

Effect of allopurinol and oxypurinol treatment on apoptosis in an experimental testicular torsion model

Emine Bilaloglu,¹ Levent Duman,² Yalcin Erzurumlu,^{3,4} Onur Ertunc,⁵ Yeliz Kart²

¹Department of Pediatric Surgery, Bayburt State Hospital, Bayburt-Türkiye

²Department of Pediatric Surgery, Suleyman Demirel University Faculty of Medicine, Isparta-Türkiye

³Department of Biochemistry, Suleyman Demirel University Faculty of Pharmacy, Isparta-Türkiye

⁴Department of Drug Research and Development, Institute of Health Sciences, Suleyman Demirel University, Isparta-Türkiye

⁵Department of Pathology, Gazi University Faculty of Medicine, Ankara-Türkiye

ABSTRACT

BACKGROUND: The aim of this study was to investigate whether allopurinol and oxypurinol treatment could mitigate oxidative stress and germ cell apoptosis in testicular ischemia-reperfusion (IR) injury.

METHODS: Thirty-two male rats were divided into four groups: Group 1 (Sham-Operated, n=8), in which the testicle was exposed but torsion was not performed; Group 2 (IR + Saline, n=8), in which torsion/detorsion was applied to the left testicle and 1 mL of normal saline was administered; Group 3 (IR + Allopurinol, n=8), in which torsion/detorsion was applied to the left testicle and 50 mg/kg allopurinol was administered; and Group 4 (IR + Oxypurinol, n=8), in which torsion/detorsion was applied to the left testicle and 50 mg/kg oxypurinol was administered. On postoperative day 28, left testicular tissue samples were collected, and total antioxidant status (TAS), total oxidant status (TOS), and oxidative stress index (OSI) levels were measured. Additionally, the gene expression levels of Bax, B-cell lymphoma 2 (Bcl-2), endothelial nitric oxide synthase (eNOS), and vascular endothelial growth factor A (VEGF-A) were analyzed.

RESULTS: Allopurinol and oxypurinol significantly decreased OSI levels ($p<0.001$). Oxypurinol was found to be significantly more effective in reducing oxidative stress ($p<0.001$). Both allopurinol and oxypurinol significantly reduced Bax gene expression levels ($p<0.001$). Treatment with allopurinol ($p=0.009$) and oxypurinol ($p=0.001$) significantly increased Bcl-2 levels. Additionally, both agents significantly reduced the apoptosis index ($p<0.001$). Allopurinol ($p_1=0.007$, $p_2<0.001$) and oxypurinol ($p_{1,2}<0.001$) treatments significantly increased eNOS and VEGF-A gene expression levels.

CONCLUSION: Allopurinol and oxypurinol reduce oxidative stress in the testis following IR injury, with oxypurinol demonstrating a greater antioxidant effect. Both treatments also reduce apoptosis by contributing positively to the eNOS and VEGF-A-mediated repair processes. Therefore, allopurinol and oxypurinol may serve as potential therapeutic agents for clinical application in testicular torsion.

Keywords: SIllopurinol; ischemia; oxypurinol; reperfusion; testicular torsion.

INTRODUCTION

Testicular torsion is a serious condition that, if not treated promptly, can lead to long-term consequences such as testicular atrophy and infertility. As the degree of torsion increases,

blood flow to the testis decreases, resulting in ischemic damage to the tissue. This condition requires emergency surgical detorsion to prevent further ischemic injury.^[1] However, surgical detorsion itself also promotes the generation of reactive oxygen species (ROS), leading to additional tissue dam-

Cite this article as: Bilaloglu E, Duman L, Erzurumlu Y, Ertunc O, Kart Y. Effect of allopurinol and oxypurinol treatment on apoptosis in an experimental testicular torsion model. *Ulus Travma Acil Cerrahi Derg* 2026;32:229-237.

Address for correspondence: Emine Bilaloglu

Department of Pediatric Surgery, Bayburt State Hospital, Bayburt, Türkiye

E-mail: dreminebilaloglu@gmail.com

Ulus Travma Acil Cerrahi Derg 2026;32(3):229-237 DOI: 10.14744/tjtes.2025.50636

Submitted: 30.05.2025 Revised: 01.11.2025 Accepted: 15.12.2025 Published: 10.03.2026

OPEN ACCESS This is an open access article under the CC BY-NC license (<http://creativecommons.org/licenses/by-nc/4.0/>).



age. Germ cell apoptosis, which is significantly triggered by oxidative stress, has been detected in experimental models. Testicular germ cell apoptosis plays an important role in determining the prognosis of testicular torsion.^[2]

Nitric oxide (NO) is a free radical present in nearly every tissue and plays a crucial role in regulating vascular tone, particularly within the cardiovascular system. Additionally, it exhibits anti-inflammatory and antioxidant properties, enabling it to interact readily with ROS in both physiological and pathological processes. Studies investigating ischemia-reperfusion (IR) injury in various biological systems have shown that NO reduces oxidative damage in tissues by modulating cellular processes that protect cells and tissues from oxidative stress.^[3]

There are three distinct isoforms of nitric oxide synthase (NOS), the enzyme responsible for NO synthesis, each encoded by a different gene locus: neuronal (nNOS), inducible (iNOS), and endothelial (eNOS). eNOS is a structural isoform and is localized primarily to the membrane, accounting for approximately 90%. NO synthesized by eNOS in the endothelium diffuses into smooth muscle cells, leading to muscle relaxation. Although eNOS activity has been demonstrated in various tissues and organs (including mast cells, platelets, pancreatic β -cells), its primary source is the vascular endothelial cells.^[4] Furthermore, several studies have shown that eNOS is expressed in Leydig and Sertoli cells, as well as in rare degenerated germ cells in the testis. It has also been demonstrated that germ cells in testes overexposed to IR damage express eNOS.^[5] The excessive expression of eNOS suggests that NO plays a regulatory role in apoptosis. Recent studies have shown that NO exhibits strong anti-apoptotic activity and exerts tissue-protective effects in damage caused by IR, which represents a highly interesting and potentially significant area of research.^[4]

Cell death is one of the fundamental physiological processes occurring in all living organisms. Programmed cell death occurs through three main pathways: apoptotic cell death, autophagic cell death, and necroptotic cell death. Currently, two major pathways have been defined for apoptosis: the extrinsic (death receptor) pathway and the intrinsic (mitochondrial) pathway. Although these two programmed cell death mechanisms are not completely independent, they share similarities in the proteins that regulate the process and can influence one another depending on the stimulus that triggers cell death. The intrinsic pathway is activated by intracellular signals released when cells are under stress and proceeds through the release of proteins from the mitochondrial intermembrane space. B-cell lymphoma 2 (Bcl-2) is a protein with both proapoptotic and antiapoptotic functions that plays a crucial role in the intrinsic apoptotic pathway; approximately 25 different types have been identified to date. Bcl-2 proteins are central regulators of the intrinsic apoptotic pathway and determine whether a cell will undergo apoptosis. Bax (Bcl-2 associated X apoptosis regulator) is a proapoptotic member of the Bcl-2 family capable of forming pores in the outer mi-

tochondrial membrane. These molecules are involved in the initiation of apoptosis.^[6,7]

During embryonic development, vascular endothelial growth factor (VEGF) plays a significant role in regulating angiogenesis in various physiological processes, including neovascularization, wound healing, ovulation, the menstrual cycle, blood pressure regulation, and pregnancy. VEGF was first identified as a permeability factor in tumor studies. Since then, it has been shown to be one of the key molecules in endothelial cell proliferation and angiogenesis. It exerts this effect by promoting cell migration and facilitating new vessel formation.^[8] VEGF is one of the primary factors responsible for the formation and proliferation of endothelial cells. It also stimulates the expression of anti-apoptotic proteins in endothelial cells. NO plays an important role in the angiogenesis and increased vascular permeability induced by VEGF. A study conducted in mice lacking iNOS and eNOS enzymes demonstrated a reduction in the effectiveness of VEGF.^[9]

Allopurinol was initially used for cancer treatment but was found to be ineffective. Later, its role in purine metabolism and its identification as a xanthine oxidase inhibitor led to its approval for the treatment of hyperuricemia and gout in 1966.^[10]

Xanthine oxidase converts allopurinol into its main metabolite, oxypurinol. Both compounds are analogs of the purine bases xanthine and hypoxanthine. They inhibit the production of uric acid, the end product of purine catabolism, and reduce superoxide generation by inhibiting xanthine dehydrogenase activity. At low concentrations, allopurinol acts as both a substrate and a competitive inhibitor of the enzyme, whereas at high concentrations, it functions as a non-competitive inhibitor. Oxypurinol, the active metabolite of allopurinol, is a non-competitive inhibitor of the enzyme. The formation of oxypurinol accounts for most of the pharmacological effects of allopurinol, and its prolonged presence in tissues is significant.^[10]

Compared to allopurinol, oxypurinol has been shown to possess various properties, including antioxidant, anti-inflammatory, and cell death-preventive effects. Escobar et al.^[11] demonstrated that oxypurinol protects against oxidative damage in acute pancreatitis. In an IR study conducted in rats, oxypurinol was shown to have a protective effect on the myocardium.^[11]

Effective treatment following testicular torsion may provide protection against the adverse effects of IR injury.^[12] In this study, we investigated whether treatment with allopurinol and oxypurinol could reduce oxidative stress and germ cell apoptosis in rat testes following IR.

MATERIALS AND METHODS

The study was approved by the Institutional Review Board for the Care and Use of Laboratory Animals (this study was

approved by the Suleyman Demirel University Animal Experiments Local Ethics Committee date: 12.04.2023, decision no: 148). All procedures were conducted in accordance with the ARRIVE guidelines (Animal Research: Reporting of In Vivo Experiments), the UK Animals (Scientific Procedures) Act 1986 and its associated guidelines, EU Directive 2010/63/EU for animal experimentation, and the National Research Council's Guide for the Care and Use of Laboratory Animals.

Experimental Groups

In this study, 32 male Wistar albino rats weighing between 259 and 465 g were used. The animals were housed under standardized conditions and kept in separate cages. The rats were randomly divided into four groups:

- Group 1 (Sham-Operated, n=8): The testicle was exposed, but torsion was not performed.
- Group 2 (IR-Saline, n=8): Torsion/detorsion was applied to the left testicle, and normal saline was administered.
- Group-3 (IR-Allopurinol, n=8): Torsion/detorsion was applied to the left testicle, and allopurinol was administered.
- Group 4 (IR-Oxypurinol, n=8): Torsion/detorsion was applied to the left testicle, and oxypurinol was administered.

Preparation of Allopurinol and Oxypurinol

The raw materials for the preparation of the parenteral forms of allopurinol (HY-B0219-500) and oxypurinol (HY-19657-200) were obtained from the MedChemExpress LLC (New Jersey, USA). The drugs were prepared under sterile conditions according to the manufacturer's recommendations and were administered at a dose of 50 mg/mL.

Surgical Procedure and Sample Harvesting

After 12 hours of fasting, the rats were weighed and anesthetized via intraperitoneal injection of ketamine hydrochloride (90 mg/kg) and xylazine hydrochloride (10 mg/kg). Using sterile surgical techniques, a midline scrotal incision was made, and the left testicle was exposed. In the IR-Saline, IR-Allopurinol, and IR-Oxypurinol groups, a torsion model was created by rotating the testicle and its cord 720° clockwise for two hours. The testicle was then fixed to the scrotum with a 5/0 silk suture to prevent detorsion, and the scrotum was closed. Thirty minutes before the detorsion procedure, 1 mL of normal saline was administered intraperitoneally to the IR-Saline group, whereas allopurinol and oxypurinol were administered intraperitoneally at a dose of 50 mg/kg to the IR-Allopurinol and IR-Oxypurinol groups, respectively. After two hours of ischemia, the scrotal incision was reopened, detorsion procedure was performed, and the incision was closed in all IR groups. During the reperfusion period, the rats were allowed free access to standard chow and water. On the 28th day of the experiment, all animals were sacrificed, and the left testicle of each animal was harvested and stored at -80°C until further analysis.

Determination of Total Antioxidant Status, Total Oxidant Status, and Oxidative Stress Index

Total antioxidant status (TAS) and total oxidant status (TOS) were measured using colorimetric methods with TAS and TOS kits kit (REL Assay Diagnostics, Türkiye), following the manufacturer's instructions. The TAS assay was calibrated with Trolox, and results were expressed as $\mu\text{mol Trolox equivalent/L}$ ($\mu\text{mol Trolox eq/L}$). TOS levels were determined based on the oxidation of ferrous ions to ferric ions in an acidic medium in the presence of various oxidative species. The analyses were performed using three different portions of the same tissue sample, and each portion was assessed in three technical replicates.

TAS and TOS results were presented in the graph as fold changes compared to the control group. The oxidative stress index (OSI) values were calculated as the ratio of TOS to TAS.

Quantitative Reverse Transcription Polymerase Chain Reaction (RT-qPCR)

Total RNA was isolated from minced rat testis tissues using the Monarch® Total RNA Miniprep Isolation Kit (New England Biolabs) according to the manufacturer's instructions. RNA purity and quality were measured using a microspectrophotometer (Allsheng). Approximately 500 ng of RNA from each sample was converted into complementary DNA (cDNA) using the iScript™ cDNA Synthesis Kit (Bio-Rad) following the manufacturer's protocol. qRT-PCR was performed on a CFX Connect Real-Time PCR System (Bio-Rad) using iTaq Universal SYBR® Green Supermix (Bio-Rad) according to the manufacturer's instructions. Primer sequences for rat endothelial nitric oxide synthase (eNOS), vascular endothelial growth factor-A (VEGF-A), Bax, Bcl-2, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were generated using the NCBI BLAST interface. Primer sequence information is provided in Table 1. Relative gene expression levels were normalized to GAPDH expression, and quantification was performed using the $\Delta\Delta\text{Ct}$ method.

Statistical Analysis

Statistical analyses were conducted using SPSS 23.0 (IBM Inc., Chicago, IL, USA). Tukey's Honestly Significant Difference (HSD) test and one-way analysis of variance (ANOVA) were used to compare measurements between the study groups for statistical significance. Normality was assessed using the Kolmogorov-Smirnov test, and homogeneity of variance was evaluated with Levene's test. Continuous variables with normal distribution were presented as mean \pm standard deviation (SD). The level of statistical significance was set at $p < 0.05$.

RESULTS

TAS, TOS, and OSI Levels

A significant increase in TAS levels was observed in the IR-Saline ($p=0.002$), IR-Allopurinol ($p<0.001$), and IR-Oxypurinol ($p<0.001$) groups compared with the sham-operated group.

Table 1. Quantitative real-time polymerase chain reaction (qRT-PCR) primer sequences

| Gene | Primer Direction | Primer Sequence |
|--------|------------------|-------------------------------|
| BAX | Forward | 5'-GCAGAGGATGATTGCTGATGT-3' |
| | Reverse | 5'-CCTTGAGCACCAGTTTGCTA-3' |
| BCL2 | Forward | 5'-GTGGATGACTGAGTACCTGAAC-3' |
| | Reverse | 5'-GAGACAGCCAGGAGAAATCAA-3' |
| eNOS | Forward | 5'-GTGAAGGCGACTATCCTGTATG-3' |
| | Reverse | 5'-CATGCTCTAGGGATACCACATC-3' |
| VEGF-A | Forward | 5'-GGAAGAGAGAGAGAGAGAGAGAC-3' |
| | Reverse | 5'-GACTGGTCCGATGAAAGATCC-3' |
| GAPDH | Forward | 5'-CAAGTCATCCCAGAGCTGAA-3' |
| | Reverse | 5'-CATGTAGGCCATGAGGTCCAC-3' |

Table 2. Total antioxidant status (TAS), total oxidant status (TOS), and oxidative stress index (OSI) values of the groups (values are presented as mean±standard deviation)

| Group | TAS (mmol Trolox Eq/L) | TOS ($\mu\text{mol H}_2\text{O}_2$ Eq/L) | OSI |
|----------------|----------------------------------|---|-----------------------------------|
| Sham-operated | 1.06±0.15 ^a | 3.86±1.02 ^a | 3.71±1.07 ^a |
| IR-Saline | 1.52±0.11 ^b (p=0.002) | 31.19±1.88 ^b (p<0.001) | 20.35±2.19 ^b (p<0.001) |
| IR-Allopurinol | 2.41±0.38 ^c (p<0.001) | 15.93±0.69 ^c (p<0.001) | 6.72±0.86 ^c (p<0.001) |
| IR-Oxypurinol | 3.84±0.15 ^d (p<0.001) | 10.94±0.90 ^d (p<0.001) | 2.85±0.27 ^a (p=0.554) |

^{a,b,c,d} Different superscript letters indicate statistically significant differences. TAS: Total antioxidant status; TOS: Total oxidant status; OSI: Oxidative stress index.

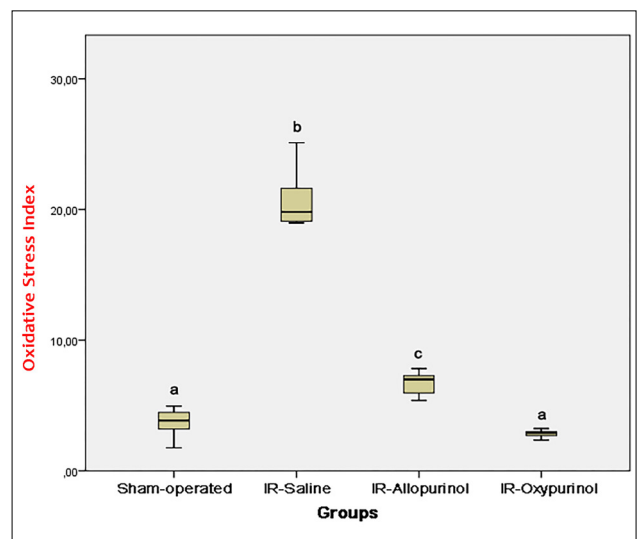
Allopurinol and oxypurinol treatments significantly increased TAS levels compared to the IR-Saline group (p<0.001). Oxypurinol increased TAS levels significantly more than allopurinol (p<0.001) (Table 2).

Compared to the sham-operated group, TOS levels were significantly higher in all IR groups (p<0.001). TOS levels were significantly lower in the IR-Allopurinol and IR-Oxypurinol groups when compared to the IR-Saline group (p<0.001). Oxypurinol decreased TOS levels significantly more than allopurinol (p<0.001) (Table 2).

Compared to the sham-operated group (p<0.001), OSI levels were significantly higher in the IR-Saline and IR-Allopurinol groups. However, no significant difference was observed between the IR-Oxypurinol and sham-operated groups (p=0.554). Both allopurinol and oxypurinol treatments significantly reduced OSI values compared to the IR-Saline group (p<0.001). Oxypurinol was more effective than allopurinol in reducing oxidative stress (p<0.001) (Table 2, Fig. 1).

Bax, Bcl-2 Gene Expression Levels, and Bax/Bcl-2 Ratio

Bax gene expression levels were significantly increased in the IR-Saline group compared to the other groups (p<0.001). Al-

**Figure 1.** Oxidative stress index (OSI) levels among the groups.

lopurinol and oxypurinol treatments significantly decreased Bax gene expression levels compared to the IR-Saline group (p<0.001). No significant difference was found between the IR-Allopurinol and IR-Oxypurinol groups (p=0.171) (Table 3).

Table 3. Bax, Bcl-2 gene expression levels and Bax/Bcl-2 ratios of the groups (values are presented as mean±standard deviation)

| Group | Bax | Bcl-2 | Bax/Bcl-2 |
|----------------|----------------------------------|----------------------------------|----------------------------------|
| Sham-operated | 1.00±0.08 ^a | 1.00±0.07 ^a | 1.00±0.10 ^a |
| IR-Saline | 1.43±0.13 ^b (p<0.001) | 0.48±0.08 ^b (p=0.002) | 3.01±0.48 ^b (p<0.001) |
| IR-Allopurinol | 1.10±0.07 ^a (p=0.277) | 0.92±0.45 ^a (p=0.917) | 1.40±0.59 ^a (p=0.193) |
| IR-Oxypurinol | 0.98±0.13 ^a (p=0.990) | 1.02±0.21 ^a (p=0.999) | 0.98±0.09 ^a (p=0.999) |

^{a,b} Different superscript letters indicate statistically significant differences.

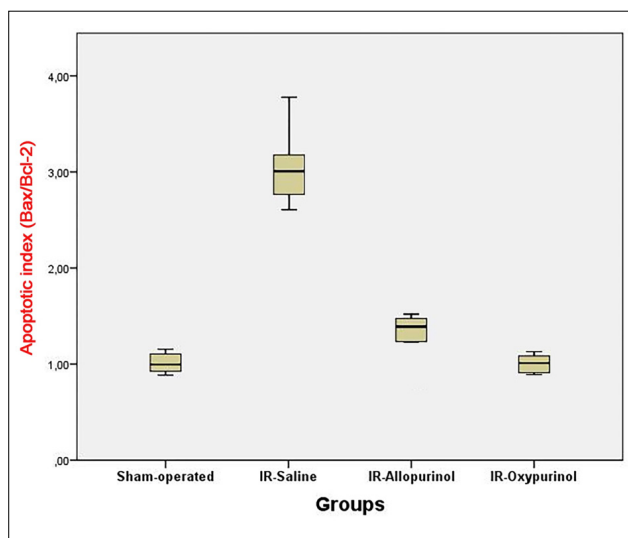


Figure 2. Apoptotic index values among the groups.

Table 4. Endothelial nitric oxide synthase (eNOS) and vascular endothelial growth factor A (VEGF-A) gene expression levels of the groups (values are presented as mean±standard deviation)

| Group | eNOS | VEGF-A |
|----------------|----------------------------------|----------------------------------|
| Sham-operated | 1.00±0.09 ^a | 1.00±0.08 ^a |
| IR-Saline | 1.24±0.16 ^b (p=0.006) | 1.20±0.18 ^a (p=0.342) |
| IR-Allopurinol | 1.48±0.11 ^c (p<0.001) | 1.74±0.18 ^b (p<0.001) |
| IR-Oxypurinol | 1.60±0.16 ^c (p<0.001) | 2.42±0.27 ^c (p<0.001) |

^{a,b,c} Different superscript letters indicate statistically significant differences.

Bcl-2 gene expression levels were significantly decreased in the IR-Saline group compared to the other groups (p=0.002). Compared to the IR-Saline group, allopurinol (p=0.009) and oxypurinol (p=0.001) treatments significantly increased Bcl-2 gene expression levels. No significant difference was found between the IR-Allopurinol and IR-Oxypurinol groups (p=0.863) (Table 3).

The Bax/Bcl-2 ratio (apoptotic index) was significantly increased in the IR-Saline group compared to the other groups (p<0.001). Compared to the IR-Saline group (p<0.001), both allopurinol and oxypurinol treatments significantly decreased the Bax/Bcl-2 ratio. No significant difference was found between the IR-Allopurinol and IR-Oxypurinol groups (p=0.159) (Table 3, Fig. 2).

eNOS and VEGF-A Gene Expression Levels

Compared to the sham-operated group, eNOS gene expression levels were significantly higher in the IR-Saline (p=0.006), IR-Allopurinol (p<0.001), and IR-Oxypurinol (p<0.001) groups. Allopurinol (p=0.007) and oxypurinol (p<0.001) treatments significantly increased eNOS gene expression levels compared to the IR-Saline group. No significant difference was found between the IR-Allopurinol and IR-Oxypurinol groups (p=0.306) (Table 4).

VEGF-A gene expression levels were significantly higher in the IR-Allopurinol and IR-Oxypurinol groups compared to the sham-operated group (p<0.001), whereas no significant difference was found between the IR-Saline group and sham-operated groups (p=0.342). Allopurinol and oxypurinol treatments significantly increased VEGF-A gene expression levels compared to the IR-Saline group (p<0.001). Oxypurinol increased VEGF-A gene expression levels significantly more than allopurinol (p<0.001) (Table 4).

DISCUSSION

The present study aimed to investigate the protective effects of allopurinol and oxypurinol on germ cell apoptosis induced by testicular IR in rats. The findings indicate that testicular IR significantly induces germ cell apoptosis in rats, whereas allopurinol and oxypurinol activate repair mechanisms by reducing apoptosis.

The testes are highly susceptible to ischemic damage due to the terminal nature of their blood supply and the lack of arterial anastomoses. The duration of testicular torsion is directly proportional to the extent of damage. The salvage rate of the testis is 90% in patients who undergo surgical detorsion within six hours.^[13] After this critical period, the salvage rate decreases significantly. However, it is unclear whether

testicular function is fully preserved even after early surgical detorsion. Even a few hours of untreated torsion can damage testicular tissue and lead to functional loss. Experimental studies have shown that when the twisted testis is left in place after unilateral testicular torsion, damage to the blood-testis barrier may occur, affecting both testes and potentially reducing fertility. Testicular torsion followed by detorsion has been shown to induce the formation of ROS, which can result in germ cell apoptosis and DNA damage in the testis. Oxidative stress negatively affects sperm concentration and motility and is therefore considered a significant cause of male infertility. Studies have demonstrated that detorsion can impair the exocrine functions of the testis, cause changes in sperm morphology, and lead to decreased sperm motility and count. Therefore, additional therapeutic strategies are needed following surgical detorsion.^[14] Effective treatment following testicular torsion can provide protection against the detrimental effects of IR. Various drugs have been successfully tested in animal models to reduce the adverse effects of IR in testicular torsion.^[15] The fundamental mechanism of these agents is the regulation of blood flow after injury, reduction of germ cell apoptosis, and minimization of oxidative stress. These agents have largely been used in experimental torsion models. However, there are also studies in which treatment was administered before torsion.^[16] Since testicular torsion is an unpredictable condition, initiating appropriate treatment after torsion is more meaningful in terms of clinical applicability. Testicular torsion is an emergency situation, and in our clinical practice, the time from diagnosis to emergency testicular detorsion is approximately 30 minutes. Therefore, we determined this period based on the assumption that these drugs could be administered immediately after diagnosis and exert a protective effect on the testicles until surgery. Despite the promising results of experimental studies, the greatest challenge is translating one or more of these agents into clinical use. In this context, since allopurinol and oxypurinol, which were used as antioxidants in our study, are already clinically prescribed for conditions such as hyperuricemia and gout, their application in the clinical management of testicular torsion may be more feasible.

Allopurinol, a xanthine oxidase inhibitor, is a urate-lowering drug used in the treatment of hyperuricemia and gout. Various studies have demonstrated the protective effects of allopurinol on the testis after IR in experimental models.^[17] Oxypurinol, the active metabolite of allopurinol, has been reported to have stronger biological properties, including antioxidant, anti-inflammatory, and anti-apoptotic activities in various pathological conditions. Oxypurinol protects against oxidative damage in acute pancreatitis and regulates pro-inflammatory genes.^[18] Another study showed that oxypurinol protects cardiac tissue from IR damage in rats.^[19] In our opinion, no study has yet investigated the effects of allopurinol and oxypurinol on germ cell apoptosis in testicular torsion. Therefore, the results of this study add novel information to the current literature.

The mechanisms underlying testicular damage following IR have not yet been fully elucidated. The main pathophysiological events in testicular torsion are ischemia caused by twisting of the spermatic cord and reperfusion injury resulting from surgical correction of the torsion. IR injury involves neutrophil migration, the formation of pro-inflammatory cytokines and adhesion molecules, lipid peroxidation, apoptosis, anoxia, and changes in microvascular blood flow.^[12] The most important factor initiating cytotoxic events after reperfusion is the ROS produced by xanthine oxidase (XO). ROS increases membrane permeability and disrupts membrane integrity through the oxidation of lipids in cellular and mitochondrial membranes.^[20] Our study showed that allopurinol and oxypurinol, which are XO inhibitors, reduce oxidative stress in the testis after IR, and that oxypurinol is a more effective antioxidant than allopurinol.

Experimental data have shown that germ cells in the testis are highly sensitive to ischemia and are susceptible to damage following IR. The most important pathological mechanism in this injury is germ cell apoptosis. Due to oxidative damage, significant germ cell apoptosis has been observed in experimental testicular torsion/detorsion models. Clinical and animal studies have shown that apoptosis can lead to germ cell loss and reduced spermatogenesis. Apoptosis is a physiological process by which the body eliminates unwanted cells and serves as the primary defense mechanism against damaged cells.^[4,5] Various regulatory genes involved in apoptosis have been identified.

Studies have shown that the Bcl-2 protein family, including pro-apoptotic (Bax, Bak, Bid, and Bim) and anti-apoptotic (Bcl-2, Bcl-xL) molecules, plays an important role in regulating germ cell apoptosis.^[21] Some studies in various experimental models have revealed that the Bax/Bcl-2 system is crucial for the maintenance of normal spermatogenesis.^[22] In addition to their roles in normal testicular physiology, Bax and Bcl-2 proteins have also been found to be significant in the development of various testicular disorders, such as testicular hyperthermia, cryptorchidism, and radiation-induced testicular damage.^[23] In the present study, Bax and Bcl-2 gene expression levels were measured to assess the level of germ cell apoptosis in testicular tissue using qRT-PCR. A significant increase in the apoptotic index was identified in the IR-Saline group compared to the sham-operated group. This result demonstrates that oxidative damage leads to germ cell apoptosis in testicular tissue. The results of the present study also showed that treatment with allopurinol and oxypurinol administered after IR effectively reduces germ cell apoptosis. However, no difference was observed between the two drugs in terms of anti-apoptotic properties.

Nitric oxide, which has dual effects on both cell survival and death, plays a vital role in testicular disorders, including testicular torsion and inflammation. However, the functions of NOS in testicular torsion are currently not well understood.^[24] NOS is an enzyme family that catalyzes the production

of NO from L-arginine. There are three subtypes: neuronal NOS (nNOS), endothelial NOS (eNOS), and inducible NOS (iNOS).^[25] Previous studies have shown that eNOS, in particular, accelerates angiogenesis under ischemic stress conditions.^[26] Phosphorylation of eNOS leads to its activation and NO production. The potential importance of NO in IR stems from its role as an antioxidant in both physiological and pathological processes, as it readily interacts with ROS and reduces oxidative damage in various biological systems.^[27] NO has both toxic and protective effects following IR injury. High levels of NO produced by iNOS and nNOS can cause cell damage or death. However, NO produced by eNOS has been shown to reduce oxidative damage in various biological systems. NO has also exhibited anti-inflammatory effects by mediating several inflammatory processes, such as preventing neutrophil infiltration and reducing pro-inflammatory cytokine levels.^[28] These anti-inflammatory effects contribute to tissue protection against IR damage. Additionally, degenerative germ cells in the testis have been shown to excessively express eNOS,^[29] suggesting that eNOS and eNOS-mediated NO play a role in germ cell apoptosis. It has also been observed that NO inhibits recombinant human caspases, a family of cysteine proteases that play a critical role in the initiation and execution of apoptosis, in a dose-dependent manner.^[30] In this study, eNOS levels were found to be significantly increased in all IR groups compared to the sham-operated group. This result indicates that the tissue enters a repair process after IR. Moreover, allopurinol and oxypurinol increased eNOS levels significantly more than the IR-Saline group. These findings suggest that treatment with allopurinol and oxypurinol after IR may positively contribute to the repair process through antioxidant and anti-apoptotic effects by increasing eNOS-mediated NO production.

Angiogenesis is a physiological process that enables the formation of new vessels from pre-existing vascular structures in response to ischemia. VEGF is the most important factor in the angiogenic process. In previous studies, exogenous local injection of VEGF has been shown to improve neovascularization in both mice and humans.^[31] An experimental study on renal IR demonstrated that increased VEGF expression is associated with reduced renal damage through enhanced new blood vessel formation.^[32] The presence of VEGF in normal testicular tissue, prostate, and seminal vesicles, as well as its high levels in semen, suggests that it plays an important role in male reproductive physiology.^[33] In testicular tissue, VEGF contributes to both angiogenesis and steroidogenesis in Leydig cells. It also maintains the permeability of testicular blood vessels by regulating testicular function.^[34] In a study conducted on rats, VEGF protein expression was reported to be significantly decreased in the testicular IR group, while VEGF levels increased in the treatment group, leading to increased vascularization and reduced damage.^[35] In the present study, allopurinol and oxypurinol treatment significantly increased VEGF-A gene expression levels compared to the sham-operated and IR-Saline groups. This result suggests that

allopurinol and oxypurinol treatment enhances vascularization in testicular tissue after IR. Theoretically, increased vascularization may reduce testicular damage; however, further studies including pathological tissue scoring are needed to confirm this conclusion.

Our study has several limitations. First, as an experimental study, the sample size was limited. Second, testicular size was not measured, as we initially planned to focus on pathology and apoptosis. Histopathological examination could not be performed due to a technical problem. Finally, since hormone levels were not evaluated, we were only able to demonstrate the effects of the drugs on reducing infertility in the long term. Further studies are needed on this issue. We believe that our study may serve as a pioneer for future research.

CONCLUSION

In conclusion, testicular torsion caused a significant increase in germ cell apoptosis due to elevated testicular oxidative stress. Allopurinol and its active metabolite, oxypurinol, were shown to reduce oxidative stress in the testis after IR, and oxypurinol was found to be a more effective antioxidant than allopurinol. Treatment with allopurinol and oxypurinol was also shown to be effective in reducing germ cell apoptosis. In this respect, allopurinol and oxypurinol are potential agents for clinical applications in testicular torsion.

Ethics Committee Approval: This study was approved by the Suleyman Demirel University Animal Experiments Local Ethics Committee (Date: 12.04.2023, Decision No: 148).

Peer-review: Externally peer-reviewed.

Authorship Contributions: Concept: E.B., L.D.; Design: E.B., L.D.; Supervision: E.B., L.D.; Resource: E.B., L.D.; Materials: E.B., L.D., O.E.; Data collection and/or processing: E.B., L.D., Y.E., O.E., Y.K.; Analysis and/or interpretation: E.B., L.D., Y.K.; Literature review: E.B., L.D.; Writing: E.B.; Critical review: L.D., Y.K.

Conflict of Interest: None declared.

Financial Disclosure: This study was supported by Suleyman Demirel University internal funds (TTU-2023-9063).

REFERENCES

1. Yagmurdu H, Ayyildiz A, Karaguzel E, Ogus E, Surer H, Caydere M, et al. The preventive effects of thiopental and propofol on testicular ischemia-reperfusion injury. *Acta Anaesthesiol Scand* 2006;50:1238—43. [\[CrossRef\]](#)
2. Anim JT, Kehinde EO, Prasad A, Varghese R. Morphological responses of the rabbit testis to ischemic/reperfusion injury due to torsion. *Urol Int* 2005;75:258—63. [\[CrossRef\]](#)
3. Phillips L, Toledo AH, Lopez-Neblina F, Anaya-Prado R, Toledo-Pereyra LH. Nitric oxide mechanism of protection in ischemia and reperfusion injury. *J Invest Surg* 2009;22:46—55. [\[CrossRef\]](#)
4. Sahay S, Gupta M. An update on nitric oxide and its benign role in plant responses under metal stress. *Nitric Oxide* 2017;67:39—52. [\[CrossRef\]](#)
5. Zini A, O'Bryan MK, Magid MS, Schlegel PN. Immunohistochemical localization of endothelial nitric oxide synthase in human testis, epidid-

- ymis, and vas deferens suggests a possible role for nitric oxide in spermatogenesis, sperm maturation, and programmed cell death. *Biol Reprod* 1996;55:935—41. [\[CrossRef\]](#)
6. Singh R, Letai A, Sarosiek K. Regulation of apoptosis in health and disease: the balancing act of BCL—2 family proteins. *Nat Rev Mol Cell Biol* 2019;20:175—93. [\[CrossRef\]](#)
 7. Peña-Blanco A, García-Sáez AJ. Bax, Bak and beyond - mitochondrial performance in apoptosis. *FEBS J* 2018;285:416—431. [\[CrossRef\]](#)
 8. Heidenreich R, Röcken M, Ghoreschi K. Angiogenesis drives psoriasis pathogenesis. *Int J Exp Pathol* 2009;90:232—48. [\[CrossRef\]](#)
 9. Fukumura D, Gohongi T, Kadambi A, Izumi Y, Ang J, Yun CO, et al. Predominant role of endothelial nitric oxide synthase in vascular endothelial growth factor-induced angiogenesis and vascular permeability. *Proc Natl Acad Sci U S A* 2001;98:2604—9. [\[CrossRef\]](#)
 10. Crowell JW, Jones CE, Smith EE. Effect of allopurinol on hemorrhagic shock. *Am J Physiol* 1969;216:744—8. [\[CrossRef\]](#)
 11. Yang JJ, Finn WF. Effect of oxypurinol on cyclosporine toxicity in cultured Ea, Llc-Pk1 and mdck cells. *Ren Fail* 1998;20:85—101. [\[CrossRef\]](#)
 12. Cuzzocrea S, Riley DP, Caputi AP, Salvemini D. Antioxidant therapy: a new pharmacological approach in shock, inflammation, and ischemia/reperfusion injury. *Pharmacol Rev* 2001;53:135—59. [\[CrossRef\]](#)
 13. Ringdahl E, Teague L. Testicular torsion. *Am Fam Physician* 2006;74:1739—43.
 14. Törzsök P, Steiner C, Pallauf M, Abenhardt M, Milinovic L, Plank B, et al. Long-term follow-up after testicular torsion: prospective evaluation of endocrine and exocrine testicular function, fertility, oxidative stress and erectile function. *J Clin Med* 2022;11:6507. [\[CrossRef\]](#)
 15. Cay A, Alver A, Küçük M, Işık O, Eminağaoğlu MS, Karahan SC, et al. The effects of N—acetylcysteine on antioxidant enzyme activities in experimental testicular torsion. *J Surg Res* 2006;131:199—203. [\[CrossRef\]](#)
 16. Unsal A, Eroglu M, Avci A, Cimentepe E, Guven C, Derya Balbay M, et al. Protective role of natural antioxidant supplementation on testicular tissue after testicular torsion and detorsion. *Scand J Urol Nephrol* 2006;40:17—22. [\[CrossRef\]](#)
 17. Abasiyanik A, Dağdönderen L. Beneficial effects of melatonin compared with allopurinol in experimental testicular torsion. *J Pediatr Surg* 2004;39:1238—41. [\[CrossRef\]](#)
 18. Escobar J, Pereda J, Arduini A, Sandoval J, Moreno ML, Pérez S, et al. Oxidative and nitrosative stress in acute pancreatitis. Modulation by pentoxifylline and oxypurinol. *Biochem Pharmacol* 2012;83:122—30. [\[CrossRef\]](#)
 19. LoBalsamo L, Bergsland J, Lajos P, Feldman MJ. Prevention of reperfusion injury in ischemic-reperfused hearts by oxypurinol and allopurinol. *Transpl Int* 1989;2:218—22. [\[CrossRef\]](#)
 20. Reilly PM, Schiller HJ, Bulkley GB. Pharmacologic approach to tissue injury mediated by free radicals and other reactive oxygen metabolites. *Am J Surg* 1991;161:488—503. [\[CrossRef\]](#)
 21. Boise LH, Gottschalk AR, Quintans J, Thompson CB. Bcl-2 and Bcl-2-related proteins in apoptosis regulation. *Curr Top Microbiol Immunol* 1995;200:107—21. [\[CrossRef\]](#)
 22. Knudson CM, Tung KS, Tourtellotte WG, Brown GA, Korsmeyer SJ. Bax-deficient mice with lymphoid hyperplasia and male germ cell death. *Science* 1995;270:96—9. [\[CrossRef\]](#)
 23. Embree-Ku M, Venturini D, Boekelheide K. Fas is involved in the p53-dependent apoptotic response to ionizing radiation in mouse testis. *Biol Reprod* 2002;66:1456—61. [\[CrossRef\]](#)
 24. Ozturk H, Buyukbayram H, Ozdemir E, Ketani A, Gurel A, Onen A, et al. The effects of nitric oxide on the expression of cell adhesion molecules (ICAM-1, UEA-1, and tenascin) in rats with unilateral testicular torsion. *J Pediatr Surg* 2003;38:1621—7. [\[CrossRef\]](#)
 25. He F, Xu BL, Chen C, Jia HJ, Wu JX, Wang XC, et al. Methylophipogonone A suppresses ischemia/reperfusion-induced myocardial apoptosis in mice via activating PI3K/Akt/eNOS signaling pathway. *Acta Pharmacol Sin* 2016;37:763—71. [\[CrossRef\]](#)
 26. Huang D, Wang FB, Guo M, Li S, Yan ML, Yu T, et al. Effect of combined treatment with rosuvastatin and protein kinase C β inhibitor on angiogenesis following myocardial infarction in diabetic rats. *Int J Mol Med* 2015;35:829—38. [\[CrossRef\]](#)
 27. Wang L, Chen Q, Li G, Ke D. Ghrelin ameliorates impaired angiogenesis of ischemic myocardium through GHSR1a-mediated AMPK/eNOS signal pathway in diabetic rats. *Peptides* 2015;73:77—87. [\[CrossRef\]](#)
 28. De Caterina R, Libby P, Peng HB, Thannickal VJ, Rajavashisth TB, Gimbrone MA Jr, et al. Nitric oxide decreases cytokine-induced endothelial activation. Nitric oxide selectively reduces endothelial expression of adhesion molecules and proinflammatory cytokines. *J Clin Invest* 1995;96:60—8. [\[CrossRef\]](#)
 29. Akgür FM, Kiliç K, Aktuğ T, Olguner M. The effect of allopurinol pretreatment before detorting testicular torsion. *J Urol* 1994;151:1715—7. [\[CrossRef\]](#)
 30. Boyd CS, Cadenas E. Nitric oxide and cell signaling pathways in mitochondrial-dependent apoptosis. *Biol Chem* 2002;383:411—23. [\[CrossRef\]](#)
 31. Vale PR, Losordo DW, Milliken CE, Maysky M, Esakof DD, Symes JF, et al. Left ventricular electromechanical mapping to assess efficacy of ph-VEGF(165) gene transfer for therapeutic angiogenesis in chronic myocardial ischemia. *Circulation* 2000;102:965—74. [\[CrossRef\]](#)
 32. Xue J, Zhu K, Cao P, Long C, Deng Y, Liu T, et al. Ischemic preconditioning-induced protective effect for promoting angiogenesis in renal ischemia—reperfusion injury by regulating miR-376c-3p/HIF-1 α /VEGF axis in male rats. *Life Sci* 2022;299:120357. [\[CrossRef\]](#)
 33. Minutoli L, Antonuccio P, Squadrito F, Bitto A, Nicotina PA, Fazzari C, et al. Effects of polydeoxyribonucleotide on the histological damage and the altered spermatogenesis induced by testicular ischaemia and reperfusion in rats. *Int J Androl* 2012;35:133—44. [\[CrossRef\]](#)
 34. Hashimoto H, Ishikawa T, Yamaguchi K, Shiotani M, Fujisawa M. Experimental ischaemia-reperfusion injury induces vascular endothelial growth factor expression in the rat testis. *Andrologia* 2009;41:216—21. [\[CrossRef\]](#)
 35. Abdel-Aziz AM, Naguib Abdel Hafez SM. Sitagliptin protects male albino rats with testicular ischaemia/reperfusion damage: Modulation of VCAM-1 and VEGF-A. *Andrologia* 2020;52:e13472. [\[CrossRef\]](#)

DENEYSEL ÇALIŞMA - ÖZ

Deneysel testis torsiyonu modelinde allopurinol ve oksipurinol tedavisinin apoptozis üzerine etkisi

AMAÇ: Allopurinol ve oksipurinol tedavisinin testis iskemi-reperfüzyonunda (IR) oksidatif stresi ve germ hücre apoptozunu azaltıp azaltamayacağını incelemek.

GEREÇ VE YÖNTEM: Otuz iki erkek sıçan dört gruba ayrıldı: Grup 1 (Sham-Ameliyat, n=8), testis açığa çıkarıldı, ancak torsiyon uygulanmadı; Grup-2 (IR+Salin, n=8), sol testise torsiyon/detorsiyon uygulandı ve 1 ml serum fizyolojik verildi; Grup-3 (IR+Allopurinol, n=8), sol testise torsiyon/detorsiyon uygulandı ve 50 mg/kg allopurinol verildi; Grup 4 (IR+Oksipurinol, n=8), sol testise torsiyon/detorsiyon uygulandı ve 50 mg/kg oksipurinol verildi. Sol testis ameliyat sonrası 28. günde alındı ve dokuda TAS, TOS ve OSI düzeyleri ölçüldü. Ayrıca Bax, Bcl-2, eNOS ve VEGF-A gen ekspresyon düzeyleri incelendi.

BULGULAR: Allopurinol ve oksipurinol, OSI düzeylerini anlamlı şekilde azalttı ($p<0.001$). Oksipurinolün oksidatif stresi azaltmada anlamlı şekilde daha etkili olduğu bulundu ($p<0.001$). Allopurinol ve oksipurinol, Bax gen ekspresyon düzeyini anlamlı şekilde azalttı ($p<0.001$). Allopurinol ($p=0.009$) ve oksipurinol ($p=0.001$) tedavisi Bcl-2 düzeyini anlamlı şekilde artırdı. Allopurinol ve oksipurinol apoptoz indeksini anlamlı şekilde azalttı ($p<0.001$). Allopurinol ($p^1=0.007$, $p^2<0.001$) ve oksipurinol ($p^{1,2}<0.001$) tedavileri, eNOS ve VEGF-A gen ekspresyon seviyelerini anlamlı şekilde artırmıştır.

SONUÇ: Allopurinol ve oksipurinol, IR sonrası testiste oksidatif stresi azaltmada etkilidir ve oksipurinol daha güçlü bir antioksidan etkiye sahiptir. Allopurinol ve oksipurinol tedavisi, eNOS ve VEGF-A aracılı onarım sürecine olumlu katkıda bulunarak apoptozu azaltmada etkilidir. Bu açılardan, allopurinol ve oksipurinol, testis torsiyonunda klinik uygulamalar için potansiyel ajanlardır.

Anahtar sözcükler: Allopurinol; iskemi; oksipurinol; reperfüzyon; testis torsiyonu.

Ulus Travma Acil Cerrahi Derg 2026;32(3):229-237 DOI: 10.14744/tjtes.2025.50636