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Original Article

Healing Effect of Genistein on Detorsioned Rats After Experimental Ovarian Torsion

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ABSTRACT

Objective: This study aims to evaluate the antioxidant and anti-inflammatory effects of genistein in preventing damage caused by ovarian torsion. Ovarian torsion is an emergency condition characterized by the complete or partial rotation of the ovary on its supporting ligaments and can affect women of all age groups. Rapid diagnosis is crucial for preserving ovarian function and preventing associated complications. However, due to the relatively nonspecific nature of symptoms, making a diagnosis can be challenging.

Methods: In the study, 21 Sprague-Dawley female rats of about four months old, weighing 200–250 g, were used. The study involved three groups of rats: torsion (T), torsion/detorsion (T/DT), and genistein therapy following torsion/detorsion (T/DT/G). Ovarian ischemia was induced in the T and T/DT groups, with the latter undergoing detorsion after 3 hours. The T/DT/G group received genistein treatment (1 mg/kg/day) for 14 days post-detorsion. After treatment, samples were collected for histological and biochemical analysis, including Enzyme-Linked ImmunoSorbent Assay for serum samples and assessment of tissue damage and blood parameters.

Results: Our study focuses on genistein, a phytoestrogen described as having similar effects to endogenous estrogen and has gained importance and become an area of interest through advancing research.

Conclusion: This study demonstrates that genistein could be an effective protective treatment against post-ovarian torsion damage. However, more comprehensive studies are needed to establish the direct effects of genistein on the ovaries. This study presents important findings supporting the potential clinical use of genistein following ovarian torsion.

INTRODUCTION

Complete or partial ovarian torsion on its supporting ligaments can block blood flow.[1-3] Preserving ovarian function and preventing morbidities requires prompt diagnosis.[4,5]

Traditional therapy for ovarian torsion, especially black-blue instances, was salpingo-oophorectomy. New techniques have revealed that detorsion can restore viability in necrotic ovarian tissue. Salpingo-oophorectomy is less prevalent, while ovarian-preserving methods are more popular.[4-6]

Another challenge post-detorsion is the occurrence of reperfusion injury. In fact, reperfusion injury often causes more damage to ovarian tissue than ischemic injury itself. This is because while reperfusion is necessary for repair, the release of reactive oxygen species (ROS) in previously ischemic tissue exacerbates the damage. Hence, antioxidant therapies have been used in various medical fields to prevent or mitigate ischemia-reperfusion injury. Additionally, different and novel antioxidant treatment options are being explored. These antioxidant trials often include experiments with N-acetylcysteine, vitamin E, vitamin C, su-

peroxide dismutase, catalase, allopurinol, and hyperbaric oxygen therapy.[7-9]

Genistein (C15H10O5), a phytoestrogen with similar properties to natural estrogen, has grown in prominence and research.[10,11] Genistein was chosen for this study due to its well-documented antioxidant and anti-inflammatory properties, which have been shown to mitigate oxidative stress and reduce tissue damage in ischemia-reperfusion injury.

Genistein as an antioxidant: Antioxidants donate hydrogen atoms to reduce free radicals. Genistein also boosts superoxide dismutase, a potent antioxidant. Numerous in vitro and in vivo investigations reveal that genistein is the strongest isoflavone antioxidant. Genistein can directly neutralize free radicals or prevent oxidative DNA damage with antioxidant enzymes. Toda and Shirataki examined the effects of four phytoestrogens on reactive oxygen species-induced lipid peroxidation, revealing a link between isoflavone chemical structure and antioxidant function.[10,12]

MATERIALS AND METHODS

Animals

This study was discussed at the relevant ethics committee meeting on 11.04.2019 and was evaluated with the number 2019-07/09. After obtaining ethical the study was conducted and deemed ethically appropriate. The study is in compliance with the Declaration of Helsinki.

In the study, 21 Sprague-Dawley female rats, approximately four months old and weighing 200–250 g, were used. Additionally, the experimental design was conducted in accordance with the ARRIVE 2.0 guidelines. There is no conflict of interest among the authors. All stages of the study were carried out in compliance with the Helsinki Declaration.

Anesthesia

Anesthesia was provided intraperitoneally with 80 mg/kg ketamine hydrochloride and 20 mg/kg xylazine hydrochloride. In due necessity, ketamine (25 mg/kg) was repeated via checking the reflex responses to keep the anesthesia depth of the rats constant.

Procedure And Design

The rats were divided into three groups: In the torsion group (T), seven rats were exposed after laparotomy, exposing the ovaries, and tied approximately 1 cm below the adnexal structure containing the tubal and ovarian vessels to enable disintegration with 5/0 polydioxanone suture, in order to create an ovarian ischemia model. After the abdominal skin was closed, bilateral ovaries were removed by relaparotomy at the end of the third hour. Both S and T groups were sacrificed after blood and tissue samples were taken on the day of the operation. After performing the ischemia model in seven rats in the torsion/detorsion group (T/DT), the ovaries were restored by relaparotomy at the third hour, with reversible sutures removed, and the ovarian ischemia was terminated. The rats were housed in their cages for 1 week without any other treatment. Similarly, to the torsion/detorsion group, after making an ischemia model for seven rats in the Genistein therapy group (T/DT/G), a relaparotomy was performed at the third hour, the reversible suture was removed, and the ovarian ischemia was terminated while the ovaries were restored. After reperfusion of the ovaries following the 3rd hour after detorsion, the rats were placed on a predetermined genistein treatment protocol for two weeks.

Genistein Treatment Protocol: Genistein was administered subcutaneously, at a dose of 1 mg/kg/day, in a volume of 0.1 ml, from the shaved nape of the neck. The application treatment period was planned to be 14 days. Fourteen days later, relaparotomy was performed and bilateral ovaries were removed, blood and tissue samples were taken, and the rats were sacrificed.

After all blood samples were collected, serum samples were taken to the laboratory for Enzyme-Linked ImmunoSorbent Assay (Bioassay Laboratory brand trade kit, China) study. Therefore, the working team did not know which groups the values obtained belonged to. Oophorectomy was performed on rats immediately after cardiac puncture. The ovarian tissues taken were numbered in separate containers and placed in 10% formaldehyde. After fixing in formol for 24 hours, 4-micron-thick sections were prepared from the prepared paraffin blocks and stained with hematoxylin eosin (H&E). The sections prepared were examined with a light microscope in terms of ovarian ischemia-reperfusion injury and the results were evaluated semi-quantitatively. Each ovarian tissue was categorized as follows: '0: No damage, 1: Mildly damaged, 2: Moderately damaged, 3: Severely damaged', in terms of edema, follicular cell damage, vascular congestion, hemorrhage, neutrophil infiltration, and cohesion failure. This scoring system was obtained as a result of the evaluation of these criteria with a light microscope by a pathologist who did not know to which group the rats belonged.

In the study, 5 parameters from blood values were examined: 8-hydroxy-2′-deoxyguanosine (8-OHdG), Malondialdehyde (MDA), Superoxide dismutase (SOD), Glutathione Peroxidase (GSH-Px), and Anti-Mullerian Hormone (AMH).

Statistical Analysis

"E" value analysis of variance was used to determine the number of animals to be used in our study. According to this analysis, the "E" value should be between 10 and 20. The "E" value was calculated according to the following formula:

E = Total number of animals - Total number of groups.

Assuming that we will use 6 rats in each group, we will have 3 groups, one of which is the control group. Therefore, our total number of animals will be 18, and our "E" value will be 18-3=15.13 (16). An E value within the range

of 10-20 ensures that the number of animals is sufficient yet not excessive, maintaining an ideal balance between ethical considerations and scientific adequacy.

The statistical analysis of the data obtained was conducted using SPSS version 22.0 statistical package program. Individual group biochemical parameters were assessed using the 1-sample Kolmogorov-Smirnov Z test and were found to follow a normal distribution (P<0.05). Analysis of variance (ANOVA) was performed on the biochemical data to examine differences among groups. If a significant group effect was found, the Tukey Honestly Significant Difference (HSD) test was applied to determine the location of differences between groups. Statistical significance was defined as P < 0.05. Tissue damage scores were compared using nonparametric analysis, and statistical significance was determined using the Kruskal-Wallis test, followed by the Bonferroni-corrected Mann-Whitney U test. The data were expressed as means ± SD.

RESULTS

A post hoc Bonferroni correction was performed to determine which specific means differed. A post hoc Bonferroni correction was performed to determine which specific means differed.

Results for 8-OHDG values:

The T/DT/G group was statistically significantly higher than the other two groups: 2.56 (1.27), p<0.05. The result between groups T and K is statistically very similar (1.31 vs 1.83). (Table 1)

MDA Values:

The T group exhibited significantly lower mean MDA levels compared to the T/DT and T/DT/G groups (0.76(0.24), p<0.05). However, the results between the T/DT and T/DT/G groups were similar (1.22 versus 1.51). (Table 1)

GSH-PX Values:

The highest GSH-Px value was observed in the T/DT/G group, while the lowest was found in the T group. The difference in GSH-Px levels among the three groups was statistically significant (p<0.05). (Table 1)

SOD Values:

The values of Grup T/DT/G were significantly higher than the other two groups. (2.02(1.05), p<0.05). Grup T/DT was also significantly higher than Grup T (1.56 versus 0.94). (Table 1)

Table 1. Mean and standard deviation values of all biochemical markers

	Mean	Standard Deviation	Median	Minimum	Maximum	p values
8-OHDG (ng/ml)						
T	1.31	0.19	1.32	1.02	1.58	**0.018**
T/DT	1.83	0.87	1.50	0.83	3.25	
T/DT/G	2.56	1.27	1.93	1.38	4.48	
MDA (nmol/ml)						
T	0.76	0.24	0.68	0.59	1.30	**0.002**
T/DT	1.22	0.15	1.20	1.01	1.46	
T/DT/G	1.51	0.25	1.47	1.19	1.92	
GSH-PX (U/ml)						
T	38.58	27.30	30.92	22.10	99.42	**0.001**
T/DT	134.54	59.16	117.56	60.95	228.41	
T/DT/G	228.66	115.64	171.30	131.54	430.08	
SOD(ng/ml)						
T	0.94	0.11	0.93	0.79	1.16	**0.006**
T/DT	1.56	0.67	1.35	0.88	2.62	
T/DT/G	2.02	1.05	1.70	1.17	3.72	
AMH(ng/ml)						
T	1.27	0.89	0.93	0.35	2.87	**0.007**
T/DT	3.42	1.07	3.96	1.73	4.32	
T/DT/G	3.98	1.74	3.35	1.99	6.37	

Pathological Analysis of Ovarian Tissue: Histopathological evaluation revealed varying degrees of tissue damage and inflammatory response among the groups. The T/DT/G group showed milder pathological changes overall, suggesting a protective or mitigating effect of the applied intervention. Parameters such as edema, vascular congestion, neutrophil and eosinophil infiltration, follicular cell damage, hemorrhage, and cohesion failure were quantitatively and qualitatively assessed, supporting the observed group differences. Abbreviations: T: Represents the control group; T/DT: Represents the treatment group. T/DT/G: Represents the group receiving both treatment and supportive intervention. p values: Indicates the level of statistical significance in comparisons between groups. 8-OHDG, MDA, GSH-PX, SOD, and AMH: Biochemical markers analyzed in the study.

AMH Values:

The one-way ANOVA test revealed a statistically significant difference in the mean AMH (ng.ml-1) levels among the groups ($p<0.05$). However, a post hoc Bonferroni correction did not show a significant difference between the AMH levels of Grup T/DT and Grup T/DT/G (3.98 versus 3.42, $p>0.05$). (Table 1)

Edema:

Grup T/DT/G exhibited significantly lower edema compared to the other two groups ($p<0.05$). The edema levels were similar between Grup T/DT and Grup T.

Vascular Congestion:

Grup T/DT/G had no severe vascular congestion, while Grup T/DT had two cases, and Grup T had one case of severe vascular congestion. The mean vascular congestion was significantly lower in Grup T/DT/G ($p<0.05$).

Neutrophil Infiltration:

Grup T had three cases of moderate neutrophilic infiltration, while Grup T/DT and Grup T/DT/G had none. The results were similar between Grup T/DT and Grup T/DT/G.

Follicular Cell Damage:

Grup T had three cases of mild follicular cell damage, while T/DT had one case. Grup T/DT/G had no moderate damage, indicating the protective effect of genistein.

Hemorrhage:

Grup T/DT/G had two cases of moderate hemorrhage, while Grup T/DT had none. Grup T had one case of moderate hemorrhage.

Cohesion Failure:

Grup T had two cases of moderate cohesion failure, while Grup T/DT had none. Grup T/DT/G had no cases of moderate cohesion failure.

Eosinophilic Infiltration:

Grup T had the lowest level of eosinophilic infiltration, Grup T/DT had moderate, and Grup T/DT/G had severe infiltration. This indicates genistein's anti-inflammatory effect.

DISCUSSION

Our study shows that genistein has strong antioxidant and anti-inflammatory capabilities. The genistein group's higher SOD and GSH-Px levels imply improved antioxidant repair. Genistein's strong anti-inflammatory effects are shown by the considerable increase in eosinophil infiltration in Group T/DT/G, which contains cells that travel fastest to fight inflammation.[13,14]

T/DT/G had the greatest statistically significant 8-OHDG value. Given genistein's antioxidant and anti-inflammatory characteristics, we were surprised by the T/DT/G group's high scores. Our data could not be compared to literature values since no analogous research have studied 8-OHDG.[15]

MDA values show that T/DT and T/DT/G are high (relative to Group T). Because reperfusion injury damages more than ischemic injury. However, genistein did not raise MDA levels. A research by Yazıcı et al. indicated that genistein decreased MDA levels, though not significantly. Most research have shown genistein's antioxidant power, one of its key mechanisms. Group T/DT/G had considerably higher values than Group T/DT in our investigation. Genistein-treated Group T/DT/G had a higher compensatory GSH-Px value due to reperfusion damage. Genistein boosts antioxidant capacity faster. Our study's GSH-Px and SOD values supported this.[16]

Our study's AMH value was highest in Group T/DT/G, but not statistically significant. It may not be statistically significant because rats were sacrificed early. If another group was formed and sacrifice time was prolonged, the AMH score may have increased much more. This study group could not be formed due to ethical concerns and inability to quantify this beforehand.

The trial group with the least edema was T/DT/G. The other two groups had similar outcomes. Genistein's antioxidant and anti-inflammatory capabilities explain Group T/DT/G's lowest result. The anti-inflammatory action reduced rat ovarian edema. These results are consistent with the previously reported antioxidant effects of genistein in other organs, further supporting its potential role in mitigating ischemia-reperfusion injury.

Group T had the highest values for cohesion loss, bleeding, neutrophilic infiltration, and follicular cell destruction, while the detorsion groups had similar results. Genistein's main effect is not apoptosis correction. Comprehensive investigations with larger rat groups may yield more conclusive outcomes.

The body's battle against inflammation is shown by eosinophil infiltration, which is significantly higher in Group T/DT/G. Genistein speeds up eosinophil migration to fight ischemia-reperfusion damage.

Conclusion

Although the beneficial relationships of genistein on ovaries have been demonstrated, direct relationships regarding these have not been proven. To further elucidate its therapeutic potential, future studies should evaluate the efficacy of genistein at different doses and investigate its dose-dependent effects. Additionally, longer follow-up periods should be considered to assess the long-term impact of genistein on ovarian tissue recovery. Finally, clinical studies are necessary to validate these findings and determine the applicability of genistein treatment in human cases. This study supports the potential evaluation of genistein as a therapeutic agent in future research.

Ethics Committee Approval

The study was approved by the Sağlık Bilimleri University Ethics Committee (Date: 11.09.2019, Decision No: 2019-07/09).

Informed Consent

Retrospective study.

Peer-review

Externally peer-reviewed.

Authorship Contributions

Concept: E.B., H.B., G.B.B.; Design: E.B., H.B., A.Y.; Supervision: E.B., H.B., G.B.B.; Fundings: E.B., H.B., A.Y.; Materials: E.B., H.B., G.B.B.; Data collection &/or processing: E.B., H.B., A.Y.; Analysis and/or interpretation: E.B., H.B., G.B.B.; Literature search: E.B., H.B., A.Y, D.C.R.; Writing: E.B., H.B., G.B.B., D.C.R.; Critical review: E.B., H.B., A.Y., D.C.R.

Conflict of Interest

None declared.

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Deneysel Yumurtalık Torsiyonu Sonrasında Genisteinin Deforme Olmuş Sıçanlar Üzerindeki Iyileştirici Etkisi

Amaç: Bu çalışma, over torsiyonunun neden olduğu hasarı önlemede genisteinin antioksidan ve anti-enflamatuvar etkilerini değerlendirmeyi amaçlamaktadır. Over torsiyonu, ovariumun destekleyici bağları üzerinde tam veya kısmi dönmesiyle karakterize edilen ve tüm yaş gruplarındaki kadınları etkileyebilen acil bir durumdur. Over fonksiyonunu korumak ve ilişkili komplikasyonları önlemek için hızlı teşhis kritik öneme sahiptir. Ancak, semptomların nispeten spesifik olmaması nedeniyle teşhis koymak zor olabilir.

Gereç ve Yöntem: Çalışmada yaklaşık dört aylık, 200-250 gram ağırlığında 21 adet Sprague-Dawley dişi sıçan kullanılmıştır. Çalışma, torsiyon (T), torsiyon/detorsiyon (T/DT) ve torsiyon/detorsiyon sonrası genistein tedavisi (T/DT/G) olmak üzere üç sıçan grubunu içermektedir. T ve T/DT gruplarında over iskemisi indüklenmiş, T/DT grubunda ise 3 saat sonra detorsiyon gerçekleştirilmiştir. T/DT/G grubu, detorsiyondan sonra 14 gün boyunca genistein tedavisi (1 mg/kg/gün) almıştır. Tedavi sonrası, histolojik ve biyokimyasal analizler için örnekler toplanmış; serum örneklerinde Enzim Bağlantılı İmmunosorbent Analiz (ELISA) ve doku hasarı ile kan parametrelerinin değerlendirilmesi yapılmıştır.

Bulgular: Çalışmamızda, endojen östrojene benzer etkiler gösterdiği tanımlanan ve ilerleyen araştırmalarla önem kazanan bir fitoöstrojen olan genisteine odaklanılmıştır.

Sonuç: Bu çalışma, genisteinin over torsiyonu sonrası oluşabilecek hasarlara karşı etkili bir koruyucu tedavi olabileceğini göstermektedir. Ancak, genisteinin over üzerindeki doğrudan etkilerini belirlemek için daha kapsamlı çalışmalara ihtiyaç vardır. Ayrıca, bu çalışma genisteinin over torsiyonu sonrası potansiyel klinik kullanımını destekleyen önemli bulgular sunmaktadır.

Anahtar Sözcükler: Genistein; iskemi ve reperfüzyon hasarı; over torsiyonu; sıçanlar.