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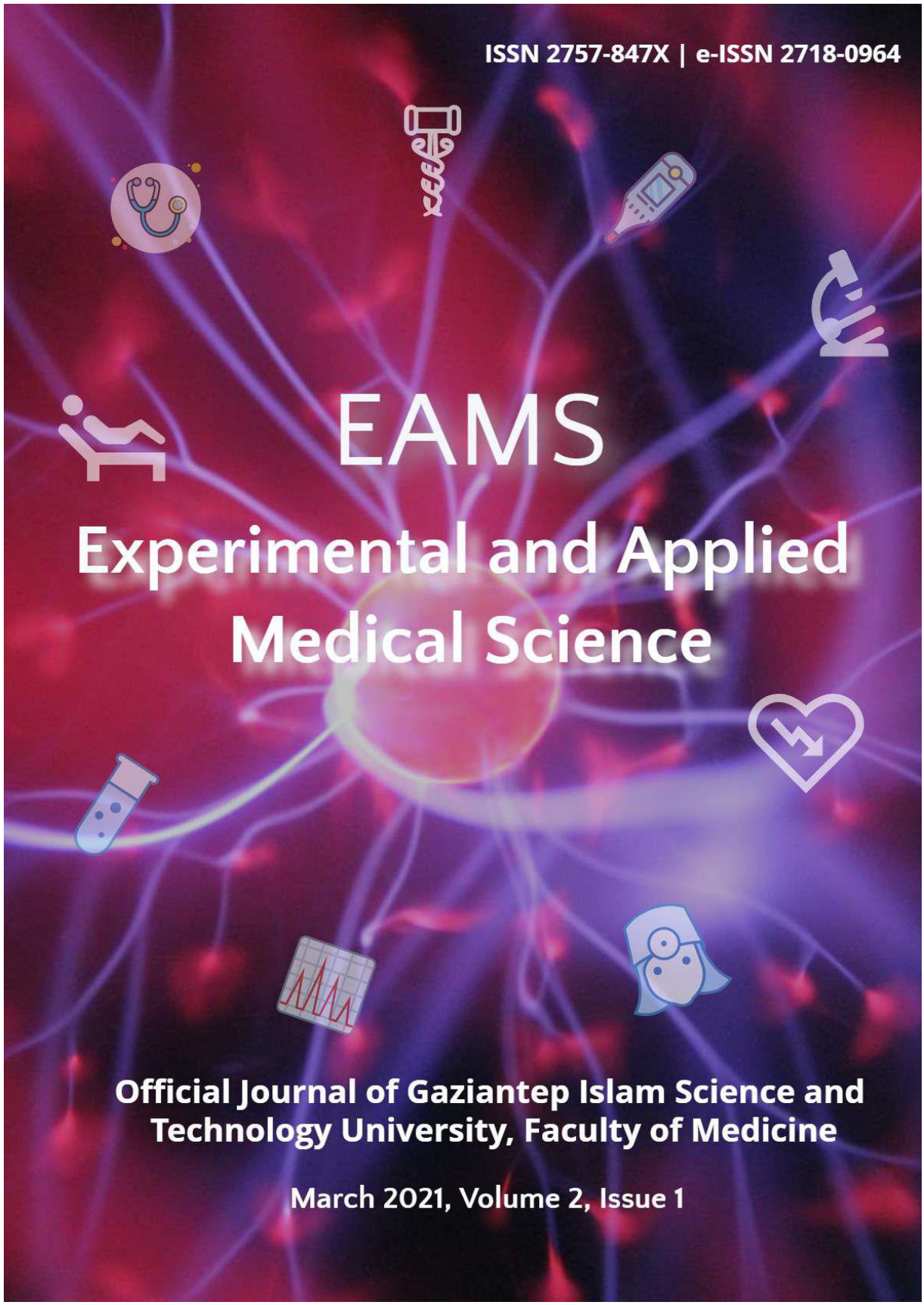

EAMS

Experimental and Applied Medical Science

Official Journal of Gaziantep Islam Science and Technology University, Faculty of Medicine

March 2021, Volume 2, Issue 1

GAZİANTEP İSLAM BİLİM VE TEKNOLOJİ ÜNİVERSİTESİ TIP FAKÜLTESİ

GAZİANTEP ISLAM SCIENCE AND TECHNOLOGY UNIVERSITY FACULTY OF MEDICINE

Experimental and Applied Medical Science

Volume 2, 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

Mediha Begüm Kayar, Asst. Prof.

Chief Editor/Baş Editör

Hamit Yıldız, Assoc. Prof.

Clerk of Editorial Office/Sorumlu Yazı İşleri Müdürü

Mehmet Göl, Asst. Prof.

Aim

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

Amaç

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

Scope

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

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

Kapsam

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

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

Ethical Principles and Publication Policy

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

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

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

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

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

Etik İlkeler ve Yayın Politikası

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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Author Guidelines

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

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

There should be a separate title page with:

a) The title

b) The authors' names

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

d) A running title

e) Corresponding author and e-mail

f) Conflict of interest

g) Acknowledgements

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

1. Abstract

2. Introduction

3. Material and Methods

4. Results

5. Discussion

Yazım Kuralları

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

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

Aşağıdakileri içeren ayrı bir başlık sayfası olmalıdır:

a) Başlık

b) Yazarların isimleri

c) Her yazarın tam adresi ile birlikte çalıştıkları laboratuarlar

d) Kısa başlık

e) İletişimdeki yazar ve iletişim bilgileri

f) Çıkar çatışması beyanı

g) Teşekkür, bilgilendirme

Tam uzunluktaki kağıdın ana gövdesi şu bölümlere ayrılmalıdır:

1. Özet

2. Giriş

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

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

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

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

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

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

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

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

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

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

specified in the metric system.

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

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

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

Example:

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

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

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

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

tablolarda istatistiksel varyasyon ölçütleri tanımlanmalıdır.

Tüm boyutlar ve ölçüler metrik sistemde belirtilmelidir.

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

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

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

Örnek:

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

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

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

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

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

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

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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, preventivemedicine 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 thebaby 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 AppliedMedical Science" sprouted, which is the academic publication of our Faculty of Medicine andin 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 phaseis 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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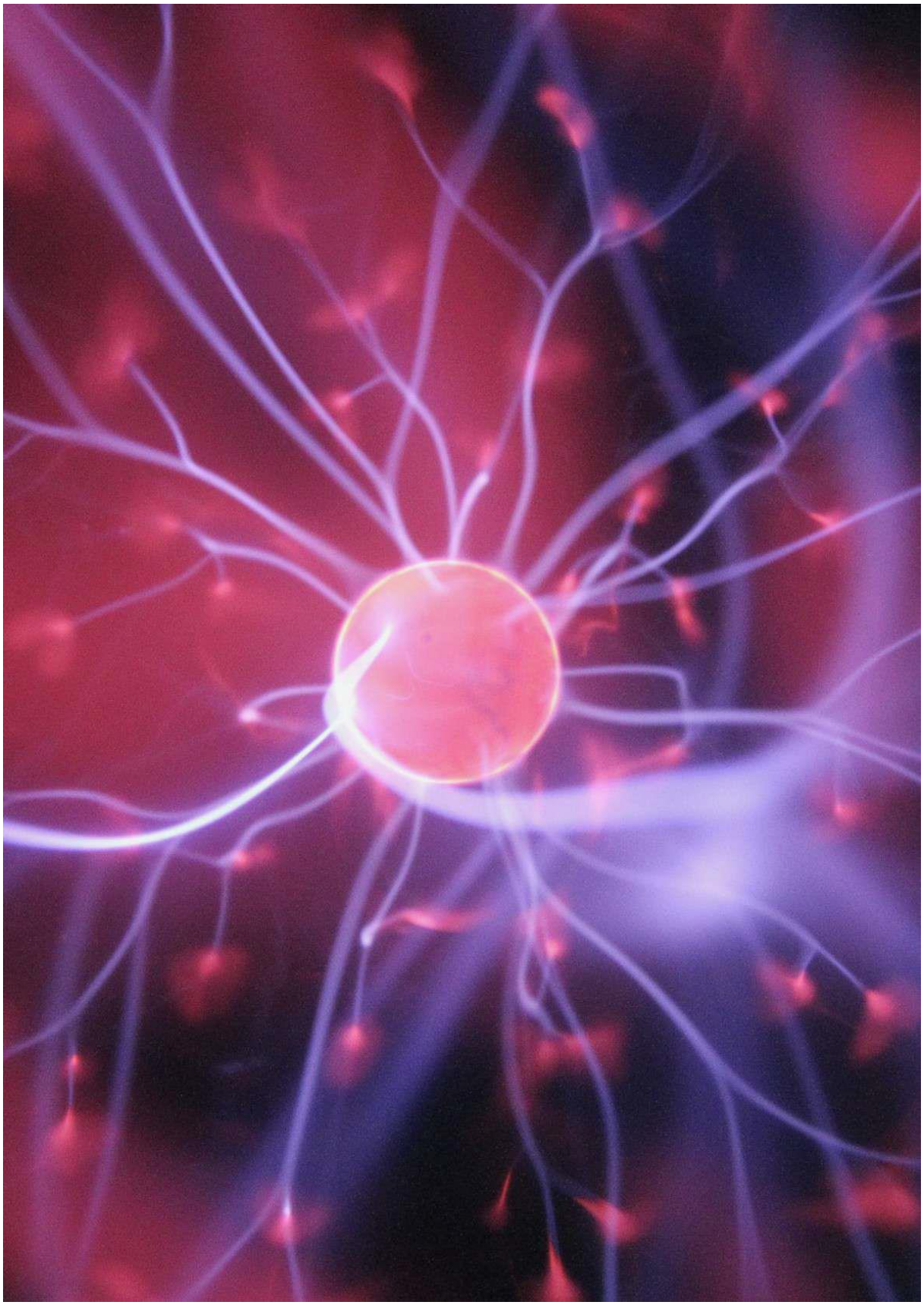

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Morphometric Analysis of the Relationship between Bigonial Width and Cranium

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Abstract

*In the present study, the purpose was to determine the relation between bigonial width and craniometric parameters. In this study, seven craniometric parameters were examined based on computed tomography images of 110 adult individuals 58 of whom were males and 52 females. These parameters were Bigonial Width (BGW) in mandible, and Maximum Cranium Width (MCW), Upper Face Width (UFW), Bizygomatic Width (BZW), Vertex-Prosthion Height (VPH), Vertex-Nasion Height (VNH), Upper Face Height (UFH) in the cranium. The difference between two groups was examined with **t**-test in independent groups. The relation between numerical variables was examined with the Pearson Correlation Coefficient. The analyses were made with IBM SPSS v.21. The significance level was taken as $p<0.05$. When the morphometric relationship between the mandible and cranium was examined, it was found that although a relation was detected between BGW and all craniometric parameters, there was a relation between BGW and MCW, UFW, BZW in male, and UFW and BZW parameters in females when gender was taken into account ($p<0.05$). No significant correlations were detected between BGW and VPH, VNH, UFH measurement values in both genders. This study revealed that there is a significant relation between BGW and UFW and BZW parameters in both genders. It is considered that the relation between the mandible and cranium can be used in the clinic when it should be considered, and the results will contribute to scientific studies.*

Key words: *Bigonial width, Mandible, Cranium, Compatibility, Computed tomography*

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Introduction

The mandible, which is a facial bone, is the movable part of the cranium, and temporal bone and temporomandibular joint allow the chewing movements of the mandible (1, 2). In joint biomechanics, when maximum chewing pressure is applied to the occlusal surfaces, this chewing pressure is transferred to the viscerocranium from the teeth, then to the basis cranii and cranium (3). With the transfer of this pressure, it is important with a clinical and anthropological viewpoint to understand how and to what extent the forces applied by chewing muscles affect the mandibular shape (4).

The fact that the mandible is one of the skeletal components of the cranium, and it was found as a separate bone in skeletal remains led to morphological and morphometric studies regarding this bone (5, 6). The mandible, which is the largest and hardest facial bone, has come to the forefront in studies conducted to identify gender as an important source of personal identification, since it keeps its integrity, usually because of its resistance to postmortem damages (7).

The important position of the mandible on the face was evaluated in studies in terms of its aesthetic, sexually dimorphic, and anthropometric aspects as a part of important functions, such as eating and as a bone very easy to recognize among skeletal remains (8, 9). When previous morphometric studies were examined, it was seen that mostly the metrical and angular parameters of the mandible were examined (10, 11). It was also found that the focus was on the gonion area of the mandible, the holding place of the chewing muscles, and the parameters of bigonial angle and bigonial width (12-15).

Although limited studies examined craniometric parameters, it was found that the relation between these parameters was not evaluated (8, 15, 16). In a previous study that evaluated cranium compatibility with the mandible, it was found that a small number of linear parameters were evaluated and were transverse only (17).

Although there are studies that investigated the morphometric features of mandible and cranium, studies that examine relations between these parameters are limited. The purpose of the present study was to evaluate the transverse and vertical parameters of the relations between bigonial width and cranium by using Computed Tomography (CT) results.

Material and methods

The images of 58 male and 52 female adult individuals who underwent head CT in the Department of Radiology of Bolu Abant Izzet Baysal University, Faculty of Medicine were examined retrospectively in the present study. The individuals who had cranium integrity and no diseases that affected their bone structures, and with no bone anomalies in CT images were included in the study. The present study was approved by Bolu Abant Izzet Baysal University, Clinical Researches Ethics Committee, with the Decision Number 2021/54.

Computed Tomography Protocol

All patients underwent CT using a 64-slice Multi-Detector Computed Tomography (MDCT) device (Revolution EVO, GE healthcare, Waukesha, WI, the USA) with the same examination protocol using 64x0.5 mm collimation scanner with a gantry rotation speed of 400 ms/rotation, range of box 450-500, image thickness 5

mm, standard pitch factor of 0.641, reconstruction interval 0.625 mm and a total exposure time 11. Each scan was obtained with a tube voltage of 120KV and 320mAs. Images have been transferred to a separate workstation (GE, Advantage Workstation 4.4) for measurements.

Obtaining Measurements of Parameters

Gonion (go), euryon (eu), frontomalar temporal (fmt), zygion (zy), nasion (n), prosthion (pr) and vertex anatomical landmarks were used in the measurement of our parameters. In the present study, seven different parameters were evaluated to determine the morphometric relations between horizontal and vertical parameters, such as bigonial width in mandible, and maximum cranium width, upper face width, bizygomatic width, vertex-prosthion height, vertex-nasion height, upper face height in the cranium, by referencing these anatomical landmarks (16, 18, 19) (Figure 1). All parameters were measured by 2 different people in 3 repetitions.

Measured parameters, abbreviations and related landmarks

	Measurement name	Abbreviation	Landmarks
1	Bigonial width	BGW	go – go
2	Maximum cranium width	MCW	eu – eu
3	Upper face width	UFW	fmt – fmt
4	Bizygomatic width	BZW	zy–zy
5	Vertex-prosthion height	VPH	Vertex – pr
6	Vertex-nasion height	VNH	Vertex – n
7	Upper face height	UFH	n – pr

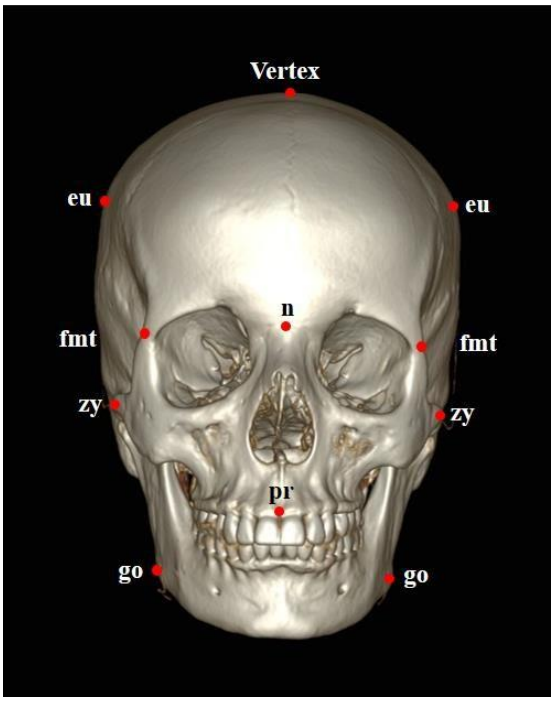

Figure 1: Frontal view of cranium showing anatomical landmarks used in the present study.

Statistical Analyses

Mean and Standard Deviation (SD) values were given for numeric variables, and numbers and percentage values were given for categorical variables in defining the data. Normality assumption was examined with the Kolmogorov-Smirnov Test. Whether there were significant differences between two groups was evaluated with the ***t****-Test in Independent Groups*. The relations between numerical variables were examined with the Pearson Correlation Coefficient. Coefficient correlation 0.0 – 0.30 were interpreted as negligible, those 0.31 – 0.50 as low, those 0.51 – 0.70 as moderate, 0.71 – 0.9 as strong and 0.91 – 1.0 as very strong. Analyses were made with IBM SPSS v.21. The significance level was taken as $p<0.05$.

Results

The CT images of 110 individuals, including 58 males with a mean age of 51.59±12.96 and 52 females with a mean age of 52.29±14.08, were evaluated retrospectively in our study. When gender groups (male 52.7%, female 47.3%) were evaluated, no significant differences were detected in terms of age (p=0.786). The demographic characteristics of the individuals who participated in the study are given in Table 1.

Table 1: Demographic characteristics of individuals.

		Number (%)	Age	p
Gender	**Female**	52 (47.3)	52.29 ± 14.08	0.786
	Male	58 (52.7)	51.59 ± 12.96	
	Total	110 (100.0)	51.92 ± 13.44	

When the measurement parameters between gender groups were examined, it was found that there were significant differences in BGW, MCW, UFW, BZW, VPH, VNH and UFH values ($p<0.001$). It was found that the measurement values of all parameters were higher in male individuals (Table 2).

Table 2: Evaluation of parameters' measurement values according to gender.

	Male (n=58)	Female (n=52)	
	Mean ± SD	**Mean ± SD**	**p**
BGW	106.01 ± 7.42	97.67 ± 6.48	**<0.001**
MCW	151.81 ± 7.17	146.44 ± 5.81	**<0.001**
UFW	108.99 ± 4.79	104.52 ± 4.35	**<0.001**
BZW	137.62 ± 6.19	128.31 ± 5.05	**<0.001**
VPH	172.33 ± 8.21	164.70 ± 7.76	**<0.001**
VNH	98.29 ± 6.76	94.63 ± 5.42	**<0.001**
UFH	73.56 ± 5.66	69.31 ± 5.72	**<0.001**

The morphometric relations between BGW in mandible and MCW, UFW, BZW, VPH, VNH, UFH parameters in cranium were examined in our study. It was found that there was a strong relation between BGW and VPH measurement values, a moderate relation with BZW, and a low relation with MCW, UFH, and a negligible and low relation with VNH (Table 3).

Table 3: Relation between bigonial width and craniometric parameters' measurement values.

	BGW	
	r	**p**
MCW	0.462	**<0.001**
UFW	0.635	**<0.001**
BZW	0.747	**<0.001**
VPH	0.362	**<0.001**
VNH	0.201	**0.035**
UFH	0.356	**<0.001**

When the relation between bigonial width and measurement values of craniometric parameters were examined according to gender, it was found that there was a strong relation between BGW and BZW in males, a moderate relation with UFW, and a low relation with MCW. Although there were moderate relations between BZW and UFW in females, the relation that was detected in MCW in males was not detected in females. It was also found that there were no correlations among VPH, VNH, UFH and BGW measurement values in males and females (Table 4).

Table 4: Relation between bigonial width and craniometric parameters' measurement values according to gender.

	Female		**Male**	
		BGW		
	r	**p**	**r**	**p**
MCW	0.209	0.138	0.416	**0.001**
UFW	0.525	**<0.001**	0.535	**<0.001**
BZW	0.508	**<0.001**	0.715	**<0.001**
VPH	0.164	0.247	0.192	0.148
VNH	0.087	0.541	0.052	0.697
UFH	0.211	0.133	0.224	0.091

Discussion

The main purpose of the present study was to examine the relation between the measurement values of the mandible's BGW and craniometric parameters with CT. The relation between the cranium is important because the mandible has several specific anatomical features, with aesthetic importance with facial bone, and due to the possibility of a matching when a separated cranium and mandible are found in skeletal remains with have impaired integrity (17, 20, 21).

When the studies conducted on mandible were examined, it was found that the parameters were evaluated directly with dry bones with the help of caliper and radiological images (9, 17, 20). We also

found that the cases used in previous studies were examined in different populations by considering the age and gender data (11, 14, 22).

When previous studies in which mandible and craniometric parameters were evaluated together were examined, 63 males were evaluated with a mean age of 48±15.5, and 57 females with a mean age of 47±15.7 with CT images. The BGW, BZW, UFH measurement values were found to be 94.94±7.12 mm; 87.52±5.25 mm, 131.73±5.20 mm; 122.73±4.08 mm, 73.15±5.41 mm; and 69.37±4.24 mm in males and females, respectively. Although it was found that there was a difference in these parameters between the gender groups ($p<0.001$), the relation between mandible and cranium morphometry was not examined in this study (16). In our study, BGW, BZW, and UFH values with the help of CT were determined to be 106.01±7.42 mm; 97.67±6.48 mm, 137.62±6.19 mm; 128.31±5.05 mm, 73.56±5.66 mm; 69.31±5.72 mm, respectively in males with an average age of 51.59±12.96 and females with a mean age 52.29±14.08. It was also found that there was a difference between gender groups in terms of measurement values ($p<0.001$); and the relation between BGW and craniometric parameters was examined in our study. A moderate and significant relation was detected between BGW measurement value and BZW measurement value ($r=0.635$; $p<0.001$), and low and significant relation was detected with UFH measurement value ($r=0.356$; $p<0.001$).

When we compared our results with the study of Gillet C et al. (16), it was found that BGW and BZW value was high in our study, and UFH value was similar. It was found that it is similar in these studies that there were differences between gender groups in terms of measurement values of these parameters in both studies. We believe that the lack of similarity in BGW and BZW values maybe because of ethnic differences, although the mean age of the cases was similar. The relation between mandible and cranium morphometry was not examined in the study conducted by Gillet C et al. (16).

In a study evaluating the cranium for the Caucasian race in males and females with a mean age of (approximately) 40, the following results were found; MCW 139.6±5.70 mm; 137.5±4.75 mm, BZW, 128.9±4.27 mm; 122.0±3.47 mm, UFH, 71.2±3.82 mm; 66.0±4.96 mm and BGW 99.9±5.71 mm; 92.1±5.17 mm. The relation between BGW and craniometric parameters was not evaluated in this study (23). When the results of this study were compared with those of Iscan et al., the measurement values of parameters were MCW 151.81±7.17 mm; 146.44±5.81 mm, BZW, 137.62±6.19 mm; 128.31±5.05 mm, UFH, 73.56±5.66 mm; 69.31±5.72 mm and BGW 106.01±7.42 mm; 97.67±6.48 mm and the mean age of 51.92±13.44 in our study in males and females who had a mean age of 51.92±13.44. Although this study was conducted directly on dry bones, and our study was conducted with the CT method, it was reported that the data obtained with these two different methods can be compared safely (24). We believe that these differences may have stemmed from the mean age difference in studies and that these studies were not conducted in the same populations.

The relation between BGW measurement value and craniometric measurement values was examined in our study; and as a result, strong relation was found with VPH ($r=0.747$; $p<0.001$), a moderate relation

with BZW (r=0.635; p<0.001), and a low relation with MCW (r=0.462; p<0.001), UFH (r=0.356; p<0.001), and a negligible and low relation with VNH (r=0.201; p=0.035). When this relation was examined according to gender, it was found that there was a strong relation (r=0.715; p<0.001) between BGW and BZW in males, a moderate relation between UFW (r=0.535; p<0.001), and low relation between MCW (r=0.416; p=0.001). A moderate relation was detected in females between BGW and BZW and UFW (r=0.508; p<0.001, r=0.525; p<0.001, respectively). Although the mandible and cranium were examined morphometrically in studies, the fact that the relation of these parameters was not examined with each other prevents the comparability of our study results (16, 23).

Conclusion

The results of our showed found that there are relations among BGW and MCW, UFW, BZW, VPH, VNH, UFH parameters; however, when gender was taken into account, there was a relation between BGW and MCW, UFW, BZW in males, and UFW and BZW parameters in females. When the mandible is found as a bone separated from the cranium in skeletal remains, the morphometric relation between the mandible and the cranium is important, also for facial reconstruction surgeries and the design of the appropriate mandible for forensic facial reconstruction. For this reason, we recommend that the number of parameters that will be investigated in the mandible and cranium is increased when future clinical trials are planned and that this relation is examined and modeled again in the relations identified here.

Conflict of interests

The authors declare no conflict of interests.

Acknowledgement

Researchers attributed equally to the study.

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Effects of Aerobic Exercise on Oxidant/Antioxidant Status in Obese Boys: A Controlled Trial

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Abstract

It is unclear how the aerobic exercise program effects oxidative stress and antioxidant defense parameters in obese children; therefore, our objective is to examine the effects of aerobic exercise on parameters of oxidative stress and antioxidant in obese boys. Our study included 10 obese boys, aged from 13 to 15, and 10 healthy boys as a control group. Before and after exercise, 10 ml blood specimens were taken from the obese boys, who exercised 3 days/week for 12 weeks. 10 ml blood specimens were also taken from the healthy control group. The oxidative stress and antioxidant defense parameter levels in blood samples were measured for both groups. In our study, it was found that the control group and obese children after aerobic exercise had significantly higher superoxide dismutase, catalase, glutathione peroxidase, and paraoxonase-1 values than those of obese boys before aerobic exercise. On the other hand, it was found that after aerobic exercise, the control group and obese children had significantly lower malondialdehyde, nitric oxide, and carbonyl values than those of obese boys before aerobic exercise. When these findings are taken into account, it may be suggested that aerobic exercise regulated oxidative stress and improved the state of antioxidant status in obese boys.

Key words: *Childhood obesity, Oxidative stress, Antioxidant, Regular exercises, Carbonyl*

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Introduction

Obesity, resulting from excessive fat storage in the body, is an unresolved clinical condition affecting the health of children and adults. Childhood obesity prevalence has increased all over the world recently, especially in the developed countries. The lack of physical activity, which has a negative effect on maintaining a healthy lifestyle, and high-calorie food intake are among the reasons for obesity (1).

The effect of oxidative stress and oxidants, which occur due to various reasons, has proven to be causing the development of various complications in obese individuals (2). Reactive oxygen species (ROS) creates oxidative damage in carbohydrates, proteins, and lipid molecules. The lipid peroxidation and carbohydrate oxidation products, formed by the action of ROS, constitute modifications in the amino acid content of protein and cause an increase in carbonyl content of plasma protein (3).

Genetic studies have found some enzyme genes affected by exercises and physical fitnesses (4). One of them is the serum paraoxonase-1 (PON-1) enzyme, which proved to have an antioxidative function in humans. HDL-related PON-1 enzyme, formed under oxidative stress, has an effect to reduce hydrogen peroxide (H_2O_2). Previous studies examining the effect of exercise on oxidative stress and antioxidative defense system mostly focused on aerobic exercise form. Aerobic exercise has been shown to strengthen the antioxidative defense system and inhibit damage caused by oxidative stress (5).

The primary components of the physiological antioxidative defense are catalase (CAT), glutathione peroxidase (GPx), and superoxide dismutase (SOD). Antioxidative protection of skeletal muscles in humans is weak; therefore, skeletal muscles may easily be subjected to oxidative stress (6). Acceleration of energy in metabolism during aerobic exercise increases the concentration of free oxygen radicals in cells, so this situation increases lipid peroxidation rate causing muscle damage (6). Nitric oxide (NO) is the most important molecule in the human body. It penetrates almost all organs from the heart to the lungs. It relaxes the vessels, decreases the risk of stroke or heart attack, and lowers blood pressure (7).

Several *in vitro* and *in vivo* human and experimental investigations demonstrated that exercise increases the production of lipid peroxidation, ROS and whole-body oxygen consumption. On the other hand, regular physical training leads to an increase in antioxidant enzyme activities (8).

The examination of benefits and arrangement of exercise programs will provide practical data for the literature to prevent heart disease, which is quite common all over the world, and strengthen antioxidant metabolism. Regular exercise is known to empower antioxidant metabolism. Although the impacts of exercise on antioxidative and oxidative stress parameters in obese adult individuals have been investigated before, in terms of these parameters, there is no satisfactory study on the effects of exercise in obese children. However, how regular exercise affects the oxidant/antioxidant status is still not completely obvious in obese children. Considering these aspects, the present investigation was conducted to evaluate the impacts of a 12-week aerobic activity program on serum levels of SOD, CAT, GPx, malondialdehyde (MDA), C=O, NO and PON-1, on 13-15 years old obese boys.

Materials and methods

Study Design

The study group consisted of 10 boys, diagnosed with obesity (aged 13-15) and receiving education in a private middle school in Nevsehir province, Turkey. Ten healthy children, who were students at the same school, gender and age-matched, were also included in the research as the healthy control group.

Data Collection

This study was carried out in Erciyes University, Faculty of Pharmacy, Biochemistry Department Laboratories and Nevşehir Special Versa Hospital. The study protocol was approved by the Ethics Committee of Medical Faculty of Erciyes University (approval number and date: 684-2012/06.11.2012). Before starting the research, GPx activity results from previous studies (9), which conduct on obese children, were taken into consideration. A sample size of 7 reaches 95% power to detect a mean of paired differences of 4.7(0.25) with a known standard deviation of differences of 3 (0.15) and with a significance level (alpha) of 0,05000 using a Wilcoxon test (A paired t-Test) assuming that distribution is normal (10). As a result of these calculations, it started to work in groups of 20 children in both groups in priority, but then some of the individuals who did not work regularly and do not fully comply with the exercise instructions were excluded from the study and finally, the examination was terminated with 10 children in both group who complied with the working procedures. No restrictions were imposed on the dietary regulation of children, and they were able to continue their eating habits.

The body weights and heights of the children included in the study were measured with G-TECH brand and GL-150 model electronic weighing and stadiometer. Specific BMI percentile curves prepared for children and adolescents, and adjusted according to their ages and sexes, were used (11).

Exercise protocol and training arrangement

The fitness levels of obese children were determined according to physical activity readiness inventory, physical activity index, and physical fitness (Table 1). In order to increase the level of physical fitness, the training intensity must be 130 beats/min and above. For this reason, moderate aerobic exercises were applied on a running band by following polar brand pulse counting time between 130-150 beats/min. The maximal oxygen using (Max VO_2) levels of obese children were determined by 20 m shuttle running test (12). It was built at optimum level of exercise in order to obtain the targeted level of fitness. For each obese child, the optimal fitness status was determined using the Karvonen formula (13).

With the help of the obtained results, a personal exercise prescription for each obese boy was created using a personal exercise protocol chart (12). In each training unit, 400 kcal of energy loss was targeted, and participants tried to reach 1200 kcal of energy loss per week. These calculations are based on the mentioned graphics, tests, and the fitness scores of obese boys (12, 13).

An exercise session consists of the following sections:

1. Warm-up (5-10 min.)
2. Condition (20-60 min) (aerobic exercises, muscle strengthening, and endurance exercises)
3. Cooldown (5-10 min.)

Table 1: Physical activity readiness inventory and physical fitness (Score = Violence x time x frequency)..

Questions	
1-Did your doctor tell you that you have a problem with your heart and that you can only do physical activity with a doctor's check?	If ones has given all the answers to no questions, ones can start training in physical activity with coach control. If ones answer yes to a question, ones can start training by consulting your doctor and your coach at the check of your doctor. If ones say yes to two or more questions, ones can only start the exercise with the supervision of your doctor.
2-Do you feel pain in your chest during physical activity?	
3-Did you feel chest pain in case you did not physical activity last month?	
4-Have you lost your balance due to dizziness or loss of consciousness?	
5-Do you have a bone or joint problem that will change your physical activity?	
6-Did your doctor recommend medication for your blood pressure or your heart?	
7-Do you have any reason not to participate in physical activity?	

Evaluation of physical activity index and physical fitness		
Intensity	**Continuance**	**Frequency**
(5) Continuous Deep Breathing and Sweating	(4) 30 min or over	(5) Everyday
(4) Intermittent Deep Breathing and Sweating (Tennis)	(3) 20-30 min	(4) 3-5 times a week
(3) Normal (Cycling)	(2) 10-20 min	(3) 1-2 times a week
(2) Normal (Volleyball)	(1) 10 min or less	(2) Several times a month
(1) Light walking etc.		(1) Less than one month

Laboratory measurements

The fasting blood specimens were also taken from the exercise group in the morning of the first day after the end of the exercise. Afterward, blood samples were allowed to clot and centrifuged at 4000 g as usual. Yellow and clear serum specimens were selected and both lipemic or haemolysed specimens were excluded. The serum specimens were kept at −70°C for evaluation of oxidative stress and antioxidative defense parameters.

Determination of SOD, CAT, GPx, and PON-1

Obese children were given aerobic exercise for 3 days a week for 12 weeks, and 10 ml blood samples were taken twice (pre-test and post-test). SOD activity was measured using the method described by Sun et al. (14). The activity of CAT was measured using the technic described by Yasmineh et al. (15). The activity of glutathione peroxidase was determined using the technic described by Paglia et al. (16). The activity of PON-1 was determined using the technic described by Eckerson et al. (17).

Measurement of Carbonyl, MDA, and NO

The levels of the protein carbonyl group were measured using the technic described by Evans et al. (18). Malondialdehyde was determined using the technic described by Yoshoiko et al. (19). Nitrite and nitrate measurements were measured using the method described by Smarason et al. (20).

Statistical Analysis

Statistical analyses were conducted using SigmaStat 3.5 and SPSS software version 15.0 statistical packages. For calculate sample size in this study, a power analysis was performed with GPower software 3.1 (Düsseldorf University, Germany). The normality of the data was evaluated by the Kolmogorov-Smirnov Test. The values of the groups were compared with the independent sample t-test. The Mann-Whitney U test was used for intergroup comparisons of continuous data. Wilcoxon test was used for pre-and post-exercise

comparison in obese children. The chi-square test was used for the evaluation of difference in the distribution of categorical variables. Statistical significance was determined as 0.05.

Results

Sociodemographic data and biochemical parameters of study groups are shown in Table 2. We found that there was no significant difference between the groups in terms of gender, age, educational status, and socio-demographic data. The ages of healthy volunteers and obese children were 13-15 (mean: 14±1.0 years), and all were male.

Table 2: Comparison of study groups in terms of sociodemographic data and biochemical parameters.

Parameters	Study Groups			Comparisons		
	Control (*n*=10)	Pre-exercise (*n*=10)	Post-exercise (*n*=10)	Control/ pre-exercise *p*	Control/ post-exercise *p*	Pre-exercise / post-exercise *p*
Age	14±1.0	14±1.0	14±1.0	1.000	1.000	1.000
Gender (G/B)	0/10	0/10	0/10	1.000	1.000	1.000
Height (cm)	170±3.45	170±2.90	170±2.90	0.985	0.985	1.000
Education time (year)	7.00±2	7.00±2	7.00±2	1.000	1.000	1.000
Weight (kg)	68±2.75	90±2.87	84±2.02	<0.001	<0.001	<0.001
BMI (kg/m^2)	23.5±2.06	31.1±1.05	29.2±1.02	<0.001	<0.001	0.059
SOD (U/ml)	9.17(9.19-9.21)	7.21(7.22-7.25)	8.88(8.93-8.95)	<0.001	0.034	<0.001
CAT (U/ml)	3.17(3.18-3.19)	2.5(2.53-2.55)	3.01(3.04-3.07)	<0.001	<0.001	<0.001
GPx (U/ml)	142.3±1.31	132.2±1.44	140.5±1.76	<0.001	0.034	<0.001
PON-1 (U/ml)	130.6±3.61	106.0±3.90	111.4±2.41	<0.001	0.004	<0.001
Carbonyl (µmol/L)	92.44±0.96	101.5±2.41	93.4±2.40	<0.001	0.547	<0.001
MDA (nmol/ml)	3.79(3.81-3.87)	4.91(4.97-4.98)	3.71(3.74-3.75)	<0.001	0.001	<0.001
NO (µmol/L)	41.8(42.4-42.7)	52.6(53.3-54.1)	39.6(40.2-41.7)	<0.001	0.055	<0.001

The data are presented as mean±standard deviation or median (25th-75th percentil) for continuous variables. BMI: Body Mass Index, CAT: Catalase, GPx: Glutathione Peroxidase, MDA: Malondialdehyde, NO: Nitric oxide, PON-1: Paraoxonase-1, SOD: Superoxide Dismutase.

The median (25^{th}-75^{th} percentile) levels of SOD and CAT of the controls were significantly higher than pre-exercise and post-exercise SOD and CAT levels of obese children ($p<0.001$). Nevertheless, the median level of (25^{th}-75^{th}percentil) SOD and CAT in obese children after exercise was found to be closer to those of the control values. The median (25^{th}-75^{th} percentile) level of SOD and CAT in obese children after exercise was shown to be significantly higher than those of pre-exercise ($p<0.001$; Table 2).

The mean levels of GPx and PON-1 in the control group were significantly higher than pre-exercise and post-exercise GPx and PON-1 levels of obese children ($p<0.001$; Figure 1). Nevertheless, the mean post-exercise GPx and PON-1 levels of obese children were found to be closer

to those of the control values (p=0.034 and p=0.004, respectively). The mean GPx and PON-1 levels of obese children after exercise were shown to be significantly higher than those of pre-exercise (p<0.001; Table 2).

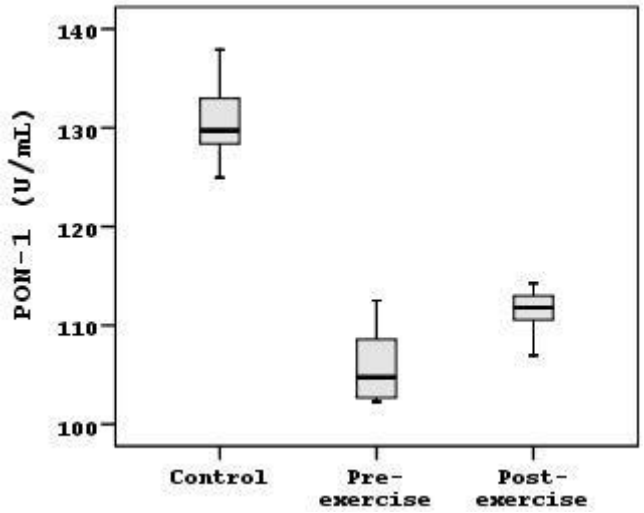

Figure 1. Comparison of study groups in terms of PON-1 levels. PON-1: Paraoxonase-1.

The mean level of carbonyl and median (25^{th}-75^{th} percentile) NO level of the control group was significantly lower than pre-exercise carbonyl and NO levels of obese children (p<0.001). On the other hand, there was no significant difference between the control group and post-exercise in terms of the carbonyl and NO levels (p=0.547 and p=0.055; Figure 2 and Figure 3, respectively). Moreover, the median (25th-75^{th} percentile) level of NO in post-exercise was lower than those of the control group (p=0.055). The mean level of carbonyl and median (25^{th}-75^{th} percentil) level of NO in obese children after exercise was found to be significantly lower than those of pre-exercise. The median (25^{th}-75^{th} percentile) MDA level of the controls was significantly lower than pre-exercise MDA levels of obese children. On the other hand, the median (25^{th}-75^{th} percentile) post-exercise MDA levels of obese children were significantly lower than those of the control group and pre-exercise obese children (p<0.001; Table 2; Figure 4).

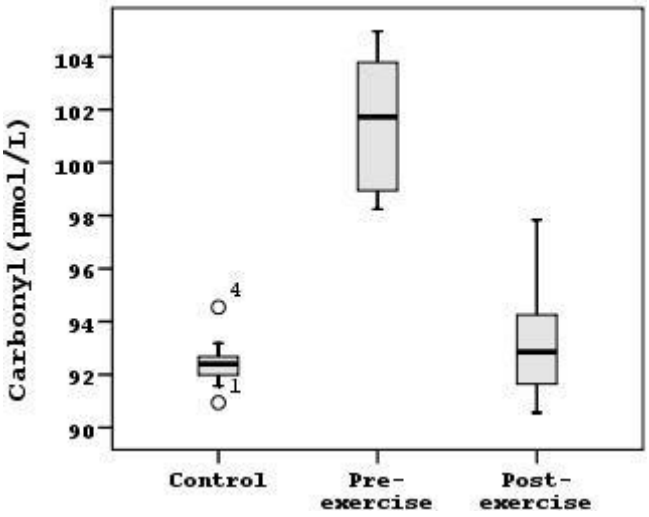

Figure 2: Comparison of study groups in terms of carbonyl levels.

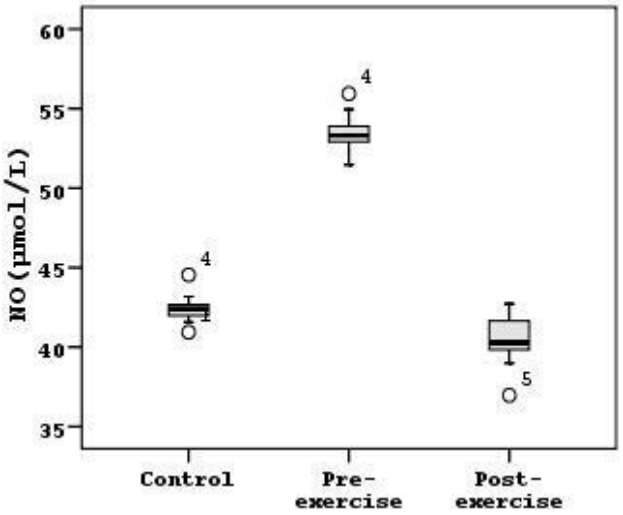

Figure 3: Comparison of study groups in terms of NO levels. NO: Nitric oxide.

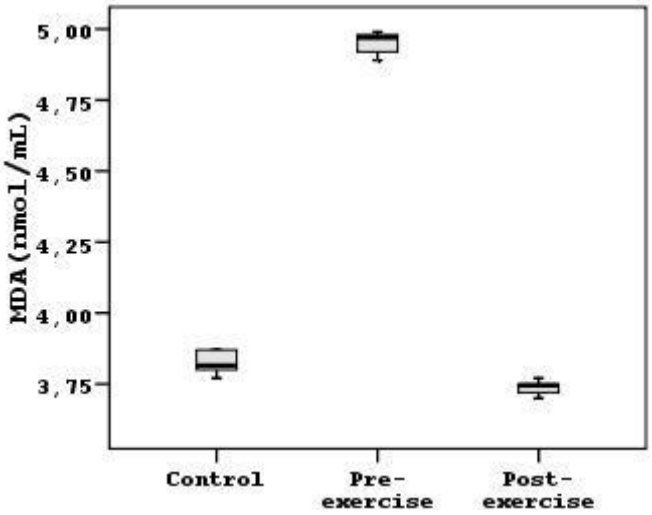

Figure 4: Comparison of study groups in terms of MDA levels. MDA: Malondialdehyde.

Discussion

The findings of the present investigation provide a demonstration of reduced oxidative stress status for obese children after 12 weeks of physical exercise as shown with an increase in activity of antioxidant enzymes but a decrease in levels of oxidative stress markers.

In the first measurements of our study, we found that the levels of SOD, CAT, GPx, and PON-1 in obese boys were significantly lower than the control group, and consistent with these findings; and we found out that obese boys had significantly higher carbonyl, MDA and NO levels than controls.

Oxidative stress occurs under physiological conditions and in pathological processes including obesity and damage to different organs (2). Similar results with our findings were reported in the group of prepubertal obese children who had a significant decrease of GPx and SOD activities, and antioxidant/oxidant status was significantly altered in obese children. Analogously, it was reported that previous studies on oxidative stress established significantly higher concentrations of MDA, ox-LDL, carbonyl, advanced oxidation protein products, and lower concentrations of enzymes with antioxidative activities, such as SOD, CAT, and GSH in children with obesity than non-obese ones (21). Rowicka et al. showed that prepubertal children with obesity already have a greater intensification of oxidative processes measured by total oxidant capacity concentrations and oxidative stress index values, while they simultaneously have lowered antioxidant defense measured by total antioxidant capacity concentrations compared with non-obese children (21). They also found a positive correlation between obesity duration and total oxidant capacity concentration, and they suggested that these findings may confirm the intensification of oxidative processes along with the duration of obesity occurrence not only in adults but also in children (21). This is concordant with the findings of Kilic et al. (22). They showed that oxidative stress markers and antioxidant activities were significantly higher in the obese group compared to the control while oxidative stress index was not distinct between the groups.

Considering these aspects, it may be suggested that our results confirm previous findings in which oxidative/antioxidant activities are altered due to obesity in children.

The second most important finding of our study is that the demonstration of the increased levels of SOD, CAT, GPx, and PON-1 in obese children after exercise is higher than those of pre-exercise.

Anaerobic and aerobic exercises in association with weight loss have been shown to be beneficial in the improvement of oxidative stress. It has been reported that oxidative stress has decreased in healthy obese adults following 24 weeks of resistance exercise (23), potentially due to decreases in total fat mass and/or increases in fat-free mass and maximal oxygen consumption (24).

Oh et al. reported that activity of the GPX enhanced following 6 months of regular exercise in obese women, while 12 weeks from moderate to high-intensity aerobic exercise reduced thiobarbituric reactive acid substances in obese individuals (25). Moreover, following regular training, acute exercise-induced increasing MDA levels were extenuated, while GPX and SOD enhanced levels than acute exercise-induced responses of pre-training.

In the present study, in compliance with the antioxidant enzyme findings, we determined that decreased levels of carbonyl, MDA, and NO were present in obese children after exercise when compared to those of pre-exercise.

To our knowledge, there is no similar study conducted on the effect of regular exercise on carbonyl, MDA and NO in obese children. Therefore, we think that our study findings will have great contributions to the literature.

Growing evidence demonstrated that regular training enhances the repair system and improves antioxidant status to recover from oxidative damage. When there was a weight loss, what caused significant skeletal and muscle-specific oxidative stress reduction in sedentary healthy obese adults was aerobic training for 3 months (26). It was exhibited by Youssef et al. that it was also adequate to have a moderate 3 months of regular exercise in the absence of weight loss to reduce training-induced increases of myeloperoxidase just after an acute bout of maximal aerobic training in obese and overweight adolescent girls when compared with pre-exercise girls (27). On the contrary, training without weight loss was not enough to enhance any oxidative stress markers in adolescents with obesity at the end of 3 months training course, in spite of making use of exercise in higher intensities (28). When handled together, powerful evidence supports a positive influence of aerobic training on redox balance. The training-induced adaptations of oxidative stress enhance the efficiency of the enzymatic antioxidant defense systems, after they lead to a greater mitochondrial capacity to scavenge free radicals.

In spite of all these, it can be suggested that regular exercise training have beneficial effects on reducing oxidative stress in our study. We can emphasize the fact that the results of this study demonstrate the positive effects of a correct exercise to be even more advantageous than the control group in terms of some oxidant parameters. In the present study, similar to oxidative stress parameter levels, the mean weight of obese children of post-exercise was significantly lower than pre-exercise obese children. On the other hand, it was stated that the antioxidant state shows differences depending on the type, size, and direction of exercise (29). Furthermore, the characteristics of the participants (gender, clinical disease status, and fitness or training levels) can affect the resultant amount of oxidization that occurs (30).

As far as we know, for the first time in this study, it was found that exercise can improve health in obese children including decreased lipid levels and protein oxidation markers, and increased antioxidant enzyme levels. However, this study has several limitations. Since this is a pilot study, we did not obtain any data about the nutritional status of study groups, and we did not make any arrangements about their diets. Moreover, the study population was small, for this reason, many more subjects are needed for the follow-up study, which should include the nutritional modification to examine the association between oxidative stress and exercise benefit.

In conclusion, the findings of this study are very interesting because it was performed strictly with obese children, who could have more oxidative stress conditions than lean subjects. Long-term exercise training regulated oxidative stress, however, improved the state of antioxidant status in obese boys. Nevertheless, examining the effect of regular exercise between different

gender groups of obese children having similar nutritional forms might be more precious and deserves further investigation.

Conflict of interest

The authors declare that no conflict of interest exists.

Acknowledgement

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Investigation of the Protective Effects of Caffeic Acid Phenethyl Ester against Cisplatin-induced Liver Damage in Rats

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Abstract

Cisplatin (CP) is used as an effective chemotherapeutic drug in the treatment of various solid tumors. However, side effects such as hepatotoxicity limit the use of the drug. We investigated the protective effects of caffeic acid phenethyl ester (CAPE), one of the active ingredients of propolis, against hepatotoxicity caused by CP treatment in the liver. 38 Wistar albino rats were divided into 4 groups. Control group was given physiological saline solution for 12 day. CP group was given a single dose of CP (7 mg/kg) on the day 7. CP+CAPE group, was given CAPE (10 µmol/kg/day) for 12 days and a single dose of CP (7 mg/kg) on day 7. CAPE group received CAPE (10 µmol/kg/day) for 12 days. Livers of rats sacrificed on the 14th day were stained with hematoxylin-eosin after histological tissue follow-up. The preparations were evaluated and scored. Rat weights were measured and recorded at the beginning and end of the experiment. CP caused significant histopathological changes in the liver. CP also prevented the increase in rat weight. CAPE played an effective role as a protective agent against the histopathological changes caused by CP and showed signs of tissue healing. Our results show that CAPE can be protective against hepatotoxicity associated with CP.

Key words: *Caffeic acid phenethyl ester, Cisplatin, Hepatotoxicity, Liver damage, Rat.*

Introduction

Cisplatin (CP) is an important antineoplastic drug used in the treatment of solid tumors (1). The drug, which was first successfully used in cancer patients in 1971, was approved by the American Food and Drug Administration (FDA) in 1978 (2). CP or cis-diamminedichloroplatinum (II) is one of the effective chemotherapeutic drugs widely used in the treatment of testicular, bladder, head, neck, lung, breast and ovarian cancers. However, its negative side effects such as nephrotoxicity, ototoxicity and cardiomyopathy limit its clinical use. (3, 4). It is widely known that CP is significantly retained in the human liver and high doses of the drug cause

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hepatotoxicity. (5). Although dose-related side effects and ototoxicity are important limiting factors in cancer treatment, recent studies have confirmed that hepatotoxicity is another important dose-limiting side effect of CP-based chemotherapy. However, there is a little research on CP-induced hepatotoxicity and the underlying mechanism remains unclear (6).

Caffeic acid phenethyl ester (CAPE) is one of the active ingredients of the pungent and fragrant propolis substance found in the extract collected by bees from plants. CAPE has been used as a folk remedy for many years (7). CAPE has many biological activities such as anticancer, antioxidant, anti-inflammatory, immune stimulator, and antifungal, antiviral and antibacterial. The mechanism of biological effects of CAPE is not fully known (8, 9). CAPE is known to be cytotoxic to many types of cancer cells, including breast cancer cells, but it has not been shown to have any toxic effects on healthy tissues (9).

In this study, we evaluated protective effects of CAPE against hepatotoxicity caused by CP, which is used as an anticarcinogenic drug in the treatment of many cancer types.

Material and methods

Animals and drug administration

The study protocol was accepted by the Experimental Animal and Local Ethics Committee of Erciyes University (decision no: 15/59). Herein, 38 male adult Wistar albino rats, weighing between 150 and 220 g, and aged between 8 and 10 weeks, were supplied by the Experimental Animal Laboratory of Erciyes University. The rats were housed at 20–22°C under a 12:12 light/dark photoperiod and fed ad libitum. The animals were randomly divided into 4 groups. Body weights of animals in each group were measured and recorded. Control group (n=8) was given a physiological saline solution (1 ml/kg/day) intraperitoneally (i.p.) for 12 day. CP group (n=10) was given a single dose of CP (Koçak Pharma, İstanbul, Turkey) (7 mg/kg) i.p. on day 7 of the experiment. The dose of CP was selected according to a previous study that demonstrated significant hepatotoxicity in rats (3). CP+CAPE group (n=10) was given CAPE (Sigma-Aldrich, St. Louis, MO, USA) (10 µmol/kg/day) i.p. for 12 days and single dose of CP (7 mg/kg) i.p. on day 7 of the experiment. The dose of CAPE was chosen according to the results of the studies in which this agent has a corrective effect (10). CAPE group (n=10) was given CAPE (10 µmol/kg/day) i.p. for 12 days. Before animals were sacrificed, the body weights were measured and recorded. At the end of the study (14th day), rats were decapitated after intraperitoneal ketamine (75 mg/kg) xylazine (10 mg/kg) anesthesia, and the liver tissues were rapidly removed. The sections obtained from the livers were stained with hematoxylin-eosin (HE) and histological damage was evaluated.

Hematoxylin-eosin staining

First, the 5 µm sections taken from the paraffin blocks were spread out on slides. Standard histological methods were applied to the prepared slides. The paraffin was removed with xylol and passed through a graded alcohol series and diluted. The sections were stained with H&E to observe the general histological structure. The sections were examined after passing through an increasing alcohol series and xylene. H&E staining was purchased from Nanotek Lab (Kayseri, Turkey) (11). Then, images of the slides were taken under the Olympus BX51 light microscope (Tokyo, Japan) with a DP71

model digital camera.

The liver tissue structure was examined and randomly evaluated with standard light microscopy and it was scored by the study group. While applying histopathological score, the following criteria were used; hemorrhage, necrotic hepatocytes, vacuolized hepatocytes, and the appearance of hepatocyte cords. Scoring was conducted as follows: 0: not at all, 1: 0-25%, 2: 26–50%, 3: 51–75%, and 4: 76–100% (12).

Statistical analysis

All statistical analyses were carried out using SPSS statistical software (SPSS for Windows, SPSS Inc, Chicago, IL, version 21.0). The Kolmogorov–Smirnov and Shapiro-Wilk tests were used to identify normal distribution of the data. In case of normal distribution, quantitative variables were compared using One-way analysis of variance (ANOVA) and post hoc Tukey test. Descriptive statistics were shown as mean ± standard deviation (SD). Statistical significance was defined as $p<0.05$.

Results

Light microscopic examination

Light microscopic evaluation was made on preparations stained with H&E. The preparations were evaluated at ×200 and ×400 magnifications. The tissue sections in the Control and CAPE groups have normal histological appearance. Hemorrhage, sinusoidal dilatation and irregular sinusoids were observed in the tissue sections belonging to the CP group. In addition, eosinophilic necrotic hepatocytes and vacuolization were also observed in the CP group. Hemorrhage, sinusoidal dilatation, eosinophilic hepatocytes and vacuolization were reduced in the tissue sections belonging to the CP+CAPE group when compared with the CP group. H&E staining of the liver tissues in the experimental groups are shown in the Figure 2 and Figure 3. In addition, liver histopathological scoring was significantly higher in the CP group compared to the Control group ($p<0.001$). On the contrary, it was significantly lower in the CP+CAPE group when compared with the CP group ($p<0.001$). Liver histopathological scoring did not show a significant difference among the Control, CAPE and CP+CAPE groups. The data of liver histopathological scoring are shown in Table 1 and Figure 1.

Table 1: Initial and final body weights and histopathological score among experimental groups.

	Initial Weight (g)	Final Weight (g)	Histopathological score	p value
Control	242.87 ± 15.35	273.87 ± 14.78*	0.52 ± 0.65[a]	< 0.001
CP	219.75 ± 14.92	234.62 ± 18.32	2.27 ± 1.00[b]	< 0.001
CP + CAPE	209.83 ± 17.31	218.83 ± 15.21	0.96 ± 0.90[a]	< 0.001
CAPE	210.25 ± 10.15	234.62 ± 9.89*	0.78 ± 0.78[a]	< 0.001

Data are expressed as mean ± standard deviation and $p<0.05$ was considered as significant.
***** Significant when compared to initial body weight.
[a,b] There is a significant difference between groups with different letters.

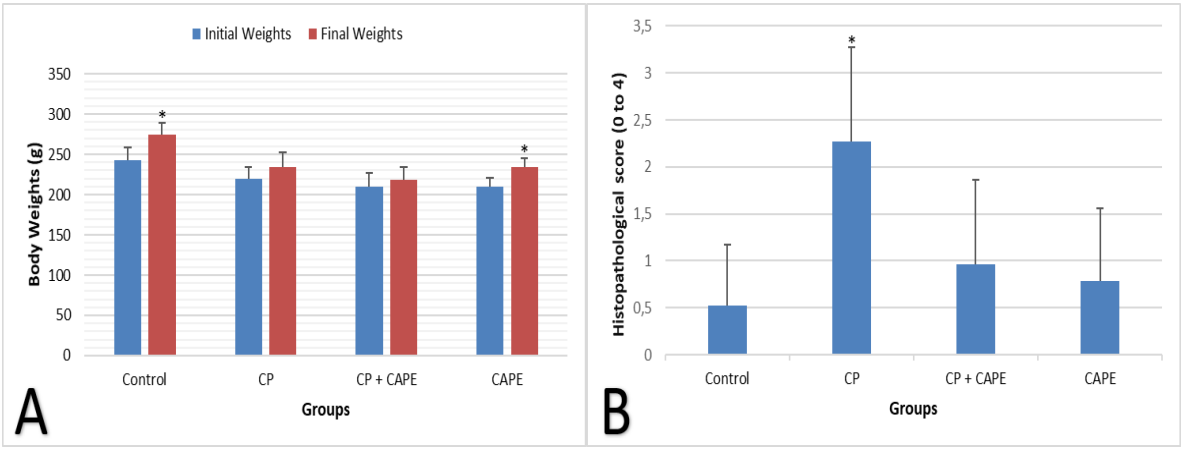

Figure 1: A: Changes in body weight of experimental animals before and after the experimental procedure. B: Liver histopathological scoring. Abbreviations: CP, Cisplatin; CAPE: Caffeic Acid Phenethyl Ester.

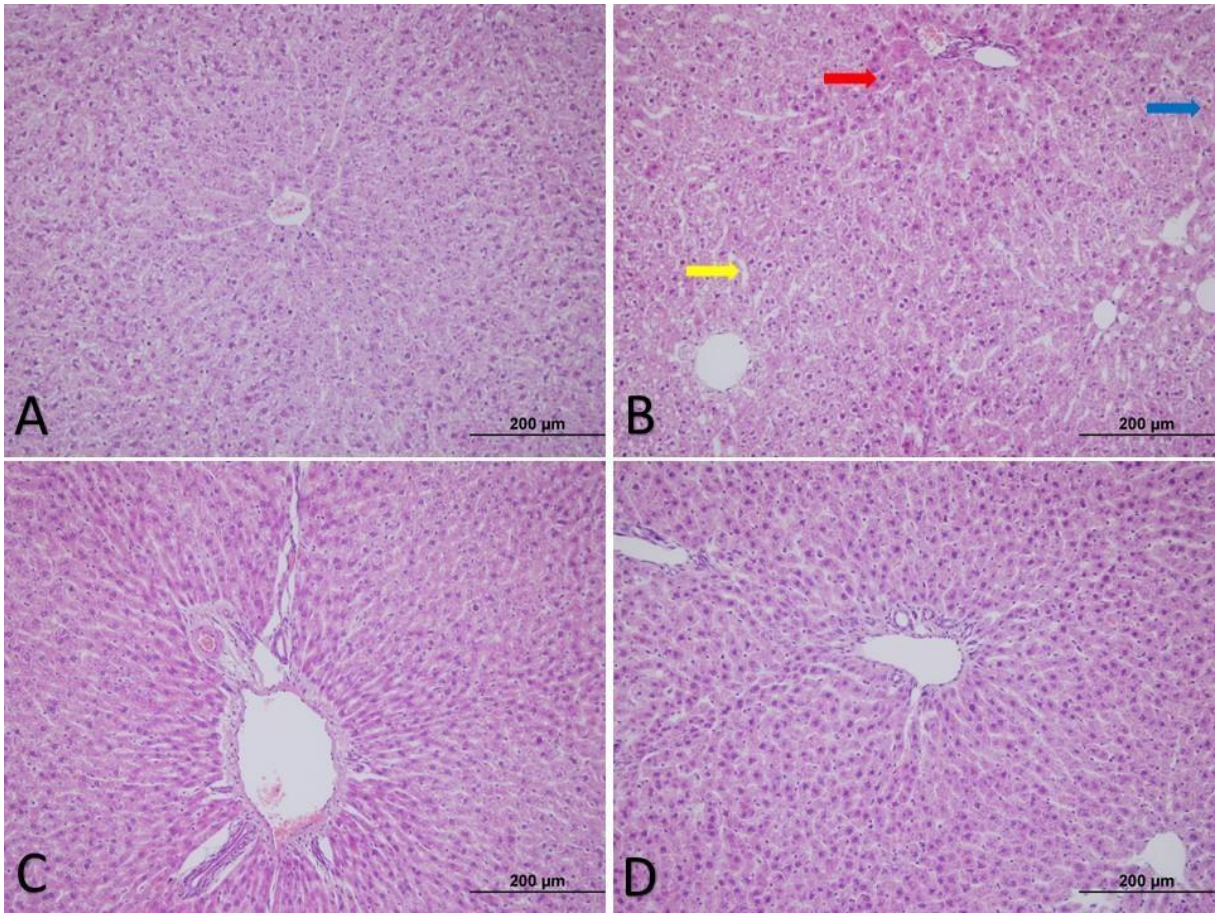

Figure 2: Control (A), CP (B), CP + CAPE (C), and CAPE (D) groups (HE staining, ×200). In the CP group, hemorrhage (blue arrow), eosinophilic cells (red arrow) and sinusoidal dilatation (yellow arrow) are shown. Abbreviations: CP, Cisplatin; CAPE: Caffeic Acid Phenethyl Ester.

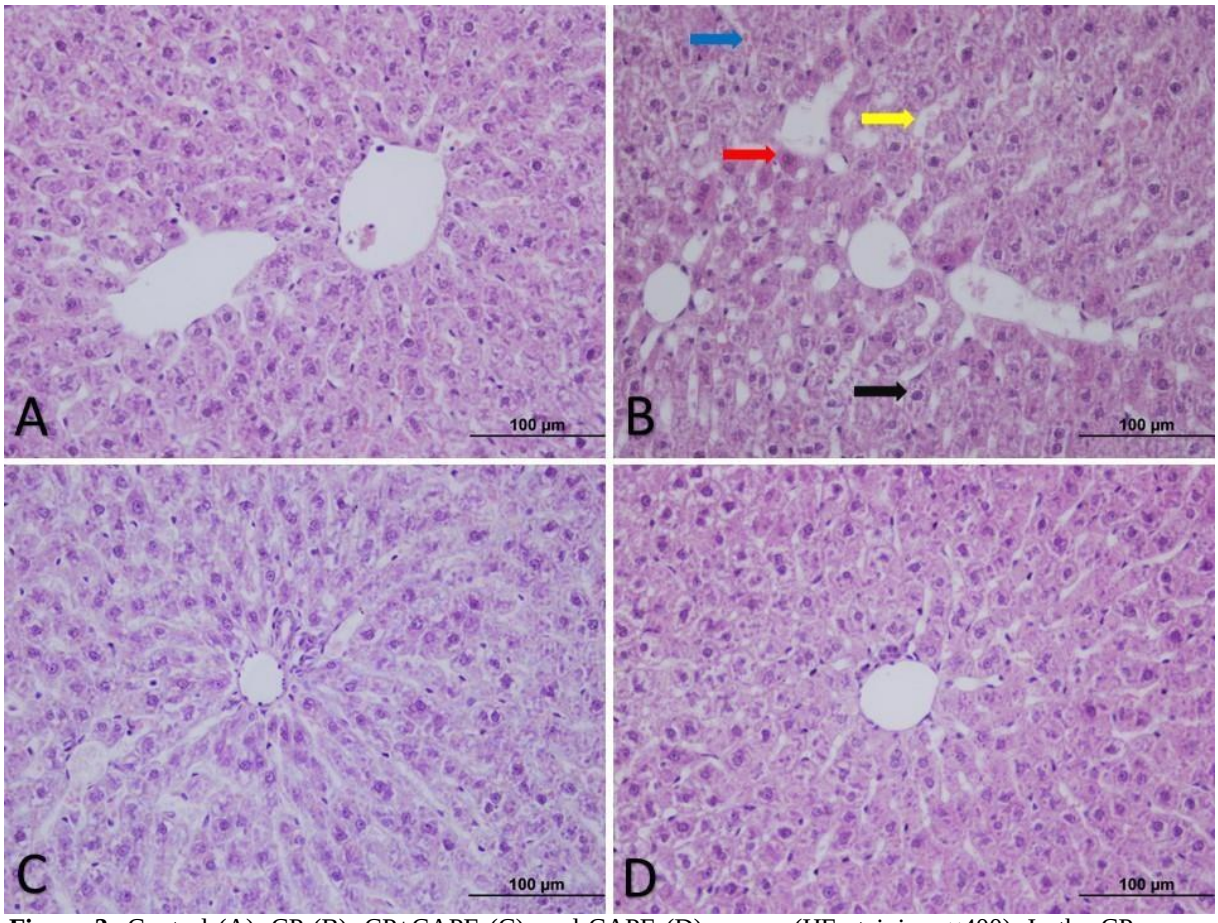

Figure 3: Control (A), CP (B), CP+CAPE (C), and CAPE (D) groups (HE staining, ×400). In the CP group, hemorrhage (blue arrow), eosinophilic cells (red arrow), sinusoidal dilatation (yellow arrow) and vacuolization (black arrow) are shown. Abbreviations: CP, Cisplatin; CAPE: Caffeic Acid Phenethyl Ester.

Evaluation of rat body weights

Body weights of the experimental animals were measured and recorded at the beginning and end of the experiment. Body weights of the animals in the Control and CAPE groups at the end of the experiment increased significantly compared to those on the first day of the experiment (p<0.001). Body weight of the animals both in the CP group and the CP+CAPE group at the end of the experiment increased when compared to those on the first day of the experiment. However, this increase was not statistically significant. The data of animal weights in the experimental groups are shown in Table 1 and Figure 1.

Discussion

Cancer is one of the most important health problems in the world and is the second leading cause of death in the United States. Cancer is defined as the rapid growth of normal cells in any part of the body out of control. These out-of-control cells can form a mass of tissue called a tumor. Depending on the type of cancer, patients are treated with methods such as radiotherapy, surgery, immunotherapy and chemotherapy (13). CP, cisplatinum or cis-diamminedichloroplatinum (II), is a well-known chemotherapeutic drug. It is used in the treatment of many types of cancer such as bladder, head and neck, lung, ovarian

and testicular cancers (3, 14). However, negative side effects of CP such as nephrotoxicity, ototoxicity, hepatotoxicity and cardiomyopathy limit its clinical use (3-5).

Bilgic et al. reported that the necrotic hepatocytes with pycnotic nuclei and eosinophilic hepatocytes in the liver tissue were observed in the single dose of CP (7 mg / kg) CP administered animals. He also stated that Kuppfer cells increased in the CP administered group. Overproduction of reactive oxygen species or disruption of antioxidant mechanisms is effective in the formation of this damage in cells. Because reactive oxygen species produced by normal metabolism are eliminated in the liver by some reactions involving enzymes such as superoxide dismutase, catalase, and GSH. Excessive reactive oxygen species cause inflammation and increase cell death by damaging DNA. (15). Boroja et al reported in their study that CP (single dose, 7.5 mg / kg) developed hepatotoxicity through hepatocyte degeneration, necrosis and lymphocyte infiltration in the liver (16). Similar to the studies in the literature, we applied a single dose of CP (7 mg / kg) to animals in our study. According to our histopathological examinations, CP caused severe histopathological effects in the liver tissues in the CP administered group. These effects were in the form of hemorrhage, sinusoidal dilatation and irregular sinusoids. Furthermore, eosinophilic stained necrotic hepatocytes and vacuolization were observed in addition to other histopathological effects. CP also affected the increase in weight of the animals fed under normal conditions. The fact that the animal weights in the CP group did not increase significantly during the experiment indicates that the drug also affected the development of the experimental animals. The negative side effects of CP, which is widely used in cancer patients, on the liver tissue and the disruption of the increase in animal weight limit its use. Elimination of the side effects caused by CP with various agents will increase the preference of using the drug in the treatment of the disease.

Interesting pharmacological activities from other phenolic components of propolis have been reported in recent studies (17). For this purpose, we investigated the effectiveness of CAPE, one of the active ingredients of propolis, whose effectiveness in many tissues and organs has been investigated. CAPE is one of the active ingredients of propolis and has been used as a folk remedy for many years. CAPE has been shown to inhibit the growth of different types of transformed cells (7). The mechanism of these biological effects of CAPE is not fully known (8, 9).

Ferreira et al. applied CAPE at doses of 1, 5, 10, 25 and 50 μmol against CP-induced neurotoxicity in cell culture models and reported that cell viability increased with a dose of 10 μmol (19). Yigit et al. compared the protective effects of low-dose doxycycline and CAPE, reported that CAPE was more protective than doxycycline at low doses. They attributed this effect to the anti-inflammatory, anti-oxidant and anti-apoptotic properties of CAPE (20). Salmas et al. reported that oxidative changes in kidney tissue of chronic hypertensive rats can be prevented by CAPE administration (21). In our previous study, we observed that CAPE (10 μmol / day) was significantly protective against testicular damage caused by CP (22). According to the literature, CAPE has been shown to have therapeutic effect on many tissues. In our current

study, we investigated the protective effects of CAPE, one of the active ingredients of propolis, against liver damage caused by CP. According to our results, hemorrhage, sinusoidal dilatation, eosinophilic hepatocytes and vacuolization were reduced in tissue sections belonging to the CP + CAPE group. Even in many tissue sections, these harmful effects have disappeared completely. In addition, sinusoids displayed a more regular appearance. Histopathological scoring of the liver was also significantly decreased in the CP + CAPE group compared to the CP group. This shows that there is a significant reduction in hemorrhage, sinusoidal dilatation, eosinophilic hepatocytes, vacuolization and irregular sinusoids in the tissue. Although CAPE had a corrective effect on the liver, it did not work as an effective agent in increasing animal weight. Long-term effects on metabolism should be investigated in the further studies.

As a result, it was determined that CP also caused serious histopathological damage in the liver. In this study, it has been shown that CAPE can be protective and corrective against the liver damage caused by CP. Considering the ease of obtaining CAPE and its effectiveness in the clinic, researching the underlying mechanisms will contribute to the literature and public health.

Conflict of interest statement

The authors declare that they have no conflict of interest.

Acknowledgments

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Review Article

PCOS Animal Models: An Approach Induced By Dehydroepiandrosterone

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Abstract

Polycystic ovary syndrome (PCOS) is one of the most common metabolic and endocrine diseases in women. Researchers generally use animal models in order to observe mechanisms of PCOS. One of the PCOS animal model is dehydroepiandrosterone (DHEA)-induced PCOS model. In the present review, DHEA-induced PCOS animal models are investigated according to species, age, number of groups, animal weight, DHEA amount applied, solvent, solvent amount, treatment days and injection way.

Key words: *Dehydroepiandrosterone, Polycystic Ovary Syndrome, Animal models*

1. Introduction

Polycystic ovary syndrome (PCOS) is one of the most common metabolic and endocrine diseases in women of reproductive age. While the metabolic symptoms of the disease are insulin resistance, obesity and increase in cardiovascular risk factors, endocrine symptoms are hyperandrogenaemia, oligomenorrhea, amenorrhea and hirsutism (1). Mechanisms regulating follicular development are disrupted due to the change in the balance of endocrine system in PCOS patients, morphological changes of ovaries are observed. High luteinizing hormone (LH) level disrupts the interaction between granulosa cells and oocytes, maturation of follicules and oocyte. It also causes antral follicles to remain small (2). International guidelines for the Assessment and Management of PCOS progressed PCOS identification during last three decades. In 1990, National Institutes of Health (NIH) constituted criteria for polycystic ovary syndrome as hyperandrogenism, oligo-ovulation and exclusion for other types of etiologies like Cushing's syndrome, hyperprolactinemia (3). In 2003, Rotterdam criteria are revised again to diagnose polycystic ovary syndrome. The Rotterdam criteria were expanded as oligo- or anovulation, clinical and/or biochemical signs of hyperandrogenism and polycystic ovaries. A clinical diagnosis involving at least 2 out of the 3 Rotterdam requirements is supported by the International Guidelines for the Evaluation and Treatment of PCOS (4). Prevalence of PCOS is changing according to populations based on using

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NIH or Rotterdam criteria. Available treatments for PCOS: inhibiting increased androgen secretion, improving menstrual dysfunction, preserving the endometrium, fertility to promote and alleviating metabolic disfunctions (5). Each of these is vital to the health and welfare of PCOS patients. Therefore, treatments mostly relieve symptoms of PCOS. More researches are needed to enlighten molecular and pathophysiological mechanisms of PCOS.

Researchers generally use animal models in order to observe mechanisms of PCOS. One of the PCOS animal model is dehydroepiandrosterone (DHEA)-induced PCOS model.

Dehydroepiandrosterone -Induced PCOS model

DHEA is one of the most common circulating steroid hormones and it acts as a precursor for testosterone. DHEA is primarily produced by the adrenal cortex and to the small degrees by the testes and ovaries (6).

PCOS rodent models are generated to help us understand mechanisms and focus on clinical therapies of PCOS. Their size, life span and physiological resemblance make them favoured organisms for PCOS research. There are agents applied on laboratory animals to produce PCOS. One of the androgen therapies in laboratory animals, DHEA have been commonly used to cause PCOS-like phenotypes. The pathophysiology of human PCOS is currently being investigated using with it. After DHEA treatment, female rats/mice form follicular cysts and become anovulatory like human PCOS features (7, 8). In addition to, DHEA influences the usual function of hypothalamus-pituitary-ovarian axis structure through Luteinizing hormone (9). While DHEA-induced PCOS model demonstrate limitations as PCOS is genetic based disease also, the use of the model enables potential biomarkers and therapies for women with PCOS to be researched and tested.

This review will focus on some characteristics of DHEA-induced PCOS models which have provided comprehensive knowledge of complex mechanisms underlying PCOS.

2. Materials and Methods

2.1. Species

This review searched data by using PubMed (https://www.ncbi.nlm.nih.gov/pubmed/) and Google Scholar (https://scholar.google.com/). The following words were looked for in the papers:' PCOS',' polycystic ovary syndrome',' animal model', 'Dehydroepiandrosterone' and 'DHEA'. This review excluded studies of animal models with other androgen or agents. All the animals mentioned in this study are female. According to Pubmed and Google Scholar, 90 publication were analysed in terms of species. According to data, all studies focused on rodent family. Results suggest that most used animal type is rats with 52 articles. 38 of the 52 studies were done with Sprague Dawley rats. 37 of the studies chose mice. One data were not available. In fact, it is shown that Sprague Dawley rats were most commonly preferred in PCOS models induced by DHEA. Strain of rats were Sprague Dawley and Wistar albino, strains of mice were ranked as C57BL/6J, BALB/C, Parkes, B6D2F1 and CD1 (Table 1 – 2, Figure 1).

Table 1: Strain of rats in DHEA-induced PCOS models

Strains of rats	Researchers
Sprague Dawley rats	Jong Hee Choi et al (10)
Wistar Albino rats	Takuya Misugi et al (11)

Table 2: Strain of mice in DHEA-induced PCOS models

Strains of mice	Researchers
C57BL/6J mice	Qiyang Shen et al (12)
BALB/c mice	Shabnam Bakhshalizadeh et al (13)
Parkes mice	Anusha Singh et al (14)
B6D2F1 mice	Fatemeh Eini et al (15)
CD1 mice	Shu-Yun Li et al (16)
	Giovanna Di Emidio et al (17)

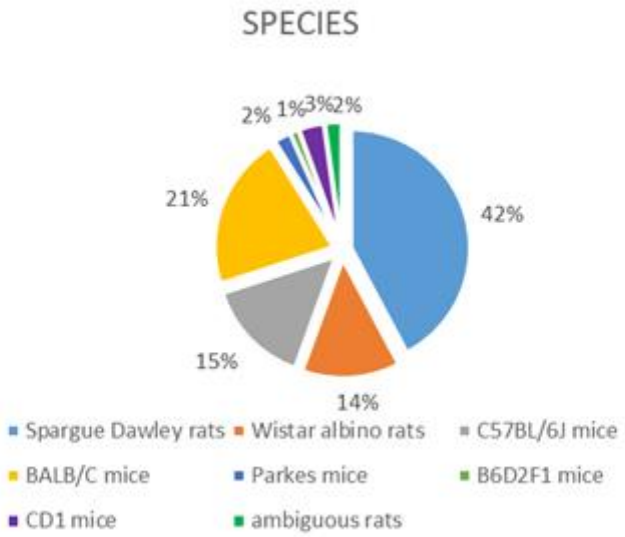

Figure 1: Percentile of species used in DHEA induced PCOS model.

2.2. Age

High DHEA levels during both prenatal and early postnatal life cause a wide variety of PCOS characteristics. In the studies subject to this review showed different age of rodents. From 20 days to 3 months, age of rodents varies. Researchers mostly chose 21 days old animals for research. In 40 of 90 articles, 21-day-old female mice and rats were used. The second most used age was 25 days with 21 publications. The remaining order of the data is 20,22,23,24,28,30,40,42 days and 3 months. Some of the research did not show any age (Table 3, Figure 2).

Table 3: Age range of the rodents used in DHEA induced PCOS model.

Age of rats/mice	Researchers
20-24 days old Sprague-Dawley rats	Lingying Wen et al (18)
21 days old Wistar albino rats	Selenay Furat Rencber et al (19)
28 days old CD1 mice	Giovanna Di Emidio et al (17)
23-30 days old BALB/c mice	María Emilia Solanoa et al (20)
40 days old BALB/c mice	Irene Tessaro et al (21)
42 days old Sprague Dawley rats	Jong Hee Choi et al (10)
3 months Sprague Dawley rats	Hengxia Zhao et al (22)

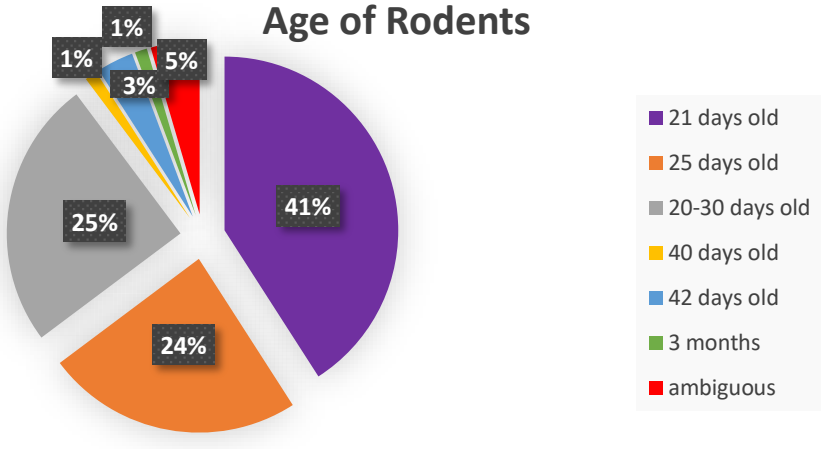

Figure 2: Percentile of rodents used in DHEA induced PCOS model in different age groups.

2.3. Number

According to searched articles, number of animals varies significantly. Range of number in each group is 6-109. Therefore, this study suggests that researchers have not used any specific number of rodents (Table 4).

Table 4: Numbers of animals used in DHEA induced PCOS model.

Numbers of groups	Researchers
6 C57BL/6 mice	Ya-Li Yang et al (23)
15 Sprague Dawley rats	Yanhua Shi et al (24)
56 and 109 Sprague Dawley rats	Eun-Jeong Kim et al (8)
48 BALB/c mice	Gordon Kyei et al (25)
30 Wistar albino rats	Wei Wang et al (26)
10 CD1 mice	Shu-Yun Li et al (16)

2.4. Animal Weight

According to 90 articles, 60 articles did not mention about any rodent weight. The initial weight in rats was at least 16 grams, while the highest weight was 200 grams. When looking at the mice, the lowest weight was 12 grams, and the highest weight was 20 grams (Table 5).

Table 5: Weight of rodents used in DHEA induced PCOS model.

Animal weight	Researchers
12-13gram mice	Gordon Kyei et al (25)
50-70gram rats	Wei Wang et al (26)
120-180gram rats	Ahmed Kabel et al (27)
50-105gram rats	Abeer M. Rababa'h et al (28)

2.5. DHEA amount

PCOS was induced by administration of dehydroepiandrosterone according to body weight of rats and mice. Developing a PCOS model in animals experimentally with DHEA application was attempted for the first time in the 1960s. Roy et al tried different doses of DHEA subcutaneously on female rats. These doses were 1,5 mg/kg, 3 mg/kg, 4 mg/kg, 6 mg/kg. 3 mg/kg and 6 mg/kg dose were similar after administration according to follicule stimulating hormone, luteinizing hormone, and prolactin levels (29). In 1978, Ward et all found out that 6 mg/100 g body weight of DHEA application demonstrated significantly improved levels of FSH, elevated levels of prolactin and depressed serum LH (30). Anovulation, polycystic ovaries and hyperandrogenism, features of PCOS, have been observed after administration of 6 mg/100 g (60 mg/kg) body weight (BW) DHEA. (31-33). Therefore 78 out of 90 articles chose 6mg/100 g body weight DHEA to apply rodents. 6 out of 90 articles used 6 mg/kg DHEA on rodents. Two articles used 2,2 mg/day and 7.5 mg/day in DHEA subcutaneous implantation. 30 mg/kg body weight DHEA was used in one article. Rest of the data were not available (Figure 3).

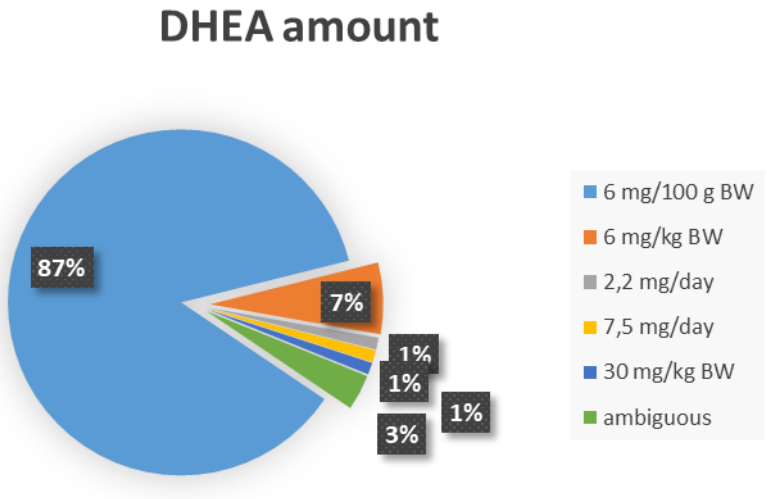

Figure 3: Percentile of dose ranges used in DHEA induced PCOS model.

2.6. Solvent

In experimental groups, PCOS was induced by administration of dehydroepiandrosterone dissolved in different solvents according to body weight of rats and mice. Researchers chose sesame oil, olive oil, peanut oil, corn oil, neutral oil, tea oil, soybean oil, castor oil and phosphate buffer solution. 63 out of 90 articles chose sesame oil as a vehicle. 4 articles have soybean oil, 4 articles used corn oil, 2 articles used olive oil. In some articles, vehicle data is not available (Table 6).

2.7. Solvent Amount

Researchers administered DHEA in different amount of solvents into the study group of rats/mice via different ways of injections. In 34 of the 90 studies, 0.2 ml different types of oil were chosen as solvent. 0.1 ml oil was applied in 22 studies. In 17 studies, oil was diluted with ethanol. Ethanol may have been used to reduce oil viscosity. In these studies, 0.01 ml 95% ethanol was mixed in oil. 3 studies used 0.05 ml oil. Rest of the data were not available (Table 7, Figure 4).

Table 6: Types of solvents applied in DHEA induced PCOS model.

Solvent	Researchers
Sesame oil	Eun-Jeong Kim et al (8)
Olive oil	Gengxiang Wu et al (34)
Peanut oil	Junyu Zhai et al (35)
Corn oil	Olugbemi T. Olaniyan et al (36)
Neutral oil	María Emilia Solanoa et al (20)
Tea oil	Lingying Wen et al (18)
Soybean oil	Lingjun Sun et al (37)
Castor oil	Dan-ni Zhou et al (38)
Phosphate buffer solution	Yan Peng et al (39)

Table 7: Solvent amount applied with DHEA in DHEA induced PCOS model.

Solvent Amount	Researchers
0.01 mL 95% ethanol and corn oil	Vaibhave Ubba et al (40)
0.01 ml 95% ethanol and 0.09 ml sesame oil	Aylin Yaba et al (41)
0.05 ml sesame oil	Ying Huang et al (42)
0.1 ml sesame oil	Lei Dou et al (43)
0.2 ml sesame oil	Yurdun Kuyucu et al (44)

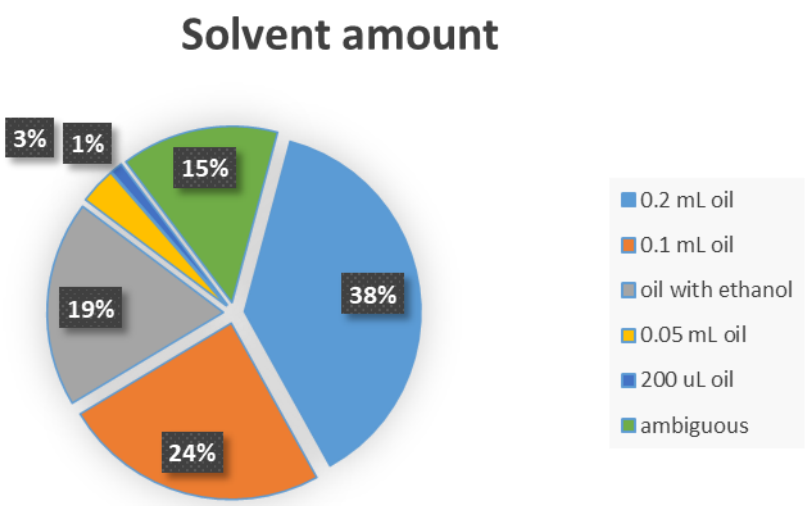

Figure 4: Solvent amounts used in PCOS model to administer DHEA.

2.8. Treatment Days

Differences in the timing of DHEA overload exposure contribute to improvements in the expression of PCOS characteristics found in models of this study. DHEA administration triggers cystic changes in the ovaries of female rats/mice in combination with acyclic ovulation and anovulation (7, 8). In 1991, Everett Anderson et al tried different exposure time of DHEA (0, 10,15,20,25, and 30 days) and found out that follicles after 10 days of DHEA treatment were undergoing atresia and the actual number of follicular cysts occurring in a single ovary is in 20-30 days (45). Therefore 52 out of 90 publications chose 20 days of DHEA treatment as developmental stage of follicular cysts is likely to be a key determinant. 21 out of 92 chose 20 – 30 days, most of them were 21 days treatment. 6 publications chose 15 days, and 4 publications were 35 days. Two studies used DHEA subcutaneous implantation for 60 and 90 days. In one article, researchers applied 12 weeks of DHEA treatment. Rest of the data was 7 days and ambiguous (Table 8, Figure 5).

Table 8: Treatment durations conducted with DHEA induced PCOS model.

Treatment days	Researchers
7 days and 15 days of DHEA injection	Hiroyuki Honnma et al (46)
20 days of DHEA injection	Gordon Kyei et al (25)
21 days of DHEA injection	Ya-Li Yang et al (23)
28 days of DHEA injection	Yurdun kuyucu et al (44)
35 days of DHEA injection	Daojuan Wang et al (32)
12 weeks of DHEA injection	Kok-Min Seow et al (47)

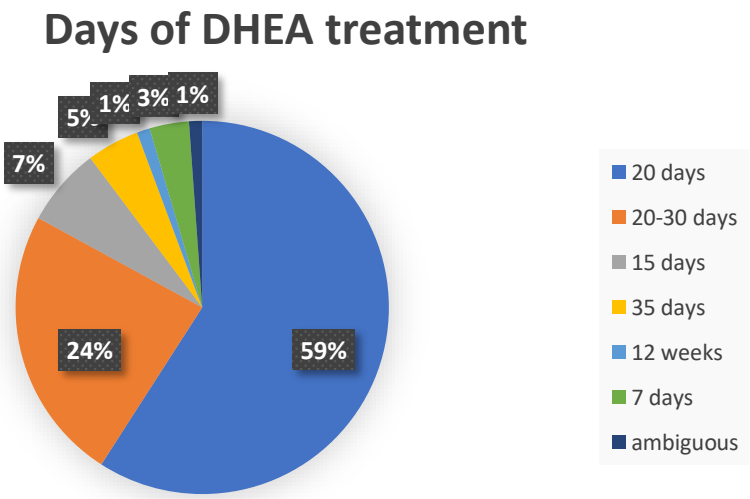

Figure 5: Percentile of different treatment durations conducted with DHEA induced PCOS model.

2.9. Injection Way

Researchers administered DHEA into the study groups of rats/mice via different ways of injections. 83 out of 90 articles used subcutaneous way to produce PCOS. DHEA was injected by unknown way in 3 articles (48-50). While hypodermic injection was used in 2 articles (51, 52), intramuscular injection was used in one article (53). Animals were fed with DHEA in one article (54).

3. Conclusion

Overall, our understanding of PCOS pathogenesis and the development of new PCOS therapeutics is supported by DHEA-induced PCOS models. In the present study DHEA induced PCOS animal models are investigated according to species, age, number of groups, animal weight, DHEA amount applied, solvent, solvent amount, treatment days and injection way. This study might therefore contribute to PCOS research and its development.

4. Conflict of interests

The author declares no conflict of interests.

5. Acknowledgement

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