EXPERIMENTAL STUDY

Effects of lupeol on experimental testicular ischemia-reperfusion damage in rats

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ABSTRACT

BACKGROUND: Infertility and organ loss are potential consequences of testicular torsion, a urological emergency. This study aimed to evaluate the impact of lupeol on testicular ischemia-reperfusion damage.

METHODS: Thirty adult male Sprague-Dawley rats were randomly assigned to five groups: Control (C), Lupeol (L), Ischemia (Isc), Treatment 1 (T1), and Treatment 2 (T2). In the study groups, detorsion was applied to the left testicles following the induction of 720-degree testicular torsion for two hours. In the T1 and T2 groups, 100 mg/kg of lupeol was administered intraperitoneally 30 minutes before and immediately after detorsion. At the sixth hour, blood and testicular tissue samples were collected from each rat. Measurements included serum interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α), tissue glutathione (GSH), malondialdehyde (MDA), and caspase-3 levels. Histopathological analysis was performed to assess the Johnsen Tubular Biopsy Score (JTBS).

RESULTS: Levels of caspase-3 (2.74±0.32), MDA (1.71±0.26), IL-6 (4.92±0.57), and TNF-α (113.18±29.77) were elevated in Group Isc compared to Group C and showed a significant reduction in Group T2 (2±0.67, 1.16±0.36, 3.95±0.17, and 106.13±12.49, respectively) and particularly in Group T1 (1.65±0.50, 0.95±0.143, 80±0.35, and 104.86±8.42, respectively) (p=0.001). However, while TNF-α levels decreased in both treatment groups, the difference was not statistically significant (p=0.768). GSH levels decreased in Group Isc (140.63±25.71) but increased in Group T2 (211.58±95.05) (p=0.753) and particularly in Group T1 (219.9±48.21) (p=0.078). The JTBS was lowest in Group Isc (7.67±0.25). However, improvements were observed in both treatment groups (8.93±0.16 and 8.82±0.22, respectively) (p=0.001).

CONCLUSION: This study, the first to use lupeol in an experimental testicular torsion model, demonstrated its antioxidant, anti-inflammatory, anti-apoptotic, histopathological damage-reducing, and protective effects.

Keywords: Testicular torsion; treatment; lupeol; rat; experimental.

INTRODUCTION

Ischemia is the complete absence of oxygen in the body due to insufficient blood flow to tissues or organs. The restoration of blood flow following ischemia, known as reperfusion, often exacerbates ischemia-induced tissue damage.[1] Testicular torsion occurs when a testicle rotates around the spermatic cord, leading to compromised testicular blood flow. If left untreated, the resulting ischemia can cause irreversible testicular damage and infertility.[2] Testicular torsion can occur at any age, but, it predominantly affects newborns and adolescents in a bimodal distribution, with an annual incidence of 1 in 4,000 cases.[3]

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Under normal physiological conditions, most oxygen entering cells is reduced to water in the electron transport chain (ETC). However, 1-2% of oxygen may be converted into superoxide due to electron leakage from the ETC. These superoxides are usually effectively neutralized by the superoxide dismutase (SOD) and catalase (CAT) enzymes. Ischemia is accompanied by hypoxia, which reduces high-energy phosphate synthesis by causing mitochondrial ETC dysfunction. This leads to metabolic acidosis, disruption of intracellular ionic balance, and cell swelling. During the reperfusion phase, blood flow is restored, resulting in reoxygenation of the ischemic tissue. However, the increase in oxidant substances and the decrease in antioxidative capacity disrupt the oxidative balance, leading to an elevated production of free oxygen radicals (FORs). FORs create oxidative stress by inducing endothelial dysfunction, DNA damage, and a local inflammatory response. The biochemical mechanisms underlying ischemia-reperfusion (IR) injury are driven by the production of reactive oxygen species (ROS). Xanthine oxidase mediation, mitochondrial ETC, nitric oxide synthase, and phagocytic cell mediation are some of the metabolic mechanisms that can produce ROS. A cytokine storm resulting in cellular damage and death can be a pathophysiological consequence of these oxidative stress and inflammatory cascades. IR injury occurs as a result of these complex processes. The reperfusion phase is dynamic and can continue for several days.[4-6]

To prevent future testicular damage and infertility, studies have been conducted using various agents to address testicular IR damage. The triterpenoid lupeol is naturally found in fruits, vegetables, and medicinal plants. It possesses a variety of pharmacological properties, including antibacterial, anti-inflammatory, antioxidant, and anticancer effects.[7] Lupeol has been used to treat osteosarcoma, myocardial and cerebral ischemia, bladder cancer, prostate cancer, colorectal cancer, liver cancer, and lung cancer.[4] Studies have shown that oral and intraperitoneal administration of lupeol (up to 100 mg/kg) at various time intervals (e.g., short-term, daily, weekly, and/or monthly) does not cause any systemic toxicity in animals. In fact, when lupeol was topically applied for 28 days, it did not affect skin epidermal histology or gene expression in rat wounds.[8] Lupeol exerts its anti-inflammatory effects by inhibiting inflammatory cytokines such as tumor necrosis factor-α (TNF-α); interleukin (IL)-1α, IL-2, IL-4, and IL-6; and prostaglandin 6. Additionally, it exhibits antioxidant effects by increasing the levels of antioxidants such as SOD, CAT, and glutathione (GSH).[9] However, to date, no study has demonstrated these effects of lupeol in testicular IR injury. Hence, this study aimed to evaluate the biochemical and histopathological effects of lupeol on IR injury using the testicular torsion-detorsion model.

MATERIALS AND METHODS

This study was conducted in July 2023 at the experimental research center of our university. And was approved by the Gazi University Animal Experiments Local Ethics Committee (E-66332047),

Thirty adult male Sprague-Dawley rats, each weighing 300-350 g, were included and randomly assigned to five groups, with six subjects in each group. Intramuscular xylazine hydrochloride (5 mg/kg; Alfazyne 2%, Ege Vet, Türkiye) and ketamine hydrochloride (50 mg/kg; Ketalar, Eczacıbaşı, Türkiye) were used during the surgery, which was performed under sterile conditions.

Experimental Testicular Ischemia Model

The left testicle was rotated 720 degrees clockwise (two full turns) and fixed to the scrotum with 4/0 silk suture. After two hours of testicular ischemia, the testicle was detorsioned, and reperfusion was allowed for four hours. The study included the following five groups:

- **Control Group (Group C):** Testicular tissue and blood samples were obtained through a scrotal incision under anesthesia without any additional procedure.

- **Lupeol Group (Group L):** A lupeol solution (100 mg/kg, intraperitoneal [i.p.]) was injected. After one hour, testicular tissue and blood samples were obtained through a scrotal incision. As mentioned previously, lupeol administration at 100 mg/kg has been shown to be non-toxic. There is no study indicating how long lupeol remains in the testis after intraperitoneal administration or when it reaches maximum concentration. However, in the pharmacokinetic study conducted by Khatal and More, lupeol was administered to rats at doses of 1 mg/kg intravenously (iv) and 30 mg/kg orally. Lupeol exhibited slow and poor absorption when administered orally, with the time required to achieve peak plasma concentration (Tmax) at 4.67 hours (h), T1/2 of 8.66 h, and a maximum plasma concentration (Cmax) of 133.33 ng/mL. In contrast, lupeol showed a faster effect when administered intravenously, with a Tmax of 0.14 h, a T1/2 of 0.084 h, and a Cmax of 12,485.69 ng/mL.[10] Therefore, in Group L, testicular tissue was removed at the first hour, recognizing that lupeol would not cause any tissue damage and to minimize the effects of prolonged anesthesia and prolonged exposure of the rat scrotum.

- **Testicular Ischemia Group (Group Isc):** After the testicular IR model, testicular tissue and blood samples were obtained at the sixth hour.

- **Treatment Group I (Group T1):** Lupeol solution (100 mg/kg, i.p.) was injected 30 minutes before testicular detorsion, and testicular tissue and blood samples were obtained at the sixth hour.

- **Treatment Group II (Group T2):** Lupeol solution (100 mg/kg, i.p.) was injected immediately after testicular detorsion, and testicular tissue and blood samples were obtained at the sixth hour.

Under deep anesthesia, intracardiac blood was drawn from each rat before euthanasia to collect samples for biochemical and histological analyses.

Biochemical Analysis

For serum TNF-α and IL-6 measurement, rat blood was collected in yellow-cap gel tubes and allowed to stand for 30 minutes. It was then centrifuged at 3000 rpm for 10 minutes.

Testicular tissues were homogenized in phosphate-buffered saline (pH 7.2), and the homogenates were centrifuged at 3500 rpm for 15 minutes. The levels of GSH, malondialdehyde (MDA), and caspase-3 were determined from the supernatants obtained after centrifugation. Before analysis, the serum and homogenate supernatants were transferred to Eppendorf tubes and stored at -80°C. Serum TNF-α and IL-6 levels, as well as testicular tissue GSH, MDA, and caspase-3 levels, were analyzed using the Bioassay Technology Laboratory Human kit with the enzyme-linked immunosorbent assay method. The catalog numbers were as follows: TNF-α (Cat No: E0764Ra), IL-6 (Cat No: E0135Ra), GSH (Cat No: E1100Ra), MDA (Cat No: E0156Ra), and caspase-3 (Cat No: E1648Ra). Their units of measurement were: TNF-α (ng/L), IL-6 (ng/L), GSH (mg/L), MDA (nmol/L), and caspase-3 (ng/mL).

Histopathological Analysis

Testicular specimens were fixed in a 10% formaldehyde solution for 24 hours. Tissue slices were placed in cassettes and processed for routine tissue follow-up. The samples were then embedded in paraffin and stained with hematoxylin and eosin (H&E) at a thickness of 4-µ. The stained sections were examined under a light microscope (Olympus Bx50) at magnifications of ×12.5, ×40, ×100, ×200, and ×400 (objective lens ×10 and ocular lens ×10). For sample evaluation, only the Johnsen Tubular Biopsy Score (JTBS) was used, assigning a score from 1 to 10 for each seminiferous tubule observed and analyzed. In the sections stained with H&E, a total of 10 seminiferous tubules were scored per section.[11]

Statistical Analysis

Statistical analysis was conducted using SPSS version 22. The appropriateness of the histogram and probability graphs for normal distribution was assessed using the one-way analysis of variance test. For normally distributed data, descriptive analyses were performed using means and standard deviations. Tukey and Tamhane tests were used for pairwise comparisons based on the significance of the distribution. A 5% error threshold ($p<0.05$) was considered statistically significant.

RESULTS

The highest MDA level was observed in the Group Isc (1.71±0.26), while the lowest level was recorded in Group T1 (0.95±0.14). The intergroup statistical comparison of MDA levels yielded a p value of 0.001. Statistically significant differences were observed in the mean MDA levels between Group Isc and Groups C, L, T1, and T2 (p=0.047, p=0.002, p=0.001, and p=0.031, respectively) (Table 1). However, no statistically significant differences were found in the treatment groups compared to Groups C and L (p=0.603, p=1, p=1, and p=0.747, respectively) (Table 1).

The highest caspase-3 level was observed in the Group Isc (2.74±0.32), while the lowest level was recorded in Group C (1.09±0.10). As a result of the intergroup statistical comparison of caspase-3 levels, a p value of 0.001 was observed. Statistically significant differences were observed in caspase-3 levels between Group Isc and Group C, Group L, Group T1, and Group T2 (p=0.001, p=0.001, p=0.001, and p=0.047, respectively) (Table 1). Additionally, a statistically significant difference was observed between Group Isc and Group T2 compared to Group C (p=0.001 and p=0.009, respectively) (Table 1). However, no statistical difference was found in Group T1 compared to Groups C and L (p=0.196 and p=0.573, respectively) (Table 1). In contrast, a statistically significant difference was found in Group T2 compared to Groups C and L (p=0.009 and p=0.050, respectively).

Table 1. Average oxidative stress marker levels in testicular tissue samples

	Group C	Group L	Group Isc	Group T1	Group T2	p values
MDA	1.20±0.45	0.95±0.13	1.71±0.26*	0.95±0.14*	1.16±0.36*	0.001
Caspase-3	1.09±0.10	1.28±0.29	2.74±0.32@	1.65±0.50@	2±0.67@	0.001
Glutathione	271.53±116.43	268.66±234.71	140.63±25.71&	219.9±48.21&	211.58±95.05&	0.399
IL-6	4±0.16	3.73±0.10	4.92±0.57®	3.80±0.35®	3.95±0.17®	0.001
TNF-α	100.21±9.78	104.96±15.23	113.18±29.77€	104.86±8.42€	106.13±12.49€	0.768
JTBS	9.70±0.16	9.62±0.14	7.67±0.25¥	8.93±0.16¥	8.82±0.22¥	0.001

TNF-α: ng/L; IL-6: ng/L; GSH: mg/L; MDA: nmol/L; Caspase-3: ng/mL.
Statistically significant differences were detected in Groups T1 and T2 compared to Group Isc (except for IL-6 in T2 vs. Isc) (p=0.001 and p=0.031)*, (p=0.001 and p=0.047)@, (p=0.031 and p=0.070)®, and (p=0.001 and p=0.001)¥.
No statistically significant differences were detected in Groups T1 and T2 compared to Group Isc. However, reductions were observed in the T groups (p=0.078 and p=0.753)& and (p=0.913 and p=0.950)€.

The highest GSH level was observed in Group C (271.53±116.43), while the lowest level was recorded in Group Isc (140.63±25.71). The intergroup statistical comparison of GSH levels yielded a p value of 0.399, indicating no significant difference among the groups. In contrast to Group Isc, the GSH levels were higher in the T1 and T2 groups; however, the difference was not statistically significant (Table 1). Additionally, no statistically significant difference was observed in the treatment groups compared to Groups C and L (p=0.987, p=1, p=0.987 and p=1, respectively) (Table 1).

The highest IL-6 level was observed in Group I (4.92±0.57), while the lowest level was recorded in Group T1 (3.80±0.35). The intergroup statistical comparison of IL-6 levels yielded a p value of 0.001. A statistically significant difference was observed between Group L and Group T1 compared to Group Isc (p=0.033 and p=0.031, respectively). Furthermore, IL-6 levels in the two treatment groups were lower than those in the ischemia group (Table 1). However, no statistical difference was found between the treatment groups compared to Groups C and L (p=0.936, p=1, p=1, and p=0.28, respectively) (Table 1).

The highest TNF-α level was observed in Group Isc (113.18±29.77), while the lowest level was recorded in Group C (100.21±9.78). The intergroup statistical comparison of TNF-α levels yielded a p value of 0.768, indicating no significant difference among the groups. In contrast to the ischemia group, the values in the treatment groups were lower; however, the difference was not statistically significant (Table 1). Similarly, no statistically significant difference was observed in the treatment groups compared to Groups C and L (p=0.989, p=0.973 and p=1, respectively) (Table 1).

The highest JTBS was observed in Group C (9.70±0.16), while the lowest was recorded in Group Isc (7.67±0.25) (Figs. 1 and 2). The intergroup statistical comparison of JTBS yielded a p value of 0.001. Lupeol was found to reduce testicular damage by preserving seminiferous tubule function and sperm cell integrity. A statistically significant difference was observed between Groups C and Isc, as well as between Groups T1 and T2 (p=0.001) (Fig. 3). In addition, a statistically significant difference (p=0.001) was observed between the ischemia group and all other groups. A statistically significant difference was also found in the treatment groups compared to Groups C and L (p=0.001). Table 1 shows that there was no statistically significant difference between Group T1 and Group T2 (p=0.896).

Furthermore, no statistically significant difference was observed between Groups T1 and T2 for any of the parameters examined (p>0.05).

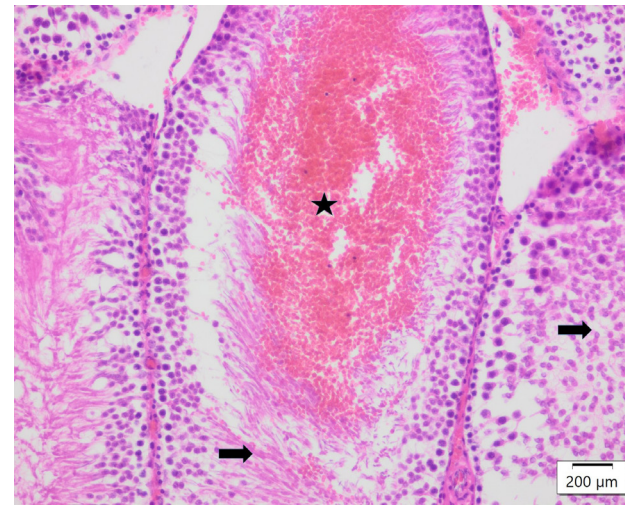

Figure 2. Decreased spermatogenic activity compared to the normal Johnsen Tubular Biopsy Score (JTBS) in Group I (H&E, ×200).

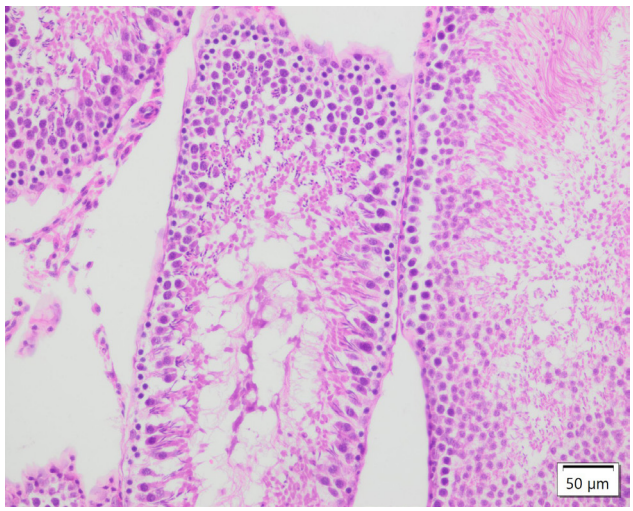

Figure 1. Normal spermatogenic activity in Group C (Hematoxylin and eosin [H&E], ×200).

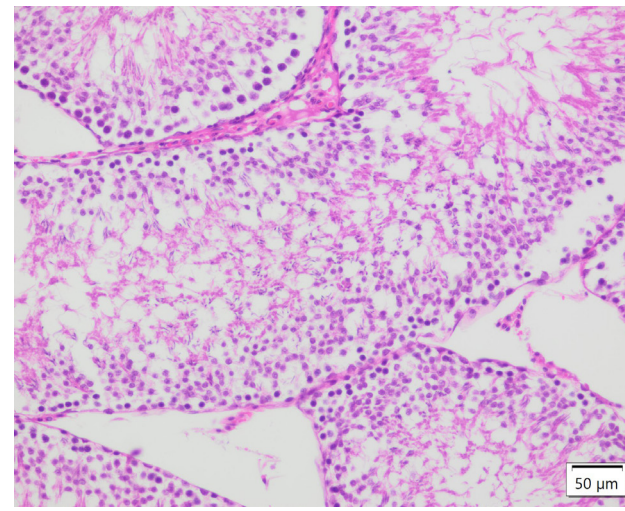

Figure 3. The Johnsen Tubular Biopsy Score (JTBS) showing relatively improved spermatogenic activity in Group T1 (H&E, ×200).

DISCUSSION

Testicular torsion occurs when the spermatic cord twists around its axis, leading to a reduction or absence of testicular blood flow. This urological emergency requires immediate therapeutic intervention. Since most of the damage occurs within the first 4-8 hours of testicular ischemia, early diagnosis and intervention are crucial for both testicular preservation and the protection of future fertility. The severity of testicular damage is directly proportional to the degree and duration of torsion. Additionally, testicular injury during torsion is influenced by reperfusion damage following ischemia and detorsion, as well as the subsequent inflammatory process. A review of the literature indicates that numerous experimental studies have investigated various pharmacological agents to prevent IR damage following testicular torsion. These agents include hydrogen sulfide, vitamin C, fentanyl, Ginkgo biloba, astaxanthin, syringic acid, cordycepin, and pentoxifylline.[12-19]

Over the past 40 years, numerous in vitro and preclinical animal studies have demonstrated the anti-inflammatory, antioxidant, anticancer, antibacterial, antiprotozoal, antiproliferative, antiangiogenic, and cholesterol-lowering properties of lupeol.[7,20] While lupeol has been reported to exhibit antioxidant, anti-inflammatory, and histopathological healing properties in other organs, this study is the first to explore its potential in the treatment of testicular torsion. In this testicular torsion model, lupeol demonstrated its antioxidant, anti-inflammatory, and tissue-healing effects, positively influencing JTBS by reducing MDA, IL-6, TNF-α, and caspase-3 levels while increasing GSH levels. Rats serve as suitable subjects in the experimental testicular IR injury model.

A study has shown that maximum hemorrhagic necrosis occurs at 360-540 degrees of extravaginal torsion, while ischemia begins at torsion degrees exceeding 540 degrees.[21] In clinical practice, testicular torsions typically occur counterclockwise and involve a 720-degree rotation. Experimental studies have demonstrated that testicular blood flow can be restored within 1-3 hours after detorsion; however, irreversible damage begins to occur in cases where torsion persists beyond four hours.[3] Additionally, because the spermatic cord of the left testicle is longer than that of the right, testicular torsion is more commonly observed on the left side.[3] Previous research utilizing a rat model of testicular torsion has demonstrated that a two-hour, 720-degree rotation of the testis followed by reperfusion results in a significant increase in testicular lipid peroxidation products, nitric oxide content, and myeloperoxidase activity, which serves as a marker of neutrophil accumulation.[22] Turner also observed complete infarction in testicular tissues four hours after a two-hour, 720-degree rotation of the testis, followed by reperfusion.[23] Previous research conducted by our team has similarly demonstrated that testicular injury occurs both biochemically and histopathologically after two hours of torsion followed by a four-hour reperfusion period.[24,25] Therefore, the same durations of torsion and reperfusion were adopted in this study.

In ischemia, which occurs following the interruption of blood flow to any tissue, anaerobic metabolism becomes predominant, leading to a decrease in adenosine triphosphate (ATP) production and an increase in antioxidant substance production. The impairment of aerobic metabolism, metabolic acidosis, and disruption of intracellular calcium balance contribute to the increased formation of FORs. During the reperfusion phase, when blood flow is restored after detorsion, purine metabolites metabolized by xanthine oxidase lead to excessive FOR formation. The mitochondrial ETC, nicotinamide adenine dinucleotide phosphate (NADPH) oxidase system, uncoupled nitric oxide synthase system, and xanthine oxidase system all contribute to FOR production.[4-6] The increasing levels of ROS in the environment can damage lipids, proteins, and DNA, resulting in cellular dysfunction. However, this damage can be mitigated by reducing FOR production or enhancing antioxidant defenses.[26]

Various drugs have been used to demonstrate antioxidant effects in the testicular torsion model. For instance, Yuksel et al.[12] examined the effects of hydrogen sulfide on MDA, GSH, SOD, and ferric acid using a one-hour 720-degree torsion model. Jafari et al.[27] investigated the effects of memantine on testicular torsion by analyzing MDA, GSH, and CAT levels. The antioxidant effects of the drugs used in these two studies were demonstrated by reducing oxidant parameters that increase during ischemia, and these effects also manifested as histopathological improvement. Lupeol is a triterpene found naturally in medicinal plants as well as in a variety of vegetables and fruits such as peppers, tomatoes, figs, mangoes, and strawberries.[20] It has demonstrated antioxidant effects in models of middle cerebral artery occlusion, selenite-induced cataract, myocardial ischemia, and cerebral ischemia.[7] Additionally, lupeol suppresses FOR formation in acetaminophen- and aflatoxin-induced liver damage and hypercholesterolemia-associated kidney damage.[28-30] Reportedly, its antioxidant effect is achieved by reducing MDA levels and increasing GSH levels. Similarly, in this study, the antioxidant activity of lupeol was demonstrated by its ability to reduce MDA and increase GSH. This effect was more pronounced in the T1 group (administered lupeol 30 minutes before detorsion) than in the T2 group (administered immediately after detorsion).

Numerous studies have demonstrated that testicular IR injury results in an increase in proinflammatory cytokines, including TNF-α, IL-6, and IL-1α, indicating a potential role for these cytokines in the inflammatory process.[31,32] Lee et al.[33] investigated the effect of lupeol on colitis and concluded that it reduces IL-6, IL-12, and TNF-α levels through its inhibitory effect on macrophages. Furthermore, lupeol has been shown to reduce the overexpression of proinflammatory cytokines, including TNF-α, interferon-gamma (IFN-α), IL-6, and IL-2, by inhibiting the expression of inflammatory genes and proteins.[7] Consistent with previous findings, we observed a considerable increase in TNF-α and IL-6 levels in the testicular ischemia group. However, following lupeol treatment, these

levels decreased, with a statistically significant reduction observed for IL-6 (p=0.001). Although a decrease in TNF-α levels was observed in both treatment groups, particularly in the T1 group, where lupeol was administered before detorsion, the difference was not statistically significant (p=0.768). In a study examining the antinociceptive effect of lupeol in mice, pretreatment with lupeol (50 and 100 mg/kg) inhibited hyperalgesia and localized increases in TNF-α and IL-1b levels; however, the same effect was not observed when lupeol was administered postoperatively.[34]

A frequent physiological occurrence during healthy spermatogenesis is the apoptosis of spermatogenic cells.[35] However, extensive spermatogenic cell death is typically a consequence of the pathogenic process of testicular IR. If testicular dysfunction worsens, it may lead to male infertility.[36] While numerous biomarkers reliably measure apoptosis, the most commonly assessed markers include p-53, caspase-3, B-cell lymphoma 2 (Bcl-2), and Bcl-2-associated X protein (Bax) due to their critical roles in the apoptotic process.[20] Lipid peroxidation-induced membrane instability leads to the release of mitochondrial cytochrome-C into the cytoplasm. The resulting cytochrome-C facilitates apoptosome formation.[37] Apoptosis occurs when this apoptosome activates the initiator caspase-9 and the effector caspase-3.[38,39] Moreover, it has been demonstrated that lupeol downregulates caspase-3 in an experimental cerebral IR model through the phosphatidylinositol 3-kinase/protein kinase B (PI3K/Akt) signaling pathway.[20,36] In our experimental testicular torsion model, consistent with previous research, caspase-3 levels increased in the ischemia groups but decreased statistically significantly in the lupeol treatment groups (p=0.001).

Overproduction of ROS during testicular reperfusion damages the cellular genome, induces oxidative stress in the testicular parenchyma, and triggers cell death by activating caspase cascades.[40] Increased necrosis in testicular tissue is directly associated with these changes. The damage caused by testicular torsion is evaluated using histopathological, biochemical, and fertility assessment methods. Since ischemic conditions primarily affect spermatogenesis, testicular damage caused by torsion was evaluated using the JTBS, which is considered a significant parameter in the histological assessment of spermatogenesis.[11,41] In our study, a significant decrease in JTBS scores was observed in the torsion groups, suggesting that testicular damage also developed histopathologically. The formation of values close to normal, although not as high as in the control group, in the testicular tissues removed after six hours of lupeol treatment reflects its histopathological healing effects. Although the values in the T1 group were higher than those in the T2 group, the difference was not statistically significant. In this experiment, lupeol protected tubular function by reducing damage to the seminiferous tubules and helped preserve fertility by enhancing spermatogenic cell maturation to a higher and near-normal level. The effect of lupeol on testicular torsion and reperfusion injury has not been previously investigated. One of the few studies in the literature, conducted by Gupta et al.[42], examined the effects of Thevetia peruviana (Apocynaceae) extract, which contains α-amyrin acetate, lupeol acetate, α-amyrin, α-amyrin, lupeol, and thevetigenin. When administered orally to rats at 10 mg/kg for two months, the extract led to a reduction in spermatocytes, secondary spermatocytes, round spermatids, and mature Leydig cells. In addition, Leydig cell nuclear diameter, seminiferous tubular diameter, Sertoli cell area, sperm density, and motility decreased, demonstrating both an anti-spermatogenic and contraceptive effect. However, that study did not focus on testicular torsion and involved long-term oral administration of lupeol for two months. Furthermore, lupeol was not administered as an isolated agent. In our study, MDA and caspase-3 levels decreased with lupeol treatment in torsioned testicular tissues, while GSH levels increased, demonstrating both antioxidant and antiapoptotic effects. Although tissue examination was not possible due to budget constraints, lupeol also exhibited anti-inflammatory effects by reducing serum IL-6 and TNF-α levels. Additionally, while the JTBS in the torsion group was at the level of undifferentiated early spermatids, it remained at the level of late spermatids in the lupeol-treated groups (Table 1). As the first study to investigate this subject, our findings indicate that lupeol suppressed the release of proinflammatory cytokines and ROS, maintained redox balance, reduced cell damage, exerted both anti-inflammatory and antioxidant effects, and prevented cell damage, ultimately providing a protective effect on testicular tissue and fertility. However, these findings should be supported by long-term studies with additional parameters.

The duration of action of lupeol was 6.44±0.85 hours in the trials, and at doses ranging from 30 to 2000 mg/kg, no toxic effects were observed in the animals. As a result, the trial was completed in six hours, and a dose of 100 mg/kg of medication was used.[43] Lupeol did not affect skin epidermal histology or gene expression in rats when applied topically for approximately one month.[8] Lupeol was administered orally at 100 mg/kg in a rat model of aflatoxin B1-induced peroxidative hepatic damage, 2000 mg/kg orally for T lymphocyte suppression in mice, and 40 mg/kg intraperitoneally in a human pancreatic cancer cells model.[29,44,45] However, the primary limitations of this study include its small sample size due to budget constraints and its completion within a single day. If additional groups were included to assess long-term effects, both the late effects of testicular IR injury and the prolonged effects of lupeol could be evaluated. Therefore, more comprehensive studies should be conducted in the future.

CONCLUSION

This study demonstrated the potential of lupeol as an antioxidant, anti-inflammatory, and antiapoptotic agent, while also highlighting its protective and damage-reducing properties against histopathological damage in an experimental testicular torsion model. These protective effects were more pronounced in the groups where lupeol was administered before detorsion.

Ethics Committee Approval: The study was approved by the Gazi University Animal Experiments Local Ethics Committee (Date: 27.12.2022, Decision No: E-66332047).

Peer-review: Externally peer-reviewed.

Authorship Contributions: Concept: A.A., R.K., C.K., S.E.; Design: A.A., R.K., C.K., S.E., A.K.; Supervision: Al.A. Z.T., K.S.; Data collection and/or processing: A.A., R.K., A.K.; Analysis and/or interpretation: M.A.İ, G.Y.A.; Literature review: S.E., A.K., A.A.; Writing: A.A., R.K., C.K.; Critical review: Al.A., Z.T., K.S.

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DENEYSEL ÇALIŞMA - ÖZ

Lupeol'ün sıçanlarda deneysel testiküler iskemi/reperfüzyon hasarı üzerine etkileri

AMAÇ: Kısırlık ve organ kaybı, ürolojik acil durum olan testis torsiyonunun olası sonuçlarıdır. Bu araştırmada lupeolün testiküler iskemi reperfüzyon hasarı üzerindeki etkisini göstermeye çalıştık.
GEREÇ VE YÖNTEM: 30 adet yetişkin erkek Spraque Dawley sıçanı randomize edilerek Kontrol(C), Lupeol(L), İskemi(I), Tedavi 1(T1) ve Tedavi 2(T2) gruplarına ayrıldı. Çalışma gruplarında sol testislere 2 saat boyunca 720 derece testis torsiyonu oluşturularak detorsiyon uygulandı. T1 ve T2 gruplarına detorsiyondan 30 dakika önce ve hemen sonra 100 mg/kg Lupeol intraperitoneal olarak enjekte edildi. Altıncı saatte her sıçandan kan ve testis dokusu örnekleri alındı. Serum interlökin-6(IL-6) ve tümör nekroz faktörü-α (TNF-α), doku glutatyonu (GSH), malondialdehit (MDA) ve kaspaz 3 ölçümleri için kan alındı. Johnsen Tübüler Biyopsi Skorunu (JTBS) değerlendirmek için histopatolojik analiz yapıldı.
BULGULAR: Kaspaz 3 (2.74±0.32), MDA (1.71±0.26), IL-6 (4.92±0.57) ve TNF-α (113.18±29.77) değerleri Grup I'de Grup C'ye göre artarken T2'de (2±0.67, 1.16±0.36, 3.95±0.17 ve 106.13±12.49) ve özellikle T1 grubunda (1.65±0.50, 0.95±0.143, 80±0.35 ve 104.86±8.42) belirgin azalma saptandı (p=0.001). Ancak istatistiksel olarak anlamlı bir fark olmamasına rağmen her iki tedavi grubunda da TNF-α'da azalma vardı (p=0.768). GSH düzeyleri Grup I'de azalırken (140.63±25.71), T2 grubunda (211.58±95.05) (p=0.753) ve özellikle T1 grubunda (219.9±48.21) artma gözlendi (p=0.078). JTBS en düşük skoru Grup I'de görülürken (7.67±0.25), tedavi gruplarında iyileşme gözlendi (8.93±0.16 ve 8.82±0.22), (p=0.001).
SONUÇ: Lupeol'ün ilk kez deneysel testis torsiyon modelinde kullanıldığı bu çalışmada antioksidan, antienflamatuvar ve antiapoptotik etkilerinin yanı sıra histopatolojik hasarı azaltıcı ve koruyucu etkileri de ortaya konmuştur.

Anahtar sözcükler: Testis torsiyonu, tedavi, lupeol, sıçan, deneysel

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