Kanunu'na göre; yayına kabul edilen makalelerin her türlü yayın hakkı dergiyi yayınlayan kuruma aittir. Ancak makalelerdeki düşünce ve öneriler tamamen yazarların sorumluluğundadır. https://www.gibtu.edu.tr/Medya/Birim/Dosya/20210818145630_406d24df.pdf

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Author Guidelines

Submission of a paper will be taken to imply that it has not previously been published and that it is not being considered for publication elsewhere. Decision as to publication of papers submitted to the Experimental and Applied Medical Science will be based on the opinion of the Editorial Board as to the significance and originality of the work.

Manuscripts should be prepared electronically using an appropriate “office word” compatible text-processing package, formatted for A4 size, double-spaced throughout, and using a “Times New Roman” 12 point font. Articles must be written in English. Abstracts must be written in both Turkish and English. Text should flush left, and not be justified. Words should not be hyphenated. Pages should be numbered sequentially.

There should be a separate title page with:

a) The title

b) The authors' names

c) The laboratory of origin, with complete address of each author

d) A running title

e) Corresponding author and e-mail

f) Conflict of interest

g) Acknowledgements

The main body of full-length paper should be divided into:

1. Abstract

2. Introduction

3. Material and Methods

4. Results

5. Discussion

Yazım Kuralları

Bir çalışmanın dergimize gönderilmesi için bu çalışmanın daha önce yayınlanmamış veya başka bir akademik dergide şu anda yayınlanmak üzere değerlendirilmiyor olması koşulu ile mümkündür. Experimental and Applied Medical Science’a gönderilen her türlü çalışmanın yayınlanmasına ilişkin karar, Yayın Kurulu'nun çalışmanın önemi ve özgünlüğü konusundaki görüşüne dayanacaktır.

Çalışmalar, ya “office word” programı ile ya da bu program ile uyumlu uygun bir metin işleme programı kullanılarak, A4 boyutunda hazırlanmalı, baştan sona çift aralıklı ve “Times New Roman” tarzında 12 punto yazı tipi kullanılarak elektronik ortamda yazılmalıdır. Makaleler İngilizce yazılmalıdır. Özetler hem Türkçe hem de İngilizce olarak yazılmalıdır. Metin iki yana yaslandırılmamalı, sadece sola yaslanmamalıdır. Kelimeler kısa çizgi ile hecelenmemelidir. Sayfalar sırayla numaralandırılmalıdır.

Aşağıdakileri içeren ayrı bir başlık sayfası olmalıdır:

a) Başlık

b) Yazarların isimleri

c) Her yazarın tam adresi ile birlikte çalıştıkları laboratuarlar

d) Kısa başlık

e) İletişimdeki yazar ve iletişim bilgileri

f) Çıkar çatışması beyanı

g) Teşekkür, bilgilendirme

Tam uzunluktaki kağıdın ana gövdesi şu bölümlere ayrılmalıdır:

1. Özet

2. Giriş

6. Conclusion
7. Conflict of interest
8. Acknowledgement
9. References

In general, there are no specific word lengths for any manuscript. The general principle is that a manuscript can be as long as necessary to communicate clearly and most effectively the scientific message, but should be as short as possible to achieve a complete presentation of the information without undue repetition or redundancy.

In the *Materials and Methods* section, the source of all compounds, equipment or software should be identified by the full name of the supplier, city, state/country. The chemical names of any drug should precede the trade name.

Papers describing animal experiments must define species, strain, sex, age, supplier and number of animals used. An ethical statement concerning the use of animals, or the details of ethical approvals, consent and recruitment of human subjects should be clearly stated. *Results* and *Discussion* can be broken down into subsections for improving the comprehensibility. The Results should not repeat methodological details and should avoid the discussion of the data.

The results of statistical tests should be incorporated in the body of the text, typically in the *Results* section, rather than in figure legends. Adequate description of statistical analysis should be provided. Statistical measures of variation in the text, illustrations and tables, should be identified. All dimensions and measurements must be

3. Gereç ve Yöntemler
4. Sonuçlar
5. Tartışma
6. Bağlam
7. Çıkar çatışması
8. Teşekkür, bilgilendirme
9. Kaynaklar

Genel olarak, herhangi çalışma için şart koşulan belirli bir kelime sayısı/metin uzunluğu yoktur. Genel ilke; bir makalenin bilimsel mesajı açık ve etkili bir şekilde iletmek için gerektiği kadar uzun olabileceği, ancak gereksiz tekrar veya fazlalık olmadan bilgilerin eksiksiz bir sunumunu elde etmek için mümkün olduğunca kısa olması gerektiğidir.

Gereçler ve Yöntemler bölümünde, tüm bileşiklerin, malzemelerin veya yazılımların kaynağı, tedarikçinin tam adı, şehir, eyalet/ülke ile tanımlanmalıdır. Herhangi bir ilacın kimyasal isimleri ticari isminden önce gelmelidir.

Hayvan deneylerini açıklayan makaleler, tür, soy, cinsiyet, yaş, tedarikçi ve kullanılan hayvan sayısını açıkça tanımlamalıdır. Hayvanların kullanımına ilişkin bir etik beyan veya insan deneklerin etik kurul onayları, bilgilendirilmiş onamları ve çalışmaya dâhil edilmelerine ilişkin ayrıntılar açıkça belirtilmelidir. *Sonuçlar ve Tartışma* bölümleri, anlaşılırlığı artırmak için alt bölümlere ayrılabilir. Sonuçlar, metodolojik ayrıntıları tekrarlamamalı ve verilerin tartışılmasından kaçınmalıdır.

İstatistiksel testlerin sonuçları, şekillerin altındaki açıklama kısımlarından ziyade metnin gövdesine, tipik olarak Sonuçlar bölümüne dâhil edilmelidir. İstatistiksel analizin yeterli bir şekilde açıklaması sağlanmalıdır. Metinde, resimlerde ve

specified in the metric system.

All subscripts, superscripts, Greek letters and unusual characters must be clearly identified.

In the text, abbreviations should be used consistently. Abbreviations should be defined on first use.

References should be designed in “Vancouver” style. While writing references, “Times New Roman” 10 point font should be used. Multiple authors should be separated by a comma. If there are more than three authors, after the 3rd author, "et al." should be inserted without a comma for both article and book references. If reference is made from a chapter in a book and there are many authors belonging only to this chapter, the title and chapter of the book are indicated, the first three of the chapter authors are written, and "et al." statement is added for subsequent authors.

Example:

1. Perell KL, Nelson A, Goldman RL, et al. Fall risk assessment measures: an analytic review. The journals of gerontology Series A, Biological sciences and medical sciences. 2001;56(12):M761-6.

2. Ha H, Han C, Kim B. Can Obesity Cause Depression? A Pseudo-panel Analysis. Journal of preventive medicine and public health = Yebang Uihakhoe chi. 2017;50(4):262-7.

3. Çekmen MB, Turgut M, Türköz Y, et al. Nitrik Oksit (NO) ve Nitrik Oksit Sentaz (NOS)'ınFizyolojik ve Patolojik Özellikleri. Türkiye Klinikleri Journal of Pediatrics. 2001;10(4):226-35.

4. Parlakpınar H, Örüm MH, Acet A. Kafeik asit fenetil ester (KAFE) ve miyokardiyal

tablolarda istatistiksel varyasyon ölçütleri tanımlanmalıdır.

Tüm boyutlar ve ölçüler metrik sistemde belirtilmelidir.

Tüm alt simgeler, üst simgeler, Yunan harfleri ve olağandışı karakterler açıkça tanımlanmalıdır.

Metinde kısaltmalar tutarlı bir şekilde kullanılmalıdır. Kısaltmalar ilk kullanımda tanımlanmalıdır.

Kaynaklar “Vancouver” tarzında yazılmalıdır. Kaynaklar yazılırken, “Times New Roman” 10 punto kullanılmalıdır. Birden çok yazar virgülle ayrılmalıdır. Hem makale hem de kitap referanslarında, eğer üçten çok yazar varsa, 3. Yazardan sonra virgül ve “et al.” ifadesi kullanılmalıdır. Kitapta bir bölümden referans yapılıyorsa ve sadece bu bölüme ait çok sayıda yazar varsa, kitabın başlığı ve bölümü belirtilip, bölüm yazarlarının ilk üçü yazılıp ve ardından sonraki yazarlar için “et al.” ifadesi eklenmelidir.

Örnek:

1. Perell KL, Nelson A, Goldman RL, et al. Fall risk assessment measures: an analytic review. The journals of gerontology Series A, Biological sciences and medical sciences. 2001;56(12):M761-6.

2. Ha H, Han C, Kim B. Can Obesity Cause Depression? A Pseudo-panel Analysis. Journal of preventive medicine and public health = Yebang Uihakhoe chi. 2017;50(4):262-7.

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iskemi reperfüzyon (Mİ/R) hasarı. İnönü Üniversitesi Sağlık Bilimleri Dergisi 2012; 1: 10-5.
5. Yıldırım AB. The effects of maternal hypothyroidism on the immunoreactivity of cytochrome p450 aromatase in the postnatal rat testes. 2015; Doctoral thesis.
6.https://hsgm.saglik.gov.tr/depo/birimler/kanserdb/istatistik/Trkiye_Kanser_statistikleri_2016.pdf (Last access date: 21.09.2020).
7. Kuran O, İstanbul, Filiz Kitabevi. Sistematik Anatomi. 1983 p. 76-9.
8. Abbas AK, Andrew H Lichtman, Shiv Pillai. Cellular and Molecular Immunology. 6th ed. Philadelphia: Saunders Elsevier; 2007 p. 121-56.
Submit illustrations as separate files, only as TIFF or EPS files, with a minimum resolution of 300dpi.
Tables of numerical data should each be typed with double spacing on separate pages numbered in sequence in numerals, provided with a heading, and referred to in the text, as Table 1, Table 2, etc. Each table should have a brief but descriptive heading. Explanatory matter should be included in footnotes to the table.
We accept electronic supplementary material to support and enhance your scientific research. Supplementary files offer the author additional possibilities to publish supporting applications, movies, animation sequences, high-resolution images, background datasets, sound clips and more.

4. Parlakpınar H, Örüm MH, Acet A. Kafeik asit fenetil ester (KAFE) ve miyokardiyal iskemi reperfüzyon (Mİ/R) hasarı. İnönü Üniversitesi Sağlık Bilimleri Dergisi 2012; 1: 10-5.
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6.https://hsgm.saglik.gov.tr/depo/birimler/kanserdb/istatistik/Trkiye_Kanser_statistikleri_2016.pdf (Last access date: 21.09.2020).
7. Kuran O, İstanbul, Filiz Kitabevi. Sistematik Anatomi. 1983 p. 76-9.
8. Abbas AK, Andrew H Lichtman, Shiv Pillai. Cellular and Molecular Immunology. 6th ed. Philadelphia: Saunders Elsevier; 2007 p. 121-56.
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Sayısal veri tablolarının her biri, sayılarla sırayla numaralandırılmış bir başlık ile birlikte ve metinde Tablo 1, Tablo 2, vb. olarak atıfta bulunulmuş halde, ayrı sayfalarda çift aralıkla hazırlanmalıdır. Her tablonun kısa ama açıklayıcı bir başlığı olmalıdır. Tablo dipnotlarında açıklayıcı hususlara yer verilmelidir.
Bilimsel araştırmalarınızı desteklemek ve geliştirmek için elektronik ek materyaller kabul edilmektedir. Ek dosyalar, yazara, destekleyici uygulamaları, filmleri, animasyon dizilerini, yüksek çözünürlüklü

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görüntüleri, arka plan veri kümelerini, ses kayıtlarını ve daha fazlasını yayınlamak için ek olanaklar sunmaktadır.

Başvuru sırasında çıkar çatışmasının ve mali destek konularının açıklanması elzemdir. Yazarlar, makalelerinin yayımlanma tarihinden önce ortaya çıkabilecek ek çıkar çatışmalarını veya bulunan mali destekleri dergiye bildirmekle yükümlüdür. Tüm yazarlar, makalelerinin konusuyla doğrudan ilgili olsun ya da olmasın, tüm olası çıkar çatışmalarını ve mali desteği bireysel olarak açıklamalıdır.

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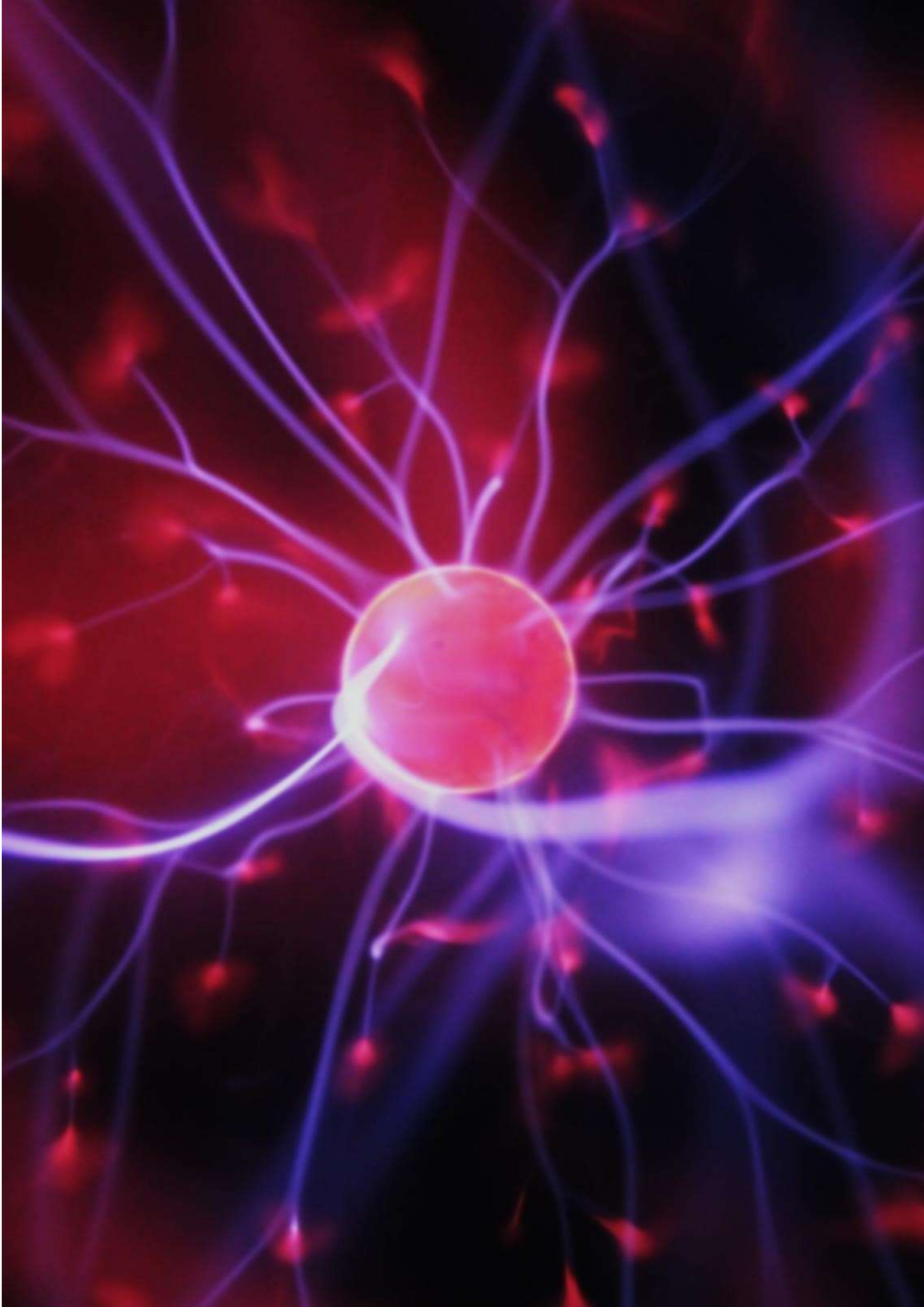

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Antioxidative Strategy in Traumatic Brain Injury: Role of Low-Molecular-Weight Antioxidants

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Abstract

Objective*: Traumatic brain injury (TBI) is a major cause of mortality and disability worldwide. This study was designed to investigate the beneficial and neuroprotective role of some Low-Molecular-Weight antioxidants (LMWA) in the treatment of TBI in albino rats.*

Methods*: TBI was induced in adult albino rats using the weight-drop method. A total of 70 Rats was used and were divided into 12 treatment groups, a traumatized non-treated group (TNT) and a Non-traumatized non-treated group (NTNT). There were 5 rats per group. Each of the treatment groups received 22.5 or 45 mg/kg of dimethyl sulfoxide (DMSO), Alpha Lipoic acid (ALA), Uric acid (UA), vitamin C (VC), vitamin E (VE), or Mannitol. Treatment was started 30 min after the trauma and continued for 21 days.*

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To evaluate the functional outcomes, the modified neurological severity score (mNSS) was calculated. The antioxidant enzymes [superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx)], and malondialdehyde (MDA) were assayed to evaluate oxidative stress (OS).

***Results**: At 7 days post-TBI, the antioxidant-treated groups exhibited significant ($p<0.05$) improvements in neurological scores compared to the traumatized non-treated group (TNT). The treated groups showed a significant ($p<0.05$) increase in the activities of antioxidant enzymes (SOD, CAT and GPx) and a significant ($p<0.05$) decrease in the concentration of MDA compared with the TNT group.*

***Conclusion**: These promising results suggest that the use of low-molecular-weight antioxidants may be a useful neuroprotective strategy in the treatment of TBI. However, further studies should investigate the molecular mechanisms of these antioxidants on TBI pathophysiology and functional outcome.*

***Key words**: Traumatic brain injury, Oxidative stress, Low-Molecular-Weight Antioxidants, Malondialdehyde, Neurological score*

Introduction

Traumatic brain injury (TBI) is characterized as an alteration of cerebral function or abrupt mechanical impairment of brain tissue caused by an external force, subsequently leading to biochemical cascades that may exacerbate the injury (1). Though the extent of damage is not always immediately apparent, the severity of brain damage can vary depending on the type of external force applied and may range from minor to critical (2). Life threatening complications can develop even after mild injuries (3). Mortality rates in developed nations are observed to range between 20-30%, while developing nations can experience rates as high as 90% (4). The socioeconomic impacts of TBI are of noteworthy importance, as it constitutes one of the foremost causes of mortality and morbidity among individuals in the young adult demographic (5). The cause of TBI involves primary and secondary injury processes leading to neurodegenerative and disabling effects. Primary injury can be simple or complex but leads to a series of molecular events resulting in secondary injury.

These events include depolarization, ionic homeostasis perturbations, neurotransmitter release, mitochondrial dysfunction, inflammation, and free radical release, contributing to chronic neurodegeneration and neurological impairment (6).

The production of free radicals after TBI is a well-researched aspect of secondary brain injury (7). A free radical is a chemical entity that possesses one or more unpaired electrons, and this unpaired electron is accountable for its reactivity (8). Reactive oxygen species (ROS) and reactive nitrogen species (RNS), which consist of both free radicals and decomposable compounds that can generate free radicals, are commonly produced after TBI through various mechanisms and play a role in the pathogenesis of TBI by exacerbating secondary injury mechanisms and inducing OS (9). The role of OS in acute central nervous system injury is significant, as the generation of ROS and RNS after brain injury leads to tissue damage and cell death via various pathways (7). Mammalian cells have inherent antioxidant mechanisms, namely superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), which function to shield the cells from an overabundance of free radicals (10).

Antioxidants refer to chemical compounds which serve to inhibit the oxidation of other chemicals. These substances play a pivotal role in safeguarding essential cell components by neutralizing the detrimental impact of free radicals (11). The first and paramount mechanism of antioxidant defense systems to counteract ROS involves the utilization of enzymatic antioxidants such as SOD, CAT, and GPX (12). When a TBI occurs, the body's endogenous antioxidants experience an increase in activity level. However, the sheer intensity of free radicals overwhelms the antioxidant system's ability to counteract the detrimental impact of these molecules (13). Exogenous administration of compounds possessing antioxidant properties, such as vitamins, minerals (selenium, zinc), or albumin, can offer supplementary safeguarding measures (14). Natural antioxidants and other compounds that can counteract free radicals are crucial for preventing OS, indicating that augmenting the endogenous antioxidant defense mechanism may be neuroprotective following injury (15,16). Neuroprotection is vital in the treatment of TBI to prevent or reverse secondary brain injury and avoid further neurological decline caused by the release of toxic free radicals and neuronal necrosis (17). Neuroprotective agents that can limit secondary tissue loss and/or improve behavioral outcomes can be of therapeutic value in acute brain injury (18). However, as of the present moment, the

quest to establish efficacious neuroprotective agents has yet to yield any fruitful results. Nevertheless, the pressing need to continue said quest for neuroprotective agents that are truly effective remains paramount (15). The aim of this research work is to validate the beneficial and neuroprotective role of some Low-Molecular-Weight antioxidants in TBI.

Materials and Methods

Animals

All the experimental rats were apparently healthy albino rats weighing 200-250g. They were obtained from the Animal House of the Biological Sciences Department, Usmanu Danfodiyo University, Sokoto, Nigeria for this study. The rats were allowed to acclimatize to the research laboratory condition and were subjected to a 12-hours light/12-hour dark schedule. The rats were fed with growers' mash of vital® feed and allowed to clean drinking water *ad libitum.*

Experimental Design

Number of rats used in this work was 70 which were randomly divided into 12 treated groups, 1 positive control group to be traumatized non-treated (TNT), and 1 normal control group that is non-traumatized non-treated (NTNT). Each group has five rats. Rats in the treatment groups were induced with TBI and treated with six different antioxidants (in two different doses) for 21 days. Table 1 below shows the groupings of the work. This work was approved by the board of postgraduate school of Usman Danfodiyo University Sokoto after meeting national and international standard care of animals used was in accordance with institution guidelines.

Induction of TBI

Head injury was induced in the entire experimental animals except in the negative control group by weight drop method using an acceleration impact device according to Mamarou et al. (19).

Neurological Assessment

Animal were examined and scored with a modified neurological severity score (mNSS) by the modified method of Scallert *et al.* (20). The composite score of 18 points was comprised of five distinct components, namely, consciousness and respiration, cranial nerve function, motor function, sensory function, and coordination. A total of 18 assessments were conducted to evaluate these functions, wherein one point was allocated for the failure to perform a task, while zero points were given for success. The scores ranged from zero for healthy, uninjured animals to a maximum of

18, indicating severe neurological dysfunction with a failure in all tasks. The mNSS, immediately after the trauma, is indicative of the initial severity of injury. Following the initial evaluation of mNSS, the rats were assigned to treatment groups, which were evenly distributed to ensure homogenous grouping.

Sample collection

The rats were subjected to anesthesia via chloroform contained within a glass jar, and subsequent to this, blood was collected through the process of cardiac puncture and the serum was then obtained. The extraction of the brain from the skull was carried out for the purpose of histopathological and biochemical examination.

Table 1: The design of the experiment

Group	Trauma induction	Treatment	Dose
1	Yes	Vitamin E	22.5 mg/kg
2	Yes	Vitamin E	45 mg/kg
3	Yes	Vitamin C	22.5 mg/kg
4	Yes	Vitamin C	45 mg/kg
5	Yes	uric acid	22.5 mg/kg
6	Yes	Uric acid	45 mg/kg
7	Yes	DMSO	22.5 mg/kg
8	Yes	DMSO	45 mg/kg
9	Yes	α- Lipoic acid	22.5 mg/kg
10	Yes	α- Lipoic acid	45 mg/kg
11	Yes	Mannitol	22.5 mg/kg
12	Yes	Mannitol	45 mg/kg
TNT	Yes	NO	0 mg/kg
NTNT	NO	NO	0 mg/kg

Key; TNT – traumatized non-treated, NTNT- non-traumatized non-treated

Analysis of Oxidative Stress

In the present investigation, markers of oxidative stress were examined in the serum and brain tissues of rats. The assessment of antioxidant enzymes, namely superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX), as well as the evaluation of lipid peroxidation byproduct malondialdehyde (MDA), were performed using Cayman's Assay Kits with the following batch numbers: 706002 for SOD, 707002 for CAT, 703102 for GPX, and 700870 for MDA. The SOD assay utilizes a tetrazolium salt to detect the superoxide radicals generated by the reaction between hypoxanthine and xanthine oxidase (XO). Briefly, Two wells were designated as standard and sample. To each well 200µl of the diluted radical detector, 10µl each of prepared standard to the serum were added to the standard well and sample well respectively. Twenty microliter (20µl) of diluted xanthine oxidase was added to both standard and sample wells to initiate the reaction. The plate was shaken for a few seconds and covered with cover plate. The plate was then incubated on a shaker at room temperature for 20 minutes and absorbance was read at 450nm using Rayto RT 2100C plate reader (21). CAT is determined by the reaction of catalase with methanol in the presence of an optimal concentration of H2O2. The resulting formaldehyde is analyzed spectrophotometrically using 4-amino-3-hydrazino-5-mercapto-1,2,4-triazole (Purpald) as the chromogen. Briefly, Three wells were designated as sample, standard and control. To each well, 100µl of assay buffer and 30µl of methanol were added. To standard well, 20µl of prepared standard (Formaldehyde Standards) were added and to sample well 20µl of serum were added. 20µl of H2O2 were added to each well to initiate the reaction. The plate was covered with lid and incubated on a shaker for 20 minutes at room temperature. To each well, 30µl of potassium hydroxide were added to terminate the reaction and 30µl of purpald were then added. The plate was covered once again and incubated for 10 minutes at room temperature on a shaker. Once again, to each well, 10µl of potassium periodate were added, covered and incubated for 5 minutes on a shaker. The absorbance was read at 540nm using Rayto RT 2100C plate reader (22). GPx activity is measured indirectly through a coupled reaction with glutathione reductase, which allows for the assessment of glutathione peroxidase activity. The reduction of hydroperoxides by glutathione peroxidase leads to the formation of oxidized glutathione, which is subsequently regenerated to its reduced state by glutathione reductase and NADPH. Briefly, three wells were designated as

sample, non-enzymatic and positive control. To sample well, 100µl of assay buffer, 50µl of co-substrate mixture and 20µl of serum were added. To non-enzymatic well, 120µl of assay buffer and 50µl of co-substrate mixture were added and to positive control well 100µl of assay buffer, 50µl of co-substrate mixture and 20µl of diluted GPx were added. The reaction was initiated by adding 20µl of cumenehydroperoxide to each well and the plate was carefully shaken for a few seconds. The absorbance was read at 340nm using Rayto RT 2100C plate reader (23). The MDA assay is based on the reaction between thiobarbituric acid and MDA, resulting in the formation of an MDA-TBA2 adduct with strong absorption at 535nm. Briefly, Into two test tubes labeled sample and standard, 100µl of serum/ brain tissue homogenate and standard were added respectively and treated with 100µl of TCA (trichloroacetic acid) reagent. Then 800 µl of color reagent (106 mg thiobarbituric acid, 10 ml TBA-acetic acid solution and 10 ml NaOH) was added to each test tube and vortex. Tubes were heated in boiling water for one hour and cooled on ice to stop reaction and incubated for ten minutes on ice. After ten minutes, tubes were centrifuge for another ten minutes at 4000 rpm and stabilized at room temperature for 30minutes. After transferring 200 µl of the supernatant to the plate absorbance was read at 540 nm using the plate reader (24).

Statistical Analysis

The statistical software package GraphPad Prism 9 was utilized to conduct an analysis of the results. The data was presented as means with standard deviation. The statistical method of one-way analysis of variance (ANOVA) was employed to analyze the data. In the event that the F values were deemed significant, the Tukey post-hoc test was utilized to compare the groups.

Results

This work evaluated the possible therapeutic potentials of Vitamin C, Vitamin E, α-Lipoic acid, Uric acid, DMSO, and Mannitol in rat model of TBI.

Neurological assessment

Fig. 1 shows the outcome of the neurological assessment (mNSS) of all the experimental groups. No neurological changes were observed in the NTNT rats. There were noteworthy improvements in the neurological response in the rats treated with the antioxidants as indicated by their mNSS. The TNT group did not show significant improvement in their neurological score.

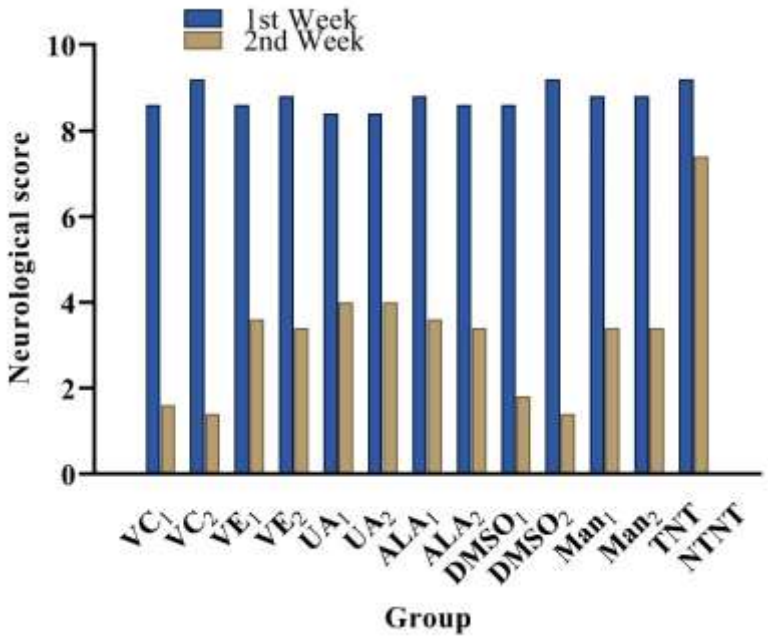

Fig. 1 Modified Neurological Severity Score of Rats; VC_1. – Vitamin C 22.5 mg/kg, VC_2- Vitamin C 45 mg/kg, VE_1-Vitamin E 22.5 mg/kg, VE_2- Vitamin E 45 mg/kg, UA_1- Uric acid 22.5 mg/kg, UA_2- Uric acid 45 mg/kg, ALA_1 – α-Lipoic acid 22.5 mg/kg, ALA_2- α-Lipoic acid 45 mg/kg, $DMSO_1$ – dimethyl sulfoxide 22.5 mg/kg $DMSO_2$ – 45 mg/kg, Man_1- Mannitol 22.5 mg/kg, Man_2 – Mannitol 45 mg/kg, TNT- traumatized non-treated, NTNT – Non traumatized non-treated

The Effect of Supplementation of TBI Rats with LMWA on the Activity of Serum SOD

Fig. 2 depicts the outcomes of the serum SOD level in groups treated with antioxidants. The findings suggest that TBI incurred a noteworthy decline ($p<0.05$) in the enzyme's activity. However, administering antioxidants at 22.5mg/kg and 45mg/kg increased the SOD activity significantly ($p<0.05$). The antioxidants increased SOD activity in a dose-dependent manner. The results indicate that DMSO and Vitamin C exhibited significantly ($p<0.05$) greater efficacy on the enzyme activity than all other antioxidants.

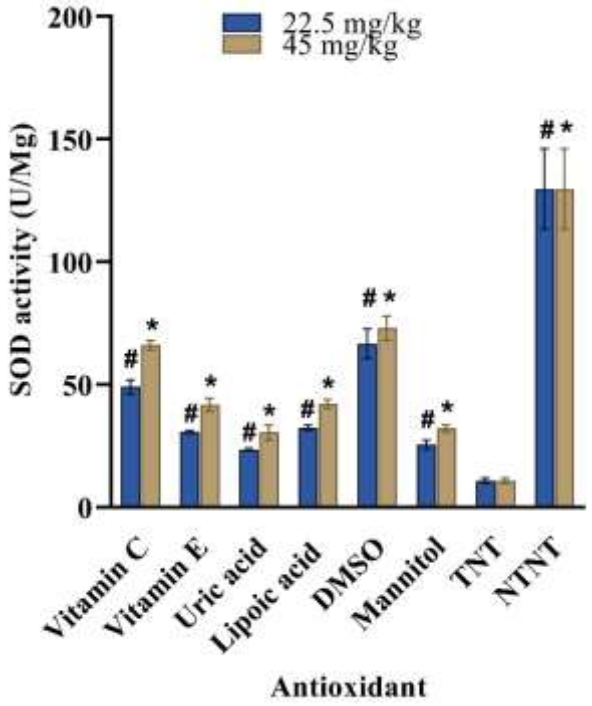

Fig. 2 Effects of LMWA on the Activity of SOD in Serum; SOD- Superoxide dismutase, DMSO- Dimethyl sulfoxide, TNT- Traumatized non- treated, NTN- Non-traumatized non- treated. Values are significantly different ($^{\#}p<0.05$) compared to TNT at 22.5 mg/kg and ($^{*}p<0.05$) compared to TNT at 45 mg/kg

The Effect of Supplementation with LMWA on the Activity of Serum CAT

Fig. 3 displays the results of administering LMWA supplements on CAT activity in TBI rats. The findings reveal that the enzyme's activity significantly decreased ($p<0.05$) due to TBI.

However, administering antioxidants at 22.5 and 45 mg/kg doses significantly ($p<0.05$) increased the enzyme's activity. Furthermore, the activity displayed a dose-dependent pattern, with the exception of Vitamin E, Lipoic acid, and mannitol groups, which exhibited a similar effect. Vitamin C and DMSO produced significantly higher effects on the enzyme activity compared to the other antioxidants.

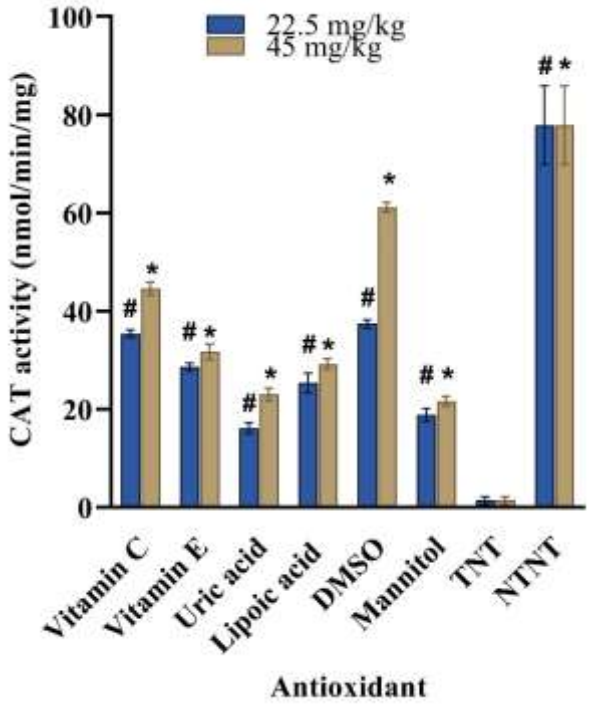

Fig. 3 Effects of LMWA on the Activity of CAT in Serum; CAT- Catalase, DMSO- Dimethylsulfoxide, TNT- Traumatized non-treated, NTN- Non-traumatized non-treated. Values are significantly different ($^{\#}p<0.05$) compared to TNT at 22.5 mg/kg and ($^{*}p<0.05$) compared to TNT at 45 mg/kg

The Effect of Supplementation with LMWA on the Activity of Serum GPx

The effects of LMWA on the activity of GPx in serum was presented in Fig. 4. The results showed that TBI caused significant ($p<0.05$) decrease in the activity of the enzyme. Administration of the antioxidant at 22.5 and 45 mg/kg, significantly ($p<0.05$) increased the activity in a concentration dependent manner. The result also indicated that DMSO and Vitamin C had significantly higher effects compared to the remaining treatment while DMSO had significantly higher effect than Vitamin C.

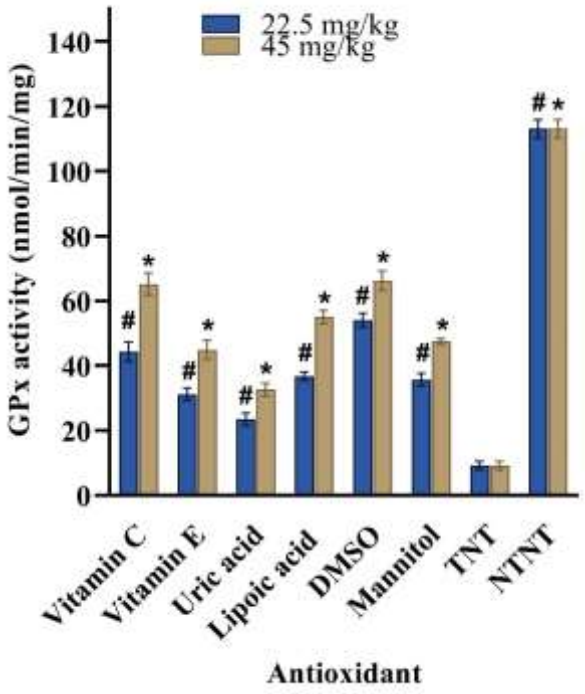

Fig. 4 Effects of LMWA on the Activity of GPX in Serum; GPX- Glutathione peroxidase, DMSO- Dimethylsulfoxide, TNT- Traumatized non-treated, NTN- Non-traumatized non-treated. Values are significantly different ($^{\#}p<0.05$) compared to TNT at 22.5 mg/kg and ($^{*}p<0.05$) compared to TNT at 45 mg/kg

The Effect of LMWA Supplementation on the Serum MDA Level

Fig. 5 shows the effects of LMWA on lipid peroxidation. The results indicated that TBI caused significant ($p<0.05$) increase in the concentration of MDA in the TNT group. After supplementation with the antioxidants in two different doses, the concentration of MDA decreased significantly ($p>0.05$) in all the treated groups in a concentration dependent manner. Vitamin C and DMSO at 22.5 mg/kg had no significant difference between their effects on MDA but both had significantly higher effect compared to all other groups. At 45 mg/kg DMSO had higher effect than Vitamin C which also had higher effect compared to the rest.

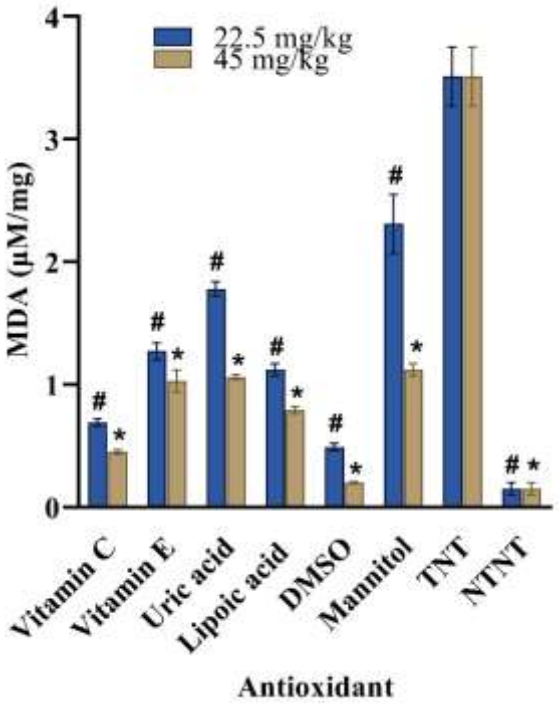

Fig. 5 Effects of LMWA on the Level of MDA in the Serum of experimental rats; MDA- Malondialdehyde, DMSO- Dimethyl sulfoxide, TNT- Traumatized non-treated, NTN- Non-traumatized non-treated. Values are significantly different ($^{\#}p<0.05$) compared to TNT at 22.5 mg/kg and ($^{*}p<0.05$) compared to TNT at 45 mg/kg

The Effect of LMWA Supplementation on the Brain Tissue Activity of SOD

The results presented in Fig. 6 shows the activity of SOD in brain tissue of TBI rats treated with LMWA. The result indicated that TBI caused significant ($p<0.05$) decrease in the activity of the enzyme. Supplementation significantly ($p<0.05$) increased the activity except 22.5 mg/kg of Uric acid and mannitol. Comparison between the groups indicated that the highest activity was observed in DMSO and Vitamin C treated groups.

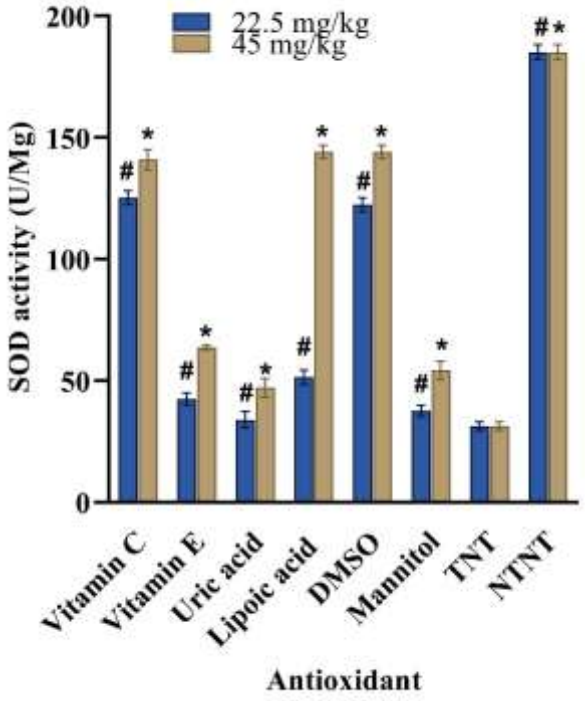

Fig. 6 Effects of LMWA on the SOD Activity in Brain Tissue; SOD- Superoxide dismutase, DMSO- Dimethyl sulfoxide, TNT- Traumatized non- treated, NTN- Non-traumatized non-treated. Values are significantly different ($^{\#}p<0.05$) compared to TNT at 22.5 mg/kg and ($^{*}p<0.05$) compared to TNT at 45 mg/kg

The Effect of Supplementation with LMWA on the Brain Tissue Activity of CAT

Fig. 7 shows the result of the effect of LMWA on brain tissue level of CAT. The result indicated that TBI caused significant ($p<0.05$) decrease in the activity of the enzyme.

Supplementation of the antioxidants (22.5 and 45 mg/kg) increased the activity significantly ($p<0.05$) except in the groups treated with 22.5mg/kg of Uric acid and mannitol. The activity increased in a dose dependent manner. The enzyme activity in Vitamin C group is not statistically different from the DMSO group.

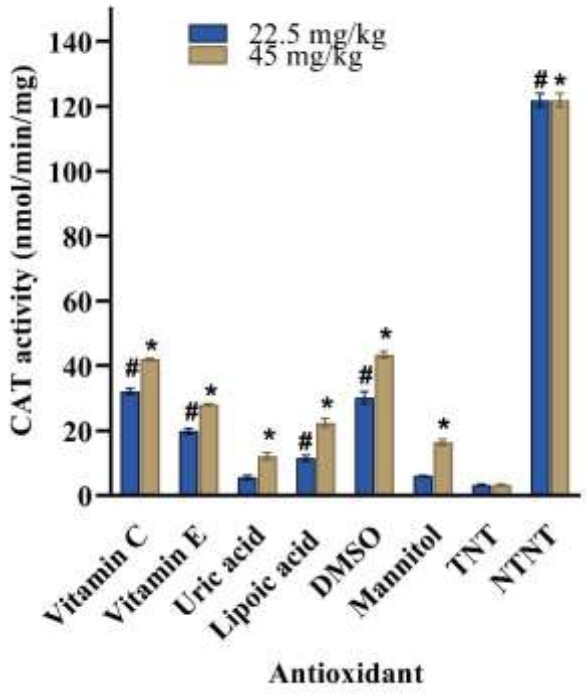

Fig. 7 Effects of LMWA on the CAT Activity in Brain Tissue; CAT- catalase, DMSO- Dimethyl sulfoxide, TNT- Traumatized non-treated, NTNT non traumatized non-treated. Values are significantly different ($^{\#}p<0.05$) compared to TNT at 22.5 mg/kg and ($^{*}p<0.05$) compared to TNT at 45 mg/kg

The Effect of Supplementation with LMWA on the Brain Tissue Activity of GPX

The results in Fig. 8 showed that TBI caused significant ($p<0.05$) decrease in the activity of the enzyme while administration at 22.5 mg/kg and 45 mg/kg BW of the antioxidants significantly ($p<0.05$) increased the activity in a dose-dependent manner except in uric acid. At 22.5 mg/kg, Vitamin C and DMSO had similar increasing effect on the enzyme activity while Lipoic acid compared to DMSO had significantly lower effect. Vitamin E, Uric acid and mannitol at the dose of 22.5 mg/kg showed statistically similar effects on the enzyme activity. Supplementation at 45 mg/kg indicated that Vitamin E and Lipoic acid have no significant difference between their effects on GPX activity.

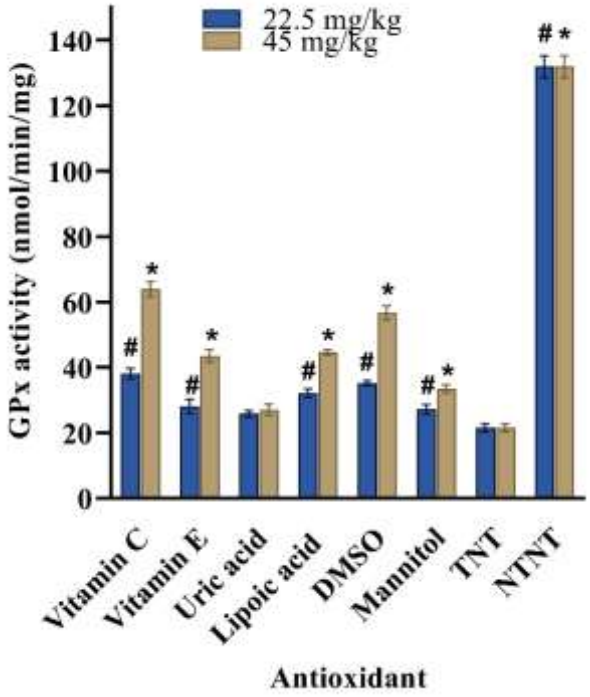

Fig. 8 Effects of LMWA on the GPX Activity in Brain Tissue; GPX- Glutathione peroxidase, DMSO- Dimethyl sulfoxide, TNT- Traumatized non-treated, NTNT- Non traumatized non-treated. Values are significantly different ($^{\#}p<0.05$) compared to TNT at 22.5 mg/kg and ($^{*}p<0.05$) compared to TNT at 45 mg/kg

The Effect of Supplementation with LMWA on Brain Tissue MDA Level

The results in Fig. 9 indicated that TBI caused significant ($p<0.05$) increase in the level of MDA in the brain of TNT rats. After administration of the antioxidants in two different doses, the concentration of MDA decreased significantly ($p<0.05$) in all the treated groups in a concentration dependent manner when compared to TNT group. Vitamin C and DMSO showed higher effects than all the treatment groups.

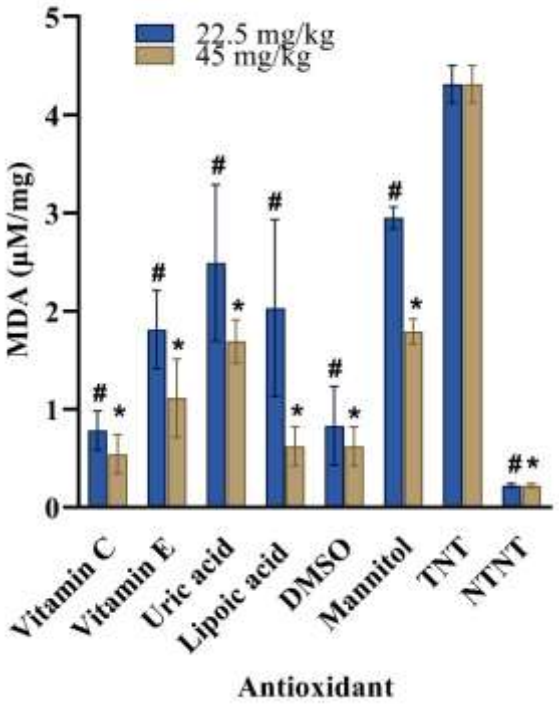

Fig. 9 Effects of LMWA on the Level of MDA in Brain Tissue; MDA- Malondialdehyde, DMSO- Dimethyl sulfoxide, TNT- Traumatized non-treated, NTNT- Non traumatized non-treated Values are significantly different ($^{\#}p<0.05$) compared to TNT at 22.5 mg/kg and ($^{*}p<0.05$) compared to TNT at 45 mg/kg

Discussion

In this study, the neurological assessment was conducted to include only animals with moderate scores (mNSS of 8-10) to ensure homogeneity in injury distribution and trauma level after induction (Fig.1). The results of the assessment indicated significant improvement in all treated groups (from an average score of 8-9 to 4-1), whereas the untreated group showed only minimal improvement (from an average score of 9 to 7). The mitigating effects of antioxidants on oxidative damage may be linked to the observed reestablishment of neurological function in the groups receiving treatment. This is due to the fact that TBI induces both structural damage and functional impairments through OS, which consequently impacts neurological function (25).

In this study, the assessment of OS was conducted by means of quantifying the levels of SOD, CAT, GPx, and MDA, which serve as indicators of enzymatic antioxidant activity and lipid peroxidation. Our findings indicated a substantial reduction in the activities of SOD, CAT, and GPx, and a corresponding increase in the concentration of MDA in both the serum and brain tissues of TNT rats, in comparison to NTNT rats (refer to Fig. 2-9). Such observations suggest that the induced TBI

leads to oxidative stress. However, the administration of vitamin C, vitamin E, α-Lipoic acid, Uric acid, DMSO, and Mannitol, in two different doses, showed an alleviation of the induced OS in a dose-dependent manner (refer to Fig. 2-9).

Treatment vitamin C resulted in a noteworthy increase ($p< 0.05$) in the activities of SOD, CAT and GPx, and a reduction in the concentration of MDA in the serum and brain tissues of the treated groups, in comparison to the TNT group (refer Fig. 2-9). These effects were observed to be dose-dependent. It is plausible that these benefits are attributed to the potential of vitamin C to counteract free radicals and mitigate their oxidative activity on lipids, proteins, and nucleic acids, which in turn leads to the suppression of the antioxidant system and the accumulation of MDA. It may also be attributed to the revitalization of vitamin E and glutathione by vitamin C, which exhibit remarkable efficacy against ROS as indicated by Denniss et al. (26). In this study, it was also observed that vitamin C exhibited a higher degree of antioxidant effect, second only to DMSO. This finding may be attributable to its robust scavenging and reduction potential, as well as it's ability to enhance various substances that possess antioxidant properties, such as α-Lipoic acid, vitamin E, Uric acid and other mild antioxidants.

The administration of vitamin E at doses of 22.5 and 45 mg/kg to rats with TBI led to a significant increase ($p<0.05$) in the activities of SOD, CAT, and GPx in both serum and brain tissues, while there was a significant decrease ($p<0.05$) in the concentration of MDA in these tissues, as observed in Fig. 2–9. Moreover, this outcome was dose-dependent, which could be attributed to the particular characteristics of vitamin E as a highly relevant chain-breaking antioxidant that is abundantly present in cells and mitochondria membranes, where the generation of ROS also occurs, and their dysfunction results in the excessive release of free radicals. Thus, it is possible that its mode of action was through the inhibition of lipid peroxidation and OS in these vital locations of free radical production, as indicated by Inci et al. (27). Ehizuelen et al. (28) has indicated that vitamin E, a lipid-soluble antioxidant, effectively averts the formation of lipid peroxide. It is also widely recognized that, aside from its direct impact on ROS, vitamin E can interact with a range of antioxidants such as vitamin C, GSH, and β-carotene to potentiate synergistic activity. As a result, these antioxidants facilitate the regeneration of vitamin E, which in turn enhances its therapeutic efficacy (29).

The outcomes of this research demonstrated that groups administered with Uric acid

exhibited a significant ($p<0.05$) elevation in the activities of SOD, CAT, GPx, and a significant ($p<0.05$) reduction in the level of MDA in serum and brain tissue when compared to their TNT counterpart (as illustrated in Fig. 2–4). This effect was found to increase in a dose-dependent manner. This can be attributed to the ability of uric acid to prevent OS through the scavenging of free radicals and chelating metal ions, which promote the development of free radicals, as indicated by Watanabe et al. (30). The decline in MDA caused by Uric acid can be attributed to its inhibitory role against lipid peroxidation and inflammatory reaction. The latter can result in increased ROS levels and tissue oxidation. According to Jagroop et al. (31), urate has been found to be effective in preventing lipid peroxidation, whereas its anti-inflammatory effect has been reported by Hooper et al. (32).

The application of α-Lipoic acid resulted in a notable enhancement of the diminished levels of SOD, CAT, and GPx caused by free radicals as compared to the TNT group (Fig. 2-9). Additionally, the use of α-Lipoic acid led to a meaningful decrease in MDA levels among the treated groups in contrast to the TNT group, with a dose-dependent effect observed wherein higher doses yielded greater efficacy. Notably, these outcomes are potentially attributed to the chelation of metal ions by α-Lipoic acid, which in turn impedes the metal-dependent production of free radicals, as supported by Patwa et al. (33). The ability of α-Lipoic acid to recycle vitamin C and vitamin E, both potent free radical scavengers as reported by Jones et al. (34) and Packer et al. (35), may be responsible for its observed effects. Furthermore, it has been demonstrated that α-Lipoic acid metabolites possess anti-inflammatory properties, according to Mei and You-wen's study (36). When pro-inflammatory cells and substances are mobilized to the site of injury, they trigger the release of more ROS, which can lead to OS. Modulating this reaction can help to mitigate the negative effects of OS.

In the present study, DMSO administration resulted in a significant ($p< 0.05$) elevation of antioxidant enzymes (SOD, CAT, GPx) levels in both serum and brain tissue of treated rats, when compared to the TNT group (Fig. 2-9) in a does-dependent manner. Additionally, DMSO exhibited a decreasing effect on the MDA level, with the concentration being reduced to the level observed in the NTNT rats at a dose of 45 mg/kg in serum. The observed effects of DMSO could be attributed to its ability to scavenge free radicals (37), which might have prevented the accumulation of free radicals that consumed the enzymatic

antioxidants and reduce their levels. As a result, DMSO's scavenging effect could have minimized the chain reaction oxidation of lipids by free radicals.

In the present study, the administration of mannitol resulted in a significant ($p< 0.05$) increase in the serum levels of SOD, CAT, and GPx, along with a decrease in the serum concentration of MDA, in a dose-dependent manner in the treated groups compared to the TNT group (Fig. 2-9). These beneficial effects were also observed in brain tissue. The observed antioxidant effect of mannitol can be attributed to its free radical scavenging properties. This is supported by previous findings by Kalemci et al. (38) who demonstrated the free radical scavenging effect of mannitol. Mannitol finds extensive usage in the reduction of cerebral edema during TBI. The accumulation of water in the brain results in an increase in intracranial pressure, which leads to a decrease in cerebral blood flow, thereby worsening tissue damage as a consequence of brain ischemia. Hence, the anti-edema effect of mannitol might have contributed to its antioxidant effect observed in this study (39).

The findings that were obtained from the present study indicate that the Low-Molecular-Weight antioxidants that were examined exhibited promising and beneficial effects concerning neuroprotection, as well as the modulation of Oxidative Stress (OS), thereby ameliorating neurological deficits in the experimental rats. Additional studies must be conducted to investigate the molecular mechanisms of these antioxidants concerning TBI pathophysiology and functional outcomes.

Conclusion

To conclude, after TBI induction, a remarkable decrease in antioxidant enzyme activities was observed, accompanied by an increase in MDA level, indicating the occurrence of oxidative stress. The administered antioxidants demonstrated their neuroprotective and neurorestorative potential by enhancing the antioxidant capacity, inhibiting lipid peroxidation, and improving the neurological deficits. These encouraging outcomes suggest that low-molecular-weight antioxidants may hold potential in the management of TBI.

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Conflict of Interest

The authors have no relevant financial or non-financial interests to disclose.

Ethical approval

This study was performed in line with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving animals. Approval was granted by the Ethics Committee of Usman Danfodiyo University Sokoto. Animal care was done by following institutional guidelines.

Author Contribution

Lawal Suleiman Bilbis and Ibrahim Bulama contributed to the conception. Material preparation, investigation and data collection were performed by Ibrahim Bulama. Nasiru Suleiman curated the data. Yusuf Saidu and Yusuf Yakubu provide supervision. Umar Faruk Saidu performed the formal analysis and prepared Figures 1-9; Abdullahi Yahaya Abbas, Umar Faruk Saidu, and Nasiru Ismail Jinjiri wrote the first draft of the manuscript. Umar Faruk Saidu and Ibrahim Bulama wrote the final version of the manuscript. All authors contributed to manuscript revision, read and approved the final manuscript.

Data Availability Statement

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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The "Eye of the tiger sign" in Progressive Supranuclear Palsy: Is it a coincidence or not?

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Abstract

Progressive supranuclear palsy (PSP); is a neurodegenerative disorder involved in atypical parkinsonism syndromes. The classical clinical presentation is postural instability, falls, downward paralysis, frontal dementia, and symmetric akinetic-rigid parkinsonism. The atrophy of the mesencephalon in magnetic resonance imaging (MRI) is an important marker in diagnosing the disease. Recently, a few PSP cases reported the "eye of the tiger" sign on MRI. The "eye of the tiger" sign, in globus pallidus, is a sign that bilaterally symmetrically located low signal intensity and central longitudinal hyperintensity are observed. While previously a specific finding for the pantothenate kinase-associated neurodegeneration (PKAN), it is no longer considered specific because of the reported cases of non-PKAN with the "eye of the tiger" sign such as neuropherritinopathy, multi-system atrophy, corticobasal degeneration. In this report, we aimed to contribute to the literature by presenting two PSP cases in which the "eye of the tiger" sign was observed.

Key words*: Progressive supranuclear palsy; the "eye of the tiger" sign; iron accumulation*

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Background

Progressive supranuclear palsy (PSP) is a degenerative disorder of the central nervous system and one of the Parkinson-plus syndromes. The classic clinical features of PSP are postural instability, falls, supranuclear palsy with downgaze paralysis, frontal dementia, and symmetric akinetic-rigid parkinsonism (1).

Despite several diagnostic studies, the diagnosis of PSP is made only by history, neurological examination, and magnetic resonance imaging (MRI). In MRI studies, distinctive atrophy of mesencephalon tegmentum is a sign of disease (2). Recent MRI studies have shown the "eye of the tiger" sign in some PSP patients (3). The eye of the tiger sign is typically seen in iron accumulation diseases such as Pantothenate Kinase-Associated Neurodegeneration (PKAN) and neuroferritinopathy (4). In this article, we aimed to present two PSP patients with the "eye of the tiger sign" finding on MRI.

Case 1

An 83-year-old man admitted to the nephrology department with acute renal failure was referred to our clinic because of disorientation, hallucinations, and loss of consciousness. He had been suffering from forgetfulness for the last three years. His daily activities worsened, and he has dysphagia, dysarthria, and behavioral changes. His son had noticed that he had significantly slowed down while walking and sometimes fell. For the last year, complaints of urinary incontinence have appeared. His previous records indicated ongoing problems with myelodysplastic syndrome for the last year. On neurologic examination, he was apathic. Upward gaze paralysis, hypomimia, antecollis, axial rigidity, and bradykinesia were present. No tremor was evident on examination. No pyramidal symptoms were detected. His standardized mini-mental test score was 14/30 (Orientation: 3/10, memory: 3/3, attention and calculation: 0/5, recall: 0/3, language 8/9). On cerebral MRI, a "hummingbird sign "in the sagittal T1 section and a "mickey mouse" appearance in the axial T2 section were seen (Figure 1).

In addition, the eye of the tiger sign on the axial T2 section and iron accumulation in globus pallidus, substantia nigra, and red nucleus on SWI (Susceptibility Weighted Imaging) was seen (Figure 2).

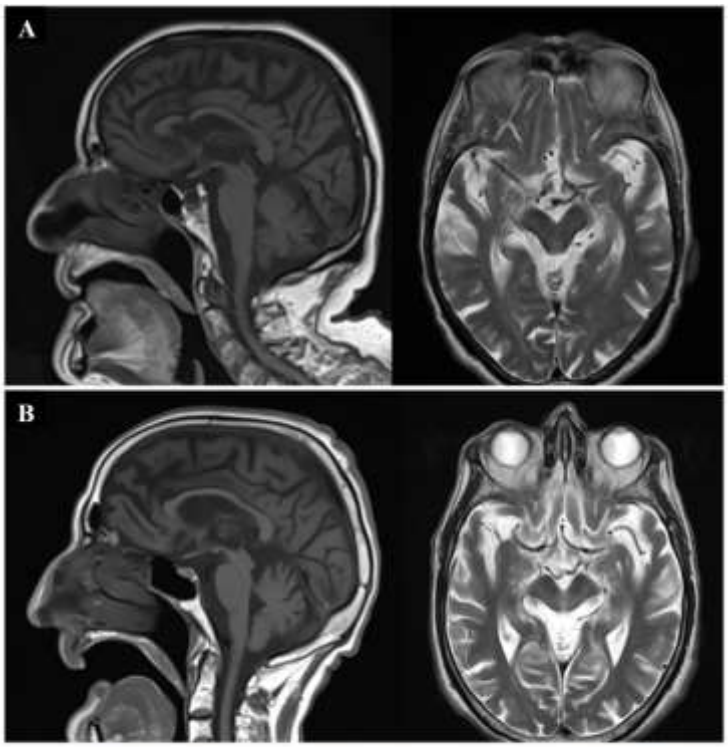

Figure 1: A; Case 1, **B;** Case 2, In cerebral MRI, it has shown" hummingbird sign" at mesencephalon in T1 midsagittal section examination, and "mickey mouse sign" in axial T2 section.

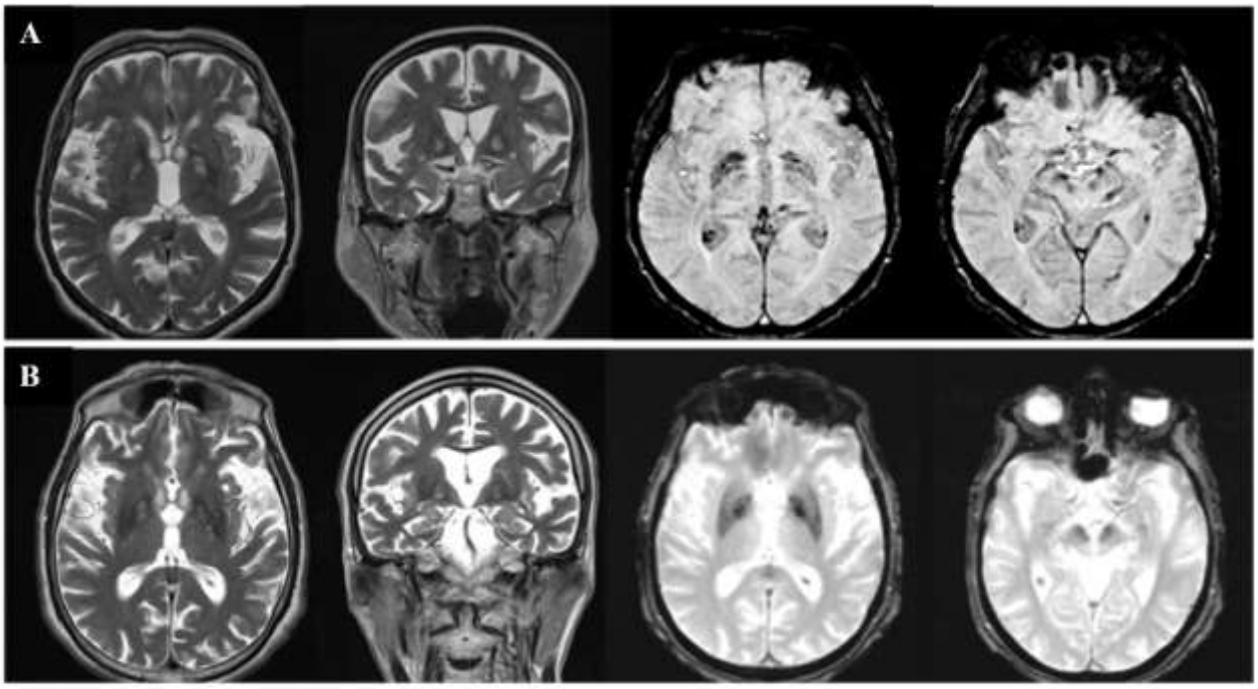

Figure 2: A; Case 1, **B;** Case 2, Cerebral MRI examination shows a view consistent with the eye of the tiger sign in the left-sided axial T2 section and next to the coronal section of the same area. In the two sections on the right, observed view consistent iron accumulation in axial examinations of case 1 with SWI and case 2 with gradient echo in bilateral globus pallidus, substantia nigra, and red nucleus.

The patient was diagnosed with PSP-Richardson syndrome (RS) according to the 2017 MDS (The Movement Disorder Society) criteria, and his UPDRS (Unified Parkinson's Disease Rating Scale) score was 71 points. 62.5 mg madopar (combination of levodopa and benserazide hydrochloride) three times per day was given to the patient, and after one week, his UPDRS score decreased to 50 points.

Case 2

A 77-year-old man presented to our clinic with a 5-month history of dizziness, complaining of walking difficulties, dysarthria, and dysphagia. He reported that he would suddenly fall without a trigger. He did not have any disease in his history. On examination, he had dysarthria, hypophonia, palilalia, and dysphagia. There was moderate bilateral rigidity, bradykinesia, and reduced blinking frequency and facial expression. He had impaired vertical gaze, predominantly on upward gaze. He had a stooped posture and reduced arm swing without a tremor on gait examination. On the pull test, he had significant retropulsion and would have fallen if not caught by the examiner. His blood tests, including ferritin and ceruloplasmin, were within normal limits. On cerebral MRI, a "hummingbird sign "in the sagittal T1 section and a "mickey mouse" appearance in the axial T2 section were seen (Figure 1). The eye of the tiger sign on axial T2 and iron accumulation in globus pallidus, substantia nigra, and red nucleus were also seen on gradient echo (Figure 2). According to the 2017 MDS criteria, the patient was diagnosed with PSP-RS, and his UPDRS score was 54. After a mild response to the levodopa test, a combination of levodopa and carbidopa 125/12,5 mg three times per day and rasagiline 1 mg per day was begun.

Discussion

The "eye of the tiger sign" is a radiological sign. This sign indicates abnormally low signal intensity and central longitudinal hyperintensities symmetrically located in the globus pallidus in T2-weighted MR images. This sign is the most prominent radiological feature of iron accumulation in the brain, formerly called Hallervorden-Spatz disease. Iron is not present in the central nervous system at birth; however, it occurs throughout life in healthy adults. The highest concentration is reached in the globus pallidus, primarily in the form of the metalloprotein ferritin (5).

Differential diagnosis of symmetrical basal ganglion involvement, except PKAN, includes mitochondrial diseases (Leigh disease), methylmalonic acidemia, Wilson's disease, toxin exposure (CO, Methanol, Cyanide), and hypoxic damage. The

exciting part of our cases was MRI findings, including the "eye of the tiger sign" and iron deposition in the bilateral basal ganglia and the substantia nigra (Figure 2) (6). Nevertheless, our patients' history, age, and other clinical findings were inconsistent with these situations.

The eye of the tiger sign was observed in almost all patients with PKAN. This sign was also observed in some patients with a multi-system atrophy-parkinsonian type (MSA-P) (7), corticobasal degeneration (CBD) (8), and pure akinesis (9). Davie CA et al. also reported the "eye of the tiger sign" in a few PSP patients, as in our cases. Iron accumulation was not evaluated in MSA, pure akinesia, and CBD patients in these cases (7-9). Tokunori ve Ikeda has reported a case of atypical parkinsonism in which iron accumulation was evaluated and seemed to be the "eye of the tiger sign"; there was no evidence of iron deposition. For this reason, they called this sign the "pseudo eye of the tiger sign" and emphasized the need for research on iron accumulation in these cases (10).

Besides, Akashi et al. described a 60-year-old male patient who was clinically diagnosed with PSP and detected changes of spesific to the PKAN and changes in the typical PSP in the postmortem examinations (11). Then, Yamamoto et al. conducted a case of a patient with the characteristics of both pathologies has been reported (12).

Iron accumulation secondary to myelodysplastic syndrome accompanied by PSP disease as coincidence may be developed in the first case. Iron accumulation occurs due to excessive iron absorption from the intestinal tract caused by chronic anemia and recurrent blood transfusions. Therefore, iron accumulation exists in both parenchymal tissues and reticuloendothelial system. Even if there is no transfusion in these patients, iron accumulation occurs (13). Both cases had no transfusion history, and the ferritin level was within the normal range. In the second case, there was no history of disease-causing iron accumulation. It was demonstrated iron deposition with MRI in both patients indirectly.

Conclusion

These cases bring to mind a question "Are the patients with PSP with the "eye of tiger sign" another part of a spectrum of iron accumulation diseases." Another question is, "Does iron accumulation originate secondary to the accompanying diseases or increased iron accumulation due to advanced age?". If iron accumulation is due to advanced age in case, why do we not see the same sign in all patients with PSP? We

only think more case reports and studies can resolve this uncertainty.

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Investigatıon of Inflammatory Marker Levels in Overactive Bladder Patients

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Abstract

***Aim**: To investigate whether inflammation plays a role in the pathogenesis of patients diagnosed with overactive bladder (OAB).*

***Material-Method**: Patients who applied to the urology outpatient clinic with a preliminary diagnosis of OAB between March 2022 and September 2023 and were diagnosed were prospectively included in the study. With the OAB V8 scores (0-40) and the anticholinergics used, the number of urgency attacks, pollakiuria and nocturia were also recorded. Blood group, complete blood count, inflammation markers and biochemical values recorded.*

Patients who presented to the outpatient clinic with non-OAB were included as the control group. Data recorded at baseline were compared between groups.

***Results**: A total of 198 patients were included in the study (OAB group n: 99, control group n: 99). No statistically significant difference was observed between the groups in terms of gender and blood group, hemoglobin, hematocrit, lymphocyte, platelet, AST, ALT, albumin, neutrophil-lymphocyte ratio (NLR), lymphocyte-monocyte ratio (LMR), platelet-lymphocyte ratio (PLR), Deritis, MLR and CRP/albumin ratio.*

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The mean age of the OAB group was found to be significantly higher than the control group (p<0.001). The mean WBC, neutrophil, monocyte and CRP values of the control group were found to be statistically significantly higher than the OAB group.

As a result of the multivariate analysis, only age was found to be a predictor for OAB.

***Conclusion**: According to the results of our study, inflammation markers used in our study were found to be low in OAB patients.*

***Key words**: Overactive bladder, inflammation, neutrophils*

Introduction

Overactive bladder is a syndrome characterised by urinary urgency, usually accompanied by frequency and nocturia, with or without UUI, in the absence of UTI or other obvious pathology (1). Diagnosis is based on symptoms. It is a chronic disease that significantly reduces quality of life.

Many theories have been proposed to explain the pathophysiology. The main known mechanism is detrussor hypersensitivity or imbalance in the inhibitory and excitatory pathways of the bladder. Many biomarkers have been identified that are thought to be effective in its pathogenesis (2). These include nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), prostoglandins, cytokines and CRP (3, 4).

Based on the assumption that inflammation plays an aetiological role in OAB, some authors have investigated the role of urinary cytokines in patients with OAB and whether they can be considered as a biomarker for the disease (5, 6). Biomarkers aim to aid diagnosis in OAB, monitor disease progression, and potentially assess response to treatment. However, there is no strong data on the usability of these biomarkers in the evaluation of diagnosis and response to treatment in the studies conducted so far.

In our study, we aimed to investigate whether inflammation markers play a role in the diagnosis of OAB patients.

Material-Method

Ethics committee approval dated 11.05.2023 and numbered 2023-10/1 was obtained from Erzincan Binali Yıldırım University Faculty of Medicine Ethics committee for the study.

Between May 2022 and September 2023, patients between the ages of 18 and 60 who were admitted to the urology outpatient clinic with a preliminary diagnosis of OAB and diagnosed with OAB were prospectively included in the study. OAB was diagnosed according to the criteria defined by the International Continence Society (7). The study was conducted in accordance with the principles of the 2008 Helsinki Declaration.

Demographic data including age, gender and BMI were recorded. Demographic data of the patients were recorded. With the OAB V8 scores (0-40) and the anticholinergics used, the number of urgency attacks, pollakiuria and nocturia were also recorded. The OAB-V8 form is a form consisting of 8 questions with each question being scored between 0-5 and a total score of 40 points. A total score above 8 is considered significant (8). Blood group, complete blood count and biochemical values, inflammation markers (NLR, PLR, LMR, CRP/Albumin ratio and De ritis ratios) were recorded from peripheral blood at outpatient clinic visits.

Patients with urinary tract infection, systemic inflammation, renal function test disorders, patients with a history of previous urological surgery, patients with a history of urological malignancy, patients with bladder stones, benign prostatic hyperplasia and urethral stricture were excluded from the study. Patients with haematological diseases that might affect haemogram parameters were also excluded from the study. As a control group, patients who were admitted to the outpatient clinic for other reasons and who did not have OAB symptoms were included. Haemogram and biochemical values of these patients were also recorded.

The data recorded at baseline were compared between the groups.

Statistical Analysis

Statistical analysis was performed using IBM SPSS version 21 (IBM Corp., Armonk, NY, USA). Variables were expressed as mean ± standard deviation and percentage. The Kolmogorov-Smirnov test was applied to examine normal distribution. Differences between 2 groups were analysed by Mann-Whitney U test. Chi-square test was used to compare qualitative data. Logistic regression analysis was used to identify predictors of OAB. Predictive

accuracy for diagnosis was assessed by area under the curve (AUC) of ROC analysis. G-Power 3.1.9.4 statistical power analysis programme was used to calculate the sample size of the study. $p < 0.05$ was considered statistically significant.

Results

According to the power analysis results (two-way correlation, type-1 error rate (α)=0.05, power of the study (1-β)=0.80 and effect size=0.52), a sufficient number of patients were included in each group (n=99). A total of 198 patients were included in the study. 99 patients were included in the OAB group and 99 patients were included in the control group.

The mean age of all patients was 52.3±15 years and the mean OAB-V8 score was 11.9±9.9. Male/female ratio was 101/98.

No statistically significant difference was observed between the groups in terms of gender and blood group (Table 1). No statistically significant difference was observed between the groups in terms of haemoglobin, haematocrit, lymphocyte, platelet, AST, ALT, albumin, LMR, PLR, De ritis, MLR and CRP/albumin ratio. The mean age of the OAB group was significantly higher than the control group ($p<0.001$). Mean WBC, neutrophil, monocyte and CRP values of the control group were significantly higher than those of the OAB group (p values were 0.007; 0.024; 0.010; 0.048, respectively) (Table 2).

In the multivariate analysis, only age was found to be a predictor for AAM (Table 3).

Table 1: Comparison of blood group and gender of the groups

	OAB group(n:99)	**Control group(n:99)**	**p value**
Gender (n/%)			0.394
Male	47 (47.4%)	54 (54.5%)	
Female	52 (52.6%)	45 (45.5%)	
Blood group (n/%)			0.761
A+	23 (23.2%)	21 (21.2%)	
A-	17 (17.2%)	21 (21.2%)	
B+	18 (18.1%)	13 (13.1%)	
B-	11 (11.1%)	13 (13.1%)	
AB+	5 (5.1%)	2 (2.2%)	
AB-	5 (5.1%)	3 (3%)	
0+	11 (11.1%)	16 (16.1%)	
0-	9 (9.1%)	10 (10.1%)	

Table 2: Comparison of inflammation markers between groups

Parameters (ort±SS)	OAB group (n:99)	Control group (n:99)	p value
Age (years)	57.5±13.7	47±14.5	**<0.001**
BMI (kg/m2)	25.2±1.4	25.4±1.5	0.393
OAB-V8 score	21.4±3.8	2.4±1.8	**<0.001**
Urgency attacks	2.7±0.7	0.05±0.22	**<0.001**
Hg (g/dL)	13.9±1.4	14.2±1.8	0.273
Hct	41.4±4	41.7±4.8	0.619
WBC (10^3/µL)	7.2±2.5	8.4±3.3	**0.007**
Neutrophils (10^3/µL)	4.5±2.3	6.1±6.3	**0.024**
Lymphocytes (10^3/µL)	2±0.6	2.1±0.8	0.173
Platelets (10^3/µL)	243±62	252±54	0.267
Monocyte (10^3/µL)	528±217	624±292	**0.010**
AST (U/L)	22.2±7.1	24.7±12.8	0.085
ALT (U/L)	23.9±23.9	24.4±17.3	0.884
CRP (mg/dL)	5.7±10.07	11.3±26.5	**0.048**
Albumin (g/dL)	41±5	41.9±4	0.163
NLR	2.9±3.9	3.6±4.7	0.28
PLR	139.7±102.2	152±156.8	0.513
LMR	4.6±4.4	3.9±1.7	0.145
De ritis rate	1.1±0.4	1.2±0.6	0.424
MLR	0.3±0.2	0.3±0.3	0.267
CRP/Albumin rate	0.1±0.2	0.2±0.7	0.056

BMI: Body mass index, OAB-V8: Overactive bladder form, Hg: Haemoglobin, HCT: Haematocrit, WBC: White blood cell, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, CRP: C reactive protein, NLR: Neutrophil to lymphocyte ratio, PLR: Platelet lymphocyte ratio, LMR: Lymphocyte monocyte ratio, MLR: Monocyte lymphocyte ratio De ritis: AST/ALT

Table 3. Multivariate analysis

Parameters	OR	95% CI	p value
Age	1.055	1.031-1.079	**<0.001**
Neutrophils	1.000	1.000-1.000	0.126
CRP	0.976	0.948-1.004	0.094

CRP: C reactive protein

According to ROC analysis, a cut-off value of 49.5 (years) for age had a sensitivity of 76% and a specificity of 57% for predicting AAM (AUC: 0.705, 95%CI=0.632-0.777, p<0.001) (Figure 1).

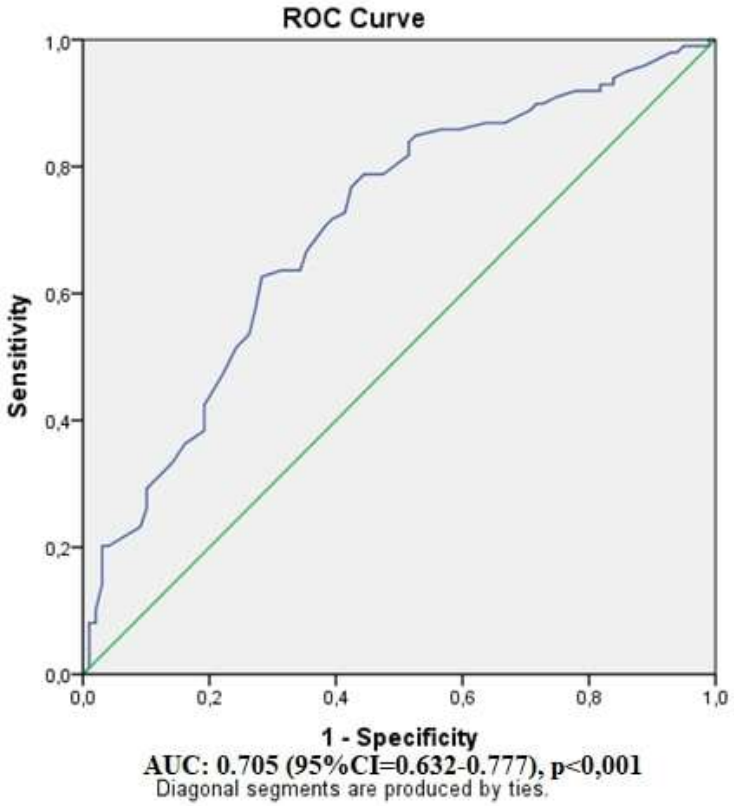

Figure 1: ROC curve for age in the diagnosis of AAM

Discussion

Overactive bladder is a pathology involving very complex processes involving the bladder urothelium, bladder nerves and central nervous system. The pathology causing this disease has not been elucidated so far. It is important to determine the underlying causes in order to provide safer and more effective treatment options (9). In our study, we investigated whether inflammation markers play a role in the pathogenesis of OAB. According to our results, inflammation markers were found to be lower in OAB patients than in the control group. In addition, the mean age of the patients in the OAB group was statistically significantly higher than that in the control group.

In our study, the mean age of the OAB group was 57.5 years, while the mean age of the control group was 47 years. Studies have shown that OAB symptoms increase with advancing age in both men and women (10). In the study conducted by Benli, the mean age of the OAB group was found to be significantly higher than that of the control group patients (11).

Involuntary contractions of detrussor muscle fibres play a role in the pathogenesis of overactive bladder. Although it is thought that stimuli originating from urothelium and suburothelium are the initiating factor of involuntary contractions, the cause has not been elucidated yet. Inflammation is one of the reasons to be emphasised. Kupelyan et al. in their study of 2301 male and 3202 female patients, showed a consistent association between increased CRP levels and OAB among both men and women. Among men, an increase in symptoms was observed above CRP>3 mg/L, whereas among women, an increase in symptoms was observed above CRP>1 mg/L (12).

In the present study, no correlation was found between NLR, LMR, PLR and LMR, which are well-known markers of inflammation, and OAB. There are contrary results to our findings in the literature. Çulha et al. 77 OAB and 80 control group patients, NLR and CRP values were found to be higher in the OAB group compared to the control group (13). In a study of 4394 South Korean women, the association of NLR with OAB was investigated. Among this group, 432 patients were diagnosed with OAB. In the analysis, the mean NLR value in the OAB group was found to be higher than the other group. In addition, NLR was found to be associated with the severity of OAB (14).

Similar to our findings in the literature, there are also studies supporting that inflammation does not play a role in OAB patients. Tyagi et al. concluded that PDGF, IL-1β, CCL2, CXCL1, CXCL8, and CXCL10 levels were not different in the OAB group compared to the control group (15). Similarly, Pennycuff et al. 38 OAB and 29 healthy volunteers concluded that NGF, BDNF, Substance p and CGRP levels were not different between the two groups and that NGF and BDNF levels increased with age (16). In a study investigating CRP levels in BPH patients, Inamura et al. showed that CRP was not associated with storage function. The authors emphasised that the cause of OAB exacerbation may be due to a cause other than elevated CRP levels (17). Considering that ischaemia plays an important role in the pathogenesis of OAB, tissue damage markers (NGF, BDGF) are expected to increase as a result of denervation of bladder nerve fibres.

Since the inflammation markers we analysed in our study are non-specific markers, variability in serum levels may be expected for many reasons. We can explain the discrepancies between our results and the literature with this situation.

One of the limitations of our study is that it was performed with a small number of patients. The second limitation is that we used non-specific inflammation markers. The study of more specific inflammation markers analysed from urine may provide more accurate results.

Conclusion

In conclusion, in our study, no elevated levels of inflammation markers were found in patients with OAB. In order to elucidate the relationship between OAB and inflammation, more specific markers analysed in urine and studies with a higher number of patients are needed.

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Conflict of Interest: Regarding this study, the authors and/or their family members do not have any relationship with scientific and medical committee membership or members, consultancy, expertise, employment status in any company, shareholding and similar situations that may have the potential for conflict of interest.

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Determination of Mutagenic Potentials of Diarylmethylamine Based Imine Compounds By Ames Test And Computational Molecular Docking

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Abstract

In recent years, studies that investigate the effects of chemical compounds on organisms have increased in direct proportion to their widespread use. In this study, four different bidentate imine ligands and bidentate imine ligands+Cu(II) complexes were synthesized from the bioactive synthetic diarylmethylamine compound. After the ligands and metal complexes obtained were purified using chromatographic and analytical methods, their mutagenic effects were investigated with the Ames/Salmonella test system. In addition, interactions of four different Cu(II) complexes with B-DNA were evaluated with molecular docking analysis.

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Accordingly, the results indicated a significant increase in the colonies formed in the presence (+S9) and absence (-S9) of the metabolic activation system, meaning a mutagenic effect against strain TA98 and TA100 strains in general.

Key words: *Diarylmethylamine, Imine Compounds, Salicylaldehyde, Cu (II) Complexes, Salmonella/Microsome Test, Molecular Docking.*

1. Introduction

While the Industrial Revolution provided technological development, it also caused chemical pollution in the world. All living organisms are dealing with these pollutants in a slightly greater amount every day. There are many chemicals taken directly or indirectly in food consumed in daily life, inhaled air, clothes worn and drugs used for survival purposes (1). Living organisms are also exposed to these chemical pollutants, either directly or indirectly. Some of the chemicals do not cause any changes in the DNA structure of the living organism, or the resulting damage can be repaired through DNA repair enzymes, while some damage cannot be overcome. These damages in the living organism can cause mutations, recombinational changes, or structural chromosome errors. If these changes occur in reproductive attacks, they are passed on to subsequent generations, while if they occur in somatic attacks, they cause cancer (2). Various mutagenicity, toxicity, carcinogenicity and teratogenicity determination test systems have been developed to determine the effects of chemicals on living organisms. The studies, devoted to understanding the effects of chemical products on ecosystem and human health, are safety tests. For this purpose, back mutation tests are performed first (eg Ames). Additionally, medicines containing natural and synthetic chemical active substances are subjected to many safety tests considering the effects on human health and ecosystem before they are placed on the market (2, 3, 4, 5).

The Ames test system is a commonly used test to determine point mutations in DNA, such as modification, addition, or deletion of one or more base pairs. This test system uses *Salmonella typhimurium* strains (TA98 and TA100) derived by *in vitro* mutation from the LT2 ancestral strain (6).

It was first improved by Ames in 1975 (7, 8, 9, 10). TA100 has base change mutation, while TA98 has frameshift mutation. Because of these mutations of TA98 and TA100 strains, both strains cannot synthesize histidine (his-). The principle of the test system is based on the ability of strains TA98 and TA100 to re-synthesize histidine after exposure to various chemicals or test substances. Point mutations and frameshift mutations have been reported to induce tumor formation in both humans and animals due to base change mutations in tumor suppressor genes or oncogenes, and these mutations are also known to underlie many genetic diseases (6).

Schiff bases containing an azomethine group (-CH=N-) have been studied for a long time due to their high biological activity profiles (11). Numerous information on the properties of synthetic Schiff bases of potential biological interest suggests that some of these compounds have been identified and used as models for a number of systems **(**12, 13, 14, 15, 16).

Metal complexes are widely used in the treatment of cancer, arthritis and diabetes (17). Copper (II) complexes, for example, are known to be effective against joint inflammations and also to exhibit anti-ulcer activity (18, 19). This is significant because gastrointestinal irritation often hinders the treatment of other antiarthritic drugs. This suggests that copper's acidic anti-inflammatory agents play a role in preventing gastrointestinal damage (19).
The purpose of this study is to determine the mutagenic potential of four new Schiff base derivatives-Cu(II) complexes with the Ames/*Salmonella* test system. In this study, molecular docking analysis was performed using Autodock 4.2 for the purpose of the deeper perspective on the binding poses of four different bidentate imine ligands-Cu(II) complexes with B-DNA.

2. Materials and methods

All chemicals and solvents were of high quality and purchased from commercial suppliers (Aldrich or Merck). Elemental analyses (C, H, N) were carried out using Costech ECS 4010 (CHN). Infrared spectra were obtained using KBr disc (4000-400 cm^{-1}) on a PerkinElmer Spectrum 100 FT-IR. ^{1}H and ^{13}C NMR (Nuclear Magnetic Resonance) spectra were recorded on a Bruker 400 MHz instrument and TMS was used as an internal standard. Below presents the methodological concerns in detail:

2.1. Phenyl(p-tolyl)methanamine

The amine 2 was synthesized from benzoic acid following a detailed set of literature procedures (20, 21, 22). ^{1}H-NMR and ^{13}C-NMR data are in compromise with the literature data (22).

2.2. The method for the synthesis of imine compounds (3a-d).

The imine compounds were successfully prepared by following the well-described literature method. The ^{1}H-NMR data and ^{13}C-NMR data are in compatibility with literature (22).

2.3. The method for the synthesis of metal complexes (3a-d-Cu)

The complexes were prepared according to a known procedure. The Schiff base ligand (0.21 g; 0.0006 mol) was dissolved in MeOH (20 mL) solution and the metal salts [$CuCl_2.2H_20$ (0.107 g; 0.0006 mol) were added to the mixture of MeOH (20 mL) and the solution was refluxed for about 48 h. After confirming that the reaction was completed by thin layer chromatography, it was cooled to room temperature. The reaction mixture was evaporated; it was purified by filtration in a 3:1 hexane / ethyl acetate solvent mixture. The purity of the complexes was checked by TLC (Thin Layer Chromatography) studies.

3a-Cu: Color: Brown. melting point: 198-200°C. FTIR: (υ_{max} cm^{-1}): 3445, 3344, 3164, 1605, 1534, 1408, 1251, 995, 845, 696, 558 cm^{-1}. Anal. Calcd for $C_{22}H_{20}ClCuNO_2$: C, 61.54; H, 4.69; N, 3.26. Found C, 61.58; H, 4.71; N, 3.28.

3b-Cu: Color: Light brown. melting point: 98-102°C. FTIR: (υ_{max} cm^{-1}): 3410, 3153, 3010, 2920, 2577, 1592, 1506, 1374, 1279, 1194, 1096, 830, 777, 705, 549 cm^{-1}. Anal. Calcd for $C_{22}H_{20}ClCuNO_2$: C, 61.54; H, 4.69; N, 3.26. Found C, 61.52; H, 4.67; N, 3.27.

3c-Cu: Color: Brown. melting point: 150-152°C. FTIR: (υ_{max} cm^{-1}): 3421, 3159, 3038, 2923, 1606, 1522, 1453, 1242, 1185, 1094, 1028, 812, 703, 550 cm^{-1}. Anal. Calcd for $C_{21}H_{18}ClCuNO_2$: C, 60.72; H, 4.37; N, 3.37. Found C, 60.69; H, 4.38; N, 3.36.

3d-Cu: Color: Brick red. melting point: >250°C. FTIR: (υ_{max} cm^{-1}): 3452, 3351, 2922, 2224, 1608, 1542, 1509, 1447, 1385, 1260, 1230, 1036, 847, 806, 701, 555 cm^{-1}. Anal. Calcd for $C_{21}H_{18}ClCuNO_2$: C, 60.72; H, 4.37; N, 3.37. Found C, 60.71; H, 4.38; N, 3.36.

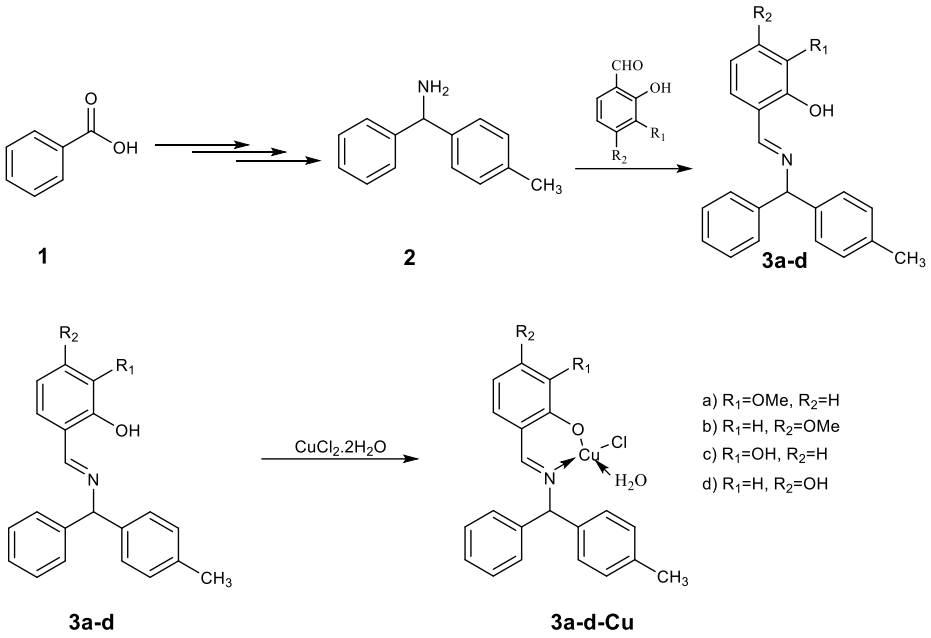


Figure 1. Synthesis scheme of imine compounds and metal complexes

2.4. The culture of the bacterial strains TA98 and TA100

2.4.1. Salmonella/Microsome test (Ames)

The Salmonella microsome test system is one of the most preferred short-term genotoxic tests to detect the mutagenic potential of chemicals due to its rapid results and low cost. In the experiment TA98 and TA100 strains of *Salmonella*

typhimurium LT2 ancestral strain developed with *in vitro* mutations were used. Prior to experimentation, both strains were checked for the presence of strain-specific markers as suggested by Maron and Ames. The standard plate insertion test was investigated both in the presence and absence of a mixture of *Salmonella typhimurium* TA98 and TA100. The same experiment was performed in the S9 mixture as suggested by Maron and Ames Ames (8).

2.4.2. Bacterial strains

TA98 and TA100 were purchased commercially (J.L. Swezey, Curator, ARS Patent Culture Collection, Microbial Genomics, and Bioprocessing Research Unit, North University Street, Peoria, Illinois 61604, USA.). To the detection of frameshift mutagens and base-pair substitution mutagens, TA100 and TA98 strain are used respectively. Before the bacterial strains were used in the experiment, the strains were checked for the

presence of strain-specific markers as suggested by Maron and Ames Ames (8).

2.4.3. Mutagenicity assay

In order to determine the mutagenic effects of the test substances, TA98 and TA100 strains were examined in environments with and without S9 Ames (8). The S9 factor mix was used as 1/10 of the total volume for each plate. Test substances were prepared at concentrations of 0.80, 0.40, 0.20, 0.10, and 0.05 μg/plate (Solvent distilled water). For medium with S9, 2-AF (2-amino fluorene) was used as a positive mutagen (20 μg/plate) (in strains TA98 and TA100). Without the S9 medium, 4-NPD (4-nitro phenylenediamine) was used as the positive mutagen (200 μg/plate). All experiments were performed at two different times and in five replicates for each sample.

2.4.4. Preparation of S9

The fraction with and without S9 was prepared according to the literature Ames (8). S9 tablets were purchased commercially (Roche, Cat. no: 1.745.425). Freshly prepared S9 fraction was stored at -35ºC. The S9 mix was freshly prepared before each experiment.

2.5. *In silico* molecular docking analysis

Molecular docking analyses were performed, using AutoDock 4.2 (23), to predict possible binding sites on the B-DNA (PDB code: 1BNA) crystal structure of four different bidante imine ligands and their Cu(II) complexes synthesized from the bioactive synthetic diarylmethylamine compound). The crystal structure of the 1.9 Å resolution B-DNA molecule was chosen as the target (receptor) molecule. AutoDockTools (ADT) was used to prepare the parameters before starting the docking analysis of the receptor and ligand molecules. Nonpolar hydrogen atoms of B-DNA and ligand molecules were combined and the non-polar hydrogen atoms were removed. Gasteiger charge were detected according to Ricci and Netz (24, 25). B-DNA and ligand structures were saved in PDBQT format. The grid box and grid spacing were set 60 × 60 × 60 Å and 0.375 Å, respectively. Dockings were constructed from 50 GA (Genetic Alghorithm) runs using an initial population of up to 150 individuals, $5x10^5$ energy evaluation counts, a maximum of 27,000 generation. Mutation and transmission rates performed at 0.02 and 0.8, respectively. 4 different ligand molecules' 100 docking results were examined. Autodock was clustered and ranked for receptor/ligand all possible binding modes and according to the free energy of binding (kcal/mol) of the conformation with the lowest binding free energy and the best docking pose. The best docking pose between ligands and B-DNA using the AutoDock 4.2 output file was analyzed in BIOVIA Discovery Studio Visualizer 2016 (26).

2.6. Statistical significance

To determined the significance of the between control revertants and revertants of treated groups, used t-test in SPSS.

3. Results

Viability test results suggested that the highest concentration was 0.8 μg/plate. For that reason, other concentrations of 0.05, 0.10, 0.20, 0.40, and 0.80 μg/plate were chosen for each test compound in the mutagenicity assay, respectively.

In our study, all test substances were showed significant mutagenic effects on TA98 and TA100 strains. Mutagenicity test results were given in Table 1.

Table 1. The mutagenicity of 3a-Cu, 3b-Cu, 3c-Cu and 3d-Cu on *S. Typhimurium* TA98 and TA100 strains in the presence or absence of S9 mix.

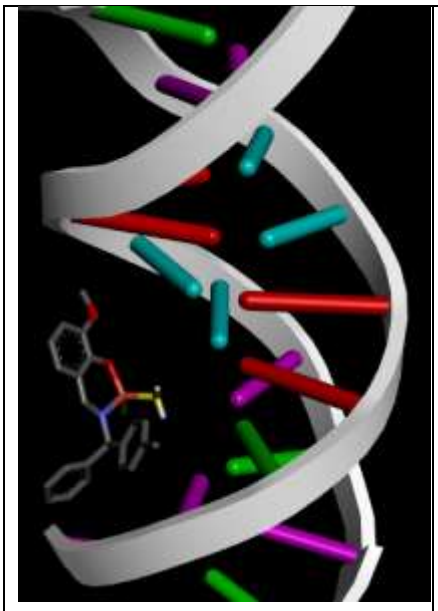
Test substances	Cont.	TA 98		TA 100	
	μg/plt.	– S9	+ S9	– S9	+ S9
Spontaneous Control	-	9.67±2.27	11.49±1.87	103.2±10.9	100.00±9.59
NPD		3001±172			
2-AF			3409±239		739.2±39.8
SA				644.8±50.2	
	0.80	26.34±3.40**	15.56±2.78	131.00±4.15**	52.14±4.86***
	0.40	31.19±1.84***	15.16±1.98	137.00±7.84*	47.26±7.09***
3a-Cu	0.20	22.40±1.86**	13.77±3.39	120.08±10.4	37.69±5.10***
	0.10	13.90±3.40	8.11±1.33*	110.32±9.12	31.70±2.74***
	0.05	13.40±1.67	7.59±3.40*	81.89±7.22**	41.22±15.23**
	0.80	26.51±2.48**	170.20±16.07***	28.73±3.00**	477.2±95.0***
	0.40	30.87±2.48***	144.00±12.34***	32.43±4.10**	489.5±92.3***
3b-Cu	0.20	28.17±2.77**	131.42±17.70**	29.18±2.91**	248.02±32.9**
	0.10	21.97±2.26**	85.0±12.89**	23.02±2.00**	200.6±22.67*
	0.05	21.00±2.58*	65.83±5.41***	18.13±1.87*	131.5±13.9
	0.80	35.37±1.78***	36.83±2.33***	121.17±2.32**	60.00±2.35
	0.40	28.33±2.17***	31.67±4.60**	90.33±3.99**	95.17±7.11
3c-Cu	0.20	27.33±1.23***	23.33±2.60**	93.50±3.98**	93.17±5.51
	0.10	29.33±2.54***	19.00±1.81**	74.0±4.03**	76.50±5.79*
	0.05	14.33±1.38**	15.00±1.37*	59.00±5.99***	67.2±10.9*
	0.80	29.17±3.63***	26.33±1.98***	138.70±3.10***	132.50±4.50***
	0.40	31.17±3.46***	29.33±3.65**	131.80±5.70**	126.00±2.30***
3d-Cu	0.20	27.00±2.44**	28.50±1.13***	124.70±4.10***	110.17±7.82*
	0.10	26.67±3.01*	27.33±1.00**	89.00±4.02*	98.33±9.75*
	0.05	16.67±1.94	18.67±1.93*	83.33±8.92	84.33±8.49***

*: P<0.05; **: P<0.01; ***: P<0.001

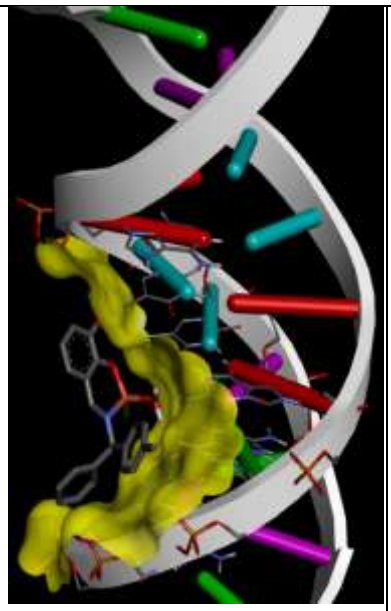
NPD: 4-nitro-o-phenylenediamine, 2AF: 2-Aminoflourene, SA: Sodium aside

The interaction of between target molecule (crystallographic B-DNA) and the 3a-Cu, 3b-Cu and 3c-Cu ligands based on gibbs free binding energies (kcal/mol) is shown in the B-DNA major groove (Fig. 1., 1a, 2a and 3a) while 3d-Cu ligand was found to bind to the B-DNA' minor groove (Fig. 2., 4a). Linkages with a mean distance measure (RMSD value) between the atoms of the B-DNA molecule of less than 2 Å were evaluated. At the same time, the lowest negative free binding energy (ΔG binding) was calculated for 3a-Cu, 3b-Cu, 3c-Cu and 3d-Cu ligands. Since the results were -5.09, -5.34, -4.46 and -6.73 kcal/mol, the interaction of ligands with B-DNA was thought to be significant. The best docking poses, including H-bonds of ligands and B-DNA, were shown in different poses (Figure 2).

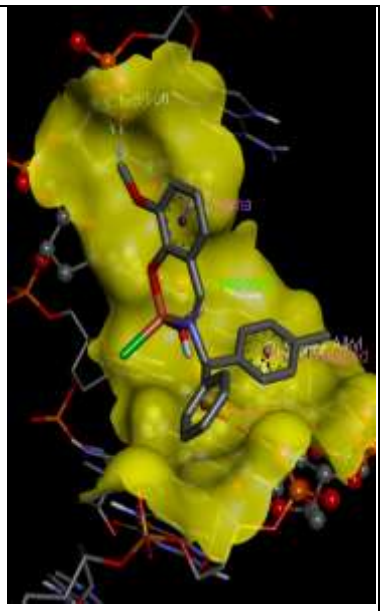
1-3a-Cu



1-a

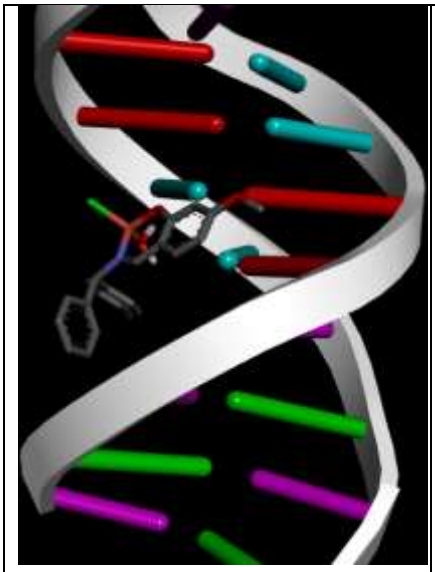
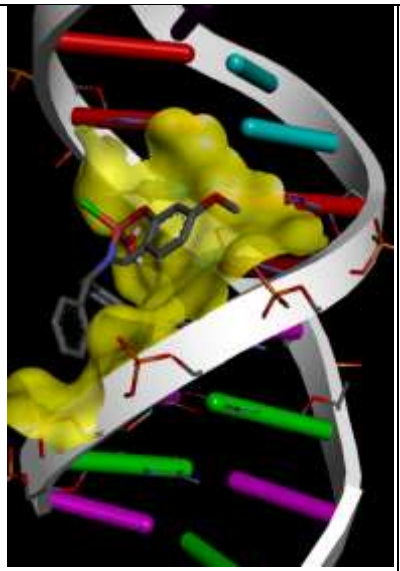
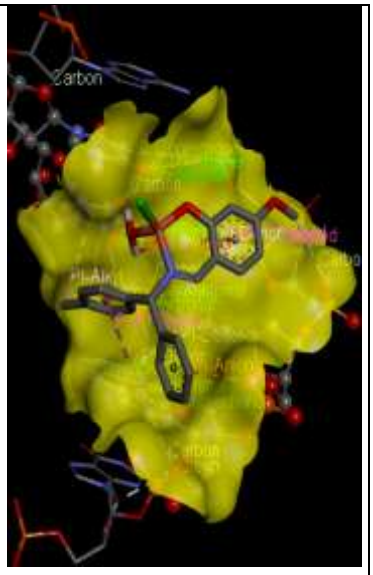


1-b

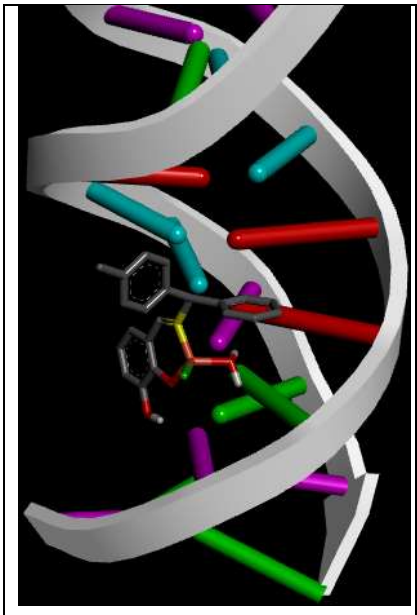
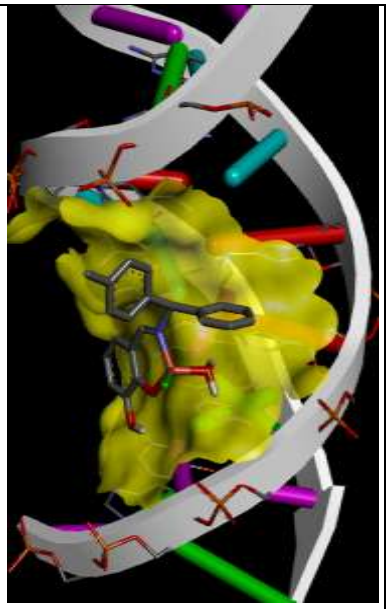
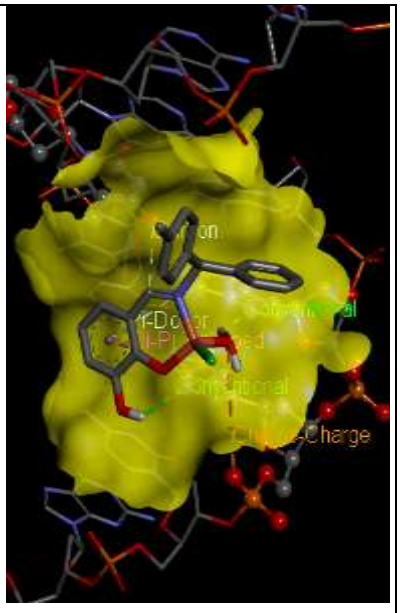


1-c

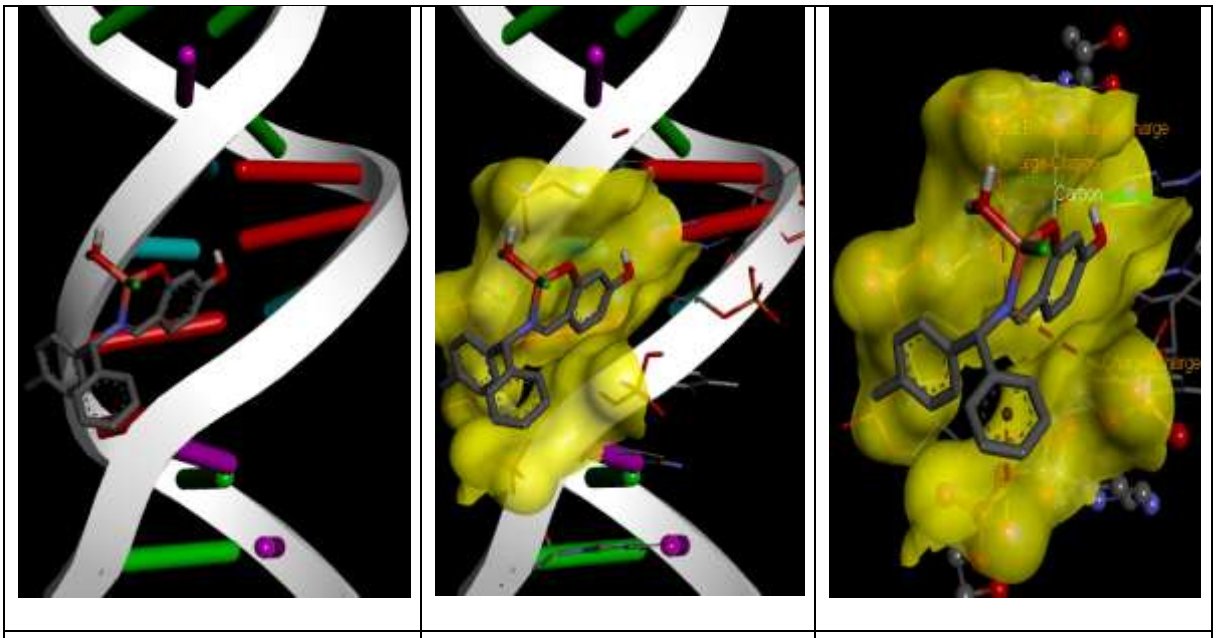
2-3b-Cu

		
2-a	**2-b**	**2-c**

3-3c-Cu

		
3-a	**3-b**	**3-c**

4-3d-Cu



4-a	**4-b**	**4-c**

Figure 2: Docking data display the interaction between four different bidentate imine ligands and their Cu(II) complexes and DNA (Pdb Code: 1BNA). 1:**3a-Cu**, 2: **3b-Cu**, 3: **3c-Cu** 4: **3d-Cu**, a: Best docking pose, b: Receptor ligand intraction with H-Bond surface, c: Solvent hydrogen bond donor/acceptor surface with other bond types.

4. Discussion

In our study, the 3a-Cu, 3b-Cu, 3c-Cu and 3d-Cu test substances caused a significant increase in the number of colonies returning in the presence (+S9) and absence (-S9) of the metabolic activation system in strains TA 98 and TA100.

The gibbs free binding energies of 3a-Cu, 3b-Cu, 3c-Cu and 3d-Cu chemicals, which were selected as ligands in molecular docking analysis and used as test substances in the study, were found to be -5.09, -5.34, -4.46 and -6.73 kcal/mol, respectively. These values are compared with the standard threshold binding free energy (-6.0 kcal/mol) due to the importance of this procedure (27). Accordingly, it was seen that the 3d-Cu ligand was higher than the threshold energy level (Figure 2., 1a).

3a-Cu ligand caused a significant increase in concentrations of 0.40 µg/plt ($P<0.001$) and 0.05 µg/plt ($P<0.05$) in –S9 and +S9 media, respectively (strain TA98). This suggests that concentrations higher than 0.10 µg/plt in +S9 environment are toxic. The 3a-Cu ligand caused a significant increase in concentrations of 0.05 µg/plt ($P<0.01$) and 0.10 µg/plt ($P<0.001$) in –S9 and +S9 medium (strain TA100), respectively. The gibss free binding energy of the same ligand to the major groove of B-DNA was calculated as -5.09 kcal/mol (Fig. 2., 1a).

The 3b-Cu ligand caused a significant increase in concentrations of 0.40 µg/plt ($P<0.001$) and 0.05 µg/plt ($P<0.001$) in –S9 and +S9 media, respectively (strain TA98). The 3b-Cu ligand caused a significant

increase in concentrations of 0.10 µg/plt ($P<0.01$) and 0.40 µg/plt ($P<0.001$) in –S9 and +S9 medium, respectively (in strain TA100). The gibss free binding energy of the same ligand to the major groove of B-DNA was calculated as -5.34 kcal/mol (Figure 2., 2a).

The 3c-Cu ligand caused a significant increase in concentrations of 0.10 µg/plt ($P<0.001$) and 0.80 µg/plt ($P<0.001$) in –S9 and +S9 media, respectively (strain TA98). The 3c-Cu ligand caused a significant increase in concentrations of 0.05 µg/plt ($P<0.001$) and 0.05 µg/plt ($P<0.05$) in –S9 and +S9 media, respectively (strain TA100). The gibss free binding energy of the same ligand to the major groove of B-DNA was calculated as -4.46 kcal/mol (Fig. 2., 3a).

The 3d-Cu ligand caused a significant increase in concentrations of 0.40 µg/plt ($P<0.001$) and 0.20 µg/plt ($P<0.001$) in –S9 and +S9 medium, respectively (strain TA98). The 3d-Cu ligand was 0.20 µg/plt ($P<0.001$) and 0.05 µg/plt in –S9 and +S9 medium, respectively (strain TA100). ($P<0.001$) caused a significant increase in concentrations. The gibss free binding energy of the same ligand to the minor groove of B-DNA was calculated as -6.73 kcal/mol (Figure 2., 4a).

The molecular docking results are evaluated together with the reverse mutation test (Ames), it is seen that 3a-Cu, 3b-Cu and 3c-Cu ligands bind to the major groove of B-DNA and dock with a binding energy close to the threshold standard binding free energy, whereas the 3d-Cu ligand bound to the minor groove of B-DNA and was found to be clamped with a gibss free binding energy stronger than the threshold standard binding free energy (27). Our molecular docking results were consistent with the Ames test results.

Literature studies show that imidazole derivatives exhibit various pharmacological activities such as antiviral, anti-inflammatory and analgesic, antidepressant, antifungal and antibacterial, anticancer, antituberculosis and antileishmanial activity (28).

Onur S. et al. (2020) to determine antimicrobial activity, investigated the effects of imine compounds on bacterial (E. coli, S. typhimurium, S. aureus, B. cereus) and fungal (C. albicans) microorganisms. Some Imine compounds have been reported to show high activity against bacteria and fungi (22). In another study investigating insecticide properties, it was determined that a series of newly designed 4-(N, N-diarylmethylamine)furan-2(5H)-one derivatives showed strong toxic effects (34).

Schiff bases (Ni(II) complex) may show significant antimicrobial activity, and they significant activity against cancer cell lines. At the same time, these bases can exhibit strong DNA interactions (29). Metal complexes generally exhibit higher activity against microorganisms than Schiff base. This can be explained on the basis of the chelation effect, which can inhibit the role of metal-dependent proteins disrupting microbial cell homeostasis and blocking microbial nutrition, growth, and development (30). Our experimental results (significant mutagenic effects) showed differences when compared with other studies investigating the mutagenic (Ames Test) effects of Schiff bases (31, 32, 33).

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