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# EAMS

## Experimental and Applied Medical Science

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**GAZİANTEP İSLAM BİLİM VE TEKNOLOJİ ÜNİVERSİTESİ TIP FAKÜLTESİ**

**GAZİANTEP ISLAM SCIENCE AND TECHNOLOGY UNIVERSITY FACULTY OF MEDICINE**

# Experimental and Applied Medical Science

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On behalf of the Medical Faculty of Gaziantep Islam Science and Technology University
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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 are presented.

# Scope

***Experimental and Applied Medical Science*** is an open-access, internationally double-blind peer reviewed academic medical journal and 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 çalışmalarını, klinik, farmakolojik, epidemiyolojik, deontolojik çalışmalar ile beraber halk sağlığının geliştirilmesi amacı taşıyan ve sağlık hizmetleri veya sağlık sigortaları konularında araştırma makalelerini, derlemeleri, vaka sunumlarını, 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.

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Manuscripts are only considered for publication provided that they are original, not under consideration simultaneously by another journal, and 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 indicated 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 ve daha önce yayınlanmamış olmaları koşuluyla yayına kabul edilebilmesi için değerlendirmeye alınır. 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*

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

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

Processing and publication processes are free of charge. Any fee can not be requested from the authors during 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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All researchers should have contributed to the article directly either academically or scientifically. Authors should have contributed either one or a few of planning, performing, writing or reviewing of manuscript. All authors should approve the final version. It is the authors'

bölümünde, internet adresi aşağıda 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

responsibility to prepare a manuscript that meets scientific criterias.

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

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

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

All manuscripts and ecorrespondence with the editorial board must be sent to the editorial office, at 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 added in time, all kinds of publication rights of the articles accepted for publication belong to the institution that

hazırlamak yazarların sorumluluğundadır.

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 ve kaynaklar ile eğer varsa biyoistatistiksel kısımlar açık, anlaşılır ve tatminkâr şekilde açıklanmış olmalıdır.

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

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

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# Author Guidelines

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Manuscripts should be prepared electronically by using “office word” or any other text-processing package compatible with that, 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 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) 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 a maximum specific word length laid down as a condition for any manuscript. The general principle is that a manuscript should be as long as necessary to communicate the scientific message clearly and effectively at the most, but should be as short as possible to avoid undue repetition or redundancy with a complete presentation of the information.

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.

3. Gereç ve Yöntemler
4. Sonuçlar
5. Tartışma
6. Bağlam
7. Çıkar çatışması
8. 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

All dimensions and measurements must be specified in the metric system.

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

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

References should be designed in “Vancouver” style. While writing references, “Times New Roman” 10 point font should be used. Multiple authors should be separated by a comma. If there are more than three authors, after the 3rd author, "et al." should be inserted with 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

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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asit fenetil ester (KAFE) ve miyokardiyal iskemi reperfüzyon (Mİ/R) hasarı. İnönü Üniversitesi Sağlık Bilimleri Dergisi 2012; 1: 10-5.
5. Yıldırım AB. The effects of maternal hypothyroidism on the immunoreactivity of cytochrome p450 aromatase in the postnatal rat testes. 2015; Doctoral thesis.
6. https://hsgm.saglik.gov.tr/depo/birimler/kanserdb/istatistik/Trkiye_Kanser_statistikleri_2016.pdf (Last access date: 21.09.2020).
7. Kuran O, İstanbul, Filiz Kitabevi. Sistematik Anatomi. 1983 p. 76-9.
8. Abbas AK, Andrew H Lichtman, Shiv Pillai. Cellular and Molecular Immunology. 6th ed. Philadelphia: Saunders Elsevier; 2007 p. 121-56.

Submit illustrations as separate files, only as TIFF or EPS files, with a minimum resolution of 300dpi.
Tables of numerical data should each be typed with double spacing on separate pages numbered in sequence in numerals, provided with a heading, and referred to in the text, as Table 1, Table 2, etc. Each table should have a brief but descriptive heading. Explanatory matter should be included in footnotes to the table.
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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.
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.

Görseller, minimum 300 dpi çözünürlükte, yalnızca TIFF veya EPS dosyaları halinde ve ayrı dosyalar olarak gönderilmelidir.
Sayısal veri tablolarının her biri, sayılarla sırayla numaralandırılmış bir başlık ile birlikte ve metinde Tablo 1, Tablo 2, vb. olarak atıfta bulunulmuş halde, ayrı sayfalarda çift aralıkla hazırlanmalıdır. Her tablonun kısa ama açıklayıcı bir başlığı olmalıdır. Tablo dipnotlarında açıklayıcı hususlara yer verilmelidir.
Bilimsel araştırmalarınızı desteklemek ve geliştirmek için elektronik ek materyaller kabul edilmektedir. Ek dosyalar, yazara, destekleyici uygulamaları, filmleri, animasyon dizilerini, yüksek çözünürlüklü görüntüleri, arka plan veri kümelerini, ses kayıtlarını ve daha fazlasını yayınlamak için ek olanaklar sunmaktadır.
Başvuru sırasında çıkar çatışmasının ve mali

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

**Editorial Board/Editör Kurulu**

**Statistics Editor/İstatistik Editörü**

**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 many centuries ago. 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 his genuine perspective through the medicine and adapting to orders of the Quran and medicinal principles of "Tıbb-ı Nebevî", really worth mentioning here. He wrote more than 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 Medical Faculty which was brought to our country within the newly formed Gaziantep Islamic Science and Technology University, it has been just a kind of more than one year since our academic journal, the Experimental and Applied Medical Science in which we wholeheartedly believe will make a significant contribution to our academic community, sprouted. 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 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 a little more than 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

**Contents/İçindekiler**

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# In Vivo Investigation of Genotoxic and Antigenotoxic Potential of *Hypericum Perforatum* Extract in Rats

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**Abstract**

*This study was planned to reveal in vivo genotoxic, antigenotoxic and cytotoxic properties of methanolic extracts of St. John's Wort plant (Hypericum perforatum). The methanol extract of H. perforatum was administrated to rats alone or in combination with N-nitrosodimethylamine and Triclosan (positive control). The concentrations of H. Perforatum were 20, 100, 500 mg/kg and administration duration was 30 days. At the end of this period, chromosome aberration test used to determine genotoxic and antigenotoxic effects of H. perforatum. In addition, total oxidant and antioxidant levels of peripheral blood serum of rats were determined by spectrophotometric method. According to our result, H. perforatum extract did not demonstrate genotoxic effect at concentrations administrated. When compared to the negative control, the percentage of abnormal cells was increased in groups treated with test substance, N-nitrosodiethylamine and triclosan together. However, these increases were not as higher as groups treated with N-nitrosodiethylamine plus triclosan (positive control). Even, in groups treated with the test substance additional N-nitrosodiethylamine plus triclosan, the ratio of abnormal cells was inversely proportional to the increase in the concentration. The lowest ratio of abnormal cells was in the group in which the highest concentration of the H. perforatum (500 mg/kg) was applied. The highest two concentrations of the H. perforatum (100 and 500 mg/kg) significantly increased the mitotic index. In the group treated with test substance and positive control together, the mitotic index was even greater. At the same time 100 mg/kg of H. perforatum extract significantly reduced the oxidative stress.*

***Key words:*** *Hypericum Perforatum, N-nitrosodiethylamine, triclosan, cytotoxicity, genotoxicity, rat bone marrow, oxidative stress.*

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## Introduction

Including *Hypericum perforatum* (St. John's Wort) Around 490 species from Hypericaceae family, are grown in many different regions of the world, and Turkey. It is known as John's wort. During the flowering period, flowers and buds of the plant or all over the ground parts can be collected and used immediately after drying. The John's wort was used in the treatment of mental disorders in Europe and was also a very popular plant in America continent. In folkloric use, the plant's yellow stems are collected and kept in a place where the sunlight is shining until it takes a red colour in the olive oil. This oil is used commonly as a cure for burn injuries and wounds in bedridden patients (1-3). *H. perforatum* is a medicinal plant with anti-depressant properties and has anti–inflammatory effects such as arachidonate 5-lipoxygenase inhibitor and COX-1 inhibitor (4, 5). According to clinical studies, *H. perforatum* has antidepressant properties and has milder side effects (6). It has also been shown that *H. perforatum* had antiviral, anticancer and antiproliferative effects (7, 8).

The ethanolic aqueous extract of *H. perforatum* gave negative results in both in vitro and in vivo studies and did not show mutagenic potential (9). In another study, it was reported that *H. perforatum* water extract had protective effect against alkylating DNA damage induced by methylmethanesulfonate (MMS) in colon cancer cell line (HT29) and also increased the repair of alkylated DNA damage (10). In a different in vitro study, hyperforin a constituent of St John's wort extract was found to induce apoptosis by trigerring activation of caspases, and in combination with hypericin, it synergistically exerted cytotoxicity towards human malignant cell lines (11). Methanolic extract of *H. perforatum* and hypericin, the plant's active ingredient, induced apoptosis and long-lasting inhibi- tion of cell growth and decreased phototoxicity (12, 13). However, in an investigation of the effects of *H. perforatum* peptide extracts on non- small cell lung carcinoma cells (A549), lung cancer cells (H1299) and HeLa cervical cancer cells, extracts at various concentrations were administrated to cells. It was indicated that *H. perforatum* peptide extract did not have any effect on proliferation activity and growth of tumor in that study (14). N-nitrosodimethylamine (DEN), which is considered a human car- cinogen (15, 16) and plays a role in the induction of gastric, esophagus and nasopharyngeal cancers (17). Triclosan [5- chloro-2- (2, 4-ichlorophenoxy) fenol; TCS], a broad spectrum antibacterial agent used in a wide range of synthetic products like soaps, cosmetics, therapeutics and plastics (18). Both of them were used as a positive control in this study. It has been discovered that triclosan significantly accelerates the development of hepatocellular carcinoma (HCC) and acts as a liver tumor promoter when it was used with procarcin- ogen diethylnitrosamine (DEN). Triclosan treated rats were found to exhibit large increases in tumor variety, size, and incidence when compared with control groups (19).

In this study, chronically (30 days) genotoxic and cytotoxic effects of *H. perforatum* extract alone or in combination with positive control (DEN and triclosan) were investigated in rat

marrow cells. Furthermore, the effects of *H. perforatum* on oxidative stress in rat peripheral blood were also determined.

**Method**

Sprague-Dawley albino rats were used in this study. The animals were 8-12 week old and their weights varied between 180-250 gr. six male rats were used for each group. The animals were provided by Çukurova University Medical Faculty Experimental Medicine Research and Application Center (CUTF-DETAUM) and housed 12-hour light and dim cycle individually in air-conditioned cages (24 ± 2ºC) rooms. Water and nutrients were provided ad libitum. Experimental studies were conducted upon the approval of animal ethics by the local ethical committee (12/28/2015, 10).

N-Nitrosodiethylamine (CAS No: 55-18-5), Triclosan (3380-34-5) and Colchicine (CAS No: 64-86-8) were purchased from Sigma-Aldrich.

*H. perforatum* was collected from Mersin, Gözne region on July 2016 and identified by Prof. Necattin Türkmen who is botanic professor in Çukurova University, Department of Biology. The remaining plants from the study are stored in the genetics laboratory of the Biology Department of Çukurova University. Aboveground parts of *H. perforatum* (Yellow centaur) were dried with a lyophilizer. Then 15 grams of each dried tissues were weighed, mixed with 200 mL of methanol and milled with a laboratory type mill (KINEMATIC MB 800). After that, the mixture was placed in an ultrasonic bath for 45 minutes at 30 °C, centrifuged at 3500 g for 10 minutes at 20°C and filtered through a blotting paper with drying paper. This process was repeated one more time. Then, methanol was evaporated at 40 °C in a filtrate vacuum evaporator (Polyscience Rotary Vacuum) and finally the remaining extract was dissolved in distilled water.

In the literature, the lowest toxic dose (TDLo) value of *H. perforatum* alcohol extract was reported to be 500 mg / kg body weight (bw) for oral rat (20). Therefore, the highest concentration in our study was determined as 500 mg / kg. Lower concentrations were also 100 mg / kg and 20 mg / kg. For each concentration, a total of six mice were treated with test medium via intragastric gavage. The rats were given a single dose of test substance every day for 30 days and then bone marrow cell was harvested (Test group 1). In addition, serum obtained from the peripheral blood of rats was stored to -80 ºC conditions up to TAS-TOS measurement.

N-nitrosodimethylamine was dissolved in water and treated to rats intraperitoneal as single dose of 200 mg / kg body weight before the *H. perforatum* extract treatments. Triclosan was dissolved in % 70 ethyl alcohol and was sprayed onto the feed of experimental animals (0.09 mg Triclosan for 1 kg meal). In terms of equal treatment of animals, the animals were housed in different cages. After the end of the treatment period, chromosome preparation and blood serum storage were performed (Test group 2).

Two hours before the chromosome aberration test, colchicine was given to the animals intraperitoneally (3 mg / kg, bw). The current protocol for testing this study (21) has been modified and used (22). Rats were sacrificed by cervical dislocation then bone marrow was taken

from femur and transferred to warm physiological solution (0.9% NaCl, 37 °C). The resulting suspension was centrifuged at 2,000 rpm for 5 min. Supernatant was removed and hypotonic solution (0.4 KCl, 37 ºC) was added to cells and centrifuged for 10 min in room temparature. Later supernatant was removed by centrifugation and cold fixative (1/3, glacial acetic acid / methanol) was added. Bone marrow cells were fixed at room temperature (24 ± 2 ºC, 20 min) and supernatant was removed by centrifugation at the end of the time. Then this process was repeated once more. The fixed cells were dripped onto clean cold slides. Dried slides were stained with 5% Giemsa and the slides were cover slipped for microscopic evaluation. With this way, permanent slides were prepared.

In order to detect chromosome aberrations, were calculated by examining 100 metaphases for each rat and 600 metaphases for each group. To determine the mitotic index (MI), 3.000 cells per rat and 18.000 cells per group were assessed.

Total oxidant and antioxidant level testing was performed using peripheral blood se- rum. Blood was put in the tubes, centrifuged at 3.000 rpm for 10 min and serum was stored. TOS and TAS values were determined using commercial kits (Rel assay, Turkey). Calculation of the TOS values is based on the oxidation of the divalent iron ($Fe^{+2}$). Existing oxidants oxidize divalent iron (ferrous ion = $Fe^{+2}$) to trivalent iron (ferric ion = $Fe^{+3}$). The ferric ion forms a coloured complex with the chromium in an acidic medium. This colour intensity, which is proportional to the amount of oxidizing molecules present in the sample, is measured spectrophotometrically. The test is calibrated with hydrogen peroxide and the results are expressed in micromolar hydrogen peroxide equivalent (μmol equivalent H2O2 / L) per liter (23).

Determination of TAS values is based on the principle that ABTS (2, 2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) radical cation is bleached by antioxidants. The results are expressed as mmol Trolox equivalents / L (24).

Oxidative stress index (OSI) was determined using the obtained TOS and TAS values. OSI is calculated by using the following formula:

OSI = TOS / TAS (25-27).

All values were presented as mean ± standard error (SE). Shapiro-Wilk testwas used to check normal distribution of the data. One-way variance analysis (ANOVA) and post hoc Dunnett test were performed using SPSS software. In comparisons, $p \leq 0.05$ was considered significant.

**Result**

*Genotoxic Effects of Hypericum perforatum*

In this study, it was found that any concentrations did not significantly increased abnormal cell ratios compared to the negative control group. Also, these levels were significantly lower compared to the test group 2 ($P<0.001$). It was observed that *H. perforatum* had a tendency to reduce the abnormal cell percentage and that effect was mostly apparent in 100 mg/kg treatment group administered with *H. perforatum* alone (Table 1). Abnormal cell percentage determined in groups in which *H. perforatum* and N-nitrosodiethylamine and triclosan were given together was

higher than negative control However, all detected abnormalities were significantly lower than the test group 2 (P<0.001). In this study, it was observed that as *H. perforatum* concentrations increased, abnormal cell ratios were decreased accordingly. Finally, the lowest value of abnormal cell percentage in test group 2 was found at the highest concentration (500 mg/ kg) of *H. perforatum*. It was observed that these treatments (20, 100 or 500 mg / kg) of *H. perforatum* reduced the percentage of abnormal cells caused by the positive control (DEN+T) (Table1).

**Table 1:** Abnormal Cell Percentage, CA/Cell Ratio and Mitotic Index.

| Test Sub-stance | Treatment period (day) | Cons. mg/kg b.w. | Abnormal Cell Percantage ± SE[b] | CA/Cell Ratio ± SE | MI ± SE |
|---|---|---|---|---|---|
| **Negative Control** | - | 0 | 0.333 ± 0.211 | 0.0033 ± 0.0021 | 2.61 ± 0.11 |
| **Positive Control[a]** | 30 | 200 [e] | 5.167 ± 0.601 | 0.0567 ± 0.0056 | 3.36 ± 0.40 |
| **Test Group 1** | 30 | 20 | 0.333 ± 0.211 | 0.0033 ± 0,0021 | 2.94 ± 0.37 |
| | | 100 | 0,167 ± 0.167 | 0.0017 ± 0,0017 | 4.55 ± 0.22 [c] |
| | | 500 | 0.333 ± 0.211 | 0.0033 ± 0,0021 | 5.91 ± 0.47 [c] |
| **Test Group 2** | 30 | 20 + 200 [e] | 2.167 ± 0.401 [d] | 0.0250 ± 0,0056 [d] | 6.22 ± 0.22 [d] |
| | | 100 + 200 [e] | 1.000 ± 0.000 [d] | 0.0125 ±0.0025 [d] | 7.45 ± 0.07 [d] |
| | | 500 + 200 [e] | 0.833 ± 0.307 [d] | 0.0083 ± 0.0031 [d] | 6.50 ± 0.35 [d] |

[a] Positive control: Triclosan + N-nitrosodiethylamine
[b] SE: Standart error
[c] comparison with negative control is very important (p≤0.001)
[d] comparison with positive control is very important (p≤0.001)
[e] 0.09 mg / kg triclosan were given to rats with food once every 3 days.

*Cytotoxic Effects of Hypericum perforatum*

The increase in cell proliferation at all concentrations of *H. perforatum* has manifested itself as an increase in mitotic index. In the group administered with *H. perforatum* alone, the mitotic index, at the highest concentration of *H. perforatum*, was found to be significantly higher than the negative control and positive control ($P \leq 0.001$). In the test group 2, the mitotic index was found to be statistically significantly higher than positive control ($P \leq 0.001$). It has been determined that the *H. perforatum* showed a synergistic effect when administered with DEN + T (Table 1). In the literature, it is pointed out that *H. perforatum* had cytotoxic effects in most of the in vitro studies (28, 29). In contrast, in our study, we found that *H. perforatum* extract had proliferative effect in vivo. From this point of view, our study is the first to show that *H. perforatum* extract promotes cell division. For this reason, we believe that this property of *H. perforatum* should be supported by additional in vivo studies.

*Total Oxidant and Antioxidant Capacity of Hypericum Perforatum*

Total oxidant (TOS) and total antioxidant values (TAS) were measured spectrophotometrically in rat blood serum

in order to detect the oxidative effects. Only one group (100 mg / kg) had significantly lower total oxidant level than the control groups ($p<0.01$). In the test group 2, total oxidant levels showed significant reductions (except 100 mg/kg concentration) (Table 2). Total antioxidant levels were found to be reduced only in group administered with 500 mg/kg *H. perforatum* + positive control (DEN and triclosan) compared to positive control alone (DEN and triclosan) (Table 2). As a result, it was found that 100 mg/kg *H. perforatum* decreased the oxidative stress in comparison with the negative control group (Table 2).

**Table 2:** Total Oxidant and Total Antioxidant in Rat Peripheral Blood Lymphocytes Treated with *Hypericum Perforatum* Extract at Different Concentrations

| Test Sub-stance | Treatment period (day) | Cons. mg/kg bw | TOS ± SE[b] | TAS ± SE | OSI ± SE |
|---|---|---|---|---|---|
| **Negative Control** | - | 0 | 33.79 ± 4.27 | 1.51 ± 0.08 | 21.97 ± 1.73 |
| **Positive Control [a]** | 30 | 200 [I] | 26.57 ± 3.73 | 1.63 ± 0.07 | 16.22 ± 2.09 |
| **Test Group 1** | 30 | 20 | 42.04 ± 5.75 | 1.73 ± 0.10 | 23.79 ± 1.85 [h] |
| | | 100 | 18.19 ± 1.16 [d] | 1.58 ± 0.04 | 11.53 ± 0.64 [e] |
| | | 500 | 37.26 ± 2.92 | 1.68 ± 0.05 | 22.06 ± 1.38 [h] |
| **Test Group 2** | 30 | 20 + 200 [i] | 16.76 ± 1.50 [g] | 1.53 ± 0.04 | 11.02 ± 1.11 [c] |
| | | 100 + 200 [i] | 23.43 ± 3.15 | 1.59 ± 0.09 | 14.55 ± 1.45 [d] |
| | | 500 + 200 [i] | 20.36 ± 1.47 [f] | 1.40 ± 0.05 [g] | 14.54 ± 0.71 [d] |

[a] Positive control: Triclosan + N-nitrosodiethylamine
[b] SE: Standart error
[c] comparison with negative control is little important ($p\leq0.05$)
[d] comparison with negative control is important ($p\leq0.01$)
[e] comparison with negative control is very important ($p\leq0.001$)
[f] comparison with positive control is little important ($p\leq0.05$)
[g] comparison with positive control is important ($p\leq0.01$)
[h] comparison with positive control is very important ($p\leq0.001$)
[i] 0.09 mg / kg triclosan were given to rats with food once every 3 days.

## Discussion

In our study, *H. perforatum* extract significantly reduced the frequency of chromosome aberrations induced by N-nitrosodiethylamine and triclosan in bone marrow cells of healthy rats. In chromosome aberration tests in mouse and chinese hamster bone marrow cells, aqueous methanolic extract of *H.* perforatum was found to have no significant mutagenic effect (9). Some phytotoxins present in *H. perforatum* (cyanogenic glycosides, solanine and chaconine, thujone, and glycyrrhizinic acid) react with many molecules including DNA resulting in toxic effects. One of these effects that cause tumor formation is genotoxic effect (30). In a different study, it has been shown that the non-photoactivated (non-phototacticized) hypericin did not exhibit mutagenic activity in the Ames test with or without metabolic activation and did not show any protective effect against 9-aminoacridine-induced mutagenicity. In analysis with yeast Saccharomyces Cerevisiae, hypericin did not increase the frequency of mitotic crossing-over or total aberrations

in the ade (2) locus. Hypericin, while not mutagenic in the Chlamydomonas reinhardtii, has reduced methyl methane sulfonate toxicity and mutagenesis to a small extent. In a chromosome aberration assay using three mammalian cell lines, hypericin did not alter the frequency of structural chromosome aberrations and resulted in no antioxidant effect in the DPPH radical sweep test (31). Similar to our findings, it has been reported that the water extract of *H. perforatum* showed protective effect against alkylating DNA damage induced by methylmethanesulfonate (MMS) and also sup- ported the repair of alkylated DNA damage (10). Resarchers have reported a similar result and it has been found that *H. perforatum* has anti-clastogenic effect against clastogenicity, which is caused by cytotox- ic and mutagenic effect of cyclophosphamide (CYP) in onion meristem cells and rat bone marrow cells (32). In the same study, pretreatment of *H. perforatum* decreased the damage of CYP up to 76%, concurrent treatment decreased 95% and post-treatment decreased 97%. Antigenotoxic effect of *H. perforatum* on healthy cells is consistent with our findings. In summary, in the majority of genotoxicity studies conducted with the extract of *H. perforatum*, this agent appears to have a protective potential against genotoxic and mutagenic agents.

According to the results of this study, *H.* perforatum extract shows a proliferative activity. In contrast to our findings, it has been shown that apoptosis was induced by hypericin in neoplastic human leukemia cell line and thus showed cytotoxic effect (28). In a similar study in which HepG2, HepaRG and WRL-68 cell lines were exposed to *H. perforatum*, hypericin and hyperphosphoric compounds at different concentrations for 24 or 72 hours, these substances were reported to be cytotoxic in the MTT assay (29). In another study, it was reported that *H. perforatum* extract showed neither cytotoxic effect in meristematic cells of Allium cepa L. nor cytotoxic/mutagenic effect in acute treatment of intraperitoneal or subchronic gavage in animal experiments (32). Based on our findings and the literature, these results indicate that the bioactive components of *H. perforatum* show cytotoxic effect on neoplastic cell lines, but do not show such an effect on healthy cells and even proliferative effect.

Our results suggest that although *H. perforatum* extract showed no significant preventive effect on the oxidative stress induced in the blood serum of rats, 100 mg / kg concentration of *H. perforatum* extract caused significant reduction in total oxidant status and therefore decreased the oxidative stress level. On the other hand, in parallel with our findings, it has been found that *H. perforatum* extract was effective against apoptosis induced by hydrogen peroxide in PC12 cells (cell line derived from mouse adrenal medulla phaeochromocytoma) and standard extracts of *H. perforatum* may prevent DNA breakdown and cell shrinkage resulting from hydrogen peroxide activity (33). In another study, a flavonoid rich extract of *H. perforatum* was able to effectively treat oxidative stress related neurodegenerative disorders like Alzheimer's and Parkinson's disease (34). Moreover, the ability of *H. perforatum* extract to reduce oxaliplatin induced caspase-3 activity in rat astrocytes was evaluated. The extract showed a significant antioxidant effect and could reduce caspase-3 activity in rat astrocytes

(35). In our in vivo study, 100 mg/kg *H. perforatum* extract showed antioxidant effect.

## Conclusion

On the basis of the data obtained in this study, *H. perforatum* extract was not found to have genotoxic potential. Moreover, it has also been found that this extract had a pronounced antigenotoxic effect. Contrary to the studies in the literature, our findings also pointed toward a proliferative feature of *H. perforatum*. As a result, considering the antigenotoxic and cell division promoting effects of *H. perforatum*, we are of the opinion that *H. perforatum* is an effective agent that can be used in many areas.

## Conflict of Interests

The authors declare that no conflict of interest exists.

## Acknowledgements

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# Effects of Age and Sex on Cerebellum and Ventral Pons Volume - MRI Study

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**Abstract**

*Magnetic resonance image (MRI) has much importance in terms of searching aging and gender effects on brain. In this study, age and gender differenceswere aimed to be found out on cerebellar and ventral pons volumes. It is totally studied on nine cross-section images from MRI; three at transvers plane (top, middle, and bottom), three at frontal plane (front, middle, back), and three at sagittal plane (right, middle, left). T1 transvers, frontal and sagittal MRI was taken from 43 men and 57 womenany of whomdo not display any pathological symptom. Both gender groups were separated into age groups as young, middle aged and elderly in order to understand effects of aging. Areas of the cerebellum and ventral pons formation were calculated by transferring selected images to NETCAD software. The volumes were calculated in Excel program by using the values obtained from MRI and analysed by SPSS. No significant difference between genders in the ventral pons volumes. A significant size in men's right and left hemisphere volumes at transverse and frontal planes. It was determined a significant size in women's vermis of cerebellum volumes at the sagittal plane. Also, a significant reducing was observed in right hemisphere volumes, in frontal plane right – left hemisphere volumes owing to aging and, so it was found that the reducing was much more in men. The results were comparable with previous ones. NETCAD is concluded to be suitable in volume calculating with MRI.*

***Keywords:*** *Cerebellum, Ventral Pons, MRI, Volumetry, NETCAD*

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## Introduction

Morphological changes are expected to occur in different anatomical areas of the brain due to gender and aging. The aging of the brain is characterized by regional shrinkage in the gray and white matter regions (1). Magnetic resonance imaging (MRI) shows the morphological and pathological changes of intracranial anatomy. It also gives the opportunity to examine the changes that occur with aging in healthy individuals (2). The cerebellum plays an important role in normal brain function and it has structural and functional involvement in a number of neurological diseases associated with impairment of functions such as both motor and cognition, mood, and behaviour (3). In different disease states and after damage, it has become important to find concrete ways to evaluate the cerebellum. There are studies in cerebellum, which examine: 21-hydroxylase deficiency (21OHD) (4), effect of steroid hormones in congenital adrenal hyperplasia (5), the volume of cerebellum in normal fetal brain (6), the volume of cerebellum in glioma inactivated protein 1 (LGI1), the volume of cerebellum in encephalitis (7), in neurometabolic diseases such as cerebrotendinousxanthomatosis, the volume of the cerebellum nuclei (8) and the volumes of the cerebellum and cerebellar peduncles to distinguish progressive supranuclear Palsy-Richardson Syndrome from Parkinson's disease (9). As Murshed (2003) has carried out, manual drawings on MR images for the volume of cerebellum studies will take more time and subjective data will be obtained. In this study, it was aimed to calculate the cerebellum and the volume of ventral pons changes on the MR images of healthy individuals over the areas obtained with the NETCAD program.

## Method

For the present study, approval was obtained from Selçuk University Faculty of Medicine Ethics Committee with the decision number 2007/091 dated 25.04.2007.

The study was performed on the MR images of patients who applied to the neurology and neurosurgery outpatient clinics of Afyonkarahisar State Hospital for various reasons in 2007. Radiologists studied MR images of 100 adult individuals (43 males - 57 females) (aged between 15-70 years) who were not reported any disease, anomaly or damage and were evaluated as normal. Both sexes were divided into three groups according to their ages:

1- Young age group; 23 individuals (13 males, 10 females) between the ages of 15-29.

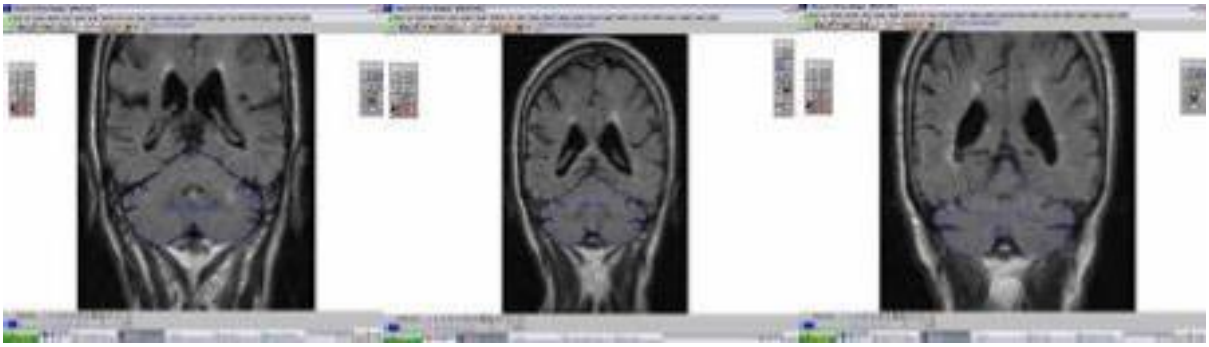
2- Middle age group; 36 individuals (12 males, 24 females) between the ages of 30-49.

3- Advanced age group; 41 individuals (18 males, 23 females) between the ages of 50-70.

For the images; spin-echo transverse using 1.5 tesla Siemens Maestro Glast brand device
(TR: 475 TE: 8,1), sagittal (TR: 375 TE: 113), frontal (TR: 8770 TE: 113); thickness in sections: transverse and frontal (4,5mm), sagittal (5mm); FOW: 500mm MATRIX: 512 * 512 GAP: 2,2 m.tesla values were received. T1-weighted

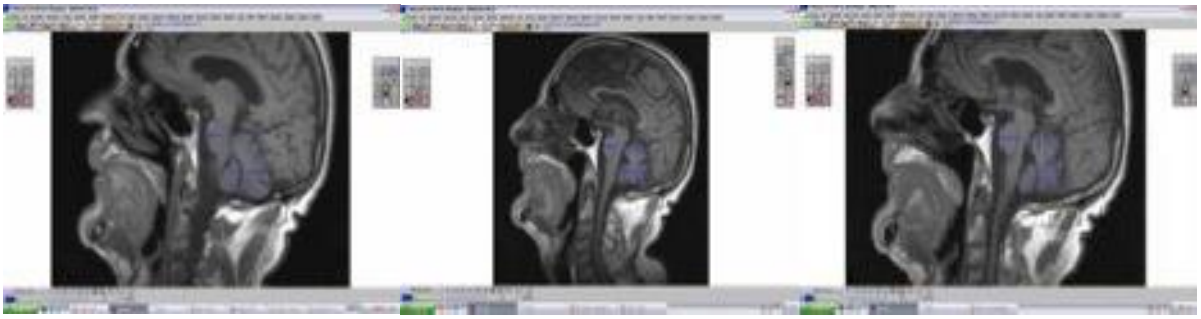
transverse, frontal and sagittal MR images were evaluated, since the anatomical structures of the cerebellum and pons without pathology were examined. NETCAD 4.0 GIS software was used to calculate the area and volume of the images included in the research.

A total of 9 images were selected from transverse, frontal and sagittal planes. These images were transferred to the NETCAD program in bitmap format. First, the borders of the hemisphere of cerebellum, vermisof cerebellum and ventral pons were drawn (Figures 1, 2, 3). The area accuracy of the software on the images was chosen as 0.01 $mm^2$. Numerically determined areas were recorded in Excel and the volumes of the following regions were calculated.



**A** **B** **C**

**Figure 1:** Field plot of the hemisphere of cerebellum (A, a lower section from the middle section; B, middle section; C, upper section from the middle section) on the cranial MRI in the transverse plane transferred to the NETCAD program.



**A** **B** **C**

**Figure 2:** Field plot of the hemisphere of cerebellum (A, the previous section from the middle section, B, the middle section, C, the next section from the middle section) in the cranial MRI in the frontal plane transferred to the NETCAD program.

1- Right hemisphere of cerebellum in the transverse plane.

2- Left hemisphere of cerebellum in the transverse plane.

3- Right-left hemisphere of cerebellum in the frontal plane.

4- Vermis of cerebellum in the sagittal plane.

5- Ventral pons in the sagittal plane.

**A** **B** **C**

**Figure 3:** Field plot of the vermis of cerebellum and ventral pons (A; the previous section from the middle section, B; the middle section, C; the next section from the middle section) in the cranial MRI in the sagittal plane transferred to the NETCAD program.

Statistical analysis of parameters according to age groups and gender was performed in SPSS 15.0 (for Windows) program. A two-way analysis of variance was used to evaluate the combined effect of age and gender. If there was a difference in terms of age groups, the bonfer's corrected one-way analysis of variance and Tucey HSD test were performed to determine in which age group the difference originated from. A student-t test was applied to determine, which age group caused the difference in gender. Spearman correlation test, which is used in non-homogeneous data, was used to determine the relationship between the values obtained from the study.

## Results

According to the volume calculations made in all three planes in the study, it was found that the right and left cerebellar volume were larger in men and the vermis of cerebellum volumes in women ($p < 0.05$) (Table 1). Also; It was determined that the volume decrease between the middle age group and the advanced age group was greater in males, and the volume decreases between the younger and middle age group in females (Table 2). No gender and age-related changes were observed in ventral pons volume. When analysed by age groups, although the decrease in right cerebellar volume was not found to be statistically significant, it was found that there was a greater decrease in men with increasing age compared to women.

**Table 1:**Comparison of cerebellar volumes (mean $mm^3$ ± SD) by sex.

| Parameters | Male (n=43) | Female (n=57) |
|---|---|---|
| **Right hemisphaerium** | 10124 ±1959 * | 9436 ± 1714 |
| **Left hemisphaerium** | 10452 ± 1193 ** | 9390 ± 1210 |
| **Total hemisphaerium** | 17613 ± 15494 * | 13741 ± 1478 |
| **Vermis** | 6264 ± 1534 ** | 7120 ± 1227 |

SD; Standart deviation, *: $p<0.05$, **: $p<0.001$.

**Table 2:** Comparison of cerebellar volumes (mean $mm^3$ ± SD) by age groups and sex.

| | **Young age** (15-29 age) | | **Middle age** (30-49 age) | | **Late age** (50-70 age) | |
|---|---|---|---|---|---|---|
| | **M (n=13)** | **F (n=10)** | **M (n=12)** | **F (n=24)** | **M (n=18)** | **F (n=23)** |
| **RH** | 10257 ± 2954 | 9755 ± 1402 | 10157 ± 1668***** | 9422 ± 1325 | 9958 ± 1218 | 9311 ± 2185 |
| **LH** | 10611 ± 865 | 9523 ± 1198 | 10571 ± 1405****** | 9147 ± 1175 | 10257 ± 1280 | 9085 ± 1259 |
| **TH** | 15815 ± 1598***** | 14367 ± 1634 | 13966 ± 1669 *a* | 13990 ± 1360 | 13800 ± 2097*a* | 13317 ± 1223 |
| **Vermis** | 7225 ± 2034 | 7367 ± 695 | 6002 ± 1129 | 7321 ± 1315****** | 6802 ± 1285 | 5745 ± 1014 *******a* |

SD; Standart deviation, *: p<0.05, **: p<0.001, ***a***; Significantly different from the young age group. RH; right hemisphaerium, LH; left hemisphaerium, TH; total hemisphaerium, M; male and F; female.

## Discussion

Thanks to the morphological measurements made in normal people, the changes in the cerebellum and subcortical structures in both aging and neurodegenerative diseases are successfully demonstrated. While MRI is widely used for brain and cerebellar morphological evaluation, advanced MRI techniques allow investigation of cerebellar microstructural and functional features. A group of researchers who calculated the volume on MR images in mice showed that the lateral cerebellar volume was higher in female mice and the medial cerebellar volume was higher in male mice (11).As a result of volumetric analysis performed on healthy people, it was shown that there was no difference between genders in the gray matter of the cerebellum, but there was a decrease in gray matter volume with aging (12).

Volumetry, voxel-based morphometry, diffusion MRI based tractography, functional MRI, perfusion, and proton MR spectroscopy are among the most commonly used techniques in the cerebellum study (3). Sumiyoshi et al. (2017) reported an increase in brain gray matter in adolescent rats in their study by using voxel-based morphometry in MRI. The highest increase was observed in the occipital cortex, amygdala, hippocampal formation and cerebellum. Voxel- based morphometry, one of the current methods used in volume calculation, is mostly preferred by T1-weighted MRI to define volume changes in normal aging and regional brain atrophy in various neurological and psychiatric diseases (14,15). Unlike previous studies, there are also studies using a Freesurfer 5.1. fully automated technique to measure the volumes of T1-weighted MR images obtained from 3T MR scanners (16). There are studies reporting that the poster inferior part of the cerebellum differs in cognitive criteria in the cerebellar volume measurements performed using the Spatially Un-neutral Infratentorial Toolbox (SUIT) in the Statistical Parametric Matching (SPM12) program of MS patients and healthy individuals, while the anterior part creates the variance in the motor-performance level (17). It was also reported that cerebellar volumetric abnormalities can make an important contribution to explain motor and cognitive performance in MS patients. Vurdem et al. (2012), who measured posterior cranial fossa and cerebellar volume in patients with type I Chiari malformation using a different method: the stereological method, could not find any statistical difference between patients with type I Chiari malformation and healthy individuals in

terms of cerebellar volumes.

Cerebellar volume is important in neurological and some genetic diseases. In a study conducted on patients with schizophrenia, it was observed that posterior vermis of cerebellum volumes decreased significantly in men (19). In a study examining the cerebellar volumes in patients with Prader-Willi syndrome (PWS), a decrease in all parts of the cerebellum was shown in patients compared to the control group, and it was suggested that this would be an important evidence for behavioural change in individuals with PWS (20). It has been reported that there is a significant decrease in white matter of the cerebellum in the volume calculations made on 7-tesla MRI images in patients with Friedreich's ataxia (FRDA), another genetic disease (21).

In a study conducted to differentiate migraine and tension-type headache, it was shown that there was a decrease in the gray matter of the cerebellum in the migraine group, thus it was reported that the cerebellar volume could be the differential diagnosis between the two types of diseases (22). Decreased cerebellar volumes were observed in both genders in smokers compared to non-smokers (23).

In our study, unlike these methods, MR images obtained from 3 planes were transferred to the NETCAD program and measurements were made. The similarity of the results obtained in our study with the results of other studies supports that the NETCAD program can be used in area and volume calculations over MRI.

## Conflict of interest

The authors declare that no conflict of interest exists.

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# Nitrotyrosine formation, iNOS and the $Na^+/K^+$-ATPase activities in sepsis: The possible effects of CAPE

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**Abstract**

*Sepsis is a response to infection characterized by the formation of highly reactive oxygen and nitrogen substances. The rat kidney was chosen for this purpose because many important inflammatory mediators, including inducible nitric oxide synthase (iNOS) and nitrotyrosine (nTyr) production, are expressed by kidney cells following either lipopolysaccharide (LPS) or bacterial challenge. The present study was aimed at investigating the relationship between nTyr formation with iNOS and $Na^+/K^+$-ATPase activities. We were also aimed at investigating the possible role of caffeic acid phenethyl ester (CAPE) on endogenous nTyr production, $Na^+,K^+$-ATPase and iNOS activities in the kidney. Kidney $Na^+/K^+$-ATPase activity were maximally inhibited 6h after LPS injection and LPS treatment significantly increased iNOS activity of kidney. The regression analysis displays negative correlation between $Na^+/K^+$-ATPase activity and nTyr levels of LPS treated animals. $Na^+/K^+$-ATPase activity were also negatively correlated with iNOS activity in LPS–treated rats. These data suggest that nitric oxide ($NO^{\cdot}$) and peroxynitrite ($ONOO^-$) contribute to the development of oxidant injury. Furthermore, the source of $NO^{\cdot}$ may be iNOS. iNOS are expressed by the kindey, and their activity may increase following LPS administration. Also, $NO^{\cdot}$ and $ONOO^-$ formation inhibited $Na^+/K^+$-ATPase activity. This results also have strongly suggested that bacterial LPS disturbs activity of membran $Na^+/K^+$-ATPase that may be an important component leading to the pathological consequences such as renal dysfunction in which the production of reactive nitrogen substance (RNS) are increased as in the case of LPS challenge. CAPE treatment was decreased nTyr production and iNOS activites and increased $Na^+/K^+$-ATPase activity. These data suggest that CAPE treatment contribute to the decrease of oxidant injury.*

***Key words:*** *Nitrotyrosine, $Na^+/K^+$-ATPase, iNOS, kidney, CAPE*

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## Introduction

The expression of inducible nitric oxide synthase (iNOS) protein is induced by lipopolysaccharide (LPS) in many cells and tissues including kidney. In other words, iNOS and cytokine production are up–regulated by LPS in the kidney (1, 2).

Reduction in glomerular filtration rate (GFR) and hypotension is associated with up-regulation of iNOS in LPS–induced septic animals (1, 3). Inner medullary collecting duct cells and renal proximal tubule can produce $NO^{\cdot}$ via expression of an iNOS isoform (1, 4).

$NO^{\cdot}$ and $ONOO^{-}$ contribute to the development of oxidant injury. The cell types responsible for $NO^{\cdot}$ and superoxide generation in the kidney in response to LPS are not known. Interestingly, proximal tubule constitutive NOS and iNOS are both capable of generating superoxide in addition to $NO^{\cdot}$ (1, 5). The production of both $NO^{\cdot}$ and superoxide increases in septic shock. The cogeneration of these molecules is known to yield $ONOO^{-}$, which preferentially nitrates tyrosine resudies of protein and non–protein orgins (1, 6). The production of nitrotyrosine (nTyr) in the kidney has been associated with several pathological conditions (1, 7). $Na^{+}/K^{+}$-ATPase is an energy utilizing transmembrane enzyme. It is responsible for the maintenance of ionic gradients of $Na^{+}$and $K^{+}$. $Na^{+}/K^{+}$-ATPase has been shown to very susceptible to free radicals and membrane lipid peroxidation (1, 8). It has been reported that $NO^{\cdot}$ derived products ($NO_2^{\cdot}$ and $ONOO^{-}$) inhibit $Na^{+}/K^{+}$-ATPase activity via the possible oxidation of thiol groups of the enzyme in many cells and tissues (1, 9-13). Previous studies have been demonstrated that $ONOO^{-}$ signaling participates in the regulation of renal $Na^{+}/K^{+}$-ATPase activity (1, 12). Furthermore, it has been demonstrated that the endogenous $NO^{\cdot}$ plays a direct inhibitory effect over $Na^{+}/K^{+}$-ATPase activity in the kidney (1, 14). It has been also reported that $NO^{\cdot}$ generated by iNOS inhibits $Na^{+}/K^{+}$-ATPase activity in an autocrine fashion (1,15).

Caffeic acid phenethyl ester (CAPE), an active component of propolis from honeybee hives, is known to have anticarcinogenic, immunomodulatory, antiinflammatory, and antioxidant properties. It has been demonstrated that CAPE is an agent which is a free radical scavenger and activates antioxidant enzymes (16).

The aim of this study is to evaluate the effects of CAPE on nTyr formation, $Na^{+}/K^{+}$-ATPase and iNOS activities in septic kidney.

## Method

Rats (250–300g) were divided into 3 groups (n=10 each group). Group 1 animals were intraperitoneally injected with saline (control group). Group 2 animals were intraperitoneally injected with LPS, 20 mg/kg single dose (LPS–treated group) (17). Group 3 animals were intraperitoneally injected with CAPE, 10 µmol/kg single dose (CAPE-treated group) following a 20 mg/kg single dose of LPS injection (18-24).

Animals were sacrified under ketamin/xylazin (60-10 mg/kg i.p single dose) anasthesia at 6h after injections (1, 25, 26). After sacrification, the kidneys were removed, washed with cold NaCI 0.9% and immediately kept frozen in liquid nitrogen. The kidney tissues were stored at -70 $^{0}$C until use.

*Measurement of 3-nitrotyrosine*

Tissue sample was homogenized in buffer

(50mM potassium-phosphate buffer, pH:7.4) and hydrolysed in 6 N HCI at 90–110 $^0$C for 18-24 h. Hydrolyzed samples were centrifugated at 3000 rpm for 10 min, the supernatants were separated for the analysis of nTyr levels. The samples were analyzed on a Agilent 1200 diode array detector HPLC apparatus. The analytical column was 5µm pore size Spherisorb ODS–2 C18 reverse–phase column (4.6×250mm, Alltech, Dearfield, IL, USA). The guard column was a C18 cartridge (Alltech, Dearfield, IL, USA). The mobile phase was 50 mmol/L sodium acetate/50mmol/L citrate/8% (v/v) methanol pH 3.1. HPLC analysis was performed under isocratic conditions at a flow rate of 1ml/min and UV detector set at 274nm. Concentrations of nTyr were calculated from nTyr standard curve and expressed as nmol/g tissue (1, 11, 25, 27, 28).

*Measurement of $Na^+/K^+$-ATPase activity*

Tissue homogenate was prepared for the $Na^+/K^+$-ATPase study using a glass–homogenizer. Homogenates were centrifuged at 3000 rpm for 5 min and supernatant was separated. $Na^+/K^+$-ATPase activity in the supernatant was determined. $Na^+/K^+$-ATPase activity was assessed by the measurement of the produced inorganic phosphate and results were expressed as spesific activity (µmol $P_i$ / h / mg protein) (1, 11, 25, 28, 29).

*Assay for nitric oxide synthase activity*

Tissues were homogenized with a buffer containing 10mM HEPES, 0.32M sucrose, 0.1mM EDTA, 1mM dithiothretiol, 10µg of soyabean tripsin inhibitör/ml, 10µg of leupeptin/ml, 2µg of aprotinin/ml and 1mg of PMSF/ml, adjusted to pH 7.4. The homogenates were then centrifuged at 100000×g for 1h. $NO^{\cdot}$ synthesis was measured by a previously described method (1, 30, 31), in which the oxidation of oxyhaemoglobin to methaemoglobin by $NO^{\cdot}$ is monitored spectrophotometrically. The absorption difference between 401 and 411 nm was continously monitored with a dual–wavelength recording spectrophotometer by using a bandwith of 2 nm, at 37$^0$C.

*Statistical calculations*

The data resulting from each group were expressed as the mean ± S.E.M. A Mann Whitney U test *t*-test was used to compare means between the two groups using SPSS 10.0. Linear regression analysis was applied where indicated. A p value < 0.05 was considered significant.

## Results

Nitrotyrosine levels, iNOS and $Na^+/K^+$-ATPase activities were shown in Table 1. In our study, nTyr levels have been hardly detected in control rat kidneys but nTyr levels have been detected markedly in kidneys of septic animals. nTyr levels were also significantly increased in the LPS-treated group when compared to the control ($P< 0.05$) (Table 1).

**Table 1:** nTyr levels and iNOS and $Na^+/K^+$-ATPase activities.

| | **iNOS (nmol/min/g tissue)** | **nTyr (nmol/g tissue)** | **$Na^+K$+ATPase (µmol Pi/h/mg protein)** |
|---|---|---|---|
| **Control (n=10)** | 0.375 ± 0.044 | Hardly detectable | 2.690 ± 0.172 |
| **LPS–treatment (n=10)** | 0.865 ± 0.118 | 4.633 ± 0.716 | 1.352 ± 0.158 |
| **CAPE–treatment (n=10)** | 0.510 ± 0.069 | 0.712 ± 0.111 | 2.765 ± 0.065 |

CAPE; Caffeic acid phenethyl ester, iNOS; inducible nitric oxide synthase, LPS; lipopolysaccharide, nTyr; 3-nitrotyrosin.

The regression analysis between $Na^+/K^+$-ATPase activitiy and nTyr levels of LPS–treated animals revealed negative correlation (Figure 1). Similarly, $Na^+/K^+$-ATPase and iNOS activities were also negatively correlated (Figure 2).

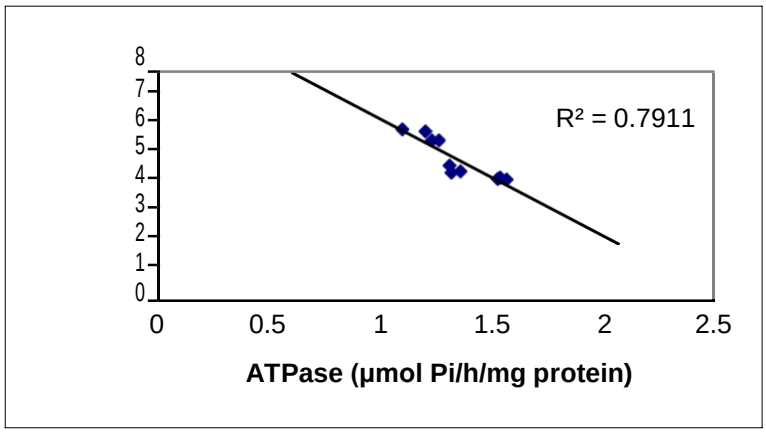


**Figure 1:** Negative correlation between $Na^+/K^+$-ATPase activity and nTyr formation in kidney of septic rats.

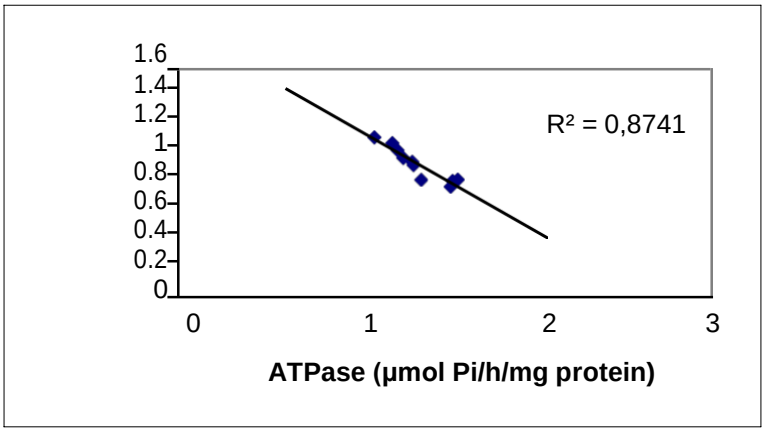


**Figure 2:** Negative correlation between $Na^+/K^+$-ATPase activity and iNOS measurements.

In our this study, $Na^+/K^+$-ATPase activity significantly decreased in LPS–treated animals compared to control animals ($P< 0.05$) (Figure 3). Although iNOS activities were low, even barely detectable in control animals, iNOS activity in LPS-treated groups were significantly increased ($P< 0.05$) (Figure 4).

In this study, $Na^+/K^+$-ATPase activity significantly increased in CAPE–treated rat kidneys compared to LPS animals ($P< 0.05$) (Figure 3). Although iNOS activity and nTyr formation were high level detectable in kidney of LPS–treated animals, these parameters were significantly decreased in CAPE–treated animals ($P< 0.05$) (Table 1).

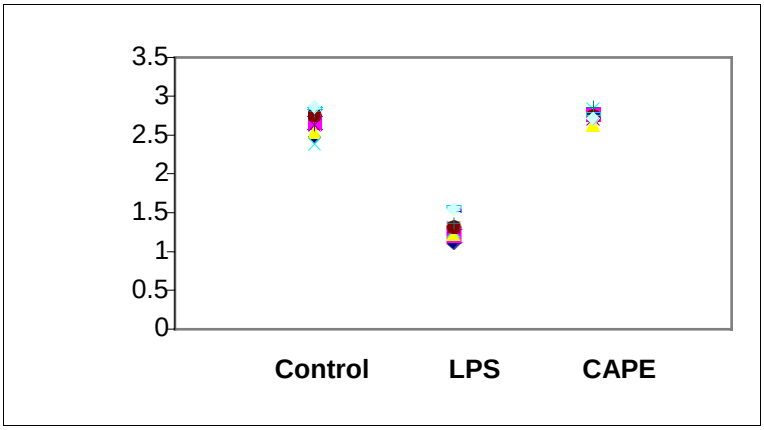


**Figure3:** $Na^+/K^+$-ATPase activities in groups.

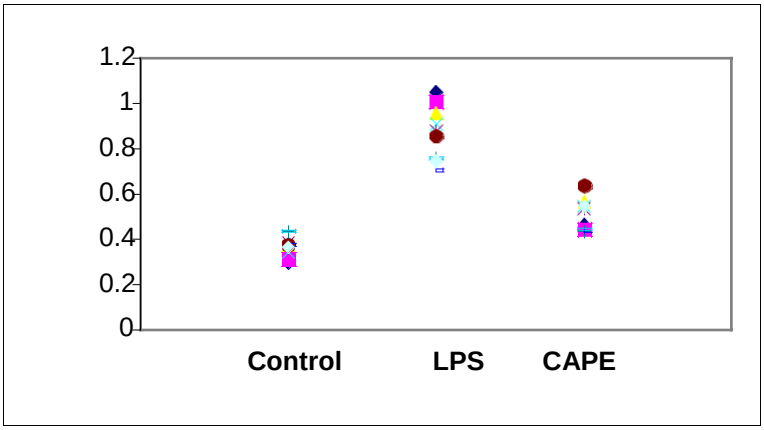


**Figure4:** $Na^+/K^+$-ATPase activities in groups.

## Discussion

In our study, there is a significant increase in iNOS activity in kidney following 6h after injection of LPS to rats. This findings is in accord with previous observations in liver (1,25), and also consistent with those of others showing that $NO^{\cdot}$ or its metabolites are significantly increased in many organs after LPS administration (1, 32-34).

It is demonstrated that iNOS mRNA induced by LPS (1, 32) and it has been reported that LPS–treatment caused increase in the amount of iNOS mRNA in many tissues (1, 35). In addition, Mayeux et al. report that proximal tubules express a calcium/calmodulin–dependent NOS activity that is increased in vivo by LPS (1, 36). Our results are also in accordance with those of previous studies on increased iNOS level in sepsis.

In this study, we indicated that excess formation of reactive nitrogen substance (RNS) is responsible for the impaired $Na^+/K^+$-ATPase activity in septic kidney. Guzman et al reported that $NO^{\cdot}$ generated by mause proximal tubule epithelial cell iNOS inhibits $Na^+/K^+$-ATPase activity (1, 15). Our results are in accordance with those of previous studies on impaired $Na^+/K^+$-ATPase activity in septic animals. A negative correlation observed between

$Na^+/K^+$-ATPase and iNOS activity as well as nTyr levels in septic animals strengthens the direct involvement of RNS. These data suggest that $ONOO^-$ signaling participates in the regulation of renal $Na^+/K^+$-ATPase activity and the endogenous $NO^\cdot$, formation due to elevated iNOS activity, plays a inhibitory role on $Na^+/K^+$-ATPase activity in the kidney. Furthermore, this finding is also in accord with previous observations in kidney (1, 12, 14). Depletion of glutathione and other protective antioxidants by RNS may greatly contribute to increasing amount of reactive species, which may also account for impaired activity of $Na^+/K^+$-ATPase (1, 37)**.** $ONOO^-$ has been shown to directy oxidize a SH group of the active site of the $Na^+/K^+$-ATPase (1, 9).

Song YS et al. reported that CAPE inhibits nitric oxide synthase gene expression and enzyme activity (38). Çelik S and Erdoğan S. demonstrated that CAPE protects brain against oxidative stress and inflammation induced by diabetes in rats (39). Kassim M. *et al* reported that CAPE is scavenger of peroxynitrite in vitro and in sepsis models (40). Çakır T et al. showed that CAPE prevents methotrexate-induced hepatorenal oxidative injury in rats (41). Our findings are in accordance with those of previous studies on antioxidant and protective effects of CAPE.

In summary, our results show that although nTyr levels and iNOS activity were increased, $Na^+/K^+$-ATPase activities were decreased in rat kidney exposed to LPS. Thus, the negative correlation of $Na^+/K^+$-ATPase activity was observed with both iNOS activity and nTyr levels in the kidney treated LPS. In conclusion, the present study have indicated that both endogenous production of $NO^\cdot$ via iNOS activity and simultanously superoxide generation are stimulated in response to LPS. Thus, $NO^\cdot$ and superoxide react spontaneously to form nitrating agent and versatile oxidant $ONOO^-$. Therefore, although $Na^+/K^+$-ATPase activity is impaired, iNOS activity is increased in response to LPS. In this sence, our this study have demonstrated that RNS-dependent kidney dysfunction also include the modification in membrane $Na^+/K^+$-ATPase, which impairs the activity of the enzyme. This event may be a crucial component leading to pathological consequences such as kidney dysfunction in which the production of RNS are increased as in the case of LPS challenge. In addition, our results also have suggested that CAPE treatment decreased kidney tissue injury in sepsis.

## Conflicting Interest

The authors have no competing interests

## Acknowledgement

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# Stevens Johnson Syndrome Like Skin Lesions in a Patient With Systemic Lupus Erythematosus After Hydroxychloroquine Treatment, a Case

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**Abstract**

*Stevens Johnson Syndrome (SJS) is a clinical syndrome with a high morbidity and mortality, characterized by exfoliation of the skin, generally due to medications, and less frequently due to infections. Most frequently sulphonamides, penicillin, and anti-convulsants were among blamed medications. Systemic lupus erythematosus (SLE) is a systemic auto-immune disorder and skin involvement is frequently seen. Hydroxychloroquine (HCQ) is an important agent which is used in the treatment of SLE and which increases survival. Its side effects include skin reactions. We present here a case of SJS like skin lesions in a 38-year-old female patient with SLE after receiving HCQ.*

***Key words:*** *Stevens Johnson Syndrome, Systemic lupus erythematosus, Hydroxychloroquine, Skin lesion.*

## Introduction

Stevens Johnson syndrome (SJS) is severe cutaneous adverse reaction, mainly caused by drugs but also related to infections and unidentified causes. It is characterized by an acute onset of erythema with detachment of the epidermis and epithelia of mucous membranes resulting in extensive areas of denuded skin (1). Stevens Johnson Syndrome and toxic epidermal necrolysis (TEN) are considered to be severity variants of the same disease entity with SJS being the milder and TEN the most severe form (2). The disease has been related to some drugs, such as the sulphonamides, penicillin, salicylates, anti-retroviral drugs and anti-convulsants, and there is an association with herpes simplex and mycoplasma pneumoniae infections (3). Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by the presence of autoantibodies that can cause tissue damage in multiple organs. The skin is the second most commonly affected organ involved in SLE, including malar rash, subacute cutaneous lupus, discoid lupus, bullous lesions, periungual erythema (4).

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Hydroxychloroquine (HCQ) is an antimalarial drug with immunosuppressive and anti-inflammatory effects that is used in the treatment of SLE, rheumatoid arthritis and other disorders of connective tissues. It has protective effects against disease progression in SLE. Side effects include gastrointestinal disturbances, headache, retinopathy and skin rash (5). There are very few cases of SJS due to HCQ in the medical literature. We present here a case of a patient with SLE in whom serious and life-threatening skin reactions like Stevens Johnson Syndrome after receiving HCQ.

**Case report**

A 38-year-old female patient was admitted with complaints of fatigue, swelling and pain in the wrists and metacarpophalangeal (MCP) joints, and redness at the face and arms which became more prominent after exposure to sunlight. She did not have any co-existent diseases, but her mother had SLE. The patient had swelling, sensitivity and limitation of motion of the right wrist, left 1st MCP, right 1st, 2nd, and 3rd MCP, and a malar rash and no other findings were detected in her physical examination. Laboratory values were as follows; hemoglobin 8.2 g/dl (12.5-13.5), leukocytes 4.500/mm³ (4500-10000), platelets 98.000/mm³ (140000-440000), urea: 11 mg/dl (10-50), creatinine 0.4 mg/dl (0.4-1.1), aspartate aminotransferase (AST) 49 IU/l (4-44), alanin aminotransferase (ALT) 56 IU/l (4-44), total protein 6.1 g/dl (6-8.2), albumin 3.3 g/dl (3.5-5.1). C-reactive protein was 15 mg/dl (0-5), erythrocyte sedimentation rate (ESR) was 34 mm/h (1-20), Rheumatoid factor (RF) 9 IU/ml (0-15). Autoantibodies were planned to be measured, and treatment with 5 mg/day prednisolon was started. The joint complaints receded and the patient was admitted again 2 weeks later. Anti citrullinated cyclic peptide (Anti-CCP) 5 IU/ml (0-15), antinuclear antibody (ANA) homogenously positive 1:1280 titres (>1/80), anti-dsDNA 5 (0-20) U/ml, complement 3 (c3) 0.723 g/l (0.9-1.8) and complement 4 (C4) was 0.249 g/l (0.1-0.4). Direct and indirect Coombs tests were positive. Normochrome normocytic erythrocytes were seen at the peripheral blood smear, with 8-9 platelets at each field. Urinalysis was normal. Anti SS-A, Anti-histone, Anti-centromer, Anti-jo1, Anti-SM, Anti-SCl-70, Anti-SS-B were negative. A diagnosis of SLE was decided with arthritis of the wrist and small joints of the hand, malar rash, photosensitivity, anemia, thrombocytopenia, positive ANA, low c3, positive Coombs. Hydroxychloroquine 400 mg/day was added to the treatment. The patient was admitted again 7 days later with fever and rash. Her temperature was 39.8°C with maculopapular and bullous lesions all over her body, which became denser on the neck, knees, chest and back, and mucositis on the lips and inside the mouth. Her blood pressure was 100/80 mmHg, pulse 70/min, and rate of breathing that was 14/min. Findings from further physical examinations were unremarkable. Laboratory examination results were not different from earlier values.

Hydroxychloroquine treatment was terminated and methyl prednisolon 1 g/day IV was administered for 3 days, which was maintained at a dose of 60 mg/day. The skin lesions disappeared in time, and the patient was discharged cured and without fever.

**Discussion**

Stevens Johnson Syndrome is a clinical picture with very high morbidity and mortality, characterized by exfoliation of the skin, generally due to medications, and less frequently to infections (3). It runs a course of epidermal exfoliation, conjunctivitis, mucosal membrane involvement and fever. While SJS is a milder disorder where less than 10% of the body surface is involved with mucous membrane erosions and vesicular lesions, TEN is a clinical entity where more than 30% of the total body surface is involved with lesions resembling superficial burns due to coalescence of erosions and vesicles. An involvement of body surface between 10-30% is considered as an overlap of SJS - TEN. The etiology is frequently associated with drug use. These medication include sulphonamides and penicillin group among antibiotics, phenytoin, carbamazepine and barbiturates among anticonvulsants, non-steroidal anti-inflammatory drugs, allopurinol and antifungals. Other causes include mycoplasma, herpes, varicella and measles infections, neoplasies, autoimmune diseases such as SLE, and radiotherapy (6). Our patient exfoliative skin lesions, fever and oral mucosal involvement is considered SJS. There are no universally accepted diagnostic criteria for Stevens Johnson syndrome/toxic epidermal necrolysis (SJS/TEN). However, the diagnosis of SJS/TEN would be appropriate in a patient with the following clinical features (7);

1) History of drug exposure or febrile illness,
2) A prodrome of acute-onset febrile illness and malaise.
3) A painful rash that progresses rapidly.
4) Erythematous macules, targetoid lesions, or diffuse erythema progressing to vesicles and bullae.
5) Positive Nikolsky sign and/or "bulla spread sign."
6) Oral, ocular, and/or genital mucositis with painful mucosal erosions.
7) Necrosis and sloughing of the epidermis of varying degree.

Systemic lupus eryhtematosus is a prototype of autoimmune diseases with a multisystem involvement. The clinical spectrum is quite variable, from skin involvement to joint involvement, and from organ involvement to a life-threatening clinical picture. The second most frequently seen organ involvement is the skin. Acute cutaneous lupus erythematosus (ACLE) can be categorized into localized ACLE, generalized ACLE and toxic epidermal necrolysis-like ACLE (8).

Hydroxychloroquine is a reliable drug which was proven to be effective in the treatment of SLE. It is an anti-malarial drug reported to have anti-inflammatory and immunosuppressive effects. The mechanisms of action of HCQ are, Toll-like receptor activation antagonism, inhibition of interferon-alpha expression, IFN-alpha mediated pathways inhibition (9). The most important toxic effect is retinal damage. For this reason, periodic ophthalmologic examinations should be done every 4-6 months during treatment. Other important side effects include fulminant hepatic failure, autotoxicity, neuromyotoxicity and skin reactions (5).

In the literature the association of SLE and SJS has numerous cases reported. Some patients with SLE present with SJS. Other SLE patients, depending on the drug they developed SJS (10, 11). Few cases have

been reported SJS associated with HCQ. The data of the German Hospital between 1990 and 2006 was evaluated in this study by Ziemer et al, and a history of SLE was detected in 17 of 1366 patients. The number of female patients was larger, with a mean age of 49.2 and increased positivity of anti-La and Anti-Ro. Also, 13 of the 17 patients were taking immunosuppressive medications, with a history of drug intake in 15 (88%). Stevens Johnson Syndrome was more frequently seen in young female patients and in those with positive Anti-Ro and Anti-La in other reported cases in the literature. Stevens Johnson Syndrome had developed in a 31-year-old patient who was being followed-up due to SLE, with two probable causing medications: co-trimoxazole and HCQ. On the other hand, HCQ is the only etiologic agent in another patient. This patient was a 50-years-old female followed-up for CLE with positive ANA and Anti-Ro. She had had skin lesions resembling erythema multiforme before the incident, after which SJS had developed with generalized mucocutaneous involvement (10). Lateef have been reported a 67 -year-old woman with SLE after treatment of HCQ developed acute generalized pustulosis exanthematous and TEN overlap. And the patient successfully treated with steroid and intravenous immunoglobulin (IVIG) therapy (12). Simsek reported a 28-year-old woman patient with SLE after the treatment of prednisolone and HCQ developed TEN like reaction (13). Lastly in Leckie's study, a 65 years old diagnosed with rheumatoid athritis for almost 9 year, after 2 weeks addition of 200mg/day of HCQ and developing SJS, it reported that she respond with steroids (14). In addition, in previous studies, there are cases shows the association of using steroid to develop SJS in SLE patients (13, 15, 16, 17, 18). Our patient had HCQ and prednisolone intake. There is a decline and disappearing of clinical complaints after prednisolone treatment, the formation of skin lesions was thought to be developed after the treatment of HCQ.

The mechanisms leading to SJS are not yet fully understood, but a modification of the structure of the drug could thus alter this presentation due to poor recognition of the drug molecule by major histocompatibility complex (MH C) or the T cell receptor (19). This recognition by the lymphocyte receptors leads to their activation (20). The other important mechanism is an increase in keratinocyte cell death. This is caused by an increased FasL and Fas expression in the keratinocytes (21). Also, immunochemical examination of skin biopsies have shown an increased expression of FasL in all patients with SLE in comparison with controls (22). A triggering agent such as a medication may increase apoptotic ligand production of keratinocytes and may cause apoptosis with Fas-FasL binding (23).

There is still no specific therapy. A satisfactory benefit from systemic steroids and immunosuppressive treatment was not reported. The recent introduction of IVIG therapy in these cases is a safe alternative treatment for these patients (24). The antibodies in IVIG were shown to bind to Fas, thus inhibiting FasL binding to Fas. Also, IVIG was found to strongly inhibit apoptosis by inhibiting in vitro Fas-FasL binding (22, 25). Our patient could be successfully treated with steroids and supportive therapy. In conclusion, SJS may occur frequently in patients with SLE. The etiology of these patients HCQ and steroid therapy should be noted that may

take place.

## Conflict of interest

The authors declare that no conflict of interest exists.

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