Check for updates

Journal of Biochemical and Molecular Toxicology

WILEY

RESEARCH ARTICLE OPEN ACCESS

Ameliorative Effects of Silymarin on Ovarian Ischemia/Reperfusion Injury via Modulation of Oxidative Stress and Inflammatory Pathways

Saime Ozbek Sebin[1] | Basak Gulakar[1] | Ayhan Tanyeli[1] | Mustafa Can Guler[1] | Elif Erbas[2] | Yasin Bayır[3] | Emir Enis Yurdgulu[3] | Selim Comakli[4] | Özlem Erdemci[1]

[1]Department of Physiology, Faculty of Medicine, Ataturk University, Erzurum, Turkey | [2]Department of Histology and Embryology, Faculty of Veterinary Medicine, Ataturk University, Erzurum, Turkey | [3]Department of Biochemistry, Faculty of Pharmacy, Ataturk University, Erzurum, Turkey | [4]Department of Veterinary Pathology, Faculty of Veterinary Medicine, Ataturk University, Erzurum, Turkey

Correspondence: Saime Ozbek Sebin (saime.ozbek@atauni.edu.tr)

Received: 6 May 2026 | **Revised:** 1 June 2026 | **Accepted:** 13 July 2026

Keywords: inflammation | ischemia/reperfusion injury | Ovarian torsion | oxidative stress

ABSTRACT

Ischemia/reperfusion (IR) injury secondary to ovarian torsion results in oxidative stress, inflammation, and structural damage to the ovary. Silymarin (SLY), a natural flavonoid complex, possesses potent antioxidant and anti-inflammatory effects. The present study was designed to evaluate the protective effects of SLY on ovarian IR injury. An experimental model of OIR was created, and animals were divided into Sham, OIR, and OIR + SLY groups. An acute single dose of silymarin (100 mg/kg) was given to the treatment group. Tissue and serum levels of TNF-α, IL-17, TGF-β1, and H_2O_2 were determined, as well as TOS. Tissue oxidative stress parameters MDA, GSH content, and SOD activity were assessed. Histopathological evaluation of ovarian tissues was done. Compared to sham controls, the OIR group exhibited significantly higher tissue and serum inflammatory cytokine levels, and oxidative stress indicators, as well as higher MDA levels and lower GSH and SOD activity ($p < 0.05$). Histological analysis revealed significant vascular congestion, bleeding, and tissue deterioration. SLY therapy significantly decreased inflammatory cytokines and lipid peroxidation while increasing antioxidant defenses ($p < 0.05$). Histopathological results also showed improved ovarian tissue integrity in the treatment group. SLY may be used as an adjuvant treatment for ovarian torsion, as it reduces oxidative stress and inflammation in OIR injury of the ovary while preserving its histological structure.

1 | Introduction

Ovarian torsion (OT) constitutes a gynecological emergency capable of impacting women across all age groups, arising when the adnexa twists around its supporting ligaments [1, 2]. It is a significant risk factor for ovarian dysfunction [3]. Torsion disrupts blood flow to the ovary, reducing tissue perfusion, thereby initiating a cascade of ischemic cellular injury. Ischemia leads to depletion of tissue energy reserves, stimulation of proteolytic enzymes, and calcium influx into the ischemic cell. Decreased mitochondrial ATP generation and alterations in the selective permeability of the cell membrane disrupt ionic balance. Inflammatory processes in the tissue begin upon reperfusion. This impairs microcirculation, leading to apoptosis and necrosis [4]. In addition, OT can lead to infertility, bleeding, intra-abdominal inflammation, and inflammatory changes in the vascular system [3, 5]. Currently, the gold standard treatment methods are two surgical procedures: laparoscopy and laparotomy [6, 7]. Surgical intervention detorses the torsioned ovary, restoring blood flow to the tissue. However, the damage resulting from reperfusion exceeds the extent of ischemic damage. Reperfusion increases oxygen levels, produces free radicals, and

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

© 2026 The Author(s). *Journal of Biochemical and Molecular Toxicology* published by Wiley Periodicals LLC.

causes inflammation [7, 8]. The balance between oxidants and antioxidants is disrupted by increased reactive oxygen species, leading to oxidative stress (OS). OS contributes to cellular damage by causing lipid peroxidation, protein oxidation, and DNA damage [9, 10]. Another consequence of increased ROS is the activation and adhesion of neutrophils [11, 12]. With reperfusion, proinflammatory cytokine levels increase, leading to the onset of an inflammatory process in the tissue [3].

Silymarin (SLY), commonly known as milk thistle, is a natural flavonoid mixture derived from Silybum marianum and belonging to the Asteraceae family. Silybin is the main active ingredient of SLY, comprising ~60%–70% of the flavonolignan mixture [13]. Other components found in the mixture are isosilibinin, silydianin, silychristin, isosilychristin, and taxifolin [14]. It possesses antioxidant and chemoprotective properties for the liver and is prescribed as a hepatoprotective agent [15]. It has been reported that SLY acts as a ROS scavenger, provides an antioxidant effect, and also has anti-inflammatory and antifibrotic effects [16, 17]. Additionally, SLY has been reported to have immunomodulatory, anti-apoptotic, antitumor [18], and neuroprotective [19] effects.

Appropriate therapeutic approaches should be developed to mitigate the enhanced reactive oxygen species (ROS) effects and inflammatory processes associated with ovarian ischemia/reperfusion (OIR) injury. This study investigated the protective effects of SLY characterized by its antioxidant, anti-inflammatory, and anti-apoptotic properties, against OIR injury.

2 | Materials and Methods

2.1 | Animals

Rats were maintained on a 12 h light/12 h dark cycle consistent with their physiological rhythm. Rats were fed standard pellet food and had free access to tap water until the day of the experiment. The study was divided into three groups, with 10 animals per group. A total of 30 Wistar albino female rats, 12–16 weeks old and weighing 200–230 g, were used. The Atatürk University Local Animal Experiments Ethics Committee granted permission for this study (2023/10-160).

2.2 | Experimental Design

Before surgical procedures, ketamine (100 mg/kg) and xylazine (10 mg/kg) anesthesia were applied to the rats intraperitoneally.

Group 1 (Sham): The peritoneum was accessed via a 1–2 cm lower abdominal incision in the experimental subjects and closed without further intervention.

Group 2 (OIR): The peritoneum was accessed by making a 1-2 cm incision in the lower abdomen of the experimental animals. Clamps were applied to the bilateral ovarian arteries, maintaining the ovarian tissue in an ischemic state for 3 h. Following ischemia, the clamps were removed, and a 3 h reperfusion period was initiated. The incision line was closed with 3-0 silk suture. After reperfusion, the rats were sacrificed by drawing blood from their hearts. Ovarian tissues were collected.

Group III (OIR + 100 mg/kg SLY): Surgical procedures in Group II were performed. 100 mg/kg SLY was administered intraperitoneally 30 min before the start of reperfusion.

After reperfusion, rats were sacrificed by collecting blood from their hearts. The collected blood was centrifuged, and the resulting serum was stored in a deep freezer at −80°C. Tissues from the sacrificed rats were then placed in a 10% formaldehyde solution for histopathological processing and stored at −80°C until biochemical analyses.

2.3 | Biochemical Analysis and Evaluation

2.3.1 | ELISA Analyses in Tissue Samples (Determination of TNF-α, IL-17, TGF-β1, H_2O_2, and TOS Levels)

Ovarian tissues, homogenized with liquid nitrogen and stored in a −80°C refrigerator until the day of the experiment, were removed and left to thaw in a 4°C refrigerator. Homogenized tissues were prepared at a ratio of 1:9 (mg tissue/mL PBS) as indicated in the kits. Each sample, prepared with 100 mg of tissue in 900 μL of PBS, was homogenized using a Tissue Lyser II device. Following homogenization, centrifugation was performed as indicated in the kits, and the supernatants were separated. All ELISA kits conforming to the manufacturer's protocols were used. At the end of the experiment, a microplate reader (Biotek Epoch) was used at the appropriate wavelengths specified in the kit protocol for measurements. Total protein analysis was performed for each sample using the Lowry method [20], and the obtained data were normalized according to the total protein amount. As a result, all concentrations are given as mean ± standard deviation (SD) per mg of protein. The ELISA and biochemical analysis kits used in this study were obtained from Sunred Biotechnology Company, Elabscience, and Rel Assay Diagnostic. Accordingly, TNF-α (Rat TNF-α, Cat. No: 201-11-0765) was obtained from Sunred Biotechnology Company; IL-17 (Rat IL-17, Cat. No: E-EL-R0566), TGF-β1 (Cat. No: E-EL-R0084), and the Hydrogen Peroxide (H_2O_2) Colorimetric Assay Kit (Cat. No: E-BC-K102-S) were obtained from Elabscience; and the Total Oxidant Status (TOS) kits were obtained from Rel Assay Diagnostic.

2.3.2 | ELISA Analysis in Serum Samples (TNF-α, IL-17, TGF-β1, H_2O_2, TOS)

Blood samples were centrifuged to separate the serum. All ELISA kits were performed according to the protocols recommended by the manufacturers. At the end of the experiment, measurements were taken using a microplate reader (Biotek Epoch) at the wavelengths specified in the kit protocol. All concentrations are given as mean ± standard deviation (SD) per mL of serum. ELISA kits were obtained from Sunred Biotechnology Company and Elabscience. Accordingly, TNF-α (Rat TNF-α, Cat No: 201-11-0765) was obtained from Sunred Biotechnology Company; IL-17 (Rat IL-17/Cat No: E-EL-R0566), and TGF-β1 (Rat TGF-β1, Cat No: E-EL-R0084) were obtained from Elabscience.

2.3.3 | Oxidative Stress Parameters in Tissue (GSH, SOD, and MDA)

Ovarian tissues, homogenized with liquid nitrogen and stored in a −80°C refrigerator until the day of the experiment, were

10990461, 2026, 8, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/jbt.71030 by YASIN BAYIR - Ataturk University , Wiley Online Library on [20/07/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

removed and left to thaw in a 4°C refrigerator. Homogenized tissues were prepared at a ratio of 1:9 (mg tissue/mL PBS) as indicated in the kits. Each sample, prepared with 100 mg of tissue in 900 μL of PBS, was homogenized using a TissueLyser II. Following homogenization, centrifugation was performed as indicated in the kits, and the supernatants were separated. Superoxide dismutase (SOD) activity was assessed using the Sun et al. [21] method; glutathione (GSH) levels via the Sedlak et al. [22] method; malondialdehyde (MDA) levels via the Ohkawa et al. [23] method; and protein content via the Lowry et al. [20] method. SOD, GSH, and MDA in the supernatant of homogenized and centrifuged samples were measured triply at room temperature with a microplate reader (Biotek Epoch) in accordance with established literature methods. The results for GSH, SOD, and MDA analyses in tissue extracts are expressed as nmol/mg protein, U/mg protein, and nmol/mg protein, respectively. Results based on the standard curve are presented as mean ± standard deviation (SD) per mg of protein.

2.4 | Statistical Analysis

The design of statistical analysis and graphs was done using Graphpad Prism 10. The analysis was made by the Tukey test, which is a post hoc multiple comparison test of one-way ANOVA. 0.1234 (ns), 0.0332 (*), 0.0021 (**), 0.0002 (***), < 0.0001 (****).

2.5 | Histopathological Analysis and Evaluation

At the conclusion of the experiment, ovarian tissues from anesthetized rats were promptly placed in 10% neutral formaldehyde solution and replenished every 24 h. The tissues remained in this solution for 72 h to ensure fixation. Thereafter, the samples were processed through a graded ethanol series (70%, 80%, 96%, and 100%), followed by xylene, and embedded in paraffin blocks. For histopathological evaluation, 5 μm-thick sections were prepared with a microtome (Leica RM2125 RTS). The sections were stained using Crossman's Modified Mallory's Triple Staining method for histopathological assessment, and tissue damage was examined under a light microscope. Microscopic evaluation was conducted with a light microscope (Nikon Eclipse i50) connected to a computer and camera. Vascular congestion, hemorrhage, edema, and follicular degeneration were assessed histopathologically using ratings of 0 (normal), 1 (mild), 2 (moderate), and 3 (severe) [2, 24, 25]. Figures 1 and 2 show the results.

3 | Results

3.1 | Biochemical Findings

3.1.1 | *Tissue TNF-α, IL-17, TGF-β1, H_2O_2, and TOS Levels*

TNF-α, IL-17, and TGF-β1 tissue levels were considerably greater in the OIR group compared to the sham group ($p < 0.05$) (Figures 1, 2, and 3).

The tissue H_2O_2 and TOS levels of ovarian tissues in the OIR group were significantly higher ($p < 0.05$) (Figures 4 and 5).

The treatment group had significantly lower levels of TNF-α, IL-17, TGF-β1, H_2O_2, and TOS than the OIR group ($p < 0.05$).

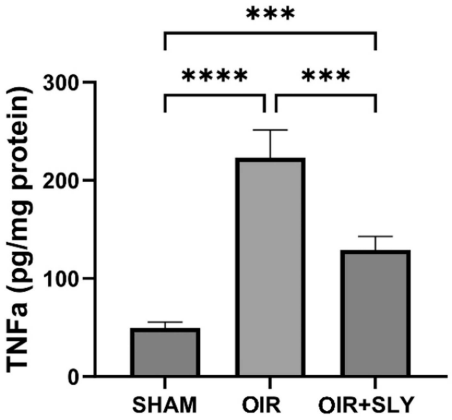

FIGURE 1 | TNF-α levels in tissue across all experimental groups. ***$p = 0.0002$; ****$p < 0.0001$.

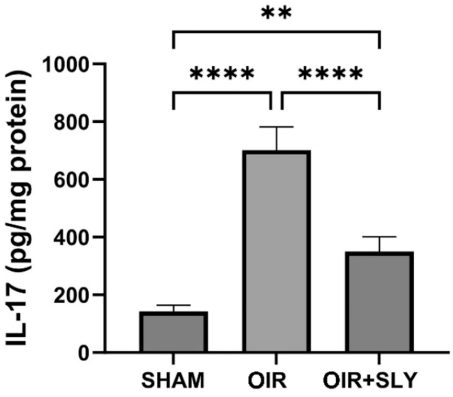

FIGURE 2 | All experimental groups' tissue IL−17 levels. **$p = 0.0021$ and ****$p < 0.0001$.

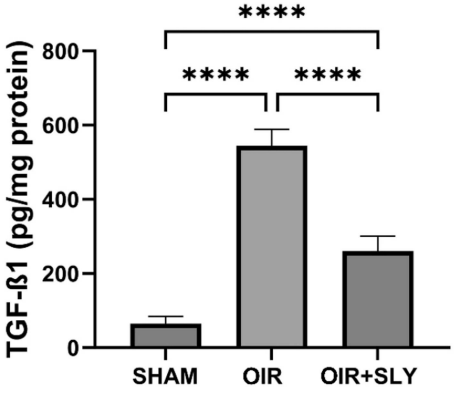

FIGURE 3 | TGF-β1 levels in tissue across all experimental groups. ****$p < 0.0001$.

3.1.2 | *Serum TNF-α, IL-17, TGF-β1, H_2O_2, and TOS Levels*

Serum levels of TNF-α, IL-17, and TGF-β1 were significantly higher in the OIR group than in the sham group ($p < 0.05$). Serum H_2O_2 and TOS levels were considerably greater in the OIR group than in the sham group ($p < 0.05$). (Figures 6–8).

10990461, 2026, 8, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/jbt.71030 by YASIN BAYIR - Ataturk University , Wiley Online Library on [20/07/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

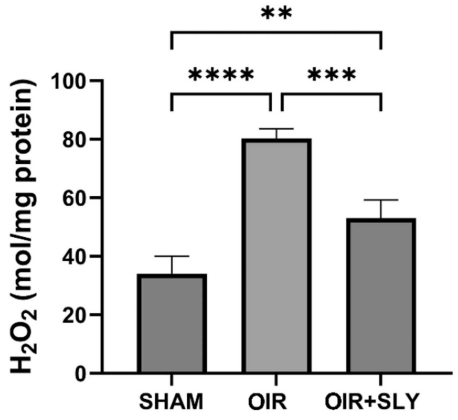

FIGURE 4 | Tissue H_2O_2 concentrations in every experimental group. $^{**}p = 0.0021$, $^{***}p = 0.0002$ and $^{****}p < 0.0001$.

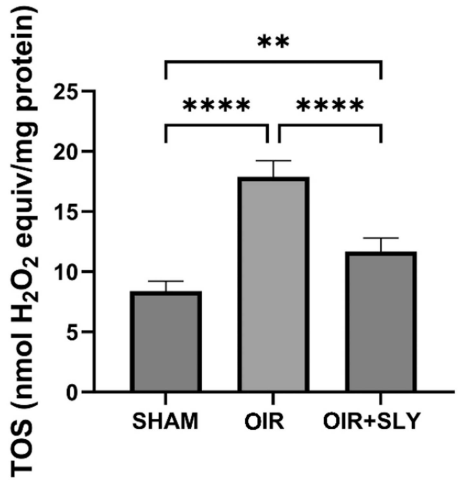

FIGURE 5 | Tissue TOS concentrations in all experimental groups. $^{**}p = 0.0021$ and $^{****}p < 0.0001$.

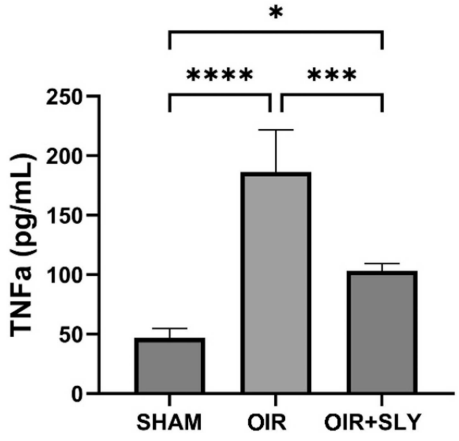

FIGURE 6 | Serum TNF-α levels among experimental groups. $^{*}p = 0.0332$, $^{***}p = 0.0002$ and $^{****}p < 0.0001$.

3.1.3 | Tissue Oxidative Stress Parameters (GSH, SOD, and MDA)

Increased lipid peroxidation was indicated by significantly higher MDA levels in the tissue ($p < 0.05$) in the OIR group compared to the sham group (Figure 11). Conversely, the OIR group had decreased GSH levels and SOD activity in the tissue ($p < 0.05$) (Figures 9 and 10).

When compared to the OIR group, treatment administration dramatically raised GSH and SOD activity while lowering MDA levels ($p < 0.05$).

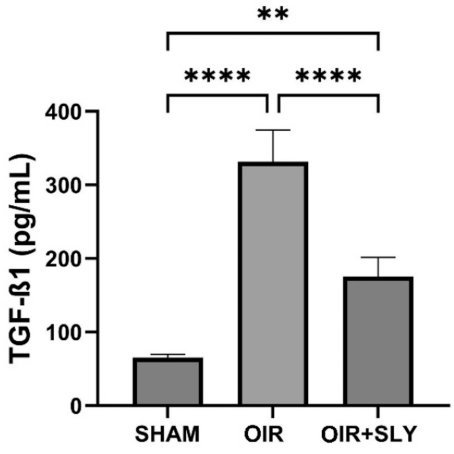

FIGURE 7 | Serum TGF-β1 levels among experimental groups. $^{**}p = 0.0021$ and $^{****}p < 0.0001$.

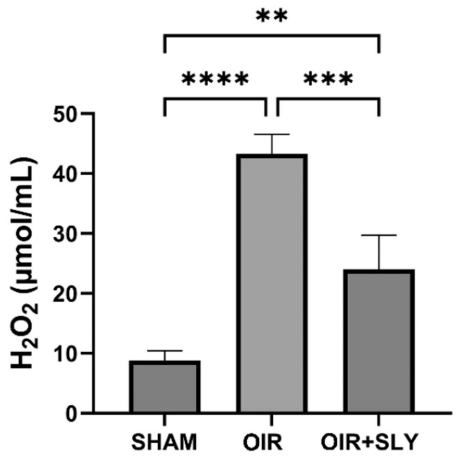

FIGURE 8 | Serum H_2O_2 levels among experimental groups. $^{**}p = 0.0021$, $^{***}p = 0.0002$ and $^{****}p < 0.0001$.

3.2 | Histopathological Findings

Representative histology pictures at 100X magnification from each research group are displayed in Figure 12. The cortex of the ovarian tissues from the Sham group showed normal follicular organization. The ovarian tissues from the OIR group showed signs of deterioration, bleeding, and diffuse vascular congestion. In comparison to the OIR group, the ovarian tissues from the OIR + SLY group showed mild vascular congestion and bleeding. Figure 13 shows a graphic representation of each group's histological examination.

4 | Discussion

Ovarian ischemia, often resulting from OT, poses a significant risk for female infertility. Early diagnosis and intervention are

10990461, 2026, 8, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/jbt.71030 by YASIN BAYIR - Ataturk University , Wiley Online Library on [20/07/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

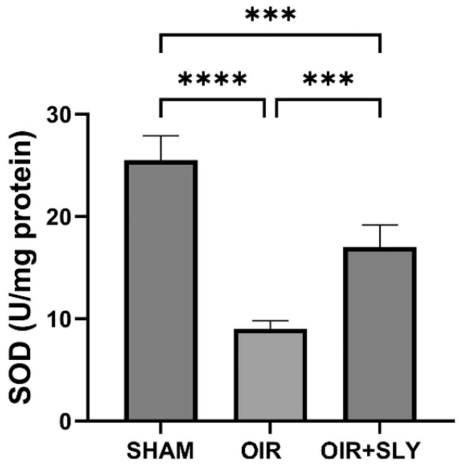

FIGURE 9 | Tissue SOD activity among experimental groups. ***$p = 0.0002$ and ****$p < 0.0001$.

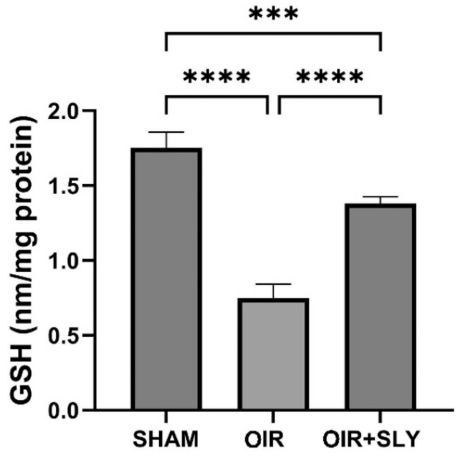

FIGURE 10 | Tissue GSH levels among experimental groups. ***$p = 0.0002$ and ****$p < 0.0001$.

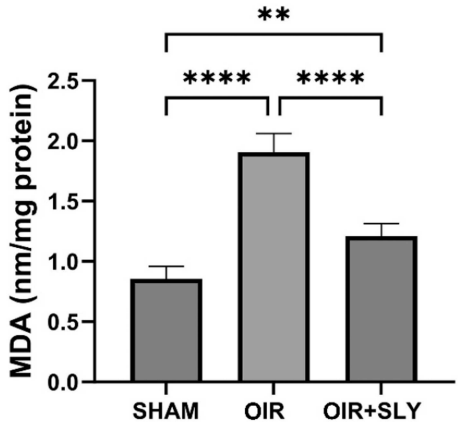

FIGURE 11 | Tissue MDA levels among experimental groups. **$p = 0.0021$ and ****$p < 0.0001$.

crucial. While current standard treatment restores blood flow to the ovaries, it is also crucial to protect the ovaries from reperfusion injury that occurs following. Consequently, this restriction initiates an ischemic cascade and cellular deprivation. The quick flow of oxygenated blood into ischemic tissues

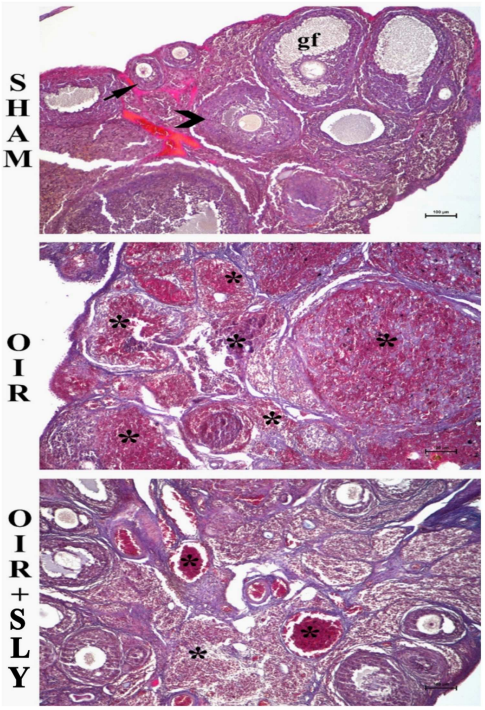

FIGURE 12 | Ovarian tissue micrographs from every group. Primary follicle, arrowhead; secondary follicle, gf: Graafian follicle; Star: Vascular congestion and bleeding. Staining: Crossman's modified Mallory's Triple Stain. 100X magnification.

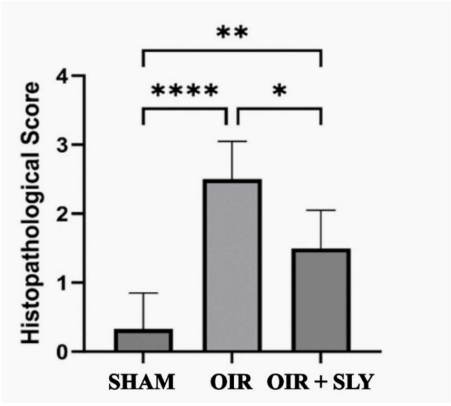

FIGURE 13 | Assessment of each group's ovarian tissue histology. Tukey's test and one-way ANOVA are used to examine the values, which are shown as mean ± SD ($n = 10$). *$p = 0.0332$, **$p = 0.0021$ and ****$p < 0.0001$.

promotes ROS production. Common ROS include H_2O_2, superoxide anion, and hydroxyl radicals. These ROS mediate reperfusion injury by causing cell membrane lipid peroxidation, which produces lethal substances, including MDA. Additionally, the release of proinflammatory cytokines is increased when neutrophils are drawn into the parenchyma past the endothelial barrier, initiating an inflammatory response [26].

The current study's biochemical analysis data revealed that the OIR group had higher levels of proinflammatory cytokines, such as TNF-α and IL-17, than the sham group. In the SLY-treated group, their levels dropped. The OIR group had significantly greater levels of the OS biomarkers MDA, H_2O_2, and TOS than

10990461, 2026, 8, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/jbt.71030 by YASIN BAYIR - Ataturk University , Wiley Online Library on [20/07/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

the sham group, but the treatment group had lower levels. The elevation of GSH and SOD levels in the SLY-treated group indicates that SLY may enhance endogenous antioxidant defense mechanisms during reperfusion. Öztürk and colleagues investigated the protective effects of SLY in an experimental study using a cerebral IR model in rats. The research data reported that the levels of the inflammatory cytokines NF-κB, IL-1β, and TNF-α were higher in the IR group and lower in the SLY-treated group. The oxidative state of ovarian tissue was evaluated by measuring the levels of SOD, MDA, and GSH. GSH and SOD levels rose in the treatment group and fell in the IR group. The IR group had higher levels of MDA, the final byproduct of lipid peroxidation, than the treatment group [27]. The article by Abbasirad et al. described the protective effect of SLY on Multiple Sclerosis, a neuroimmune disease. SLY was reported to reduce the levels of pro-inflammatory cytokine IL-17 and increase IL-10. Taken together with these data, it seems biologically plausible that SLY might exert a protective effect via the IL-17 axis in addition to its known anti-inflammatory/NF-κB suppressive effects in ischemia; however, there are no studies that have directly measured IL-17 levels in ischemia models [28]. TGF-β1 is a cytokine activated by cellular stress, inflammation, and apoptotic signals during the post-ischemia and reperfusion period. Our findings indicate that TGF-β1 levels were significantly elevated in the OIR group, but this increase was reduced in the treatment group. The reduction of TGF-β1 levels in our treatment group as compared to the ischemic group may represent regression of endogenous TGF-β1 induction due to lessening tissue damage as well as direct inhibition of the TGF-β1/Smad axis. Pharmacological blockade of the TGF-β/Smad pathway in models with ischemia and fibrosis has been linked to decreased infarct size and inhibition of inflammatory responses. Therefore, SLY treatment can be viewed as having a protective effect against inflammation and subsequent tissue injury [29].

Histopathological results showed the harmful impacts of OIR damage on ovarian structure and the protective effect of SLY treatment. The cortex of ovarian tissues in the sham group had a normal follicular structure, while there was extensive vascular blockage, bleeding, and degeneration found in the ovarian tissues of the OIR group. The treatment group's ovarian tissues showed mild vascular blockage, bleeding, and degenerative changes when compared with those found in the OIR group. These alterations indicate heightened oxidative stress, endothelial injury, and breakdown of follicular integrity. Demir and colleagues reported that in their experimental studies of mesenteric IR models, widespread fragmentation and bleeding were observed in the villous tissue of the IR group, whereas a decrease in edema and vascular congestion was noted in the SLY treatment group [30]. A study conducted on rats with a renal IR model showed marked tubular dilation, interstitial inflammation, and necrosis in the IR group. Conversely, it was stated that renal morphological damage was significantly less and tissue architecture was better in treatment groups administered SLY [31]. In this situation, SLY has been demonstrated to have marked protective effects against injury that occurs after IR.

In summary, the congruence between histopathological and biochemical results confirms that SLY reduces OIR injury through modulation of OS and inflammatory response, thus positioning it as a potential protective agent against OIR injury.

5 | Conclusion

The preventive effects of SLY against OIR damage were investigated in this work at the biochemical and histological levels. Data showed that severe OS and inflammatory response are hallmarks of OIR damage. Pro-inflammatory cytokines TNF-α, IL-17, and TGF-β1 were considerably elevated in the OIR group's tissue and serum. This indicates a very strong activation of the inflammatory cascade. At the same time, H_2O_2 and TOS level increases support the disruption of the oxidant/antioxidant balance and highlighting OS.

MDA level, which is an indicator of tissue oxidative stress, increased in the OIR group, demonstrating lipid peroxidation, while GSH and SOD levels decreased, showing suppression of endogenous antioxidant defense mechanisms. Histopathological evaluation revealed vascular congestion, hemorrhage, and tissue degeneration in the OIR group. These findings match the biochemical results.

In the group treated with SLY, the significant decrease in pro-inflammatory cytokine levels and oxidant parameters in both tissue and serum, along with an increase in antioxidant defense markers, demonstrates that SLY has strong antioxidant and anti-inflammatory effects against OIR injury. Histopathologically, a significant reduction in vascular congestion and hemorrhage was observed with SLY application, as well as partial preservation of ovarian tissue integrity.

In conclusion, this study demonstrates that silymarin attenuates oxidative stress, inflammation, and histopathological injury in an experimental ovarian ischemia/reperfusion model. Results imply that SLY may be a possible adjuvant therapeutic agent for OIR injury after developing OIR. However, more experimental and clinical studies are required concerning various doses, application periods, and molecular mechanisms before it can be applied clinically.

6 | Limitations

This study has some limitations. SLY was tested in a single dose and as a single exposure; we were unable to address alternative doses, multiple exposures, and dose-response relationships. In addition, the potential sustained impacts of SLY on ovarian function, follicular pool, and fertility were not measured. Because apoptotic, as well as other cellular, signaling pathways at the molecular level have not been studied in depth, the protective mechanisms could not be completely explained. Lastly, it is not possible to directly generalize the results of this study to clinical applications as a consequence of being limited to an experimental animal model. In addition, estrous cycle phases were not controlled, and no formal power analysis was performed, which may limit the interpretation of some biochemical and histopathological outcomes.

Author Contributions

Saime Ozbek Sebin: conceptualization, investigation, methodology, supervision, writing – review and editing, writing – original draft, validation, project administration. **Basak Gulakar:** writing – review and editing, writing – original draft, methodology, validation, conceptualization, project

10990461, 2026, 8, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/jbt.71030 by YASIN BAYIR - Ataturk University , Wiley Online Library on [20/07/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

administration, visualization. **Ayhan Tanyeli:** conceptualization, methodology, investigation, validation, supervision, project administration, writing – review and editing. **Mustafa Can Guler:** investigation, validation, methodology, conceptualization, data curation, writing – review and editing. **Elif Erbas:** formal analysis, project administration, data curation, visualization, writing – review and editing. **Yasin Bayır:** visualization, formal analysis, software, writing – review and editing. **Emir Enis Yurdgulu:** conceptualization, visualization, formal analysis, writing – review and editing, validation. **Selim Comakli:** resources, conceptualization, investigation, methodology, writing – review and editing. **Özlem Erdemci:** writing – review and editing, resources, methodology, conceptualization, investigation.

Acknowledgments

The authors used artificial intelligence (AI) tools (Grammarly Pro) to assist with grammar and spelling checks during the preparation of this manuscript.

Funding

The authors have nothing to report.

Ethics Statement

The experimental protocol received approval from the Atatürk University Experimental Animal Ethics Committee (Approval No: 2023/10-160).

Consent

All authors have approved the publication of the article.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data supporting the findings of this study are not publicly available due to confidentiality and ethical considerations, but can be obtained from the corresponding author upon reasonable request.

References

1. S. Baron and J. Mathai, *Ovarian Torsion* (StatPearls. StatPearls Publishing, 2023).

2. B. Gulakar, S. O. Sebin, E. Laloglu, et al., "New Potential Agent in Ovarian Ischemia Reperfusion Injury: Alpha Pinene," *Journal of Biochemical and Molecular Toxicology* 39, no. 6 (2025): e70318.

3. Güvenç, M. Yüksel, M. Kutlu, et al., "Protective Effects of Esculetin Against Ovary Ischemia–Reperfusion Injury Model in Rats," *Journal of biochemical and molecular toxicology* 38, no. 1 (2024): e23528.

4. M. Yardımcı, M. Göz, M. S. Aydın, N. Kankılıç, and Temiz EJBJoCS, "Antioxidant Actions of Thymoquinone, Silymarin, and Curcumin on Experimental Aortic Ischemia- Reperfusion Model in Wistar Albino Rats," *Journal of Cardiovascular Surgery* 37, no. 6 (2022): 807–813.

5. M. C. Güler, A. Tanyeli, F. N. E. Akdemir, et al., "An Overview of Ischemia–Reperfusion Injury: Review On Oxidative Stress and Inflammatory Response," supplement, *Eurasian Journal of Medicine* 54, no. Suppl 1 (2022): S62.

6. S. Yigit, I. Yesilyurt, S. Bitiktas, et al., "Therapeutic Effect of Berberis Vulgaris Fruit Extract On Histopathological Changes And Oxidative Stress Markers of Ovarian Ischemia And Reperfusion Injury In Rats," *Journal of King Saud University-Science* 36, no. 11 (2024): 103578.

7. C. Huang, M.-K. Hong, and D. C. Ding, "A Review of Ovary Torsion," *Tzu Chi Medical Journal* 29, no. 3 (2017): 143–147.

8. Q. Gao, D. Zhao, and W. He, "Protective Effects of Fisetin on Ovarian Ischemia-Reperfusion Injury in Rats via Modulation of the TLR4-MyD88-TRAF6 Signaling Pathway," *Acta Cirúrgica Brasileira* 40 (2025): e405925.

9. S. Ulusoy Tangul, T. Onat, D. Aydoğan Kirmizi, et al., "Effect of Bromelain on Ischemia-Reperfusion Injury in the Torsion Model Created in Polycystic and Normal Ovarian Tissues," *Frontiers in Pharmacology* 15 (2024): 1451592.

10. A. M. Al-Kufaishi and Al-Musawi, "NJJJoP, Diagnostic, Medicine TSi," *Oxidative Stress and Related Diseases: A Comprehensive Review* 4, no. 2 (2025): 65–70.

11. E. Türk, İ. Karaca, E. Ozcinar, et al., "The Effect of Hypothermia on Adnexal Torsion/Detorsion Injury in a Rat Ovary Model," *Journal of Pediatric Surgery* 50, no. 8 (2015): 1378–1381.

12. S. O. Sebin, B. Gulakar, A. Tanyeli, et al., "Alpha-Pinene Mitigates Renal Ischemia–Reperfusion Injury via Anti-Oxidant, Anti-Inflammatory, Anti-apoptotic Effects and Favorable eNOS/iNOS Shifts in Rats," *Toxicon* (2025): 108637.

13. K. Wellington and B. Jarvis, "Silymarin: A Review of Its Clinical Properties in the Management of Hepatic Disorders," *BioDrugs: Clinical Immunotherapeutics, Biopharmaceuticals and Gene Therapy* 15, no. 7 (2001): 465–489.

14. T. Koltai and Fliegel, "LJJoE-BIM," *Role of Silymarin in Cancer Treatment: Facts, Hypotheses, and Questions* 27 (2022): 2515690X211068826.

15. P. F. Surai, "Silymarin as a Natural Antioxidant: An Overview of the Current Evidence and Perspectives," *Antioxidants (Basel, Switzerland)* 4, no. 1 (2015): 204–247.

16. A. Gillessen and H. H. Schmidt, "Silymarin as Supportive Treatment in Liver Diseases: A Narrative Review," *Advances in Therapy* 37, no. 4 (2020): 1279–1301.

17. A. Aghemo, O. P. Alekseeva, F. Angelico, et al., "Role of Silymarin as Antioxidant in Clinical Management of Chronic Liver Diseases: A Narrative Review," *Annals of Medicine* 54, no. 1 (2022): 1548–1560.

18. M. Singh, M. M. Kadhim, A. Turki Jalil, et al., "A Systematic Review of the Protective Effects of Silymarin/Silibinin Against Doxorubicin-Induced Cardiotoxicity," *Cancer Cell International* 23, no. 1 (2023): 88.

19. A. G. Almutary, M. Y. Begum, A. Siddiqua, et al. Unlocking The Neuroprotective Potential of Silymarin: A Promising Ally In Safeguarding The Brain From Alzheimer's Disease And Other Neurological Disorders. 2025:1-23.

20. O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, "Protein Measurement With the Folin Phenol Reagent," *Journal of Biological Chemistry* 193, no. 1 (1951): 265–275.

21. Y. Sun, L. W. Oberley, and Y. Li, "A Simple Method for Clinical Assay of Superoxide Dismutase," *Clinical Chemistry* 34, no. 3 (1988): 497–500.

22. J. Sedlak and R. H. Lindsay, "Estimation of Total, Protein-Bound, and Nonprotein Sulfhydryl Groups in Tissue With Ellman's Reagent," *Analytical Biochemistry* 25, no. 1 (1968): 192–205.

23. H. Ohkawa, N. Ohishi, and K. Yagi, "Assay for Lipid Peroxides in Animal Tissues by Thiobarbituric Acid Reaction," *Analytical Biochemistry* 95, no. 2 (1979): 351–358.

24. O. Ilgen, I. Hortu, G. Ozceltik, et al., "Effects of Methylene Blue on Ovarian Torsion– Detorsion Injury in a Rat Model," *Journal of Pediatric and Adolescent Gynecology* 33, no. 5 (2020): 506–510.

25. Ş. Osmanlıoğlu, M. Arslan, R. O. Dağ, et al., "Artemisinin Reduces Acute Ovarian Ischemia-Reperfusion Injury in Rats," *Reproductive Toxicology (Elmsford, N.Y.)* 119 (2023): 108417.

26. E. Hizal, E. Uyar, E. Bingul, et al., "The Role of Antioxidant and Anti-Inflammatory Mechanisms in the Protective Effects of Vigabatrin Against Ovarian Ischemia-Reperfusion Injury in a Rat Model," *Journal of Ovarian Research* 18, no. 1 (2025): 214.

10990461, 2026, 8, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/jbt.71030 by YASIN BAYIR - Ataturk University , Wiley Online Library on [20/07/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

27. A. B. Öztürk, N. Akaras, H. Şimşek, and F. M. Kandemir, "Investigation of the Effects of Silymarin on Ovarian Ischemia Reperfusion via Nrf-2/HO-1/NQO1, Ki-67 and Wnt Signaling Pathways," *Journal of Biochemical and Molecular Toxicology* 39, no. 1 (2025): e70138.

28. F. Abbasirad, V. Shaygannejad, F. Hosseininasab, et al., "Significant Immunomodulatory and Hepatoprotective Impacts of Silymarin in MS Patients: A Double-Blind Placebo-Controlled Clinical Trial," *International Immunopharmacology* 97 (2021): 107715.

29. D. M. Mekawy, D. Sabry, and R. M. Sabry, "Abozeid NFJMBR," *Silymarin and MSC-Exosomes Ameliorate Thioacetamide-evoked Renal Fibrosis By Inhibiting Tgf-β/smad Pathway In Rats* 51, no. 1 (2024): 529.

30. M. Demir, R. Amanvermez, A. Kamalı Polat, et al., "The Effect of Silymarin on Mesenteric Ischemia-Reperfusion Injury," *Medical Principles and Practice: International Journal of the Kuwait University, Health Science Centre* 23, no. 2 (2014): 140–144.

31. H. Senturk, S. Kabay, G. Bayramoglu, et al., "Silymarin Attenuates the Renal Ischemia/Reperfusion Injury-Induced Morphological Changes in the Rat Kidney," *World Journal of Urology* 26, no. 4 (2008): 401–407.

10990461, 2026, 8, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/jbt.71030 by YASIN BAYIR - Ataturk University , Wiley Online Library on [20/07/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License