ATATURK
UNIVERSITY
PUBLICATIONS

Journal of Laboratory Animal Science and Practices

Laboratuvar Hayvanları Bilimi ve Uygulamaları Dergisi

Official Journal of Atatürk University-Medical Experimental Application and Research Center

Volume 5 • Issue 2 • September 2025

EISSN 2791-8645
https://dergipark.org.tr/tr/pub/jlasp

Journal of Laboratory Animal Science and Practices

Journal of Laboratory Animal Science and Practices

AIMS AND SCOPE

Journal of Laboratory Animal Science and Practices is a scientific, open access, online-only periodical published in accordance with independent, unbiased, and double-blinded peer-review principles. The journal is an official publication of the Atatürk University, Medical Experimental Application and Research Center, and published biannually in March and September. The publication languages of the journal are Turkish and English.

Journal of Laboratory Animal Science and Practices aims to contribute to the literature by publishing manuscripts at the highest scientific level. The journal publishes research articles, reviews and case reports that are prepared in accordance with ethical guidelines.

The scope of the journal includes but not limited to relevant to the topics of laboratory animals in the fields of medical medicine, veterinary medicine, dentistry, aquaculture, science, agriculture, pharmacy and similar fields in which laboratory animals are used, and all other related interdisciplinary theoretical research.

The target audience of the journal includes researchers and specialists who are interested or working in all fields in the journal's scope.

The editorial and publication processes of the journal are shaped in accordance with the guidelines of the International Committee of Medical Journal Editors (ICMJE), World Association of Medical Editors (WAME), Council of Science Editors (CSE), Committee on Publication Ethics (COPE), European Association of Science Editors (EASE), and National Information Standards Organization (NISO). The journal is in conformity with the Principles of Transparency and Best Practice in Scholarly Publishing (doaj.org/bestpractice).

Journal of Laboratory Animal Science and Practices currently indexed in ***DOAJ, EBSCO, CABI, GALE CENGAGE, TÜBİTAK ULAKBİM TR Dizin***.

All expenses of the journal are covered by the Atatürk University. 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/en/pub/jlasp. The journal guidelines, technical information, and the required forms are available on the journal's web page.

Disclaimer

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

Open Access Statement

You can find the current version of the Instructions to Authors at https://dergipark.org.tr/en/pub/jlasp/writing-rules .

Contact (Editor in Chief) / İletişim (Baş Editör)

Hakan AYDIN

Atatürk University, Faculty of Veterinary Medicine, Department of Virology, Erzurum, Türkiye

Atatürk Üniversitesi, Veteriner Fakültesi, Viroloji Anabilim Dalı, Erzurum, Türkiye

✉ hakanayd@atauni.edu.tr

✉ hakanayd@atauni.edu.tr

https://dergipark.org.tr/en/pub/jlasp

☎ +90 442 231 70 27

Contact (Publisher) / İletişim (Yayıncı)

Atatürk University

Atatürk University, Erzurum, Türkiye

Atatürk Üniversitesi Rektörlüğü 25240 Erzurum, Türkiye

✉ ataunijournals@atauni.edu.tr

https://bilimseldergiler.atauni.edu.tr

☎ +90 442 231 15 16

Journal of Laboratory Animal Science and Practices

AMAÇ VE KAPSAM

Laboratuvar Hayvanları Bilimi ve Uygulamaları Dergisi; bağımsız, tarafsız ve çift-kör hakem değerlendirme ilkelerine bağlı yayın yapan, Atatürk Üniversitesi, Tıbbi Deneysel Uygulama ve Araştırma Merkezinin açık erişimli bilimsel elektronik yayın organıdır. Dergi, Mart ve Eylül aylarında olmak üzere yılda iki sayı olarak yayınlanmaktadır. Yayın dili Türkçe ve İngilizce'dir.
Laboratuvar Hayvanları Bilimi ve Uygulamaları Dergisi, yüksek bilimsel kaliteye sahip özgün araştırma, derleme ve olgu sunumu türündeki makalelerle literatüre katkı sunmayı amaçlamaktadır.
Dergi kapsamına; laboratuvar hayvanlarının kullanıldığı tıp hekimliği, veteriner hekimliği, diş hekimliği, su ürünleri, fen bilimleri, ziraat, eczacılık ve benzeri alanlarındaki ulusal ve uluslararası çalışmalar gibi konular girmektedir.
Derginin hedef kitlesini derginin kapsamındaki alanlarda araştırma yapan bilim insanları, amatör ve profesyonel olarak çalışanlar ve bu alana ilgi duyan tüm kişiler oluşturmaktadır.
Derginin editöryel ve yayın süreçleri, International Committee of Medical Journal Editors (ICMJE), World Association of Medical Editors (WAME), Council of Science Editors (CSE), Committee on Publication Ethics (COPE), European Association of Science Editors (EASE) ve National Information Standards Organization (NISO) kılavuzlarına uygun olarak biçimlendirilmiştir. Laboratuvar Hayvanları Bilimi ve Uygulamaları Dergisi'nin editöryel ve yayın süreçleri, Akademik Yayıncılıkta Şeffaflık ve En İyi Uygulama (doaj.org/bestpractice) ilkelerine uygun olarak yürütülmektedir.
Laboratuvar Hayvanları Bilimi ve Uygulamaları Dergisi; ***DOAJ, EBSCO, CABI, GALE CENGAGE, TÜBİTAK ULAKBİM TR Dizin*** tarafından dizinlenmektedir.
Derginin tüm masrafları Atatürk Üniversitesi tarafından karşılanmaktadır. Makale değerlendirme ve yayın işlemleri için yazarlardan ücret talep edilmemektedir. Tüm makaleler https://dergipark.org.tr/tr/pub/jlasp sayfasındaki online makale değerlendirme sistemi kullanılarak dergiye gönderilmelidir. Derginin yazım kurallarına, gerekli formlara ve dergiyle ilgili diğer bilgilere web sayfasından erişilebilir.

Sorumluluk Reddi

Dergide yayınlanan makalelerde ifade edilen bilgi, fikir ve görüşler Laboratuvar Hayvanları Bilimi ve Uygulamaları Dergisi, Baş Editör, Editörler, Yayın Kurulu ve Yayıncı'nın değil, yazar(lar)ın bilgi ve görüşlerini yansıtır. Baş Editör, Editörler, Yayın Kurulu ve Yayıncı, bu gibi yazarlara ait bilgi ve görüşler için hiçbir sorumluluk ya da yükümlülük kabul etmemektedir.

Açık Erişim Beyanı

Laboratuvar Hayvanları Bilimi ve Uygulamaları Dergisi yayınlanma modeli, Budapeşte Açık Erişim Girişimi (BOAI) bildirgesine dayanan açık erişimli bilimsel bir dergidir. Derginin arşivine https://dergipark.org.tr/tr/pub/jlasp adresinden ücretsiz olarak erişilebilir. Yazarlar Laboratuvar Hayvanları Bilimi ve Uygulamaları Dergisi'nde yayınlanmış çalışmalarının telif hakkını elinde tutar. Laboratuvar Hayvanları Bilimi ve Uygulamaları Dergisi'nin içeriği, Creative Commons Alıntı-GayriTicari (CC-BY-NC) 4.0 Uluslararası Lisansı ile yayınlanmaktadır. Söz konusu telif, üçüncü tarafların içeriği uygun şekilde atıf vermek koşuluyla, ticari olmayan amaçlarla paylaşımına ve uyarlamasına izin vermektedir.
2023 Ocak'tan itibaren dergi Creative Commons CC BY-NC 4.0 lisansıyla yayın yapmaya başlamıştır. Bu tarihten önceki dergi içeriği ücretsiz erişime açık olmakla birlikte geleneksel telif sistemiyle yayınlanmıştır.
Yazarlara Bilgi'nin güncel versiyonuna https://dergipark.org.tr/tr/pub/jlasp/writing-rules adresinden ulaşabilirsiniz.

Contact (Editor in Chief) / İletişim (Baş Editör)
Hakan AYDIN
Atatürk University, Faculty of Veterinary Medicine, Department of Virology, Erzurum, Türkiye
Atatürk Üniversitesi, Veteriner Fakültesi, Viroloji Anabilim Dalı, Erzurum, Türkiye
✉ hakanayd@atauni.edu.tr
✉ hakanayd@atauni.edu.tr
https://dergipark.org.tr/en/pub/jlasp
☎ +90 442 231 70 27

Contact (Publisher) / İletişim (Yayıncı)
Atatürk University
Atatürk University, Erzurum, Türkiye
Atatürk Üniversitesi Rektörlüğü 25240 Erzurum, Türkiye
✉ ataunijournals@atauni.edu.tr
https://bilimseldergiler.atauni.edu.tr
☎ +90 442 231 15 16

Journal of Laboratory Animal Science and Practices

İÇİNDEKİLER / CONTENTS

Araştırma Makaleleri / Research Articles

ATATÜRK ÜNİVERSİTESİ YAYINLARI
ATATURK UNIVERSITY PUBLICATIONS

Araştırma Makalesi *Research Article* DOI: 10.62425/jlasp.1539922

Ratlarda 3-Asetilpyridin ile Oluşturulan Ataksi Modelinde Aposinin'in İskemi Modifiye Albümin (İMA) Düzeyleri Üzerindeki Etkisi

The Effect of Apocynin on Ischemia Modified Albumin (IMA) Levels in the Ataxia Model Induced with 3-Acetylpyridine in Rats

Ömercan ALAT[1]
Elif DALKILINÇ[1]

[1]Atatürk Üniversitesi, Veteriner Fakültesi, Biyokimya Anabilim Dalı, Erzurum, Türkiye.

ÖZ

Serebellar ataksi koordinasyon bozukluğuna sebep olan nörodejenereatif bir hastalıktır. Bunun gibi nörodejeneratif hastalıklarda *apocynin* (APO) gibi bileşiklerin tedavi edici rolü bulunmaktadır. Yapılan çalışmanın amacı, *3-asetilpyridin* (3-AP) ile oluşturulan serebellar ataksi modelinde APO tedavisinin iskemi modifiye albümin (İMA) seviyesi üzerindeki etkisini incelemektir. Çalışmada, 200–250 gr ağırlığında *Sprague-Dawley* cinsi, 5-6 haftalık erkek ratlar kullanıldı. Ratlar, Kontrol grubu, 3-AP grubu, 3-AP + APO grubu ve APO grubu olmak üzere dört gruba ayrıldı. 3-AP uygulaması ile ratlarda serebellar ataksi modeli oluşturuldu ve tedavi için APO uygulandı. Deney sonunda anestezi altında dekapitasyon gerçekleştirilerek alınan beyin dokularında İMA seviyeleri ölçüldü. Tüm gruplara göre 3-AP uygulanan ratlarda İMA düzeylerinde istatistiksel olarak anlamlı artış saptanırken, APO uygulanan ratlarda İMA seviyelerinde istatistiksel olarak anlamlı azalış tespit edildi (p< ,001). Son yıllarda biomarker olarak İMA düzeylerinin kullanılabilirliği yönünde birçok çalışma olsa da yapılan literatür taramalarında APO'nun 3-AP uygulaması sonucu serebellar ataksi gibi nörodejeneratif hastalıklarda terapötik bir bileşik olarak kullanılabileceğine yönelik beyin İMA düzeylerine bakılan herhangi bir literature rastlanılmadığı için yapılan çalışma literatüre öncü çalışma olarak sunulmuştur. Elde edilen bulgular yapılacak çalışmalar için ışık tutar niteliktedir.

Anahtar Kelimeler: Antioksidan, Aposinin, Ataksi, Beyin, İMA.

ABSTRACT

Cerebellar ataxia is a neurodegenerative disease that causes coordination disorder. In such neurodegenerative diseases, compounds such as *apocynin* (APO) have a therapeutic role. The aim of this study is to examine the effect of APO treatment on ischemia modified albumin (IMA) level in the cerebellar ataxia model induced by *3-acetylpyridine* (3-AP). In the study, 5–6-week-old male *Sprague-Dawley* rats weighing 200-250 grams were used. Rats were divided into four groups: Control group (K), 3- 3-AP group, 3-AP + APO, and APO group. A cerebellar ataxia model was induced in rats using 3-AP administration, and APO was administered as a treatment. At the end of the experiment, brain tissues were collected via decapitation under anesthesia, and IMA levels were measured. In rats administered with 3-AP, there was a statistically significant increase in IMA levels compared to all groups, whereas rats treated with APO showed a statistically significant decrease in IMA levels (p< .001). Although there have been many studies in recent years investigating the usability of IMA levels as biomarkers, a literature search did not reveal any studies examining brain IMA levels to assess the therapeutic potential of APO in neurodegenerative diseases such as cerebellar ataxia induced by 3-AP. Therefore, this study is presented as a pioneering contribution to literature. The findings obtained shed light on future research.

Keywords: Antioxidant, Apocynin, Ataxia, Brain, IMA.

Geliş Tarihi/Received :28.08.2024
Kabul Tarihi/Accepted :26.05.2025
Yayın Tarihi/Publication Date :19.09.2025

Sorumlu Yazar/Corresponding author: Ömercan Alat
E-mail: omeralat9@gmail.com

Cite this article: Alat, Ö., & Dalkılınç, E. (2025). The Effect of Apocynin on Ischemia Modified Albumin (IMA) Levels in the Ataxia Model Induced with 3-Acetylpyridine in Rats. *Journal of Laboratory Animal Science and Practices*, *5*(2), 67-72. https://doi.org/10.62425/jlasp.1539922

Giriş

Ataksi, merkezi sinir sistemiyle bağlantılı bir nörolojik belirti olup, kas kontrolünün kaybı veya istemli hareketlerin koordinasyon eksikliği ile karakterize edilir. Özellikle serebellar ataksi, beyinciğin fonksiyonlarını olumsuz yönde etkileyen bir ataksi türüdür ve etiyolojisinin karmaşıklığı nedeniyle anlaşılması güç bir bozukluk olarak bilinir (Lastres-Becker vd., 2008; Manto ve Marmolino, 2009; Rosenthal, 2022). Ataksi, etiyolojinin baskın lokalizasyonuna göre üç kategoride sınıflandırılabilir (Claassen, 2022). Küçük veya büyük sinir liflerinin nöropatisine bağlı duyusal ataksi; (O'Malley, 2022) yarım daire kanalları ve otolit organlardaki değişiklikler veya disfonksiyonlara bağlı vestibüler ataksi; ve (van der Heijden ve Sillitoe, 2021) beyincikteki değişiklikler veya işlev bozukluklarından kaynaklanan serebellar ataksi. Buna ek olarak, ataksiler genetik, mitokondriyal, otoimmün, toksik, metabolik ve sporadik gibi farklı etiyolojik sınıflamalara da ayrılabilir (Jaques, 2022). Genetik manipülasyon veya belirli kimyasallar kullanılarak insan serebellar ataksi nöropatolojisini modellemek amacıyla çeşitli hayvan modelleri geliştirilmiştir (Aghighi vd., 2022). Örneğin, nikotinamid antimetaboliti olan nörotoksin *3-asetilpyridin* (3-AP), inferior olivary çekirdekte kalbindin ifade eden nöronları hedef alır ve hasara uğratır. Bu, serebellar nöronların fonksiyon bozukluğuna ve dejenerasyonuna yol açarak ataksi oluşumuna neden olur (Wecker vd., 2017).

Albümin birçok fizyolojik süreçte rol oynayan önemli bir proteindir ve plazmadaki toplam proteinlerin yaklaşık yarısını oluşturmaktadır (Arroyo vd., 2014). Karaciğerde üretilen albümin, kanda steroid hormonlar, yağ asitleri ve toksik maddeler dahil olmak üzere çeşitli bileşiklerin taşınmasında önemli bir rol oynar (Sbarouni vd., 2011; Kennelly vd., 2023). Albümin molekülü, içerisinde bulunan bir amino ucu aracılığıyla kobalt, nikel, bakır gibi metal iyonları ile etkileşime girer. Reaktif oksijen türleri (ROT), oksidatif stres ve asidoz gibi durumlar albüminin bu amino ucu üzerinde değişikliklere yol açarak, albüminin metal iyonlarını bağlama yeteneğini azaltır. Bu değişime uğramış albümin formu, iskemi modifiye albümin (İMA) olarak adlandırılır (Sbarouni vd., 2011; Shevtsova vd., 2021). İMA'nın miyokart iskemisi, akut böbrek hasarı, beyin iskemisi, karaciğer fibrozisi gibi çeşitli hastalıkların tanısında biyobelirteç olarak kullanılabilirliği gösterilmiştir (Tahtacı vd., 2019; Demirci vd., 2021; Tarihi vd., 2022). Ayrıca, İMA'nın periferik vertigo, akut koroner sendrom ve akut aort diseksiyonu gibi farklı klinik durumlarda da değerli bir biyobelirteç olarak kullanılabileceği gösterilmiştir (Abo Saleh vd., 2023; Xiang vd., 2023; Karakılıç vd., 2023). Bu bulgular doğrultusunda, İMA düzeylerindeki artışın doku hipoksisi ya da reperfüzyon hasarını erken dönemde yansıtan hassas bir gösterge olduğu düşünülmektedir.

Aposinin, ilk kez 1883 yılında Schmiedeberg tarafından tanıtılan doğal bir organik bileşiktir (Stefanska ve Pawliczak, 2008). Aposinin, hayvan çalışmalarında çok iyi bir güvenlik profiline sahip olup, uzun süreli tedavi kullanımı sırasında herhangi bir sağlık sorunu belirtisi göstermediği birçok çalışma tarafından desteklenmiştir(Yu vd., 2008; Simonyi, 2012).

Yeni araştırmalar, APO'nun farmakokinetik ve farmakodinamik özelliklerini tanımlamış ve bu bileşiğin büyük oranlarda ROT üretimini inhibe ettiğini göstermiştir. Ayrıca, kan-beyin bariyerini kolaylıkla aşabilen APO'nun, beyindeki oksidatif stresin azaltılmasında metabolik bozukluklar ve yaşlanma ile ilişkili potansiyel faydaları olduğu belirtilmiştir (Liu vd., 2020). APO'nun daha önce sitotoksisite, oksidatif stres ve nörodejenerasyonu başarılı bir şekilde önlemede kullanıldığı bilgisi, 3-AP ile oluşturduğumuz rat ataksi modelinde antioksidan olarak APO seçimimizde önemli bir rol oynamıştır.

Bu çalışma, 3-AP ve aposininin (APO) İMA düzeyi üzerine olan etkilerini araştırmak amacıyla yapılmıştır.

Yöntemler

Deney Hayvanları

Araştırmada ağırlıkları 200-250 gr arasında değişen 28 adet erkek *Sprague-Dawley* cinsi rat kullanıldı. Ratlar standart laboratuvar koşullarında (24 ± 2 °C, %55 ± 5 nem, 12 saat aydınlık/karanlık döngüsü) barındırıldı. Ratlar, çalışma süresince standart yem ve su ile ad libitum olarak beslendi. Çalışma başlatılmadan önce, ratların adaptasyon sağlamaları için bir hafta bekletildi. Bu çalışmamızın etik kurul onayı 23.07.2024 tarihli 168 sayılı karar ile Atatürk Üniversitesi Hayvan Deneyleri Yerel Etik Kurulu Başkanlığı tarafından onaylanmıştır.

Deneysel Tasarım

Ratlar her bir grupta 7 adet rat bulunacak şekilde 4 gruba ayrıldı.

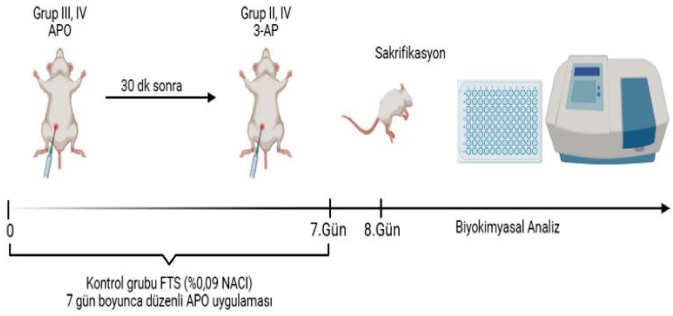
Grup I (Kontrol): Ratlara 7 gün boyunca intraperitoneal (ip) olarak 0,5 mL fizyolojik tuzlu su (%0,09 NaCl) uygulandı.

Grup II (3-AP): Ratlara, çalışmanın ilk gününde tek doz 3-AP 75 mg/kg (Cat: A21207 Sigma-Aldrich, St. Louis, MO, USA) 0,5 mL ip olarak uygulandı (Ghorbani, 2024).

Grup III (APO): Ratlara 7 gün boyunca APO 0,5 mL 20 mg/kg (Sigma-Aldrich, St. Louis, MO, USA) ip olarak uygulandı (Yücel, 2019).

Grup IV (3-AP+APO): Ratlara 3-AP 75 mg/kg ip uygulama yapılmadan 30 dk önce ve 7 gün boyunca 0,5 mL 20 mg/kg APO ip olarak uygulandı.

Deney süresince yapılan uygulamalar Şekil 1'de sunuldu.



Şekil 1. Deney sürecinde yapılan uygulamalar.

Figure 1. Applications made during the trial process.

Numunelerin Alınması

Son uygulamadan 24 saat sonra, ratlar Xylazine (8 mg/kg) ve Ketamin (60 mg/kg) anestezisi altında dekapite edildi ve beyin dokusu alındı. Biyokimyasal analiz yapılana kadar -80°C'de saklandı.

Beyin Dokusundan Homojenat Elde Edilmesi

-80°C'de saklanan beyin dokuları sıvı nitrojen kullanılarak toz haline getirildi ve 0,5 g tartıldı. Tartılan beyin dokuları, 1:10 (ağırlık/hacim) homojenat elde etmek için 0,1 mL fosfat tamponu (pH 7,4) ile QIAGEN TissueLyser LT (Qiagen, Hilden, Almanya) cihazı kullanılarak homojenize edildi ve 4°C'de 20 dakika boyunca 11.000 rpm'de santrifüj edildi. Elde edilen süpernatant kullanılarak oksidatif stres parametresi olan İMA düzeyine Bar-Or ve arkadaşlarının geliştirdiği yönteme göre bakılarak Biotek ELISA Reader (Bio-Tek µQuant MQX200 Elisa reader/USA) ile absorbansları ölçülerek İMA birimi ABSU olarak belirtildi (Bar–Or vd., 2000).

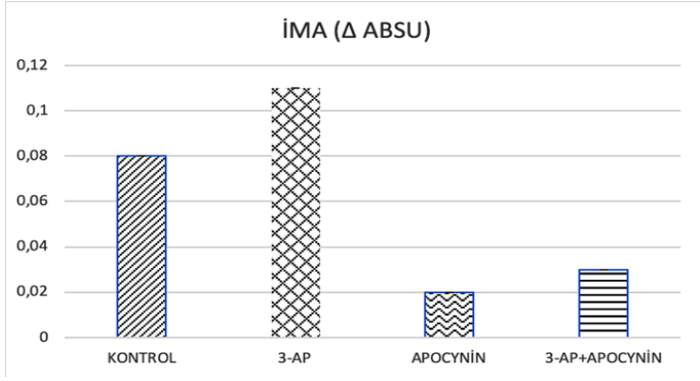
İstatistiksel Analiz

Çalışmadan elde edilen tüm veriler SPSS 20.0 paket program kullanılarak istatistiksel olarak analiz edildi. Verilerin normal dağılım gösterdiği belirlendikten sonra gruplar arası farklılıkların tespiti için tek yönlü ANOVA ve Tukey testi kullanıldı. Tüm değerler ortalama ± standart hata olarak ifade edildi ve $p< ,05$ anlamlı kabul edildi.

Bulgular

Biyokimyasal Bulgular

3-Asetilpyridin'in beyin dokusunda oluşturduğu oksidatif hasara karşı APO uygulamasının potansiyel etkileri oksidan belirteç olan İMA analizi ile değerlendirilerek bulgular Tablo 1 ve Şekil 2'de sunulmuştur.



Şekil 2. Beyin dokusu İMA (İskemi Modifiye Albumin) düzeyinin gruplara göre dağılımı. 3-AP: *3-Asetilpyridin.*

Figure 2. Distribution of IMA (Ischemia Modified Albumin) levels in brain tissue according to groups. 3-AP: *3-Acetylpyridine*.

Albüminin metal iyonlarını bağlama yeteneğini gösteren İMA seviyesi beyin dokusunda değerlendirildiğinde 3-AP uygulanan gruptaki İMA düzeyi diğer gruplara göre istatistiksel olarak yüksek bulundu ($p< ,001$). İMA düzeyi en düşük APO grubuna saptandı. 3-AP ile birlikte APO uygulanan gruptaki İMA düzeyi kontrol ve 3-AP grubuna göre istatistiksel olarak anlamlı bir düşüş gösterdi ($p< ,001$).

Tablo 1. Tüm grupların beyin dokusunda ölçülen İMA düzeyi.

Table 1. IMA levels measured in the brain tissue of all groups.

GRUPLAR	İMA (Δ ABSU)
KONTROL	0,08± 0,00^{b}
3-AP	0,11± 0,00^{a}
APO	0,02± 0,00^{c}
3-AP+APO	0,03± 0,00^{c}
P	***

Beyin Dokusu; İMA (İskemi Modifiye Albumin) Düzeyi. 3-AP: 3-asetilpyridin, APO: Aposinin. *** $p< ,001$. a, b, c: Aynı sütunda farklı harf ile gösterilen ortalamalar arası fark önemlidir.

Tartışma

Ataksi, serebellum ve diğer sinir yolları gibi beyin bölgelerindeki dejeneratif hasarlardan kaynaklanan bir durumdur. Beyin korteksinin tek çıkış noktası olan purkinje hücrelerinin işlevsiz olması ataksik hareketlerle sonuçlanır (Ashizawa ve Xia, 2016). İnsanlarda spinoserebellar ataksi, serebellar ataksi ve olivary serebellar ataksi olmak üzere çeşitli ataksi modelleri vardır (González-Tapia vd., 2024).

Birçok çalışma, 3-AP kullanılarak inferior olivary çekirdekte oluşturulan farmakolojik lezyonların, serebellar ataksilerin karakteristik motor bozukluklarını güvenilir bir şekilde taklit ettiğini belirtmektedir (Cao vd., 2020; Ghorbani vd., 2022; Ranjbar vd., 2022). Bu kimyasalın inferior olivary çekirdekteki kalbindin eksprese eden nöronlarda lezyonlara sebep olarak serebellar ataksiye benzer anomaliler gösterdiği belirtilmiştir. İnferior olivary çekirdek, glutamaterjik uyarıcı sinyalleri, purkinje hücrelerine göndererek motor fonksiyon ve nöromusküler koordinasyonun kontrolünde önemli bir rol oynayarak motor kontrol anormalliklerine ve serebellar atrofiye yol açar (Akhlaghpasand vd., 2020). Saeidikhoo ve ark.'nın yaptığı çalışmaya göre 3-AP grubunda kontrol grubuna kıyasla toplam Purkinje hücre sayısında belirgin bir düşüş olduğu belirtilmiştir (Saeidikhoo vd., 2020).

Mevcut çalışmamızda, 200–250 g ağırlığında, 5-6 haftalık *Sprague-Dawley* cinsi erkek ratlar kullanılarak 3-AP ile indüklenen serebellar ataksi modeli oluşturulmuştur. Ratlar dört gruba ayrılmıştır: Kontrol grubu, 3-AP grubu, 3-AP+APO grubu ve yalnızca APO uygulanan grup. 3-AP uygulaması ile ataksi modeli oluşturulduktan sonra, belirlenen gruplara göre antioksidan APO tedavisi uygulanmıştır. Çalışmanın sonunda ratlar dekapite edilerek beyin dokuları alınmış ve oksidatif stres belirteci olan İMA seviyeleri ölçülerek değerlendirilmiştir.

Albümin oksidatif stres, ROT üretimi ve asidoz gelişimi ile ilişkili iskemik durumlar altında bazı değişikliklere uğrar ve kobalta olan afinitesi azalır. Bu albümin çeşidine İMA adı verilir (Shevtsova vd., 2021). Yapılan son çalışmalarda serebellar ataksinin patogenezinde ROT üretimi ile antioksidan savunma sistemi arasındaki dengenin bozulduğu belirtilmiştir (Lew vd., 2022). Reaktif oksijen türleri üretimi ve antioksidan savunma sistemi arasındaki dengenin bozulması oksidatif strese neden olur (Torres-Ramos vd., 2018). Ajayi vd. (2012) çalışmasında APO'nun ROT miktarındaki artışı engellediği ifade edilirken, Mazzonetto vd. (2019) ise beyin dokusunda NADPH oksidaz tarafından üretilen ROT miktarını APO'nun inhibe ettiğini belirtmiştir. Mevcut çalışmada, Ajayi vd. (2012) ve Mazzonetto vd. (2019)'nun bulgularına uyumlu olarak, oksidatif stres belirteci olan beyin dokusunda İMA seviyesi 3-AP grubunda kontrol grubuna göre artarken, APO grubunda ise azalma göstermiştir ve 3-AP ile birlikte 20 mg/kg APO uygulanan grupta İMA seviyelerinde iyileşme gözlenmiştir; bu durum, APO'nun güçlü antioksidan etkisiyle NADPH oksidaz kaynaklı ROT üretimini inhibe ederek oksidatif stresi azaltmasından kaynaklanmaktadır.

Sonuç

Bu çalışmanın sonucu, 3-AP kaynaklı oluşan oksidatif stresin beyin dokusunda hasara yol açarak ataksiye sebep olduğunu ve APO tedavisi ile beyin hasarı üzerinde koruyucu etki sağladığını göstermiştir. Bu nedenle ROT seviyesini artıran 3-AP kaynaklı hasarı önlemek için APO'nun 20 mg/kg dozunda kullanımının İMA seviyesini azaltarak antioksidan seviyesini iyileştirmede etkili olduğu görülmüştür. Sonuç olarak bu bulgular APO'nun 3-AP kaynaklı beyin hasarına karşı koruyucu etkisinin ardındaki mekanizmaya dair yeni bakış açıları sağlayabilir. Mevcut literatürde bu konuya dair çalışmalar bulunmakla birlikte, İMA parametresine odaklanan bu araştırma, alanda bir ilk olma özelliği taşımaktadır.

Etik Kurul Onayı: Bu çalışma için etik komite onayı Atatürk Üniversitesi'nden (Tarih: 23.07.2024, Karar No: 168) alınmıştır.
Yazar Katkıları: Fikir – Ö.A, E.D.; Tasarım – Ö.A, E.D.; Denetleme – Ö.A, E.D.; Kaynaklar – Ö.A, E.D.; Malzemeler – Ö.A, E.D.; Veri Toplanması ve/veya İşlemesi – Ö.A, E.D.; Analiz ve/veya Yorum – Ö.A, E.D.; Literatür Taraması – Ö.A, E.D.; Yazıyı Yazan – Ö.A, E.D.; Eleştirel İnceleme – Ö.A, E.D.
Hakem Değerlendirmesi: Dış Bağımsız
Finansal Destek: Yazarlar bu çalışma için finansal destek almadıklarını beyan etmişlerdir.
Çıkar Çatışması: Yazarlar, çıkar çatışması bildirmemişlerdir.
Ethics Committee Approval: Ethics committee approval for this study was received from Atatürk University (Date: 23.07.24, Decision No: 168).
Author Contributions: Concept – Ö.A, E.D; Design – Ö.A, E.D; Supervision – Ö.A, E.D; Resources – Ö.A, E.D; Materials – Ö.A, E.D; Data Collection and/or Processing – Ö.A, E.D; Analysis and/or Comment – Ö.A, E.D; Literature Review- Ö.A, E.D; Writing Manuscript – Ö.A, E.D; Critical Review - Ö.A, E.D.
Peer-review: Externally peer-reviewed
Funding: The authors declared that they received no financial support for this study.
Declaration of Interests: The authors declare no conflict of interest.

Kaynaklar

Abo Saleh, A., Marouf, B., & Zrieki, A. (2023). Ischemia modified albumin (ima): a novel biomarker for the diagnosis of acute coronary syndrome. *Bulletin of*

Pharmaceutical Sciences Assiut University, 46(1), 473-481. https://doi.org/10.1007/BF02913070

Aghighi, Z., Ghorbani, Z., Moghaddam, M. H., Fathi, M., Abdollahifar, M.-A., Soleimani, M., Karimzadeh, F., Rasoolijazi, H., & Aliaghaei, A. (2022). Melittin ameliorates motor function and prevents autophagy-induced cell death and astrogliosis in rat models of cerebellar ataxia induced by 3-acetylpyridine. *Neuropeptides, 96*, 102295. https://doi.org/10.1016/j.npep.2022.102295

Ajayi, A., Yu, X., Lindberg, S., Langel, Ü., & Ström, A.-L. (2012). Expanded ataxin-7 cause toxicity by inducing ROS production from NADPH oxidase complexes in a stable inducible Spinocerebellar ataxia type 7 (SCA7) model. *BMC Neuroscience, 13*(1), 86. https://doi.org/10.1186/1471-2202-13-86

Arroyo, V., García-Martinez, R., & Salvatella, X. (2014). Human serum albumin, systemic inflammation, and cirrhosis. *Journal of Hepatology, 61*(2), 396-407. https://doi.org/10.1016/j.jhep.2014.04.012

Bar–Or, D., Lau, E., & Winkler, J. V. (2000). A novel assay for cobalt-albumin binding and its potential as a marker for myocardial ischemia—a preliminary report. *The Journal of Emergency Medicine, 19*(4), 311-315. https://doi.org/10.1016/S0736-4679(00)00255-9

Canakci, E., Karatas, A., Dereli, S., Kaya, Y., Bayrak, T., Coskun, I., Altınbaş, A., & Cebeci, Z. (2022). Association between ischemia modified albumin and myeloperoxidase levels with epicardial adipose tissue thickness in acute kidney injury in intensive care patients. *Firat Medical Journal, 27*(4), 269–276.

Cao, B.-B., Zhang, X.-X., Du, C.-Y., Liu, Z., Qiu, Y.-H., & Peng, Y.-P. (2020). TGF-β1 provides neuroprotection via inhibition of microglial activation in 3-acetylpyridine-induced cerebellar ataxia model rats. *Frontiers in Neuroscience, 14*. https://doi.org/10.3389/fnins.2020.00187

Claassen D. O. (2022). Multiple System Atrophy. *Continuum, 28*(5), 1350–1363. https://doi.org/10.1212/CON.0000000000001154

Demirci, B., Karakılıç, M. E., Coşkun, A., Yel, C., Uyanık, S. A., Ünal, K., Başpınar, İ., Çaltılı, Ç., & İnan, S. (2021). The brain ischemic volume correlation with the ischemic modified albumin level. *Bagcilar Medical Bulletin, 6*(1), 26-31. https://doi.org/10.4274/BMB.galenos.2020.10.068

Ghorbani, Z., Abdollahifar, M. A., Vakili, K., Moghaddam, M. H., Mehdizadeh, M., Marzban, H., Rasoolijazi, H., & Aliaghaei, A. (2022). Melittin administration ameliorates motor function, prevents apoptotic cell death and protects Purkinje neurons in the rat model of cerebellar ataxia induced by 3-Acetylpyridine. *Toxicon, 205*, 57-66. https://doi.org/10.1016/j.toxicon.2021.11.008

Jaques, C. S., Escorcio-Bezerra, M. L., Pedroso, J. L., & Barsottini, O. G. P. (2022). The intersection between cerebellar ataxia and neuropathy: a proposed classification and a diagnostic approach. *Cerebellum, 21*(3), 497–513. https://doi.org/10.1007/s12311-021-01275-2

Karakılıç, E., Çelikel, E., Uysal, P. I., Topçuoğlu, C., Turhan, T., & Özakın, E. (2023). Diagnostic value of ischemia-modified albumin as a biomarker in patients with peripheral vertigo at emergency department of state hospital in Ankara: A cross-sectional study. *Nigerian Journal of Clinical Practice, 26*(12), 1779–1783. https://doi.org/10.4103/njcp.njcp_1862_21

Kennelly, P. J., Botham, K. M., McGuinness, O., Rodwell, V. W., & Weil, P. A. (2023). *Harper's Illustrated Biochemistry* (32nd ed.). McGraw Hill LLC.

Lastres-Becker, I., Rüb, U., & Auburger, G. (2008). Spinocerebellar ataxia 2 (SCA2). *The Cerebellum, 7(*2), 115-124. https://doi.org/10.1007/s12311-008-0019-y

Liu, P., Cui, L., Liu, B., Liu, W., Hayashi, T., Mizuno, K., Hattori, S., Ushiki-Kaku, Y., Onodera, S., & Ikejima, T. (2020). Silibinin ameliorates STZ-induced impairment of memory and learning by up- regulating insulin signaling pathway and attenuating apoptosis. *Physiology & Behavior, 213*, 112689. https://doi.org/10.1016/j.physbeh.2019.112689

Manto, M., & Marmolino, D. (2009). Cerebellar ataxias. *Current Opinion in Neurology, 22*(4), 419-429. https://doi.org/10.1097/WCO.0b013e32832b9897

Mazzonetto, P. C., Ariza, C. B., Ocanha, S. G., de Souza, T. A., Ko, G. M., Menck, C. F. M., Massironi, S. M. G., & Porcionatto, M. A. (2019). Mutation in NADPH oxidase 3 (NOX3) impairs SHH signaling and increases cerebellar neural stem/progenitor cell proliferation. *Biochimica et Biophysica Acta - Molecular Basis of Disease, 1865*(6), 1502-1515. https://doi.org/10.1016/j.bbadis.2019.02.022

O'Malley J. A. (2022). Diagnosing common movement disorders in children. *Continuum, 28*(5), 1476–1519. https://doi.org/10.1212/CON.0000000000001187

Ranjbar, H., Soti, M., Janahmadi, M., Kohlmeier, K. A., Sheibani, V., Ahmadi-Zeidabadi, M., & Shabani, M. (2022). Modulation of the CB1 cannabinoid receptor has potential therapeutic utility in the 3-acetylpyridine cerebellar ataxia rat model. *Experimental Brain Research, 240*(9), 2339-2348. https://doi.org/10.1007/s00221-022-06415-6

Rosenthal L. S. (2022). Neurodegenerative cerebellar ataxia. *Continuum, 28*(5), 1409–1434. https://doi.org/10.1212/CON.0000000000001180

Saeidikhoo, S., Ezi, S., Khatmi, A., Aghajanpour, F., Soltani, R., Abdollahifar, M. A., Jahanian, A., & Aliaghaei, A. (2020). Effect of sertoli cell transplantation on reducing neuroinflammation-induced necroptosis and improving motor coordination in the rat model of cerebellar ataxia induced by 3-acetylpyridine. *Journal of Molecular Neuroscience, 70*(7), 1153-1163.

https://doi.org/10.1007/s12031-020-01522-x

Sbarouni, E., Georgiadou, P., & Voudris, V. (2011). Ischemia modified albumin changes - review and clinical implications. *Clinical Chemistry and Laboratory Medicine*, *49*(2), 177–184. https://doi.org/10.1515/CCLM.2011.037

Shevtsova, A., Gordiienko, I., Tkachenko, V., & Ushakova, G. (2021). Ischemia-modified albumin: origins and clinical implications. *Disease Markers, 2021*, 1-18. https://doi.org/10.1155/2021/9945424

Simonyi, A., Serfozo, P., Lehmidi, T. M., Cui, J., Gu, Z., Lubahn, D. B., Sun, A. Y., & Sun, G. Y. (2012). The neuroprotective effects of apocynin. *Frontiers in Bioscience, 4*(6), 2183–2193. https://doi.org/10.2741/535

Tahtacı, M., Başaran, M., Köseoğlu, H., Yildirim, F., Ahmedova, P., Akin, F., Alişik, M., Bolat, A., Kivrakoğlu, F., Erel, Ö., & Ersoy, O. (2019). Karaciğer fibrozisli olgularda serum iskemi modifiye albüminin değerlendirilmesi. *Akademik Gastroenteroloji Dergisi, 19*(1), 21-24. https://doi.org/10.17941/agd.723299

Torres-Ramos, Y., Montoya-Estrada, A., Cisneros, B., Tercero-Pérez, K., León-Reyes, G., Leyva-García, N., Hernández-Hernández, O., & Magaña, J. J. (2018). Oxidative stress in spinocerebellar ataxia type 7 is associated with disease severity. *The Cerebellum, 17*(5), 601-609. https://doi.org/10.1007/s12311-018-0947-0

van der Heijden, M. E., & Sillitoe, R. V. (2021). Interactions between purkinje cells and granule cells coordinate the development of functional cerebellar circuits. *Neuroscience, 462*, 4-21. https://doi.org/10.1016/j.neuroscience.2020.06.010

Wecker, L., Marrero-Rosado, B., Engberg, M. E., Johns, B. E., & Philpot, R. M. (2017). 3-Acetylpyridine neurotoxicity in mice. *NeuroToxicology, 58*, 143-152. https://doi.org/10.1016/j.neuro.2016.11.010

Xiang, J., He, L., Pen, T., & Wei, S. (2023). Ischemia modified albumin can act as an independent predictor of inhospital mortality in patients with acute aortic dissection. *Scientific Reports, 13*(1), 343. https://doi.org/10.1038/s41598-023-27659-4

Yu, J., Weiwer, M., Linhardt, R., & Dordick, J. (2008). The role of the methoxyphenol apocynin, a vascular nadph oxidase inhibitor, as a chemopreventative agent in the potential treatment of cardiovascular diseases. *Current Vascular Pharmacology, 6*(3), 204-217. https://doi.org/10.2174/157016108784911984

ATATÜRK ÜNİVERSİTESİ YAYINLARI
ATATURK UNIVERSITY PUBLICATIONS

Araştırma Makalesi *Research Article* DOİ: 10.62425/jlasp.1569687

Pinealektomi Uygulanmış Erkek Sıçanlarda Spermatolojik Parametrelerin Değerlendirilmesi

Evaluation of Spermatologic Parameters in Pinealectomized Male Rats

Tutku Can ACISU[1]
Eda ÇOBAN ERCAN[2]
Gamze UÇAK[3]
Nida BADILLI[1]
İlker ÇOBAN[4]

[1]Fırat Üniversitesi, Veteriner Fakültesi, Dölerme ve Suni Tohumlama Anabilim Dalı, Elazığ, Türkiye
[2]Fırat Üniversitesi, Tıp Fakültesi, Fizyoloji Anabilim Dalı, Elazığ, Türkiye
[3]Atatürk Üniversitesi, Veteriner Fakültesi, Dölerme ve Suni Tohumlama Anabilim Dalı, Erzurum, Türkiye
[4]İstanbul Okan Üniversitesi, Tıp Fakültesi, Fizyoloji Anabilim Dalı, İstanbul, Türkiye

ÖZ

Pineal bez, beynin orta kısmında yer alan ve melatonin hormonunun salınımından sorumlu olan yapıdır. Melatonin, reprodüktif sistemin düzenlenmesinde rol oynayan önemli hormonlardandır. Sunulan çalışmada, pinealektominin sıçanlarda sperma kalitesi üzerine etkisinin araştırılması amaçlanmıştır. Çalışmada 36 adet erkek *Sprague-Dawley* cinsi sıçan kullanıldı. Sıçanlar kontrol, sham, pinealektomi olarak her bir grupta 12 hayvan olmak üzere üç eşit gruba ayrıldı. Pinealektomi grubundan cerrahi yöntemle pineal bez uzaklaştırıldı. Sham grubunda ise pinealektomi işleminin yöntemleri uygulanıp yalnızca pineal bez uzaklaştırılmadı. Çalışma sonunda dekapitasyon sonrasında hayvanların sperma örnekleri alınarak incelendi. Sabah ve gece yapılan uygulamalar arasında anlamlı bir fark gözlenmedi. Kontrol grubunda; vücut ağırlığı, pinealektomi ve sham gruplarından daha yüksek ölçüldü ($p<$,001). Testis ağırlığında gruplar arasında anlamlı bir fark gözlenmedi. Total motilite ve sperma yoğunluğu değerleri; en yüksek kontrol grubundan elde edilirken en düşük değer, pinealektomi grubundan elde edildi ($p<$,001). En fazla anormal spermatozoon oranı pinealektomi grubunda, DNA fragmantasyonu oranı ise sham grubunda gözlendi ($p<$,001). Çalışma neticesinde; pinealektomi uygulamasının sıçan spermasında total motilite ve yoğunluğun azalmasına, DNA fragmantasyonu ve anormal spermatozoon oranının ise artmasına neden olarak sperma kalitesini olumsuz yönde etkilediği ortaya koyulmuştur.

Anahtar Kelimeler: Melatonin, Pinealektomi, Sıçan, Sperm.

ABSTRACT

The pineal gland is the structure located in the central part of the brain that is responsible for the release of the hormone melatonin. Melatonin is one of the important hormones involved in the regulation of the reproductive system. In the present study, we aimed to investigate the effect of pinealectomy on semen quality in rats. In the study, 36 male *Sprague-Dawley* rats were used. The rats were divided into three equal groups as control, sham and pinealectomy with 12 animals in each group. The pineal gland was surgically removed from the pinealectomy group. In the sham group, the methods of pinealectomy procedure were applied and only the pineal gland was not removed. At the end of the study, semen samples of the animals were taken after decapitation and analyzed. No significant difference was observed between morning and night applications. Body weight was higher in the control group than in the pinealectomy and sham groups ($p<$ .001). No significant difference was observed in testicular weight between the groups. Total motility and semen density values were highest in the control group and lowest in the pinealectomy group ($p<$ .001). The highest abnormal spermatozoon rate was observed in the pinealectomy group and the highest DNA fragmentation rate was observed in the sham group ($p<$ .001). As a result of the study, it was revealed that pinealectomy application negatively affected sperm quality by decreasing total motility and density and increasing DNA fragmentation and abnormal spermatozoon ratio in rat sperm.

Keywords: Melatonin, Pinealectomy, Rat, Sperm.

Geliş Tarihi/Received :28.10.2024
Kabul Tarihi/Accepted :21.04.2025
Yayın Tarihi/Publication Date :19.09.2025

Sorumlu Yazar/Corresponding author: Gamze Uçak
E-mail: gamzeucak@atauni.edu.tr

Cite this article: Acısu, T.C., Çoban Ercan, E., Uçak, G., Badılll, N., & Çoban, İ. (2025). Evaluation of Spermatologic Parameters in Pinealectomized Male Rats. *Journal of Laboratory Animal Science and Practices*, *5*(2), 73-80. https://doi.org/10.62425/jlasp.1569687

Giriş

Pineal bez, epifiz bezi, tüm memelilerde beynin orta kısmında gömülü vaziyette bulunan küçük, beyaz, çam kozalağı görünümündeki yapıdır (Murala vd., 2022). Pineal bez, 16. yüzyılda ruhun merkezi olarak kabul edilmiştir ancak işlevi hakkında bilgiler yetersiz kalmıştır (Aleandri vd., 1996). İnsanlar ve hayvanlar üzerinde yapılan çeşitli çalışmalarda pineal bezin sirkadiyen ve mevsimsel değişikliklerde vücudun düzenlenmesinde rol oynadığı ortaya koyulmuştur (Masson-Pévet ve Gauer, 2004). Ayrıca; vücut ağırlığının (Lerchl ve Schlatt, 1993) ve vücut ısısının (Viola vd., 2023) düzenlenmesinde, immunonöroendokrin fonksiyonlar ile tümöral büyümenin engellenmesinde de (Maestroni, 1993) pineal bezin görev aldığı bilinmektedir.

Pineal bez, çevresel sinyalleri retina aracılığıyla alarak nöroendokrin mesajlara dönüştürme özelliği gösteren tek endokrin bezdir (Binkley, 1993). Retinadan alınan sinyaller beynin ilgili merkezlerine iletilip reseptörler uyarılır ve pineal bezden melatonin hormonu salgılanır (Horodincu ve Solcan, 2023). Pineal bezin vücuttaki işlevlerinin birçoğu melatonin hormonu aracılığıyla gerçekleşmektedir. Melatonin hormonunun üretimi, karanlık-aydınlık döngüsüne bağlı olarak ritimsel olarak gerçekleşir. Karanlıkta, özellikle gece saatlerinde, melatonin hormonunun plazma konsantrasyonu en yüksek düzeye ulaşır (Reiter, 1991). Işığa maruz kalma süresi arttıkça melatonin konsantrasyonu düşmeye başlar. Gece-gündüz farkının yanı sıra mevsimsel değişikliklere bağlı olarak da aynı değişim gözlenir (Goldman ve Nelson, 2020).

Melatonin, temel etkisini kısrak, koyun gibi mevsime bağlı poliöstrik hayvanlarda çiftleşme mevsiminin başlaması ve sona erdirilmesinde gösterir. Koyunlarda üreme mevsimi, gün uzunluğunun azaldığı sonbahar-kış aylarıdır. Melatonin, koyunlarda üreme üzerinde uyarıcı etki gösterir ve gün ışığı süresi azaldığında melatonin salınımı artmaya başlar (Malpaux vd., 2020). Kısraklarda ise üreme mevsimi, gün uzunluğunun arttığı ilkbahar-yaz aylarıdır. Melatonin, kısraklarda üreme üzerinde baskılayıcı etkiye sahiptir. İlkbahar-yaz aylarında gün ışığı artınca melatonin salınımı azalır. Melatoninin dolaşımdaki konsantrasyonunun azalmasıyla baskılayıcı etki ortadan kalkar ve seksüel aktivite başlar (Diekman vd., 2002).

Erkeklerde melatonin; testis dokusunun korunmasında, Leydig hücrelerinden testosteron salınımının uyarılmasında ve Sertoli hücrelerinde enerji metabolizmasının düzenlenmesinde rol oynamaktadır (Frungieri vd., 2017; Paiva vd., 2024; Sun vd., 2020). Önceki çalışmalarda, reaktif oksijen türleri üretiminin arttığı durumlarda melatonin etkisiyle oksidatif stresin önlendiği ortaya koyulmuştur (Espino vd., 2010; Semercioz vd., 2003).

Pinealektomi, pineal bezin çıkarılması işlemine verilen addır. Önceki çalışmalarda çeşitli amaçlarla pinealektomi işlemi gerçekleştirilerek melatoninin farklı organlarda işlevleri üzerine etkileri araştırılmıştır. Pineal bez yüzeysel bir pozisyonda yer aldığı için genellikle çalışmalar sıçanlar üzerinde yürütülmüştür (Mohammadi ve Zahmatkesh, 2023). Yapılan bir çalışmada, pinealektomi uygulanan sıçanların karaciğerinde lipogenezin azaldığı ve obezitenin tetiklendiği ortaya koyulmuştur (Kim vd., 2020). Başka bir çalışmada, pinealektomize sıçanlara pilokarpin intraperitoneal enjeksiyon ile uygulanarak deneysel epilepsi oluşturulmuştur. Pinealektomize grupta, kontrol gurubuna göre daha hızlı ve daha çok sayıda nöbet oluşumu gözlenmiştir (de Lima vd., 2005). Pinealektomi uygulanan sıçanlarda oksidatif stresin indüklendiği ve glutatyon sisteminin değiştiğini ortaya koyan çalışmalar bulunmaktadır (De Butte ve Pappas, 2007). Reprodüktif sistem üzerine etkilerinin araştırıldığı çalışmalarda; pinealektomi uygulanan sıçanlarda ovulasyon oranının azaldığı, ovaryumlarda morfolojik değişikliklerin meydana geldiği saptanmıştır (Dair vd., 2008). Hamile sıçanlarda ise kan östradiol ve progesteron konsantrasyonunun artmasına neden olduğu gözlenmiştir (Nir ve Hirschmann, 1980). Bir diğer çalışmada, pinealektomize sıçanlarda endometriyal hiperplazi meydana geldiği belirlenmiştir (Pekmez vd., 2005).

Pinealektominin erkek reprodüktif sistem ve sperma üzerine etkilerinin araştırıldığı çalışmalar yetersiz kalmaktadır. Sunulan çalışmada, pinealektomi uygulanan sıçanlarda spermatolojik parametrelerin etkisinin değerlendirilmesi amaçlanmıştır.

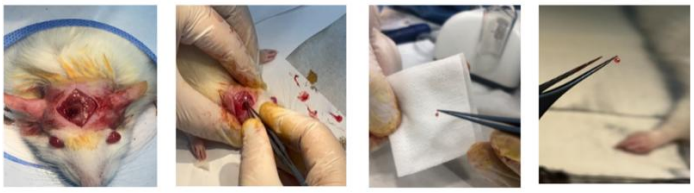
Yöntemler

Çalışma için Fırat Üniversitesi Hayvan Deneyleri Etik Kurul onayı alındı (14.08.2024 tarihli ve 2024/14-01 oturum numaralı karar). Çalışmada 250-300 gram ağırlığında 36 adet *Sprague-Dawley* cinsi erişkin erkek sıçan kullanıldı. Sıçanlar 12 saat aydınlık-12 saat karanlık ortamda ve 21±1°C sıcaklık koşullarında barındırıldı. Beslenmelerinde musluk suyu ve standart laboratuvar yemi kullanıldı ve ad libitum besleme uygulandı.

Sıçanlar, her grupta 12 hayvan olacak şekilde 3 eşit gruba ayrıldı. Grup I, kontrol grubu olarak kullanıldı. Grup II ve Grup III ise sırasıyla pinealektomi grubu ve sham grubu olarak tasarlandı. Her grup, altışar sıçan olmak üzere rastgele iki eşit gruba bölündü. Gruplardan biri sabah, diğeri ise gece sakrifiye edilmek üzere ayrı grupları temsil etmek üzere sınıflandırıldı.

Grup II'deki sıçanlara cerrahi yöntem ile pinealektomi uygulandı. Pinealektomi işleminde sıçanlar, Kuszack ve Rodin (Kuszak ve Rodin, 1977) tarafından önceden

tanımlanan yöntemle ketamin (60 mg/kg) ve ksilazin (5 mg/kg) kombinasyonu ile genel anesteziye alındı. Karınları üzerine yatırılan sıçanlar, kafa derisinden başlayıp iki göz arasından orta hat çizgisinde kafatası tabanına kadar bisturi ile kesilerek açıldı. Ekartör yardımıyla deri flepleri iki yana ayrılarak alt tabakadaki kaslar serbest hale getirildi. Tur cihazı yardımıyla dura materin derinliği boyunca delik oluşturuldu. Düz uçlu penset, oluşturulan delikten uçları açık şekilde içeri sokularak pineal bez tutuldu ve geri çekildi. Ekartör çıkarılıp deri flepleri yeniden bir araya getirilerek dikildi.



Şekil 1. Sıçanlarda cerrahi yöntem ile pineal bezin çıkarılması.

Figure 1. Surgical removal of the pineal gland in rats.

Grup III'teki sıçanlara, pineal bezin çıkarılması kısmı haricinde Grup II'ye uygulanan işlemlerin aynısı uygulandı.

Tüm hayvanlar pinealektomi işleminden 21 gün sonra canlı ağırlıkları tartılarak sakrifiye edildi. Sakrifikasyon işleminin ardından testis dokusu çıkarılarak sağ ve sol testisin ağırlıkları ayrı ayrı tartılıp kaydedildi. Testisler gibi epididimisler de sağ ve sol olarak ayrı şekilde tartıldı. Epididimisin total ağırlığının ardından, sağ kauda epididimis ayrıca tartılıp not edildi. Eklenti üreme bezlerinden vesikula seminalis ve prostatın ağırlıkları da tartılıp kaydedildi.

Sağ kauda epididimisler tartıldıktan sonra sperma eldesi için kullanıldı. Anatomik makas yardımıyla kauda epididimis parçalanarak sperma örneği steril bir petri kabındaki 5 µL tris sulandırıcısının üzerine alındı. Tris ile sulandırılmış haldeki sperma örneğinden 10 µL lam üzerine alınıp lamel ile üzeri kapatıldı ve motilite tayini için ısıtma tablası (37°C) yerleştirilen faz kontrast mikroskobunda (Celestron, Torrance, California, USA) değerlendirildi. Tüm örneklerde lam üzerindeki üç ayrı bölge incelenip ortalama bir motilite skoru belirlenerek kaydedildi.

Sperma yoğunluğunun tespitinde Varışlı ve ark. (Varisli vd., 2009) tarafından tarif edilen yöntemin modifikasyonu uygulandı. Petri kabına alınan sağ kauda epididimisler kesit atılarak ayrıştırıldı ve 37°C'de inkübasyona alındı. İnkübasyonun ardından, sperm süspansiyonu transfer pipetlerine çekilip santrifüj edildi. Bu yöntemle elde edilen saf spermadan 5 µL bir eppendorf tüpüne alınarak üzerine 995 µL distile su ilave edildi. Son karışım homojen hale getirilmek için vortekslendi. Homojen sperma karışımından 10 µL Thoma lamına alınıp spermatozoon sayısı sayılarak yoğunluk tespit edildi.

Anormal spermatozoon oranının tespiti için 20 µL sperma örneği ile 20 µL eosin-nigrosin boya (%1,67 eozin, %10 nigrosin ve 0,1 M sodyum sitrat) lam üzerine alındı (Swanson ve Bearden, 1951). Lamel yardımıyla sperma ve boya karıştırıldı, froti çekildi ve kuruması için bırakıldı. Kuruyan preparatlar ışık mikroskobun 400x büyütmesinde incelendi. Tüm örneklerde 200 adet spermatozoon incelenerek baş ve kuyruk bölümlerinde anomali olan hücreler kaydedildi.

DNA fragmantasyonu tespiti için Acridine Orange floresan boyası kullanıldı (Akarsu vd., 2024). Lam üzerine 20 µL sperma örneği alınıp froti çekildi ve kurumaya bırakıldı. Froti örnekleri, Carnoy solüsyonuna (metanol/asetik asit, 3:1) alınarak 24 saat inkübasyona bırakıldı. İnkübasyon süresi tamamlandığında örnekler, önceden hazırlanan Acridine Orange solüsyonu ile 15 dakika muamele edildi. Boyamanın ardından örnekler floresan mikroskop (Zeiss Axioscope, Almanya) ile incelendi.

Melatonin hormonunun salınımı gece saatlerinde artış gösterdiğinden, sabah ile gece uygulaması arasındaki farkın ortaya koyulması amacıyla tüm gruplarda sabah ve gece olmak üzere iki ayrı kesim işlemi uygulanarak sonuçlar elde edilmiştir.

Pineal bezin yerleşim yeri ve spesifik görüntüsü dikkate alınarak yapılan pinealektomi operasyonu, hem işlem esnasında hem de deney sonlandırılırken beyin dokusunun diseksiyonu esnasında ilgili bezin yerleşmiş olduğu beyin bölgesinden çıkarılmış olduğu görüldü. Pineal bezin diseke edilmiş olması durumunda yapılan çok sayıda çalışma ile melatonin seviyesinin düştüğü belirtilmektedir (Kennaway, 2023; Kim vd., 2020; Lee vd., 2020). Dolayısıyla pineal bezin uzaklaştırılması sonucunda melatonin seviyesinin düşmesi kaçınılmaz bir durumdur.

İstatistiksel Analiz

Çalışma sonucunda elde edilen verilerin istatistiksel analizi IBM SPSS (IBM SPSS Corp., Armonk, NY, ABD, Version 26.0) veri programı ile yapıldı. Verilerin normallik kontrolünde Shapiro-Wilk testi kullanıldı ve dağılımın normallik gösterdiği saptandı. Verilerin homojenlik kontrolü Levene's test kullanılarak yapıldı. Levene's testi sonucunda grupların homojen olduğu belirlendi. Aynı zamanda yapılan uygulamalarda gruplar arasındaki istatistiksel değerlendirmede One-Way ANOVA post-hoc Duncan Testi uygulandı. Çalışma grubunda farklı zamanlarda yapılan ölçümler arasındaki karşılaştırmalar Independent- Samples T testi ile değerlendirildi.

Bulgular

Sabah gerçekleştirilen uygulamada, vücut ağırlığında gruplar arasında anlamlı bir fark gözlenmedi. Ancak gece gerçekleştirilen uygulamada, kontrol grubunun diğer iki gruptan daha yüksek vücut ağırlığına sahip olduğu saptandı (p< ,001). Grup içinde iki uygulama karşılaştırıldığında, kontrol grubunda gece ölçülen ağırlık sabah ölçülenden daha yüksek tespit edildi (p< ,001).

Sağ testis ve sol testisin ağırlıklarında herhangi bir istatistiksel fark saptanmadı. Sağ epididimis ağırlıklarında sabah ve gece uygulamaları arasında herhangi bir fark gözlenmedi. Sabah yapılan tartımlarda, pinealektomi grubunda kontrol grubuna göre daha yüksek sonuç elde edildi. Sol epididimis ağırlıkları da, testis ağırlıklarında olduğu gibi gruplar arasında fark göstermedi. Spermanın elde edildiği sağ kauda epididimis, sabah yapılan tartımda pinealektomi grubunda kontrol ve sham gruplarına kıyasla daha yüksek ölçüldü (p< ,001).

Eklenti üreme bezlerinden vesikula seminalisin ağırlığı, gece yapılan uygulamada sham grubunda kontrol grubundan daha yüksek sonuç verdi (p< ,001). Kontrol grubunda ise sabah ağırlığı geceden daha fazla ölçüldü. Prostat bezi ise pinealektomi grubunda sabah, geceye kıyasla daha fazla ağırlıkta tespit edildi (p< ,001).

Total motilite değeri, sabah ve gece yapılan uygulamalar arasında farklılık göstermedi. Gece yapılan uygulamada gruplar arasında fark saptanmazken, sabah uygulamasında kontrol grubunun pinealektomi grubundan daha yüksek motiliteye sahip olduğu ortaya koyuldu (p< ,001).

Sperma yoğunluğunda motiliteye benzer şekilde sabah-gece uygulamaları arasında fark görülmedi. Sabah uygulamasında gruplar arasında anlamlı bir fark görülmezken gece uygulamasında kontrol grubunun yoğunluğu, sham ve pinealektomi gruplarına göre yüksek olarak saptandı (p< ,001).

Başa bağlı anormal spermatozoon oranı, sabah uygulamasında sham grubunda kontrol ve pinealektomi gruplarından yüksek tespit edildi (p< ,001). Gece uygulamasında ise en yüksek sonuç pinealektomi grubunda gözlendi. Ayrıca pinealektomi grubunda, gece elde edilen sonuçlar sabah uygulaması sonuçlarından daha yüksek belirlendi (p< ,001). Kuyruğa bağlı anormal spermatozoon oranı ise, gruplar arasında anlamlı bir fark göstermedi. Total anormal spermatozoon oranına bakıldığında, sabah uygulamasında gruplar arasında anlamlı bir fark yoktu. Gece uygulamasında ise en düşük anormal spermatozoon oranı sham grubunda gözlendi.

DNA fragmantasyonu, sabah ve gece sonuçlarında en yüksek sham grubunda, en düşük ise kontrol grubunda gözlendi (Tablo 1 ve Tablo 2).

Tablo 1. Vücut ve bazı reprodüktif organ ağırlıkları (g) (±SEM)

Table 1. Body and some reproductive organ weights (g) (±SEM)

	Vücut Ağırlığı (g)	Sağ Testis Ağırlık (g)	Sol Testis Ağırlık (g)	Sağ Epididimis Ağırlığı (g)	Sol Epididimis Ağırlığı (g)	Sağ Kauda Epididimis Ağırlığı (g)
Sham-Sabah	301,00±6,31	1,39±0,05	1,35±0,05	0,52±0,01^{ab}	0,50±0,03	0,17±0,00^{a}
Pinealektomi-Sabah	313,41±12,69	1,34±0,06	1,11±0,13	0,54±0,01^{b}	0,50±0,02	0,21±0,01^{b}
Kontrol-Sabah	300,50±14,82^{-}	1,38±0,05	1,36±0,05	0,48±0,00^{a}	0,50±0,01	0,16±0,00^{a}
Sham-Gece	315,41±14,61^{1}	1,51±0,20	1,46±0,15	0,50±0,02	0,46±0,03	0,16±0,01
Pinealektomi-Gece	316,41±10,02^{1}	1,34±0,05	1,32±0,04	0,48±0,05	0,48±0,02	0,16±0,02
Kontrol-Gece	363,33±5,99^{+2}	1,38±0,05	1,39±0,04	0,51±0,02	0,47±0,03	0,14±0,02

Aynı sütundaki farklı simgeler (a, b) sabah uygulamasında gruplar arasındaki farkı ifade eder (p< ,001). Aynı sütundaki farklı simgeler (1, 2, 3) gece uygulamasında gruplar arasındaki farkı ifade eder (p< ,001). Aynı sütundaki farklı simgeler (-, +) aynı grubun farklı zamanlarda (sabah, gece) yapılan uygulamasında gruplar arasındaki farkı ifade eder (p< ,001).

Tablo 2. Vesikula seminalis ve prostat ağırlıkları (g) ile Spermatolojik analiz sonuçları (±SEM)

Table 2. Vesicula seminalis and prostate weights (g) and Spermatological analysis results (±SEM

	Vesikula Seminalis Ağırlığı (g)	Prostat Ağırlığı (g)	Total Motilite (%)	Yoğunluk (x10^6)	Başa Bağlı Anomaliler (%)	Kuyruğa Bağlı Anomaliler (%)	Total Anormal Spermatozoon Oranı (%)	DNA Hasar Oranı (%)
Sham-Sabah	1,74±0,10	0,57±0,08	48,86±12,18^{ab}	100,33±16,41	6,16±1,10^{b}	3,00±0,73	9,16±0,74	46,16±3,30^{b}
Pinealektomi-Sabah	1,79±0,09	0,59±0,04^{-}	29,41±7,31^{a}	91,16±16,99	3,00±0,51^{-a}	5,00±1,46	8,00±1,73	39,33±3,40^{ab}
Kontrol-Sabah	1,70±0,11^{-}	0,39±0,07	62,75±5,33^{b}	124,66±8,28	3,16±0,98^{a}	2,66±0,49^{-}	5,83±0,90	31,83±3,75^{-a}
Sham-Gece	1,70±0,07^{2}	0,41±0,02	59,40±4,96	91,00±13,45^{1}	3,50±0,76^{1}	3,33±0,98	6,83±1,24^{1}	54,83±2,56^{3}
Pinealektomi-Gece	1,58±0,11^{12}	0,45±0,03^{+}	31,08±9,13	107,00±8,66^{1}	7,00±1,80^{+2}	6,33±1,33	13,33±2,41^{2}	34,16±2,37^{2}
Kontrol-Gece	1,43±0,04^{+1}	0,49±0,02	58,85±12,06	146,33±12,45^{2}	2,66±0,42^{1}	5,83±1,13^{+}	8,50±1,17^{12}	38,33±3,53^{+1}

Aynı sütundaki farklı simgeler (a, b) sabah uygulamasında gruplar arasındaki farkı ifade eder (p< ,001). Aynı sütundaki farklı simgeler (1, 2, 3) gece uygulamasında gruplar arasındaki farkı ifade eder (p< ,001). Aynı sütundaki farklı simgeler (-,+,) aynı grubun farklı zamanlarda yapılan uygulamasında gruplar arasındaki farkı ifade eder (p< ,001)

Tartışma

Pineal bez; beynin orta kısmında yer alan ve önemli endokrin aktiviteye sahip olan bir organdır. Sirkadiyen ve mevsimsel değişikliklere bağlı olarak vücuttaki fonksiyonların düzenlenmesinden pineal bez sorumludur. Pineal bez asıl fonksiyonunu melatonin hormonu salgılayarak gösterir (Forsling vd., 1993). Karanlık hormonu olarak da bilinen melatoninin salınımı ışık miktarına bağlı olarak aydınlık-karanlık döngüsünde düzenlenir. Melatonin, özellikle mevsime bağlı poliöstrik hayvanlarda (kısrak, koyun vb.) üremenin düzenlenmesinde rol oynar. Önceki çalışmalarda; pineal bezin cerrahi yöntemle dışarı alınması işlemi olan pinealektomi uygulamasının, farklı organlarda çeşitli etkiler meydana getirdiği saptanmıştır (De Butte ve Pappas, 2007; de Lima vd., 2005; Kim vd., 2020). Bu çalışmada ise pinealektominin sıçanlarda spermatolojik parametreler üzerindeki etkisi araştırılmıştır.

Pinealektomi ve sham grubuna yapılan uygulama, vücut ağırlık artışının kontrol grubuna göre daha az olmasına neden olmuştur. Cerrahi işlem sırasında meydana gelen stres, hayvanların vücut ağırlık artışını yavaşlatmıştır. Vücut ağırlıkları arasında fark oluşurken, testis ağırlıklarında anlamlı bir fark gözlenmemiştir. Sağ epididimisin hem total ağırlığında hem de kauda epididimis ağırlığında, sabah yapılan tartımlarda en yüksek sonuç pinealektomi grubundan elde edilmiştir. Ancak gece tartımlarında gruplar arasında böyle bir farka rastlanmamıştır. Aynı grubun sabah-gece tartımlarında da fark gözlenmemesi, ağırlık farkında melatonin etkisinin olmadığını göstermektedir. Önceki bir çalışma, melatonin uygulamasının epididimis ağırlığı üzerine etkisinin olmadığını gösterdiğinden, çalışma sonucunu doğrulamaktadır (Eleiwe vd., 2008). Eklenti üreme bezlerinin ağırlığı, pinealektomi uygulamasından sonra anlamlı bir fark oluşturmamıştır.

Motilite, spermatozoonların hareketliliğini ifade eder ve fertilizasyon kapasitesinin en önemli belirteçlerinden biridir (İnanç vd., 2022). Koçlar üzerinde gerçekleştirilen bir çalışmada, üreme mevsimi dışında melatonin implantı kullanıldığında sperma kinematik parametrelerinde artış gözlenmiştir (Casao vd., 2010). Melatoninin, spermatozoonlarda hem mitokondriyal homeostazı

etkileyerek ATP üretimini uyardığı hem de oksidatif strese karşı mitokondriyi koruduğu düşünülmektedir (Ortiz vd., 2011). Çalışma sonucunda en yüksek motilite değeri kontrol grubunda saptanırken en düşük motilite ise pinealektomi uygulanan grupta gözlenmiştir. Çalışmamız sonucunda pinealektomi uygulamasıyla melatonin salınımının engellenmesi, motilitenin düşmesiyle sonuçlanmıştır.

Sperma yoğunluğu, 1 ml hacimdeki spermatozoon sayısını ifade eder. Oksidatif stres oluşumu, testislerin toksik etkili maddeler ile uyarımı gibi çeşitli nedenlerle sperma yoğunluğunda azalma meydana gelmektedir (Akarsu vd., 2024; Tuncer vd., 2023). Sham ve pinealektomi gruplarına uygulanan cerrahi müdahalenin, oksidatif stres düzeylerindeki artışa bağlı olarak sperma yoğunluğunun düşmesine neden olabileceği düşünülmektedir. Kontrol grubuna kıyasla pinealektomi ve sham gruplarında belirgin bir azalma olmakla birlikte iki grubun arasında anlamlı bir fark saptanmamıştır. Elde edilen sonuç, sperma yoğunluğunun melatonin varlığından önemli ölçüde etkilenmeyip yapılan müdahaleye bağlı olarak şekillendiği sonucuna varmaktadır.

Anormal spermatozoon oranı, sperma kalitesini etkileyerek spermatozoonların işlevlerinde bozukluklar ile sonuçlanmaktadır. Özellikle başa bağlı anormal spermatozoon oranındaki artış, spermatozoon DNA'sını etkileyerek genetik materyalin aktarımını engellemektedir. Yapılan bir çalışmada, boğa spermasının dondurulması sırasında sulandırıcıya değişen konsantrasyonlarda melatonin ilave edilmiştir. Melatonin ilave edilen gruplarda kontrol grubuna kıyasla anormal spermatozoon oranında düşüş tespit edilmiştir (Ashrafi vd., 2013). Pinealektomi grubunda, özellikle gece uygulamasında gruplar arasında anlamlı bir farklılık tespit edilmiştir. Diğer gruplarda ise melatonin salınımına bağlı olarak daha düşük oranda anormal spermatozoon oluşmuştur.

DNA fragmantasyonu, DNA'da meydana gelen geri dönüşümsüz hasardır ve sperma kalitesini etkileyen başlıca etmenlerden biridir. Fareler üzerinde gerçekleştirilen bir çalışmada organofosforlu pestisitlere maruziyet sonrası melatoninin koruyucu etkileri araştırılmıştır. Çalışma sonucunda melatoninin düşük dozlarda bile DNA fragmantasyonuna karşı koruyucu etkili olduğu bilinmektedir (Mir vd., 2022; Sarabia vd., 2009). Melatoninin güçlü antioksidan etkisi sayesinde DNA yapısının korunmasında görev aldığı düşünülmektedir. Hem sabah hem de gece uygulamasında, en düşük hasar kontrol grubunda gözlenirken en yüksek hasar ise sham grubunda saptanmıştır. Pineal bezin uzaklaştırılmasının yanı sıra uygulanan cerrahi prosedürün de, sperma kalitesini etkileyerek DNA fragmantasyonu oranında ciddi bir artışa yol açtığı düşünülmektedir.

Sonuç

Sonuç olarak elde edilen bulgular değerlendirildiğinde; pinealektomi uygulaması sıçanlarda total spermatozoon motilitesinin ve sperma yoğunluğunun azalmasına neden olurken anormal spermatozoon oranının artmasına yol açmaktadır. Pineal bez çıkarılmasa dahi aynı cerrahi prosedür uygulandığından dolayı sham grubunda da kontrol grubuna kıyasla total motilitede ve yoğunlukta belirgin bir azalma, anormal spermatozoon oranı ve DNA fragmantasyonunda ise artış şekillenmiştir. Pineal bezin uzaklaştırılmasıyla sperma kalitesinde daha ciddi düşüş görülmekle birlikte pineal beze yapılan müdahalede dahi sperma kalitesinin olumsuz etkilenebildiği çalışma sonucunda ortaya koyulmuştur.

Etik Kurul Onayı: Bu çalışmanın etik kurul onayı Fırat Üniversitesi Hayvan Deneyleri Yerel Etik Kurulu'nda alınmıştır (Tarih: 14.08.2024, Karar numarası: 2024/14-01).
Yazar Katkıları: Konsept – T.C.A.; Tasarım – T.C.A., E.Ç.E.; Denetim – T.C.A.; Kaynaklar – G.U.; Malzemeler – T.C.A., N.B.; Veri Toplama ve/veya İşleme – İ.Ç., E.Ç.E., N.B.; Analiz ve/veya Yorum – T.C.A., İ.Ç.; Literatür Taraması – G.U.; Yazma – G.U.; Eleştirel İnceleme – T.C.A.
Hakem Değerlendirmesi: Dış Bağımsız
Finansal Destek: Yazarlar bu çalışma için finansal destek almadıklarını beyan etmişlerdir.
Çıkar Çatışması: Yazarlar, çıkar çatışması bildirmemişlerdir.
Ethics Committee Approval: Ethics committee approval for this study was obtained from the Local Ethics Committee for Animal Experiments of Fırat University (Date: 14.08.2024, Decision number: 2024/14-01).
Author Contributions: Concept – T.C.A.; Design – T.C.A., E.Ç.E.; Supervision – T.C.A.; Resources – G.U.; Materials – T.C.A., N.B.; Data Collection and/or Processing – İ.Ç., E.Ç.E., N.B.; Analysis and/or Interpretation – T.C.A., İ.Ç.; Literature Search – G.U.; Writing Manuscript – G.U.; Critical Review – T.C.A.
Peer-review: Externally peer-reviewed
Funding: The authors declared that they received no financial support for this study.
Declaration of Interests: The authors declare no conflict of interest.

Kaynaklar

Akarsu, S. A., Gür, C., Küçükler, S., Akaras, N., İleritürk, M., & Kandemir, F. M. (2024). Protective effects of syringic acid against oxidative damage, apoptosis, autophagy, inflammation, testicular histopathologic disorders, and impaired sperm quality in the testicular tissue of rats induced by mercuric chloride. *Environmental Toxicology*, *39*(10), 4803–4814. https://doi.org/10.1002/tox.24395

Akarsu, S. A., İleritürk, M., Küçükler, S., Akaras, N., Gür, C., & Kandemir, F. M. (2024). Ameliorative effects of sinapic acid against vancomycin-induced testicular oxidative damage, apoptosis, inflammation, testicular histopathologic disorders and decreased epididymal sperm quality. *Reproductive Toxicology, 129*, 108666.

https://doi.org/10.1016/j.reprotox.2024.108666

Aleandri, V., Spina, V., & Morini, A. (1996). The pineal gland and reproduction. *Human Reproduction Update, 2*(3), 225-235. https://doi.org/10.1093/humupd/2.3.225

Ashrafi, I., Kohram, H., & Ardabili, F. F. (2013). Antioxidative effects of melatonin on kinetics, microscopic and oxidative parameters of cryopreserved bull spermatozoa. *Animal Reproduction Science, 139*(1-4), 25-30. https://doi.org/10.1016/j.anireprosci.2013.03.016

Binkley, S. (1993). Structures and molecules involved in generation and regulation of biological rhythms in vertebrates and invertebrates. *Experientia, 49*, 648-653. https://doi.org/10.1007/bf01923946

Casao, A., Vega, S., Palacín, I., Pérez-Pe, R., Laviña, A., Quintín, F., Sevilla, E., Abecia, J., Cebrian-Perez, J., & Forcada, F. (2010). Effects of melatonin implants during non-breeding season on sperm motility and reproductive parameters in Rasa Aragonesa rams. *Reproduction in Domestic Animals, 45*(3), 425-432. https://doi.org/10.1111/j.1439-0531.2008.01215.x

Dair, E. L., Simoes, R. S., Simões, M. J., Romeu, L. R. G., Oliveira-Filho, R. M., Abi Haidar, M., Baracat, E. C., & Soares Jr, J. M. (2008). Effects of melatonin on the endometrial morphology and embryo implantation in rats. *Fertility and Sterility, 89*(5), 1299-1305. https://doi.org/10.1016/j.fertnstert.2007.03.050

De Butte, M., & Pappas, B. (2007). Pinealectomy causes hippocampal CA1 and CA3 cell loss: reversal by melatonin supplementation. *Neurobiology of Aging, 28*(2), 306-313. https://doi.org/10.1016/j.neurobiolaging.2005.12.004

de Lima, E., Soares Jr, J. M., Garrido, Y. d. C. S., Valente, S. G., Priel, M. R., Baracat, E. C., Cavalheiro, E. A., da Graça Naffah-Mazzacoratti, M., & Amado, D. (2005). Effects of pinealectomy and the treatment with melatonin on the temporal lobe epilepsy in rats. *Brain Research, 1043*(1-2), 24-31. https://doi.org/10.1016/j.brainres.2005.02.027

Diekman, M., Braun, W., Peter, D., & Cook, D. (2002). Seasonal serum concentrations of melatonin in cycling and noncycling mares. *Journal of Animal Science, 80*(11), 2949-2952. https://doi.org/10.2527/2002.80112949x

Eleiwe, S. A., Al-Taii, A. A., & Mobarak, H. J. (2008). Effect of melatonin on histology of the epididymidis of adult rat. *Iraqi Journal of Medical Sciences, 6*(2).

Espino, J., Bejarano, I., Ortiz, Á., Lozano, G. M., García, J. F., Pariente, J. A., & Rodríguez, A. B. (2010). Melatonin as a potential tool against oxidative damage and apoptosis in ejaculated human spermatozoa. *Fertility and Sterility, 94*(5), 1915-1917. https://doi.org/10.1016/j.fertnstert.2009.12.082

Forsling, M. L., Stoughton, R. P., Zhou, V., Kelestimur, H., & Demaine, C. (1993). The role of the pineal in the control of the daily patterns of neurohypophysial hormone secretion. *Journal of Pineal Research, 14*(1), 45-51. https://doi.org/10.1111/j.1600-079X.1993.tb00484.x

Frungieri, M. B., Calandra, R. S., & Rossi, S. P. (2017). Local actions of melatonin in somatic cells of the testis. *International Journal of Molecular Sciences, 18*(6), 1170. https://doi.org/10.3390/ijms18061170

Goldman, B. D., & Nelson, R. J. (2020). Melatonin and seasonality in mammals. *Melatonin*, 225-252. https://doi.org/10.1201/9781003068570-9

Horodincu, L., & Solcan, C. (2023). Influence of different light spectra on melatonin synthesis by the pineal gland and influence on the immune system in chickens. *Animals, 13*(13), 2095. https://doi.org/10.3390/ani13132095

İnanç, M. E., Güngör, Ş., Avdatek, F., Yeni, D., Gülhan, M. F., Olğaç, K. T., Denk, B., & Taşdemir, U. (2022). Thymoquinone improves motility, plasma membrane integrity and DNA integrity of frozen–thawed ram semen. *Andrologia, 54*(10), e14547. https://doi.org/10.1111/and.14547

Kennaway, D. J. (2023). The mammalian gastro-intestinal tract is a NOT a major extra-pineal source of melatonin. *Journal of Pineal Research, 75*(4), e12906. https://doi.org/10.1111/jpi.12906

Kim, M., Lee, S. M., Jung, J., Kim, Y. J., Moon, K. C., Seo, J. H., Ha, T. K., & Ha, E. (2020). Pinealectomy increases thermogenesis and decreases lipogenesis. *Molecular Medicine Reports, 22(*5), 4289-4297. https://doi.org/10.3892/mmr.2020.11534

Kuszak, J., & Rodin, M. (1977). A new technique of pinealectomy for adult rats. *Experientia, 33*, 283-284. https://doi.org/10.1007/bf02124114

Lee, B. H., Bussi, I. L., de la Iglesia, H. O., Hague, C., Koh, D. S., & Hille, B. (2020). Two indoleamines are secreted from rat pineal gland at night and act on melatonin receptors but are not night hormones. *Journal of Pineal Research, 68*(2), e12622. https://doi.org/10.1111/jpi.12622

Lerchl, A., & Schlatt, S. (1993). Influence of photoperiod on pineal melatonin synthesis, fur color, body weight, and reproductive function in the female Djungarian hamster, Phodopus sungorus. *Neuroendocrinology, 57*(2), 359-364. https://doi.org/10.1159/000126380

Maestroni, G. J. (1993). The immunoneuroendocrine role of melatonin. *Journal of Pineal Research, 14*(1), 1-10. https://doi.org/10.1111/j.1600-079X.1993.tb00478.x

Malpaux, B., Chemineau, P., & Pelletier, J. (2020). *Melatonin and reproduction in sheep and goats.* In Melatonin, (pp. 253-287). CRC Press. https://doi.org/10.1201/9781003068570-10

Masson-Pévet, M., & Gauer, F. (2004). Seasonality and melatonin receptors in the pars tuberalis in some long day breeders. *Neurosignals, 3*(2), 63-70. https://doi.org/10.1159/000109527

Mir, S. M., Aliarab, A., Goodarzi, G., Shirzad, M., Jafari, S. M., Qujeq, D., Samavarchi Tehrani, S., & Asadi, J. (2022). Melatonin: A smart molecule in the DNA repair system. *Cell Biochemistry and Function, 40*(1), 4-16. https://doi.org/10.1002/cbf.3672

Mohammadi, S., & Zahmatkesh, M. (2023). A surgical modification in the technique of rat pinealectomy. *Anatomical Science International, 98*(2), 164-175. https://doi.org/10.1007/s12565-022-00683-6

Murala, S., Nagarajan, E., & Bollu, P. C. (2022). *Melatonin*. In neurochemistry in clinical practice (pp. 149-163). Springer. https://doi.org/10.1007/978-3-031-07897-2_8

Nir, I., & Hirschmann, N. (1980). The effect of pinealectomy on serum progesterone and oestradiol levels during the last stage of pregnancy in rats. *Experientia, 36*(2), 259-260. https://doi.org/10.1007/bf01953769

Ortiz, A., Espino, J., Bejarano, I., Lozano, G., Monllor, F., García, J., Pariente, J., & Rodríguez, A. (2011). High endogenous melatonin concentrations enhance sperm quality and short-term in vitro exposure to melatonin improves aspects of sperm motility. *Journal of Pineal Research, 50*(2), 132-139. https://doi.org/10.1111/j.1600-079X.2010.00822.x

Paiva, R. V. N., Mondes, P. H. d. L., Brandão, B. d. J., Sant'Anna, J. N., Freire dos Santos, M. E., Fighera, Y. M., Santos, L. C., Markus, R. P., Fernandes, P. A. C. M., & Silva, J. F. (2024). Effects of acute hypothyroidism on plasma melatonin and Aanat and Asmt expression in the pineal gland and gonads of rats. *Frontiers in Endocrinology, 15*, 1322867. https://doi.org/10.3389/fendo.2024.1322867

Pekmez, H., Kuş, İ., Çolakoğlu, N., Ögetürk, M., Kuloğlu, T., & Sarsılmaz, M. (2005). Pinealektomili Sıçanlarda Uterusun Işık Mikroskop Düzeyde İncelenmesi. *Fırat Tıp Dergisi, 10*(3), 92-95.

Reiter, R. J. (1991). Neuroendocrine effects of light. *International Journal of Biometeorology, 35*, 169-175. https://doi.org/10.1007/bf01049063

Sarabia, L., Maurer, I., & Bustos-Obregon, E. (2009). Melatonin prevents damage elicited by the organophosphorous pesticide diazinon on mouse sperm DNA. *Ecotoxicology and Environmental Safety, 72*(2), 663-668. https://doi.org/10.1016/j.ecoenv.2008.04.023

Semercioz, A., Onur, R., Ogras, S., & Orhan, I. (2003). Effects of melatonin on testicular tissue nitric oxide level and antioxidant enzyme activities in experimentally induced left varicocele. *Neuroendocrinology Letters, 24*(1/2), 86-90.

Sun, T.-C., Li, H.-Y., Li, X.-Y., Yu, K., Deng, S.-L., & Tian, L. (2020). Protective effects of melatonin on male fertility preservation and reproductive system. *Cryobiology, 95*, 1-8. https://doi.org/10.1016/j.cryobiol.2020.01.018

Swanson, E. W., & Bearden, H. (1951). An eosin-nigrosin stain for differentiating live and dead bovine spermatozoa. *Journal of Animal Science, 10*(4), 981-987. https://doi.org/10.2527/jas1951.104981x

Tuncer, S. Ç., Akarsu, S. A., Küçükler, S., Gür, C., & Kandemir, F. M. (2023). Effects of sinapic acid on lead acetate-induced oxidative stress, apoptosis and inflammation in testicular tissue. *Environmental Toxicology, 38*(11), 2656-2667. https://doi.org/10.1002/tox.23900

Varisli, O., Uguz, C., Agca, C., & Agca, Y. (2009). Various physical stress factors on rat sperm motility, integrity of acrosome, and plasma membrane. *Journal of Andrology, 30*(1), 75-86. https://doi.org/10.2164/jandrol.107.004333

Viola, I., Canto, F., & Abecia, J. A. (2023). Effects of melatonin implants on locomotor activity, body temperature, and growth of lambs fed a concentrate-based diet. *Journal of Veterinary Behavior, 68*, 24-31. https://doi.org/10.1016/j.jveb.2023.08.004

ATATÜRK ÜNİVERSİTESİ YAYINLARI
ATATURK UNIVERSITY PUBLICATIONS

Research Article *Araştırma Makalesi* DOI: 10.62425/jlasp.1581664

Histopathological and Biochemical Investigation of the Effects of Rutin on Diclofenac-Induced Renal Toxicity in Rats

Sıçanlarda Diklofenak ile Oluşturulan Böbrek Toksisitesinde Rutin'in Etkilerinin Histopatolojik ve Biyokimyasal Olarak Araştırılması

Rabia OĞUZ KABAYEL[1]
Nurhan AKARAS[2]
Özge KANDEMİR[3]
Hasan ŞİMŞEK[4]
Fatih Mehmet KANDEMİR[5]

[1]Health Sciences University, Gülhane Health Vocational School, Department of Medical Services and Techniques, Pathology Laboratory Techniques Program, Ankara, Türkiye.
[2]Department of Histology and Embryology, Faculty of Medicine, Aksaray University, Aksaray, Türkiye.
[3]Aksaray University, Aksaray Vocational School of Technical Sciences, Department of Food Processing, Dairy and Products Technology Program, Aksaray, Türkiye.
[4] Department of Physiology, Faculty of Medicine, Aksaray University, Aksaray, Türkiye
[5] Deparment of Medical Biochemistry, Faculty of Medicine, Aksaray University, Aksaray, Türkiye.

ABSTRACT

Diclofenac (DCL), which is in the nonsteroidal anti-inflammatory drug (NSAID) category, known for its anti-inflammatory, antipyretic and analgesic properties, has a toxic effect by causing increased oxidative stress and inflammation in tissues when used for a long time. Rutin (RUT) is a flavonoid glycoside with anti-oxidant, anti-inflammatory and anti-apoptotic effects naturally found in many plants. This study aimed to investigate the effects of RUT, a natural antioxidant, on DCL-induced kidney tissue damage. 28 Wistar albino rats were divided into 4 groups: control, DCL, RUT, DCL+RUT100 groups. 100 mg/kg RUT was administered orally for 4 days, and 50 mg/kg DCL was administered intraperitoneally on the 3rd and 4th days. On the 5th day, kidney tissues were taken and oxidative stress, inflammation and apoptotic markers were analyzed by PCR (Polymerase Chain Reaction) method and histopathological analysis of the tissues was performed. Levels of DCL-induced oxidative stress, inflammation and apoptosis parameters in kidney tissues increased compared to the control group ($p<$ .001). With the application of RUT, all these DCL-related increase levels decreased ($p<$ .05). It was concluded that RUT has a potential protective effect against toxicity caused by DCL exposure in kidney tissues.

Keywords: Diclofenac, Nephrotoxicity, Rat, Rutin.

ÖZ

Antiinflamatuar, antipiretik ve analjezik özellikleriyle bilinen nonsteroid antiinflamatuar ilaç (NSAID) kategorisinde yer alan diklofenak (DCL), uzun süreli kullanımı dokularda oksidatif stres ve inflamasyon artışına sebep olarak toksik etki oluşturur. Rutin (RUT), birçok bitkide doğal olarak bulunan anti-oksidan, anti-inflamatuar ve anti-apoptotik etkilere sahip bir flavanoid glikozidtir. Bu çalışmada, doğal bir antioksidan olan RUT'nin DCL kaynaklı böbrek doku hasarı üzerine etkilerinin araştırılması amaçlanmıştır. 28 adet Wistar albino cinsi sıçan kontrol, DCL, RUT, DCL+ RUT100 grupları olmak üzere 4 gruba ayrıldı. 4 gün boyunca 100 mg/kg RUT uygulaması oral yolla verilerek bununla birlikte 3. ve 4. günlerde 50 mg/kg dozda DCL uygulaması intraperitoneal yolla yapıldı. 5. günde böbrek dokuları alındı ve PCR (Polimeraz Zincir Reaksiyonu) yöntemi ile oksidatif stres, inflamasyon ve apoptotik belirteçlerin analizi ve dokuların histopatolojik analizi yapıldı. Böbrek dokularında DCL kaynaklı oksidatif stres, inflamasyon ve apoptoz parametrelerin düzeyleri kontrol grubuna göre artmıştır ($p<$,001). RUT uygulamasıyla birlikte DCL bağlı tüm bu artış düzeylerinde azalmalar meydana gelmiştir ($p<$,05). Böbrek dokularında DCL maruziyetinin sebep olduğu toksisiteye karşı RUT'nin potansiyel koruyucu etkiye sahip olduğu sonucuna varıldı.

Anahtar kelimeler: Diklofenak, Nefrotoksisite, Rutin, Sıçan.

Geliş Tarihi/Received :08.11.2024
Kabul Tarihi/Accepted :25.04.2025
Yayın Tarihi/Publication Date :19.09.2025

Sorumlu Yazar/Corresponding author: Rabia Oğuz Kabayel
E-mail: rabia.oguzkabayel@sbu.edu.tr

Cite this article: Oğuz Kabayel, R., Akaras, N., Kandemir, Ö., Şimşek, H., & Kandemir, F. M. (2025). Histopathological and Biochemical Investigation of the Effects of Rutin on Diclofenac-Induced Renal Toxicity in Rats. *Journal of Laboratory Animal Science and Practices*, *5*(2), 81-91. https://doi.org/10.62425/jlasp.1581664

Introduction

The kidneys are anatomically and physiologically functional and are quite sensitive to chemical damage compared to other organs due to their high blood flow. The kidneys regulate metabolic functions such as water, electrolyte, and acid-base balance, and also produce hormones, contribute to blood production, and maintain extracellular fluid balance through the renin-angiotensin system while controlling arterial blood pressure (Abiola et al., 2019; Alabi & Akomolafe, 2020).

As a result, the kidneys concentrate toxic chemical substances in the filtrate and transport them along the tubular cells, and some toxic substances are bioactivated (Hickey et al., 2001). In addition, their roles in the metabolism, detoxification, storage, and excretion of drugs and metabolites make the kidneys a target organ vulnerable to damage (Alabi & Akomolafe, 2020; Hickey et al., 2001).

Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) are among the most commonly prescribed medications with well-known nephrotoxic effects. Diclofenac (DCL), which falls under the NSAID category, is a phenylacetic acid derivative used worldwide by more than 30 million people daily for its anti-inflammatory, antipyretic, and analgesic properties in the treatment of pain, inflammation, degenerative joint disease, rheumatoid arthritis, dysmenorrhea, and trauma (Abiola et al., 2019; Sivaraj and Umarani, 2018). Despite the therapeutic benefits of DCL, it is known to cause nephrotoxicity, cardiotoxicity, hepatotoxicity, gastrointestinal toxicity, and pulmonary toxicity even when used at low doses (Alabi & Akomolafe, 2020).

DCL has a mechanism that leads to the inhibition of cyclooxygenase (COX) enzymes and a decrease in prostaglandin release through the activity of arachidonic acid (Uehara et al., 2016). Recent evidence suggests that the inhibition of COX enzymes may lead to oxidative stress (Thai et al., 2023). The toxicity caused by DCL targets mitochondria by triggering the production of reactive oxygen species (ROS), which leads to apoptosis and DNA damage. While this toxicity can be prevented by the increased expression of antioxidant enzymes against cellular damage caused by ROS, inflammation can be treated by suppressing the activity of the COX enzyme (Prince, 2018). Since the toxic effects of DCL largely arise through oxidative damage mechanisms, scientists have emphasized the importance of natural antioxidants as a solution in the treatment of DCL-induced toxicities (Alabi & Akomolafe, 2020; Prince, 2018). Therefore, it is believed that the use of natural antioxidant compounds will provide significant protection against DCL toxicity.

Flavonoids are a group of natural polyphenolic compounds found in plants; they possess various biological effects and enhance antioxidant enzyme capacity by facilitating the detoxification of free radicals (Alhoshani et al., 2017; Kandemir et al., 2022). Rutin (RUT), which is among flavonoid glycosides, is found in many plants and herbal foods such as oats, buckwheat, tea, pomegranate, apricot, cherry, grapefruit, plum, orange, passionflower, asparagus, grape, fig, and Ruta graveolens, from which it derives its name (Alhoshani et al., 2017; Kandemir et al., 2020). Known as vitamin P, rutoside, quercetin-3-rutinoside, and soforin, this flavonoid has various protective effects under both in vitro and in vivo conditions (Alhoshani et al., 2017; Sirotkin, 2024). In addition to its anti-inflammatory, anti-apoptotic, autophagy-inhibiting, and antioxidant biological effects, it also exhibits pharmacological effects such as nephroprotective, hepatoprotective, anti-allergic, anti-mutagenic, anti-nociceptive, anti-arthritic, anti-cancer, anti-diabetic, anti-ulcer, anti-cholinergic, anti-antibacterial, antifungal, antiviral, and superoxide radical scavenging properties (Çağlayan et al., 2019; Gür & Kandemir, 2023).

In the literature, there is insufficient information regarding the protective effect of RUT against DCL-induced kidney toxicity. Therefore, considering the role of ROS in the toxicity mechanism of DCL, the aim is to investigate the potential defensive effects of RUT, known for its protective properties (antioxidant, anti-apoptotic, and anti-inflammatory), against DCL-induced kidney damage. In this context, oxidative stress, inflammation, and apoptosis markers in kidney tissues will be analyzed using biochemical and molecular methods, and histopathological evaluations will be conducted.

Methods

Experimental Animals

In this study, 28 Wistar albino rats with an average weight of 200-250 grams and aged 10-12 weeks were used, obtained from the KONÜDAM Experimental Medicine Application and Research Center at Konya Necmettin Erbakan University. The animals were kept in a controlled room at a constant temperature of 24-25°C with a 12-hour light-dark cycle (07:00-19:00 light; 19:00-07:00 dark) in cages. The rats were fed with normal drinking water and standard rat chow. They were allowed to rest in their cages for one week to acclimate to the environment before the

experiments began. The ethical approval for this study was granted by the Local Ethics Committee for Animal Experiments of the KONÜDAM Experimental Medicine Application and Research Center at Konya Necmettin Erbakan University, with the decision number 2024-079 dated 25.09.2024.

Experimental Applications

In the study, a total of 28 Wistar albino rats were used, and they were divided into 4 groups with 7 rats in each group. The determination of doses was based on information from the literature (Çağlayan et al., 2019; Varışlı et al., 2023).

1-Control Group: Rats were administered saline solution orally for 4 days, with oral administration on the 3rd and 4th days.

2-RUT Group: RUT was administered orally at a dose of 100 mg/kg for 4 days.

3-DCL Group: DCL was administered intraperitoneally at a dose of 50 mg/kg on the 3rd and 4th days.

4-DCL + RUT100 Group: RUT was administered orally at a dose of 100 mg/kg for 4 days, and DCL was administered intraperitoneally at a dose of 50 mg on the 3rd and 4th days.

Sample Collection

Twenty-four hours after the last application (day 5), the animals were decapitated under light sevoflurane anesthesia, and kidney and blood samples were collected. Blood samples were taken, centrifuged at 3500 rpm for 10 minutes, and stored at -20°C until biochemical analyses. A portion of the kidney tissues was collected for biochemical analyses and stored at -20°C until the analyses were conducted.

Analysis of lipid peroxidation marker

The degree of lipid peroxidation in kidney tissues was assessed by measuring the absorbance of the color generated by the reaction of malondialdehyde (MDA) with thiobarbituric acid at 532 nm. Tissues were homogenized in 1.15% potassium chloride using a homogenizer (Tissue Lyser II, Qiagen). The homogenates were then centrifuged for 15 min at +4°C and 1000g, and the supernatant was used. The technique developed by Kandemir et al. was used to determine MDA levels (Kandemir et al., 2022).

Reverse Transcription PCR (RT-PCR) Analysis

The effects of DCL damage and RUT application on the relative mRNA transcript levels of the gene regions listed in Table 1 were examined using the qRT-PCR technique in kidney tissues collected at the end of the experiment. Total RNA was isolated from the tissues, and cDNA synthesis was performed from the obtained total RNA. The prepared cDNAs, along with primer sequences and MasterMix, were combined to carry out the reaction. The mixture was run in a real-time PCR thermal cycler according to the duration and temperature cycles specified by the manufacturer's instructions. After the completion of the cycles, gene expressions were normalized to β-Actin and evaluated using the 2-ΔΔCT method (Livak & Schmittgen, 2001).

Table 1. Primer Sequences

Tablo 1. Primer Dizileri

Gene	Sequences (5'-3')	Accession no or references PUBMED ID
Cu-Zn SOD	F: AGTCCCGCCCCTTCTAAAAC R: CAATGGCCTCTGTGTAGCCC	PMID: 22057777
CAT	F: ATGGCAACTGTCCCTGAACT R: AGTGACACTGCCTTCCTGAA	PMID: 22057777
GPx	F: CTCGAGTGACAAGCCCGTAG R: ATCTGCTGGTACCACCAGTT	PMID: 22057777
NF-κB	F: AGTCCCGCCCCTTCTAAAAC R: CAATGGCCTCTGTGTAGCCC	NM_001276711.1
TNF-α	F: CTCGAGTGACAAGCCCGTAG R: ATCTGCTGGTACCACCAGTT	NM_012675.3
Caspase -3	F: ACTGGAATGTCAGCTCGCAA R: GCAGTAGTCGCCTCTGAAGA	NM_012922.2
Bax	F: TTTCATCCAGGATCGAGCAG R: AATCATCCTCTGCAGCTCCA	NM_017059.2
Bcl-2	F: GACTTTGCAGAGATGTCCAG R: TCAGGTACTCAGTCATCCAC	NM_016993.2
PERK	F: GATGCCGAGAATCATGGGAA R: AGATTCGAGAAGGGACTCCA	NM_031599.2
ATF-6	F: TCAACTCAGCACGTTCCTGA R: GACCAGTGACAGGCTTCTCT	NM_001107196.1
KIM-1	F:TGGCACTGTGACATCCTCAGA R: GCAACGGACATGCCAACATA	PMID: 32794300
AQP-2	F: AGCTGCCTTCTATGTGGCT R: GCGTTGTTGTGGAGAGCATT	NM_012909.2
β-Actin	F: CAGCCTTCCTTCTTGGGTATG R: AGCTCAGTAACAGTCCGCCT	NM_031144.3

SOD: Superoxide Dismutase, CAT: Catalase, GPx: Glutathione Peroxidase, NF-κB: Nuclear Factor kappa B, TNF-α: Tumor Necrosis Factor-alpha, Caspase-3: Cysteine Aspartate Specific Protease-3, Bax: Bcl-2 Associated X Protein, Bcl-2: B-cell Lymphoma 2, PERK: Protein Kinase R-like Endoplasmic Reticulum Kinase, ATF-6: Activating Transcription Factor 6, KIM-1: Kidney Injury Molecule-1, AQP-2: Aquaporin 2.

Histopathological Analysis

Kidney tissues were collected from anesthetized rats and fixed in a 10% neutral buffered formalin solution for 48 hours. The kidney tissues were washed under running water for one night in accordance with routine tissue processing procedures, and then subjected to dehydration through a series of increasing alcohol concentrations (70% for 1 hour, 80% for 1 hour, 96% for 1 hour, and 99% for 1 hour). The tissues passed through the alcohol series were placed in xylene for a total of one hour in three stages and then treated with paraffin for infiltration. The tissues were subsequently embedded in metal blocks during the blocking stage and formed into solid blocks. Paraffin blocks were sectioned into 4-5 micrometer slices on glass slides using a semi-automatic microtome. The sections on the slides were stained with Hematoxylin and Eosin, a routine tissue stain, for examination. The stained sections were then analyzed using a binocular light microscope and photographed with a camera.

Statistical Analysis

The statistical analysis of the data obtained from the study was conducted using the IBM SPSS software (version 20.0; IBM Corp., North Castle, NY). The Shapiro-Wilk test was used to analyze the sample size, which was less than 50. Since the data had a normal distribution and there were more than two groups, Tukey's multiple comparison test and one-way analysis of variance (ANOVA) were used to compare differences between groups. The results were presented as mean ± SE, with $p< .05$ considered to be statistically significant.

Results

Oxidative Stress Findings

When MDA, one of the markers showing the oxidant status of kidney tissues, was compared with the control group, DCL increased MDA levels ($p< .001$). However, RUT treatment has been determined to reduce MDA levels (Table 2, Figure 1). The mRNA transcript levels of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) in kidney tissue were analyzed to assess antioxidant levels (Table 2, Figure 1). In the DCL group, the mRNA transcript levels of SOD, CAT, and GPx decreased compared to the control group ($p< .001$). In the DCL+RUT group, the mRNA transcript levels of SOD, CAT, and GPx increased compared to the DCL group (SOD: $p< .001$, CAT: $p< .01$, GPx: $p< .05$). Accordingly, DCL suppressed the expressions of antioxidant enzymes (SOD, CAT, GPx) in kidney tissue compared to the control group ($p< .001$). These results indicate that DCL may cause tissue damage by reducing the antioxidant capacity in kidney tissues. However, it was observed that RUT treatment increased antioxidant enzyme expressions in the tissue and reduced oxidative damage caused by DCL.

Table 2. MDA Levels and SOD, CAT, GPx mRNA Transcript Levels in Kidney Tissue in All Groups

Tablo 2. Tüm Gruplardaki Böbrek Dokusunda MDA Düzeyleri ve SOD, CAT, GPx mRNA Transkript Düzeyleri

	MDA	SOD	CAT	GPx
Control	17.49±0.48^{a}	1.00±0.02^{c}	1.00±0.01^{c}	1.00±0.06c
RUT	17.18±0.40^{a}	1.13±0.01^{c}	1.12±0.03^{c}	1.07±0.03c
DCL	27.56±0.45^{c}	0.16±0.01^{a}	0.31±0,01^{a}	0.24±0.01^{a}
DCL+RUT	21.19±0.49^{b}	0.60±0.01^{b}	0.55±0.02^{b}	0.79±0.05^{b}

Superscript letters (a, b, c) indicate the difference between groups.$p < .001$

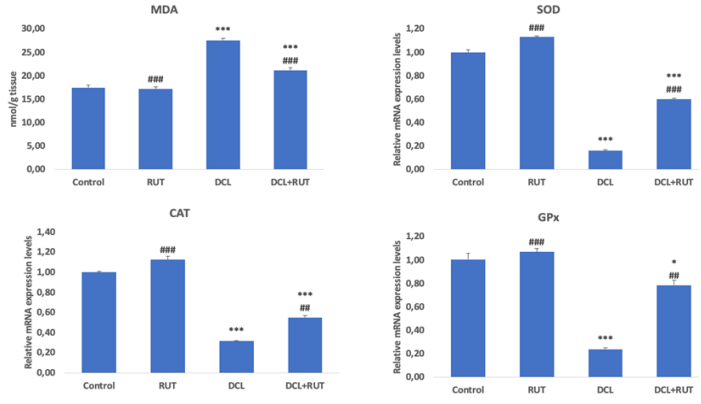


Figure 1: MDA Levels and mRNA Transcript Levels of SOD, CAT, GPx in Kidney Tissues of Rats in Experimental Groups. n=7. ***$p< .001$, *$p< .01$, $p< .05$: Control vs. others; ###$p< .001$, ##$p< .01$, # $p< .05$: DCL vs. others; not significant. (DCL: Diclofenac, RUT: Rutin, MDA: Malondialdehyde, SOD: Superoxide Dismutase, CAT: Catalase, GPx: Glutathione Peroxidase)

Şekil 1: Deney Gruplarındaki Sıçanların Böbrek Dokularındaki MDA Düzeyleri ve SOD, CAT, GPx'in mRNA Transkript Düzeyleri. n=7. ***$p<$,001, *$p<$,01, $p<$,05: Kontrol ve diğerleri; ###$p<$,001, ##$p<$,01, # $p<$,05: DCL ve diğerleri; önemli değil. (DCL: Diklofenak, RUT: Rutin, MDA: Malondialdehit, SOD: Süperoksit Dismutaz, KAT: Katalaz, GPx: Glutatyon Peroksidaz)

Inflammation Findings

To observe the effects of DCL and RUT applications on the inflammatory response in kidney tissues, the mRNA transcript levels of nuclear factor kappa B (NF-κB) and tumor necrosis factor-alpha (TNF-α) were analyzed (Table

3, Figure 2). Accordingly, it was determined that DCL application triggered inflammation by causing an increase in the mRNA transcript levels of NF-κB and TNF-α in the kidneys (p< .001). It was observed that the therapeutic application of DCL+RUT reduced the mRNA transcript levels of NF-κB and TNF-α in kidney tissue (NF-κB: p< .01, TNF-α: p< .001).

Table 3. PERK, ATF-6, NF-κB and TNF-α mRNA Transcript Levels in Kidney Tissue in All Groups.

Tablo 3. Tüm Gruplarda Böbrek Dokusundaki PERK, ATF-6, NF-κB ve TNF-α mRNA Transkript Düzeyleri.

	PERK	ATF-6	NF-kB	TNF-α
Control	1.00±0.09 [a]	1.00±0.39 [a]	1.00±0.01 [a]	1.00±0.07 [a]
RUT	0.93±0.07 [a]	0.85±0.01 [a]	0.90±0.03 [a]	0.86±0.01 [a]
DCL	5.59±0.16 [c]	4.49±0.07 [c]	4.82±0.29 [c]	5.70±0.10 [c]
DCL+RUT	2.99±0.15 [b]	2.36±0.03 [b]	3.27±0.09 [b]	3.22±0.06 [b]

Superscript letters (a, b, c) indicate the difference between groups. p <.001

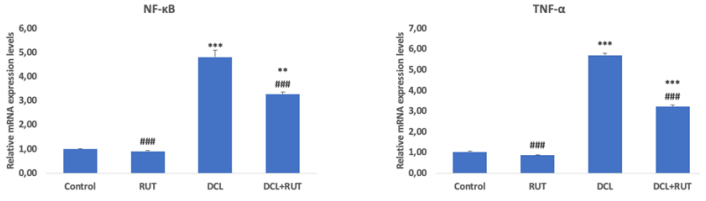


Figure 2: mRNA Transcript Levels of NF-κB and TNF-α in Kidney Tissue of Rats in Experimental Groups. n=7. ***p< .001, *p< .01, p< .05: Control vs. others; ###p< .001, ##p< .01, # p< .05: DCL vs. others; not significant. (DCL: Diclofenac, RUT: Rutin, NF-κB: Nuclear Factor kappa B, TNF-α: Tumor Necrosis Factor-alpha)

Şekil 2: Deney Gruplarındaki Sıçanların Böbrek Dokusunda NF-κB ve TNF-α'nın mRNA Transkript Düzeyleri. n=7. ***p< ,001, *p< ,01, p< ,05: Kontrol ve diğerleri; ###p< ,001, ##p< ,01, # p< ,05: DCL ve diğerleri; önemli değil. (DCL: Diklofenak, RUT: Rutin, NF-κB: Nükleer Faktör kappa B, TNF-α: Tümör Nekroz Faktörü-alfa)

Apoptosis Findings

Table 4 and Figure 3 shows the mRNA transcript levels of cysteine aspartate-specific protease-3 (Caspase-3), Bcl-2-associated X protein (Bax), and B-cell lymphoma 2 (Bcl-2) in rat kidney tissues. According to RT-PCR analysis, DCL was shown to induce Bax and caspase-3 mRNA expressions and to cause apoptosis in the kidney by inhibiting Bcl-2 mRNA expression (p< .001). It was observed that DCL+RUT treatment reduced the mRNA transcript levels of Caspase-3 and Bax in kidney tissue while increasing Bcl-2 mRNA expression (Caspase-3: p< .001, Bax: p< .001, Bcl-2: p< .01). Furthermore, the administration of RUT to the animals provided significant tissue protection by inhibiting the apoptotic pathway in the kidneys.

Table 4. Caspase-3, Bax and Bcl-2 mRNA Transcript Levels in Kidney Tissue in All Groups.

Tablo 4. Tüm Gruplarda Böbrek Dokusundaki Kaspaz-3, Bax ve Bcl-2 mRNA Transkript Düzeyleri.

	Caspase-3	Bax	Bcl-2
Control	1.00±0.16 [a]	1.00±0.01 [a]	1.00±0.01 [c]
RUT	0.91±0.02 [a]	0.93±0.02 [a]	1.09±0.03 [c]
DCL	4.85±0.14 [c]	4.23±0.09 [c]	0.39±0.01 [a]
DCL+RUT	2.79±0.03 [b]	1.99±0.04 [b]	0.66±0.03 [b]

Superscript letters (a, b, c) indicate the difference between groups. p <.001

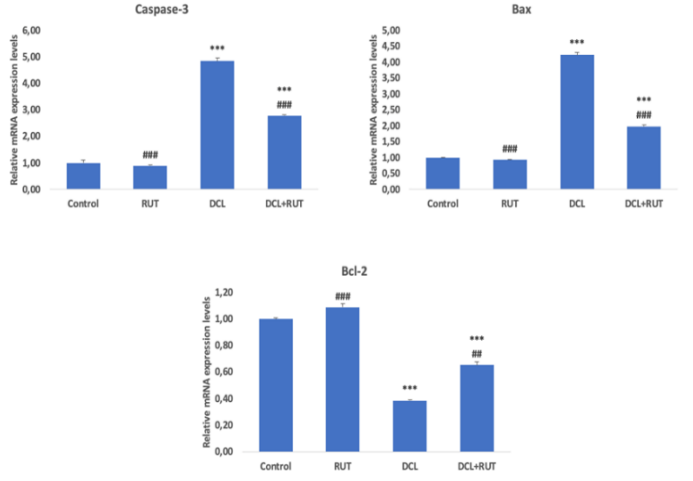


Figure 3: mRNA Transcript Levels of Caspase-3, Bax, and Bcl-2 in Kidney Tissue of Rats in Experimental Groups. n=7. ***p< .001, *p< .01, p< .05: Control vs. others; ###p< .001, ##p< .01, #p< .05: DCL vs. others; ns not significant. (DCL: Diclofenac, RUT: Rutin, Caspase-3: Cysteine Aspartate Specific Protease-3, Bax: Bcl-2 Associated X Protein, Bcl-2: B-cell Lymphoma 2)

Şekil 3: Deney Gruplarındaki Sıçanların Böbrek Dokusunda Kaspaz-3, Bax ve Bcl-2'nin mRNA Transkript Düzeyleri. n=7. ***p< ,001, *p< ,01, p< ,05: Kontrol ve diğerleri; ###p< ,001, ##p< ,01, # p< ,05: DCL ve diğerleri; önemli değil. (DCL: Diklofenak, RUT: Rutin, Kaspaz-3: Sistein Aspartat Spesifik Proteaz-3, Bax: Bcl-2 İlişkili X Proteini, Bcl-2: B Hücreli Lenfoma 2)

Kidney Damage Biomarker Findings

Table 5 and Figure 4 shows the mRNA transcript levels of kidney injury molecule-1 (KIM-1) and aquaporin-2 (AQP-2) in rat kidney tissues. According to RT-PCR analysis, the increase in KIM-1 mRNA transcript expression in the DCL group indicated kidney damage and an inflammatory response (p< .001). On the other hand, the application of RUT in conjunction with DCL was observed to reduce the mRNA transcript levels, thereby suppressing the inflammatory response (p< .001). It was determined that the mRNA expression of AQP-2 significantly decreased due to damage in the kidneys of the DCL-treated group (p< .001). DCL improved the mRNA expression and regulation of AQP-2 in the kidneys of rats treated with RUT (p< .01). This finding suggests that RUT may alleviate the decrease in AQP-2 mRNA expression caused by DCL exposure, potentially providing both protective and reparative effects (p< .01).

Table 5. KIM-1 and AQP-2 mRNA Transcript Levels in Kidney Tissue in All Groups.

Tablo 5. Tüm gruplarda böbrek dokusundaki KIM-1 ve AQP-2 mRNA Transkript Düzeyleri.

	KIM-1	AQP-2
Control	1.00±0.05 [a]	1.00±0.07 [c]
RUT	0.84±0.03 [a]	1.23±0.04 [c]
DCL	3.67±0.11 [c]	0.25±0.01 [a]
DCL+RUT	2.01±0,01 [b]	0.45±0.01 [b]

Superscript letters (a, b, c) indicate the difference between groups. p < .001

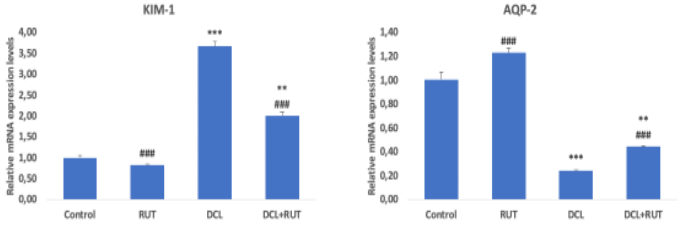


Figure 4: mRNA Transcript Levels of KIM-1 and AQP-2 in Kidney Tissue of Rats in Experimental Groups. n=7. ***p< .001, *p< .01, p< .05: Control vs. others; ###p< .001, ##p< .01, #p< .05: DCL vs. others; ns not significant. (DCL: Diclofenac, RUT: Rutin, KIM-1: Kidney Injury Molecule-1, AQP-2: Aquaporin 2)

Şekil 4: Deney Gruplarındaki Sıçanların Böbrek Dokusunda KIM-1 ve AQP-2'nin mRNA Transkript Düzeyleri. n=7. ***p< ,001, *p< ,01, p< ,05: Kontrol ve diğerleri; ###p< ,001, ##p< ,01, #p< ,05: DCL ve diğerleri; önemli değil. (DCL: Diklofenak, RUT: Rutin, KIM-1: Böbrek Hasarı Molekülü-1, AQP-2: Aquaporin 2)

Endoplasmic Reticulum Stress Findings

The mRNA transcript levels of double-stranded RNA-activated protein kinase (PKR)-like ER kinase (PERK) and activating transcription factor-6 (ATF-6) genes, which indicate ER stress in rat kidney tissues, are presented in Table 3 and Figure 5. It was observed that DCL caused ER stress by increasing the mRNA transcript levels of ATF-6 and PERK in kidney tissue (p< .001). On the other hand, it was noted that DCL+RUT treatment suppressed the mRNA transcript levels of PERK and ATF-6 in the kidney tissue (PERK: p< .001, ATF-6: p< .01).

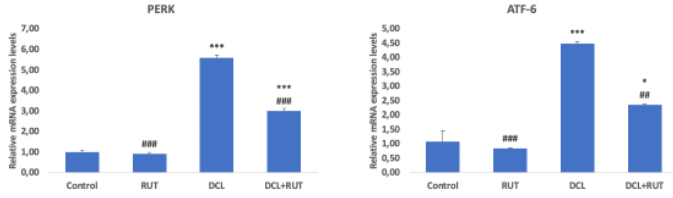


Figure 5: mRNA Transcript Levels of ATF-6 and PERK in Kidney Tissue of Rats in Experimental Groups. n=7. ***p< .001, *p< .01, p< .05: Control vs. Others; ###p< .001, ##p< .01, #p< .05: VPA vs. Others; ns not significant. (DCL: Diclofenac, RUT: Rutin, ATF-6: Activating Transcription Factor-6, PERK: Protein Kinase R-like Endoplasmic Reticulum Kinase)

Şekil 5: Deney Gruplarındaki Sıçanların Böbrek Dokusunda ATF-6 ve PERK'nin mRNA Transkript Düzeyleri. n=7. ***p< ,001, *p< ,01, p< ,05: Kontrol ve Diğerleri; ###p< ,001, ##p< ,01, #p< ,05: VPA ve Diğerleri; önemli değil. (DCL: Diklofenak, RUT: Rutin, ATF-6: Aktive Edici Transkripsiyon Faktörü-6, PERK: Protein Kinaz R-benzeri Endoplazmik Retikulum Kinaz)

Histopathological Findings

The histopathological findings of kidney tissues subjected to H&E staining from four different groups are presented in Figure 6. In the images of the control group rats, the cortex and medulla appeared to have a normal histological structure. The cortex displayed a normal appearance characterized by glomeruli surrounded by convoluted nephron tubules. Additionally, Malpighian corpuscles with normal histological morphology were present, and the structure of the proximal and distal tubules was intact. Only the group that received RUT maintained the normal architecture of the kidney tissue. In the DCL group,

shrunken and atrophic glomeruli in the cortical labyrinth, an enlarged Bowman's space compared to the control, vascular congestion, edema in the interstitium, and inflammatory cell infiltration were notable. Furthermore, DCL induced damage in the proximal and distal renal tubules, leading to the loss of brush borders, epithelial degeneration, and eosinophilic changes in their lumens. In rats treated with RUT, however, improvement in tubular damage was observed compared to the DCL group. In the DCL+RUT group, only mild occlusion in blood vessels and occasional shrunken granules were noted.

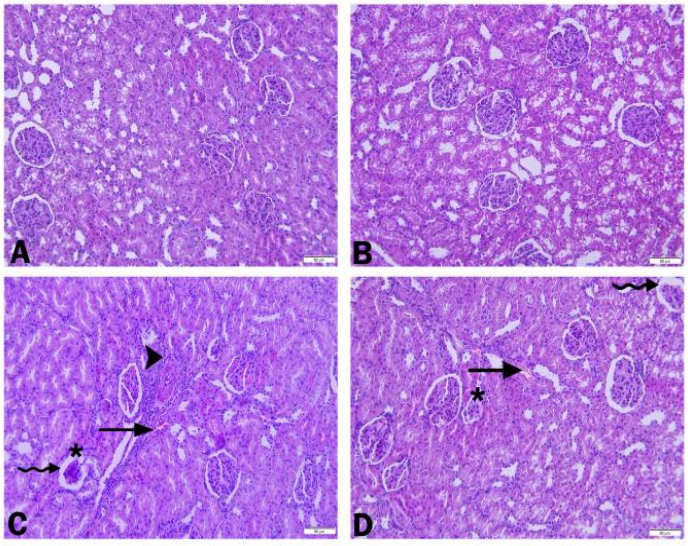


Figure 6: Histopathological Photomicrography of Kidney Sections Stained with Hematoxylin and Eosin (200x). Control (A) and RUT (B) groups show normal histological structure of glomeruli and renal tubules in the cortex. In the DCL (C) group, there is a reduction and degeneration of renal glomeruli (*) with the expansion of the Bowman's space (curved arrow), inflammatory cell infiltration (arrowhead), and vascular congestion (arrow). The DCL + RUT (D) group shows slightly expanded Bowman's space (curved arrow) with reduced and degenerated renal glomeruli (*), and mild vascular congestion (arrow). (DCL: Diclofenac, RUT: Rutin)

Şekil 6: Hematoksilen ve Eozinle boyanmış böbrek kesitlerinin histopatolojik fotomikrografisi (200x) Kontrol (A) ve RUT (B) verilen gruplarının böbrek kesitlerinde, kortekste glomerulus ve renal tübüllerin normal histolojik yapısını gösterir. DCL (C) uygulanan grupta bowman boşluğunun genişlemesiyle (kıvrık ok) büzülmüş ve dejenere olmuş renal glomerülleri (*), inflamatuar hücre infiltrasyonu (ok başı), vasküler konjesyon (ok) gösterir, DCL + RUT (D) grubunda hafif bowman boşluğunun genişlemesiyle (kıvrık ok) büzülmüş ve dejenere olmuş renal glomerüller (*), hafif vasküler konjesyon (ok) gösterir. (DCL: Diklofenak, RUT: Rutin)

Discussion

NSAID DCL can cause toxic effects in many tissues, particularly in the kidneys, at high doses. RUT, on the other hand, is known as a natural flavonoid with strong antioxidant, anti-inflammatory, and anti-apoptotic properties. In this study, the protective effects of RUT against nephrotoxicity induced by DCL were investigated.

An increase in ROS production has been identified as a fundamental cause of various organ dysfunctions in studies conducted (Caglayan et al., 2019; Keles et al., 2014). Furthermore, it has been reported that foods with high antioxidant content in the diet can reduce kidney function losses (Çömez et al., 2024). RUT is known as an antioxidant that has the ability to reduce ROS accumulation and protect cells from damage to prevent oxidative injury. Therefore, in this study, the protective effect of RUT against oxidative stress induced by DCL was investigated by examining the activities of SOD, GPx, and CAT in kidney tissue. It has been reported that organisms prevent cell damage caused by free radicals through mechanisms involving various antioxidant enzymes such as SOD, CAT, and GPx, detoxifying hydrogen peroxide formed in the cells (Akarsu et al., 2023; Çömez et al., 2024). In the study, it was determined that the levels of these three antioxidant enzymes significantly decreased in the kidney tissues of the DCL group compared to the control group. This finding is consistent with the results of a study by Abiola et al., which showed a decrease in SOD, CAT, and GPx enzyme activities in the kidneys of rats treated with DCL, leading to nephrotoxicity by suppressing the antioxidant defense system (Abiola et al., 2019). On the other hand, a study by Kandemir et al. reported that RUT provides significant protection against oxidative stress by increasing the activities of antioxidant enzymes in the kidneys (Kandemir et al., 2022). In our study, the activities of SOD, CAT, and GPx enzymes in the kidney tissues of the DCL+RUT group significantly increased compared to the DCL group. Therefore, it can be said that SOD, CAT, and GPx enzyme activities help prevent oxidative damage caused by DCL in the kidney tissues of rats exposed to DCL. It was concluded that the four hydroxyl groups in the RUT structure probably replenish GSH stores by scavenging free radicals, increase antioxidant enzymes by up-regulating the expression of SOD, CAT, GPx, and reduce MDA levels by alleviating oxidative stress.

Apoptosis, known as programmed cell death, is a pathway directed by various physiological and pathological stimuli and is triggered as oxidative stress levels increase (Akaras et al., 2023; Ayhan et al., 2020; Şimşek et al., 2023). ROS, which play a significant role in apoptosis, are produced in

the mitochondria (Akaras et al., 2023a; Çağlayan et al., 2019). The effect of ROS increases mitochondrial membrane fluidity and permeability (Akaras et al., 2023b). In the apoptosis pathway, these structural and biological changes in the mitochondria are regulated by apoptotic (Bax) and anti-apoptotic (Bcl-2) proteins. While Bcl-2 stabilizes the mitochondrial membrane, Bax increases its permeability (Akaras et al., 2023a; Akaras et al., 2023b). The apoptosis process progresses based on the balance of these proteins. When this balance is disrupted, cytochrome C released from the cytoplasm forms an apoptosome complex with Apaf-1. This complex induces caspase-9, subsequently activating caspase-3, thereby initiating apoptosis (Akaras et al., 2023b; Şimşek et al., 2023). Studies have reported that the disruption of the balance in the Bax/Bcl-2 ratio in kidney tissues leads to increased mitochondrial membrane permeability, resulting in cytochrome C release and caspase-3 activation, thereby reinforcing the notion that this apoptotic effect occurs via the inhibition of the intrinsic pathway mediated by mitochondria (Şimşek et al., 2023). According to our findings, the mRNA transcript levels of the apoptotic genes caspase-3 and Bax increased in the DCL-treated group, while the mRNA transcript level of the anti-apoptotic Bcl-2 gene decreased. These findings suggest that kidney cells inhibit apoptosis via the mitochondrial pathway. In toxicity models created in different organs using various chemicals, RUT has been reported to inhibit apoptotic proteins like caspase-3 and induce anti-apoptotic proteins like Bcl-2, demonstrating an anti-apoptotic effect (Akaras et al., 2023a; Çağlayan et al., 2019). In our study, RUT+DCL group reversed the mRNA levels of these genes. On the other hand, the apoptotic pathway was interrupted, probably due to the alleviating effect of RUT on oxidative stress, ER stress and inflammation. The interruption of apoptosis can be understood from the suppression of Bax and Caspase-3 expressions and the upregulation of Bcl-2 expression.

Inflammation is a biological response of the immune system that can be triggered by various factors such as toxic compounds, damaged cells, and pathogens (Chen et al., 2018). The activation of inflammatory pathways is associated with excessive ROS production (Akaras et al., 2023a). ROS directly activates NF-κB by inducing the phosphorylation following the degradation of the endogenous inhibitor IκB, after which NF-κB is transported from the cytosol to the nucleus, functioning as a transcription factor to stimulate the expression of pro-inflammatory mediators such as TNF-α and inflammatory cytokines like IL-1β (Guo et al., 2024; Kandemir et al., 2022). Previous studies on the reliability of DCL have highlighted that overproduction of ROS can increase the level of NF-κB and induce the expression of pro-inflammatory mediators such as TNF-α resulting (Kankılıç et al., 2024; Wadie et al., 2021). The inhibition of NF-kB is therapeutically important in preventing inflammatory conditions (Bal Taştan et al., 2023). According to the findings of this study, DCL was found to cause an increase in NF-κB and TNF-α mRNA transcript levels, triggering inflammation in kidney tissues, as also confirmed by histological findings. When RUT was administered alongside DCL, a reduction in all these inflammation parameters was observed. Similarly, it has been reported that RUT application prevents inflammation by reducing NF-κB and TNF-α levels (Çağlayan et al., 2019). Kandemir et al. also demonstrated the anti-inflammatory effect of RUT in their in vivo studies with rats (Akaras et al., 2023a; Gür & Kandemir, 2023; Kandemir et al., 2022). Consequently, it is thought that the pathways induced by ROS trigger nephrotoxicity when activated by DCL, while RUT exhibits an anti-inflammatory effect by counteracting this nephrotoxicity through its antioxidant properties.

The kidneys play a vital role in maintaining the body's electrolyte and water balance (Kwon et al., 2013). The ultrafiltrate from the glomerulus (containing substances such as water, sodium chloride, bicarbonates, glucose and amino acids) passes into the proximal tubules, where a significant portion of the kidney's reabsorption function occurs. The reabsorption of water takes place through aquaporin-1 (AQP-1) expressed on the apical and basolateral surfaces of the proximal tubules, and primarily through AQP-2 expressed in collecting duct cells (Musah et al., 2024). Therefore, AQP-2 plays an important role in urine concentration and the body's water balance (Kwon et al., 2013; Musah et al., 2024). Kidney dysfunction and nephrotoxicity significantly affect AQP levels, and it has been reported that AQP-2 levels in the kidney tissues of rats exposed to various toxic agents are significantly reduced (Kwon et al., 2013; Wang et al., 2024). KIM-1, a type I transmembrane glycoprotein, is almost absent in healthy kidney tissue but is highly expressed in damaged proximal and renal tubular cells (Karağaç et al., 2024). Previously, KIM-1 has been reported to be used as a biomarker in both tissue and urine to determine kidney damage caused by various toxic substances (Kim & Moon, 2012). In nephrotoxicity models created in the kidneys using different chemical agents, KIM-1's mRNA and protein levels have been reported to show high expression (Çomaklı et al., 2022; Karağaç et al., 2024). In the current study, it was determined that DCL-induced nephrotoxicity causes kidney dysfunction, indicated by an increase in KIM-1 expression and a decrease in AQP-2 expression. Experimental evidence suggests that RUT restores AQP-2 and KIM-1 levels, indicating its potential use to alleviate the damage caused by DCL.

The endoplasmic reticulum (ER) is involved in protein

biosynthesis, carbohydrate metabolism, drug detoxification and calcium storage in eukaryotic cells (Ricciardi & Gnudi, 2020). The accumulation of misfolded proteins in the ER in response to stimuli such as oxidative stress, metabolic and ischemic damage leads to endoplasmic reticulum stress (Akaras et al., 2024a; Foufelle & Fromenty, 2016; Kandemir et al., 2021).

ER stress triggers the unfolded protein response (UPR), activating PKR-like ER kinase (PERK), transcription factor 6 (ATF6), and inositol-requiring enzyme 1 (IRE1) to maintain ER homeostasis. When the UPR response is prolonged, cells enter the apoptosis process, leading to significant tissue damage (Akaras et al., 2023c; Akaras et al., 2024b; Kandemir et al., 2021). This response is facilitated by PERK, which reduces the influx of proteins into the ER, alleviating its burden, while ATF6 promotes protein folding and translation, thereby reducing protein accumulation within the ER (Yuan et al., 2024). A study suggested that, alongside important mechanisms such as mitochondrial dysfunction and oxidative stress, endoplasmic reticulum (ER) stress may play a critical role in drug-induced cellular damage, leading to harmful effects such as lipid accumulation, cytolysis, cell death, and inflammation (Foufelle & Fromenty, 2016). In the current study, it was observed that DCL increased the mRNA transcript levels of the ATF-6 and PERK genes, which, consistent with the literature, led to ER stress (Varışlı et al., 2023). Considering the connection between ER stress and oxidative stress, it was hypothesized that RUT could be effective against ER stress; results indicated that RUT could indirectly alleviate ER stress by suppressing oxidative stress. This is supported by the significant reduction in the mRNA transcript levels of the ATF-6 and PERK genes in kidney tissue following RUT treatment. This is thought to be due to the possible reason that DCL-induced ROS causes the accumulation of misfolded proteins due to the acceleration of oxidation of cysteine residues during peroxidation of polyunsaturated fatty acids and formation of disulfide bonds in the ER, thus causing ER stress. Nevertheless, RUT may have reversed this mechanism in the ER by clearing ROS from the kidneys of rats.

The histopathological examination of the kidney plays a critical role in detecting DCL-induced kidney damage. Histopathological evaluations revealed structural abnormalities in kidney tissues from rats, including significantly shrunken and atrophic glomeruli in the cortical labyrinth, expanded Bowman spaces, vascular congestion, prominent edema in the interstitium, and inflammatory cell infiltration. Additionally, degenerative changes such as loss of brush borders in proximal and distal renal tubules, epithelial cell degeneration, and accumulation of eosinophilic material in tubular lumens were observed. The histopathological findings of our study indicate that RUT treatment contributes to the repair of these structural damages caused by DCL.

Conclusion

Our biochemical and histopathological findings strongly confirm that DCL induces toxicity in the kidneys through oxidative stress, inflammation, apoptosis, and endoplasmic reticulum (ER) stress. Additionally, it has been identified that this toxicity leads to a significant decrease in aquaporin-2 (AQP-2) levels. Furthermore, it has been concluded that the antioxidant, anti-inflammatory, and anti-apoptotic effects of RUT are effective against DCL-induced nephrotoxicity, suggesting that RUT holds promise as a potential therapeutic agent for the treatment of kidney toxicity. However, further comprehensive studies are needed to fully understand the underlying molecular mechanisms of RUT's effects.

Ethics Committee Approval: Ethics committee approval was received for this study from the Necmettin Erbakan University, Decision No: 2024-079, Date: 25.09.2024.

Author Contributions: Concept - R.O.K., N.A., F.M.K.; Design - R.O.K., N.A., Ö.K., H.Ş., F.M.K.; Supervision - R.O.K., N.A., F.M.K.; Resources - R.O.K., N.A., Ö.K., H.Ş., F.M.K.; Materials - R.O.K., N.A., Ö.K., H.Ş., F.M.K.; Data Collection and/or Processing - R.O.K., N.A., Ö.K., H.Ş., F.M.K.; Analysis and/or Interpretation - R.O.K., N.A., Ö.K., H.Ş., F.M.K.; Literature Search - R.O.K., N.A., Ö.K., H.Ş., F.M.K.; Writing Manuscript - R.O.K., N.A.;Critical Review - R.O.K., N.A., Ö.K., H.Ş., F.M.K.; Other – R.O.K., N.A., Ö.K., H.Ş., F.M.K.

Peer-review: Externally peer-reviewed.

Funding: Financial support has not been received.

Declaration of Interests: The authors declare that they have no competing interest.

References

Abiola, Ti.S., Adebayo, O.C., & Babalola, O.O. (2019) Diclofenac-induced kidney damage in wistar rats: involvement of antioxidant mechanism. *Journal of Biosciences and Medicines, 7*, 44-57. https://doi.org/10.4236/jbm.2019.712005

Akaras, N., Kandemir, F. M., Şimşek, H., Gür, C., & Aygörmez, S. (2023a). Antioxidant, antiinflammatory, and antiapoptotic effects of rutin in spleen toxicity induced by sodium valproate in rats. *Türk Doğa Ve Fen Dergisi, 12*(2), 138-144. https://doi.org/10.46810/tdfd.1299663

Akaras, N., Gur, C., Kucukler, S., & Kandemir, F. M. (2023b). Zingerone reduces sodium arsenite-induced nephrotoxicity by regulating oxidative stress, inflammation, apoptosis and histopathological changes. *Chemico-Biological Interactions, 374*, 110410. https://doi.org/10.1016/j.cbi.2023.110410

Akaras, N., Ileriturk, M., Gur, C., Kucukler, S., Oz, M., & Kandemir, F. M. (2023c). The protective effects of chrysin on cadmium-induced pulmonary toxicity; a multi-biomarker approach. *Environmental Science and Pollution Research İnternational, 30*(38), 89479–89494. https://doi.org/10.1007/s11356-023-28747-8

Akaras, N., Gür, C., Caglayan, C., & Kandemir, F. M. (2024a). Protective effects of naringin against oxaliplatin-induced testicular damage in rats: Involvement of oxidative stress, inflammation, endoplasmic reticulum stress, apoptosis, and histopathology. *Iranian Journal of Basic Medical Sciences, 27*(4), 466-474. https://doi.org/10.22038/IJBMS.2024.73824.16048

Akaras, N., Kucukler, S., Gur, C., Ileriturk, M., & Kandemir, F. M. (2024b). Sinapic acid protects against lead acetate-induced lung toxicity by reducing oxidative stress, apoptosis, inflammation, and endoplasmic reticulum stress damage. *Environmental Toxicology, 39*(7), 3820–3832. https://doi.org/10.1002/tox.24255

Akarsu, S. A., Gür, C., İleritürk, M., Akaras, N., Küçükler, S., & Kandemir, F. M. (2023). Effect of syringic acid on oxidative stress, autophagy, apoptosis, inflammation pathways against testicular damage induced by lead acetate. *Journal of Trace Elements in Medicine and Biology, 80*, 127315. https://doi.org/10.1016/j.jtemb.2023.127315

Alabi, Q. K., & Akomolafe, R. O. (2020). Kolaviron diminishes diclofenac-induced liver and kidney toxicity in wistar rats via suppressing inflammatory events, upregulating antioxidant defenses, and improving hematological indices. *Dose-Response, 18*(1), 1559325819899256. https://doi.org/10.1177/1559325819899256

Alhoshani, A. R., Hafez, M. M., Husain, S., Al-Sheikh, A. M., Alotaibi, M. R., Al Rejaie, S. S., Alshammari, M. A., Almutairi, M. M., & Al-Shabanah, O. A. (2017). Protective effect of rutin supplementation against cisplatin-induced Nephrotoxicity in rats. *BMC Nephrology, 18*(1), 194. https://doi.org/10.1186/s12882-017-0601-y

Caglayan, C., Kandemir, F. M., Darendelioğlu, E., Yıldırım, S., Kucukler, S., & Dortbudak, M. B. (2019). Rutin ameliorates mercuric chloride-induced hepatotoxicity in rats via interfering with oxidative stress, inflammation and apoptosis. *Journal of Trace Elements in Medicine and Biology, 56*, 60-68. https://doi.org/10.1016/j.jtemb.2019.07.011

Caglayan, C., Kandemir, F. M., Yildirim, S., Kucukler, S., & Eser, G. (2019). Rutin protects mercuric chloride-induced nephrotoxicity via targeting of aquaporin 1 level, oxidative stress, apoptosis and inflammation in rats. *Journal of Trace Elements in Medicine and Biology, 54*, 69-78. https://doi.org/10.1016/j.jtemb.2019.04.007

Chen, L., Deng, H., Cui, H., Fang, J., Zuo, Z., Deng, J., Li, Y., Wang, X., & Zhao, L. (2017). Inflammatory responses and inflammation-associated diseases in organs. *Oncotarget, 9*(6), 7204–7218. https://doi.org/10.18632/oncotarget.23208

Çomaklı, S., Kandemir, F. M., Küçükler, S., & Özdemir, S. (2022). Morin mitigates ifosfamide induced nephrotoxicity by regulation of NF-kappaB/p53 and Bcl-2 expression. *Biotechnic & Histochemistry, 97*(6), 423–432. https://doi.org/10.1080/10520295.2021.2021449

Çömez, M., Cellat, M., Kuzu, M., Uyar, A., Türk, E., Kaya, Y. S., Etyemez, M., Gökçek, İ., & Güvenç, M. (2024). The effect of tyrosol on diclofenac sodium-induced acute nephrotoxicity in rats. *Journal of Biochemical and Molecular Toxicology, 38*(1), e23582. https://doi.org/10.1002/jbt.23582

Foufelle, F., & Fromenty, B. (2016). Role of endoplasmic reticulum stress in drug-induced toxicity. Pharmacology *Research & Perspectives, 4*(1), e00211. https://doi.org/10.1002/prp2.211

Guo, Q., Jin, Y., Chen, X., Ye, X., Shen, X., Lin, M., Zeng, C., Zhou, T., & Zhang, J. (2024). NF-κB in biology and targeted therapy: new insights and translational implications. *Signal Transduction and Targeted Therapy, 9*(1), 53. https://doi.org/10.1038/s41392-024-01757-9

Gür, C., & Kandemir, F. M. (2023). Molecular and biochemical investigation of the protective effects of rutin against liver and kidney toxicity caused by malathion administration in a rat model. *Environmental Toxicology, 38*(3), 555-565. https://doi.org/10.1002/tox.23700

Hickey, E. J., Raje, R. R., Reid, V. E., Gross, S. M., & Ray, S. D. (2001). Diclofenac induced in vivo nephrotoxicity may involve oxidative stress-mediated massive genomic DNA fragmentation and apoptotic cell death. *Free Radical Biology and Medicine, 31*(2), 139-152. https://doi.org/10.1016/S0891-5849(01)00560-3

Kandemir, F. M., Ileriturk, M., & Gur, C. (2022). Rutin protects rat liver and kidney from sodium valproate-induce damage by attenuating oxidative stress, ER stress, inflammation, apoptosis and autophagy. *Molecular Biology Reports, 49*(7), 6063-6074. https://doi.org/10.1007/s11033-022-07395-0

Kandemir, F. M., Caglayan, C., Aksu, E. H., Yildirim, S., Kucukler, S., Gur, C., & Eser, G. (2020). Protective effect of rutin on mercuric chloride-induced reproductive damage in male rats. *Andrologia, 52*(3), e13524. https://doi.org/10.1111/and.13524

Kandemir, F. M., Ileriturk, M., & Gur, C. (2021). Rutin protects from destruction by ınterrupting the pathways playing a role in the possible damage mechanism of sodium valproate in the liver and kidney tissues of rats. *Research Square*. https://doi.org/10.21203/rs.3.rs-1184419/v1

Kankılıç, N. A., Şimşek, H., Akaras, N., Gür, C., İleritürk, M., Küçükler, S., Akarsu, S. A., & Kandemir, F. M. (2024). Protective effects of naringin on colistin-induced damage in rat testicular tissue: Modulating the levels of Nrf-2/HO-1, AKT-2/FOXO1A, Bax/Bcl2/Caspase-3, and Beclin-1/LC3A/LC3B signaling pathways. *Journal of Biochemical and Molecular Toxicology, 38*(2), e23643. https://doi.org/10.1002/jbt.23643

Karağaç, M. S., Yeşilkent, E. N., Kizir, D., Öztürk, N., Isıyel, M., Karadaş, H., Tosun, H., Karaman, M., Ceylan, H., & Demir,

Y. (2024). Esculetin improves inflammation of the kidney via gene expression against doxorubicin-induced nephrotoxicity in rats: In vivo and in silico studies. *Food Bioscience, 62*, 105159. https://doi.org/10.1016/j.fbio.2024.105159

Keleş, O.N., Serpil, C., Çığşar, G., Çolak, S., Erol, H.S., Akaras, N., Erdemci, B., Bilgin, B.Ç., Can, İ., Ünal, B., & Halıcı, M.B. (2014) Hepatoprotective effects of B-1, 3-(D)- glucan on bortezomib-induced liver damage in rats. *Kafkas Universitesi Veteriner Fakultesi Dergisi, 20*(6):929-38. https://doi.org/10.9775/kvfd.2014.11413

Kim, S. Y., & Moon, A. (2012). Drug-induced nephrotoxicity and its biomarkers. *Biomolecules & Therapeutics, 20*(3), 268-272. https://doi.org/10.4062/biomolther.2012.20.3.268

Kwon, T. H., Frøkiær, J., & Nielsen, S. (2013). Regulation of aquaporin-2 in the kidney: A molecular mechanism of body-water homeostasis. *Kidney Research and Clinical Practice, 32*(3), 96-102. https://doi.org/10.1016/j.krcp.2013.07.005

Livak, K. J., & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods, 25*(4), 402–408. https://doi.org/10.1006/meth.2001.1262

Musah, S., Bhattacharya, R., & Himmelfarb, J. (2024). Kidney disease modeling with organoids and organs-on-chips. *Annual Review of Biomedical Engineering, 26*. https://doi.org/10.1146/annurev-bioeng-072623-044010

Prince, S. E. (2018). Diclofenac-induced renal toxicity in female Wistar albino rats is protected by the pre-treatment of aqueous leaves extract of Madhuca longifolia through suppression of inflammation, oxidative stress and cytokine formation. *Biomedicine & Pharmacotherapy, 98,* 45-51. https://doi.org/10.1016/j.biopha.2017.12.028

Ricciardi, C. A., & Gnudi, L. (2020). The endoplasmic reticulum stress and the unfolded protein response in kidney disease: Implications for vascular growth factors. *Journal of Cellular and Molecular Medicine, 24*(22), 12910-12919. https://doi.org/10.1111/jcmm.15999

Sirotkin, A. V. (2024). Positive effects of rutin on female reproduction. *Reproduction in Domestic Animals, 59*(2), e14540. https://doi.org/10.1111/rda.14540

Sivaraj, R., & Umarani, S. (2018). Diclofenac-induced biochemical changes in nephrotoxicity among male Albino rats. *International Journal of Basic & Clinical Pharmacology, 7*(4), 640-643. https://doi.org/10.18203/2319-2003.ijbcp20181162

Şimşek, H., Akaras, N., Gür, C., Küçükler, S., & Kandemir, F. M. (2023). Beneficial effects of Chrysin on Cadmium-induced nephrotoxicity in rats: Modulating the levels of Nrf2/HO-1, RAGE/NLRP3, and Caspase-3/Bax/Bcl-2 signaling pathways. *Gene, 875*, 147502. https://doi.org/10.1016/j.gene.2023.147502

Tanyeli, A., Eraslan, E., Güler, M., Kurt, N., & Akaras, N. (2020). Gossypin protects against renal ischemia-reperfusion injury in rats. *Kafkas Universitesi Veteriner Fakultesi Dergisi, 26*(1). https://doi.org/10.9775/kvfd.2019.22396

Taştan Bal, T., Akaras, N., Demir, Ö., & Ugan, R. A. (2023). Protective effect of astaxanthin and metformin in the liver of rats in which the polycystic ovary syndrome model was formed by giving letrozole. *Iranian Journal of Basic Medical Sciences, 26*(6), 688–694. https://doi.org/10.22038/IJBMS.2023.68032.14872

Thai, P. N., Ren, L., Xu, W., Overton, J., Timofeyev, V., Nader, C. E., Haddad, M., Yang, J., Gomes, A. V., Hammock, B. D., Chiamvimonvat, N., & Sirish, P. (2023). Chronic Diclofenac Exposure Increases Mitochondrial Oxidative Stress, Inflammatory Mediators, and Cardiac Dysfunction. *Cardiovascular Drugs and Therapy, 37*(1), 25–37. https://doi.org/10.1007/s10557-021-07253-4

Uehara, Y., Murata, Y., Shiga, S., & Hosoi, Y. (2016). NSAIDs diclofenac, indomethacin, and meloxicam highly upregulate expression of ICAM-1 and COX-2 induced by X-irradiation in human endothelial cells. *Biochemical and Biophysical Research Communications, 479*(4), 847-852. https://doi.org/10.1016/j.bbrc.2016.09.120

Varışlı, B., Caglayan, C., Kandemir, F. M., Gür, C., Ayna, A., Genç, A., & Taysı, S. (2023). Chrysin mitigates diclofenac-induced hepatotoxicity by modulating oxidative stress, apoptosis, autophagy and endoplasmic reticulum stress in rats. *Molecular Biology Reports, 50*(1), 433-442. https://doi.org/10.1007/s11033-022-07928-7

Wadie, W., Abdel-Razek, N. S., & Salem, H. A. (2021). Phosphodiesterase (1, 3 & 5) inhibitors attenuate diclofenac-induced acute kidney toxicity in rats. *Life Sciences, 277*, 119506. https://doi.org/10.1016/j.lfs.2021.119506

Wang, L., Sun, W., Ma, X., Griffin, N., & Liu, H. (2024). Perfluorooctanoic acid (PFOA) exposure induces renal filtration and reabsorption disorders via down-regulation of aquaporins. *Toxicology Letters, 392*, 22-35. https://doi.org/10.1016/j.toxlet.2023.12.003

Yuan, S., She, D., Jiang, S., Deng, N., Peng, J., & Ma, L. (2024). Endoplasmic reticulum stress and therapeutic strategies in metabolic, neurodegenerative diseases and cancer. *Molecular Medicine, 30*(1), 40. https://doi.org/10.1186/s10020-024-00808-9

ATATÜRK ÜNİVERSİTESİ YAYINLARI
ATATURK UNIVERSITY PUBLICATIONS

Research Article *Araştırma Makalesi* DOI: 10.62425/jlasp.1639844

How Is the Monitoring of the Health Status of Laboratory Animals Conducted in Our Country and Is the Awareness of Researchers on This Issue Sufficient?*

Ülkemizde Deney Hayvanlarının Sağlık Durumu İzlemi Nasıl Yürütülüyor ve Bu Konu Hakkında Araştırmacıların Farkındalığı Yeterli mi?*

Ayşe Meltem SEVGİLİ[1]
Burak KAYABAŞI[1]
Yakup TATAR[2]

[1]Department of Physiology, Gazi University, Faculty of Medicine, Ankara, Türkiye
[2]Department of Physiology, TOBB Economy and Technology University, Faculty of Medicine, Ankara, Türkiye

ABSTRACT

The microbiological status of laboratory animals can substantially influence research outcomes. Although legal regulations mandate the supervision of laboratory animal health, to our knowledge, no study has been conducted to examine how laboratory animal units carry out health monitoring of the animals. Similarly, researchers' interests and attitudes regarding this issue is unknown. With our study, we aimed to fill these gaps. We used two separate questionnaires. The first aimed to gather information about health screening programs in approved laboratory animal units, while the second aimed to assess researchers' knowledge and attitudes regarding animal health monitoring programs. Out of the 120 units in our country 42 (35%) contributed to our study. Over 60% of these laboratory animal units lack microbiological monitoring practices. Out of 8 units housing specific pathogen-free animals, 7 adhere to the required microbiological monitoring program. While 85% of the units conducting microbiological monitoring perform fecal examinations, only 53% serologically analyses the blood. Overall, our researchers' knowledge levels and awareness regarding the microbiological monitoring of laboratory animals are inadequate. Meanwhile, researchers who held a certificate had slightly higher awareness compared to those who did not. In summary, it has been observed that the microbiological monitoring of laboratory animals in our country is inadequate, and researchers don't request sufficient information from the units on this issue. Training programs, including undergraduate or postgraduate courses, may prove more effective than certificate programs. Additionally, it would be beneficial to incorporate provisions in our legislation that outline the methodology for microbiological monitoring of conventional animals.

Keywords: Laboratory animals, Laboratory animal units, Microbiological monitoring.

ÖZ

Deney hayvanlarının mikrobiyolojik durumu, araştırma verilerini önemli düzeyde etkileyebilir. Ülkemizde yasal düzenlemeler deney hayvan sağlığının gözetimini zorunlu tutmakla birlikte bildiğimiz kadarı ile günümüze kadar deney hayvanı ünitelerinin hayvanların sağlık gözlemini nasıl yürüttüklerini inceleyen bir çalışma yapılmamıştır. Benzer şekilde ülkemizde araştırmacıların bu konuya olan ilgi ve tutumlarını değerlendiren bir çalışma da bulunmamaktadır. Çalışmamız ile bu boşlukları doldurmayı amaçladık. Çalışmada iki ayrı anket kullandık. İlki onaylı deney hayvan ünitelerinin sağlık tarama programları hakkında bilgi toplamayı amaçlarken, ikincisi araştırmacıların yürütülmesi gereken hayvan sağlık izlem programlarıyla ilgili bilgi düzeyi ve tutumlarının değerlendirmesini amaçlamaktaydı. Ülkemizde bilimsel amaçlar ile sık kullanılan kemirgenleri bulunduran 120 üniteden 42 (%35)'si çalışmamıza katkı sağlamıştır. Bu deney hayvanları ünitelerinin en az %60'ı hiçbir şekilde mikrobiyolojik gözetim yapmamaktadır. Spesifik patojen ari (Specific Pathogen Free-SPF) hayvan bulunduran 8 üniteden 7'si mevzuatın öngördüğü mikrobiyolojik gözetim programını uygulamaktadır. Mikrobiyolojik izlem yapan ünitelerin %85'i gaita incelemesi yaparken ancak %53'ü kandan serolojik analiz yapmaktadır. Genel olarak araştırmacıların deney hayvanlarının mikrobiyolojik gözetimi konusundaki bilgi düzeyleri ve farkındalıklarının yeterli olmadığı söylenebilir. Diğer taraftan sertifika sahibi olan araştırmacıların olmayanlara göre farkındalıkları biraz daha yüksekti. Sonuç olarak ülkemizde deney hayvanlarının mikrobiyolojik izleminin yeterli düzeyde olmadığı ve araştırmacıların bu konuda ünitelerden yeterli bilgi talep etmedikleri görülmüştür. Bu konularda gelişme sağlanması için sertifika programlarından öte daha geniş kapsamlı lisans ya da lisans üstü ders gibi eğitimler daha verimli olabilir. Ayrıca mevzuatımıza konvansiyonel hayvanların mikrobiyolojik izleminin nasıl yapılması gerektiğini belirleyecek maddeler eklenmesinin faydası olacaktır.

Anahtar kelimeler: Deney hayvanları, Deney hayvanları üniteleri, Mikrobiyolojik izlem.

*The data from this study were presented as an oral presentation at the 5th National Congress of Laboratory Animal Science (September 14–16, 2023 – Istanbul).
*Bu çalışmaya ait veriler 5. Ulusal Laboratuvar Hayvanları Bilimi Kongresinde (14-16 Eylül 2023 – İstanbul) sözlü sunum olarak sunulmuştur.

Geliş Tarihi/Received :14.02.2025
Kabul Tarihi/Accepted :03.07.2025
Yayın Tarihi/Publication Date :19.09.2025

Sorumlu Yazar/Corresponding author: Ayşe Meltem Sevgili
E-mail: msevgili@gazi.edu.tr

Cite this article: Sevgili, A.M., Kayabası, B., & Tatar, Y. (2025). How Is the Monitoring of the Health Status of Laboratory Animals Conducted in Our Country and Is the Awareness of Researchers on This Issue Sufficient?. *Journal of Laboratory Animal Science and Practices*, 5(2), 92-101. https://doi.org/10.62425/jlasp.1639844

Introduction

In any scientific research, investigators bear the responsibility to adhere to ethical principles throughout the research process and to ensure the validity and reliability of the data they present. Obtaining reliable and valid data begins with the rigorous control of all known variables to the greatest extent possible, excluding the variable under investigation. The most significant of these is the standardization of experimental animals that enables highly reproducible results (Çelik et al., 2023). Initiating scientific studies involving experimental animals with healthy specimens is a fundamental principle. Consequently, health monitoring (HM) programs for laboratory rodents must serve as a foundational step in preserving animal health and ensuring the validity of biomedical research data (Burkholder et al., 2012). On the other hand, the ethical importance of health monitoring in laboratory animals should not be overlooked. The 3R principle, which constitutes the foundation of ethical standards for the use of laboratory animals, emphasizes the implementation of alternative experimental methodologies whenever feasible (Replacement), the minimization of the number of animals used in research without compromising the scientific value of the study (Reduction), and the assurance of animal welfare before, during, and after the experimental procedures (Refinement) (Çelik et al., 2023; Tüfek & Özkan, 2018). The standardization achieved through regular health monitoring of laboratory animals contributes to reducing data variability in experimental outcomes, thereby enabling a reduction in the number of animals required for research (Öbrink et al., 2000). Furthermore, ensuring optimal health is essential for the welfare of laboratory animals and represents a fundamental aspect of animal rights (Ergün, 2011).

Publications on pathogens affecting laboratory animals began in 1947 and have expanded over the years both in number and scope. The increase in these publications first led to the establishment of the Animal Care Panel in 1950, followed by the International Council for Laboratory Animal Science (ICLAS) in 1956, the American Association for Laboratory Animal Science (AALAS) in 1967, and the Federation of European Laboratory Animal Science Associations (FELASA) in 1978. In 2011 and 2014, through the collaborations established by FELASA with ICLAS and AALAS, all aspects related to the use of laboratory animals in experiments were assessed within a scientific framework, and efforts were made to develop a common language. FELASA's most recent revision of its recommendations on the microbiological health monitoring of laboratory animals was published in 2014 (Mähler et al., 2014).

Numerous groups of microorganisms are responsible in the occurrence of infections in rodents and rabbits. Most infections do not manifest with overt clinical symptoms. Therefore, the absence of clinical signs holds only limited diagnostic value. Numerous examples demonstrate the influence of microbes on the physiology of experimental animals, encompassing behavior, growth rate, relative organ mass, and immunological response. Effects of many organisms on research outcomes are reviewed elsewhere (Baker, 1998; Connole et al., 2000; Nicklas et al., 1999). Infections, regardless of the presence of clinical symptoms, can confound scientific outcomes, increase biological and experimental variability, and lead to a greater demand for animal use. For instance, *mouse hepatitis virus*, despite causing no clinical signs in immunocompetent animals, can alter liver enzyme levels, induce anemia or leukopenia, and even reduce the incidence of diabetes (Nicklas et al., 1999). Likewise, *Trichosomoides crassicauda*, a widely prevalent urinary tract parasite in rats, often remains undetected due to the lack of overt clinical symptoms. However, its ability to induce hematuria can compromise the reliability of findings, particularly in studies focusing on the urinary system (Sevgili et al., 2010). Moreover, certain infections in laboratory animals can also be transmitted to humans (zoonoses) (Colby & Zitzow, 2018; Gül et al., 2013; Uçak, 2024).

Therefore, regular health monitoring of laboratory animals is essential and highly valuable. Our study aims to examine the current status of laboratory animal health monitoring in our country and to evaluate researchers' approaches toward this issue.

Methods

Approval for this study was obtained from the Gazi University Clinical Research Ethics Committee (27.01.2020-117). Our study was conducted using two separate surveys. The first survey aimed to assess how units approved by the Ministry of Agriculture and Forestry in our country conduct the health monitoring of laboratory animals. The survey, consisting of 24 questions, was prepared and administered via Google Forms. In designing the survey questions, the current legal framework served as the primary reference point, and detailed questions related to microbiological monitoring were incorporated (Deneysel ve Diğer Bilimsel Amaçlar Için Kullanılan Hayvanların Refah ve Korunmasına Dair Yönetmelik, 2011). The survey included questions assessing the conformity of the physical infrastructure of laboratory animal units with legislative requirements, the frequency, responsible personnel, and methods of general health monitoring of animals, whether these monitoring activities were conducted within the framework of the strategic plan and surveillance program mandated by the legislation, and the frequency and methodologies employed for microbiological monitoring.

As of January 2023, a total of 192 laboratory animal

facilities had been granted operational permits, with 120 of them (%62.5) housing rodent species such as mice, rats, guinea pigs, and rabbits. Rest of the facilities (72 out of 192) are housing either aquamarine species or veterinary clinics. The survey, along with the ethics committee approval for our study, was sent to all units using the email addresses provided in the list published by the Ministry of Agriculture and Forestry. One month later, units that had not yet responded, called individually. Direct contact was successfully established with 74 of the units. During the consultations, the objectives of the study were explained, and efforts were made to encourage unit managers to participate in the survey. The survey remained accessible on Google Forms for an additional three months, after which the data were retrieved from the system for analysis.

The second survey was designed to assess researchers' level of awareness regarding the subject. Comprising 15 questions, the survey was developed and administered via Google Forms. This survey included questions evaluating the demographic characteristics of the participating researchers, their research backgrounds, their awareness of general health monitoring of laboratory animals, their experiences related to health monitoring of laboratory animals in the studies they participated in, and their awareness of the standards of the training they received. The survey was disseminated through multiple channels, a total of 99 participants completed the survey.

Statistical Analysis

For the statistical evaluation, the Jamovi software was utilized. In addition to descriptive analyses, the Mann-Whitney U test was applied for pairwise group comparisons, while the Kruskal-Wallis test was used for multiple group comparisons. Furthermore, Pearson correlation analysis was conducted to examine relationships between variables. The difference was considered statistically significant when $p < .05$.

Results

Despite extensive efforts, direct contact was successfully established only with 74 out of 120 (%58) facilities housing commonly used rodents in research. Among these, a total of 42 facilities (35%) participated in the study. The majority of these facilities (71.4%) were affiliated with public universities. Six responding facilities belonged to private institutions outside universities, representing all (100%) of the private units authorized by the Ministry of Agriculture and Forestry to house laboratory animals. When evaluated in terms of the care and housing rooms required by the regulations, the physical conditions of the 42 participating facilities were generally found to be adequate. However, it was determined that 13 facilities (31%) lacked a designated room for housing sick and injured animals, 10 facilities (24%) did not have a post-operative care room, 9 facilities (21%) were missing an operating room, and 8 facilities (19%) lacked a preparation room for procedures.

It was determined that general health monitoring of the laboratory animals was conducted at least once per day in 39 of the 42 (92.8%) participating facilities. Among the remaining units, one facility performed general health checks every three days, while two facilities conducted these assessments on a weekly basis. In 40 (95.2%) of the facilities, these evaluations were carried out either directly by a veterinarian or under veterinary supervision, whereas in two facilities, general health monitoring was solely the responsibility of veterinary technicians.

In alignment with regulatory requirements, 95.1% (39/42) of the participating facilities reported having a strategy for regular health monitoring of animals, while 73.2% (30/42) had a defined strategy for new animal intake procedures. However, only 48.8% (20/42) of the facilities had a strategy about health disorder management program in place, and merely 31.7% (13/42) had a strategy for microbiological surveillance program.

While 2 of the 13 units (15.3%) with a “microbiological surveillance program strategic plan” did not have a “microbiological surveillance program” and did not perform microbiological surveillance, it was noteworthy that there were 5 units that had and implemented a microbiological surveillance program despite not having a strategic plan, and 1 unit that performed microbiological surveillance despite not having both a strategic plan and a surveillance program (Figure 1). The total number of units conducting microbiological surveillance amounted to 17, representing only 40.5% of the survey participants.

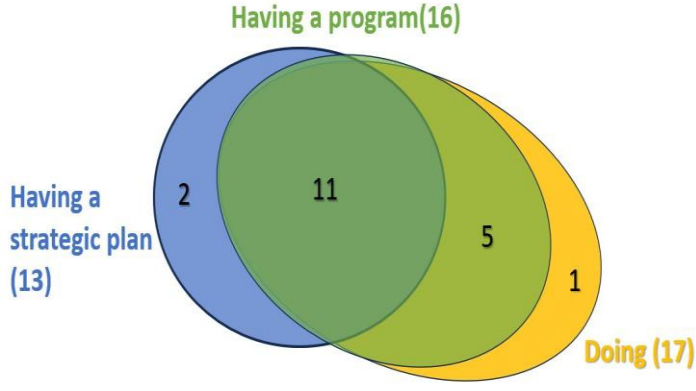


Figure 1. Microbiological surveillance status of the units.

Şekil 1. Ünitelerin mikrobiyolojik gözetim durumu.

When asked about the types of materials used for microbiological surveillance, it was observed that two units

that had previously stated they did not conduct microbiological surveillance identified material types, while one unit that had indicated performing microbiological surveillance did not specify any material type. The distribution of the 17 (40.5%) units that provided material descriptions is presented in Table 1. A particularly notable response among those provided to this question was from a unit that had previously indicated not conducting microbiological surveillance, stating: "Since we are a supplier organization, the researchers responsible for the study and the institutions providing the laboratory animals conduct the examinations."

Table 1: Distribution of Material Types Used for Microbiological Surveillance.

Tablo 1: Mikrobiyolojik gözetim amacı ile kullanılan materyal tiplerinin dağılımı.

Among the 13 (30.9) units that collected blood samples, 9 (%21.4) conducted screenings using microbiological ELISA

Blood Only	4 units
Feces Only	4 units
Feces + Saliva	1 units
Blood + Feces	3 units
Blood + Feces + Oral Mucosa + Fur Samples	1 units
Blood + Feces + Urine	2 units
Blood + Feces + Urine + Environmental Monitoring	1 units
Blood + Feces + Sentinel Animal (Blood + Feces + Fur Samples + Oral Mucosa)	1 units

or PCR, whereas the remaining four performed biochemical screenings and/or blood counts; therefore, they did not conduct a microbiological evaluation. 13 (30.9) units collecting fecal samples analyzed them for internal parasites.

While three units didn't respond to "Do you conduct microbiological monitoring during the admission process for new animals that you receive?" question, 23 out of 42 unites (55%) answered "no." Of the units that responded "yes" (38%; 16/42), 14 of them also conducted microbiological surveillance on their own animals, while two units stated that, despite the absence of routine microbiological monitoring within the facility, such monitoring was performed specifically for newly admitted animals. The final questions of our survey directed at laboratory animal facilities were: "Does your facility house Specific Pathogen-Free (SPF) animals?" and, if so, "Are you able to properly implement the screening program specified in the regulatory guidelines for SPF animals?". Among the surveyed facilities, only 19% (8/42) reported housing SPF animals, and nearly all of these facilities (7/8) indicated that they were able to conduct the required inspections in accordance with regulatory standards.

Additionally, our study assessed researchers' awareness of the health status of laboratory animals through a second survey. A total of 99 researchers participated in the survey. Of the respondents, 70% were female, and interest from early-career researchers was notably higher (Figure 2).

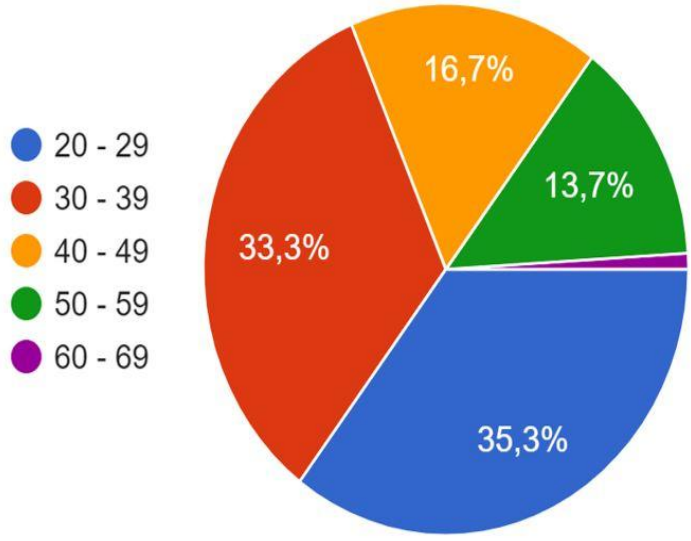


Figure 2. Age distribution of participants.

Şekil 2. Katılımcıların yaş dağılımları.

Among the participants, 86% were employed in medical faculties or research hospitals. While 68% held a laboratory animal use certificate, 8% of these individuals had never participated in any research studies. A total of 70% of all participants had been involved in studies utilizing laboratory animals, with 47% of these individuals having participated in such studies for over six years, while only 5.1% had been involved for a duration of less than six months. The responses to the question designed to assess researchers' awareness levels regarding the animals that could be used in scientific research are presented in Table 2.

Table 2: Researchers' awareness levels regarding animals that can be used in scientific studies.

Tablo 2: Araştırmacıların bilimsel çalışmalarda kullanılabilecek hayvanlarla ilgili farkındalık düzeyi.

Animal Characteristics	Usable (Number of People)	Not Usable (Number of People)
Stray animals (cats, dogs, etc.)	13	79
Owned domestic animals (cats, dogs, rabbits, etc.)	12	80
Owned farm animals (cows, sheep, chickens, etc.)	17	76
Wild-caught animals (frogs, birds, mice)	24	70
Animals obtained from experimental animal breeders	98	1
Genetically modified animals	78	15
Animals with an infectious condition	16	76
Animals that are carriers of an infectious agent	25	68
Animals infested with endoparasites	20	72
Animals infested with ectoparasites	20	73
Pathogen-free animals	80	16

* The areas highlighted with a gray background in the table indicate the usage status of animals with the specified characteristics according to our legislation.

The areas marked with a gray background in the table indicate the use of animals with the specified characteristics for experimental and other scientific purposes according to our regulations. Among the 11 characteristics defined in the question, the majority of our researchers chose the correct option according to our regulations for 5 of them, while the vast majority leaned towards the incorrect option for 6 of them. Overall, more than 70% of our participants believe that no animals, whether owned or unowned, should be used in scientific studies except for that breed by animal research units. Similarly, more than 68% of the participants believed that laboratory animals carrying pathogens could not be used in experiments. When the data were classified based on whether researchers held a laboratory animal use certificate, no significant difference was observed between the groups (p> .05). Likewise, prior experience in studies involving laboratory animals did not result in any significant differences between groups (p> .05). Analyses conducted based on age distribution also did not reveal any significant variations (p> .05). The only statistically significant difference we were able to identify was related to the use of owned animals, where women expressed the opinion that they were "not usable" at a higher rate than men (p< .05).

In response to the question "How often would you like the general health status of the animals to be checked at a minimum?" 64 participants answered "daily" (Figure 3). Forty-five of these individuals were certificate holders. Therefore, only 66% of the certified researchers deemed daily checks necessary, as required by the regulations. When the data were analyzed based on certification status, age distribution, gender, experience in laboratory animal use, and duration of laboratory animal use among experienced researchers, none of these factors created a statistically significant difference.

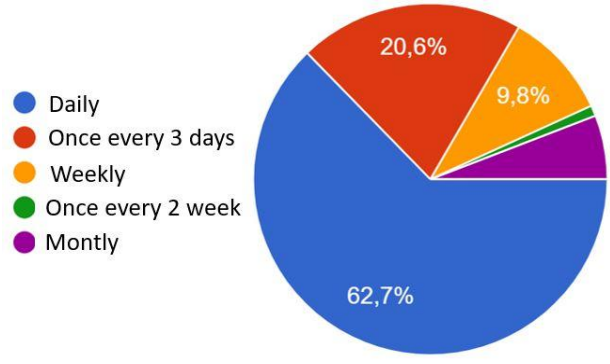


Figure 3. Researchers' expectations regarding the frequency of general health monitoring of animals.

Şekil 3. Araştırmacıların hayvanların genel sağlık durumu izleminin sıklığı hakkında beklentisi.

In response to the question "When using laboratory animals, do you obtain information from the producer/supplier regarding the health status of the animals?", 26 participants answered "no". Among the respondents, two individuals answered the follow-up question, "If your answer to the previous question is YES, what type of data is provided regarding the health status of the animals?" They indicated receiving information on Age and Species/Breed. Additionally, one participant reported receiving microbiological screening data and genetic information. All 66 participants who responded "yes" to the initial question indicated that they were provided with information on Age, Sex, and Species/Breed. Among these respondents, 29 researchers reported receiving additional data on the genetic characteristics of the animals, 18 indicated that microbiological screening data were provided, and four noted that they obtained information on general health status assessments. Interestingly, 7 researchers who held a laboratory animal use certificate and had participated in studies involving animal experimentation left both questions unanswered.

To the question "Have you incidentally detected any health problems in the animals you used for experiments?", 26 researchers answered "yes'', while 24 answered "no'', and 49 researchers left it unanswered. The conditions observed by those who responded "yes'' are listed in Table 3.

Table 3: Health problems incidentally identified by researchers in experimental animals.

Tablo 3: Araştırmacıların deney hayvanlarında tesadüfen fark ettikleri sağlık problemleri.

Health Problem	Number of Reports
Infection	8
Parasite	5
Tumor – Cyst – Organ anomaly	8
Obesity	2
General debility	2
Pulmonary hemorrhage	1
Worse outcome in the control group	1
Sudden unexpected death	1

Only four participants (4%) answered "yes" to the questions "Did you feel the need to check whether the animals you would use carried any pathogens that could affect the study results?" and "Did you check whether the animals you would use carried any pathogens that could affect the study results?". All of the researchers who answered "yes" were women, and three of them were over the age of 50. Considering that 26 researchers had previously incidentally detected health problems in their studies, the notably low number of affirmative responses to these questions is particularly striking. Further analyses revealed that only three of the researchers who had incidentally encountered health problems felt the need to verify the presence of potential pathogens and carried out such checks. The number of researchers who left these questions unanswered was 56 and 58, respectively. Furthermore, some of the statements used by researchers when answering these questions were noteworthy.

- I did not feel the need to check whether the animals carried any pathogens that could affect the study results,

- "because I assume that this has already been checked"

- "but I have significant concerns about ectoparasites"

- I checked whether the animals carried any pathogens that could affect the study results,

- "because I think there is insufficient control"

- "A swab and culture were taken due to conjunctivitis, treatment was administered, but it was unrelated to the experimental setup and was not excluded from the study."

- "I worked with conventional animals, so there was already a possibility that they could be pathogen carriers. However, during the study, they did not exhibit any changes beyond the effects of the study protocol (such as piloerection, retreating to the corner of the cage, redness around the eyes, etc.)"

While creating the options for the question "What do you think the minimum training for researchers should include?", we used the 10 items from the 10th appendix of our regulation, "Minimum Training Standards for Those Using Animals in Procedures." 56% of all our participants, and 63% (43/68) of our certified participants, marked all the options. 33% of all our participants and 22% (25/68) of our certified participants found training on the use of humane endpoints unnecessary. The data is summarized in Table 4.

Table 4: Researchers' view on the minimum training content.

Tablo 4: Araştırmacıların asgari eğitim içeriği ile ilgili görüşleri.

	Not Necessary (Number of People)	
	Total	Certified
Animal behavior, housing, and enrichment	19	5***
Animal health management and hygiene	7	3
Anesthesia, analgesic methods, and euthanasia	8	0***
Alternative methods, reduction, and refinement requirements (3R)	21	9*
Designing Procedures and Projects Based on Appropriate Environment for Animals	23	10*
Use of humane endpoints	33	15**
Recognition of species-specific pain, distress, and suffering in common laboratory animals	11	3*
Basic knowledge of species-specific biology, anatomy, physiology, reproduction, genetics, and genetic modifications	12	7
Human-animal relationships, ethical considerations on the use of animals for scientific purposes, and ethical principles regarding the value of life	10	5
National legislation on the production, transportation, management, care, and use of animals for scientific purposes	17	8

* <0.05 ** <0.01 *** <0.001

Discussion

Perhaps the most significant finding of our study was the hesitancy of laboratory animal units and researchers to participate. Although we had obtained ethical approval and despite explicitly stating in the “voluntary consent form” that the identities of the units or participants would not be disclosed under any circumstances, we frequently encountered concerns such as “what if something happens to us for filling out this survey?” Unfortunately, the primary reason behind the limited participation—only 42 out of 120 rodent laboratory animal units—was this type of fear. Among the 74 units contacted directly, 32 units still chose not to participate. This led us to believe that, had they taken part, their responses would have further lowered the overall averages observed in our findings.

In general, the physical conditions of the units are a point that is strictly controlled according to our regulations, thus ensuring that the applying unit has sufficient infrastructure in terms of care and accommodation rooms before a work permit is granted. It was observed that all the units participating in our survey, despite some deficiencies, have sufficient physical conditions for a unit of their size. Our current regulations have also emphasized multiple times that the health status of laboratory animals should be monitored daily by veterinarians, and according to the results of our study, it appears that this legal requirement has been largely adhered to. On the other hand, approximately 70% of our units do not have a strategy and program for microbiological oversight, which is not questioned before granting work permits. This situation, despite being included in our legal regulations, shows that our units can be deficient in matters that are not questioned before granting work permits.

In our current legislation regarding laboratory animals, it is not specified which pathogens should be screened for laboratory animals other than microbiologically defined animals (conventional animals). However, many studies conducted in our country to date have shown that some of the pathogens recommended for screening in the FELASA guidelines have been detected in laboratory animals (Beyhan et al., 2010; Biyikoglu, 1996; Çetinkaya et al., 2017; İçil & Erbaş, 2024; Polat et al., 2024). Among these, İçil and Erbaş (İçil & Erbaş, 2024) demonstrated the presence of *Helicobacter species* in feces and colon samples through their screening in experimental animal units in the Aegean region. They detected *H. Typhlonius*, which is especially recommended for screening in FELASA guidelines, in 72% of the mice.

Most of the studies conducted in Türkiye have focused on the prevalence of parasitic species such as *Aspiculuris, Syphacia, Trichomonas,* and *Hymenolepsis.* In the study conducted by Beyhan and colleagues (Beyhan et al., 2010) on laboratory animals, a 100% prevalence of helminth infections was observed in the feces of mice and an 81% prevalence in the feces of rats. In these animals, *Syphacia species, Aspicularis tetraptera, Hymenolepis species,* and *Trichosomoides crassicauda* were identified. The identification of these species in studies conducted on laboratory animals in our country, as recommended for screening in the FELASA guidelines, indicates the need for a standardized screening program in this regard. However, our study has shown that these types of microbiological screenings are conducted by at most 40% of licensed units. This rate is already very low considering that the need is 100%, and when considering the hesitance and the rate of participation in the survey, it can even be seen as an optimistic figure. Another striking finding in our study was that out of the 17 units that claimed to conduct screenings, 13 performed stool examinations, and among them, 4 evaluated only stool samples. This situation can probably be attributed to the fact that direct fecal examination and internal parasite evaluation are almost cost-free and accessible, whereas the high cost of blood and culture medium-required studies is a significant factor. On the other hand, the fact that the only unit in our country conducting screenings using sentinel animals is a state university indicates that sufficient resources can be found when necessary for this process.

Of the units participating in the study, 2 do not conduct regular screenings on their own animals but have stated that they do so for animals newly joining the unit from outside. This situation could be a cost-related issue, or it might stem from the misconception that if the incoming animals are regularly checked, the source that could carry pathogens into the unit is already controlled, and the unit is being protected in this way. As the literature also shows, pathogen contamination in a rodent colony can originate not only from other rodents but also from the consumables used in the unit (bedding, feed, etc.) and from the staff (Lytvynets et al., 2013). The current FELASA recommendations on the microbiological monitoring of rodent colonies include periodic serological assessments using blood samples, internal parasite screening from fecal samples, external parasite screening from skin and fur samples, and necropsies on deceased animals. According to our study, there are only 2 units that meet these recommendations.

On the other hand, the fact that units housing Specific Pathogen-Free (SPF) animals—the only group for which microbiological monitoring requirements are explicitly defined in our legislation—are able to conduct this monitoring effectively demonstrates that microbiological surveillance can be successfully implemented when legally mandated and financial resources can be allocated when deemed necessary. In the second part of our study, at least 68% of participants indicated that they expect the animals used in their research to be “SPF” if not “pathogen free”. However, only 19% of the units participating in our study, or at most 7% of all officially approved units in the country, actually house SPF animals. Therefore, it would not be inaccurate to state that scientific research in our country is predominantly conducted using conventional animals.

A total of 99 researchers completed the second questionnaire, 68% of them were under the age of 40 suggests a high level of interest among younger researchers, which is an encouraging finding. On the other hand, the fact that 70% of the participants are women reveals that gender is also a factor in interest in this subject.

When the responses of our participants to the survey were examined, it was observed that their views on which animal species could be used in scientific studies were not always in accordance with our regulations. While our researchers' view that stray animals should not be used is in line with our regulations, their view that owned animals cannot be used is not. The inability to use owned animals in scientific studies could be a factor that severely restricts clinical research in veterinary medicine and could negatively affect their health by preventing access to experimental treatment options. On the other hand, while 75% of our researchers believe that animals that are carriers of infectious agents cannot be used in studies, this situation is quite common and even considered natural for conventional animals. Similarly, 20% or fewer of our researchers consider the use of infected or infected animals appropriate. This situation is neither realistic nor necessary. As we mentioned among our findings, two of our researchers have actually expressed the necessary scientific approach very correctly. While conjunctivitis does not constitute a valid reason for excluding an animal from a study as long as it is unrelated to the research subject and remains treatable, it is a correct approach to be aware that pathogens may be found in conventional animals. However, in the latter case, it should not be overlooked that certain pathogens capable of affecting all animals within the colony uniformly may introduce confounding factors into the study outcomes (Nicklas et al., 1999).

While a large majority of our researchers believe that pathogen-free animals should be used in studies, 66 participants reported obtaining information regarding

animal health status from suppliers. Considering the total number of researchers involved in experimental studies, this figure may initially appear satisfactory; however, upon detailed examination of the data these participants requested, it was observed that 64 of these researchers obtained only information such as age, species, and breed, while the majority did not inquire about detailed data recommended by research guidelines such as ARRIVE (Animal Research: Reporting of In-vivo Experiments) (Kilkenny et al., 2010a) which could influence the outcomes of their studies. The ARRIVE guidelines, first published in 2010, emphasize that information reflecting the health status of animals used in experiments is an essential part of experimental studies and underline the necessity of evaluating their impacts on experimental outcomes (Kilkenny et al., 2010b; Pritchett-Corning et al., 2014). Many journals that accept publications involving animal research now require information reporting to comply with the ARRIVE guidelines.

26 researchers reported incidentally detecting a health issue in experimental animals, aside from routine health monitoring. Despite encountering such frequent problems, the absence of microbiological screening may result in the complete oversight of hard-to-detect conditions that could influence study outcomes at a subclinical level in terms of physiological, behavioral, and biochemical parameters. The most frequently encountered incidental findings were infections and tumors, cysts, or organ anomalies. An interesting point is that, among the 26 researchers who incidentally identified a pathological condition unrelated to their study, only two had checked whether the animals used in their research carried pathogens that could potentially affect the study outcomes.

Our participants' views on the training received by those who will use animals in procedures during certification were, unfortunately, not entirely aligned with our legislation. A reassuring aspect of this issue was the significant difference observed between certified and non-certified individuals, indicating that the training was at least somewhat effective. The most striking impact of certification training was the change in participants' approaches to anesthesia, analgesia, and euthanasia methods. While 26% of non-certified individuals considered training in these areas unnecessary, 100% of certified individuals deemed it essential. Another notable point was that certification training made a significant difference in the use of humane endpoints. However, it is quite disappointing that 22% of our certified participants still considered this unnecessary.

Conclusion

In conclusion, at least 60% of experimental animal units in our country do not conduct any form of microbiological surveillance. This issue not only significantly impacts the reliability of research data but also increases variability in collected data, potentially leading to excessive use of animals in experiments. Although the current regulations mandate health monitoring and emphasize that units should have strategic plans and programs in this regard, the fact that only 17% of all facilities housing laboratory rodents perform microbiological surveillance suggests that the obligation to develop strategic plans does not serve as an effective directive. On the other hand, the ability to monitor SPF (Specific Pathogen-Free) animals indicates that developing national guidelines for microbiological surveillance of conventional animals could be an efficient approach.

Perhaps the most crucial point for researchers is the necessity of always keeping in mind that ensuring the reliability of data and strict adherence to ethical principles in our studies is our responsibility. We cannot absolve ourselves of this responsibility with a statement like “because I assume that this has already been checked “.

Ethics Committee Approval: The necessary permission for this research was obtained from the Gazi University Clinical Research Ethics Committee on 27.01.2020-117.

Author Contributions: Concept - AMS; Design – AMS, BK, YT; Supervision - AMS; Resources - NONE; Materials – AMS, BK; Data Collection and/or Processing - AMS, BK, YT; Analysis and/or Interpretation - AMS, BK; Literature Search - AMS, BK, YT; Writing Manuscript - AMS, BK, YT; Critical Review - AMS, BK, YT

Peer-review: Externally peer-reviewed.

Funding: The authors did not recieve any funding for this study.

Declaration of Interests: The authors have no conflict of interest to report.

References

Baker, D. G. (1998). Natural pathogens of laboratory mice, rats, and their effects on research. *Clinical Microbiology Reviews, 11*(2), 231–266. https://doi.org/10.1128/CMR.11.2.231

Beyhan, Y. E., Gürler, A. T., Bölükbaş, C. S., Açici, M. & Umur, S. (2010). Helminths of some laboratory animals detected by necropsy and fecal examination. *Acta Parasitologica Turcica, 34*(2), 98–101.

Bıyıkoğlu, G. (1996). Bazı Laboratuvar Hayvanlarında Dışkı Bakılarında Saptanan Helmintler. *Etlik Veteriner Mikrobiyoloji Dergisi, 8*(4), 137-146.

Burkholder, T., Foltz, C., Karlsson, E., Linton, C. G., & Smith, J. M. (2012). Health Evaluation of Experimental Laboratory Mice. *Current Protocols in Mouse Biology, 2*, 145–165. https://doi.org/10.1002/9780470942390.mo110217

Colby, L. A. & Zitzow, L. (2018). Applied Institutional

Approaches for the Evaluation and Management of Zoonoses in Contemporary Laboratory Animal Research Facilities. *ILAR Journal, 59*(2), 134–143. https://doi.org/10.1093/ilar/ily016

Connole, M. D., Yamaguchi, H., Elad, D., Hasegawa, A., Segal, E. & Torres-Rodriguez, J. M. (2000). Natural pathogens of laboratory animals and their effects on research. *Medical Mycology, 38*, 59–65. https://doi.org/10.1080/mmy.38.s1.59.65

Çelik, A., Baksi, N. & Güneli, M. E. (2023). Deney Hayvanları Araştırmalarında Standardizasyonun Yeri ve Önemi: Geleneksel Derleme. *Uludağ Üniversitesi Tıp Fakültesi Dergisi, 49*(1), 125–132. https://doi.org/10.32708/uutfd.1216412

Çetinkaya, H., Taş, T., & Vuruşaner, C. (2017). Determination of the Parasites in the Faeces of Laboratory Animals by Using Flotation Technique in Istanbul, Turkey. *Journal of Istanbul Veterinary Sciences, 1*(2), 35-39. https://doi.org/10.30704/http-www-jivs-net.322964

Ergün, Y. (2011). Deney Hayvanı Refahı. *Arşiv Kaynak Tarama Dergisi, 20*(1), 55–68.

Gül, Y., İssi, M., & Gül Baykalır, B. (2013). Araştırma Laboratuvarlarında Biyogüvenlik, Zoonotik Hastalıklar ve Tıbbi Atıkların Bertarafı. *Atatürk Üniversitesi Veteriner Bilimleri Dergisi, 8*(1), 81-96.

İçil, N. İ. & Erbaş, G. (2024). The Prevalence of Helicobacter spp . in Licensed Experimental Animal Facilities in Aegean Region , Turkey. *Journal of Laboratory Animal Science and Practices, 4*(1), 33–43. https://doi.org/10.5281/zenodo.10894467

Kilkenny, C., Browne, W., Cuthill, I. C., Emerson, M. & Altman, D. G. (2010a). Animal research: Reporting in vivo experiments: The ARRIVE guidelines. *British Journal of Pharmacology, 160*(7), 1577–1579. https://doi.org/10.1111/j.1476-5381.2010.00872.x

Kilkenny, C., Browne, W. J., Cuthill, I. C., Emerson, M. & Altman, D. G. (2010b). Improving bioscience research reporting: The arrive guidelines for reporting animal research. *PLoS Biology*, *8*(6), 6–10. https://doi.org/10.1371/journal.pbio.1000412

Lytvynets, A., Langrova, I., Lachout, J. & Vadlejch, J. (2013). Detection of pinworm eggs in the dust of laboratory animals breeding facility, in the cages and on the hands of the technicians. *Laboratory Animals*, *47*(1), 71–73. https://doi.org/10.1258/la.2012.012060

Mähler Convenor, M., Berard, M., Feinstein, R., Gallagher, A., Illgen-Wilcke, B., Pritchett-Corning, K., & Raspa, M. (2014). FELASA recommendations for the health monitoring of mouse, rat, hamster, guinea pig and rabbit colonies in breeding and experimental units. *Laboratory Animals, 48*(3), 178–192. https://doi.org/10.1177/0023677213516312

Nicklas, W., Hornberger, F. R., Illgen-Wilcke, B., Jacobi, K., Kraft, V., Kunstyr, I., Mahler, M., Meyer, H. & Pohlmeyer-Esch, G. (1999). Implications of infectious agents on results of animal experiments. Report of the working group on hygiene of the Gesellschaft fur Versuchstierkunde - Society for Laboratory Animal Science (GV-SOLAS). *Laboratory Animals*, *33*(4), 39-87. https://doi.org/10.1258/002367799780639987

Öbrink, K. J., Rehbinder, C. & Waller, M. (2000). Animal definition: a necessity for the validity of animal experiments? *Laboratory Animals*, *34*(2), 121–130. https://doi.org/10.1258/002367700780457608

Polat, E., Bostan Yörü, H., Kalay, Y. & Akça, S. (2024). Laboratuvar Hayvanlarında Tarama Programlarının Önemi Ve Bazı Önemli Viral Ajanların Araştırılması. *Laboratuvar Hayvanları Bilimi ve Uygulamaları Dergisi*, *4*(2), 91–100. https://doi.org/10.62425/jlasp.1484805

Pritchett-Corning, K. R., Prins, J.-B., Feinstein, R., Goodwin, J., Nicklas, W. & Riley, L. (2014). AALAS/FELASA working group on health monitoring of rodents for animal transfer. *Journal of the American Association for Laboratory Animal Science*, *53*(6), 633–640. http://www.ncbi.nlm.nih.gov/pubmed/25650968

Sevgili, A., Özel, G., Karamercan, M., Doğanay, A., Atilla, P., Çakar, N., & Balkancı, Z. (2010, Eylül). Sıçanda non-travmatik hematüri: tanınız nedir? *36. Ulusal Fizyoloji Kongresi Bildiri Kitabı* (s. 156)

Tüfek, H. & Özkan, Ö. (2018). 4R Rule in Laboratory Animal Science. *Commagene Journal of Biology, 2*(1), 55–60. https://doi.org/10.31594/commagene.389909

Uçak, M. (2024). Animal Experiments and Laboratory Safety within the Scope of Occupational Health and Biosafety. *Duzce Medical Journal, 26*(S1), 19–23. https://doi.org/10.18678/dtfd.1504050

ATATÜRK ÜNİVERSİTESİ YAYINLARI
ATATURK UNIVERSITY PUBLICATIONS

Research Article *Araştırma Makalesi* DOI: 10.62425/jlasp.1660043

Memory and Learning in WAG/Rij and Sprague Dawley Rats: Investigating the Effect of "Racial Experience," Especially on Predisposition to Epilepsy

WAG/Rij ve Sprague Dawley Sıçanlarında Hafıza ve Öğrenme: "Irksal Deneyimin" Özellikle Epilepsi Yatkınlığının Üzerindeki Etkisinin Araştırılması

Burcu ÇEVRELİ[1]
Öznur Özge ÖZCAN[2]

[1]Department of Physiology, Faculty of Medicine, Neuropsychopharmacology Practice and Research Center, Üsküdar University, İstanbul, Türkiye
[2]Department of Molecular Biology and Genetics, Faculty of Engineering and Natural Sciences, Üsküdar University, Istanbul, Türkiye

ABSTRACT

This research sought to investigate how genetic variations influence learning, short-term memory, and long-term memory in rats. In particular, it compared *WAG/Rij* (WR*)* rats, which are naturally prone to epilepsy, with *Sprague-Dawley* (SD) rats. A total of 24 male rats, consisting of 12 SD and 12 WR rats, were evaluated using an eight-arm radial maze to examine spatial memory and the retention of learning over time. No significant differences were observed in working memory error (WME) at 48, 72, 96, 120, and 144 hours ($p > .05$), and similar results were found for reference memory error (RME). However, WR rats made significantly more RME than SD rats at 48 hours ($p = .0111$, 95% CI: -1.606 to -0.2178). SD rats also completed the maze significantly faster at 96 hours ($p = .0094$) and 120 hours ($p = .0383$) than WR rats. Additionally, on the 4th day of the acquisition trial, WR rats made significantly more total error than SD rats ($p = .0045$). This research offers fresh perspectives on the variations in learning and memory across different rat strains within various behavioral models. Although SD rats gave better results in short-term memory and faster results in the process of completing the task compared to WR rats, further research is recommended in different behavioral patterns.

Keywords: Racial experience, Reference memory, Spatial learning, Working memory.

ÖZ

Bu araştırmada genetik varyasyonların sıçanlarda öğrenmeyi, kısa süreli hafızayı ve uzun süreli hafızayı nasıl etkilediğinin araştırılması amaçlandı. Özellikle, doğal olarak epilepsiye yatkın olan *WAG/Rij* (WR) sıçanlarını *Sprague-Dawley* (SD) sıçanlarıyla karşılaştırmıştır. 12 SD ve 12 WR sıçanından oluşan toplam 24 erkek sıçan, uzaysal hafızayı ve zaman içinde öğrenmenin tutulmasını incelemek için sekiz kollu bir radyal labirent kullanılarak değerlendirilmiştir. 48, 72, 96, 120 ve 144. saatlerde çalışma belleği hatalarında (WME) anlamlı bir fark gözlenmemiştir ($p > ,05$) ve referans bellek hataları (RME) için benzer sonuçlar bulunmuştur. Bununla birlikte, WR sıçanları 48. saatte SD sıçanlarına göre anlamlı derecede daha fazla RME yapmıştır ($p = ,0111$, %95 GA: -1,606 ila -0,2178). SD sıçanları ayrıca labirenti 96 saatte ($p = ,0094$) ve 120 saatte ($p = ,0383$) WR sıçanlarından önemli ölçüde daha hızlı tamamladı. Ek olarak, edinim denemesinin 4. gününde, WR sıçanları SD sıçanlarından önemli ölçüde daha fazla toplam hata yaptı ($p = .0045$). Bu araştırma, çeşitli davranış modelleri içindeki farklı sıçan türleri arasında öğrenme ve bellekteki farklılıklar hakkında yeni bakış açıları sunmaktadır. SD sıçanları kısa süreli bellekte daha iyi sonuçlar ve görevi tamamlama sürecinde WR sıçanlarına kıyasla daha hızlı sonuçlar vermiş olsada, farklı davranış kalıplarında daha fazla araştırma yapılması önerilmektedir.

Anahtar kelimeler: Çalışma belleği, Irksal deneyim, Mekansal öğrenme, Referans belleği.

Geliş Tarihi/Received :17.03.2025
Kabul Tarihi/Accepted :03.09.2025
Yayın Tarihi/Publication Date :19.09.2025

Sorumlu Yazar/Corresponding author: Burcu Çevreli
E-mail: burcu.cevreli@uskudar.edu.tr

Cite this article: Çevreli, B., & Özcan, Ö.Ö. (2025). Memory and Learning in WAG/Rij and Sprague Dawley Rats: Investigating the Effect of "Racial Experience," Especially on Predisposition to Epilepsy. *Journal of Laboratory Animal Science and Practices*, *5*(2), 102-109. https://doi.org/10.62425/jlasp.1660043

Introduction

Rats are the most commonly used animal group in scientific research, particularly in biotechnology and health-related studies. Rats, which vary in size and tail length, are widely used in various fields, including basic medicine, pharmacology, food science, and behavioral research (Gou et al. 2024). Many of the inbred rat strains used today can be traced back to the *Wistar Albino* lineage. Among these, the *Sprague Dawley* (SD) rat is the most frequently employed species in pharmaceutical research, particularly in the United States and Japan (Caine et al. 2023). In comparison to laboratory mice, fewer rat species are commonly used in biomedical studies. Another widely utilized strain is the well-established *Wistar Albino* rat. Additionally, the *Wistar Albino Glaxo* from *Rijswijk* (WAG/Rij) rat, originally developed as an epilepsy model, has since been employed in the study of various related conditions. This inbred strain, known as WAG/Rij (WR), is specifically associated with genetic absence epilepsy, a non-convulsive form of the disorder (Sitnikova, 2024). Various breeds of laboratory animals are commonly used in behavioral studies; however, it is essential to consider that inherent traits may vary due to genetic differences between strains (Bárdos et al., 2024).

Behavior is closely linked to brain function, and variations in cognitive abilities among different strains can affect experimental outcomes, potentially leading to inconsistencies across research groups (Sarmiento et al. 2024). Therefore, it is essential to conduct behavioral phenotyping on laboratory animals from various strains to ensure that results are reliable and comparable (Kovarova et al. 2025). In studies of learning and behavior, related and wild-type strains of albino rats are commonly produced and utilized in neurobiological and physiological research, particularly concerning the nervous system and learning processes. This research often translates to understanding mechanisms underlying human behavior, especially memory and learning. Given that rats and mice are similar in their natural behavior and typically inhabit underground burrows resembling complex mazes, they are particularly favored in studies focused on spatial learning and memory. Researchers often assess these learning behaviors using various maze types, such as the radial arm maze, which can be adapted through different behavioral tasks and arm configurations (Peleh et al. 2019; Wijnen et al. 2024).

Spatial working memory refers to the temporary retention of a limited amount of spatial information, allowing for immediate access and use in various cognitive processes. Spatial reference memory refers to spatial information that is consistently utilized and typically acquired through repeated training. Over time, this information becomes consolidated, making it more resistant to interference (McQuail et al. 2021). The interplay of environmental influences, genetic factors and biochemical variations in neural connections contributes to the observed differences in learning and memory capabilities among individuals and across species in both humans and animals (Gökçek-Saraç et al. 2012; Lee & Jung 2014).

Behavioral differences have been widely studied in experimental animals. Research indicates notable variations in cognitive task performance among different breeds. For instance, Jaramillo and Zador reported that comparing *Long Evans* (LE) rats and *C57Bl/6J* mice on the flexible sound-categorization task, the rat species learned the task faster than mice (Jaramillo & Zador, 2014). In another study, Blankenship et al. compared morris water task performance in rats and prairie voles. Rats demonstrated superior performance compared to prairie voles in critical aspects of the task, such as the time taken to locate the platform, the efficiency of their swim paths, and the level of directional accuracy. These differences could stem from variations in spatial cognition, stress response, physiology, or motivation among the species (Blankenship et al. 2019). The radial arm maze (RMA) has been widely utilized to investigate spatial cognition, memory, and learning in rodents (Kohler et al. 2022). Research comparing potential breed-related differences in rats, especially using the radial arm maze (RAM), is relatively limited. The RAM is beneficial for simultaneously evaluating working or reference memory. For example, Gökçek-Saraç et al. conducted a study that examined the performance of various rat breeds in the RAM. Their findings indicated that *Wistar/Sprague-Dawley* (W/SD) rats made fewer reference memory error and acquired tasks more quickly than both outbred LE and Wistar rats. Moreover, Wistar rats exhibited fewer mistakes in working memory tasks than other strains (Gökçek-Saraç et al. 2015). The WR rats are an inbred genetic epilepsy model for animal studies showing absence-like epilepsy. The established impact of epilepsy on learning and memory (De Deurwaerdère et al. 2022; Casillas-Espinosa et al. 2024), along with the limited cognitive assessments of animal models for absence epilepsy, inspired our study. We assessed the learning and memory capabilities of WR rats against age-matched Sprague-Dawley control rats using a thoroughly validated RAM. To date, no studies have directly compared spatial and working memory between WR rats and SD rats.

This study aimed to examine variations in working and reference spatial memory at both the individual and breed levels between two widely used laboratory rat strains such as WR and SD rats, by utilizing various performance metrics in the RAM.

Methods

This study involved a total of 24 male rats divided 12 male SD and 12 male WR rats, each 15 months old and weighing between 350-400 grams, with a maximum age difference of 10 days between individuals. All procedures adhered to the guidelines established by the U.S. National Institutes of Health as outlined in the Guide for the Care and Use of Laboratory Animals (OECD 423). Two male rats were housed in standard cages using sawdust under a 12-h light/dark cycle. Water and ad libitum were provided. Their diet consisted of pellet food formulated to meet their physiological needs. Prior to the initiation of the experimental procedures, an official application was submitted to the Üsküdar University Animal Experiments Ethics Committee, and approval was obtained (Approval Date: 21.12.2023, Approval Number: 2023-09).

Eight-Arm Radial Maze

This approach is commonly employed in behavioral studies to assess spatial memory. It features eight horizontal arms, each measuring 57x11 cm, that extend evenly from a central platform elevated 80 cm off the ground. Each arm is equipped with an automatic door that stands 20 cm tall at its entrance. The entire structure, including the platform and doors, is constructed from opaque gray Plexiglas. The maze features eight distinct visual cues, with four near the central platform and four on the walls of the arms. These cues vary in shape (square, rectangle, circle, and triangle) and color (yellow, green, purple, and red). The setup is illuminated from above, ensuring visibility. At the end of each arm, food rewards consisting of beet sugar-coated cornflakes are placed. The test comprises three main stages: (1) a three-day habituation phase, where subjects undergo a 15-minute exploration period to become familiar with the maze, (2) an acquisition phase lasting eight days, during which two consecutive five-minute trials are conducted daily, and (3) an experimental phase that includes a single five-minute trial performed at intervals of 48, 72, 96, 120, and 144 hours following the last session. During all phases except for the initial exploration, food rewards are placed in only four of the arms. At the beginning of each trial, the rats are briefly restrained for 30 seconds before being placed on the central platform, where they can move freely until all the food is retrieved. The trial lasts for five minutes. A rat's visit to an arm is recorded when all four paws enter it. If the rat goes into an arm that was previously inaccessible, it is classified as a reference memory error (RME). Conversely, if the rat re-enters an arm that it has already visited, this is considered a working memory error (WME). Performance is assessed by measuring the time taken to locate the four accessible arms and by tallying the total occurrences of working, reference, and overall memory error during the trials (Kohler et al. 2014).

Statistical Analysis

Data analysis was conducted using GraphPad Prism 10.0 (GraphPad Software, San Diego, CA). Normality of the distribution was conducted by descriptive analysis. To examine time-dependent differences between groups, mixed-effects ANOVA was used followed by LSD post hoc test. Statistical significance was defined as $p< .05$. The results are presented as mean ± SEM.

Results

Performance Data Across Groups

The study examined performance indicators including total error and total completion time. Additionally, WME and RME were evaluated between WR and SD rat strains and the different time frames were compared. All results given in Table 1.

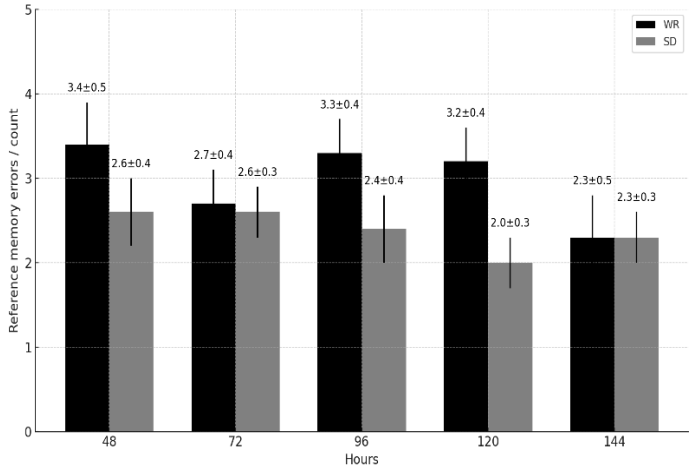


Figure 1. The results of eight arm radial maze which illustrate the reference memory error. Data are presented as mean ± S.E.M., and a mixed-effects ANOVA was conducted, followed by post hoc LSD tests. *SD:* with *Sprague-Dawley* (SD) rats, *WR: WAG/Rij* rats.

Şekil 1. Referans bellek hatalarını gösteren sekiz kollu radyal labirentin sonuçları. Veriler ortalama ± S.E.M. olarak sunulmuştur ve karışık-desen ANOVA kullanılmış, ardından post hoc LSD testleri yapılmıştır.

Table 1. Comparison of reference memory error, working memory error, session duration and total errors/count results on different acquisition trial days measured between SD and WR breeds in the eight-arm radial maze. A mixed-effects ANOVA *p< .05. *SD:* with *Sprague-Dawley* (SD) rats, *WR: WAG/Rij* rats.

Tablo 1. Sekiz kollu radyal labirentte SD ile WR ırkları arasında ölçülen farklı edinim deneme günlerindeki referans bellek hatası, çalışma belleği hatası, oturum süresi ve toplam hata/sayım sonuçlarının karşılaştırılması. Karışık-desen ANOVA. *p< ,05.

Reference memory errors/count	**Predicted (LS) mean difference**	**95% CI of difference**	**p value**
48 h	-0.7879	-1.968 to 0.3927	.1862
72 h	-0.1212	-1.302 to 1.059	.8375
96 h	-0.9773	-2.158 to 0.2033	.1026
120 h	-1.167	-2.347 to 0.01392	.0527
144 h	0.01515	-1.165 to 1.196	.9795
Working memory errors/Count			
48 h	-0.9118	-1.606 to -0.2178	.0111*
72 h	-0.06219	-0.7562 to 0.6318	.8579
96 h	0.2031	-0.4909 to 0.8971	.5593
120 h	-0.2944	-0.9884 to 0.3996	.3982
144 h	0.3924	-0.3016 to 1.086	.2615
Session duration			
48 h	-5.485	-40.18 to 29.21	.7522
72 h	-12.52	-47.22 to 22.17	.4719
96 h	-46.65	-81.35 to -11.96	.0094*
120 h	-36.77	-71.46 to -2.069	.0383*
144 h	-26.17	-60.86 to 8.529	.1361
Total errors/count			
1 Day	-0.9167	-2.790 to 0.9565	.3329
2 Day	-1.25	-3.123 to 0.6231	.1878
3 Day	-0.6667	-2.540 to 1.206	.4806
4 Day	-2.75	-4.623 to -0.8769	.0045*
5 Day	-0.5833	-2.456 to 1.290	.537
6 Day	0.25	-1.623 to 2.123	.7911
7 Day	-0.3333	-2.206 to 1.540	.724

A mixed-effects ANOVA *p<.05. *SD:* with *Sprague-Dawley* (SD) rats, *WR: WAG/Rij* rats.

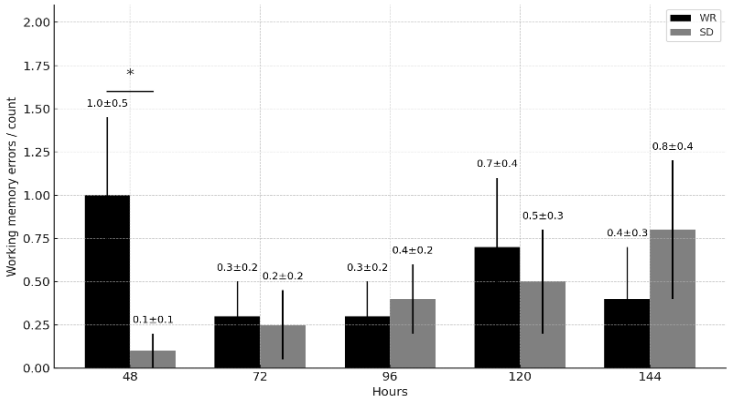


Figure 2. The results of eight arm radial maze which working memory error. Data are presented as mean ± S.E.M., and a mixed-effects ANOVA was conducted, followed by post hoc LSD tests. *p< .05. *SD:* with *Sprague-Dawley* (SD) rats, *WR: WAG/Rij* rats.

Şekil 2. Çalışma belleği hataları olan sekiz kollu radyal labirentin sonuçları. Veriler ortalama ± S.E.M. olarak sunulmuştur ve karışık-desen ANOVA kullanılmış, ardından post hoc LSD testleri yapılmıştır. *p< ,05.

Results of mixed-effects ANOVA showed the RME during the learning stage, with training day as a within-subjects factor and experimental group. There was no significant main effects for training hours [F(4, 44) = 1.033, *p*= .4010], group differences [F(1, 11) = 3.840, *p*= .0759], or the interaction between group and training hours [F(4, 39) = 0.8872, *p*= .4806] (Figure 1). These findings suggest that RME rates remained consistent throughout the training hours and did not differ significantly between the groups.

Results of mixed-effects ANOVA showed the WME during the learning phase, maintaining training day as a within-subjects factor and experimental group. There was no significant main effects for training hours [F(4, 44) = 0.5565, *p*= *.6953*], group differences [F(1, 11) = 0.7564, *p*= .4030], or the interaction between training hours and group [F(4, 39) = 2.151, *p*= .0927] (Figure 2). This indicates that WME rates did not exhibit significant differences during the training intervals of 72, 96, 120, and 144 hours, and no notable disparities were found between the groups. However, at the 48-hour mark, the WR group had significantly higher WME rates compared to the SD group (*p = .0111*, t = 2.639, 95% CI: -1.606 to -0.2178).

A mixed-effects ANOVA was performed to examine the total time taken (-on duration) during the training day, using training day as a within-subjects factor and experimental group as a between-subjects factor. The analysis did not reveal any significant main effects for hours [F(4, 44) = 2.389, *p*= .0653], session duration [F(1, 11) = 3.159, *p*= .1032], or the interaction between session duration and hours [F(4, 39) = 2.480, *p*= .0597] (Figure 3). These results suggest that the total time taken remained stable across training hours, with no significant differences between groups at 48, 72, and 144 hours. However, the SD group completed the 8-arm radial arm maze in a shorter duration compared to the WR group at 96 hours (*p*= .0094, t = 2.701, 95% CI: -81.35 to -11.96) and at 120 hours (*p*= .0383, t = 2.128, 95% CI: -71.46 to -2.069).

Additionally, another mixed-effects ANOVA indicated that the total error made during the completion of the 8-arm radial arm maze varied at different time points following the acquisition trial (Figure 4). A significant main effect was identified for acquisition days [F(7, 77) = 12.24, *p*< .0001] and for total error/count [F(1, 11) = 6.917, *p*= .0234]. However, no significant main effect was found for the interaction between acquisition trial and total error/count [F(7, 77) = 0.9147, *p*= .4999] (Figure 2). Importantly, the WR group made a significantly more total error than the SD group during the fourth acquisition trial day (*p*= .0045, t = 2.923, 95% CI: -4.623 to -0.8769).

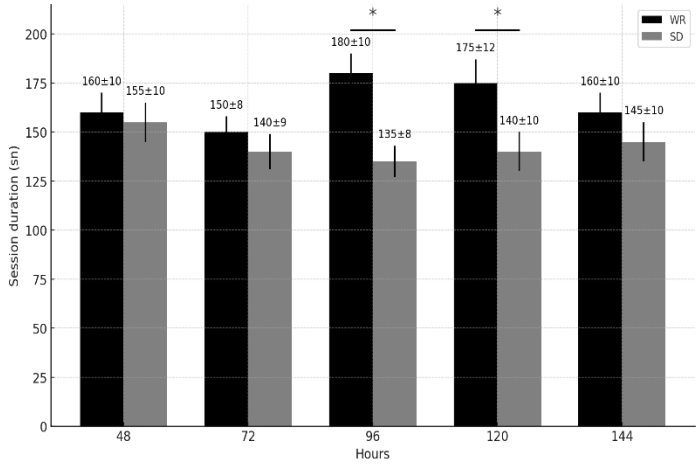


Figure 3. The total time for the session duration to complete the 8-arm radial arm maze. Data are presented as mean ± S.E.M., and a mixed-effects ANOVA was conducted, followed by post hoc LSD tests. *$p<$ .05. *SD:* with *Sprague-Dawley* (SD) rats, *WR: WAG/Rij* rats.

Şekil 3. 8 kollu radyal kol labirentini toplam tamamlama süresi. Veriler ortalama ± S.E.M. olarak sunulmuştur ve karışık-desen ANOVA kullanılmıştır, ardından post hoc LSD testleri yapılmıştır. *$p<$,05.

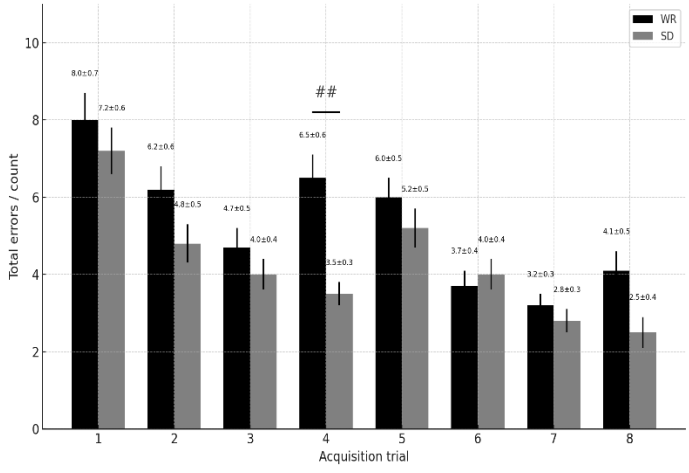


Figure 4. Total error count to complete the 8-arm radial arm maze was detected at different time points after the conclusion of the acquisition trial. Data are presented as mean ± S.E.M., and a mixed-effects ANOVA was conducted, followed by post hoc LSD tests. ##*$p<$* .005. *SD:* with *Sprague-Dawley* (SD) rats, *WR: WAG/Rij* rats.

Şekil 4. Toplam hata sayısı. Edinim denemesinin tamamlanmasından sonra farklı zaman noktalarında 8 kollu radyal kol labirentini tamamlamak için tespit edildi. Veriler ortalama ± S.E.M. olarak sunuldu ve karışık-desen ANOVA kullanılmıştır, ardından post hoc LSD testleri yapıldı. # #*$p<$* ,005.

Discussion

In this study, we examined WR rats, a widely recognized epileptic strain commonly utilized in behavioral research on learning and memory, alongside SD rats. Our results revealed notable strain-specific differences in performance on the RAM task. To assess working and reference memory separately, we compared the frequency of WME and RME between the groups. Furthermore, a mixed-effects ANOVA (group × error × hours) was conducted to analyze temporal variations in these error types concerning overall task completion and total error.

To our knowledge, we present the first study in the literature to report the results of learning and memory studies on rats of different breeds, especially those covering the old age period, comparing the inbred WR rat strain, known for its epilepsy tendency, with the SD rat strain. Another important point of the study was that the results were representative of the 15-month-old, translationally elderly, and provided important information for future studies on learning and memory in the elderly. This study investigated short and long-term spatial memory retention in rats using an eight-arm radial maze. When assessing spatial memory in SD and WR rats, no significant differences were observed in WME at 48, 72, 96, 120, and 144 hours and similar results were found for RME. However, at the 48-h, WR rats exhibited significantly more RME than SD rats. Additionally, SD rats completed the eight-arm radial maze significantly faster at 96 hours and 120 hours compared to WR rats. On the fourth day of the acquisition trial, WR rats had a significantly higher total error count than SD. These findings contribute to the understanding of learning and memory variations across different rat strains in various behavioral models. While SD rats demonstrated better short-term memory and completed tasks more quickly than WR rats, further research is necessary to explore these differences in other behavioral contexts. SD rats performed better than the WR type in the three criterion difference time periods (sessions, choices, and total error for criteria). WR rats had higher WME scores than SD rats, especially in the 48 hours. There were no significant differences between RME in the comparison, perhaps indicating that epilepsy susceptibility does not contribute much to the final behavioral outcome on working memory. Arm entry error can be divided into reference memory arms or working memory arms. When recategorized errors were examined, no significant difference was found in the reference memory arms. At this point, WR rats were consistently making more error than SD in the working error arms compared to WR rats at 48 hours. Interestingly, SD rats were making more error at 96 and 144 hours, but this difference was not significant. This pattern was also seen in the working memory arms, but did not produce significant differences across races. Thus, these results support the literature suggesting that WR has only a short-term selective deficit in working memory. These results bolster the idea that there is a notable difference between working memory and long-term memory, as suggested by current research examining spatially targeted genetic modifications in the forebrain and hippocampus. By evaluating the performance of different animals in spatial learning tasks, these studies allow for the independent measurement of working and RME (8,18,19). Variations in behavior among individuals and strains may arise from different factors. While environmental influences, such as upbringing and care conditions, can affect animal behavior, differences observed among well-established laboratory strains that adhere to standard animal care practices are more likely to have a genetic basis (Junttila et al. 2022). This is evident in WR rats, which showed distinctions in total time and total error while completing the arm maze task. Limited research has explored breed-specific differences in rats, especially utilizing the RAM, a tool that allows for the concurrent assessment of both working and reference memory. For example, Gökçek-Saraç et al. analyzed RAM performance across different rat breeds. Their findings indicated that *Wistar/Sprague-Dawley* (W/SD) rats made fewer RME and acquired tasks more quickly than both outbred LE and *Wistar* rats (Gökçek-Saraç et al. 2015). Furthermore, wistar rats showed a reduced incidence of WME when compared to other rat strains. Harker and Whishaw found that LE rats excelled over *Fisher-Norway* rats in spatial learning tasks conducted in a water maze. They also reported that *Fisher-Norway* rats had better visual acuity than LE rats (Harker & Whishaw, 2002). These results are consistent with other findings indicating that rat performance on well-established functional and mental memory tasks can be strongly influenced by environmental changes present in the experimental room (Ramos, 2000). In addition, in another study comparing the *Wistar breed*, which is frequently used in learning and memory studies, it was reported that *Hooded Lister* rats had significantly fewer WME and RME than Wistar rats, according to the results of the RAM experiment (Manahan-Vaughan & Schwegler, 2011). To our knowledge, in the preclinical literature, most of the existing studies focus specifically on pharmacological efficacy in racial differences (Bryda, 2013; Gao et al. 2021; Nollen et al. 2021; Russomanno et al. 2023), and relatively few studies have addressed differences in brain and behavior-focused cognitive functions but different memory methods at adult age of rats (Ellenbroek & Youn, 2016). In general evaluation, these previous studies

compared different behavioral platforms related to spatial, working, visual, location, reference memory in different rat strains covering the adult period (Vorhees and Williams, 2024). An important aspect that warrants further discussion is the neurophysiological basis through which the epileptic predisposition of WR rats might influence cognitive and behavioral performance. WAG/Rij rats are widely recognized as a validated model of absence epilepsy, primarily characterized by spike-and-wave discharges (SWDs) originating in the somatosensory cortex and thalamocortical circuits (Sitnikova, 2024). These spontaneous SWDs, even in the absence of overt motor seizures, have been shown to disrupt cortical information processing and interfere with attentional control and working memory (De Deurwaerdère et al., 2022). Studies using EEG recordings in WR rats have demonstrated that SWDs can transiently suppress neuronal firing in prefrontal and hippocampal regions, which are critical for spatial memory and executive function. This transient disruption may lead to increased cognitive errors during maze navigation tasks that require continuous updating of spatial information and flexible decision-making. Therefore, the higher working and total errors observed in WR rats, particularly during early retention intervals, may be attributed to impaired synchronization of hippocampal-prefrontal networks due to interictal epileptic activity. Furthermore, chronic epileptiform discharges have been associated with synaptic plasticity impairments and altered expression of NMDA receptor subunits in the hippocampus of WR rats, further compromising memory consolidation processes (Gökçek-Saraç et al., 2012). Taken together, these neurophysiological abnormalities likely contribute to the subtle but significant deficits in spatial learning and working memory observed in WR rats compared to their non-epileptic SD counterparts. The results indicate the need for further research on racial differences and at different stages of life, especially in maze experiments.

Conclusion

Overall, this study highlights spatial and functional learning differences between SD and WR rat strains, which are genetically related and associated with epilepsy susceptibility, and are widely used in studies of racial experience effects in learning and behavior. This study offers fresh insights into the variations in learning and memory among different rat strains across various behavioral models. While SD rats demonstrated superior short-term memory and completed tasks more quickly than WR rats, additional research is suggested to explore these differences in other behavioral patterns.

Ethics Committee Approval: Approval for all animal experiments was obtained from the Animal Research Ethics Committee of Üsküdar University, Istanbul, Turkey. The study received ethical approval from the Local Ethics Committee of Üsküdar University on December 21, 2023, under decision number Ü.Ü-HADYEK 2023–09.

Author Contributions: Concept – BÇ, ÖÖÖ; Design - BÇ; Supervision - BÇ; Resources - BÇ; Materials - BÇ; Data Collection and/or Processing - BÇ; Analysis and/or Interpretation – BÇ; Literature Search - ÖÖÖ; Writing Manuscript – BÇ,ÖÖÖ; Critical Review – BÇ,ÖÖÖ.

Peer-review: Externally peer-reviewed.

Funding: No funding.

Declaration of Interests: The authors declare that no conflicts of any interest.

Acknowledgements: We acknowledgement to the Experimental Research Neuropyschopharmacology Application and Research Center at Üsküdar University.

References

Bárdos, B., Török, H. K., & Nagy, I. (2024). Comparison of the exploratory behaviour of wild and laboratory mouse species. *Behavioural Processes, 217*:105031. https://doi.org/10.1016/j.beproc.2024.105031

Blankenship, P. A., Normann, M. C., Donaldson, T. N., Baumeister, J., McNeal, N., Grippo, A. J., & Wallace, D. G. (2019). Making waves: Comparing Morris water task performance in rats and prairie voles. *Behavioural Brain Research, 360*, 7–15. https://doi.org/10.1016/j.bbr.2018.11.032

Bryda, E. C. (2013). The Mighty Mouse: The impact of rodents on advances in biomedical research. *Missouri Medicine, 110*(3), 207-211

Caine, S. B., Plant, S., Furbish, K., Yerton, M., Smaragdi, E., Niclou, B., Lorusso, J. M., Chang, J. Y., Bitter, C., Basu, A., Miller, S., Huang, C. Y., Komson, R., Liu, D., Behar, S., & Thomsen, M. (2023). Sprague Dawley rats from different vendors vary in the modulation of prepulse inhibition of startle (PPI) by dopamine, acetylcholine, and glutamate drugs. *Psychopharmacology, 240*(9), 2005–2012. https://doi.org/10.1007/s00213-023-06444-1

Casillas-Espinosa, P. M., Garcia-Olivares, J., Li, R., Li, C., Yu, C., Formella, A. E., & O'Brien, T. J. (2024). Huperzine A suppresses absence seizures in the genetic absence epilepsy rat from Strasbourg (GAERS) model of genetic generalized epilepsy with absence seizures. *Epilepsia Open, 9*(5):1826-1836. https://doi.org/10.1002/epi4.13016

De Deurwaerdère, P., Casarrubea, M., Cassar, D., Radic, M., Puginier, E., Chagraoui, A., Crescimanno, G., Crunelli, V., & Di Giovanni, G. (2022). Cannabinoid 1/2 Receptor Activation Induces Strain-Dependent Behavioral and Neurochemical Changes in Genetic Absence Epilepsy Rats From Strasbourg and Non-epileptic Control Rats. *Frontiers in Cellular Neuroscience, 16*, 886033. https://doi.org/10.3389/fncel.2022.886033

Ellenbroek, B., & Youn, J. (2016). Rodent models in neuroscience research: Is it a rat race? *Disease Models & Mechanisms, 9*(10), 1079-1087. https://doi.org/10.1242/dmm.026120

Gao, S., Bell, E. C., Zhang, Y., & Liang, D. (2021). Racial disparity in drug disposition in the digestive tract. *International Journal of Molecular Sciences, 22*(3), 1038. https://doi.org/10.3390/ijms22031038

Gökçek-Saraç, Ç., Karakurt, S., Adalı, O., & Jakubowska-

Doğru, E. (2012). Correlation between hippocampal levels of neural, epithelial, and inducible NOS and spatial learning skills in rats. *Behavioral Brain Research, 233*(2), 493-499. https:// doi.org/10.1016/j.bbr.2012.08.005

Gökçek-Saraç, Ç., Wesierska, M., & Jakubowska-Doğru, E. (2015). Comparison of spatial learning in the partially baited radial-arm maze task between commonly used rat strains: Wistar, Sprague-Dawley, Long-Evans, and outcrossed Wistar/Sprague-Dawley. *Learning and Behavior, 43*(1), 83-94. https://doi.org/10.3758/s13420-014-0163-9

Guitar, N. A., & Roberts, W. A. (2015). The interaction between working and reference spatial memories in rats on a radial maze. *Behavioral Processes, 112*, 100-107. https:// doi.org/10.1016/j.beproc.2014.10.007

Guo, X., Asthana, P., Zhai, L., Cheng, K. W., Gurung, S., Huang, J., Wu, J., Zhang, Y., Mahato, A. K., Saarma, M., Ustav, M., Kwan, H. Y., Lyu, A., Chan, K. M., Xu, P., Bian, Z. X., & Wong, H. L. X. (2024). Artesunate treats obesity in male mice and non-human primates through GDF15/GFRAL signalling axis. *Nature Communication, 3;15*(1):1034. https://doi.org/10.1038/s41467-024-45452-3

Harker, T. K., & Whishaw, I. Q. (2002). Place and matching-to-place spatial learning affected by rat inbreeding (Dark-Agouti, Fischer 344) and albinism (Wistar, Sprague-Dawley) but not domestication (wild rat vs. Long-Evans, Fischer-Norway). *Behavioral Brain Research, 134*(1), 467-477. https://doi.org/10.1016/s0166-4328(02)00083-9

Jaramillo, S., & Zador, A. M. (2014). Mice and rats achieve similar levels of performance in an adaptive decision-making task. *Frontiers in Systems Neuroscience, 8*, 173. https://doi.org/10.3389/fnsys.2014.00173

Junttila, S., Valros, A., Mäki, K., Väätäjä, H., Reunanen, E., & Tiira, K. (2022). Breed differences in social cognition, inhibitory control, and spatial problem-solving ability in the domestic dog (Canis familiaris). *Scientific Reports, 12*(1), 22529. https://doi.org/10.1038/s41598-022-26991-5

Kohler, J., Mei, J., Banneke, S., Winter, Y., Endres, M., & Emmrich, J. V. (2022). Assessing spatial learning and memory in mice: Classic radial maze versus a new animal-friendly automated radial maze allowing free access and not requiring food deprivation. *Frontiers in Behavioral Neuroscience, 16*, 1013624. https://doi.org/10.3389/fnbeh.2022.1013624

Kovarova, V., Bordes, J., Mitra, S., Narayan, S., Springer, M., Brix, L. M., Deussing, J. M., & Schmidt, M. V. (2025). Deep phenotyping reveals CRH and FKBP51-dependent behavioral profiles following chronic social stress exposure in male mice. *Neuropsychopharmacology, 50*(3):556-567. https://doi.org/10.1038/s41386-024-02008-9

Lee, J. W., & Jung, M. W. (2025). Memory consolidation from a reinforcement learning perspective. *Frontiers in Computational Neuroscience, 8*;18:1538741. https://doi.org/10.3389/fncom.2024.1538741

Manahan-Vaughan, D., & Schwegler, H. (2011). Strain-dependent variations in spatial learning and in hippocampal synaptic plasticity in the dentate gyrus of freely behaving rats. *Frontiers in Behavioral Neuroscience, 7*, 5-7. https://doi.org/10.3389/fnbeh.2011.00007

Manukyan, P., Romanova, E., Latanov, A., Shlepnev, P., Sharapkova, A., Garabova, N., et al. (2025). Challenges and insights of transferring animal maze studies principles to human spatial learning research. *Scientific Reports, 15*(1), 2096. https://doi.org/10.1038/s41598-025-86037-4

McQuail, J. A., Dunn, A. R., Stern, Y., Barnes, C. A., Kempermann, G., Rapp, P. R., Kaczorowski, C. C., & Foster, T. C. (2021). Cognitive reserve in model systems for mechanistic discovery: the importance of longitudinal studies. *Frontiers in Aging Neuroscience, 12,* 607685. https://doi.org/10.3389/fnagi.2020.607685

Nollen, N. L., Ahluwalia, J. S., Sanderson Cox, L., Okuyemi, K., Lawrence, D., Samuels, L., & Benowitz, N. L. (2021). Assessment of Racial Differences in Pharmacotherapy Efficacy for Smoking Cessation: Secondary Analysis of the EAGLES Randomized Clinical Trial. *JAMA Network Open, 4*(1), e2032053. https://doi.org/10.1001/jamanetworkopen.2020.32053

Peleh, T., Ike, K. G. O., Wams, E. J., Lebois, E. P., & Hengerer, B. (2019). The reverse translation of a quantitative neuropsychiatric framework into preclinical studies: Focus on social interaction and behavior. *Neuroscience & Biobehavioral Reviews, 97*, 96-111. https://doi.org/10.1016/j.neubiorev.2018.07.018

Ramos, J. M. (2000). Influence of the shape of the experimental room on spatial learning in rats. *Physiology & Behavior, 70*(3), 351-357. https://doi.org/10.1016/s0031-9384(00)00266-3

Russomanno, G., Sison-Young, R., Livoti, L. A., Coghlan, H., Jenkins, R. E., Kunnen, S. J., Fisher, C. P., Reddyhoff, D., Gardner, I., Rehman, A. H., Fenwick, S. W., Jones, A. R., Vermeil De Conchard, G., Simonin, G., Bertheux, H., Weaver, R. J., Johnson, R. L., Liguori, M. J., Clausznitzer, D., Stevens, J. L., & Copple, I. M. (2023). A systems approach reveals species differences in hepatic stress response capacity. *Toxicological Science: An Official Journal of the Society of Toxicology, 196*(1), 112–125. https://doi.org/10.1093/toxsci/kfad085

Sarmiento, L. F., Lopes da Cunha, P., Tabares, S., Tafet, G., & Gouveia, A. J. (2024). Decision-making under stress: A psychological and neurobiological integrative model. *Brain, Behavior, and Immunity - Health, 38*, 100766. https://doi.org/10.1016/j.bbih.2024.100766

Sitnikova, E. (2024). Behavioral and Cognitive Comorbidities in Genetic Rat Models of Absence Epilepsy (Focusing on GAERS and WAG/Rij Rats). *Biomedicines, 7*;*12*(1):122. https://doi.org/10.3390/biomedicines12010122

Vorhees, C. V., Williams, M. T. (2024). Tests for learning and memory in rodent regulatory studies. *Current Research in Toxicology, 18*;6:100151. https://doi.org/10.1016/j.crtox.2024.100151

Wijnen, K., Genzel, L., & van der Meij, J. (2024). Rodent maze studies: From following simple rules to complex map learning. *Brain Structure and Function, 229*(4), 823-841. https://doi.org/10.1007/s00429-024-02771-x

ATATÜRK ÜNİVERSİTESİ YAYINLARI
ATATURK UNIVERSITY PUBLICATIONS

Research Article *Araştırma Makalesi* DOI: 10.62425/jlasp.1673052

Therapeutic Potential of Cepharanthine in Ovarian Ischemia-Reperfusion Injury: Insights from a Rat Ovarian Torsion-Detorsion Model

Over İskemi-Reperfüzyon Hasarında Sefarantin'in Terapötik Potansiyeli: Rat Over Torsiyon-Detorsiyon Modelinden Elde Edilen Bulgular

Burak BİRCAN[1]
Ayhan TANYELİ[2]
Ersen ERASLAN[3]
Fazile Nur EKİNCİ AKDEMİR[4]
Şahin YAZICI[5]
Derya GÜZEL ERDOĞAN[6]
Elif POLAT[7]
Mustafa Can GÜLER[2]

[1]Department of Medical Sciences and Techniques, Healthcare Vocational School, Osmaniye Korkut Ata University, Osmaniye, Türkiye
[2]Department of Physiology, Faculty of Medicine, Atatürk University, Erzurum, Türkiye
[3]Department of Physiology, Faculty of Medicine, Bandırma Onyedi Eylül University, Bandırma, Türkiye
[4]Department of Physiology, Erzurum Medicine Faculty, University of Health Sciences, Erzurum, Türkiye
[5]Department of Obstetrics and Gynecology, Erzurum City Hospital, Erzurum, Türkiye
[6]Department of Physiology, Faculty of Medicine, Sakarya University, Sakarya, Türkiye
[7]Department of Nutrition and Dietetics, Faculty of Health Sciences, Erzurum Technical University, Erzurum, Türkiye

ABSTRACT

This study evaluated the protective effect of Cepharanthine (CEP) against ovarian ischemia-reperfusion (I/R) injury induced by torsion-detorsion in rats, and its effects on histopathological damage and markers of oxidative stress and inflammation. Twenty-four female Sprague-Dawley rats were randomized into three experimental groups: sham, T/D, and CEP 10 mg/kg. The study examined ovarian tissue samples to measure oxidative stress biomarkers, such as malondialdehyde (MDA), myeloperoxidase (MPO), and superoxide dismutase (SOD), as well as proinflammatory mediators, specifically tumor necrosis factor-alpha (TNF-α) and interleukin-1 beta (IL-1β). The T/D group exhibited significant oxidative stress, characterized by elevated MDA and MPO levels and low SOD activity, along with heightened inflammatory responses, as indicated by elevated TNF-α and IL-1β levels ($p < .05$ vs. Sham). CEP administration mitigated these deleterious effects, significantly reducing oxidative stress and inflammatory cytokine levels while restoring SOD activity ($p < .05$ vs. T/D). CEP effectively attenuated oxidative stress and inflammation in ovarian I/R injury. The preclinical findings support the potential therapeutic effect of CEP, but further studies are needed for clinical applications.

Keywords: Cepharanthine, Inflammation, Ischemia reperfusion, Oxidative stress, Ovarian torsion detorsion.

ÖZ

Bu çalışma, Sefarantin'in (CEP)'in, sıçanlarda torsiyon–detorsiyon ile oluşturulan over iskemi–reperfüzyon (İ/R) hasarına karşı koruyucu etkisini ve histopatolojik hasar ile oksidatif stres ve inflamasyon göstergeleri üzerindeki etkisini değerlendirmiştir. Yirmi dört dişi Sprague-Dawley sıçanı rastgele üç deney grubuna ayrılmıştır: sham, T/D ve CEP 10 mg/kg. Çalışma, over doku örneklerinde malondialdehit (MDA), miyeloperoksidaz (MPO) ve süperoksit dismutaz (SOD) gibi oksidatif stres biyobelirteçleri ile tümör nekroz faktör-alfa (TNF-α) ve interlökin-1 beta (IL-1β) gibi proinflamatuar mediyatörleri ölçmüştür. T/D grubu, yüksek MDA ve MPO seviyeleri ile düşük SOD aktivitesi ile karakterize önemli oksidatif stres ve yüksek TNF-α ve IL-1β seviyelerinin gösterdiği artmış inflamatuar yanıtlar sergiledi. CEP uygulaması bu zararlı etkileri azalttı, oksidatif stresi ve inflamatuar sitokin seviyelerini önemli ölçüde azaltırken SOD aktivitesini restore etti. CEP, over İ/R hasarında oksidatif stres ve inflamasyonu etkili bir şekilde azaltmıştır. Elde edilen preklinik bulgular, CEP'nin potansiyel terapötik etkisini desteklemektedir, ancak klinik uygulamalar için ileri çalışmalara ihtiyaç vardır.

Anahtar kelimeler: İnflamasyon, İskemi reperfüzyon, Oksidatif stres, Over torsiyon detorsiyon, Sefarantin.

Geliş Tarihi/Received :09.04.2025
Kabul Tarihi/Accepted :02.09.2025
Yayın Tarihi/Publication Date :19.09.2025

Sorumlu Yazar/Corresponding author: Mustafa Can Güler
E-mail: mustafacan.guler@atauni.edu.tr

Cite this article: Bircan, B., Tanyeli, A., Eraslan, E., Ekinci Akdemir, F.N., Yazıcı, Ş., Güzel Erdoğan, D., Polat, E., & Güler, M.C. (2025). Therapeutic Potential of Cepharanthine in Ovarian Ischemia-Reperfusion Injury: Insights from a Rat Ovarian Torsion-Detorsion Model. *Journal of Laboratory Animal Science and Practices*, *5*(2), 110-118. https://doi.org/10.62425/jlasp.1673052

Introduction

Adnexal torsion (AT) is a critical gynecological emergency that poses significant risks to reproductive health and fertility (Stickland & Phillips, 2021). It occurs when the ovary and sometimes the fallopian tube twists around a central axis formed by the infundibulopelvic and tubo-ovarian ligaments. This torsional event results in at least one complete rotation, leading to compromised blood flow to the affected structures (Thiyagalingam et al., 2024). Given that acute pelvic pain is one of the most common reasons for emergency gynecological consultations, AT remains a condition of considerable clinical importance (Franco et al., 2023). Timely diagnosis of AT is crucial for preventing severe complications, such as loss of ovarian function or adnexal necrosis, which can significantly impact fertility, even in young women (Kumari et al., 2024). In this context, the surgical detorsion itself can precipitate a distinct pathological process—ischemia–reperfusion (I/R) injury—which may further amplify ovarian damage.

The pathophysiology of AT is primarily driven by an ischemic process that can rapidly progress to severe vascular complications if not promptly managed (Baron & Mathai, 2025). Torsion disrupts normal ovarian perfusion, causing venous and lymphatic congestion, leading to ovarian swelling and pelvic fluid accumulation (Dixon et al., 2025). If arterial circulation is also compromised, hemorrhagic necrosis ensues, further complicating the clinical scenario (Amirbekian & Hooley, 2014). Given the rapid progression of these pathological changes, delays in diagnosis and intervention can substantially worsen patient outcomes.

Surgical management remains the mainstay of treatment for AT, aiming to untwist the affected adnexa and restore perfusion. However, even when timely intervention is performed, I/R injury continues to pose a significant clinical challenge (Armin Akış et al., 2024). I/R injury arises following the restoration of blood flow after a temporary interruption in oxygen supply (ischemia), triggering a cascade of inflammatory responses and oxidative stress, which can exacerbate tissue damage and impair ovarian function (Tanyeli et al., 2022).

Current investigations have concentrated on exploring promising treatment options to mitigate I/R-induced damage (Erbaş et al., 2024; Yigit et al., 2024). Notably CEP has garnered attention as a potential therapeutic agent owing to its diverse range of reported pharmacological effects. These include antibacterial, antioxidant, anticancer, and anti-inflammatory activities (Liang et al., 2022; Liu et al., 2023).

Oxidative stress is characterized by the accumulation of ROS beyond cellular tolerance. It leads to molecular and tissue damage, contributing to the I/R injury pathophysiology (Ekinci Akdemir et al., 2024). Endogenous free radicals generated during oxidative metabolism can induce significant cellular and DNA damage if left unchecked (Güler et al., 2023). CEP is known for its potent oxidative stress preventive capacity by decreasing ROS levels, which are excessively produced at sites of inflammation (Chen et al., 2023). The protective effects of CEP are attributed to its membrane-stabilizing and antiperoxidative properties, which counteract oxidative stress and inflammation (Liu et al., 2023). Such findings highlight CEP's potential as a therapeutic candidate to alleviate I/R-associated ovarian tissue injury.

Here, we evaluated the protective potential of CEP in a rat model of ovarian T/D-related I/R injury. We hypothesized that CEP could attenuate I/R-induced damage, thereby preserving ovarian structure and function.

Methods

Chemicals

The disinfection process involved the application of a 10% povidone-iodine solution (Batticon; Adeka). Anesthetic induction was achieved using xylazine hydrochloride (Rompun®, Bayer, Istanbul) and ketamine (Ketalar®, Pfizer, Istanbul). The compound CEP (Figure 1, purity ≥ 98.0%, CAS Number: 481-49-2) was procured from Sigma Aldrich (Germany).

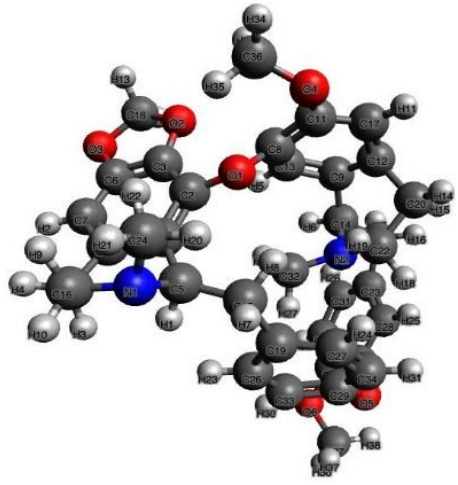


Figure 1. 3D chemical structure of cepharanthine (Created with Avogadro version 1.2.0., http://avogadro.cc/).

Şekil 1. Sefarantin'in (CEP) 3D kimyasal yapısı (Avogadro sürüm 1.2.0 ile oluşturulmuştur, http://avogadro.cc/).

Ethics Committee Approval

In accordance with the ethical principles of the Declaration of Helsinki, the study protocol received approval from the Ethics Committee (Approval No: 5, Date: 30.06.2017) at Atatürk University. All experimental animals were sourced from the Laboratory Animal Research and Application Center at Atatürk University.

Animals

The animals were housed in standard rat cages under controlled environmental conditions, with room temperature, 55± 5% humidity level, and a 12-hour light-dark cycle. They had unrestricted access to standard pellet food and water *ad libitum*. All rats were fasted overnight and free to reach water before the experimental model application.

Preoperative Preparation

The rats were positioned in a supine anatomical posture and secured for the procedure. The abdominal area was carefully shaved to ensure a clean surgical field. Disinfection was performed using a 10% povidone-iodine solution. Anesthesia was induced with a combination of ketamine and xylazine to provide adequate sedation and analgesia. The administered ketamine/xylazine dosage (100/15 mg/kg body weight (BW), intraperitoneally (i.p.)) was selected based on a previously established experimental rat model (Güler et al., 2024).

Animals and Experimental Design

24 female *Wistar albino* rats (12–16 weeks old, 200–250 g) were divided into three groups (n=8). Figure 2 summarizes the experimental process.

Group I (Sham): A median laparotomy incision (1–2 cm) was made, and the incision was closed using 3/0 silk sutures without inducing torsion-detorsion (T/D) or administering any pharmacological intervention (Figure 2a).

Group II (T/D): After performing the abdominal incision, the ovaries, ovarian vessels, and fallopian tubes were rotated 360° clockwise to induce bilateral ovarian torsion. The torsion was maintained for 3 hours using atraumatic microvascular clamps. Following the torsion phase, the clamps were removed to restore blood flow for an additional 3 hours (detorsion phase), and the incision was closed (Figure 2b) (Armin Akış et al., 2024).

Group III (CEP 10 mg/kg): All procedures were performed as in the T/D group. In addition, a 10 mg/kg CEP was administered i.p. 30 minutes before detorsion (Figure 2c). The CEP dosage was determined according to prior experimental studies (Zhao et al., 2020).

After completing the 3-hour detorsion period, anesthesia was re-administered, and the rats were euthanized using cervical dislocation. The blood samples were obtained via cardiac puncture, and ovarian tissue samples were harvested for biochemical analysis.

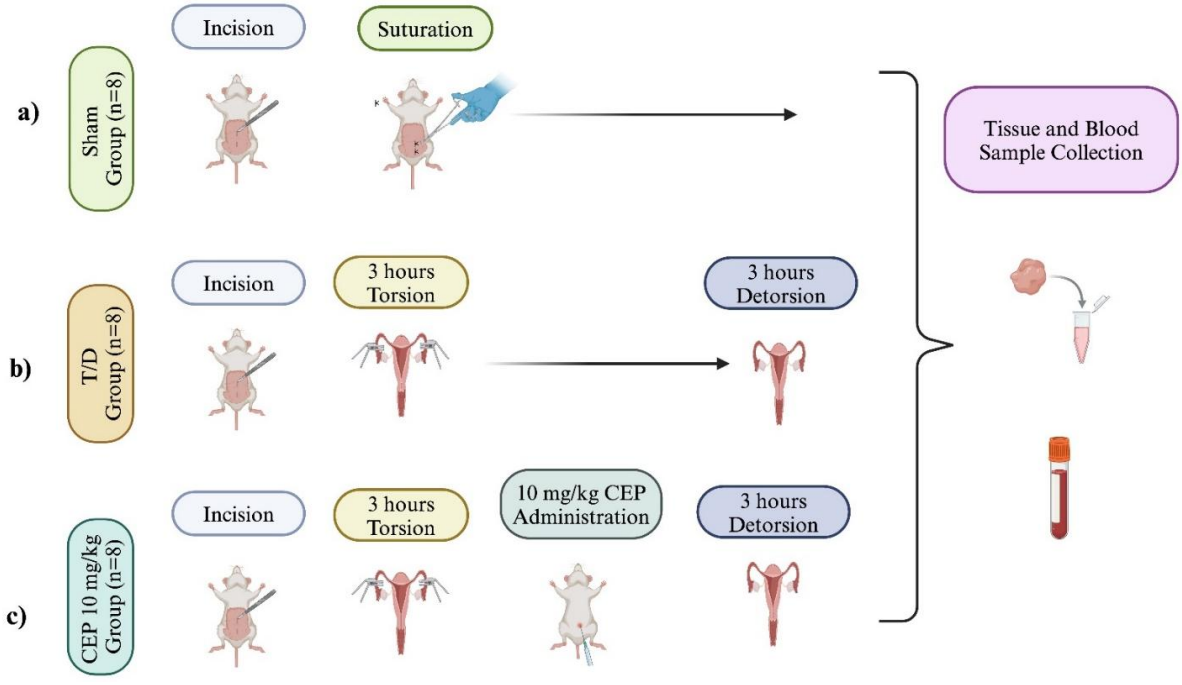


Figure 2. The summarization of the experimental process, a) the sham group; only incision and suturation was performed, b) the T/D group; the T/D model was performed, c) the CEP 10 mg/kg group; 10 mg/kg CEP was administered i.p. 30 minutes before detorsion (Created in https://BioRender.com, CEP= Cepharanthine, T/D= Torsion/detorsion).

Şekil 2. Deneysel sürecin özeti, a) sham grubu; yalnızca insizyon ve sütürasyon yapılmıştır, b) T/D grubu; T/D modeli uygulanmıştır, c) CEP 10 mg/kg grubu; detorsiyondan 30 dakika önce 10 mg/kg CEP intraperitoneal olarak uygulanmıştır (https://BioRender.com ile oluşturulmuştur, CEP= Sefarantin, T/D= Torsiyon/detorsiyon).

Biochemical Analysis

A high-speed homogenizer (IKA, Staufen, Germany) was used to homogenize 100 mg of ovarian tissue samples on ice at 10.000 rpm for 5 minutes. The homogenization was carried out in 2 mL of 10% phosphate buffer solution. After homogenization, the samples were centrifugated at 4°C, 3.000×g for 30 minutes. The resulting supernatant was collected for biochemical assessments, including malondialdehyde (MDA), myeloperoxidase (MPO), total oxidant status (TOS), total antioxidant status (TAS), and superoxide dismutase (SOD). Additionally, proinflammatory cytokines, interleukin-1 beta (IL-1β), and tumor necrosis factor-alpha (TNF-α) were quantified with a rat-specific ELISA kit (Elabscience, Wuhan, China).

MDA Determination

MDA levels were quantified following the method outlined by Ohkawa et al. (1979). Tetramethoxypropane served as standard, and lipid peroxidation levels were reported in nanomoles of MDA. The interaction between MDA and thiobarbituric acid

(TBA) was assessed spectrophotometrically using a PowerWave™ XS Biotek spectrophotometer.

For the assay, the supernatant was mixed with butylated hydroxytoluene (BHT) in methanol, followed by the addition of TBA solutions and phosphoric acid. The mixture was vortexed and incubated at 95°C for 60 minutes to allow the reaction. Following incubation, the samples were centrifuged for 3 minutes at 10.000 × *g*. The supernatant was carefully collected, transferred to a cuvette, and analyzed for absorbance at 532 nm using a spectrophotometer.

MPO Activity Assay

MPO activity was found via the technique proposed by Bradley et al. (1982). The tissue suspensions were subjected to centrifugation for 15 minutes at 40.000×g, and the obtained supernatant was utilized for analysis. This assay is based on the oxidative reaction between MPO and *o*-dianisidine in the presence of hydrogen peroxide (H_2O_2), forming a yellow-orange complex. The absorbance of this complex was assessed spectrophotometrically at 460 nm using a PowerWave™ XS Biotek spectrophotometer.

SOD Activity Assay

A method described by Sun et al. was preferred for determining the SOD activity (Sun et al., 1988) This assay is based on the formation of a blue formazan dye, which is produced when xanthine and superoxide radicals reduce nitro blue tetrazolium (NBT) in the presence of xanthine oxidase. The reaction product's optical density (OD) was measured at 560 nm using a PowerWave™ XS Biotek spectrophotometer. A single unit of SOD activity was characterized as the enzyme amount necessary to reduce NBT reduction by 50%.

The Analysis of Oxidative Stress Index (OSI), Total Antioxidant Status (TAS), and Total Oxidant Status (TOS)

The TAS assay operates on the principle that antioxidants in the sample diminish the dark blue-green 2,2'-azinobis radical to its reduced form. A reduction in absorbance at 660 nm is indicative of the total antioxidant capacity. TAS levels were quantified by a commercial assay kit (RL0024; Rel Assay Diagnostics, Gaziantep, Türkiye).

In the TOS assay, the ferrous ion-chelator complex is oxidized by oxidizing agents in the sample, producing ferric ions that form a colored complex in an acidic medium. The intensity of the resulting color was measured spectrophotometrically, reflecting the total oxidant content. TOS levels were determined through a commercial kit (RL0005; Rel Assay Diagnostics, Gaziantep, Türkiye).

The OSI was calculated by dividing TOS by TAS using the formula: OSI = TOS/TAS (Akdemir et al., 2024).

Statistical Analysis

Data are summarized as mean ± standard error of the mean (SEM) for variables meeting parametric assumptions and as median [interquartile range, IQR] otherwise.

Normality was assessed with the Shapiro–Wilk test and homogeneity of variances with Levene's test. When assumptions were satisfied, one-way ANOVA followed by Tukey's HSD (which adjusts for multiple pairwise comparisons) was used. When assumptions were violated, the Kruskal–Wallis test was applied; if significant, pairwise Mann–Whitney U tests with Holm–Bonferroni multiplicity adjustment were conducted to identify group differences. Two-sided, multiplicity-adjusted $p < .05$ were considered statistically significant. Analyses were performed in SPSS v16.0 (SPSS Inc., Chicago, IL).

Results

Oxidative Stress and Antioxidant Activity Parameters

MDA, SOD Levels, and MPO Activity

Figure 3 demonstrates the MDA, SOD levels, and MPO activity. The findings validate the successful development of the T/D model. Compared to the sham group, the T/D group exhibited a significant increase in MDA and MPO levels (Figure 3A and Figure 3C, $p < .05$). 10 mg/kg CEP treatment resulted in a marked reduction in MDA and MPO levels compared to the T/D group (Figure 3A and Figure 3C, $p < .05$).

Additionally, The T/D group exhibited significantly declined SOD levels compared to the sham group (Figure 3B, $p < .05$), indicating oxidative stress induction. Treatment with 10 mg/kg CEP significantly elevated in SOD levels relative to the T/D group (Figure 3B, $p < .05$), suggesting an enhancement in antioxidant defense mechanisms. These findings indicate that 10 mg/kg CEP mitigated oxidative stress by reducing lipid peroxidation and MPO activity while enhancing antioxidant enzyme (SOD) levels.

TAS, TOS and OSI Levels

The variations in total antioxidant and oxidant levels closely paralleled the trends observed in MDA, SOD, and MPO levels. The T/D group demonstrated significantly low TAS levels relative to the sham group (Figure 4A, $p < .05$). However, administration of 10 mg/kg CEP significantly elevated TAS levels compared to the T/D group (Figure 4A, $p < .05$).

Furthermore, TOS and OSI levels increased in the T/D group relative to the sham group (Figure 4B and Figure 4C, $p < .05$). However, treatment with 10 mg/kg CEP significantly reduced TOS and OSI levels compared to the T/D group (Figure *4B and Figure 4C,* $p < .05$).

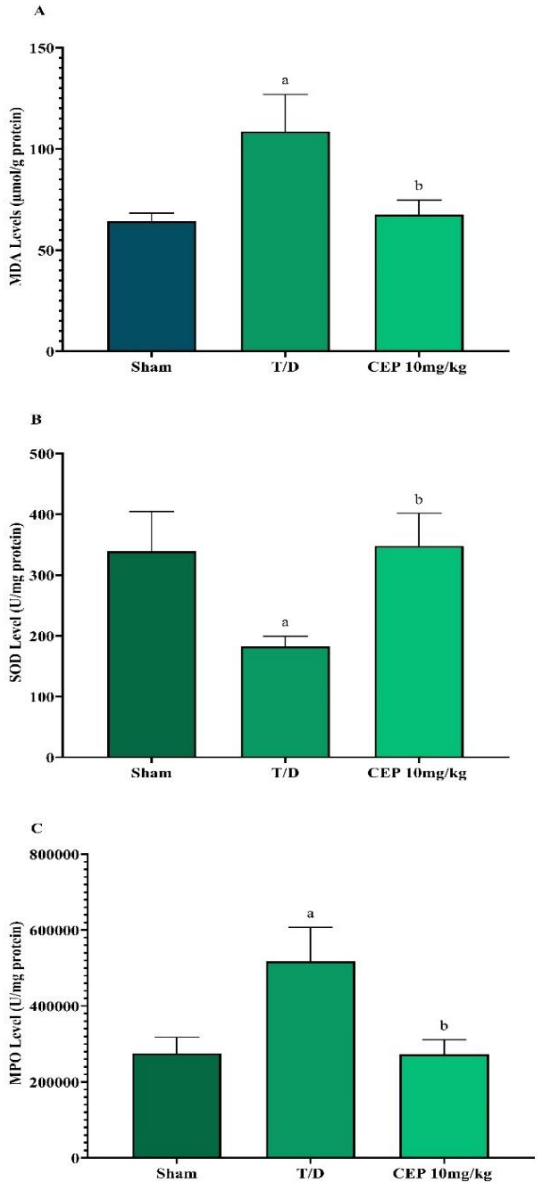


Figure 3. The levels of MDA and the activities of MPO and SOD in the different experimental groups. Data are expressed as SEM. Different superscript letters indicate statistically significant differences between groups ($p < .05$). Groups sharing the same letter are not significantly different (^{a} = indicates a significant difference compared to the sham group, ^{b} = represents a significant difference between the 10 mg/kg CEP group and the T/D group. MDA= Malondialdehyde, MPO= Myeloperoxidase, SOD= Superoxide dismutase, CEP= Cepharanthine, SEM= Standard error of the mean, T/D= Torsion/detorsion).

Şekil 3. Farklı deney gruplarındaki MDA düzeyleri ve MPO ile SOD enzim aktiviteleri. Veriler SEM olarak ifade edilmiştir. Farklı üst simge harfleri gruplar arasında istatistiksel olarak anlamlı farklılıkları göstermektedir ($p <$,05). Aynı harfi paylaşan gruplar anlamlı ölçüde farklı değildir (^{a} = T/D grubunda sham grubuna kıyasla anlamlı

artışı belirtir, [b]= 10 mg/kg CEP grubunda T/D grubuna kıyasla anlamlı azalmayı belirtir. MDA= Malondialdehit, MPO= Miyeloperoksidaz, SOD= Süperoksit dismutaz, CEP= Sefarantin, SEM= Ortalama standart hatası, T/D= Torsiyon/detorsiyon).

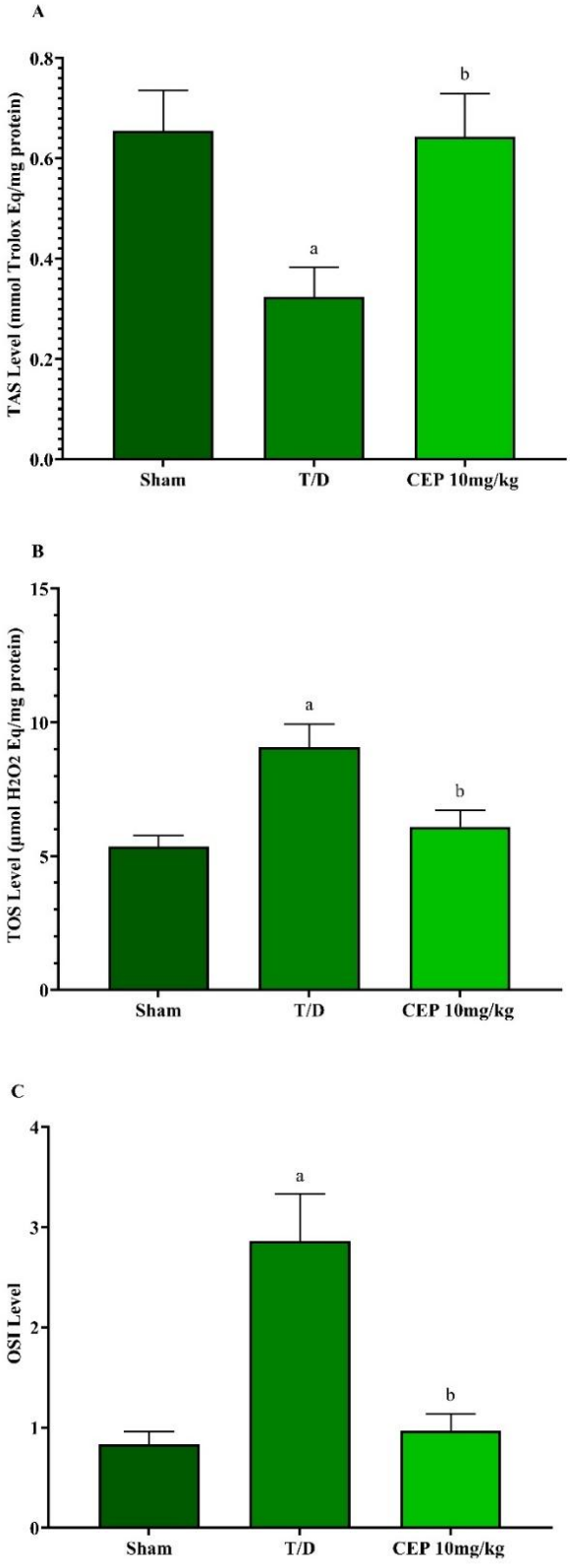


Figure 4. The levels of TAS, TOS, and OSI in the different experimental groups. Data are expressed as SEM. Different superscript letters indicate statistically significant differences between groups (p< .05). Groups sharing the same letter are not significantly different ([a] = indicates a significant difference compared to the sham group, [b] = represents a significant difference between the 10 mg/kg CEP group and the T/D group. TAS= Total antioxidant status, TOS= Total oxidant status, OSI= Oxidative stress index, CEP= Cepharanthine, SEM= Standard error of the mean, T/D= Torsion/detorsion).

Şekil 4. Farklı deney gruplarındaki TAS, TOS ve OSI düzeyleri. Veriler SEM olarak ifade edilmiştir. Farklı üst simge harfleri gruplar arasında istatistiksel olarak anlamlı farklılıkları göstermektedir (p< ,05). Aynı harfi paylaşan gruplar anlamlı ölçüde farklı değildir ([a] = T/D grubunda sham grubuna kıyasla anlamlı artışı belirtir, [b]= 10 mg/kg CEP grubunda T/D grubuna kıyasla anlamlı azalmayı belirtir. TAS= Toplam antioksidan statüsü (kapasitesi), TOS= Toplam oksidan statüsü (kapasitesi), OSI= Oksidatif stres indeksi, CEP= Sefarantin, SEM= Ortalama standart hatası, T/D= Torsiyon/detorsiyon).

Proinflammatory Cytokines (TNF-α and IL-1β)

In our study, TNF-α and IL-1β levels were evaluated by ELISA to assess the changes in proinflammatory cytokines. TNF-α and IL-1β showed elevated levels in the T/D group compared to the sham group and CEP treatment alleviated TNF-α and IL-1 levels (Table 1, Figure 5A and Figure 5B, p< .05).

Table 1. Comparison of IL-1β and TNF-α levels among the sham, T/D, and CEP 10 mg/kg groups.

Tablo 1. Sham, T/D ve CEP 10 mg/kg grupları arasında IL-1β ve TNF-α düzeylerinin karşılaştırılması.

Group	TNF-α (pg/mg protein)	IL-1β (pg/mg protein)
Sham	32.5 ± 2.1	28.7 ± 1.9
T/D	65.8 ± 3.4[a]	59.3 ± 2.8[a]
CEP 10 mg/kg	41.2 ± 2.6[b]	37.6 ± 2.3[b]

TNF-α and IL-1β levels in different groups. Data are expressed as SEM. Different superscript letters indicate statistically significant differences between groups (p< .05). [a] = indicates a significant difference compared to the sham group, [b] = represents a significant difference between the 10 mg/kg CEP group and the T/D group.

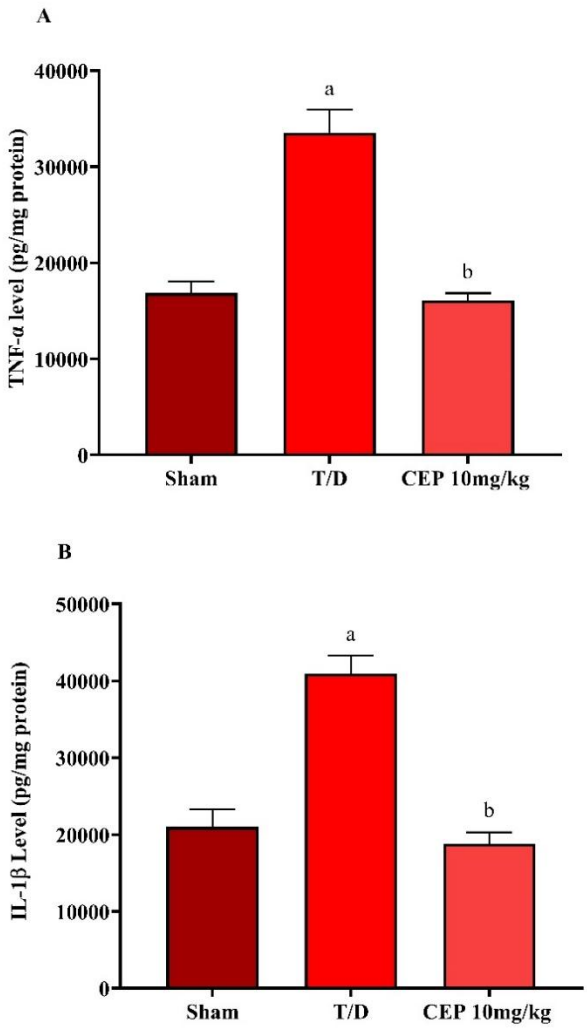


Figure 5. Comparison of IL-1β and TNF-α levels among the sham, T/D, and CEP 10 mg/kg groups. Data are expressed as SEM. Different superscript letters indicate statistically significant differences between groups (p< .05). Groups sharing the same letter are not significantly different ([a] = indicates a significant difference compared to the sham group, [b] = represents a significant difference between the 10 mg/kg CEP group and the T/D group. IL-1β= Interleukin-1 beta, TNF-α= Tumor necrosis factor-alpha, CEP= Cepharanthine, SEM= Standard error of the mean, T/D= Torsion/detorsion).

Şekil 5. Sham, T/D ve CEP 10 mg/kg grupları arasında IL-1β ve TNF-α düzeylerinin karşılaştırılması. Veriler SEM olarak ifade edilmiştir. Farklı üst simge harfleri gruplar arasında istatistiksel olarak anlamlı farklılıkları göstermektedir (p< ,05). Aynı harfi paylaşan gruplar anlamlı ölçüde farklı değildir ([a] = T/D grubunda sham grubuna kıyasla anlamlı artışı belirtir, [b]= 10 mg/kg CEP grubunda T/D grubuna kıyasla anlamlı azalmayı belirtir. IL-1β= İnterlökin-1 beta, TNF-α= Tümör nekroz faktörü-alfa, CEP= Sefarantin, SEM= Ortalama standart hatası, T/D= Torsiyon/detorsiyon).

Discussion

This research focused on evaluating the protective properties of CEP on ovarian T/D-related I/R injury in a rat model. With the continuous advancements in organ-preserving surgical techniques across various medical fields, addressing I/R injuries remains a critical concern. The prevention and mitigation of these injuries have been extensively documented in the literature (Aktepe et al., 2024; Osmanlıoğlu et al., 2023; Ulug et al., 2024). However, effective therapeutic strategies to counteract the oxidative and inflammatory damage induced by I/R injury in ovarian tissue remain limited.

Ovarian torsion is a gynecological emergency that, when treated via detorsion, frequently results in I/R injury, emphasizing the necessity for protective interventions (Güler et al., 2020). This condition is characterized by a cascade of oxidative stress and inflammation, leading to substantial tissue damage. Previous studies have primarily focused on key biochemical markers associated with I/R injury, SOD, MDA, MPO, TAS, TOS, IL-1β, and TNF-α. For instance, Tanyeli et al. (2022) demonstrated that ovarian I/R injury increases oxidative stress markers such as OSI, MPO, and pro-inflammatory cytokines like IL-1β and TNF-α while reducing antioxidant enzyme levels in distant organs such as the lung. Similarly, Arslan et al. (2023) reported that I/R injury in ovarian tissue is associated with elevated levels of MDA, MPO, TOS, and inflammatory cytokines, coupled with a reduction in TAS levels, which was ameliorated by the administration of *Passiflora incarnata*.

CEP has been recognized for its pharmacological activities, like free lipid peroxidation inhibition and radical scavenging (Liang et al., 2022). The ability of CEP to counteract oxidative damage is primarily attributed to its role in neutralizing ROS, thereby preventing cellular injury. Additionally, its anti-inflammatory effects stem from the modulation of cytokine release and inhibition of inflammatory signaling pathways, which may be crucial in mitigating I/R-induced ovarian damage (Kao et al., 2015; Liu et al., 2023).

In this study, CEP administration led to a notable decrease in oxidative stress and inflammatory markers. Specifically, CEP treatment lowered MPO and MDA levels, enhanced SOD activity, and diminished pro-inflammatory cytokines IL-1β and TNF-α. These findings strongly indicate that CEP

prevented by attenuating oxidative damage and suppressing the inflammatory response. Given its dual mechanism of action, CEP could represent a promising pharmacological intervention in managing ovarian T/D.

The potential clinical implications of these findings are substantial. Ovarian T/D is a critical condition that requires prompt surgical intervention, yet the associated I/R injury can lead to significant morbidity, including loss of ovarian function (Güler & Tanyeli, 2020). The incorporation of CEP as an adjunctive treatment could help preserve ovarian viability and improve post-detorsion outcomes in affected individuals.

Nevertheless, this study has certain limitations. While CEP consistently attenuated histopathological injury and modulated oxidative–inflammatory markers in this rat T/D model, translation to clinical ovarian torsion requires caution. First, CEP was administered before reperfusion; clinically, a feasible window is the perioperative period (on admission or immediately before detorsion). Second, the dose, route, and pharmacokinetics for this indication in humans remain undefined and warrant dose-finding and safety studies. Future work should extend endpoints beyond acute injury to ovarian reserve and fertility outcomes. Finally, the interaction of CEP with standard surgical care should be evaluated in randomized preclinical designs to inform early-phase clinical trials.

Conclusion

This research represents the first attempt to investigate the potential of CEP in mitigating ovarian T/D-induced I/R injury. The observed anti-inflammatory and antioxidant effects highlight its therapeutic potential for gynecological emergencies involving T/D-related tissue injury. Additional studies are needed to clarify its exact mechanisms of action and to establish its clinical applicability. These findings lay the groundwork for future investigations into using CEP and other natural compounds to protect ovarian function in the setting of I/R injury.

Ethics Committee Approval: In accordance with the ethical principles of the Declaration of Helsinki, the study protocol received approval from the Ethics Committee (Approval No: 5, Date: 30.06.2017) at Atatürk University. All experimental animals were sourced from the Laboratory Animal Research and Application Center at Atatürk University.

Author Contributions: Conception: B.B., A.T., E.A., F.N.E.A., Ş.Y., D.G.E., E.P., M.C.G.; Design: B.B., A.T., E.A., F.N.E.A., Ş.Y., D.G.E., E.P., M.C.G.; Supervision: B.B., A.T., E.A., M.C.G.; Fundings: B.B., A.T., E.A., M.C.G.; Data Collection and/or Processing: B.B., A.T., E.A., F.N.E.A., Ş.Y., D.G.E., E.P., M.C.G.; Analysis: E.A., E.P., M.C.G.; Literature Review: B.B., A.T., E.A., M.C.G.; Writing: B.B., A.T., E.A., F.N.E.A., Ş.Y., D.G.E., E.P., M.C.G.; Critical Review: B.B., A.T., E.A., F.N.E.A., Ş.Y., D.G.E., E.P., M.C.G.

Peer-review: Externally peer-reviewed.

Funding: None.

Declaration of Interests: None declared.

References

Akdemir, F. N. E., Güler, M. C., Eraslan, E., Tanyeli, A., & Yildirim, S. (2025). Assessment of sinapic acid's protective effects against ethanol-induced gastric ulcers in rats. *Naunyn-Schmiedeberg's Archives of Pharmacology, 398*(6), 7133–7145. https://doi.org/10.1007/s00210-024-03733-0

Aktepe, R., Ucuncu, Y., Tumkaya, L., Mercantepe, T., Topcu, A., Uydu, H. A., & Atak, M. (2024). Long-term protective effects of lamotrigine in a rat ovarian ischemia-reperfusion model. *Tissue and Cell*, *86*, 102297. https://doi.org/10.1016/j.tice.2023.102297

Amirbekian, S., & Hooley, R. J. (2014). Ultrasound Evaluation of Pelvic Pain. *Radiologic Clinics of North America*, *52*(6), 1215-1235. https://doi.org/10.1016/j.rcl.2014.07.008

Armin Akış, P., Tanyeli, A., Ekinci Akdemir, F. N., Güler, M. C., Şebin, S., Eraslan, E., Laloğlu, E., & Çomaklı, S. (2024). Costunolide, an effective agent against oxidative damage, apoptosis and autophagy in the ovarian torsion/detorsion model. *Archives of Physiology and Biochemistry,* 1-9. https://doi.org/10.1080/13813455.2024.2407548

Arslan, S., Aşır, F., Özkorkmaz, E. G., Azizoğlu, M., Basuguy, E., Okur, M. H., Otçu, S. M. Ö., Karabel, M. A., Kaplan, İ., & Aydoğdu, B. (2023). Protective effects of passiflora incarnata on ovarian ischemia/reperfusion damage in rats with ovarian torsion. *Turkish Journal of Trauma & Emergency Surgery*, *29*(12), 1344–1350. https://doi.org/10.14744/tjtes.2023.52986

Baron, S. L., & Mathai, J. K. (2025). Ovarian Torsion. In *StatPearls*. StatPearls Publishing LLC.

Bradley, P. P., Priebat, D. A., Christensen, R. D., & Rothstein, G. (1982). Measurement of cutaneous inflammation: estimation of neutrophil content with an enzyme marker. *Journal of Investigative Dermatology*, *78*(3), 206-209. https://doi.org/10.1111/15231747.ep12506462

Chen, G., Wen, D., Shen, L., Feng, Y., Xiong, Q., Li, P., & Zhao, Z. (2023). Cepharanthine Exerts Antioxidant and Anti-Inflammatory Effects in Lipopolysaccharide (LPS)-Induced Macrophages and DSS-Induced Colitis Mice. *Molecules*, *28*(16). https://doi.org/10.3390/molecules28166070

Dixon, A., Bell, D., & Le, L. (2025). *Ovarian torsion*. https://radiopaedia.org/articles/9540

Ekinci Akdemir, F. N., Güler, M. C., Eraslan, E., Tanyeli, A., & Yildirim, S. (2024). Caftaric acid attenuates kidney and remote organ damage induced by renal ischemia-reperfusion injury. *Scientific Reports*, *14*(1), 31385. https://doi.org/10.1038/s41598-024-82912-8

Erbaş, E., Özkanlar, S., Yeşildağ, A., Kara, A., & Senthil Kumar, K. J. (2024). Syringic acid loaded silver nanoparticles protects ovarian ischemia-reperfusion injury in rats by inhibiting endoplasmic reticulum stress-

mediated apoptosis. *Journal of Drug Delivery Science and Technology*, *99*, 105944. https://doi.org/10.1016/j.jddst.2024.105944

Franco, P. N., García-Baizán, A., Aymerich, M., Maino, C., Frade-Santos, S., Ippolito, D., & Otero-García, M. (2023). Gynaecological Causes of Acute Pelvic Pain: Common and Not-So-Common Imaging Findings. *Life*, *13*(10). https://doi.org/10.3390/life13102025

Güler, M. C., Akpinar, E., Tanyeli, A., Çomakli, S., & Bayir, Y. (2023). Costunolide prevents renal ischemia-reperfusion injury in rats by reducing autophagy, apoptosis, inflammation, and DNA damage. *Iranian Journal of Basic Medical Sciences*, *26*(10), 1168-1176. https://doi.org/10.22038/ijbms.2023.71779.15596

Güler, M. C., & Tanyeli, A. (2020). Role of Hyperoside on Ovarian Tissue Damage Created by Ovarian Torsion Detorsion. *New Trends in Medicine Sciences*, *1*(1), 1-5.

Güler, M. C., Tanyeli, A., Eraslan, E., Çelebi, Ö., Çelebi, D., Çomakli, S., Yurdgülü, E. E., & Bayir, Y. (2024). Alleviating sepsis: Revealing the protective role of costunolide in a cecal ligation and puncture rat model. *Iranian Journal of Basic Medical Sciences*, *27*(5), 567-576. https://doi.org/10.22038/ijbms.2024.75372.16335

Güler, M. C., Tanyeli, A., Eraslan, E., & Ekinci Akdemir, F. N. (2020). Role of 6-Shogaol Against Ovarian Torsion Detorsion-Induced Reproductive Organ Damage. *New Trends in Medicine Sciences*, *1*(1), 29-34.

Kao, M.-C., Chung, C.-Y., Chang, Y.-Y., Lin, C.-K., Sheu, J.-R., & Huang, C.-J. (2015). Salutary effects of cepharanthine against skeletal muscle and kidney injuries following limb ischemia/reperfusion. *Evidence-Based Complementary and Alternative Medicine*, *2015*(1), 504061. https://doi.org/10.1155/2015/504061

Kumari, A., Mishra, G., Parihar, P., Dudhe, S., Bhangale, P., Agrawal, R., & Shinde, D. (2024). Ovarian Torsion Leading to Necrosis: A Case Report of Acute Abdominal Pain in a 25-Year-Old Female. *Cureus*, *16*. https://doi.org/10.7759/cureus.67507

Liang, D., Li, Q., Du, L., & Dou, G. (2022). Pharmacological Effects and Clinical Prospects of Cepharanthine. *Molecules*, *27*(24). https://doi.org/10.3390/molecules27248933

Liu, K., Hong, B., Wang, S., Lou, F., You, Y., Hu, R., Shafqat, A., Fan, H., & Tong, Y. (2023). Pharmacological Activity of Cepharanthine. *Molecules*, *28*(13). https://doi.org/10.3390/molecules28135019

Ohkawa, H., Ohishi, N., & Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry*, *95*(2), 351-358. https://doi.org/10.1016/0003-2697(79)90738-3

Osmanlıoğlu, Ş., Arslan, M., Dağ, R. O., Yığman, Z., Ceyhan, M. Ş., Er, F., & Kavutçu, M. (2023). Artemisinin reduces acute ovarian ischemia-reperfusion injury in rats. *Reproductive Toxicology*, *119*, 108417. https://doi.org/10.1016/j.reprotox.2023.108417

Stickland, A. E. J., & Phillips, C. (2021). Gynaecological causes of acute abdominal pain. *Obstetrics, Gynaecology & Reproductive Medicine*, *31*(2), 48-52. https://doi.org/10.1016/j.ogrm.2020.12.005

Sun, Y., Oberley, L. W., & Li, Y. (1988). A simple method for clinical assay of superoxide dismutase. *Clinical Chemistry*, *34*(3), 497-500.

Tanyeli, A., Guzel Erdogan, D., Comakli, S., Polat, E., Guler, M. C., Eraslan, E., & Doganay, S. (2022). Therapeutic effects of apocynin on ovarian ischemia-reperfusion induced lung injury. *Biotechnic & Histochemistry*, *97*(7), 536-545. https://doi.org/10.1080/10520295.2022.2036368

Thiyagalingam, S., Petrosellini, C., & Mellon, C. (2024). Adnexal Torsion in the Third Trimester. *Cureus*, *16*(5), e60836. https://doi.org/10.7759/cureus.60836

Ulug, P., Nayki, U., Mammadov, R., Bulut, S., Tastan, T. B., Coban, T. A., & Suleyman, H. (2024). Protective Effect of Ramipril Against Oxidant and Proinflammatory Cytokine Damage Induced by Ischemia-Reperfusion in Ovarian Tissue in Rats. *Transplantation Proceedings*, *56*(1), 215-222. https://doi.org/10.1016/j.transproceed.2023.11.016

Yigit, S., Yesilyurt, I., Bitiktas, S., Aksu Kilicle, P., Duysak, L., Yayla, M., Toktay, E., Eyerci, N., Ali Bingol, S., Gezer, A., Necmiye Kaci, F., Taskin, E., Esma Akdogan, G., Cilgin, H., & Alper Kahraman, A. (2024). Therapeutic effect of Berberis vulgaris fruit extract on histopathological changes and oxidative stress markers of ovarian ischemia and reperfusion injury in rats. *Journal of King Saud University - Science*, *36*(11), 103578. https://doi.org/10.1016/j.jksus.2024.103578

Zhao, J., Piao, X., Wu, Y., Liang, S., Han, F., Liang, Q., Shao, S., & Zhao, D. (2020). Cepharanthine attenuates cerebral ischemia/reperfusion injury by reducing NLRP3 inflammasome-induced inflammation and oxidative stress via inhibiting 12/15-LOX signaling. *Biomedicine & Pharmacotherapy*, *127*, 110151. https://doi.org/10.1016/j.biopha.2020.110151

ATATÜRK ÜNİVERSİTESİ YAYINLARI
ATATURK UNIVERSITY PUBLICATIONS

Research Article *Araştırma Makalesi* DOI: 10.62425/jlasp.1678282

Effects of Melatonin on FSH/LH/Testosterone and StAR/P450scc/17β-HSD3 Pathways in Acrylamide-Induced Testicular Toxication in Rats

Ratlarda Akrilamid İle İndüklenen Testiküler Toksikasyonda Melatoninin FSH/LH/Testosteron ve StAR/P450scc/17β-HSD3 Yolakları Üzerine Etkileri

Samet TEKİN[1]
Yusuf DAĞ[1]
Furkan AYKURT[1]
Merve BOLAT[1]
İsmail BOLAT[2]
Burak Batuhan LAÇİN[1]
Burak ÇINAR[3]
Mohamad WARDA[1,4]

[1]Department of Physiology, Atatürk University Faculty of Veterinary Medicine, Erzurum, Türkiye
[2]Department of Pathology, Atatürk University Faculty of Veterinary Medicine, Erzurum, Türkiye
[3]Department of Pharmacology, Atatürk University Faculty of Medicine, Erzurum, Türkiye
[4]Department of Biochemistry Faculty of Veterinary Medicine, Cairo University, Egypt

ABSTRACT

Acrylamide (ACR) is a toxic compound formed during high-temperature food processing known to cause reproductive toxicity. This study investigated the protective effects of melatonin (MEL) on ACR-induced testicular toxicity, focusing on hormonal regulation and steroidogenic pathways. 40 adult male Wistar rats were divided into five groups: control, ACR (50 mg/kg), ACR+MEL10 (ACR + 10 mg/kg MEL), ACR+MEL20 (ACR + 20 mg/kg MEL), and MEL20 (20 mg/kg MEL only). Treatments were administered via intragastric gavage for 14 days. Serum follicle-stimulating hormone (FSH), luteinizing hormone (LH), and testosterone levels were measured together with testicular steroidogenic enzyme levels (StAR, P450scc, 17β-HSD3) by ELISA. Histopathological analysis was used to assess spermatogenic integrity by Johnsen scoring. ACR exposure significantly decreased serum FSH, LH, and testosterone levels ($p < .001$). There was a concomitant decrease in StAR, P450scc, and 17β-HSD3 expression due to this exposure. Histological evaluation revealed severe spermatocyte degeneration, necrosis, and impaired spermatogenesis. MEL administration ameliorated these effects in a dose-dependent manner. Although ACR+MEL10 partially treated hormonal and enzymatic levels ($p < .05$), ACR+MEL20 treated these parameters to a level close to control levels ($p > .05$). MEL also attenuated histopathological damage, preserved seminiferous tubule integrity, and spermatogenic function. These findings showed that MEL restored steroidogenic enzyme activity and hormonal balance through its protective effects. MEL supplementation may be a potential treatment strategy against environmental toxicants affecting male reproductive health. Advanced studies are needed to fully elucidate the molecular mechanisms of MEL's protective effects.

Keywords: Acrylamide, Hormone, Melatonin, Steroidogenesis, Testis.

ÖZ

Akrilamid (ACR), oksidatif stres ve üreme toksisitesine neden olduğu bilinen yüksek sıcaklıkta gıda işleme sırasında oluşan toksik bir bileşiktir. Bu çalışmada, hormonal düzenleme ve steroidojenik yollara odaklanılarak melatoninin (MEL) ACR kaynaklı testis toksisitesi üzerindeki koruyucu etkileri araştırıldı. 40 yetişkin erkek Wistar sıçanı beş gruba ayrıldı: kontrol, ACR (50 mg/kg), ACR+MEL10 (ACR + 10 mg/kg MEL), ACR+MEL20 (ACR + 20 mg/kg MEL) ve MEL20 (sadece 20 mg/kg MEL). Tedaviler 14 gün boyunca intragastrik gavaj yoluyla uygulandı. Serum folikül uyarıcı hormon (FSH), luteinize edici hormon (LH) ve testosteron seviyeleri, testis steroidojenik enzim seviyeleri (StAR, P450scc, 17β-HSD3) ile birlikte ELISA yoluyla ölçüldü. Histopatolojik analiz, Johnsen puanlaması yoluyla spermatogenik bütünlüğü değerlendirmek için kullanıldı. ACR maruziyeti serum FSH, LH ve testosteron seviyelerini önemli ölçüde düşürdü ($p < ,001$). Bu maruziyete bağlı StAR, P450scc ve 17β-HSD3 ekspresyonunda eş zamanlı bir azalma oldu. Histolojik değerlendirme şiddetli spermatosit dejenerasyonu, nekroz ve bozulmuş spermatogenez olduğunu ortaya koydu. MEL uygulaması bu etkileri doza bağlı bir şekilde iyileştirdi. ACR+MEL10, hormonal ve enzimatik seviyeleri kısmi olarak tedavi etse de ($p < ,05$), ACR+MEL20 bu parametreleri kontrol seviyelerine yakın bir düzeyde tedavi edilmesini sağladı ($p > ,05$). MEL ayrıca histopatolojik hasarı hafifletti, seminifer tübül bütünlüğünü ve spermatogenik işlevi korudu. Bu bulgular, MEL'in koruyucu etkileriyle steroidojenik enzim aktivitesini ve hormonal dengeyi geri kazandırdığını gösterdi. MEL takviyesi, erkek üreme sağlığını etkileyen çevresel toksik maddelere karşı potansiyel bir tedavi stratejisi olabilir. MEL'in koruyucu etkilerinin moleküler mekanizmalarını tam olarak ortaya koymak için ileri düzey çalışmalara ihtiyaç vardır.

Anahtar kelimeler: Akrilamid, Hormon, Melatonin, Steroidogenez, Testis.

Geliş Tarihi/Received :17.04.2025
Kabul Tarihi/Accepted :11.08.2025
Yayın Tarihi/Publication Date :19.09.2025

Sorumlu Yazar/Corresponding author: Samet Tekin
E-mail: samet.tekin@atauni.edu.tr

Cite this article: Tekin, S., Dağ, Y., Aykurt, F., Bolat, M., Bolat, İ., Laçin, B.B., Çınar, B., & Warda, M. (2025). Effects of Melatonin on FSH/LH/Testosterone and StAR/P450scc/17β-HSD3 Pathways in Acrylamide-Induced Testicular Toxication in Rats. *Journal of Laboratory Animal Science and Practices*, 5(2), 119-125. https://doi.org/10.62425/jlasp.1678282

Introduction

Heat treatment affects the quality and microbiological safety of food with the potential for the formation of heat-treated contaminants. One of these toxic contaminants is acrylamide (ACR), which has become increasingly important in recent years. (Mogol & Gökmen, 2016; Tareke et al., 2002). ACR starts to form once heat treatment exceeds 120°C (Halford et al., 2012), accompanied by decreased moisture (Batuwita et al., 2025). After exposure to acrylamide, it is rapidly absorbed by the gastrointestinal system. It then spreads to many tissues such as the heart, liver, brain and kidneys, and even crosses the blood-brain, blood-placenta and blood-testis barriers. Once acrylamide enters the body, it is converted to glycamide and causes damage in this form. One of the tissues affected by this damage is the reproductive system. Glycamide can induce oxidative stress, inflammation, and, in severe cases, apoptosis in these tissues. As a result, it can cause pathologies in organ physiology (Gao et al., 2022; Lebda et al., 2014; Yan et al., 2023).

Mating, fertilization and sperm transport within the uterus are significantly reduced in ACR-exposed rats (Tyl et al., 2000). Wang H et al. reported that when male rats were administered ACR, growth retardation, a decrease in sperm sources in the epididymis and histopathological lesions in the testes occurred (Wang et al., 2010). The synthesis of testosterone in the testes is controlled by luteinizing hormone (LH), which is synthesized in the anterior pituitary gland and acts on the Leydig cells. LH regulates steroidogenesis by binding to LH receptors on the membrane of Leydig cells. In steroidogenesis, the StAR protein carries cholesterol from the cytoplasm to the mitochondria, the first step in testosterone synthesis. Enzymes such as 17β-HSD3 and p450scc participate in the conversion of cholesterol to testosterone (Afzal et al., 2024; Walker & Cheng, 2005).

Light is sensed by photoreceptors on the retina and stimulates the pineal gland through a complex system that includes retinohypothalamic fibers, suprachiasmatic nuclei, hypothalamus-pineal fibers and the peripheral nervous system. Stimulation of the pineal gland regulates the synthesis and secretion of melatonin (Frungieri et al., 2017). The most important environmental factor regulating the function of the pineal gland is light duration. In rats, melatonin (MEL) synthesis via the pineal gland is limited to the absence of light and is synthesized rhythmically. Light suppresses melatonin synthesis via the pineal gland (Jimenez-Jorge et al., 2007). Leydig cells are sensitive to melatonin and therefore play an important role in the male reproductive system, especially in the function of the testes. Melatonin regulates the synthesis of androgens through melatonin receptors located around Leydig cells. Melatonin has regulatory effects on the testes protective properties, through its protective properties (Yu et al., 2018). It was designed to identify a previously unstudied and intriguing topic. This study aimed to add to the literature by determining the extent to which a vital hormone such as melatonin is effective in this type of damage.

Methods

Materials

Acrylamide (CAS Number: 79--06--1) and melatonin (CAS Number: 73--31--4) were obtained from Sigma Aldrich Chemical Co. (St. Louis, MO, USA). Follicle-stimulating hormone (FSH) (Cat. No: 201-11-0183), luteinizing hormone (LH) (Cat. No: 201-11-0180), testosterone (Cat. No: 201-11-0564), StAR (Cat. No: 201-11-2668), and 17β-HSD3 (Cat. No: 201-11-6283) kits for rat serum were purchased from SunRedBio (SunRedBio, Shanghai, China). A P450scc (MBS733760) ELISA kit was purchased from MyBioSource.

Animals and Experimental Design

Total A total of 40 healthy 240-250 gram, 12-week-old adult Wistar rats were used in our study. The cages used were meticulously cleaned before the study. The animals were kept in rooms with 12-hour light and 12-hour dark cycles. At the same time, the temperature (21±3°C) and humidity (40–50%) were adjusted. The rats had unlimited access to food and water throughout the study. The study was approved by the Atatürk University Experimental Animals Local Ethics Committee before the study was initiated. Our study was conducted at Atatürk University Medical Experimental Application and Research Center (Protocol Date:10/03/2025-Protocol Number: 59/2025). The applications were performed at the time corresponding to the animals' 12-hour dark cycle.

A total of 40 rats were divided into 5 different groups. The 8 animals in each group were divided into 2 separate cages as the cages were suitable for 4 animals. Experimental groups were designed as 1-Control (The group was given at 1 ml of distilled water), 2- ACR (The group given ACR at a dose of 50 mg/kg), 3- ACR+MEL10 (The group given ACR at a dose of 50 mg/kg and MEL at a dose of 10 mg/kg), 4-ACR+MEL20 (The group given ACR at a dose of 50 mg/kg and MEL at a dose of 20 mg/kg), 5- MEL20 (The group given MEL at a dose of 20 mg/kg). All applications were made to each group for 14 days, intragastrically. Acrylamide and melatonin were dissolved in distilled water and administered.

Preparation of Serum Samples

Blood was collected from animals and centrifuged using a BD Vacutainer SST II Advance serum tube in a refrigerated centrifuge. (Beckman Coulter). The obtained serum was transferred to another tube and stored in a -20°C cabinet. FSH, LH and testosterone levels in the serum were measured via ELISA. The protocol was adjusted according to the catalog sent by the manufacturer.

Homogenization of Tissues

80 mg of right testicular tissue from each rat was placed in Eppendorf tubes and filled with 2 mL of distilled water. The tubes were then placed in the MagNA Lyser device. The device was set to 4000 rpm for 75 seconds. After the tissues were homogenized, they were placed in a centrifuge and centrifuged at 5000 rpm for 10 min, after which the supernatants were transferred to different tubes. StAR, 17β-HSD3 and p450scc measurements were performed via ELISA. The protocol was performed according to that sent by the manufacturer.

Histopathological examinations

The left side of the testicular tissue obtained as a result of the study was fixed with a 10% buffered formaldehyde solution and blocked with paraffin following routine tissue observation procedures. Serial 5 µm thick sections were taken from the blocked tissues. The sections were stained with hematoxylin and eosin (H&E) to determine damage to spermatocytes. In addition, Jonhsen scoring was performed according to the arrangement and number of spermatocytes (Table 1). Again, degeneration, necrosis, edema and hyperemia findings in spermatocytes were examined histopathologically, and the findings were evaluated as negative (-), mild (+), moderate (+++) and severe (++++) (Bolat et al., 2024).

Statistical analyses

After the study was completed, one-way ANOVA and Tukey's test were applied to the ELISA data obtained (p< .05 was considered significant). All these analyses were performed using GraphPad Prism 8.0.1 software.

The GraphPad Prism 8.0.2 program was used for statistical analysis of the histopathological data (p< .05 was considered significant). The nonparametric Kruskal–Wallis test was used to determine group interactions, and the Mann–Whitney U test was used to determine differences between groups.

Table 1. Jonhsen scoring criteria for testicular tissues

Tablo 1. Testis dokuları için Jonhsen skorlama kriterleri

Score	Definition
1	No cells
2	Sertoli cells without germ cells
3	Spermatogonies only
4	Several spermatocytes
5	Many spermatocytes
6	A few primitive spermatids
7	Many primitive spermatids
8	Several mature spermatids
9	Many mature spermatids
10	Full spermatogenesis

Results

Hormonal assessment in testicular damage caused by ACR

Measurements of FSH, LH, and testosterone levels

The serum FSH, LH and testosterone levels were measured via ELISA and are shown in Figure 1.

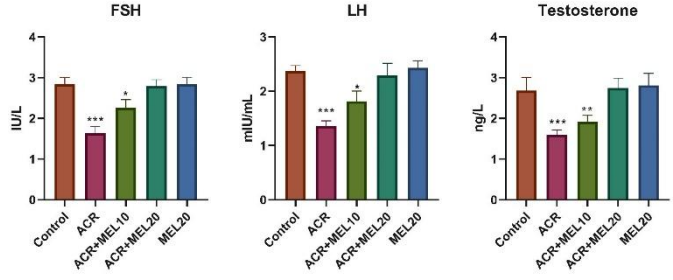


Figure 1. FSH (Follicle-Stimulating Hormone), LH (Luteinizing hormone) and testosterone levels in the serum of ACR (Acrylamide) and MEL (Melatonin) treated rats. The groups were set as Control, ACR (Acrylamide 50mg/kg), ACR+MEL10 (Acrylamide 50mg/kg + Melatonin 10mg/kg), ACR+MEL20 (Acrylamide 50mg/kg + Melatonin 20mg/kg) and MEL20 (Melatonin 20mg/kg). (There are statistically significant differences between the control group and the values expressed with different symbols. ***p< .001 **p< .01 *p< .05; n=8)

Şekil 1. ACR (Akrilamid) ve MEL (Melatonin) uygulanan sıçanların serumundaki FSH (Folikül Uyarıcı Hormon), LH (Lüteinizan hormon) ve testosteron seviyeleri. Gruplar Kontrol, ACR(Akrilamid 50mg/kg), ACR+MEL10(Akrilamid 50mg/kg + Melatonin 10mg/kg), ACR+MEL20 (Akrilamid 50mg/kg + Melatonin 20mg/kg) ve MEL20 (Melatonin 20mg/kg) olarak ayarlandı. (Kontrol grubu ile farklı sembollerle ifade edilen değerler arasında istatistiksel olarak anlamlı farklılıklar vardır. ***p< ,001 **p< ,01 *p< ,05; n=8)

The serum FSH, LH and testosterone levels were significantly lower in the ACR-treated groups than in the control group (p< .001). The serum FSH and LH levels were greater in the ACR+MEL10 group than in the ACR group. However, there was still a significant difference between these groups and the control group (p< .05). Testosterone levels were greater in the ACR+MEL10 groups than in the ACR group, but there was a significant difference between the ACR+MEL10 groups and the control group (p< .01). The serum FSH, LH and testosterone levels in the ACR+MEL20 and MEL20 groups were not significantly different from those in the control group (p> .05).

Measurement of Steroidogenic Enzyme Levels

The levels of P450scc, 17β-HSD3 and StAR enzymes were determined via ELISA in homogenates obtained from testicular tissues (Figure 2).

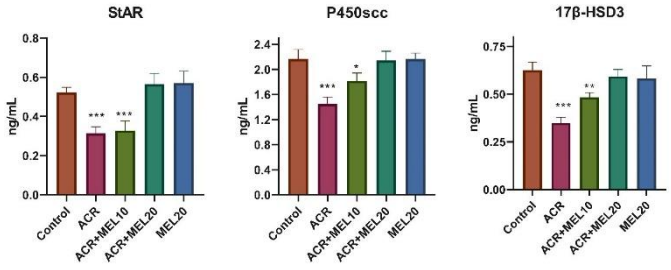


Figure 2. Protein cholesterol side-chain cleavage cytochrome P450 (P450scc), 17 beta hydroxysteroid dehydrogenase 3 (17β-HSD3) and Steroidogenic acute regulatory protein (StAR) levels in testicular tissues of ACR (Acrylamide) and MEL (Melatonin) treated rats. The groups were set as Control, ACR (Acrylamide 50mg/kg), ACR+MEL10 (Acrylamide 50mg/kg + Melatonin 10mg/kg), ACR+MEL20 (Acrylamide 50mg/kg + Melatonin 20mg/kg) and MEL20 (Melatonin 20mg/kg). (There are statistically significant differences between the control group and the values expressed with different symbols. ***p< .001 **p< .01 *p< .05; n=8)

Şekil 2. ACR (Akrilamid) ve MEL (Melatonin) uygulanan sıçanların testis dokularında protein kolesterol yan zincir bölünmesi sitokrom P450 (P450scc), 17 beta hidroksisteroid dehidrogenaz 3 (17β-HSD3) ve Steroidojenik akut düzenleyici protein (StAR) düzeyleri. Gruplar Kontrol, ACR(Akrilamid 50mg/kg), ACR+MEL10(Akrilamid 50mg/kg + Melatonin 10mg/kg), ACR+MEL20 (Akrilamid 50mg/kg + Melatonin 20mg/kg) ve MEL20 (Melatonin 20mg/kg) olarak ayarlandı. (Kontrol grubu ile farklı sembollerle ifade edilen değerler arasında istatistiksel olarak anlamlı farklılıklar vardır. ***p< ,001 **p< ,01 *p< ,05; n=8)

StAR, P450scc and 17β-HSD3 levels were significantly lower in the ACR-treated groups than in the control group (p< .001). The StAR levels obtained from the ACR+MEL10 group were close to those of the ACR group, and there was no significant difference between them (p> .05). The p450scc levels were greater in the ACR+MEL10 group than in the ACR group. However, there was a significant difference between these two groups and the control group (p< .05). 17β-HSD3 levels were significantly different between the ACR+MEL10 groups and the control group (p< .01). StAR, P450scc and 17β-HSD3 levels in the ACR+MEL20 and MEL20 groups were not significantly different from those in the control group (p> .05).

Histopathologic findings

There were no pathological findings during histopathological examination in the control or MEL20 groups. In the ACR group, severe degeneration and necrosis were observed in spermatocytes, with marked vascular hyperemia and significant edema in the intertubular spaces. In the ACR+MEL10 and ACR+MEL20 groups, these findings were significantly decreased in a dose-dependent manner (Figure 3). The scoring of the histopathological findings, Jonhson scoring and statistical analysis results are presented in Figure 4.

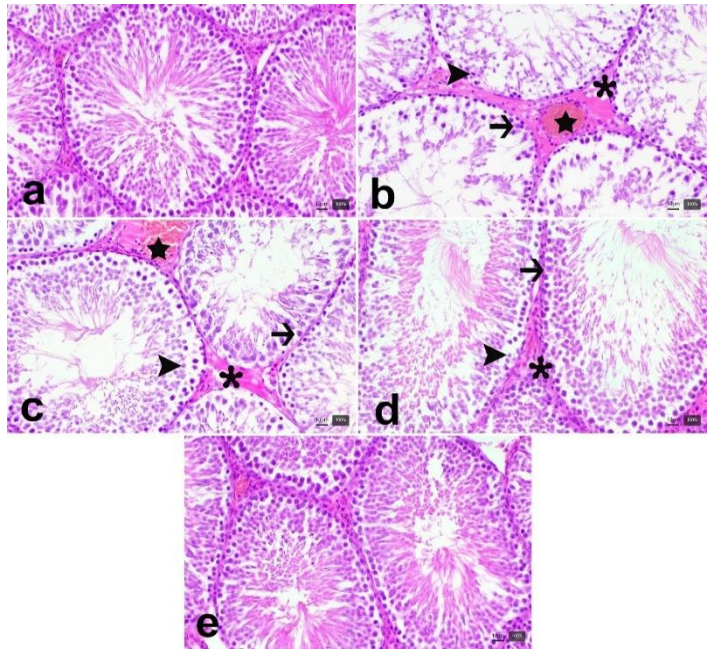


Figure 3. The groups were set as Control(a), ACR (Acrylamide 50mg/kg)(b), ACR+MEL10 (Acrylamide 50mg/kg + Melatonin 10mg/kg)(c), ACR+MEL20 (Acrylamide 50mg/kg + Melatonin 20mg/kg)(d) and MEL20 (Melatonin 20mg/kg)(e). Degeneration in spermatocytes is indicated by arrows, necrosis in spermatocytes by arrowheads, hyperemia in vessels by asterisks and edema in intertubular spaces by asterisks. Hematoxylin Eosin (H&E), scale bar: 10 µm; objective: 20X; zoom: 100%.

Şekil 3. Gruplar Kontrol(a), ACR(Akrilamid 50mg/kg)(b), ACR+MEL10(Akrilamid 50mg/kg + Melatonin 10mg/kg)(c), ACR+MEL20 (Akrilamid 50mg/kg + Melatonin 20mg/kg)(d) ve MEL20 (Melatonin 20mg/kg)(e) olarak ayarlandı. Spermatositlerdeki dejenerasyon oklarla spermatositlerdeki

nekroz ok başlarıyla, damarlardaki hiperemi yıldız işaretleriyle ve intertübüler boşluklardaki ödem yıldız işaretleriyle gösterilmiştir. Hematoksilen Eozin (H&E), ölçek çubuğu: 10 µm; objektif: 20X; yakınlaştırma: 100%.

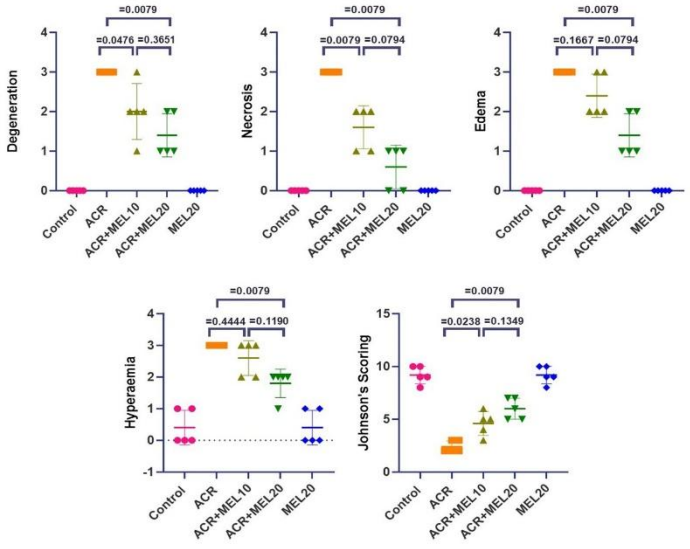


Figure 4. Histopathologic scoring, Jonhson scoring and statistical analysis were performed on testicular tissues after Hematoxylin Eosin (H&E) staining. Kruskal–Wallis and Mann–Whitney U tests were used. The groups were set as Control, ACR (Acrylamide 50mg/kg), ACR+MEL10 (Acrylamide 50mg/kg + Melatonin 10mg/kg), ACR+MEL20 (Acrylamide 50mg/kg + Melatonin 20mg/kg) and MEL20 (Melatonin 20mg/kg).

Şekil 4. Testis dokuları Hematoksilen Eozin (H&E) ile boyandıktan sonra histopatolojik skorlama, Jonhson skorlaması ve istatistiksel analiz yapıldı. Kruskal-Wallis ve Mann-Whitney U testleri kullanılmıştır. Gruplar Kontrol, ACR(Akrilamid 50mg/kg), ACR+MEL10(Akrilamid 50mg/kg + Melatonin 10mg/kg), ACR+MEL20 (Akrilamid 50mg/kg + Melatonin 20mg/kg) ve MEL20 (Melatonin 20mg/kg) olarak ayarlandı.

Discussion

ACR, a toxic chemical frequently used in the synthesis of polymers, is frequently formed during high-temperature treatment of carbohydrate-containing foods. ACR exposure has toxic effects on the immune, reproductive and nervous systems (Farouk et al., 2021; Zamani et al., 2017). ACR exposure causes damage in the testes, leading to qualitative and quantitative impairment of spermatozoa, as well as impaired testicular androgenesis (Farag et al., 2021). We focused on this topic in our study and reported that MEL has protective effects.

Testosterone, synthesized from Leydig cells, and FSH, synthesized from the anterior pituitary gland, are both essential for the production of sperm and for male reproductive system functions (Esteves & Humaidan, 2025). The synthesis of testosterone is initiated by LH, which is synthesized from the anterior pituitary gland and has a receptor on Leydig cells. After stimulation of the signaling cascade, cholesterol is transported to the inner membrane of mitochondria by the enzyme StAR. The enzyme P450scc also enables the conversion of cholesterol into pregnenolone. Many enzymes are involved in the synthesis of testosterone from pregnenolone. Some of them are 3-βHSD, Cytochrome P450 17α Hydroxylase/17,20 Lyase (CYP17) and 17β-hydroxysteroid dehydrogenase 3 (17β-HSD3) (Chen et al., 2009). This enzymatic circulation provides the synthesis of testosterone as an end product. Serum testosterone levels were significantly lower in the ACR group than in the control group, which is in agreement with the findings of previous studies (Lebda et al., 2014; Yassa et al., 2014). Low serum testosterone levels can have many different causes. They may be caused by damage to Leydig cells or by disturbances in testicular steroidogenesis (Yassa et al., 2014; Yildizbayrak & Erkan, 2018). In our study, we found that the low testosterone levels observed in the groups treated with ACR showed improvement in the groups treated with MEL10, but improved significantly in the MEL20 groups in particular, demonstrating the protective effects of MEL.

FSH acts on seminiferous tubules and plays a role in spermatogenesis. This level decreased significantly in the ACR group. Folarin et al. reported that FSH levels decreased with respect to testicular toxicity after Roundup herbicide exposure in male albino rats (Owagboriaye et al., 2017). MEL has a regulatory role in testicular function (Li & Zhou, 2015; Mogol & Gökmen, 2016). LH affects Leydig cells in the testes and has a direct effect on testosterone synthesis. LH, whose level is directly suppressed in the ACR groups, also suppresses testosterone synthesis.

We detected a decrease in the level of P450scc, which plays a very important role in the regulation of steroidogenesis, in response to ACR administration. This decrease was similarly observed in a study by Kumar et al. (Kumar et al., 2009). The StAR enzyme is involved in the transport of cholesterol in the inner membrane of mitochondria and has a very important role in steroidogenesis. We found that the level of this enzyme, like P450scc, decreased in response to ACR administration. The expression of 17β-HSD3, an important enzyme involved in the synthesis of testosterone from pregnenolone, decreased in response to ACR administration. Many different studies have shown that substances that cause testicular damage change these levels (Kumar et al., 2009; Murugesan et al., 2007; Rovira et al., 2012). Many studies have shown that melatonin has

protective effects on these steroidogenic enzymes (Li & Zhou, 2015; Mukherjee & Haldar, 2014; Tijmes et al., 1996). In our study, as in many other studies, we found that ACR treatment reduced StAR, P450scc, and 17β-HSD3, and that MEL10 had a partial protective effect on these enzymes, while MEL20 had a strong protective effect.

In various studies, ACR has been shown to cause degenerative and necrotic changes in spermatocytes due to its toxic effects on testicular tissues. ACR application results in a decrease in the number of spermatocytes and prevents spermatogenesis (Gul et al., 2021; Mokhlis et al., 2023). On the other hand, the protective effects of MEL on testicular tissues have been proven in various toxicity studies, and it has been revealed that MEL enhances spermatogenesis while preventing damage to spermatocytes (Adiguzel et al., 2024; Erdem et al., 2024). In this study, MEL10 significantly alleviated the severe suppression of spermatogenesis caused by ACR, in which degenerative and necrotic changes were observed together in spermatocytes. However, MEL20 was found to be more effective in bringing these values closer to control levels.

Conclusion

This study provides strong evidence that melatonin effectively mitigates acrylamide-induced testicular toxicity by restoring hormonal balance, enhancing steroidogenic enzyme activity, and preserving testicular histology. The protective effects of melatonin appear to be dose dependent, with higher doses yielding nearly complete restoration of testicular function. Given its potent protective properties, melatonin has emerged as a promising therapeutic agent for counteracting reproductive toxicity induced by environmental toxicants. These findings underscore the need for further research into the clinical applications of melatonin in reproductive medicine and toxicology. Future studies should explore its long-term efficacy, optimal dosing, and potential synergistic effects with other protective compounds.

Ethics Committee Approval: Ethics committee approval was received for this study from the ''Local Ethics Committee of Atatürk University Animal Experiments'' (Protocol Date:10/03/2025-Protocol Number: 59/2025)

Author Contributions: Design and Supervision- S.T., Y.D., F.A., M.B., İ.B., B.B.L., B.C.; Data Collection and/or Processing- S.T., Y.D., F.A., M.B., İ.B., B.B.L., B.C., M.W.; Analysis and/ or Interpretation – S.T., M.W.; Literature Search – S.T., M.W.; Writing Manuscript – S.T., M.W.

Peer-review: Externally peer-reviewed.

Funding: This study was not funded by any institution.

Declaration of Interests: The authors declare that they have no competing interest.

References

Adiguzel, C., Karaboduk, H., & Uzunhisarcikli, M. (2024). Protective Role of Melatonin Against Abamectin-Induced Biochemical, Immunohistochemical, and Ultrastructural Alterations in the Testicular Tissues of Rats. *Microscopy and Microanalysis, 30*(5), 962-977. https://doi.org/10.1093/mam/ozae080

Afzal, A., Zhang, Y., Afzal, H., Saddozai, U. A. K., Zhang, L., Ji, X. Y., & Khawar, M. B. (2024). Functional role of autophagy in testicular and ovarian steroidogenesis. *Frontiers in Cell and Developmental Biology, 12*. https://doi.org/10.3389/fcell.2024.1384047

Batuwita, B. K. H. H., Jayasinghe, J. M. J. K., Marapana, R. A. U. J., Jayasinghe, C. V. L., & Jinadasa, B. K. K. K. (2025). Reduction of Asparagine and Reducing Sugar Content, and Utilization of Alternative Food Processing Strategies in Mitigating Acrylamide Formation-A Review. *Food and Bioprocess Technology, 18*(3), 2101-2144. https://doi.org/10.1007/s11947-024-03565-z

Bolat, İ., Bolat, M., Kiliçlioğlu, M., Okur, S., Gölgeli, A., Gözegir, B., Çomakli, S., Yildirim, S., Sağlam, Y. S., & Warda, M. (2025). Investigation of the Effects of Boric Acid against Post Operative Testicular Adhesion Caused by Experimental Laporotomy in Rats. *Biological Trace Element Research, 203*(6), 3181–3194. https://doi.org/10.1007/s12011-024-04400-4

Chen, H., Ge, R. S., & Zirkin, B. R. (2009). Leydig cells: From stem cells to aging. *Molecular and Cellular Endocrinology, 306*(1-2), 9-16. https://doi.org/10.1016/j.mce.2009.01.023

Erdem, E., Sapmaz, T., Aras, S., Şevgin, K., Başol Baki, K., Topkaraoğlu, S., Güler, E. M., Baydili, K. N., Tekayev, M., Özkan, B. N., Dumlu, F. S., & Irkorucu, O. (2024). Melatonin administration in testicular damage caused by low and high-dose rate radiotherapy: An experimental study. *Radiation Physics and Chemistry, 220*, 111692. https://doi.org/10.1016/j.radphyschem.2024.111692

Esteves, S. C., & Humaidan, P. (2025). The role of luteinizing hormone activity in spermatogenesis: from physiology to clinical practice. *Reproductive Biology and Endocrinology, 23*. https://doi.org/10.1186/s12958-024-01333-4

Farag, O. M., Abd-Elsalam, R. M., El Badawy, S. A., Ogaly, H. A., Alsherbiny, M. A., & Ahmed, K. A. (2021). Portulaca oleracea seeds' extract alleviates acrylamide-induced testicular dysfunction by promoting oxidative status and steroidogenic pathway in rats. *BMC Complementary Medicine and Therapies, 21(*1), 1-15. https://doi.org/10.1186/s12906-021-03286-2

Farouk, S. M., Gad, F. A., Almeer, R., Abdel-Daim, M. M., & Emam, M. A. (2021). Exploring the possible neuroprotective and antioxidant potency of lycopene against acrylamide-induced neurotoxicity in rats' brain. *Biomedicine and Pharmacotherapy, 138*, 111458. https://doi.org/10.1016/j.biopha.2021.111458

Frungieri, M. B., Calandra, R. S., & Rossi, S. P. (2017). Local Actions of Melatonin in Somatic Cells of the Testis. *International Journal of Molecular Sciences, 18*(6), 1170. https://doi.org/10.3390/ijms18061170

Gao, J. G., Jiang, Y., Zheng, J. T., & Nie, L. W. (2022). Pubertal exposure to acrylamide disrupts spermatogenesis by interfering with meiotic progression in male mice. *Toxicology Letters, 358*, 80-87. https://doi.org/10.1016/j.toxlet.2022.01.014

Gul, M., Kayhan Kustepe, E., Erdemli, M. E., Altinoz, E., Gozukara Bag, H. G., Gul, S., & Gokturk, N. (2021). Protective effects of crocin on acrylamide-induced testis damage. *Andrologia, 53*(9), e14176. https://doi.org/10.1111/and.14176

Halford, N. G., Curtis, T. Y., Muttucumaru, N., Postles, J., Elmore, J. S., & Mottram, D. S. (2012). The acrylamide problem: a plant and agronomic science issue. *Journal of Experimental Botany, 63(*8), 2841-2851. https://doi.org/10.1093/jxb/ers011

Jimenez-Jorge, S., Guerrero, J. M., Jimenez-Caliani, A. J., Naranjo, M. C., Lardone, P. J., Carrillo-Vico, A., Osuna, C., & Molinero, P. (2007). Evidence for melatonin synthesis in the rat brain during development. *Journal of Pineal Research, 42*(3), 240-246. https://doi.org/10.1111/j.1600-079X.2006.00411.x

Kumar, V., Chakraborty, A., Kural, M. R., & Roy, P. (2009). Alteration of testicular steroidogenesis and histopathology of reproductive system in male rats treated with triclosan. *Reproductive Toxicology, 27*(2), 177-185. https://doi.org/10.1016/j.reprotox.2008.12.002

Lebda, M., Gad, S., & Gaafar, H. (2014). Effects of lipoic Acid on acrylamide induced testicular damage. *Mater Sociomed, 26*(3), 208-212. https://doi.org/10.5455/msm.2014.26.208-212

Li, C., & Zhou, X. (2015). Melatonin and male reproduction. *Clinica Chimica Acta, 446*, 175-180. https://doi.org/10.1016/j.cca.2015.04.029

Mogol, B. A., & Gökmen, V. (2016). Thermal process contaminants: acrylamide, chloropropanols and furan. *Current Opinion in Food Science, 7,* 86-92. https://doi.org/10.1016/j.cofs.2016.01.005

Mokhlis, H. A., Rashed, M. H., Saleh, I. G., Eldeib, M. G., El-Husseiny, A. A., Khidr, E. G., Gomaa, M. H., Gad, H. S., & Aglan, A. (2023). Hydrogen sulfide alleviates acrylamide-induced testicular toxicity in male rats. *Toxicology and Environmental Health Sciences, 15*(1), 41–51. https://doi.org/10.1007/s13530-022-00156-3

Mukherjee, A., & Haldar, C. (2014). Photoperiodic regulation of melatonin membrane receptor (MT1R) expression and steroidogenesis in testis of adult golden hamster, Mesocricetus auratus. *Journal of Photochemistry and Photobiology*, *140,* 374-380. https://doi.org/10.1016/j.jphotobiol.2014.08.022

Murugesan, P., Muthusamy, T., Balasubramanian, K., & Arunakaran, J. (2007). Effects of vitamins C and E on steroidogenic enzymes mRNA expression in polychlorinated biphenyl (Aroclor 1254) exposed adult rat Leydig cells. *Toxicology, 232*(3), 170-182. https://doi.org/10.1016/j.tox.2007.01.008

Owagboriaye, F. O., Dedeke, G. A., Ademolu, K. O., Olujimi, O. O., Ashidi, J. S., & Adeyinka, A. A. (2017). Reproductive toxicity of Roundup herbicide exposure in male albino rat. *Experimental and Toxicologic Pathology, 69*(7), 461-468. https://doi.org/10.1016/j.etp.2017.04.007

Rovira, J., Diekmann, F., Ramirez-Bajo, M. J., Banon-Maneus, E., Moya-Rull, D., & Campistol, J. M. (2012). Sirolimus-associated testicular toxicity: detrimental but reversible. *Transplantation, 93*(9), 874-879. https://doi.org/10.1097/TP.0b013e31824bf1f0

Tareke, E., Rydberg, P., Karlsson, P., Eriksson, S., & Tornqvist, M. (2002). Analysis of acrylamide, a carcinogen formed in heated foodstuffs. *Journal of Agricultural and Food Chemistry, 50*(17), 4998-5006. https://doi.org/10.1021/jf020302f

Tijmes, M., Pedraza, R., & Valladares, L. (1996). Melatonin in the rat testis: evidence for local synthesis. *Steroids, 61*(2), 65-68. https://doi.org/10.1016/0039-128x(95)00197-x

Tyl, R. W., Marr, M. C., Myers, C. B., Ross, W. P., & Friedman, M. A. (2000). Relationship between acrylamide reproductive and neurotoxicity in male rats. *Reproductive Toxicology, 14*(2), 147-157. https://doi.org/10.1016/s0890-6238(00)00066-6

Walker, W. H., & Cheng, J. (2005). FSH and testosterone signaling in Sertoli cells. *Reproduction, 130*(1), 15-28. https://doi.org/10.1530/rep.1.00358

Wang, H., Huang, P., Lie, T., Li, J., Hutz, R. J., Li, K., & Shi, F. (2010). Reproductive toxicity of acrylamide-treated male rats. *Reproductive Toxicology, 29*(2), 225-230. https://doi.org/10.1016/j.reprotox.2009.11.002

Yan, F., Wang, L., Zhao, L., Wang, C., Lu, Q., & Liu, R. (2023). Acrylamide in food: Occurrence, metabolism, molecular toxicity mechanism and detoxification by phytochemicals. *Food and Chemical Toxicology, 175*, 113696. https://doi.org/10.1016/j.fct.2023.113696

Yassa, H. A., George, S. M., Refaiy Ael, R., & Moneim, E. M. (2014). Camellia sinensis (green tea) extract attenuate acrylamide induced testicular damage in albino rats. *Environmental Toxicology, 29*(10), 1155-1161. https://doi.org/10.1002/tox.21846

Yildizbayrak, N., & Erkan, M. (2018). Acrylamide disrupts the steroidogenic pathway in Leydig cells: possible mechanism of action. *Toxicological and Environmental Chemistry, 100*(2), 235-246. https://doi.org/10.1080/02772248.2018.1458231

Yu, K., Deng, S. L., Sun, T. C., Li, Y. Y., & Liu, Y. X. (2018). Melatonin Regulates the Synthesis of Steroid Hormones on Male Reproduction: A Review. *Molecules, 23*(2), 447. https://doi.org/10.3390/molecules23020447

Zamani, E., Shaki, F., AbedianKenari, S., & Shokrzadeh, M. (2017). Acrylamide induces immunotoxicity through reactive oxygen species production and caspase-dependent apoptosis in mice splenocytes via the mitochondria-dependent signaling pathways. *Biomedicine and Pharmacotherapy, 94*, 523-530. https://doi.org/10.1016/j.biopha.2017.07.033