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Volume 1, Issue 1

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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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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.

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.

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.

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

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

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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.

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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.

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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.

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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.

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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

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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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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.

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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.
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Disclosure of conflict of interest and financial support is required at the time of submission. The authors are responsible

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.
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Publishing Board/Yayın Kurulu

The Chancellor's Message

Dear Students and Academicians,

Islam has placed a huge emphasis on medicine, since the beginning. According to the Islamic opinion, obeying certain medicinal recommendations is indispensable for a Muslim for both his and all society's good. Recently, the world has lived through unfortunate memories because of the pandemic. That is the neither the first nor the last threat for humanity. Hadiths narrated by Islamic scholars were even able to shed light on how to be at war with contagious diseases, epidemics or pandemics, for many centuries. Our beloved prophet, beloved servant of Allah (C.C), Hz. Muhammed said that "If you hear of a plague somewhere, do not enter into there. If the plague occurs in your place, do not leave there", narrated by famous Islamic scholar Buhârî. This most fundamental principle for the fight against epidemics still remains valid today.

All advices regarding the medicine internalised from verses of the Quran, hadiths and the life of Hz. Muhammed are actually a set of principles, named as "Tıbb-ı Nebevî". Tıbb-ı Nebevî means medicinal principles and remarks of our prophet, Hz. Muhammed. It acts as a guideline for Muslims in certain major medical entities, such as general medicine, preventive medicine and treatment approaches. Hadith mentioned above obviously points out certain principles of preventive medicine. Besides, there are others, for instance, in a verse of the Quran, Allah (C.C) Almighty orders that mothers should breastfeed their babies for two years. Today, scientists announce a number of research studies revealing the benefits of breast milk and they suggest that a baby should be breastfed for two years provided that the baby should take only breast milk, not any other food supplement, during the first six months of the life.

We can find out lots of medicinal principles mentioned in the Quran or hadiths narrated by Islamic scholars. Also, Islamic world has managed to train honoured medical scientists during ages. One of famous medical scholars of his period was Ibn Sînâ who is well known with its original perspective into the medicine and adapting to orders of the Quran and medicinal principles of "Tıbb-ı Nebevî", really worth mentioning here. He wrote more 100 books in the fields of medicine and philosophy and these were utilised in Europe as reference books until 18th century.

I believe in that Gaziantep Islam, Science and Technology University Medical Faculty will be inspired by this great medicinal and cultural richness and will take its place in the modern medical world. I wish great success to the Medical Faculty Journal "Experimental and Applied Medical Science".

Wish you all the best

Prof. Dr. Mehmet Nihat Hatipoğlu
Chancellor of Gaziantep Islam Science and Technology University

Chief Editor's Message

Dear Readership,

While struggles continue at full speed to start education and training in our Faculty of Medicine which was brought to our country within the newly formed Gaziantep Islamic Science and Technology University, it has been one year since the "Experimental and Applied Medical Science" sprouted, which is the academic publication of our Faculty of Medicine and in which we wholeheartedly believe will make a significant contribution to our academic community. We are very happy to deliver the fifth issue of our academic magazine to our readership in print, as well as in electronic form.

Nowadays, academic studies are accelerating, multiplying and diversifying. The need for channels where scientific studies, opinions and ideas can be freely expressed and easily shared with experts, researchers or postgraduate students who are still in the learning phase is increasing day by day. "Experimental and Applied Medical Science" has adopted it as a principle from the first day to bring together original and up-to-date studies, stimulating scientific views and ideas from every field of medicine that will potentially increase the quality of life with its readers both from home and abroad. With this fifth issue of our journal, we will continue to publish in English 4 (four) times a year, more than thirty manuscripts , in different types, research articles, case reports, reviews, etc. will have already been published and met with our readers. Recently, researchers have begun to understand the importance of having their studies published in international double-blind peer-reviewed journals. Since the first day of its publication, "Experimental and Applied Medical Science" has subjected the manuscripts which have been received, to an international double-blind peer reviewed evaluation process. For this reason, we aim not only to evaluate the manuscripts submitted with an aspect in which we decide whether the manuscript deserves to be publishing or not, but also to help researchers improve their educational or academic lives by providing on-the-spot feedback.

We are also happy that "Experimental and Applied Medical Science" which is only at the beginning of the road, has come a long way in a short time. In its 1 (one) year academic publication life, it has already started to be followed in nearly ten national or international indexes.

I would like to express my gratitude to our editorial and publishing boards, the esteemed academics who chose "Experimental and Applied Medical Science" for their manuscripts to have been submitted, all our readers, and our Rectorate for their unwavering support. I wish "Experimental and Applied Medical Science" the best success in its publication life.

Best Regards…

Chief Editor
Hamit Yıldız, Assoc. Prof.
Gaziantep University, Faculty of Medicine, Department of Internal Medicine

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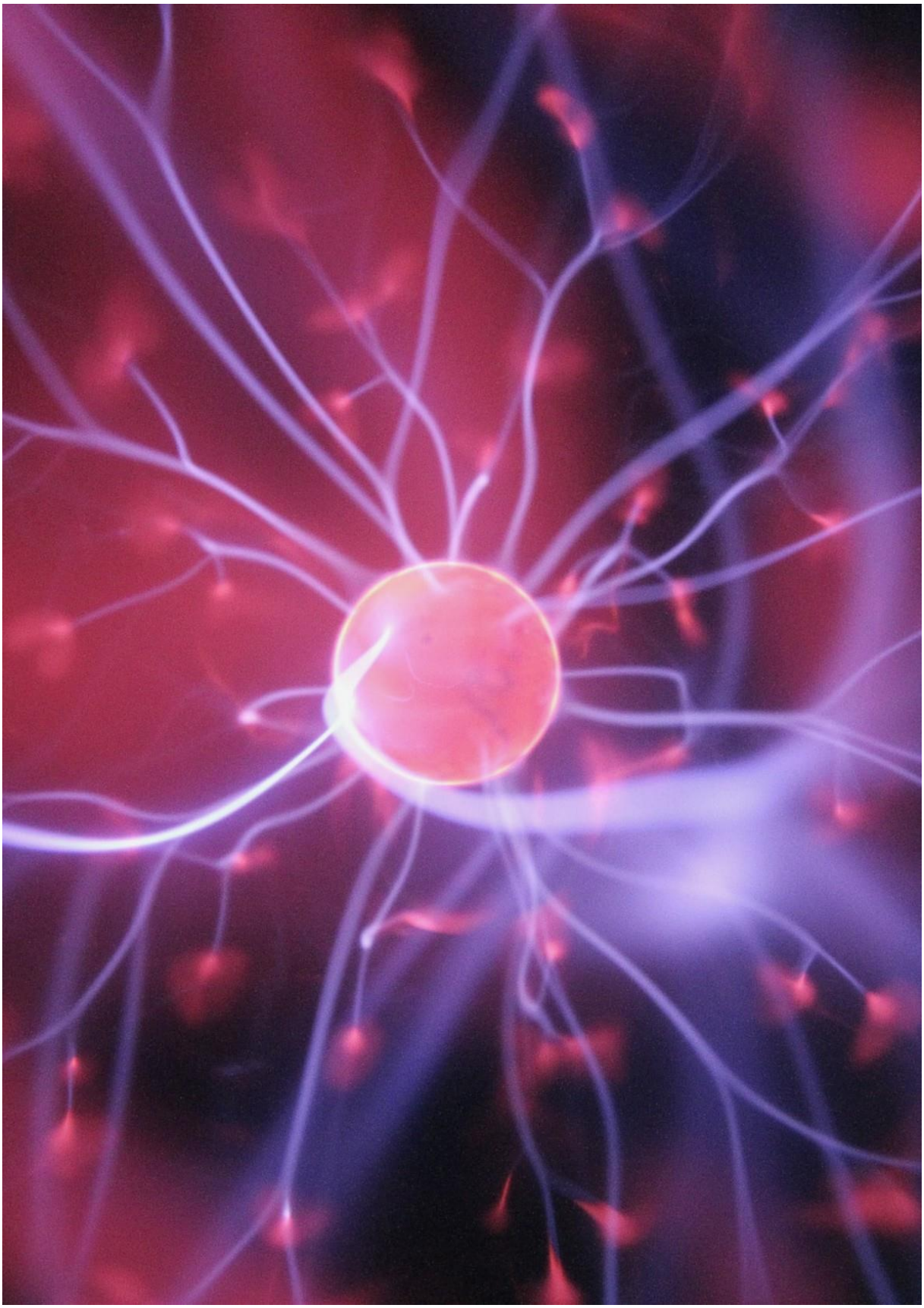

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Elderliness and Obesity

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Abstract

World becomes more and more elderly; on the other hand, obesity is on the rise. The frequency of obesity is exceedingly high in geriatric individuals and it is associated with many diseases as well as leading to a decrease in quality of life and disability in the elderly. The aim of this study is to compare Comprehensive Geriatric Assessment of geriatric obese and non-obese patients. 39 normal weight and 41 obese patients who applied to Geriatric Clinic for various reasons were included in the study. Basic activities of daily Life, instrumental activities of daily living, Tinetti balance and gait assessment, time up and go, mini mental state examination, mini nutritional assessment, Yesavage geriatric depression and Pittsburgh sleep quality indices tests were applied to the patients and also gait speed and hand grip strength of each patient was determined. Consequently, obese elderly patients were found to be deteriorated in some physical abilities and functions which are needed to maintain daily life such as gait and balance, muscle strength, certain daily life activities and mood and sleep quality compared to normal weight.

Key words: *Elderliness, Obesity, Life quality.*

Introduction

Elderly period of the life with its all difficulties is the most challenging issue facing the medicine. As many regressions in all physiological systems are encountered during this period, the most correct advices should be provided for elderly individuals and the best treatment approaches should be exhibited. Obesity can cause morbidity, functional disability and even early deaths as a global problem that can be seen in all age groups, in all developed and developing countries. Obesity is already defined as excess body fat which might result in many diseases and even premature death of individual. Obesity is related to several diseases including diabetes mellitus, hypertension, dyslipidaemia, coronary

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artery disease, heart disease and stroke, osteoarthritis, breathing problems such as sleep apnoea and some cancers. It is very well known that the risk of death because of all causes increases as an individual moves towards peak of the range of moderate to severe obesity (1). Besides, it reduces life expectancy, obesity decreases the number of years to be lived without cardiovascular diseases (2). Approximately, 32% of adults in USA are obese, 20% of them are obese in Canada and it is 30.5% in Turkey (3-5). The prevalence of obesity has been steadily increasing among older age group in both developed and developing countries. 39.3% of individuals aged 65 and above are displaying abdominal obesity in Turkey and nearly 80% percent of them have a waist to hip ratio beyond acceptable range determined by World Health Organization (WHO) (6).

The aim of this study is to comprehensively compare the obese and non – obese elderly individuals by using certain assessment tools which are uniquely applied in outpatient clinics, hospitals, and nursing homes. Older adults are already under the effect of many medical, psychiatric, or social troubles. Most probably, obesity furnishes elderly individuals with some additional adverse outcomes independent of already present medical, psychiatric, or social circumstances.

Method

39 normal weight and 41 obese male elderly patients who applied to geriatric outpatient clinics for various reasons were included in the study. The study with a research protocol number of 296 was approved by the Gaziantep University Local Research Ethics Committee. Basic activities of daily life (BADLs), instrumental activities of daily living (IADLs), Tinetti balance and gait assessment, time up and go, mini mental state examination, mini nutritional assessment, Yesavage geriatric depression and Pittsburgh sleep quality indices tests were applied to the patients and also gait speed and hand grip strength of each patient was determined. Whether the descriptive statistical values in two groups are normally distributed or not was revealed before each comparison. All descriptive statistics were found to be normally distributed and expressed as mean ± standard deviation. Student t test was used for comparisons of normally distributed descriptive values. Two sided values of $p<0.05$ were considered as statistically significant.

Results

There was no statistically significant difference between two groups in terms of age and gender. Basic activities of daily life (BADLs), mini mental state examination (MMSE) and mini nutritional assessment test scores also displayed no significance. Waist circumferences, hip circumference, mid – upper arm circumference and body mass index scores were noteworthily higher in obese group, as it is readily expected. Not surprisingly, non – obese group had better scores in other assessment tests (Table 1).

Table 1: General features and results of tests (p<0.05 is accepted as statistically different)

	Non – obese (n=39)	Obese (n=41)	p
Age	71.18 ± 6.02	71.54 ± 5.80	p>0.05
Gender (M/F)	20/19	21/20	p>0.05
Waist Circumference (cm)	97.57 ± 9.84	108.92 ± 8.60	p=0.001
Hip Circumference (cm)	105.32 ± 7.35	118.65 ± 9.60	p=0.001
Mid – Upper Arm Circumference (cm)	29.57 ± 2.31	33.00 ± 3.30	p=0.001
Body Mass Index (kg/m^2)	26.11 ± 2.59	33.49 ± 3.42	p=0.001
Gait Speed (m/s)	0.88 ± 0.37	0.62 ± 0.33	p=0.002
Hand Grip Strength (kg)	26.18 ± 10.45	18.26 ± 10.43	p=0.001
Basic Activities of Daily Life	17.62 ± 4.50	21.58 ± 5.22	p>0.05
Instrumental Activities of Daily Living	6.42 ± 1.26	5.68 ± 1.36	p=0.015
Tinetti Balance and Gait Assessment	23.18 ± 4.15	20.43 ± 4.41	p=0.006
Time Up and Go (s)	10.35 ± 4.83	13.24 ± 5.28	p=0.014
Mini Mental State Examination	24.84 ± 4.73	24.80 ± 4.51	p>0.05
Yesavage Geriatric Depression Scale	2.89 ± 0.51	4.68 ± 0.65	p=0.035
Mini Nutritional Assessment	11.42 ± 2.45	11.05 ± 2.75	p>0.05
Pittsburgh Sleep Quality Index	4.74 ± 2.35	7.24 ± 3.92	p=0.001

Discussion

There are many kinds of Comprehensive Geriatric Assessment tools each of which is prepared for different healthcare settings and to meet different needs, eventually to manage frail geriatric patients. In our study, obese and non – obese groups did not display any significance in terms of age and gender. But there were different scores worth mentioning here between groups when we use certain tools to assess geriatric patients.

First, there was no difference between BADLs scores. However, BADLs tool is used to assess the most basic daily activities such as bathing, dressing, maintaining continence, toileting, grooming, feeding and transferring. However, mainly instrumental activities of daily living (IADLs) scores of individuals should be examined to consider someone has the ability of maintaining an independent household or not. Someone might be adequate in BADLs tool but cannot live independently in community. Mental goodness of individual is demonstrated by using IADLs. Individuals with mild cognitive impairment (MCI) who have impairments performing IADLs are more likely to develop dementia (7). Obese geriatric patients had poor IADLs scores in our study. In our study, non – obese patients could not have a mean score above 24. But obese patients have significantly lower scores than the non – obese patient.

The autonomy and independence are substantial for every person. One of the most basic approaches to evaluate independence and physical frailty of an elderly is undoubtedly measurement of gait speed. The mean gait speed of non – obese elderly individuals in our study was comparable to previous studies (8). The gait speed of obese group was significantly lower than non – obese group. Some scientists propose grip strength as a biomarker of present health status of an elderly because there is a strong association between hand grip strength and strength of other muscles in the body and how

influential the muscle actions of an elderly individual (9). As it could be expected, hand grip strength of obese elderly individuals was significantly lower in our study.

Fall prevention is also a substantial issue for management of geriatric patients in clinical practice. Tinetti balance and gait assessment tool provides the most precious fall risk evaluation in the elderly (10, 11). According to this test tool, individuals score 24 and above are considered to have a low fall risk. Those of individuals score between 19 and 23 have a moderate risk and individuals score 18 or lower have a high fall risk.

One of the other important testing tools to evaluate the fall risk of an individual is the time up and go test. Indeed, observation of gait quality of an elderly is a suggestive examination method in clinical practice. Most of the time, gait assessment is ignored in physical examination by practitioners, even when fall is a one of major complaints of a patient. The time up and go test consists of certain period evaluations. How much time an elderly individual needs to get up from a chair, walk 3 meters, turn back, and sit on the chair is inquired during the TUG test. Time less than 15 s is considered a plausibly low fall risk for that patient (12, 13). In our study, both the obese and non – obese groups had better mean values than 15 s. Indeed, non – obese group was significantly much better than obese group.

Assessment of mental status, especially in patients with neurobehavioral disorders, provides valuable clues for both diagnosing and treatment. This assessment should be certainly following physical and neurological examination. The MMSE truly tests episodic memory and orientation of the patient. According to MMSE scores, there was no significant difference between groups, in our study. Mini mental state examination test is frequently used screening test for MCI diagnosis (14). 20 – 22% of 71 year olds were reported as mild cognitive impairment (MCI) & cognitive impairment, no dementia, in the United States (15). Despite the fact that, mid-life overweight or obesity is a prominent risk factor prompting Alzheimer's disease (AD) or vascular type of dementia, some studies propose no difference between cognitive functions of obese and non – obese people (16). Body mass index is alone a predictor of temporal lobe atrophy (17). Obesity, independent of any other risk factor, is known to increase the risk of MCI (18, 19). It doubles the risk of AD and mid-life obesity absolutely increase the risk of dementia in later life (20). However, there are also some studies displaying no association between obesity and dementia. In a recent retrospective cohort study, finding precisely contradicts the hypothesis that the mid-life obesity is proposed to lead to dementia in later life (21).

Besides dementia, depression is also known to be prevalent among elderly individuals. Depression among the elderly aged 65 and above, with no previous history of depression is named as late-onset depression. With a progressively increasing old age population worldwide, diagnosing depression in older adults is extremely important, because they may display different symptoms than middle-aged individuals and need diverse treatment modalities. Also an association between obesity and depression has been frequently established (22). It is often proposed that social isolation due to stigmatization of obese individuals can prompt depression. It is found that increases in BMI result in increased depression days or days having had while an individual is being depressed

(23). According to our study, obese elderly adults were within the mild depression range of Yesavage depression scale, whereas non – obese elderly adults were in normal range.

The mini nutritional assessment is an uncomplicated and extremely sensitive tool for nutritional screening and assessment (24). It particularly provides rapid assessment of nutritional status of the elderly in outpatient and inpatients clinics and nursing homes. With this test, elderly individuals who are in malnutrition or at risk of malnutrition can be determined. All participants in our study were below the range accepted as normal nutritional status. Mean of both groups were just below 12 points which is accepted as lower border of the normal range and there was no significant difference between groups.

It is also very well known that subjective sleep quality deteriorates with aging. Certain medical and psychiatric problems may contribute to that deterioration, too (25, 26). Obese elderly individuals in our study had obviously a poor sleep quality in comparison to non – obese ones.

In our study, obese patients are obviously found to have a lower performance than non – obese ones in gait speed, hand grip strength, instrumental activities of daily living, Tinetti balance and gait assessment, Time up and go, Yesavage geriatric depression scale, and Pittsburgh sleep quality tests. The relationship between obesity and mortality and whether we should advice patient to lose weight in elderly period, have still carried on remaining controversial. There are studies implicating a U – shaped relationship between obesity and mortality in elderly. It is mostly found that obesity rather than overweight results in a moderately high risk of mortality in elderly period. Also, threshold value at which BMI confers increased mortality risk to the elderly is found to be higher than that of younger adults. This is considered as "obesity paradox" (27-29). In our study, it seems that the obesity readily reduces quality of life.

Conclusion:

At the end of a comprehensive geriatric assessment, some pathfinder findings which can be arousal for a practitioner who examine an obese elderly were obtained. Obesity results in some additional consequences which certainly decrease the quality of life in the elderly other than outcomes of any medical and psychiatric circumstances.

Conflict of interest

The authors declare that no conflict of interest exists.

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References

1. Calle EE, Thun MJ, Petrelli JM, et al. Body-mass index and mortality in a prospective cohort of U.S. adults. The New England journal of medicine. 1999;341(15):1097-105.
2. Pardo Silva MC, De Laet C, Nusselder WJ, et al. Adult obesity and number of years lived with and without cardiovascular disease. Obesity. 2006;14(7):1264-73.
3. Ogden CL, Carroll MD, Curtin LR, et al. Prevalence of overweight and obesity in the United States, 1999-2004. Jama. 2006;295(13):1549-55.
4. Sokar-Todd HB, Sharma AM. Obesity research in Canada: literature overview of the last 3 decades. Obes Res. 2004;12(10):1547-53.
5. Ardahan M, Konal E. The prevalence of hypertension and obesity and effective factors: A cross-sectional bazaar study. JPMA The Journal of the Pakistan Medical Association. 2019;69(7):1018-21.

6. Pekcan AG, Samur G, Dikmen D, et al. Population based study of obesity in Turkey: results of the Turkey Nutrition and Health Survey(TNHS)-2010. Progress in Nutrition. 2017;19(3):248-56.
7. Guo HJ, Sapra A. Instrumental Activity of Daily Living (IADL). StatPearls. Treasure Island (FL)2020.
8. Busch Tde A, Duarte YA, Pires Nunes D, et al. Factors associated with lower gait speed among the elderly living in a developing country: a cross-sectional population-based study. BMC geriatrics. 2015;15:35.
9. Bohannon RW. Grip Strength: An Indispensable Biomarker For Older Adults. Clinical interventions in aging. 2019;14:1681-91.
10. 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.
11. Kopke S, Meyer G. The Tinetti test: Babylon in geriatric assessment. Zeitschrift fur Gerontologie und Geriatrie. 2006;39(4):288-91.
12. Browne W, Nair BKR. The Timed Up and Go test. The Medical journal of Australia. 2019;210(1):13-4 e1.
13. Podsiadlo D, Richardson S. The timed "Up & Go": a test of basic functional mobility for frail elderly persons. Journal of the American Geriatrics Society. 1991;39(2):142-8.
14. Onwuekwe I. Assessment of mild cognitive impairment with mini mental state examination among adults in southeast Nigeria. Annals of medical and health sciences research. 2012;2(2):99-102.
15. Plassman BL, Langa KM, Fisher GG, et al. Prevalence of cognitive impairment without dementia in the United States. Annals of internal medicine. 2008;148(6):427-34.
16. Conforto RM, Gershman L. Cognitive processing differences between obese and nonobese subjects. Addictive behaviors. 1985;10(1):83-5.
17. Gustafson D, Lissner L, Bengtsson C, et al. A 24-year follow-up of body mass index and cerebral atrophy. Neurology. 2004;63(10):1876-81.
18. Hassing LB, Dahl AK, Pedersen NL, et al. Overweight in midlife is related to lower cognitive function 30 years later: a prospective study with longitudinal assessments. Dementia and geriatric cognitive disorders. 2010;29(6):543-52.
19. Dahl A, Hassing LB, Fransson E, et al. Being overweight in midlife is associated with lower cognitive ability and steeper cognitive decline in late life. The journals of gerontology Series A, Biological sciences and medical sciences. 2010;65(1):57-62.
20. Anstey KJ, Cherbuin N, Budge M, et al. Body mass index in midlife and late-life as a risk factor for dementia: a meta-analysis of prospective studies. Obesity reviews : an official journal of the International Association for the Study of Obesity. 2011;12(5):e426-37.
21. Qizilbash N, Gregson J, Johnson ME, et al. BMI and risk of dementia in two million people over two decades: a retrospective cohort study. The lancet Diabetes & endocrinology. 2015;3(6):431-6.
22. Luppino FS, de Wit LM, Bouvy PF, et al. Overweight, obesity, and depression: a systematic review and meta-analysis of longitudinal studies. Archives of general psychiatry. 2010;67(3):220-9.
23. 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.
24. Guigoz Y, Vellas B. The Mini Nutritional Assessment (MNA) for grading the nutritional state of elderly patients: presentation of the MNA, history and validation. Nestle Nutrition workshop series Clinical & performance programme. 1999;1:3-11; discussion -2.
25. Buysse DJ, Reynolds CF, 3rd, Monk TH, et al. The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. Psychiatry research. 1989;28(2):193-213.
26. Wang P, Song L, Wang K, et al. Prevalence and associated factors of poor sleep quality among Chinese older adults living in a rural area: a population-based study. Aging Clin Exp Res. 2020;32(1):125-31.
27. Mathus-Vliegen EM. Obesity and the elderly. Journal of clinical gastroenterology. 2012;46(7):533-44.
28. Janssen I, Mark AE. Elevated body mass index and mortality risk in the elderly. Obesity reviews : an official journal of the International Association for the Study of Obesity. 2007;8(1):41-59.
29. Chapman IM. Obesity paradox during aging. Interdisciplinary topics in gerontology. 2010;37:20-36.

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Effects of Thymoquinone on Blood Parameters in Doxorubicin Cardiotoxicity

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Abstract

Thymoquinone is known to alleviate cardiotoxic effects of doxorubicin. This study was conducted to investigate the effects of thymoquinone on biochemical parameter changes in cardiotoxicity caused by doxorubicin (DOX). 18 Wistar albino male rats were used in our study. The rats were divided into three groups as control, doxorubicin, and doxorubicin + thymoquinone (DOX + TQ) group. The control group received one-time saline from the tail vein on the 5th day of the experiment. On the 5th day of the experiment, the DOX group received a single dose of 45 mg / kg DOX from the tail vein. The DOX + TQ group received 50 mg / kg thymoquinone by gavage for seven days and a single dose of 45 mg / kg DOX from the tail vein on the 5th day of the experiment. Rats were sacrificed under ketamine-xylazine anaesthesia and total blood samples were taken and biochemical parameters were evaluated on the last day of the experiment. According to the biochemical data, in DOX group in while the creatine kinase myocardial band (CK-MB), interleukin-6 (IL-6) interleukin-18 (IL-18), total oxidant status (TOS) and malondialdehyde (MDA) levels increased, the total antioxidant status (TAS) decreased compared to the control group. Compared to the DOX group, while the value of CK-MB, IL-6, IL-18, TOS, and MDA decreased, TAS increased in the DOX + TQ group. Results of our study shows that the deteriorative effects of doxorubicin on blood parameters can be corrected by thymoquinone.

Keywords: *Doxorubicin, Thymoquinone, Cardiotoxicity.*

Introduction

Doxorubicin (DOX or Adriamycin) was isolated for the first time in the 1960s and is among the most effective anticancer agents developed so far. It is classified in the cytotoxic antibiotics used in the treatment of many child and adult cancers such as haematological cancers, solid tumours, breast cancer, soft tissue sarcomas and lymphoma (1). However, acute, and chronic side effects

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can occur in patients with cancer receiving DOX treatment. Acute side effects such as myelosuppression, nausea, vomiting, and arrhythmias are reversible and can be clinically controlled. Although DOX provides successful results in many cancer treatments, it causes irreversible cardiomyopathy as a chronic side effect and consequently heart failure. These chronic side effects have poor prognosis (2). Although it has good therapeutic potentials, the main limitation of DOX is due to its cardiotoxic side effects (3).

The mechanism of action of doxorubicin on tumour cells differs from the mechanisms that cause cardiotoxicity. The anti-malignant effects on tumour cells occur through the inhibition of the enzyme topoisomerase II, formation of reactive oxygen species and, consequently, DNA damage. In the mechanism that causes cardiotoxicity, it increases the reactive oxygen species (ROS) and triggers oxidative stress and lipid peroxidation and causes a decrease in the level of sulfhydryl groups and antioxidants (3).

There are many mechanisms that are thought to be responsible for the damage caused by DOX in the heart. Among them, various mechanisms have been proposed such as oxidative stress, myofibrillary disruption, intracellular calcium regulation disruption, apoptosis (1, 4). Decrease in antioxidant and sulfhydryl groups and increase in reactive oxygen species and lipid peroxidation shows that oxidative stress is crucial in DOX-induced cardiotoxicity (5-7). We used thymoquinone (TQ), an antioxidant substance that can be effective in preventing DOX cardiotoxicity, in this study.

Although many antioxidant substances have been used to prevent DOX cardiotoxicity, studies are ongoing to find the most ideal substance. In this study, thymoquinone, a phytochemical compound found in the oil of the plant nigella sativa was used to prevent DOX cardiotoxicity. Many properties of TQ (2-isopropyl-5-methyl-1,4-benzoquinone), the most important bioactive component found in the essential oil of nigella sativa seed, have been reported. It has been reported in conducted studies that nigella sativa and its components have anti-carcinogenic, anti-tumour, anti-ulcerogenic, anti-bacterial, anti-inflammatory and analgesic, antioxidant, hypoglycaemic and immune-enhancing effects (8). The protective effects of TQ have been reported as a “protective agent” by examining the toxicity caused by cyclosporine A (9), isoproterenol (10, 11), cyclophosphamide (12), and in ischemia-reperfusion models (13-15).

The fact that DOX has exceptionally good therapeutic potential makes it indispensable in cancer treatments. However, the use of this drug has been limited because of its most important side effect, cardiotoxicity. In this study, we investigated the effects of thymoquinone, which has an antioxidant feature, on blood parameters to reduce the toxic effects of DOX. In this way, it is aimed to use DOX, which is restricted in use, with less side effects in chemotherapy.

Materials and Methods

Experimental Animals

This study was approved by Celâl Bayar University (CBU) Faculty of Medicine Local Ethics Committee of Experimental Animal use with the decision dated 09/06/2015 and numbered 77.637.435-51. 18 adult Wistar albino rats of 200-300 grams of each were

used in the study. Experimental animals were obtained from CBU Experimental Animals Practice and Research Centre and kept in the same laboratory conditions during the study. During the experiment, they were kept in a 12-hour light-12-hour dark cycle and provided access to standard water and food (ad libitum nutrition). The rats were housed in the laboratory, which averaged 22 ± 2 °C room temperature and 40 ± 20% humidification. All experiments were carried out in accordance with the rules of Animal Ethics Committee and Welfare of the Experimental Animals.

18 rats were divided into 3 groups. There were 6 rats in each group. The DOX + TQ group received 50 mg / kg TQ with gavage for seven days and a single dose of 45 mg / kg DOX from the tail vein on the 5th day of the experiment. The control group received saline from tail vein on the 5th day of the experiment. The DOX group received a single dose of 45 mg / kg DOX (6, 7) from the tail vein on the 5th day of the experiment. Rats were sacrificed on the last day of the experiment, their total blood was collected and processed for the biochemical parameters. The experiment schedule is shown in figure 1.

	1st day	2nd day	3rd day	4th day	5th day	6th day	7th day	8th day
Control group (n=6)	saline infusion							Sacrification
DOX group (n=6)	DOX Infusion							
DOX + TQ group (n=6)	DOX infusion							
	Received TQ for seven days							

Figure 1: Experiment Schedule. TQ; thymoquinone, DOX; doxorubicin.

Chemicals

Thymoquinone and doxorubicin hydrochloride were obtained from Sigma-Aldrich, 0.9% NaCl from pharmacy, ketamine and xylazine from CBU Experimental Animal Research and Practice Centre.

Anaesthesia and Sampling

Ketamine (100 mg / kg) and xylazine (10 mg / kg) anaesthesia was applied to the rats on the last day of the experiment (8th day). Anaesthesia depth was evaluated with a foot pedal reflex. After deep anaesthesia, the thorax was opened with an abdominal incision and total blood was taken from their hearts. While plasma samples were obtained from the collected blood, erythrocytes were separated and stored at –20 °C until analysis was performed. Superoxide dismutase (SOD) glutathione peroxidase (GPx) and catalase (CAT) antioxidant enzyme activities and MDA analyses were performed in erythrocyte hemolysate, while other parameters were measured in serum.

Biochemical Parameters

Nitric oxide (NO), hypoxia-induced factor-1 α (HIF-1α), CK-MB, IL-1, IL-6, and IL-18 levels were measured by ELISA tests. Analysis of total oxidant and antioxidant status were measured by TAS and TOS

technique developed by Erel (16) (17). MDA analysis, on the other hand, determined with the method reported by Yoshioka et al. (18), Erythrocyte CAT, SOD and GPx activities were determined by the method described by Nadif et al. (19).

Statistical analysis

SPSS 22.0 (Statistical Package for Social Sciences) statistics program was used for statistical evaluations. The evaluation of the data was determined by one-way analysis of variance (ANOVA). Tukey test, which is one of the multiple comparison (post hoc) tests, was used to determine which groups were statistically different. Mean and standard deviations of the measurements obtained from all subjects and calculated variables were found. $p < 0.05$, $p < 0.01$ were accepted as statistically significant.

Results

The results of the biochemical parameters and statistical evaluations measured in the blood samples taken from the groups are shown in Table 1.

Table 1: Serum biochemical parameters (mean ± SD: mean ± standard deviation).

	Control (n=6) Mean ± SD	**DOX (n=6) Mean ± SD**	**DOX+TQ (n=6) Mean ± SD**
HIF 1α (ng/ml)	3.51 ± 0.17	3.81 ± 0.63	3.22 ± 0.64
IL-1 (pg/ml)	72.18 ± 11.08	81.08 ± 13.76	69.78 ± 10.02
SOD (U/mg Hb)	1.86 ± 0.82	1.22 ± 0.37	1.79 ± 0.54
GPx (U/mg Hb)	1.80 ± 0.95	1.45 ± 0.33	1.83 ± 0.65
NO (µmol/L)	61.42 ± 11.56	75.71 ± 18.07	64.50 ± 6.86
CAT (k/g Hb)	45.70 ± 29.32	35.66 ± 8.04	44.22 ± 22.84

Effect of Thymoquinone on Myocyte Damage Marker

As shown in Figure 2 (a), the serum level of CK-MB, an important marker of myocardial damage, increased significantly in the DOX group compared to the control group ($p < 0.05$). However, when compared to the DOX group, the CK-MB level of the DOX + TQ group decreased significantly ($p < 0.05$).

Effects of Thymoquinone on Pro-inflammatory Cytokines

Serum levels of pro-inflammatory cytokines IL-6 and IL-18 are shown in Figure 2 (b) and (c). Compared with the control group, there was a significant increase in both IL-6 ($p < 0.05$) and IL-18 ($p < 0.01$) levels in the DOX group. On the other hand, in the DOX + TQ group, there was a decrease in both IL-6 and IL-18 levels compared to the group given only DOX ($p < 0.05$).

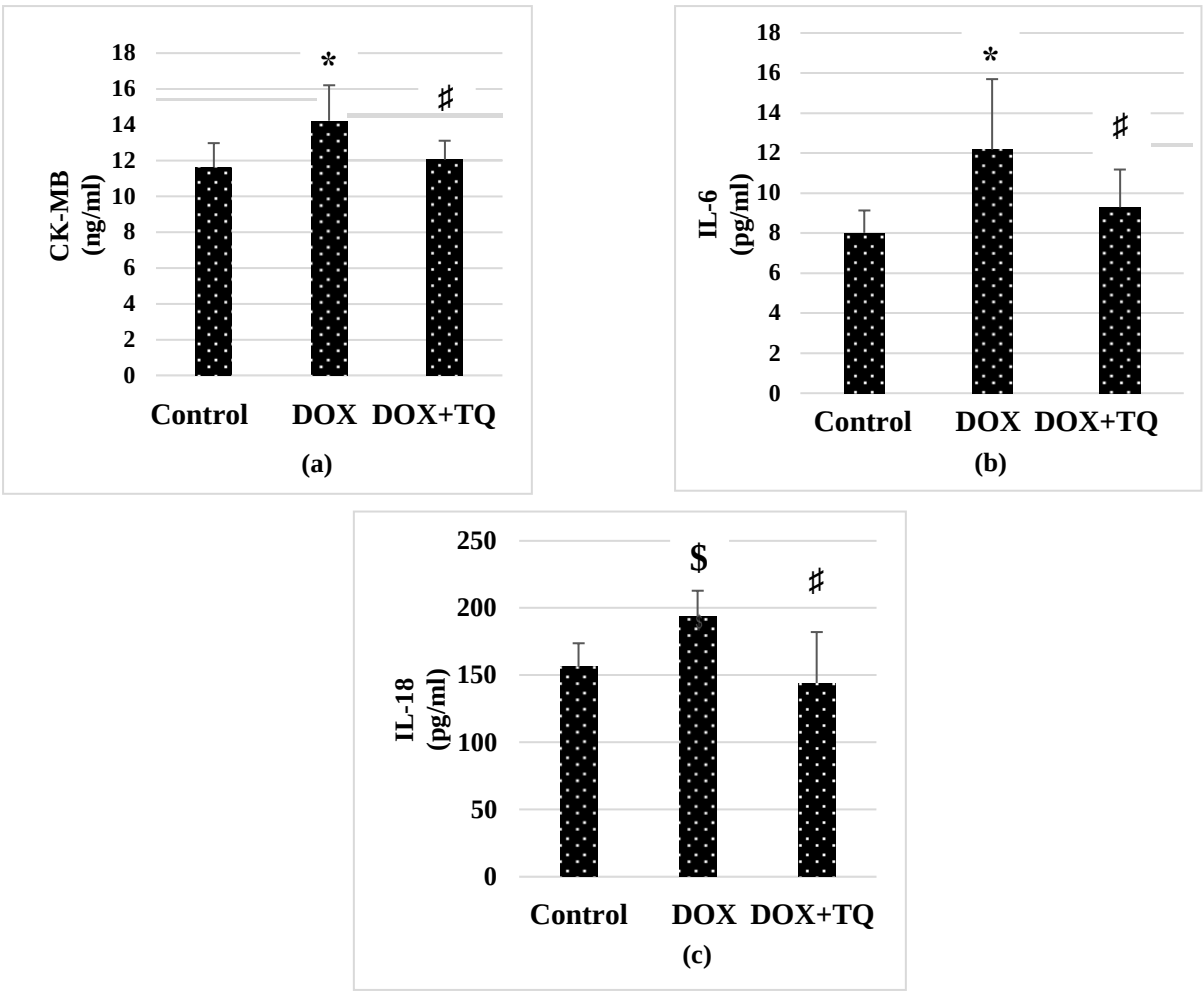

Figure 2: The effect of thymoquinone on serum CK-MB, IL-6, and IL-18 levels. *: Significant compared to the control group ($p < 0.05$), $: Significant compared to the control group ($p < 0.01$), #: Significant compared to the DOX group ($p < 0.05$).

Effect of Thymoquinone on Total Antioxidant and Total Oxidant Levels

TAS and TOS were analysed in serum from all experimental groups. As shown in Figure 3 (a) and (b), while the TAS decreased significantly ($p < 0.01$), the TOS increased significantly ($p < 0.05$) in the DOX group compared to the control group. While the TOS decreased ($p < 0.05$), the TAS increased ($p < 0.05$), when the DOX + TQ group compared to the DOX group.

A significant increase in the level of MDA, the end product of lipid peroxidation, was observed in the DOX group compared to control group ($p < 0.01$). The serum MDA levels decreased in the DOX + TQ group compared to the DOX group ($p < 0.05$), [Figure 3 (c)].

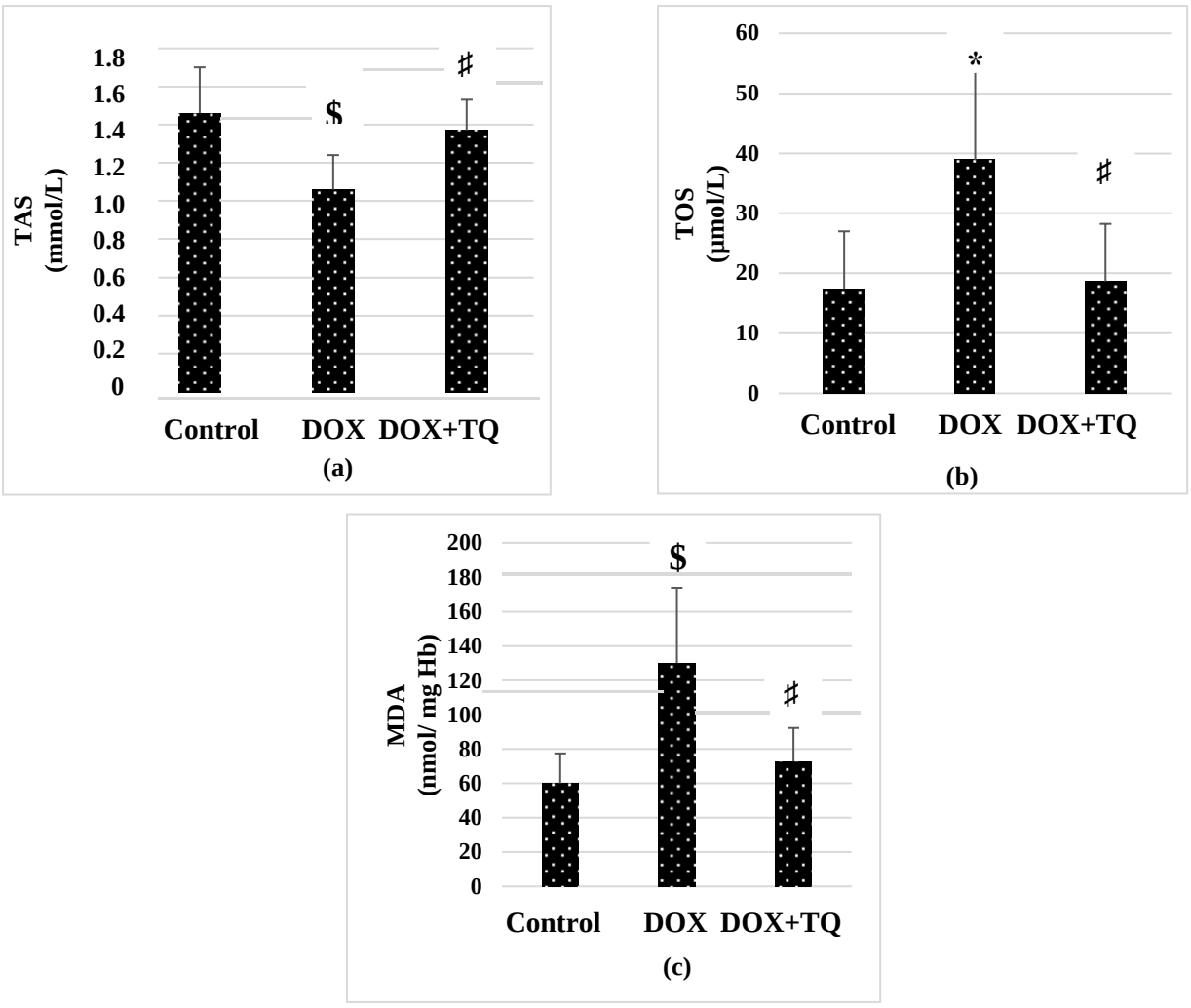

Figure 3: Effect of thymoquinone on serum TOS, TAS and MDA levels. *: Significant compared to the control group ($p < 0.05$), $: Significant compared to the control group ($p < 0.01$), #: Significant compared to the DOX group ($p < 0.05$).

Discussion

This study set out with the aim of assessing the importance of thymoquinone in preventing the crucial side effects of doxorubicin. Doxorubicin is a broad–spectrum anticancer agent used in many cancer treatments such as Hodgkin lymphoma, bladder, breast, stomach, lung, ovary, thyroid cancers, and soft tissue sarcomas. It has long been known that the most important side effect is cardiotoxicity, and its use has been limited due to this side effect. CK-MB, which is one of the enzymes that leak into the bloodstream when cardiac tissue damage occurs, is used as a cardiac marker in the diagnosis of cardiotoxicity (3). CK-MB is a plasma cardiac biomarker that helps in the laboratory diagnosis of heart attack as well.

DOX administration causes damage to the myocardial cell membrane or increase in permeability of the cell membrane resulting in CK-MB leakage into the blood (3). In our study, we revealed that CK-MB, an important

cardiac marker and showing cardiac damage in the acute period, increased significantly only in the group receiving DOX. Compared to the DOX group, we found that the level of this marker in the DOX + TQ group significantly decreased in serum and approached the control group. Previous studies confirm our study results. It has been reported that CK-MB increase due to DOX (3), cyclophosphamide (12), ischemia reperfusion (14) and streptozotocin-induced diabetes (20) decreased significantly with TQ. This might be due to the high antioxidant property of TQ, which reduces the heart muscle myofibril damage caused by DOX and consequently preventing CK-MB iso-enzyme leakage from cardiac tissue to blood. IL-18, a cytokine synthesized by Kupffer cells, has been reported to activate macrophages. In patients with acute myocardial infarction (MI) it has been reported to be activated the T cells and macrophages. It is assumed that IL-18 attend in the development of myocardial dysfunction via activation of immune cells. In one study, plasma IL-18 concentrations were found to be significantly higher in acute MI patients than in control patients. According to the results of the study, it was commented that high IL-18 concentration may be a new indicator of heart damage in the development of acute MI (21).

Production of reactive oxygen species (ROS) originating from doxorubicin usage causes oxidative stress and consequently cardiomyopathy (22). ROS activates pro-inflammatory cytokines. Compared to the control group, we found that the levels of IL-6 and IL-18, which are pro-inflammatory cytokines, increased significantly in the DOX group. We showed that the levels of IL-6 and IL-18 cytokines decreased in the DOX + TQ group. It was reported in previous studies that increase in the level of DOX-induced IL-2 (3) and the increase in the level of IL-6 in diabetes-induced cardiomyopathy (20) were prevented by TQ. We think that TQ prevents the increase in pro-inflammatory cytokine levels by preventing the increase in ROS level (by neutralizing ROS).

Increased reactive oxygen species from DOX usage cause oxidative stress and consequently lipid peroxidation. MDA occurs because of lipid peroxidation and is an important indicator of damage caused by free radicals. Free radicals result in peroxidation of membrane phospholipids, causing changes in membrane permeability. DOX disrupts membrane functions by increasing free radicals and causes cardiotoxicity. In this study, a significant increase in the level of MDA occurred in the group receiving DOX. MDA levels decreased in the group receiving thymoquinone. In previous studies, it was reported that the increase of MDA in cardiotoxicity caused by DOX (3), isoproterenol (11), ethanol (23), carbon tetrachloride (24), and diabetes (20) decreased by TQ. TQ may reduce oxidative stress and peroxidation of lipid membranes by neutralizing reactive oxygen species with its antioxidant properties.

DOX administration leads to increased ROS production in the body, cell dysfunction and impairment of cell membrane integrity. There are several antioxidant mechanisms in living organisms. The antioxidant system contains antioxidant enzymes and non-enzymatic antioxidants. Oxidant and antioxidant system balance is meticulously protected in the organism. When oxidants increase and antioxidants decrease, oxidative damage

occurs in biological structures. The term "oxidative stress" refers to the deterioration of the pro-oxidant/pro-antioxidant balance in the body in favour of pro-oxidants. Since individual measurement of antioxidant levels is time consuming and expensive, all antioxidant levels should be measured in the evaluation of the oxidant status in vivo. Total oxidant status and total antioxidant status are particularly important in terms of evaluating the stress of the body. TOS and TAS parameters are a combination of all oxidant and antioxidant parameters such as MDA, glutathione peroxidase and catalase (25). In this study, we used the TAS and TOS technique developed by Erel to determine oxidant and antioxidant levels (17).

Compared to the control group, we found that TOS increased significantly and TAS decreased only in the group receiving DOX. While TAS was statistically increased in the group receiving TQ, TOS decreased. The balance deteriorated in favour of oxidants because of DOX administration was reversed by TQ. Our study results show that TQ is an important bioactive component that can play a role in maintaining the DOX-induced deteriorating oxidant-antioxidant balance. Our cells have various adaptation mechanisms to protect itself against oxygen deprivation. Cellular responses to hypoxia are mediated by hypoxia-induced factor-1 (HIF-1). HIF-1α provides transcription of proteins that respond cellularly to hypoxia (26). HIF-1α activation plays an important role in cellular protection and metabolic changes from the outcomes of oxygen deprivation during myocardial ischemia. An increase in HIF-lα level has been reported to be one of the first adaptive responses at the molecular level in myocardial ischemia (27). The expression of HIF-1α was also previously report to increase with the progression of heart failure in rats with myocardial infarction or hamsters with cardiomyopathy. The level of transcription factor HIF-lα has been showed to increase significantly in the group receiving DOX compared to the control group (28). In other study have been reported no to be change in HIF-1α expression in DOX-treated mouse heart (29). In our study, the HIF-1α level measured in rat blood sample increased in the group treated with DOX. Compared to the DOX group, the level of HIF-1α decreased in the group receiving DOX + TQ. However, these differences were not statistically significant. NO is a free radical that has been play role in the aetiology of doxorubicin cardiotoxicity. In one study, DOX therapy has been shown to significantly increase plasma NO concentration. According to the results of the study, there was a relationship between plasmatic NO levels and histopathological myocardial damage. Therefore, it has been suggested that plasmatic NO levels can be used as a biomarker for DOX-induced myocardial damage (30). Fogli et al. had suggested that there is a decrease in cardiac contractility due to overproduction of reactive oxygen radicals and NO, and the disruption in NO synthesis due to anthracyclines plays an important role in cardiotoxicity (31). In our study, serum NO concentrations of rats treated with DOX increased compared to the control group. The serum NO level of the rats receiving TQ decreased compared to the DOX group. However, these findings were not statistically significant.

In conclusion, the present study has gone some way towards enhancing our understanding of the effects of thymoquinone

in the prevention of DOX-induced cardiotoxicity in rats. Further research is needed to determine the effectiveness of thymoquinone more clearly.

Conflicts of Interest

The authors declare that they have no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

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References

1. Raj S, Franco VI, Lipshultz SE. Anthracycline-induced cardiotoxicity: A review of pathophysiology, diagnosis, and treatment. Current Treatment Options in Cardiovascular Medicine. Current Science Inc. 2014; 16: 315-28.
2. Singal PK, Li T, Kumar D, et al. Adriamycin-induced heart failure: mechanism and modulation. Molecular and Cellular Biochemistry. 2000; 207: 77–85.
3. Alam MF, Khan G, Safhi MM, et al. Thymoquinone ameliorates doxorubicin-induced cardiotoxicity in swiss albino mice by modulating oxidative damage and cellular inflammation. Cardiology Research and Practice. 2018; 1–6.
4. Takemura G, Fujiwara H. Doxorubicin-induced cardiomyopathy. From the cardiotoxic mechanisms to management. Progress in Cardiovascular Diseases. 2007; 49(5): 330–52.
5. Chatterjee K, Zhang J, Honbo N, et al. Doxorubicin cardiomyopathy. Cardiology. 2010; 115: 155–62.
6. Oz E, Erbaş D, Sürücü HS, et al. Prevention of doxorubicin-induced cardiotoxicity by melatonin. Molecular and Cellular Biochemistry. 2006; 282: 31–7.
7. Oz E, İlhan MN. Effects of melatonin in reducing the toxic effects of doxorubicin. Molecular and Cellular Biochemistry. 2006; 286: 11–5.
8. Bacak Güllü E, Avcı G. Timokinon: Nigella Sativa'nın biyoaktif komponenti. Veterinary Journal. 2013; 6(1): 51–61.
9. Uz E, Uz B, Selcoki Y, et al. Cardioprotective effects of nigella sativa oil on cyclosporine A-induced cardiotoxicity in rats. Basic & Clinical Pharmacology & Toxicology. 2008; 103: 574–80.
10. Randhawa MA, Alghamdi MS, Maulik SK. The effect of thymoquinone, an active component of nigella sativa, on isoproterenol induced myocardial injury. Pak J Pharm Sci. 2013; 26(6): 1215-9.
11. Ojha S, Azimullah S, Mohanraj R, et al. Thymoquinone protects against myocardial ischemic injury by mitigating oxidative stress and inflammation. Evidence-Based Complementary and Alternative Medicine. 2015; 1–12.
12. Nagi MN, Al-Shabanah OA, Hafez MM, et al. Thymoquinone supplementation attenuates cyclophosphamide-induced cardiotoxicity in rats. Journal of Biochemical and Molecular Toxicology. 2011; 25(3): 135–42.
13. Seif AA. Nigella sativa attenuates myocardial ischemic reperfusion injury in rats. Journal of Physiology and Biochemistry. 2013; 69: 937–44.
14. Xiao J, Ke ZP, Shi Y, et al. The cardioprotective effect of thymoquinone on ischemia-reperfusion injury in isolated rat heart via regulation of apoptosis and autophagy. Journal of Cellular Biochemistry. 2018; 119: 7212–8.
15. Aydin MS, Kocarslan A, Kocarslan S, et al. Thymoquinone protects end organs from abdominal aorta ischemia / reperfusion injury in a rat model. Braz J Cardiovasc Surg. 2015; 30(1): 77–83.
16. Erel O. A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. Clinical Biochemistry. 2004; 37: 277–85.
17. Erel O. A new automated colorimetric method for measuring total oxidant status. Clinical Biochemistry. 2005; 38: 1103–11.
18. Yoshioka T, Kawada K, Shimada T, et al. Lipid peroxidation in maternal and cord blood and protective mechanism against activated-oxygen toxicity in the blood. American Journal of Obstetrics and Gynecology. 1979; 135: 372–6.

19. Nadif R, Bourgkard E, Dusch M, et al. Relations between occupational exposure to coal mine dusts, erythrocyte catalase and Cu++Zn++ superoxide dismutase activities, and the severity of coal workers' pneumoconiosis. Occupational and Environmental Medicine. 1998; 55: 533–40.
20. Atta MS, El-Far AH, Farrag FA, et al. Thymoquinone attenuates cardiomyopathy in streptozotocin-treated diabetic rats. Oxidative Medicine and Cellular Longevity. 2018; 1-10.
21. Seta Y, Kanda T, Tanaka T, et al. Interleukin 18 in acute myocardial infarction. Heart. 2000; 84: 668.
22. Zheng J, Lee HCM, Bin Sattar MM, et al. Cardioprotective effects of epigallocatechin-3-gallate against doxorubicin-induced cardiomyocyte injury. European Journal of Pharmacology. 2011; 652: 82–8.
23. Arslan SO, Gelir E, Armutcu F, et al. The protective effect of thymoquinone on ethanol-induced acute gastric damage in the rat. Nutrition Research. 2005; 25: 673–80.
24. Mansour MA. Protective effects of thymoquinone and desferrioxamine against hepatotoxicity of carbon tetrachloride in mice. Life Sciences. 2000; 66(26): 2583–91.
25. Yuluğ E, Turedi S, Karaguzel E, et al. The short term effects of resveratrol on ischemia-reperfusion injury in rat testis. Journal of Pediatric Surgery. 2014; 49: 484–9.
26. Spagnuolo RD, Recalcati S, Tacchini L, et al. Role of hypoxia-inducible factors in the dexrazoxane-mediated of cardiomyocytes from doxorubicin-induced toxicity. British Journal of Pharmacology. 2011;163: 299–312.
27. Zhao HX, Wang XL, Wang YH, et al. Attenuation of myocardial injury by postconditioning: role of hypoxia inducible factor-1α. Basic Res Cardiol. 2010; 105: 109–18.
28. Ichihara S, Yamada Y, Kawai Y, et al. Roles of oxidative stress and Akt signaling in doxorubicin cardiotoxicity. Biochemical and Biophysical Research Communications. 2007; 359: 27–33.
29. Soni H, Pandya G, Patel P, et al. Beneficial effects of carbon monoxide-releasing molecule-2 (CORM-2) on acute doxorubicin cardiotoxicity in mice: Role of oxidative stress and apoptosis. Toxicology and Applied Pharmacology. 2011; 253: 70–80.
30. Guerra J, De Jesus A, Santiago-Borrero P, et al. Plasma nitric oxide levels used as an indicator of doxorubicin-induced cardiotoxicity in rats. The Hematology Journal. 2005; 5: 584–8.
31. Fogli S, Nieri P, Breschi MC. The role of nitric oxide in anthracycline toxicity and prospects for pharmacologic prevention of cardiac damage. Faseb Journal. 2004; 18: 664-75.

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Histological Examination of Rat Heart Tissue with Chronic Diabetes

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Abstract

Diabetes mellitus causes structural and functional impairment of the system in the organism by affecting various organs and structures. In this study, we aimed to examine the changes in female rat heart tissue histologically by creating experimental diabetes. 16 female adult rats were used in the study. Rats were randomly divided into two groups as control and 3–month diabetes. The diabetes group was formed from subjects with blood glucose levels above 250mg/dl 72 hours after 40 mg / kg streptozotocin administration. At the end of the experiment, the heart tissues of the subjects were removed and taken into formaldehyde solution. To examine the histological structure, haematoxylin–eosin, and immunohistochemically neuronal nitric oxide synthase (nNOS) and inducible nitric oxide synthase (iNOS) were stained. Heart tissue sections belonging to the control group had histologically normal appearance. In the diabetes group heart tissue sections, vacuolization in some cells and eosinophilic increase in the cytoplasm of some cells were observed. The nNOS and iNOS immunoreactivity was observed to be decreased in the diabetes group compared to the control group, but the decrease was not statistically significant. As a chronic disease, DM causes histological damage to the heart tissue. The resulting damage causes a decrease in nNOS and iNOS expression. It is important to maintain NOS enzyme levels to protect tissue from the harmful effects of diabetes and ensure normal physiological conditions.

Keywords: *Diabetes mellitus, Heart, nNOS, iNOS.*

Introduction

Diabetes mellitus is a serious disease that affects large masses worldwide. Risk factors such as hypertension, coronary artery diseases, hypercholesterolemia increase the rate of heart failure in diabetic

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patients (1). Also, regardless of coronary artery disease or hypertension, DM can cause cardiomyopathy by causing ventricular dysfunction (2).

DM triggers oxidative stress by causing prolonged hypoglycaemia and causes disruption of protein, lipid and carbohydrate metabolism in the cell and production of reactive oxygen species (3). The biosynthesis of NO, a free radical, is provided by nitric oxide synthase (NOS) (4). NOS has two basic isoforms: constitutive NOS (cNOS) and inducible NOS (iNOS). The cNOS enzyme consists of two basic isoforms. These are neuronal NOS / NOS1 and endothelial NOS / NOS3. iNOS is also known as NOS2 (5, 6). NOS enzymes are found in three isoforms: NOS1 / nNOS, NOS2 / iNOS and NOS3 / eNOS (7). It has been reported that all three NOS isoforms are found in mammalian cardiac tissue, and they modulate oxygen consumption, substrate use, hypertrophy, apoptosis and regenerative potential in cardiac cell biology (8).

DM shows its effect on cardiovascular diseases due to irregular free radical production in the cell. In this study, it was aimed to evaluate the changes in the heart tissue of female rats with experimental chronic diabetes on nNOS and iNOS immunoreactivity.

Methods

Animals

Sexually mature 12-weeks-old male Wistar rats, obtained from the Hakan Çetinsaya Experimental and Clinic Research Centre, Erciyes University, Kayseri, Turkey, were used for this study. They were housed in plastic cages in a well-ventilated rat house and allowed ad libitum access to food and water and kept at a 12-h light: dark cycle. All the animals received humane care according to the standard guidelines. The study protocol was accepted by the Erciyes University Experimental Animal and Local Ethics Committee (decision no: 12/105/2012). The rats were randomly assigned to two groups. This Control group (n=8) and Diabetes group (n=8).

Diabetes was induced in 12-week-old female Wistar rats by intraperitoneal injection of STZ (40 mg/kg) (Sc-200719, Santa Cruz Biotechnology, CA, USA) (9). Hyperglycaemia was confirmed 72 h after streptozotocin injection by measuring glucose levels in the blood obtained from the tail vein, using a glucometer. Animals with mean plasma glucose levels higher than 250 mg/dL were considered diabetic. Diabetes group at sacrificed 12 weeks after streptozotocin injection (10). At the end of the experimental period, the animals were killed by decapitation under intraperitoneal ketamine (75 mg/kg) + xylazine (10 mg/kg) anaesthesia. After decapitation, the heart tissues were quickly removed and were fixed. To evaluate the normal histological structure, haematoxylin-eosin (H-E) staining was performed.

Immunohistochemistry

To determine the differences in expression of nNOS and iNOS in heart tissue, the avidin-biotin-peroxidase method was used for marking. Paraffin sections (5 µm) were deparaffinized in xylene. The sections were rehydrated, rinsed in deionized water and antigen retrieval was carried out by microwave treatment in 0.01 M sodium citrate buffer (pH 6.0) at 95°C for 5 min. The slides were then cooled rapidly at room temperature for 20 min. The sections were washed with phosphate-buffered saline (PBS) and endogenous peroxidase activity was inhibited by 3% H_2O_2 in methanol for 10 min. For the next stages the ABC staining system using colouring kit was

used. All cross sections were washed with PBS and then to make sure to block outside the antigenic fields, block serum was applied for 20 minutes at room temperature. The histological sections were then incubated with nNOS (Pierce antibody product, PA3-032A1/200 dilution) and iNOS (Pierce antibody product, PA3-030A, 1/200 dilution) primary antibodies overnight at 4 ˚C. After washing with PBS, the sections were incubated with biotinylated secondary antibodies. The immunoreaction was amplified with the streptavidin–avidin–peroxidase complex, and the sections were visualized using 3,3P-diaminobenzidine tetrahydrochloride (DAB) and lightly counterstained with haematoxylin (11). Ten different areas were evaluated in terms of expression using the image J program.

Statistical analysis

All statistical analyses were carried out by using GraphPad Prism version 7.00 for Mac, GraphPad Software, La Jolla, California, USA. D'Agostino Pearson omnibus test was used to identify the normal distribution of the data. In the situation of quantitative variables with abnormal distribution, variables were compared using independent two-sample t-test with Mann-Whitney test. Quantitative variables with normal distribution were compared by using independent two-sample t-test with Welch's test. The data were expressed as 'median (min-max)'. $p<0.05$ was considered as statistically significant.

Results

Histological results

The control group heart tissue sections had a normal histological appearance. Myocardial muscle fibers were smooth, H-E staining was normal. In the diabetes group heart tissue sections, vacuolization was observed in some cells and eosinophilic increase in the cytoplasm of some cells Figure 1.

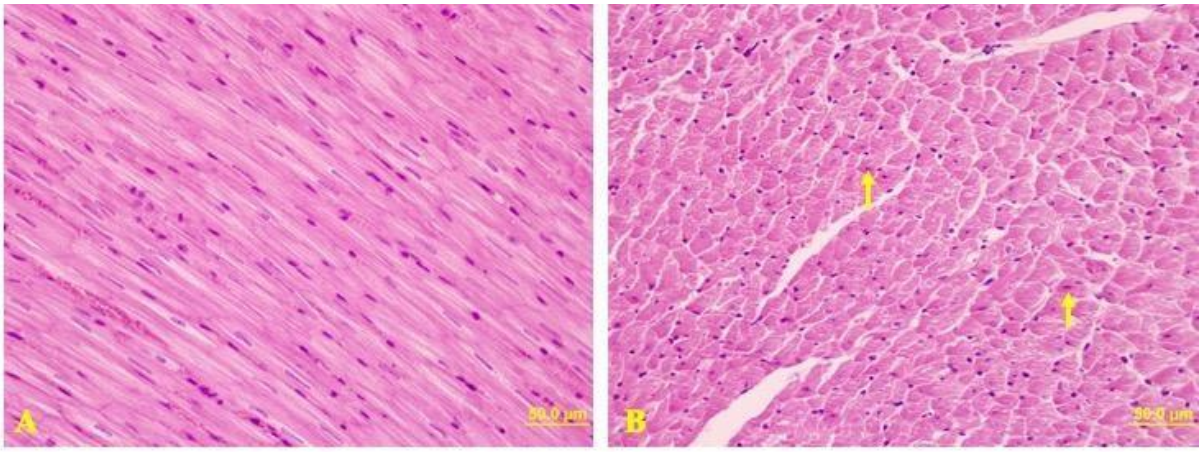

Figure 1. H-E staining of heart tissue. A) Control group, B) Diabetes group. The yellow arrows indicate increased eosinophilia. Scala bar 50 □m. Abbreviation: H-E; Haematoxylin-eosin.

Immunohistochemistry results

Expression of nNOS and iNOS in heart tissue sections was observed in both the control and diabetes groups. Both nNOS (p <0.097) and iNOS (p <0.079) expressions decreased in the diabetes group compared to the control group. The decrease in both enzyme expression was not statistically

significant. Expression of enzymes was mostly observed in the cytoplasm of heart muscle cells. Pictures and graphics of nNOS and iNOS immunoreactivity are given in Figure 2.

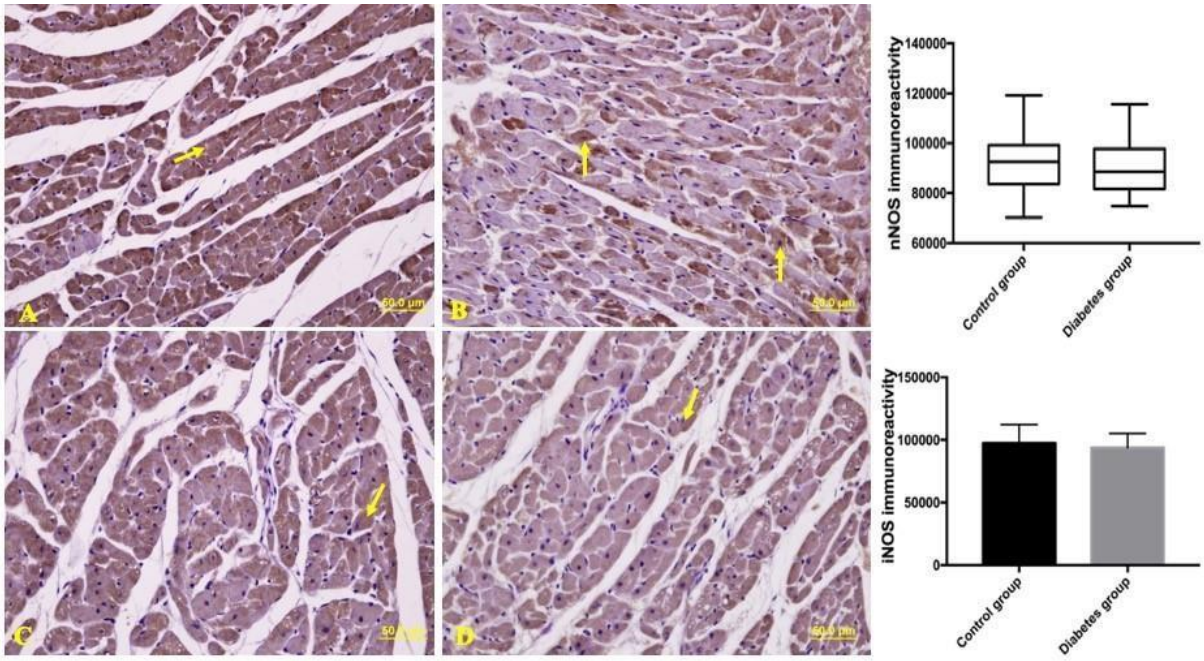

Figure 2. nNOS and iNOS immunohistochemistry staining. A) Control group, B) Diabetes group nNOS expression and immunoreactivity graph. C) Control group, D) Diabetes group iNOS expression and immunoreactivity graph. Yellow arrows indicate nNOS and iNOS expressions. Scala bar 50 □m. Abbreviations: nNOS; neuronal nitric oxide synthase, iNOS: inducible nitric oxide synthase.

Discussion

In the study, we found that DM causes myocardial damage in the heart tissue and changes in the expression levels of nNOS and iNOS. It has been reported that cardiomyocyte disintegration and cells with pyknotic nuclei are observed after the damage caused by diabetes in the heart tissue (12, 13). We found similar findings in this study, including in our previous study that we created experimental diabetes with streptozotocin (9).

DM causes a decrease in cardiac output, arterial blood pressure and heart rate, especially due to hyperglycaemia. Especially in endothelial cells, by suppressing the response to vasoactive agents, it triggers endothelial dysfunction and increases the risk of cardiac disease almost five times (14, 15). DM causes myocardial damage due to oxidative stress with increased reactive oxygen and nitrogen types. Three major free-radical sources in diabetic myocardium are mitochondria, nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and NOS (15). NOS enzyme family is necessary for the production of nitric oxide (NO) from L-arginine (16). NO is a free-radical since it has an unpaired electron in its final orbit and can be covalently bonded with other molecules (17). NOS enzymes are nNOS, iNOS and eNOS and are available in a variety of cell and tissue types. NO especially passes from the endothelium and reaches to smooth muscle cells in the

vascular wall, increasing the formation of cGMP and causing relaxation and vasodilation of smooth muscles (18). DM triggers endothelial dysfunction in the emergence of vascular diseases by reducing this bioavailability of NO (19). Decreased NO bioavailability causes an increase in reactive oxygen species in the cell, triggering an increase in oxidative stress. Briefly, DM causes a breakdown in the electron transport chain that occurs in mitochondria due to hyperglycaemia and increased fatty acid in the cell. Thus, decreased ATP production leads to decreased NOS enzyme activity and this decrease in NOS enzyme activity leads to dysfunction in the cell by causing decreased NO production and increased superoxide radicals (20). In our study, we used the heart tissues of rats exposed to diabetes for 12 weeks. According to our immunohistochemistry staining results, there was a decrease in nNOS and iNOS immunoreactivity in diabetic heart tissue compared to the Control group. We think that nNOS and iNOS synthesis decreases in the cell due to increased hyperglycaemia. In our previous experimental diabetes study, all three NOS isoforms (nNOS, iNOS, and eNOS) were reduced in the diabetes group (9). A decrease in both serum NO and constitutive NOS (cNOS) (nNOS and eNOS) levels has been reported after diabetes induced by applying a high-fat diet (21). Contrary to our study, there are studies showing that plasma NO and iNOS gene expression increases (22). Diabetic rats have been reported to have endothelial dysfunction associated with decreased aortic NO and cNOS activity, in contrast to increase NO and iNOS reactivity in cardiac tissue (23). Accordingly, the decrease in both nNOS and iNOS expression in the heart tissue indicates that endothelial dysfunction is triggered in the tissue. In this case, increased NOS enzyme levels in the heart tissue does not indicate that endothelial dysfunction started in the tissue. On the contrary, due to the reduction of these enzyme levels, NO bioavailability is eliminated and oxidative stress increases in the heart tissue. According to our results, chronic diabetes causes a decrease in both nNOS and iNOS enzyme levels in the heart tissue, inhibiting the production of beneficial NO, causing diabetic vascular complications in the heart tissue.

Conflict of interest

The authors declare that no conflict of interest exists.

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References

1. Kenny HC, Abel ED. Heart Failure in Type 2 Diabetes Mellitus. Circ Res. 2019;124(1):121-41.
2. Lee HW, Lee SJ, Lee MY, et al. Enhanced cardiac expression of two isoforms of matrix metalloproteinase-2 in experimental diabetes mellitus. PLoS One. 2019;14(8):e0221798.
3. Mutavdzin S, Gopcevic K, Stankovic S, et al. The Effects of Folic Acid Administration on Cardiac Oxidative Stress and Cardiovascular Biomarkers in Diabetic Rats. Oxid Med Cell Longev. 2019;2019:1342549.
4. Stuehr DJ, Kwon NS, Nathan CF, et al. N omega-hydroxy-L-arginine is an intermediate in the biosynthesis of nitric oxide from L-arginine. J Biol Chem. 1991;266(10):6259-63.
5. Lowenstein CJ, Dinerman JL, Snyder SH. Nitric oxide: a physiologic messenger. Ann Intern Med. 1994;120(3):227-37.

6. Moncada S, Palmer RM, Higgs EA. Nitric oxide: physiology, pathophysiology, and pharmacology. Pharmacol Rev. 1991;43(2):109-42.
7. Alderton WK, Cooper CE, Knowles RG. Nitric oxide synthases: structure, function and inhibition. Biochem J. 2001;357(Pt 3):593-615.
8. Massion PB, Pelat M, Belge C, et al. Regulation of the mammalian heart function by nitric oxide. Comp Biochem Physiol A Mol Integr Physiol. 2005;142(2):144-50.
9. Karabulut D, Ulusoy HB, Kaymak E, et al. Therapeutic effects of pentoxifylline on diabetic heart tissue via NOS. Anatol J Cardiol. 2016;16(5):310-5.
10. Sönmez MF, Karabulut D, Kilic E, et al. The effects of streptozotocin-induced diabetes on ghrelin expression in rat testis: biochemical and immunohistochemical study. Folia histochemica et cytobiologica. 2015;53(1):26-34.
11. Sonmez MF, Kilic E, Karabulut D, et al. Nitric oxide synthase in diabetic rat testicular tissue and the effects of pentoxifylline therapy. Syst Biol Reprod Med. 2016;62(1):22-30.
12. Abdel-Hamid AA, Firgany Ael D. Atorvastatin alleviates experimental diabetic cardiomyopathy by suppressing apoptosis and oxidative stress. J Mol Histol. 2015;46(4-5):337-45.
13. Li J, Peng L, Du H, et al. The Protective Effect of Beraprost Sodium on Diabetic Cardiomyopathy through the Inhibition of the p38 MAPK Signaling Pathway in High-Fat-Induced SD Rats. Int J Endocrinol. 2014;2014:901437.
14. Nasrolahi O, Khaneshi F, Rahmani F, et al. Honey and metformin ameliorated diabetes-induced damages in testes of rat; correlation with hormonal changes. Iran J Reprod Med. 2013;11(12):1013-20.
15. Ansley DM, Wang B. Oxidative stress and myocardial injury in the diabetic heart. J Pathol. 2013;229(2):232-41.
16. Palmer RM, Moncada S. Anovel citrulline-forming enzyme implicated in the formation of nitric oxide by vascular endothelial cells. Biochem Biophys Res Commun. 1989;158(1):348-52.
17. Kılınç A, Kılınç K. Nitirik oksit Biyolojik Fonksiyonları ve Toksik Etkileri. Ankara: Palme Yayıncılık. 2003:1-50.
18. Çekmen MB, Turgut M, Türköz Y, et al. Nitrik Oksit (NO) ve Nitrik Oksit Sentaz (NOS)'ınFizyolojik ve Patolojik Özellikleri. Turkiye Klinikleri Journal of Pediatrics. 2001;10(4):226-35.
19. Hoang HH, Padgham SV, Meininger CJ. L-arginine, tetrahydrobiopterin, nitric oxide and diabetes. Curr Opin Clin Nutr Metab Care. 2013;16(1):76-82.
20. Hamilton SJ, Watts GF. Endothelial dysfunction in diabetes: pathogenesis, significance, and treatment. Rev Diabet Stud. 2013;10(2-3):133-56.
21. Li M, Fang H, Hu J. Apelin13 ameliorates metabolic and cardiovascular disorders in a rat model of type 2 diabetes with a highfat diet. Mol Med Rep. 2018;18(6):5784-90.
22. Atta MS, El-Far AH, Farrag FA, et al. Thymoquinone Attenuates Cardiomyopathy in Streptozotocin-Treated Diabetic Rats. Oxid Med Cell Longev. 2018;2018:7845681.
23. Said MA. Vitamin D attenuates endothelial dysfunction in streptozotocin induced diabetic rats by reducing oxidative stress. Arch Physiol Biochem. 2020:1-5.

Investigation of Toxic Effects of Heavy Metal Level in Atatürk Dam

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Abstract

This study was conducted on the water of the largest reservoir in Turkey, the Atatürk Dam on the Euphrates River in the South-eastern Anatolia Region. Although there is only one waste-water treatment plant in Adıyaman province, industrial and agricultural wastewater is discharged into streams from various points without being treated, and these streams flow into the Atatürk Dam. Between June and August 2019, the levels of some toxic heavy metals (Cd, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Se and Zn) were examined with an ICP-MS device in water samples taken from 5 stations of Atatürk Dam, contaminated by waste water from Adıyaman province. Sampling areas were selected considering the pollution conditions. The highest mean metal levels in µl / l were; 1108.77 ± 3506.23 (Zn), 1529.34 ± 558.39 (Cr), 1507.92 ± 592.9 (Ni), 905.56 ± 329.36 (Cu), 22.34 ± 70.66 (Se), 45.5 ± 50.43 (Co) , 4.43 ± 6.92 (Pb) and 0,016322.11 ± 0,017 (Al) ,Cd and As were not detected. The metal levels were affected by domestic and industrial activities in the study areas and the highest metal levels of all the samples were higher in the polluted areas where wastewater was discharged without treatment. A significant difference was found the in terms of P values between the Cu element and Zn (0,955), the element Al, Zn, Pb and Se (0.629; 0.821;0629), the element Cr, Zn and Se (0.631; 0.631), the Co and Zn, Se (0.821), the element Ni, Zn, Pb and Se (0.873; 0.531; 0.88739). From the metal values obtained, the classifications were determined as 4th quality water pollution of Cr, Se and Ni metals, 3rd quality water pollution of Co metal and other 2nd and 1st degree water pollution according to the criteria of the Intracontinental Water Pollution Control regulations. Leaving untreated wastewater in the Atatürk Dam has the potential to harm freshwater environments and the life attached to them.

Keywords: *Atatürk dam, Water, Sediment, Heavy metal, Pollution*

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Introduction

As a result of human activity such as urbanisation and industrialisation, there has been an increase in recent years in the deposition of heavy metals in water ecosystems and this can cause severe ecological damage because of toxicity and the bio accumulative properties in the food chain. High metal concentrations in water, sediment and organisms can be well above the defined legal values (1).

The toxic substances defined as the 20 most dangerous on earth include zinc (Zn), copper (Cu), mercury (Hg), nickel (Ni), chrome (Cr) and lead (Pb), and these are known to be extremely dangerous chemicals for the environment and show the most significant water pollution. Several human diseases are known to be related to increased heavy metal pollution. For example, Hg causes neurological effects, cadmium (Cd) and Pb cause carcinogenic effects, Cr leads to mutations in genetic material, and Cu causes anaemia (2, 3). One of the most significant dangers threatening human and animal health and the current natural balance is environmental problems originating from heavy metals, and these problems are seen to be emerging in ever increasing dimensions.

The Atatürk Dam was constructed on the Euphrates River in the Southeast Anadolu region of Turkey. As the largest reservoir in the country, the basic function is the provision of water and the production of hydroelectricity. Intensive trout farming is also conducted in the reservoir and recently mass fish deaths have been seen (4, 5).

The aim of this study was to determine the accumulated levels of heavy metals (Al, Cr, Co, Ni, Cu, Zn, As, Se, Cd, Pb) which could have caused these deaths in the Atatürk Dam, and to compare these analysis values with the values of the intracontinental water source quality of the Water Pollution Control Regulations published in the Official Gazette.

Method

Study Location

Water samples were taken from 5 different stations which flow into the Atatürk Dam within the borders of Adiyaman province in the South-east Anadolu region (Figure 1). The samples were taken between June and August 2019, taking the pollution conditions of the areas into consideration.

Station 1: Adıyaman Gerger District, Budaklı Village Reservoir
Station 2: Adıyaman Centre, Boğazözü Village Girik Stream
Station 3: Adıyaman Burmapınar Village Kahta Stream
Station 4: Adıyaman Centre, İnceBağ Village (Miroğlu) Reservoir
Station 5: Adıyaman Çelikhan

Sample Collection and analysis

The water was taken from each point, as one container for each of the elements-heavy metals to be analysed (4). The process of straining solid particles in waste-water samples was applied using Whatman (No. 42) filter paper. To prevent organisms in the water starting a chemical reaction by fragmenting the heavy metals, 2 ml pure HNO_3 was added to the samples, and a pH <2 was obtained in the water. Heavy metal analyses of the water samples performed in the Advanced Technology Training, Research and Application Centre laboratory of Mersin University using an Agilent 7500 ce series ICP-MS device (Tokyo, Japan) (6).

Figure 1: Study Area and Station locations

The concentrations of Cu, Fe, Zn, Cr, Ni, Cd, As and Pb elements were determined in the samples. The heavy metal calibrations of the ICP-MS device and device accuracy were applied with international certificated standards (NIST standards). Each analysis was made 3 times on the ICP-MS device, and the mean value of these 3 repetitions was calculated. If the relative standard deviation of these three repetitions was >3, the analyses were repeated. By taking a standard reading of every 10 samples during the analyses, accuracy of the device was maintained until the end of the analyses.

Results

The highest mean levels of metals determined in the water samples of this study were (µl/l) ; 1108.7 (Zn), 0.016 (Al), 1529.4 (Cr), 1507.92 (Ni), 905.56 (Cu), 22.3 (Se), 45.5 (Co) and 4.43 (Pb). Cd and as were not determined. Cd, As were not determined in any sample. Zn and Se were determined in samples from Station 4 (11087.68 ppb) (Table 1, 2, 3).

Table 1: Heavy metal values (ppb) determined in the water according to the sampling stations. Values are given as mean ± standard deviation (SD).

	Al	Cr	Co	Ni	Cu	Zn	Cd	Pb	As	Se
Station 3	37318±22	2804±52	52 ±3	2909±81	1545±43	0	0	0	0	0
Station 2	28418 ±92	1999±9	48±11	2041±24	1142±13	0	0	3±1	0	0
Station 2	9570±41	1770±91	43±43	1782±62	931±91	0	0	17±94	0	0
Station 1	0	1132±6	19±94	1145±18	562±42	0	0	0	0	0
Station 1	0	1065±33	18±52	1063±11	535±02	0	0	0	0	0
Station 4	1535±01	1329±14	183±7	1383±38	780±16	0	0	15±12	0	0
Station 5	50897±66	1269±12	21±67	1295±81	954±07	0	0	0	0	0
Station 4	121303±9	1114±22	17±43	1033±88	825±75	0	0	81±8	0	0
Station 5	17689±3	1732±49	33±72	1368±49	1228±6	11087±68	0	0	0	223±44
Station 3	5661±2	1075±16	16±.2	1055±65	550±11	0	0	0	0	0

Table 2: Descriptive statistics for the heavy metal values determined in the water (ppb).

	N	Minimum	Maximum	Mean ± SD
Al	10	0.00	50897.66	16322.11 ± 17361.51
Cr	10	1065.33	2804.52	1529.34 ± 558.39
Co	10	16.20	183.70	45.5 ± 50.43
Ni	10	1033.88	2909.81	1507.92 ± 592.9
Cu	10	535.02	1545.43	905.56 ± 329.36
Zn	10	0.00	11087.68	1108.77 ± 3506.23
Cd	10	0.00	0.00	0 ± 0
Pb	10	0.00	17.94	4.43 ± 6.92
As	10	0.00	0.00	0 ± 0
Se	10	0.00	223.44	22.34 ± 70.66

Table 3: Correlations between the heavy metal measurements determined in the water. r; Spearman rank correlation coefficient, n: sample number, *; 0.05 significance level and **; 0.01 significance level.

		Cr	Co	Ni	Cu	Zn	Pb	Se
Al	R	0.608	0.316	0.450	0.857**	0.175	-0.082	0.175
	P	0.062	0.374	0.192	0.002	0.629	0.821	0.629
	N	10	10	10	10	10	10	10
Cr	R	1.000	0.842**	0.939**	0.867**	0.174	0.273	0.174
	P		0.002	0.000	0.001	0.631	0.445	0.631
	N	10	10	10	10	10	10	10
Co	R		1.000	0.915**	0.600	0.058	0.376	0.058
	P			0.000	0.067	0.873	0.285	0.873
	N			10	10	10	10	10
Ni	R			1.000	0.709*	0.058	0.225	0.058
	P				0.022	0.873	0.531	0.873
	N				10	10	10	10
Cu	R				1.000	0.406	0.020	0.406
	P					0.244	0.955	0.244
	N					10	10	10
Zn	R					1.000	-0.261	
	P						0.466	
	N						10	

Discussion

In this study were (µl/l) ; 1108.7 (Zn), 0.016 (Al), 1529.4 (Cr), 1507.92 (Ni), 905.56 (Cu), 22.3 (Se), 45.5 (Co) and 4.43 (Pb) determined , Cd and As were not determined. In a study by Fırat et al (4), in the muscle tissue of fish caught in Atatürk Dam in the Adıyaman Sitilce District, the maximum values of Zn, Cu, Pband Cr were 22.58, 0.75, 0.29, and 0.13 µl/g dry weight, respectively. In the current study, the values obtained of these metals were lower, which was thought to be due to the positive effect of the waste-water treatment plants having come into operation. In another study, accumulated levels of heavy metals were

examined in water samples taken from the freshwater Çıldır Lake, and the results obtained determined Mn>Fe>Zn>Pb>Cu>Cd in the water (7). Şahin et al (8) investigated the heavy metal levels in the Karakaya Reservoir from the two different stations of Arguvan and Battalgazi. In the samples taken from the Arguvan area, the metal ratios were determined as As> Fe> Cd> Mn> Pb> Cr> Ni>Zn>Cu>Co, and in the Battalgazi area as As>Cd>Fe>Mn>Pb>Cr>Co>Ni>Cu>Zn (8). In the current study, Cd and as were not determined in any sample, and Zn and Se were determined in the samples from Station 4 (11087.68 ppb).

Metal levels in the study areas are affected by domestic and industrial activities, and the highest metal levels of all the samples were seen to be higher in the polluted areas where waste-water is discharged without treatment (9).(9). According to the Intracontinental Water Pollution Control Management criteria, the metal values obtained were classified as 4th quality water pollution for Cr, Se and Ni, 3rd quality water pollution for Co, and 2nd and 1st quality water pollution for the other metals.

As a result of natural or human activities such as urbanisation and industrialisation, there has been an increase in recent years of the concentrations of heavy metals in water ecosystems (rivers, lakes, seas) and these cause severe ecological damage because of the bio accumulative property and toxicity in the food chain. The high metal concentrations in water, sediment and organisms can be much higher than the defined legal values (1, 10-12).

The toxic substances defined as the 20 most dangerous on earth include zinc (Zn), copper (Cu), mercury (Hg), nickel (Ni), chrome (Cr) and lead (Pb), and these are known to be extremely dangerous chemicals for the environment and show the most significant water pollution (13). Several human diseases are known to be related to increased heavy metal pollution. For example, Hg causes neurological effects, cadmium (Cd) and Pb cause carcinogenic effects, Cr leads to mutations in genetic material, and Cu causes anaemia (2, 3, 14).

Environmental problems formed because of modern-day industrial activities threaten the balance of nature and human and animal health, and these problems are seen to be emerging in ever increasing dimensions. The Atatürk Dam was constructed on the Euphrates River in the Southeast Anadolu region of Turkey As the largest reservoir in the country, the basic function is the provision of water and the production of hydroelectricity. Intensive trout farming is also conducted in the reservoir and recently mass fish deaths have been seen (15-18).

In conclusion ,the results of this study have demonstrated that waste-water discharged without being treated to Atatürk Dam creates a high rate of heavy metal pollution and has the potential to harm the freshwater environment and the life in it.

Conflict of interests:

The author(s) declared no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

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References

1. Monroy M, Maceda-Veiga A, de Sostoa A. Metal concentration in water, sediment and four fish species from Lake Titicaca reveals a large-scale environmental concern. The Science of the total environment. 2014;487:233-44.
2. Rashed M. Biomarkers as Indicator for Water Pollution with Heavy Metals in Rivers, Seas and Oceans. 2004.
3. Uysal K, Yılmaz E, Köse E. The Determination of Heavy Metal Accumulation Ratios in Muscle, Skin and Gills of Some Migratory Fish Species by Inductively Coupled Plasma-optical Emission Spectrometry (ICP-OES) in Beymelek Lagoon (Antalya/Turkey). Microchemical Journal. 2008;90(1):67-70.
4. Fırat Ö, Fırat Ö, Çoğun H, et al. Atatürk Baraj Gölü'nün Kirli ve Temiz Bölgelerinden Yakalanan Balıkların (Silurus triostegus Heckel, 1843, Chalcalburnus tarichi Pallas, 1811, Chondrostoma regium Heckel, 1843, Carassius carassius Linnaeus, 1758) Dokularındaki Ağır Metal Düzeylerinin Karşılaştırılması. Süleyman Demirel Üniversitesi Eğirdir Su Ürünleri Fakültesi Dergisi. 2018;14(3):173-83.
5. Uçkun A, Yoloğlu E, Uçkun M. Seasonal Monitoring of Metals in Water, Sediment and Mussel (Unio mancus) from Atatürk Dam Lake (Euphrates River). . Van Veterinary Journal. 2017;28(2):75-83.
6. Cataldo D, Colombo JC, Boltovskoy D, et al. Environmental toxicity assessment in the Parana river delta (Argentina): simultaneous evaluation of selected pollutants and mortality rates of Corbicula fluminea (Bivalvia) early juveniles. Environmental pollution. 2001;112(3):379-89.
7. Baltacı B. Ar water of Çıldır Lake, bottom sediment where was revealed the mussels (Anadonta cynea Linnaeus,1758) concentration levels of some heavy metals researching: Kafkas University; 2011.
8. Şahin A, Sünbül M, Küçükyılmaz M. Karakaya Baraj Gölü'ndeki Tatlı Su Midyesi (Unio elongatulus eucirrus Bourguignat 1860)'nin Ağır Metal Düzeylerinin Belirlenmesi. Fırat Üniversitesi Fen Bilimleri Dergisi. 2016;28(1):13-9.
9. Kalıpcı E, Cüce. H, Toprak S. Damsa Barajı (Nevşehir) Yüzey Suyu Kalitesinin Coğrafi Bilgi Sistemi ile Mekansal Analizi. Karaelmas Fen ve Mühendislik Dergisi. 2017;7(1):312-9.
10. Küçükyılmaz M, Uslu G, Birici N. Karakaya Baraj Gölü Su Kalitesinin İncelenmesi. Yunus Araştırma Bülteni. 2017;17(2):145-55.
11. MacDonald DD, Ingersoll CG, Berger TA. Development and evaluation of consensus-based sediment quality guidelines for freshwater ecosystems. Archives of environmental contamination and toxicology. 2000;39(1):20-31.
12. Minareci O, Çakır M. Determination of Detergent, Phosphate, Boron and Heavy Metal Pollution in Adıgüzel Dam Lake (Denizli/Turkey). . Iğdır University Journal of the Institute of Science and Technology. 2018;8(1):61-7.
13. Güldiren O, Tekin-Ozan S. Seyhan Baraj Gölü (Adana)'nün Suyunda ve Sedimentindeki Bazı Ağır Metallerin Mevsimsel Değişimi. Süleyman Demirel Üniversitesi Yalvaç Akademi Dergisi. 2017;2(1):99-111.
14. Dural M, Göksu M, Özak A. Investigation of heavy metal levels in economically important fish species captured from the Tuzla lagoon. Food chemistry. 2007;102(1):415-21.
15. Alhas E, Oymak SA, Karadede Akin H. Heavy metal concentrations in two barb, Barbus xanthopterus and Barbus rajanorum mystaceus from Ataturk Dam Lake, Turkey. Environmental monitoring and assessment. 2009;148(1-4):11-8.
16. Fırat Ö. Evaluation of metal concentrations in fish species from Atatürk Dam Lake (Adıyaman, Turkey) in relation to human health. . Fresenius Environmental Bulletin. 2016;25(9):3629-34.
17. Karadede H, Oymak SA, Unlu E. Heavy metals in mullet, Liza abu, and catfish, Silurus triostegus, from the Ataturk Dam Lake (Euphrates), Turkey. Environment international. 2004;30(2):183-8.
18. Olgunoğlu İ, Bayhan Y, Olgunoğlu M, et al. Adıyaman İlinde Balık Eti Tüketim Alışkanlıklarının Belirlenmesi. Gıda Teknolojileri Elektronik Dergisi. 2014;9(1):21-5.

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Effect of Antihistamine Levocetirizine Dihydrochloride on Cytogenetic Markers

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Abstract

Levocetirizine dihydrochloride is the active ingredient of Alerinit, a second-generation antihistamine, used in the treatment of allergic diseases. This study was carried out to determine the probable genotoxic and cytotoxic effects of levocetirizine using chromosomal abnormality (CA) and micronucleus (MN) tests in human peripheral lymphocytes. In this study, cell cultures were treated with 2, 4, 8 and 16 µg / ml concentrations determined by preliminary study of Levocetirizine during 24 and 48 hours. As a result of our study, we observed that Levocetirizine does not cause any significant change compared to control in CA / Cell rate, abnormal cell percentage and mitotic index values, MN frequency, binuclear micronucleus cell rate and nuclear division index values at within the all studied concentrations and treatment periods. According to this research; levocetirizine dihydrochloride has no genotoxic and cytotoxic effects.

Keywords*: Chromosome Aberration, Cytotoxicity, Genotoxicity, Micronucleus, Levocetirizine Dihydrochloride*

Introduction

Allergy is the hypersensitivity of the immune system to several substances called allergens. The concept of allergy was first described by the Austrian Clemens Von Pirquet in 1906 as “the body's response to foreign substances” (1). Allergic reactions have increased in developed countries due to the decrease in the diseases caused by infection and parasites, together with the environmental effects, but also the increase of individual hygiene and the lack of some

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immune system needed agents (2, 3). Antihistamines are drugs that bind to histamine receptors instead of histamine and block the receptors and thus eliminate the effects such as itching, and pain caused by histamine. Although these drugs are currently used as the first choice in the treatment of allergic diseases, H1 antihistamines are among all drugs are among the most widely used drugs (4, 5). There are different genotoxic studies on antihistamines. Since it does not significantly affect chromosome abnormality (CA) and micronucleus (MN) frequency in rats, fexofenadine has been reported to be not genotoxic (6). Terfenadine it was determined Chinese hamster that it did not affect MN frequency in V79 cells (7). Mizolastine, S. typhimurium has been reported to have no genotoxic effect in the presence and absence of liver enzyme complex in the Ames assays performed with strains TA98, TA100, TA1535, TA1537, as well as with E. coli WP2 uvrA (8). Cetirizine was not genotoxic in the Ames test system, CA in human lymphocytes *in vitro* and *in vivo* MN test systems in male rats (7). Astemizole did not induce SCE in human peripheral lymphocytes (9). Astemizole was not carcinogenic in long-term carcinogenicity testing with Wistar rats and Swiss rats (10). Roshdy (11) applied levocetirizine on pregnant mice and embryos during pregnancy to evaluate the active ingredient for cytotoxicity and mutagenicity. As a result, it has been reported that oral administration of levocetirizine at a recommended concentration during pregnancy can cross the placenta and cause mutagenic and cytotoxic effects on both mothers and embryos. Although this study was long-term, pregnant mice were regularly given an appropriate dose of levocetirizine daily. There is only one genotoxic study with levocetirizine, which is a frequently used antihistaminic drug group worldwide. More research should be done with this group of drugs on how antihistamines affect human genetic structure. Therefore, we determined the genotoxic and cytotoxic effects of levocetirizine in human peripheral lymphocytes by chromosome aberration and micronucleus tests.

Material and Method

The techniques of Evans (12) and Perry and Thomson (13) were followed for preparation of the CA test with minor modifications. This study was designed to follow IPCS guidelines (14). Whole blood (0.2 mL) from four healthy donors (two male and two female, non-smokers, aged 22-25 years, blood samples not pooled) was added to 2.5 mL chromosome medium B (Biochrom, F5023) supplemented with 10 µg/mL bromodeoxyuridine (Sigma, B5002). The cultures were incubated at 37ºC for 72h, and then treated with 2, 4, 8, 16 µg/mL concentrations of levocetirizine dihydrochloride dissolved in distilled water, for 24h (levocetirizine dihydrochloride added 48h after initiating culture) and 48h (levocetirizine dihydrochloride added 24h after initiating culture). A negative control and a positive control (mitomycin, 0.1 µg/mL) were also used. Colchicine (0.06 µg/mL) was present for the last 2h of culture. At the end of the incubation, cells were harvested by centrifugation at 2000 rpm for 5min then the cells were treated with 0.4 % KCl as the hypotonic solution for 20 min and with fixative (methanol; glacial acetic acid 3:1) for 10 min at room temperature (22ºC±1, fixative treatments were repeated three times). The cell suspension was centrifuged at 1200 rpm for

10 min after each fixative treatment. After last fixative process, the cells were dropped on cold glass slides. After drying at room temperature for overnight, according to the standard method, the slides were stained using 5 % giemsa stain for CA test. MI was determined by scoring 3000 cells from each donor for each concentration. The percentage of cells, showing structural chromosome alteration, was obtained by calculating the percentage of the aberrant metaphases from each concentration and treatment period. CAs were evaluated in 100 well-spread metaphases per donor (totally 400 metaphases per concentration). CAs were classified as structural aberrations structural CAs were classified as chromatid and chromosome type abnormalities (breaks sister chromatid union, chromatid exchange, ring chromosome fragments and dicentric chromosomes). For the determination of genotoxicity, only the structural and numerical CAs were taken into consideration. For the analysis of Micronucleus (MN) binucleated lymphocytes, 0.2 mL of fresh whole blood (1/10 heparinized) was used to establish the cultures were incubated for 68h (15). The cells were treated with various concentration (2, 4, 8, 16 µg/mL) of levocetirizine dihydrochloride for 24h and 48h treatment periods. To block cytokinesis, cytochalasin-B (6 µg/mL) was added to culture 24h before the end of total incubation time. Finally, the cells were harvested by centrifugation at 2000 rpm for 5 min and process continued as mentioned above for preparation of CA slides, except for a 5 min hypotonic treatment at 37ºC. The cells were fixed once with cold fixative (1:glacial acetic acid, 5:methanol, 6:0.9%NaCl) for 20 min and the twice with second cold fixative (1:glacial acetic acid, 5:methanol) for 15 min after the fixation process, the cultured cells were centrifuged at 1200 rpm for 10 min. Finally, the slides were stained with 5 % giemsa. The number of micronucleated cells and total number of micronucleus present in 1000 binuclear cells (for each of the control and treated cultures) were determined. Also, a total of 1000 cells (4000 cells for each treatment concentration) were scored to calculate the nuclear division index (NDI) for the cytotoxicity of Levocetirizine dihydrochloride using the following equation (M1: mononucleated cells; M2: binucleated cells; M3 and M4: multinucleated cells and N is the total number of cells scored) (16). One-way ANOVA (Dunnet's test) was utilized for establishing the statistical significance of all parameters. CA, MN cells, MI, NDI data obtained from microscopic analysis were compared with control groups using SPSS (17.0) at $p \leq 0.05$. To find out the concentration response relation in treated groups, the regression analysis was performed.

Results

In our study, we observed a slight increase in the frequency of abnormalities per cell compared to the control in all concentrations examined because of 24-hour treatment of levocetirizine in human peripheral lymphocytes. However, this increase was not statistically significant ($p > 0.05$). On the other hand, in human peripheral lymphocytes treated with levocetirizine at two concentrations (8 and 16 µg / ml) for 48 hours, a slight decrease was observed when the abnormal cell frequency was compared with the control, but this decrease was not statistically significant (Table 1) ($P > 0.05$).

Mitotic index (MI) value was determined by examining the effect of all doses of this active substance on mitosis because of treatment of human peripheral lymphocytes with levocetirizine for 24 and 48 hours. MI values were compared with the control group and levocetirizine caused a slight decrease in MI which was not statistically significant (p> 0.05). The effect of all doses of levocetirizine at 24 and 48 hours treatment time on MN frequency was compared with control; it was reported that this active substance caused a slight increase in MN frequency, but this increase was not statistically significant (P> 0.05).

Table 1: Abnormal cell and micronucleus finding in levocetirizine-treated human lymphocytes. MMC; Mitomycin C, PC; positive control, MN; micronucleus, SE; standard error.

Test Substance	**Treatment**		**Abnormal Cells (%) ± SE**	**MN (%) ± SE**
	Duration (hr)	**Conc. (µg/mL)**		
Control	24	-	3.50 ± 1.56	3.50±0.50
MMC (PC)	24	0.1	21.75 ± 2.15	23.75±2.75
Levocetirizine	24	2	5.25 ± 2.25	6.25 ± 2.06
	24	4	5.00 ± 1.78	6.00 ± 1.96
		8	5.50 ± 2.60	6.005 ± 1.78
		16	5.25 ± 2.36	8.75 ± 2.84
Control	48	-	3.50 ± 1.55	3.50 ± 0.50
MMC(PC)	48	0.1	40.40 ± 2.19	40.51 ± 2.12
Levocetirizine	48	2	4.00 ± 2.04	5.25 ± 0.85
	48	4	3.50 ± 1.44	5.75 ± 1.89
	48	8	3.25 ± 0.25	9.50±4.91
	48	16	2.00 ± 1.00	4.50±1.94

Furthermore, although all doses of levocetirizine active agent at both 24- and 48-hour treatment periods slightly increased binuclear micronucleus (BNMN), this increase was not statistically significant compared to control. (P> 0.05) Although all 24-hour doses of levocetirizine slightly reduced NDI compared to control, this reduction was not statistically significant (Table 2) (p> 0.05).

Table 2: Mitotic index (MI) and nuclear division index (NDI) findings in levocetirizine–treated lymphocytes. MMC; Mitomycin C, PC; positive control, SE; standard error.

Test Substance	Treatment		MI ± SE	NDI ± SE
	Duration (hr)	Conc. (µg/mL)		
Control	24	-	0.049 ± 0.017	1.074 ± 0.004
MMC (PC)	24	0.1	0.02 ± 0.01	0.1 ± 0.01
Levocetirizine	24	2	0.042 ± 0.018	1.051 ± 0.014
	24	4	0.032 ± 0.017	1.057 ± 0.010
		8	0.040 ± 0.020	1.034 ± 0.009
		16	0.036 ± 0.018	1.065 ± 0.011
Control	48	-	0.049 ± 0.017	1.074 ± 0.004
MMC(PC)	48	0.1	0.06 ± 0.01	0.2 ± 0.02
Levocetirizine	48	2	0.044 ± 0.013	1.078 ± 0.008
	48	4	0.037 ± 0.015	1.062 ± 0.007
	48	8	0.040 ± 0.017	1.075 ± 0.006
	48	16	0.043 ± 0.019	1.085 ± 0.004

Discussion

In the present study, the genotoxic and cytotoxic effects of levocetirizine were investigated in human peripheral lymphocytes *in vitro*. In this study using chromosome aberration and micronucleus techniques, there was no induction in CAs and MN frequencies. Antihistamines have been used by patients for many years. The fact that a drug that should be taken regularly every day is not genotoxic is especially important for human health. Contrary to the genotoxic effect of many drugs in both community bias and previous studies, it is promising that levocetirizine does not have a genotoxic effect. Antihistamines have not been reported to have genotoxic, cytotoxic, or carcinogenic effects, not only in our study but also in many previous studies. Doxylamine has not been shown a mutagenic effect in Ames test. Zeiger et al. (17), Clemastine has been disclosed to affect micronucleus frequency in mice (7). Cyproheptadine has been reported to have no effect on CA value in human lymphocytes (18). In this study, negative results of doxylamine were obtained *in vitro* in human lymphocytes and in SCE test in fetal mouse cells (19). Methdilazine was negative in the Ames test with various strains of S.typhimurium in the presence and absence of S9 metabolic activation (20). Methdilazine has been reported not to induce CA in CHO cells (21). Triprolidine S. typhimurium TA97, TA98, TA100 and TA104 strains were reported to be non-mutagenic in the Ames test in the presence and absence of S9 fraction (22). Triprolidine has been reported to have no carcinogenic effect on long-term carcinogenicity testing in B6C3F1 mice and F344 rats (23). Chlorphenamine gene mutation test in mouse lymphoma L5178Y cells showed negative results (24). Chlorphenamine F344 in long-term carcinogenicity test with rats the results were negative (25). Promethazine has been reported to have no genotoxic effect in CHO cells (21). Promethazine was found not to induce CA in human leukocytes *in vitro*.

Gocke, (26). In Ames tests of Mizolastine with S.typhimurium TA98, TA100, TA1535, TA1537 and E.coli WP2 uvrA, there was no genotoxic effect in the presence and absence of liver enzyme complex (8). Terfenadine has been reported to not increase MN frequency *in vitro* in Chinese hamster V79 cells (7). Tripelennamine was not genotoxic in the gene mutation test in mouse lymphoma L5178Y cells (27). Astemizole has been reported to have no carcinogenic effect on Wistar rats and Swiss rats (10). It was reported that terfenadine did not increase the MN frequency *in vivo* and was not mutagenic in the Ames test with S. typhimurium strains (28).

As can observed, the lack of genotoxic effects of many antihistamines, especially levocetirizine, is promising, but there is only one genotoxic study of levocetirizine. Therefore, to have a clearer genotoxic effect of antihistamines, studies on these drugs are needed. Many studies using different techniques will support the clarification of the results.

Conclusion

In the present study, it was shown that levocetirizine not induced the structural chromosomal aberrations, frequency of micronucleus and MI and NDI. It can be concluded that levocetirizine has not genotoxic and cytotoxic effects.

Conflict of interest

The author(s) declared no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

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References

1. Von Pirquet C. Allergie. Annals of allergy. 1946;4(5):388-90.
2. Hertzen LC. The hygiene hypothesis in the development of atopy and asthma--still a matter of controversy? QJM : monthly journal of the Association of Physicians. 1998;91(11):767-71.
3. Zeyrek FY, Zeyrek CD. Alerjik Hastalıklar ve Parazitoz Turlish Society of Parasitology. 2006;2(30):135-40.
4. Kaufman DW, Kelly JP, Rosenberg L, et al. Recent patterns of medication use in the ambulatory adult population of the United States: the Slone survey. Jama. 2002;287(3):337-44.
5. O'Donoghue M, Tharp MD. Antihistamines and their role as antipruritics. Dermatologic therapy. 2005;18(4):333-40.
6. Physicians Desk Reference. 59 ed: Thomson PDR; 2004.
7. Snyder RD. A review and investigation into the mechanistic basis of the genotoxicity of antihistamines. Mutation research. 1998;411(3):235-48.
8. Iwase Y, Uno Y, Ohkouchi A, et al. Mutagenicity and clastogenicity studies with mizolastine. Yakuri To Chiryo - Japanese Pharmacology and Therapeutics. 1998:663-72.
9. Tucker JD, Auletta A, Cimino MC, et al. Sister-chromatid exchange: second report of the Gene-Tox Program. Mutation research. 1993;297(2):101-80.
10. Benze J, Gypen L, Vandenberghe J, et al. Carcinogenicity studies of astemizole in mice and rats. Cancer research. 1995;55(23):5589-94.
11. Roshdy HM. Cytogenetic and Mutagenic Effects of Levocetirizine and Montelukast alone or in combination on pregnant mice and embryos. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 2015;6(3):979-89.
12. Evans HJ, O'Riordan ML. Human peripheral blood lymphocytes for the analysis of chromosome aberrations in mutagen tests. Mutation research. 1975;31(3):135-48.
13. Perry PE. The methodology of sister chromatid exchanges. In: Kilbey BJ, Legator M, Nichols W, Ramel C, editors.1984. p. 495-529.
14. Albertini RJ, Anderson D, Douglas GR, et al. IPCS guidelines for the monitoring of genotoxic effects of carcinogens in humans. International Programme on Chemical Safety. Mutation research. 2000;463(2):111-72.
15. Rothfuss A, Schutz P, Bochum S, et al. Induced micronucleus frequencies in peripheral

lymphocytes as a screening test for carriers of a BRCA1 mutation in breast cancer families. Cancer research. 2000;60(2):390-4.
16. Eastmond DA, Tucker JD. Identification of aneuploidy-inducing agents using cytokinesis-blocked human lymphocytes and an antikinetochore antibody. Environmental and molecular mutagenesis. 1989;13(1):34-43.
17. Zeiger E, Anderson B, Haworth S, et al. Salmonella mutagenicity tests: III. Results from the testing of 255 chemicals. Environmental mutagenesis. 1987;9 Suppl 9:1-109.
18. Hite M, Algon J, Peck HM. The effect of cyproheptadine on the chromosomes of human lymphocytes in vitro. Arzneimittel-Forschung. 1977;27(6):1203-6.
19. Muller L, Korte A, Madle S. Mutagenicity testing of doxylamine succinate, an antinauseant drug. Toxicology letters. 1989;49(1):79-86.
20. Mortelmans K, Haworth S, Lawlor T, et al. Salmonella mutagenicity tests: II. Results from the testing of 270 chemicals. Environmental mutagenesis. 1986;8 Suppl 7:1-119.
21. Galloway SM, Armstrong MJ, Reuben C, et al. Chromosome aberrations and sister chromatid exchanges in Chinese hamster ovary cells: evaluations of 108 chemicals. Environmental and molecular mutagenesis. 1987;10 Suppl 10:1-175.
22. Hansen EB, Jr., Heflich RH, Korfmacher WA, et al. Microbial transformation of the antihistaminic drug triprolidine hydrochloride. Journal of pharmaceutical sciences. 1988;77(3):259-64.
23. Greenman DL, Sheldon W, Schieferstein G, et al. Triprolidine: 104-week feeding study in rats. Fundamental and applied toxicology : official journal of the Society of Toxicology. 1995;27(2):223-31.
24. McGregor DB, Brown AG, Howgate S, et al. Responses of the L5178Y mouse Lymphoma cell forward mutation assay. V: 27 coded chemicals. Environmental and molecular mutagenesis. 1991;17(3):196-219.
25. Lijinsky W. Induction of tumours in rats by feeding nitrosatable amines together with sodium nitrite. Food Chem Toxicol. 1984;22(9):715-20.
26. Gocke E. Review of the genotoxic properties of chlorpromazine and related phenothiazines. Mutation research. 1996;366(1):9-21.
27. Oberly TJ, Yount DL, Garriott ML. A comparison of the soft agar and microtitre methodologies for the L5178Y tk +/- mouse lymphoma assay. Mutation research. 1997;388(1):59-66.
28. Gibson JP, Huffmann KW, Newberne JW. Preclinical safety studies with terfenadine. Arzneimittel-Forschung. 1982;32(9a):1179-84.