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Protective effects of Silymarin on testicular torsion/detorsion in rats

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Abstract. – OBJECTIVE: The present research aimed to study the possible protective effects of Silymarin on testicular I/R injury in a rat model evaluated through histopathology and biochemical parameters.

MATERIALS AND METHODS: This research investigated the impact of Silymarin on IR damage in male Wistar albino rats. Animals were divided into three groups: group 1 (sham), group 2 (IR), and group 3 (IR+Silymarin).

RESULTS: There were no notable differences in the levels of malondialdehyde (MDA), myeloperoxidase (MPO), and glutathione (GSH) across the three groups (p=0.260, p=0.486 and p=0.803, respectively). Contrarily, the total antioxidant status (TAS) levels exhibited significant variations between groups (p=0.001). The total oxidant status (TOS) levels also differed significantly between groups (p=0.004). The tissue evaluations uncovered substantial differences in the Johnson score, which is used to gauge testicular damage. A distinct contrast was seen between Group 1 and Group 2, and also between Group 2 and Group 3, with an all-encompassing p-value lower than 0.01. The same significant disparities were found for the percentages of Bax and Annexin V immunostaining (p<0.01 for each), reflecting the inflammation and apoptosis brought about by ischemia-reperfusion and the protective effects of the treatment.

CONCLUSIONS: The outcomes of the current investigation showed that Silymarin could be a valuable agent for reducing testicular tissue damage following I/R injury.

Key Words:
Silymarin, Ischemia-reperfusion, Testicular torsion, Testicular damage, Spermatogenesis.

Introduction

Testicular torsion represents a critical urological emergency, characterized by the testicle rotating around the spermatic cord's axis[1]. This condition often results in testicular harm, predominantly occurring in newborns, children, and young adults, affecting one in every 4,000 individuals within these age groups annually[2]. The severity of long-term outcomes, such as infertility, is largely determined by the extent and length of time the torsion persists[3]. The injury to the testicle extends beyond the initial damage caused by ischemia, continuing due to ischemic reperfusion (I/R) injury[4]. The main harmful change during testicular I/R is caused by oxidative stress, which results from excess production and accumulation of reactive oxygen species (ROS) that are not effectively cleared out[5]. This causes damage to the cell membrane through lipid peroxidation. Apart from affecting the membrane lipids, these unchecked free radicals also damage other cell parts like carbohydrates, DNA, and proteins, triggering detrimental biochemical responses[6]. Additionally, past research[7] has shed light on the fact that antioxidant substances possessing the ability to neutralize free radicals may play a beneficial role in thwarting I/R-induced damage in various organs, particularly in the testes.

Silymarin is a polyphenolic compound derived from milk thistle (*Silybum marianum*). The primary constituent of Silymarin is silybin, which is largely responsible for its phytochemical benefits. Numerous studies[8] have highlighted Silymarin's abilities, showcasing its antioxidant, anti-inflammatory, anti-

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neoplastic, antifibrotic, and immunomodulatory attributes. Acting as an antioxidant, silymarin counters ROS, neutralizes free radicals, and bolsters the body's natural antioxidant defenses by enhancing vital antioxidant enzymes like glutathione and superoxide dismutase[9]. The combined antioxidant and anti-diabetic properties of both metformin and silymarin have been explored in past research[10].

The present research aimed to study the possible protective effects of Silymarin on testicular I/R injury in a rat model evaluated through histopathology and biochemical parameters.

Materials and Methods

This study explored the effects of Silymarin on ischemia-reperfusion injury in male Wistar albino rats. In total, 21 rats, each 12 weeks old and with an average weight of 235 g, were utilized in accordance with the institution's guidelines and the National Research Council's standards for the Care and Use of Laboratory Animals. Ethical approval for the investigation was provided by the Dicle University Animal Studies Ethical Committee (approval number: 2023/08, date: 30.03.2023).

The rats were housed under controlled conditions with a temperature ranging from 20-23°C and a 12-hour light/dark cycle. They were fed standard pellets and had unrestricted access to water. The animals were randomly divided into three distinct groups: Group 1 (sham), Group 2 (IR), and Group 3 (IR+Silymarin). In Group 1, all surgical interventions except for testicular torsion-detorsion were conducted to establish baseline values for all measured parameters. The testicle was removed through a scrotal incision and then repositioned in its typical location within the scrotum, with no torsion applied. Group 2 underwent testicular torsion and detorsion without medication, while Group 3 received a single dose of 300 mg/kg of Silymarin [Catalog No.: S0292-106, Company Name: Sigma Aldrich] intraperitoneally just before the ischemia.

In Groups 2 and 3, ischemia-reperfusion injury was intentionally inflicted on the rat testes through a specific technique. This involved extracting the testis *via* an inguinoscrotal incision, twisting it 720 degrees clockwise, and then affixing it to the scrotum for two hours using a 5.0 prolene suture. The objective was to create a controlled model of testicular torsion to assess the effects of ischemia and the subsequent reperfusion. After ischemia, the testis was untwisted and left in its normal position for four hours to evaluate reperfusion damage. Ultimately, orchiectomy was carried out to obtain testicular tissue for histological analysis, and blood was collected through cardiac puncture for biochemical evaluation.

All surgical interventions were performed under appropriate anesthesia and sterile conditions. The rats were anesthetized with a combination of two drugs, xylazine hydrochloride (Rompun 2%, Bayer, Turkey) and ketamine hydrochloride (Alfamine 10%, Ege Vet, Istanbul, Turkey), at dosages of 10 mg/kg and 50 mg/kg respectively, administered intraperitoneally. Xylazine hydrochloride functioned as a calming agent and muscle relaxant, while ketamine hydrochloride provided dissociative anesthetic effects.

Biochemical Evaluation

After obtaining blood samples through cardiac puncture, they were promptly transported to the biochemistry laboratory on ice. The samples were then spun at 4,000 revolutions per minute for 5 minutes, resulting in the isolation of the serum. Tests were conducted to measure the levels of TAS, TOS, MDA, GSH, and MPO. Utilizing an Abbott Architect C16000 auto analyzer, (Siemens Diagnostics, Tarrytown, NY, USA), the TAS and TOS concentrations were determined using commercially available kits provided by Rel Assay Diagnostics from Gaziantep, Turkey, and *via* automated colorimetric techniques as formulated by Erel[11,12]. The TAS results are expressed in units of micromolar trolox equivalent per liter, and the TOS results are reported in micromolar hydrogen peroxide equivalent per liter. The MDA content was gauged using a spectrophotometric procedure that relies on the color alteration resulting from the reaction between thiobarbituric acid and MDA, as previously outlined[13]. In a similar manner, MPO activity was assessed spectrophotometrically, as previously cited[14]. To evaluate the activity of glutathione peroxidase (GSH-Px), the methodology recommended by Paglia and Valentine[15] was employed. This technique examines the enzyme's ability to facilitate the transformation of reduced glutathione (GSH) into its oxidized form (GSSG) in the presence of hydrogen peroxide.

Immunohistochemical Examination

Testicular tissues were extracted for histological examination, and dissected samples were further scrutinized for histological assessment. The samples were submerged in zinc-formalin, and then dehydrated through a series of graded alcohols before being embedded in paraffin wax. Sections of

5 µm thickness were cut from the paraffin blocks and stained with hematoxylin-eosin dye along with immune staining. The testicular sections were dewaxed, rehydrated through graded alcohol, and rinsed in distilled water. To inhibit endogenous peroxidase activity, 3% hydrogen peroxide (H_2O_2) was applied to the slides. After washing in PBS, sections were incubated with an anti-blood brain barrier (catalog No.: 836804, Biolegend, San Diego, CA, USA). Annexin V (catalog No.: 0902012, Boster Biology Tech., 1/100) and Bax (catalog No.: 15970 and 17069, Biorbyt, 1/100) overnight at +4°C. Sections were biotinylated and allowed to react with streptavidin peroxidase solution (Thermo Fisher, Waltham, MA, USA) for 15 minutes. After another wash with PBS, diaminobenzidine (DAB) chromogen was employed to detect color changes. The reactions were halted with PBS, and the sections were counterstained with hematoxylin dye. The slides were then mounted and visualized using a Zeiss Imager A2 light microscope (Appletonwoods, Birmingham B38 8SE, UK). All images were analyzed and quantified through ImageJ software. The intensity of protein expression staining was assessed using Image J software (version 1.53, http://imagej.nih.gov/ij), with measurements based on the method outlined by Crowe and Yue[16]. Spermatogenesis was histologically rated for each sample across ten fields according to the method established by Johnsen[17]. A Johnsen score of 10 signifies optimal spermatogenesis activity, while a score of 1 denotes a total absence of germ cells. The histological evaluations were conducted by two independent, blinded experts (Table I).

Statistical Analysis

The data were statistically analyzed using IBM SPSS 25.0 software (IBM Corp., Armonk, NY, USA). To determine the distribution of the data, the Shapiro-Wilk and Kolmogorov-Smirnov tests were applied. In cases where the data followed a normal distribution, the results were expressed as the mean and standard deviation, and the ANOVA test was utilized. If the distribution was not normal, the data were reported as the median along with the interquartile range (IQR). For comparisons across multiple groups, the non-parametric Kruskal-Wallis test was employed, and due to the limited sample size within the groups, the post-hoc Dunn test was conducted. A p-value lower than 0.05 was considered indicative of statistical significance.

Results

The levels of MDA were consistent among all groups, with average values of 1.36±0.55, 1.26±0.15, and 1.06±0.25 in groups 1, 2, and 3, respectively (p=0.260). Similarly, there were no notable differences in the levels of MPO and GSH across the three groups (p=0.486 and p=0.803, respectively). Contrarily, the TAS levels exhibited significant variations between groups 2 and 3 (p =0.002), and groups 1 and 3 (p=0.006), with an overall p-value of 0.001. The TOS levels also differed significantly between groups 2 and 3 (p=0.009), as well as groups 1 and 2 (p=0.007), displaying an overall p-value of 0.004. The tissue evaluations uncovered substantial differences in the Johnson score, which is used to gauge testicular damage. A distinct contrast was seen between Group 1 and Group 2, and also between Group 2 and Group 3, with an all-encompassing p-value lower than 0.01. The same significant disparities were found for the percentages of Bax and Annexin V immunostaining (p<0.01 for each), reflecting the inflammation and apoptosis brought

Table I. Johnsen scoring for spermatogenesis.

Johnsen biopsy parameters	Score
Regular, dense spermatogenesis and tubule structure	10
Dense spermatozoa in the lumen but irregularity in the spermatogenic line	9
The small number of spermatozoa present in the lumen	8
No spermatozoa in the lumen but spermatids are present	7
The low number of spermatids	6
No spermatozoa and spermatids but dense spermatocytes	5
Low number of spermatocytes	4
Only Spermatogonia available	3
There are no germ cells	2
No germ cells or Sertoli cells	1

Table II. Biochemical and immunohistopathological parameters of all groups.

	Group 1	Group 2	Group 3	*p*-value	Meaningful comparisons (intergroup)
Blood					
MDA	1.36±0.55	1.26±0.15	1.06±0.25	0.260	
MPO	12.01±2.83	11.83±0.76	10.47±2.70	0.486	
GSH	183.44±66.94	166.45±43.82	167.78±40.69	0.803	
TAS	0.65±0.08	0.62±0.12	0.99±0.27	0.001	2 and 3 (p=0.002); 1 and 3 (p=0.006)
TOS	14.20±8.49	136.58±104.19	22.42±18.26	0.004	2 and 3 (p=0.009); 1 and 2 (p=0.007)
Tissue					
Johnson score	9 (9-10)	3 (1.25-3)	7 (6-8)	<0.01	1 and 2; 2 and 3
*Bax immunostaining**	12.73%	39.22%	21.01%	<0.01	1 and 2; 2 and 3
*Annexin V immunostaining**	13.88%	42.50%	32.90%	<0.01	1 and 2; 2 and 3

*Percentage. Malondialdehyde (MDA), Myeloperoxidase (MPO), Glutathione (GSH), Total Antioxidant Status (TAS), Total Oxidant Status (TOS).

about by ischemia-reperfusion and the protective effects of the treatment (Table II).

Figure 1 illustrates the hematoxylin and eosin staining of testicular tissues. In the samples from the sham group, the structure of the testicular tissue appeared normal, with the membranes of the seminiferous tubules and the spermatogenesis line presenting a natural appearance. The Leydig cells were found within the interstitial space. In contrast, the IR group exhibited degeneration and apoptosis in the spermatogenic cells, a thickening of the seminiferous tubule membranes, and the emergence of fibrosis in the interstitial area. Additionally, pycnotic changes were noted in the Leydig cell nuclei. On the other hand, the group receiving IR+Silymarin treatment showed signs of recovery in the histopathological damage caused by IR. This was marked by the preservation of seminiferous tubule integrity, the regular arrangement of cells in the spermatogenesis line,

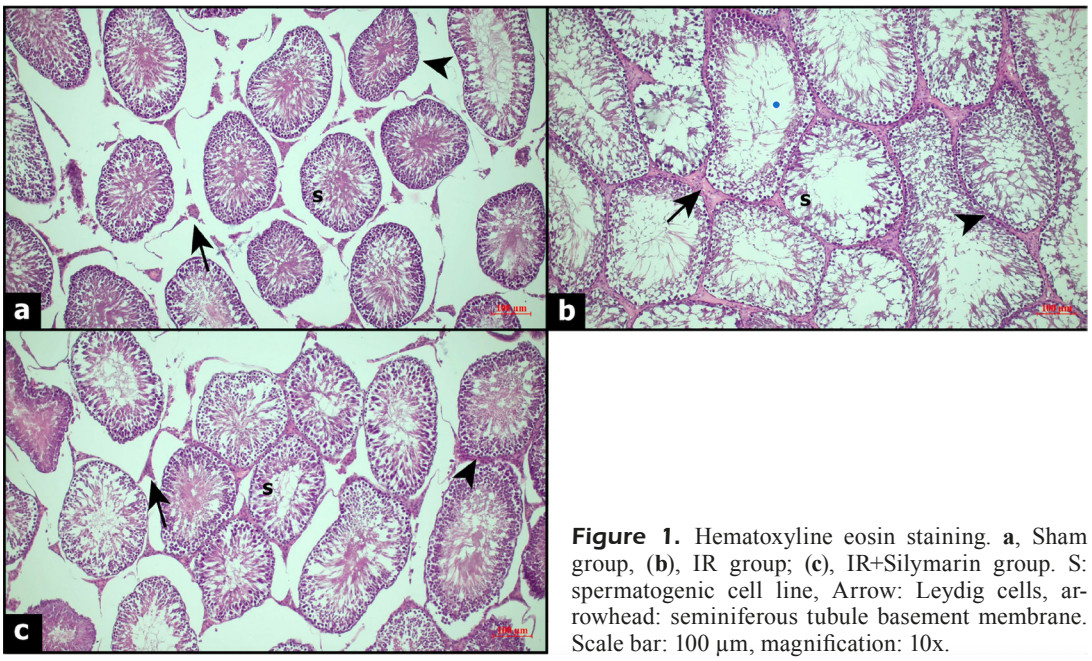

Figure 1. Hematoxyline eosin staining. **a**, Sham group, (**b**), IR group; (**c**), IR+Silymarin group. S: spermatogenic cell line, Arrow: Leydig cells, arrowhead: seminiferous tubule basement membrane. Scale bar: 100 µm, magnification: 10x.

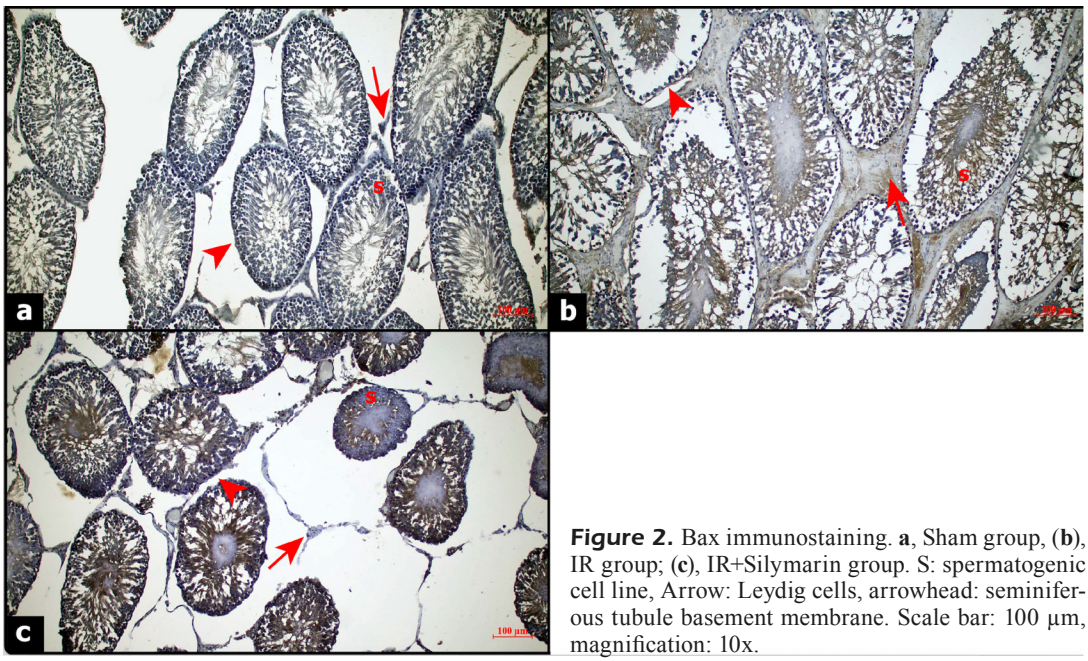

Figure 2. Bax immunostaining. **a**, Sham group, (**b**), IR group; (**c**), IR+Silymarin group. S: spermatogenic cell line, Arrow: Leydig cells, arrowhead: seminiferous tubule basement membrane. Scale bar: 100 µm, magnification: 10x.

and a near-normal appearance of interstitial blood vessels and Leydig cells after the IR injury.

Figure 2 displays the immunoreactivity of Bax in testicular tissues. In the sham group, there was a lack of Bax expression in the epithelium of seminiferous tubules, spermatogenic cells, and the interstitial space. However, IR damage triggered the apoptotic pathway, leading to a noticeable increase in Bax expression. This immune response to Bax was intensely present in spermatogenic cells, the interstitial area, and the seminiferous tubule membranes. The use of Silymarin, due to its antiapoptotic properties, led to a reduction in proapoptotic Bax expression, thereby promoting cell survival. Consequently, a decline in Bax immune reactivity was observed in the seminiferous tubules, spermatogenic cells, and Leydig cells.

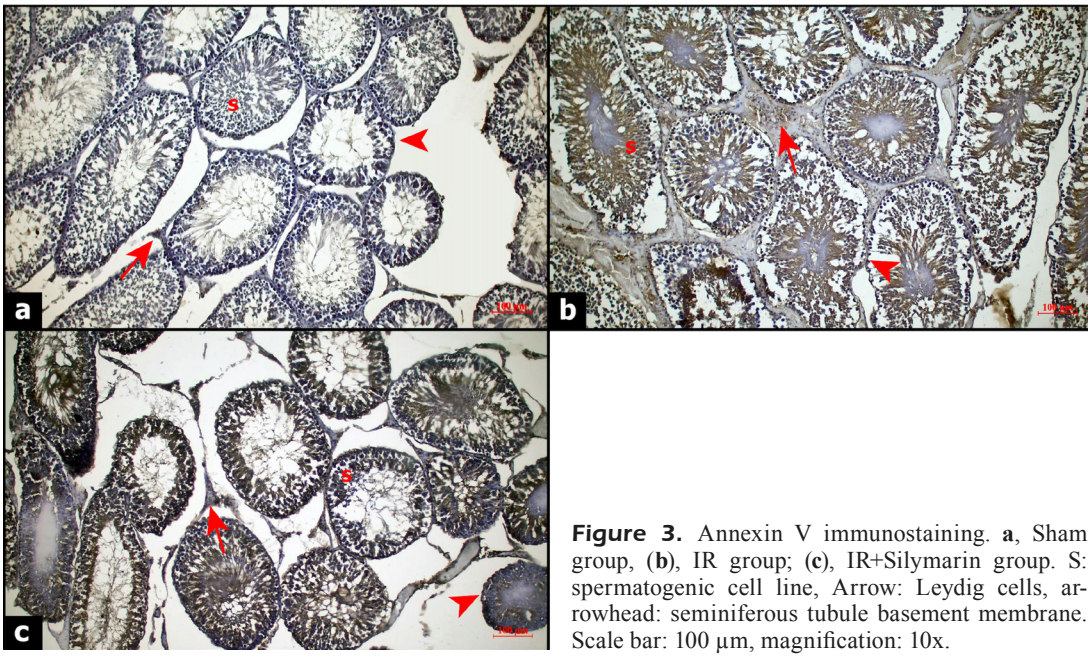

Figure 3. Annexin V immunostaining. **a**, Sham group, (**b**), IR group; (**c**), IR+Silymarin group. S: spermatogenic cell line, Arrow: Leydig cells, arrowhead: seminiferous tubule basement membrane. Scale bar: 100 µm, magnification: 10x.

Figure 3 shows the immunoreactivity of Annexin V in testicular tissues. In the sham group, there was mostly a lack of Annexin V immune response, with negative reactions detected in the epithelium of seminiferous tubules, spermatogenic cell lines, and Leydig cells. However, in the IR group, intense expression of Annexin V was seen in spermatogenic cells, the interstitial area, and the seminiferous tubule membranes, as a result of the apoptotic pathway being activated by IR. Thanks to Silymarin's antiapoptotic property, there was a noticeable reduction in Annexin V immune reaction, a marker typically associated with apoptotic cells. This led to a decrease in Annexin V expression in the seminiferous tubules, spermatogenic cells, and Leydig cells.

Discussion

Testicular torsion is a critical urological situation in which the blood supply to the organ is significantly cut off, often leading to its degeneration and shrinkage. The sole effective remedy after such an event is immediate surgical intervention[2]. Yet, even when surgery is conducted promptly, around 25% of cases can still experience testicular damage due to ischemia-reperfusion (I/R) injury. This I/R injury in the testicle is associated with an increase in the production of reactive oxygen species (ROS), resulting in oxidative stress that surpasses the cell's natural defenses to combat it[2,3]. Essentially, the oxidative stress instigated by I/R injury creates an imbalance in the restoration of cellular oxygen delivery and mitochondrial respiration. Fortunately, the harshness of the oxidative stress and tissue injuries caused by torsion and detorsion can be mitigated through the application of exogenous antioxidants. In this context, various substances, including diclofenac, hesperidin, omeprazole, cordycepin, roflumilast, ibuprofen, arbutin, thymoquinone, and syringic acid, have demonstrated[4,18-23] effectiveness in safeguarding the testes from ischemia-reperfusion-induced harm.

The research conducted by Okur et al[4] discovered that in rats exposed to I/R, cordycepin had a notable effect on testicular tissue, specifically lowering tumor necrosis factor-alpha (TNF-α) and MDA levels, boosting TAS, and cutting down TOS when compared to the I/R group that did not receive cordycepin. This points to a possible defense mechanism against damage caused by I/R to the testicles. In a study by Gökçe et al[22], the testicular IR group was found to have increased levels of TOS, OSI, and MDA compared to the control group. However, treatment with Thymoquinone (TQ) managed to diminish these levels of MDA, TOS, and OSI, although it did not alter TAC and MPO activity. Furthermore, Ganjiani et al[23] focused their investigation on the way crocin might safeguard against testicular torsion/detorsion in rats. Their findings revealed that crocin had a substantial role in lessening injuries, indicating potential therapeutic applications for this particular medical condition. However, our study is unique in that it is the first to explore the shielding effects of Silymarin on testicular ischemia-reperfusion by assessing Johnsen's scoring for spermatogenesis.

Silymarin, a flavonoid derived from *Silybum marianum* seeds, possesses various therapeutic attributes, including anti-inflammatory and anti-cancer effects. Its powerful antioxidant capabilities allow it to regulate intracellular glutathione and maintain the stability of the cell membrane[8,9]. These antioxidant qualities stem from its polyphenolic structure, along with a methoxy group located on one of its phenolic rings[9,24]. As an antioxidant substance, Silymarin acts to neutralize free radicals and interacts directly with elements of the cell membrane[8,9,24,25]. This action helps to prevent oxidative harm to the lipid constituents of the cell membrane, which are essential for preserving its inherent fluidity. Belhan et al[9] conducted a study on the impact of Silymarin on oxidative DNA damage and inflammatory markers in rats experiencing testicular torsion/detorsion. The research demonstrated that Silymarin effectively reduced ischemia/reperfusion injury in testicular tissues, highlighting its potential as a therapeutic agent in protecting against oxidative stress and inflammation in such medical emergencies. Faraji et al[26] determined that Silymarin, recognized for its strong antioxidant properties, has the ability to offset the negative impacts of cadmium chloride on the testis histopathology, testosterone levels, indicators of oxidative stress, and enzymes responsible for antioxidant defense in mice. Our research found no significant differences in MDA, MPO, and GSH levels among the three groups. However, TAS and TOS levels varied, indicating oxidative stress variations, especially between specific groups. The Johnson score, assessing testicular damage, also differed significantly. The protective effects of Silymarin treatment were shown in inflammation, apoptosis, and histopathology analyses, and further confirmed by immunostaining for Bax and Annexin V, demonstrating reduced inflammation and apoptosis after Silymarin administration.

Limitations

Notwithstanding the significant insights gained, the research presented several limitations. Firstly, the sample size was notably restricted. Secondly, the study exclusively evaluated a solitary Silymarin dosage. Thirdly, it lacked longitudinal outcome metrics. Fourthly, it is imperative to acknowledge potential variations in individual responses to treatment. Fifthly, being rooted in a rat model raises questions about direct applicability to human scenarios. Sixthly, there was an absence of a comparative analysis with other protective agents.

Conclusions

Silymarin, a flavonoid derived from *Silybum marianum* seeds, is utilized broadly due to its various remedial properties. The outcomes of the current investigation showed that Silymarin could be a valuable agent for reducing testicular tissue damage following I/R injury.

Ethics Approval

The Dicle University Animal Studies Ethical Committee granted ethical clearance for the research (approval number: 2023/08, date: 30.03.2023) approved the studies protocol.

Availability of Data and Materials

All data generated or analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author.

Conflict of Interest

The authors declare that they have no conflict of interest.

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Authors' Contributions

Conceptualization, MA, SA, EGÖ, FA, EB, and MHO.; methodology, MA, MHO, BA, MAK, and İK; software, MA, SA, EGÖ, FA, EB, BA; validation, MA, SA, EGÖ, FA, EB, BA, and İK; formal analysis, MA, SA, EGÖ, FA, EB, and BA; investigation, ma, SA, EGÖ, FA, EB, MHO, BA, MAK, and İK.; resources, MA, EGÖ, FA, and İK; data curation, SA, EGÖ, FA, EB, MHO, BA, MAK, and İK.; writing–original draft preparation, MA, SA, EB, BA, and MHO; writing–review and editing, MA, SA; visualization EGÖ, FA, EB, and MHO.; supervision, MA, SA, EGÖ, FA, İK and MHO; project administration, SA; funding acquisition, SA, EGÖ, FA. All authors have read and agreed to the published version of the manuscript.

References

1) Tehrani HG, Heidari N, Mardanian F, Mehrabian F, Naghshineh E, Motamedi N. Comparing the effect of three trigger methods (human HCG, combination of recombinant HCG with GnRH agonist, and double recombinant HCG) on the fertility outcome of patients with poor ovarian response in ovulation stimulation cycle (IVF/ICSI). Acad J Health Sci 2023; 38: 101-106.
2) Arslan S, Azizoğlu A, Kamci TO, Basuguy E, Aydogdu B, Okur MH, Karabel M, Onen A. The value of hematological inflammatory parameters in the differential diagnosis of testicular torsion and epididymo-orchitis in children. Exp Biomed Res 2023; 6: 212-217.
3) Li ZM. Role of antioxidants in preventing testicular ischemia reperfusion injury: a narrative review. Eur Rev Med Pharmacol Sci 2022; 26: 9126-9143.
4) Okur MH, Arslan S, Aydogdu B, Zeytun H, Basuguy E, Arslan MS, Ibiloglu I, Kaplan I. Protective Effect of Cordycepin on Experimental Testicular Ischemia/Reperfusion Injury in Rats. J Invest Surg 2018; 31: 1-8.
5) Ghasemnejad-Berenji M, Ghazi-Khansari M, Pashapour S, Jafari A, Yazdani I, Ghasemnejad-Berenji H, Saeedi Saravi SS, Sadeghpour S, Nobakht M, Abdollahi A, Mohajer Ansari J, Dehpour AR. Synergistic effect of rapamycin and metformin against germ cell apoptosis and oxidative stress after testicular torsion/detorsion-induced ischemia/reperfusion in rats. Biomed Pharmacother 2018; 105: 645-651.
6) McMichael M, Moore RM. Ischemia-reperfusion injury pathophysiology, part I. J Vet Emerg Crit Care 2004; 14: 231-241.
7) Ganjiani V, Ahmadi N, Divar MR, Sharifiyazdi H, Meimandi-Parizi A. Protective effects of crocin on testicular torsion/detorsion in rats. Theriogenology 2021; 173: 241-248.
8) Taghiabadi E, Imenshahidi M, Abnous K, Mosafa F, Sankian M, Memar B, Karimi G. Protective Effect of Silymarin against Acrolein-Induced Cardiotoxicity in Mice. Evid Based Complement Alternat Med 2012; 2012: 352091.

9) Belhan S, Yıldırım S, Kayıkcı C, Kömüroğlu A, Özdek U, Kuşcu Y. Investigation of the effect of silymarin on oxidative DNA damage and inflammatory markers in ischemia/reperfusion injury following experimental testicular torsion/detorsion in rats. Turk J Zool 2021; 45: 267-276.
10) Darbandi M, Darbandi S, Agarwal A, Sengupta P, Durairajanayagam D, Henkel R, Sadeghi MR. Reactive oxygen species and male reproductive hormones. Reprod Biol Endocrinol 2018; 16: 87.
11) Erel O. A novel automated method to measure total antioxidant response against potent free radical reactions. Clin Biochem 2004; 37: 112-119.
12) Erel O. A new automated colorimetric method for measuring total oxidant status. Clin Biochem 2005; 38: 1103-1111.
13) Esterbauer H, Cheeseman KH. Determination of aldehydic lipid peroxidation products: Malonaldehyde and 4- hidroxynonenal. Meth Enzymol 1990; 186: 407-421.
14) Bradley PP, Priebat DA, Christensen RD, Rothstein G. Measurement of cutaneous inflammation: estimation of neutrophil content with an enzyme marker. J Invest Dermatol 1982; 78: 206-209.
15) Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med 1967; 70: 158-169.
16) Crowe AR, Yue W. Semi-quantitative Determination of Protein Expression using Immunohistochemistry Staining and Analysis: An Integrated Protocol. Bio Protoc 2019; 9: e3465.
17) Johnsen SG. Testicular biopsy score count--a method for registration of spermatogenesis in human testes: normal values and results in 335 hypogonadal males. Hormones 1970; 1: 2-25.
18) Sarikaya K, Kölükçü E, Unsal V, Özdemir S. Protective Effects of Syringic Acid on Ischemia-Reperfusion Injury in Testicular Torsion: An Experimental Study in a Rat Model Cureus 2023; 15: e42390.
19) Özgür BC, Surer H, Yücetürk CN, Karakan T, Özer E, Ogus E. The protective effect of roflumilast and ibuprofen on testicular ischemia-reperfusion injury: An experimental study. Ulus Travma Acil Cerrahi Derg 2022; 28: 730-735.
20) Demir EA, Demir S, Kazaz IO, Kucuk H, Alemdar NT, Buyuk A, Mentese A, Aliyazicioglu Y. Arbutin abrogates testicular ischemia/reperfusion injury in rats through repression of inflammation and ER stress. Tissue Cell 2023; 82: 102056.
21) Khezri MR, Ghasemnejad-Berenji H, Ghasemnejad-Berenji M. Protective effects of empagliflozin on testicular injury induced by torsion/detorsion in adult male rats. J Pediatr Urol 2023; 19: 545.e1-545.e7.
22) Gökçe A, Oktar S, Koc A, Gonenci R, Yalcinkaya F, Yonden Z, Duru M. Protective effect of thymoquinone in experimental testicular torsion. Urol Int 2010; 85: 461-465.
23) Ganjiani V, Ahmadi N, Divar MR, Sharifiyazdi H, Meimandi-Parizi A. Protective effects of crocin on testicular torsion/detorsion in rats. Theriogenology 2021; 173: 241-248.
24) Yardımcı M, Göz M, Aydın MS, Kankılıç N, Temiz E. Antioxidant Actions of Thymoquinone, Silymarin, and Curcumin on Experimental Aortic Ischemia-Reperfusion Model in Wistar Albino Rats. Braz J Cardiovasc Surg 2022; 37: 807-813.
25) Pasala PK, Uppara RK, Rudrapal M, Zothantluanga JH, Umar AK. Silybin phytosome attenuates cerebral ischemia-reperfusion injury in rats by suppressing oxidative stress and reducing inflammatory response: In vivo and in silico approaches. J Biochem Mol Toxicol 2022; 36: e23073.
26) Faraji T, Momeni HR, Malmir M. Protective effects of silymarin on testis histopathology, oxidative stress indicators, antioxidant defence enzymes and serum testosterone in cadmium-treated mice. Andrologia 2019; 51: e13242.