International Journal of Pharmacology

ISSN 1811-7775

science alert

ansinet
Asian Network for Scientific Information

OPEN ACCESS International Journal of Pharmacology

ISSN 1811-7775
DOI: 10.3923/ijp.2025.181.188

Research Article
Biochemical and Histopathologic Investigation of the Effect of Rilmenidine on Ovarian Ischemia-Reperfusion Injury in Rats

[1]Betul Kalkan Yilmaz, [2]Seval Bulut, [1]Nesrin Yilmaz, [3]Nurinisa Yucel, [4]Betul Cicek, [5]Berrin Goktug Kadioglu, [6]Nuri Bakan, [7]Ali Sefa Mendil and [2]Halis Suleyman

[1]Department of Gynecology and Obstetric, Faculty of Medicine, Erzincan Binali Yildirim University, Erzincan, Turkey
[2]Department of Pharmacology, Faculty of Medicine, Erzincan Binali Yildirim University, Erzincan, Turkey
[3]Pharmacy Services Program, Vocational School of Health Services, Erzincan Binali Yildirim University, Erzincan, Turkey
[4]Department of Physiology, Faculty of Medicine, Erzincan Binali Yildirim University, Erzincan, Turkey
[5]Department of Obstetrics and Gynecology, Erzurum Regional Training and Research Hospital, Health Sciences University, Erzurum, Turkey
[6]Department of Medical Biochemistry, Faculty of Medicine, Erzincan Binali Yildirim University, Erzincan, Turkey
[7]Department of Pathology, Faculty of Veterinary Medicine, Erciyes University, Kayseri, Turkey

Abstract

Background and Objective: Rilmenidine is an antihypertensive drug with sympatholytic properties as well as antioxidative and anti-inflammatory properties. To investigate the biochemical and histopathological effects of Rilmenidine on Ovarian Ischemia-Reperfusion (I/R) injury in a rat model, this study aims to determine its potential protective role against oxidative stress and tissue damage associated with ovarian IR injury. **Materials and Methods:** The 24 albino Wistar female rats were divided into four groups: Healthy (HG), sham-operation (SOG), ovarian ischemia-reperfusion (OIRG) and Rilmenidine+ovarian ischemia-reperfusion (ROIRG). Ovaries in SOG, OIRG and ROIRG groups were exposed to ischemia for three hrs, followed by reperfusion for 6 days. The ROIRG rats were treated with Rilmenidine (0.2 mg/kg) daily for 6 days before ischemia. The ovaries were analyzed for oxidant, antioxidant and proinflammatory cytokines and examined histopathologically. Statistical analysis was performed using one-way ANOVA and Kruskal-Wallis tests ($p<0.05$). **Results:** The IR procedure increased tissue malondialdehyde, tumor necrosis factor-α, interleukin-6 and interleukin-1β levels and decreased total glutathione, superoxide dismutase and catalase levels ($p<0.001$). Severe histopathologic damage and 8-hydroxy-20-deoxyguanosine immunopositivity were also observed in ovarian tissues of OIRG ($p<0.001$). Rilmenidine inhibited IR-related biochemical, histopathological and immunohistochemical abnormalities ($p<0.05$). **Conclusion:** Rilmenidine may be an efficient therapeutic strategy for the protection from ovarian IR injury.

Key words: Rilmenidine, ischemia-reperfusion, ovarian damage, antioxidant, anti-inflammatory

Citation: Yilmaz, B.K., S. Bulut, N. Yilmaz, N. Yucel and B. Cicek *et al*., 2025. Biochemical and histopathologic investigation of the effect of Rilmenidine on ovarian ischemia-reperfusion injury in rats. Int. J. Pharmacol., 21: 181-188.

Corresponding Author: Halis Suleyman, Department of Pharmacology, Faculty of Medicine, Erzincan Binali Yildirim University, Erzincan, Turkey
Tel: +90 530 9211909 Fax: +90 446 2261819

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Rilmenidine is one of the first discovered second-generation central sympatholytic[1]. This orally active drug is a selective I_1 imidazoline/α_2-adrenoceptor agonist[2]. With a high safety and tolerability profile, Rilmenidine is a highly effective antihypertensive[1]. The use of imidazoline receptor agonists, including Rilmenidine, is recommended, especially in resistant hypertension[2]. Rilmenidine reduces sympathetic overactivity by activating I_1 imidazoline receptors in the rostral ventrolateral medulla oblongata and shows an antihypertensive effect by inhibiting Na^+/H^+ antiport in the kidney[3,4]. It also regulates sympathetic activity by activating α_2 adrenergic receptors and modulates inflammation-associated processes by decreasing norepinephrine release[5]. Some studies have reported that Rilmenidine reduces the production of Tumor Necrosis Factor-α (TNF-α) and Interleukin-6 (IL-6) and increases the neurotransmitter gamma-aminobutyric acid[6]. In addition, Rilmenidine has also been reported to have the ability to regulate the autophagy mechanism that removes and recycles damaged or dysfunctional components of cells[7,8]. Previous studies indicate that Rilmenidine can inhibit oxidative damage in various tissues to dose-varying degrees. High doses of Rilmenidine have been reported to reduce the overproduction of malondialdehyde (MDA), an indicator of oxidative damage[5]. Furthermore, Salman *et al.*[9] have pointed out that Rilmenidine reduces oxidative damage in uterine tissue. Rilmenidine has been reported to reduce microalbuminuria through its effects on glucose regulation, indirectly preventing lipid peroxidation (LPO) increase and alleviating oxidative stress[10]. Ischemia-reperfusion (IR) injury is a complex abnormal process that begins with the deprivation of oxygen to tissues, progresses with the generation of reactive oxygen species (ROS) and evolves into inflammatory reactions[11]. The ROS that induces oxidative stress also reacts with Deoxyribonucleic Acid (DNA) and causes oxidative DNA damage[12]. The DNA is mutated if this change cannot be repaired. The 8-hydroxy-20-Deoxyguanosine (8-OHdG) is an oxidized form of DNA[13]. Detorsion procedures are used to restore blood flow to twisted and ischemic ovaries in the clinic, which can result in ovarian IR damage[14]. It is believed that excessive amounts of molecular oxygen is supplied to the tissues during detorsion, as well as excessive amounts of ROS[15]. In addition to oxidative stress, proinflammatory cytokines such as Interleukin-1β (IL-1β), TNF-α and nuclear factor-kappa-β have been implicated in the mechanism of IR injury[16]. This information in the literature supports the idea that Rilmenidine may help to protect the ovaries from IR injury. There were no previous trials in which Rilmenidine was tested against ovarian IR damage and this trial was designed to evaluate the protective effect of Rilmenidine against ovarian IR damage in rats.

MATERIALS AND METHODS

Study area: The experimental steps of this research were carried out in the Laboratories of the Experimental Animals Application and Research Centre of Erzincan Binali Yildirim University in September, 2024.

Animals' collection: A total of 24 female albino Wistar rats (260-275 g) were purchased from Erzincan Binali Yıldırım University Experimental Animal Research and Application Center. From one week before the experiment, the animals were housed at 22±2°C with a 12 hrs automatic lighting system and fed *ad libitum*.

Ethical approval: The experimental procedure was confirmed by the local Animal Experiments Ethics Committee of Erzincan Binali Yıldırım University (Date: 25.07.2024, No: 21).

Chemicals: Rilmenidine was obtained from Servier Pharmaceuticals, France, ketamine from Pfizer Pharmaceuticals Ltd. Sti., Turkey and sevoflurane from AbbVie Trade Co., Ltd., Turkey.

Groups: The albino Wistar female rats used in the study were divided into four groups: Healthy group (HG), sham operation group (SOG), ovarian IR group (OIRG) and Rilmenidine+ovarian IR group (ROIRG).

Experimental procedure: The IR procedure was administered to the animals in an appropriate laboratory environment under sterile conditions. A dose of 60 mg/kg ketamine was given intraperitoneally (i.p.) and sevoflurane was sniffed at proper intervals to anesthetize the animals. A surgical intervention was performed during anesthesia while the animals were immobilized in the supine position[17]. In rat groups other than HG, a 2-3 cm long vertical incision was made in the lower abdomen under anesthesia and the ovaries were reached. Surgical thread was used to close the incision without treating the ovaries of the SOG group (n = 6). The right ovaries, fallopian tubes and vascular tissues of the rats in the OIRG (n = 6) and ROIRG (n = 6) were rotated clockwise one full turn, the tissues were fixed with a clamp and a 3 hrs ischemia period was induced. Then 3 hrs later, the clamps were unlocked, the ovaries deterioration and returned to

their normal position and reperfusion continued for 6 days. One hour before the application of anesthesia for surgical procedures, Rilmenidine was applied to the ROIRG at a dose of 0.2 mg/kg by oral gavage[5]. At this stage, HG (n = 6), SOG and OIRG rats were given equal volumes of pure water orally. For 6 days, Rilmenidine and pure water were given once per day. On the 7th day, all animals were sacrificed under high doses of ketamine (120 mg/kg, i.p.) anesthesia and their right ovaries were taken. The removed ovarian tissues were analyzed for MDA, Total Glutathione (tGSH), superoxide dismutase (SOD), catalase (CAT), TNF-α, IL-1β and IL-6 levels. The tissues were also examined histopathologically and immunohistochemically.

Biochemical analysis

Preparation of samples: After the weights of the samples were measured, the ovaries were cut, flash-frozen with liquid nitrogen and homogenized with a mortar and pestle. They were vortexed for 10 sec using phosphate-buffered saline (PBS) (pH 7.4), 1/10 (w/v), centrifuged at 10000 rpm for 20 min and the supernatants were collected. Samples were stored at -80°C until analysis.

Tissue MDA, GSH, SOD, CAT, TNF-α, IL-1β and IL-6 and protein analysis: The MDA, GSH and SOD analysis in tissues were performed with rat Enzyme-Linked Immunosorbent Assay (ELISA) kits following the kit instructions (product numbers 10009055, 703002 and 706002, Cayman Chemical Company, respectively). The CAT was determined according to the method recommended by Goth[18]. The levels of TNF-α (ng/mg protein), IL-1β (pg/mg protein) and IL-6 (ng/mg protein) were analyzed using kits purchased by Eastbiopharm Co. Ltd., ELISA, China. Determination of tissue protein levels was measured as described by Bradford[19].

Histopathologic method: During necropsy, the ovarian tissues were kept in a 10% formalin. The ovaries were processed through a routine alcohol-xylol step and paraffin blocks were obtained. Sections of 5 µ on poly-lysine slides were stained with Hematoxylin and Eosin (H&E) and graded semi-quantitatively as (0) Absent, (1) Mild, (2) Moderate and (3) Severe in terms of stromal hemorrhage, follicular hemorrhage and congestion in 6 different random areas.

Immunohistochemical method: Sections (5 µm) on polylysine slides were treated with xylol and alcohol. They were washed with PBS and endogenous peroxidase was inactivated with hydrogen peroxide (3%, 10 min). It was treated with antigen retrieval solution at 500 watts for 2×5 min. Tissues were washed with PBS and incubated with 8-OhdG (Santa Cruz, Cat. No. sc-66036) primary antibody (1/200, +4°C). The HRP (Thermo Fisher, Cat. No: TP-125-HL) was used as a secondary antibody in compliance with the manufacturer's directions. The chromogen used was 3,3 -diaminobenzidine. Tissues were counterstained with Mayer's hematoxylin, covered and slipped with entellan and examined by light microscopy. Immunopositivity was scored semi-quantitatively as (0) Absent, (1) Mild, (2) Moderate and (3) Severe.

Statistical analysis: The SPSS for Windows, 22.0' statistical program was used for all statistical evaluations. Numerical data were given as "Mean±Standard Deviation" ($\bar{\chi}\pm$SD). Normality of distribution was assessed by Shapiro Wilk and homogeneity of variance by Levene's test. According to the results of the tests, a one-way ANOVA test was used to evaluate the significance level of the difference between the groups and Tukey's or Games Howell's tests were preferred as *post hoc*. Ordinal data were assessed by Kruskal-Wallis test followed by Dunn's test[20]. Ordinal data were seen as median (minimum-maximum). Subsequently, Tukey HSD was performed. The $p<0.05$ was accepted as significant.

RESULTS

MDA, tGSH, SOD and CAT analysis results: In Table 1, MDA amounts were greater ($p<0.001$) and tGSH amounts were less ($p<0.001$) in the ovaries of the OIRG compared to the HG and SOG. Rilmenidine treatment suppressed the IR-related elevation of MDA and reduction of tGSH ($p<0.001$). The MDA and tGSH levels in the ROIRG were close to those in the SG ($p>0.05$).

The SOD and CAT activities were lower in the OIRG according to HG and SOG ($p<0.05$). Rilmenidine inhibited the IR-related decrease in SOD and CAT activities significantly ($p<0.001$). The SOD and CAT activities in the ovaries of Rilmenidine-treated animals were close to those of the SOG ($p>0.05$, Table 1).

TNF-α, IL-1β and IL-6 analysis results: IR surgery increased TNF-α, IL-1β and IL-6 levels in ovarian tissue compared to the HG and SOG ($p<0.05$). The levels of TNF-α, IL-1β and IL-6 in Rilmenidine-treated rats were lower than those in the OIRG ($p<0.001$). The Rilmenidine and sham groups were similar in terms of pro-inflammatory cytokine levels ($p>0.05$) (Table 1).

Histopathologic findings: The histopathological results were presented in Table 2. The table presents histopathologic and immunohistochemical findings across four groups

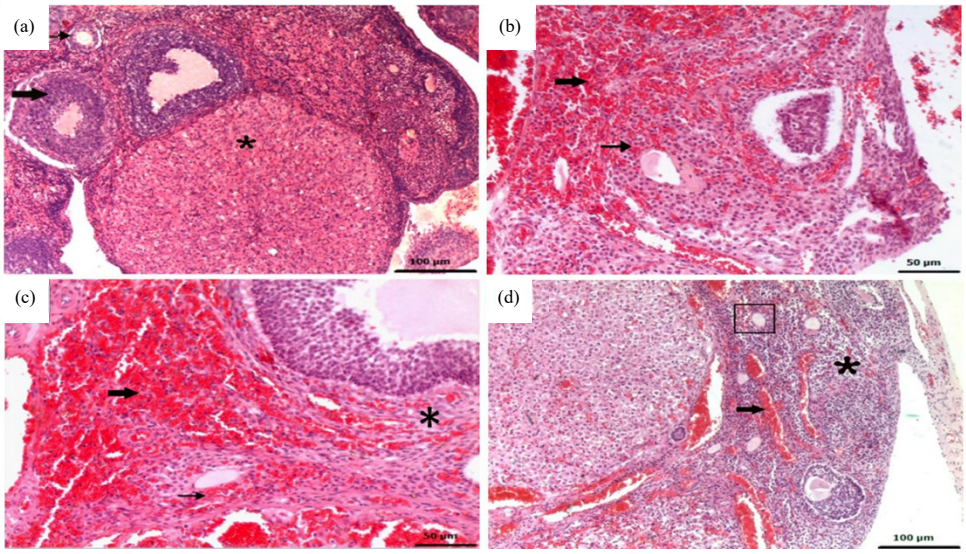

Fig. 1(a-d): Histological appearance of ovarian tissues of the experimental groups, (a) Normal histological appearance of the HG Corpus luteum (asterisk), primary follicle (thin arrow), secondary follicle (thick arrow) H&E, (b) Appearance of stromal hemorrhage (arrow) and follicular hemorrhage (thin arrow) in SOG (H&E), (c) Stromal hemorrhage (arrow), edema (asterisk) and hemorrhage in follicular granulosa cells (thin arrow) in the OIRG (H&E) and (d) Appearance of stromal hemorrhage (arrow), edema (asterisk) and follicular hemorrhage (square) in ROIRG (H&E)
HG: Healthy group, SOG: Sham operation group, OIRG: Ovarian IR group, ROIRG: Rilmenidine+ovarian IR group

Table 1: Effect of Rilmenidine on oxidant, antioxidant and proinflammatory cytokines in ovarian ischemia reperfusion model

	Group				
	HG (n = 6)	SOG (n = 6)	OIRG (n = 6)	ROIRG (n = 6)	
Biochemical parameter	Mean±Standard Deviation				F/p-values
MDA	4.57±0.24*	5.38±0.34*	7.56±0.22	5.26±0.30*,**	150.849/<0.001
tGSH	6.23±0.17*	5.36±0.38*	3.38±0.18	5.26±0.37*,**	99.131/<0.001
SOD	8.63±0.20*	7.50±0.34*	4.48±0.25	7.39±0.27*,**	259.896/<0.001
CAT	6.45±0.22*	5.22±0.20*	3.48±0.27	5.34±0.16*,**	191.088/<0.001
TNF-α	3.30±0.15*	4.61±0.45*	6.68±0.11	4.52±0.28*,**	149.821/<0.001
IL-1β	2.77±0.17*	3.80±0.09*	5.23±0.28	3.83±0.25*,**	136.975/<0.001
IL-6	2.23±0.13*	3.29±0.39*	4.91±0.19	3.35±0.56*,**	56.329/<0.001

*p<0.05 vs OIR, **p>0.05 vs SOG, MDA: Malondialdehyde, tGSH: Total glutathione, SOD: Superoxide dismutase, CAT: Catalase, TNF-α: Tumor Necrosis Factor-Alpha, IL-1β: Interleukin 1β, IL-6: Interleukin-6, HG: Healthy group, SOG: Sham operation group, OIRG: Ovarian IR group, ROIRG: Rilmenidine+ovarian IR group and statistical analysis was done with one way ANOVA/Tukey's HSD or Games-Howell tests

(HG, SOG, OIRG and ROIRG). Significant differences are observed in stromal hemorrhage, follicular hemorrhage, congestion and 8-OHdG levels, with the OIRG group showing the highest median values (3), while the HG group has the lowest (0). The differences between groups are statistically significant (p<0.001 for all parameters). Groups with similar values are marked with asterisks (*) and double asterisks (**) for clarity.

In histopathologic examinations, the ovarian tissues of SG rats had a normal histologic appearance (Fig. 1a). Moderate stromal and follicular hemorrhage was observed in the ovary of the SOG (Fig. 1b). Severe stromal and follicular hemorrhage was determined in the ovary of the OIR (Fig. 1c). However, the severity of stromal and follicular hemorrhage in the ovaries of the ROIR was moderate (Fig. 1d).

Immunohistochemical findings: The results of immunohistochemical analysis are illustrated in Table 2. As seen in Fig. 2a, no significant immunopositivity was seen in the ovaries of the HG in staining performed for 8-OhdG. Moderate 8-OhdG immunopositivity was observed in the stroma, corpus luteum and follicles of SOG (Fig. 2b) and OIRG (Fig. 2c) group ovarian tissues. However, severe 8-OhdG immunopositivity was observed in the stroma, corpus luteum and zona granulosa cells of the follicles of IR-applied ROIRG group ovarian tissue (Fig. 2d).

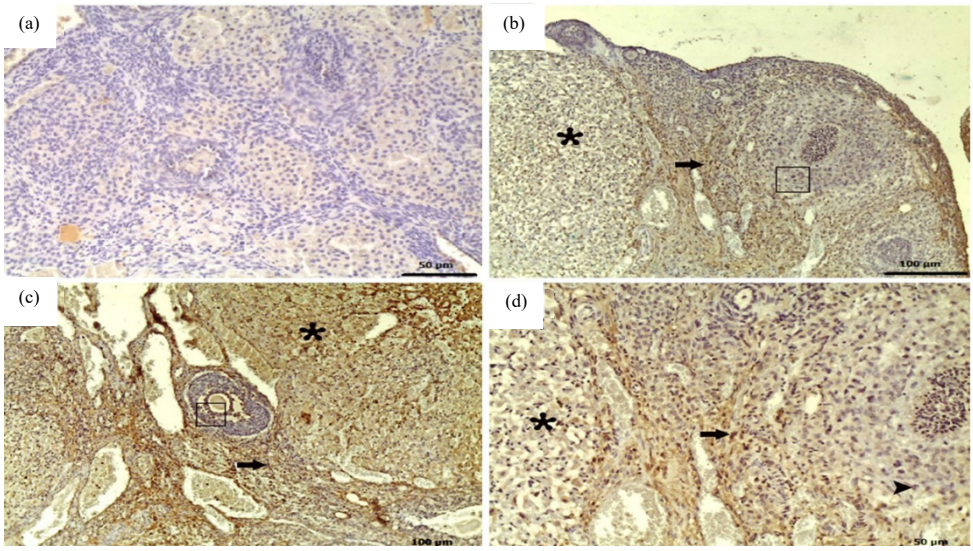

Fig. 2(a-d): 8-OHdG immunopositivity in ovarian tissues of experimental groups, (a) 8-OHdG immune negativity appearance of the HG, (b) Moderate 8-OHdG immunopositivity in corpus luteum (asterisk), stroma (arrow) and follicles (square) of SOG group, (c) Severe 8-8-OHdG immunopositivity in the corpus luteum (star), stroma (arrow) and follicles (square) of the OIRG group and (d) Moderate 8-OHdG immunopositivity in corpus luteum (star), stroma (arrow) and follicles (arrowhead) of ROIRG group

HG: Healthy group, SOG: Sham operation group, OIRG: Ovarian IR group, ROIRG: Rilmenidine+ovarian IR group and OHdG: 8-hydroxy-20-deoxyguanosine

Table 2: Effect of Rilmenidine on histopathological damage and 8-OHdG immunopositivity in ovarian ischemia-reperfusion model

	Group				
	HG (n = 6)	SOG (n = 6)	OIRG (n = 6)	ROIRG (n = 6)	
Parameter	Median (minimum-maximum)				H/p-values
Histopathologic					
Stromal hemorrhage	0 (0-1)*	2 (1-2)*	3 (2-3)	2 (1-3)*,**	118.449/<0.001
Follicular hemorrhage	0 (0-1)*	2 (1-2)*	3 (2-3)	2 (2-3)*,**	124.902/<0.001
Congestion	0 (0-1)*	2 (1-2)*	3 (2-3)	2 (1-3)*,**	118.887/<0.001
Immunohistochemical					
8-OHdG	0 (0-1)*	2 (1-3)*	3 (2-3)	2 (0-3)*,**	112.561/<0.001

Histopathological grading, 0: Absent, 1: Mild damage, 2: Moderate damage, 3: Severe damage, *p<0.05 vs OIR, **p>0.05 vs SOG, 8-OHdG: 8-hydroxy-20-deoxyguanosine, HG: Healthy group, SOG: Sham operation group, OIRG: Ovarian IR group, ROIRG: Rilmenidine+ovarian IR group, statistical analysis was done with Kruskal Walli's test-Dunn's test and OHdG: 8-hydroxy-20-deoxyguanosine

DISCUSSION

A biochemical, histopathological and immunohistochemical investigation was conducted in this study to assess the protective effects of Rilmenidine against ovarian IR injury in rats. As mentioned above, IR injury begins by depriving the tissue of oxygen and progresses with the production of ROS[11]. The ROS, also called reperfusion products, oxidize lipids in cell membranes, producing toxic products like MDA[11,21]. It is important to note that the MDA produced by the LPO reaction is itself toxic and may cause further destruction[22]. There is a wide use of MDA as an indicator of oxidative status and an increase in MDA in the ovaries indicates an increase in ROS[21]. From biochemical results, it is evident that the MDA level was significantly increased in the ovarian tissues of animals treated with IR. Oxidative stress developed in the ovarian tissue of animals subjected to IR, according to the literature. Likewise, Ingec *et al.*[23] saw that rats subjected to IR had significantly higher levels of MDA in the ovaries. It was reported by Barghi *et al.*[15] that plasma MDA levels increased following ovarian IR injury induced by torsion and detorsion methods.

Oxidative stress is evaluated not only by measuring levels of MDA but also by measuring antioxidant levels. As a result of various damage models created in living tissues, the balance between oxidants and antioxidants changes in favor of oxidants, while antioxidant levels decrease[24]. It was found that in this study, consistent with the information found in the literature, the levels of tGSH, SOD and CAT decreased as the MDA level in ovaries increased. Refaie *et al.*[25] found that histopathologic damage to ovarian tissue following IR was associated with GSH depletion. According to another study, exogenous GSH administration may be effective in preventing IR-induced tissue damage, oxidative stress and ovarian reserve loss in rats[26]. The antioxidant GSH is an essential component of mammalian cells and can directly protect cells against ROS and prooxidants[27,28].

In the study, reduced SOD and CAT activities were observed in the ovaries of the IR group. Known to be an endogenous free radical scavenger, SOD converts ROS into hydrogen peroxide[29]. The CAT catalyzes the transformation of hydrogen peroxide into water and molecular oxygen[30]. As reported by Bozkurt *et al.*[31] ovarian SOD and CAT activities decreased with IR exposure. A study by Klein *et al.*[32] demonstrated the relevance of SOD levels in protecting against IR injury. It has been documented that SOD and CAT infusion has a protective effect on the myocardium against IR injury in both hypothermic ischemia and normothermia[33].

It is well known that elevated generation of ROS in organs and tissues upsets the oxidant/antioxidant balance in the direction of the oxidants. As a result of the reaction between overproduced ROS and DNA, 8-OHdG is formed in cellular DNA as a mutant DNA derivative and is commonly referred to as a biomarker of oxidative stress[21,34]. Based on immunohistochemical results, severe 8-OHdG immunopositivity was observed in the stroma, corpus luteum and zona granulosa cells of follicles within the ovarian tissue of IR-applied animals. This indicated that IR treatment caused DNA oxidation in the ovarian tissue.

Increased ROS production in organs and tissues leads to abnormal proinflammatory cytokine production. The ROS triggers the LPO reaction in cells[11]. Feng *et al.*[35] reported that LPO-induced oxidative stress exacerbates tissue damage by accelerating the release of cytokines in tissues. Mitochondrial-derived ROS contribute to the production of IL-1β, IL-6 and TNF-α[36]. As it can be seen from experimental findings, which are consistent with the literature, the IR process elevated the levels of proinflammatory cytokines in the ovaries. Cytokines such as IL-1β, IL-6 and TNF-α produced by non-immune and immune cells play a central role in inflammation[37]. Previous studies have shown histopathologically that excessive oxidant and cytokine production causes oxidative stress and inflammatory damage in ovaries[38]. Biochemical results in the healthy, sham operation and IR groups overlapped with the histopathologic findings. In this study, significant histopathologic damage was observed in the ovarian tissue of the IR group with high levels of oxidant and proinflammatory cytokines and low levels of antioxidants.

This study examined the impact of Rilmenidine on IR-related ovarian injury. Current experimental results showed that Rilmenidine significantly reduced the IR-related elevated levels of oxidative and pro-inflammatory cytokines and the reduction of antioxidants in ovarian tissue. Furthermore, Rilmenidine significantly inhibited the deterioration of the morphology of ovarian tissue. The results of studies suggest that Rilmenidine has antioxidant properties and reduces the effects of oxidative stress[5,9]. Besides its antioxidant properties, there is also evidence that it inhibits the production of proinflammatory cytokines[6]. The fact that Rilmenidine is an antihypertensive drug was one of the key reasons why this study investigated the protective effects of it against IR injury. As it is well known, patients with hypertension who undergo surgical intervention are at an increased risk of postoperative morbidity and mortality[39]. Preoperative hypertension has been shown to increase postoperative mortality by 3.8 times compared with a normotensive patient[40,41]. Therefore, it is important to maintain normal blood pressure before and after surgery.

It may be useful to measure total oxidant, total antioxidant and anti-inflammatory cytokine levels to understand the mechanism of Rilmenidine's protective effect against IR injury.

CONCLUSION

The IR caused an increase in oxidants and 8-OHdG, a DNA damage product, in ovarian tissue. In parallel with the increase in oxidants, antioxidant levels decreased significantly. In addition, proinflammatory cytokine levels increased in ovarian tissue exposed to IR. Biochemical results were consistent with histopathologic findings. Rilmenidine antagonized the effects of IR on oxidants, 8-OHdG, proinflammatory cytokines and antioxidants in the ovarian tissue. Furthermore, it was histopathologically demonstrated that Rilmenidine protected ovarian tissue from IR injury. Experimental results revealed that Rilmenidine was effective in reducing the severity of ovarian IR injury in animals. The study results will contribute to clinical trials in which Rilmenidine is used to protect the ovary from ovarian IR injury.

SIGNIFICANCE STATEMENT

This study highlights the potential of Rilmenidine as a therapeutic agent in protecting the Ovaries from Ischemia-Reperfusion (I/R) injury. By demonstrating both antioxidative and anti-inflammatory effects, Rilmenidine offers a promising approach to mitigate the tissue damage associated with IR-induced ovarian dysfunction.

REFERENCES

1. Reid, J.L., 2000. Rilmenidine: A clinical overview. Am. J. Hypertens., 13: 106S-111S.
2. Érszegi, A., R. Viola, M.A. Bahar, B. Tóth, I. Fejes, A. Vágvölgyi and D. Csupor, 2024. Not first-line antihypertensive agents, but still effective-the efficacy and safety of imidazoline receptor agonists: A network meta-analysis. Pharmacol. Res. Perspect., Vol. 12. 10.1002/prp2.1215.
3. Mayorov, D.N., S.L. Burke and G.A. Head, 2001. Relative importance of rostral ventrolateral medulla in sympathoinhibitory action of rilmenidine in conscious and anesthetized rabbits. J. Cardiovasc. Pharmacol., 37: 252-261.
4. Karasakal, A. and E. Özdemir, 2019. Development and validation of first and zero order derivative spectrophotometric methods for the determination of rilmenidine in pharmaceutical preparations and forced degradation study. J. Res. Pharm., 23: 457-464.
5. Yalçın, M.B., E.S. Bora, A. Çakır, S. Akbulut and O. Erbaş, 2023. Autophagy and anti-inflammation ameliorate diabetic neuropathy with Rilmenidine. Acta Cirúrgica Bras., Vol. 38. 10.1590/acb387823.
6. Head, G.A., K.L. Jackson and C. Gueguen, 2019. Potential therapeutic use of neurosteroids for hypertension. Front. Physiol., Vol. 10. 10.3389/fphys.2019.01477.
7. Mercer, L.D., G.C. Higgins, C.L. Lau, A.J. Lawrence and P.M. Beart, 2017. MDMA-induced neurotoxicity of serotonin neurons involves autophagy and rilmenidine is protective against its pathobiology. Neurochem. Int., 105: 80-90.
8. Underwood, B.R., Z.W. Green-Thompson, P.J. Pugh, S.E. Lazic and S.L. Mason *et al*., 2017. An open-label study to assess the feasibility and tolerability of Rilmenidine for the treatment of Huntington's disease. J. Neurol., 264: 2457-2463.
9. Salman, S., S. Kumbasar, N. Gursan, Y. Kumtepe and B. Borekci *et al*., 2011. Investigation of the relationship of some antihypertensive drugs with oxidant/antioxidant parameters and DNA damage on rat uterus tissue. Int. J. Fertil. Sterility, 5: 96-103.
10. Farský, Š., A. Strišková and M. Borčin, 2018. Hypertension treatment in patients with metabolic syndrome and/or type 2 diabetes mellitus: Analysis of the therapy effectivity and the therapeutic inertia in outpatient study. Cardiol. Res. Pract., Vol. 2018. 10.1155/2018/8387613.
11. Yapca, O.E., B. Borekci and H. Suleyman, 2013. Ischemia-reperfusion damage. Eurasian J. Med., 45: 126-127.
12. Kobayashi, H. and S. Imanaka, 2025. Exploring potential pathways from oxidative stress to ovarian aging. J. Obstet. Gynaecol. Res., Vol. 51. 10.1111/jog.16166.
13. Chiorcea-Paquim, A.M., 2022. 8-oxoguanine and 8-oxodeoxyguanosine biomarkers of oxidative DNA damage: A review on HPLC-ECD determination. Molecules, Vol. 27. 10.3390/molecules27051620.
14. Laganà, A.S., V. Sofo, F.M. Salmeri, V.I. Palmara and O. Triolo *et al*., 2016. Oxidative stress during ovarian torsion in pediatric and adolescent patients: Changing the perspective of the disease. Int. J. Fertil. Sterility, 9: 416-423.
15. Barghi, B., M. Shokoohi, A.A. Khaki, A. Khaki, M. Moghimian and M. Soltani, 2021. Eugenol improves tissue damage and oxidative stress in adult female rats after ovarian torsion/detorsion. J. Obstet. Gynaecol., 41: 933-938.
16. Güler, M.C., A. Tanyeli, D.G. Erdoğan, E. Eraslan, S. Çomaklı, E. Polat and S. Doğanay, 2021. Urapidil alleviates ovarian torsion detorsion injury via regulating oxidative stress, apoptosis, autophagia, and inflammation. Iran. J. Basic Med. Sci., 24: 935-942.
17. Demiryilmaz, I., M.I. Turan, A. Kisaoglu, M. Gulapoglu, I. Yilmaz and H. Suleyman, 2014. Protective effect of nimesulide against hepatic ischemia/reperfusion injury in rats: Effects on oxidant/antioxidants, DNA mutation and COX-1/COX-2 levels. Pharmacol. Rep., 66: 647-652.
18. Góth, L., 1991. A simple method for determination of serum catalase activity and revision of reference range. Clin. Chim. Acta, 196: 143-151.
19. Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem., 72: 248-254.
20. Lee, S.W., 2022. Methods for testing statistical differences between groups in medical research: Statistical standard and guideline of life cycle committee. Life Cycle, Vol. 2. 10.54724/lc.2022.e1.
21. Uc, I., M. Aksoy, E. Laloglu and A. Aydin, 2024. Effects of dexmedetomidine and ketamine on oxidative stress markers in patients undergoing lower extremity surgery: A randomized, double-blind study. Int. J. Pharmacol., 20: 1301-1309.
22. Süleyman, H. and A. Özçiçek, 2020. Molecular mechanism of ischemia reperfusion injury. Arch. Basic Clin. Res., 2: 25-27.
23. Ingec, M., U. Isaoglu, M. Yilmaz, M. Calik and B. Polat *et al*., 2011. Prevention of ischemia-reperfusion injury in rat ovarian tissue with the on-off method. J. Physiol. Pharmacol., 62: 575-582.
24. Kisaoglu, A., B. Borekci, O.E. Yapca, H. Bilen and H. Suleyman, 2013. Tissue damage and oxidant/antioxidant balance. Eurasian. J. Med., 45: 47-49.

25. Refaie, M.M.M., M. El-Hussieny and S. Shehata, 2022. TLR4/NF-κB/TNFα and cAMP/SIRT1 signaling cascade involved in mediating the dose-dependent effect of cilostazol in ovarian ischemia reperfusion-induced injury. Immunopharmacol. Immunotoxicol., 44: 338-346.
26. Gürsoy, A., B.O. Ipek, A.G. Sade, K. Atasayan, E.D. Tekbaş and M.E. Sitar, 2023. Protective effects of glutathione administration on ischemia-reperfusion injury in rat ovaries. J. Exp. Clin. Med., 40: 1-6.
27. Averill-Bates, D.A., 2023. The Antioxidant Glutathione. In: Vitamins and Hormones, Litwack, G. (Ed.), Academic Press, Cambridge, Massachusetts, ISBN: 9780443157684, pp: 109-141.
28. Eldesoqui, M., L.S. Ali, A. Megahed, A.M. Ibrahim and E.M. Embaby, 2024. Effect of L-citrulline supplementation on cadmium-induced testicular dysfunction in male albino rats: Inhibition of oxidative stress and apoptosis. Int. J. Pharmacol., 20: 1339-1349.
29. Dengiz, G.O., F. Odabasoglu, Z. Halici, H. Suleyman, E. Cadirci and Y. Bayir, 2007. Gastroprotective and antioxidant effects of amiodarone on indomethacin-induced gastric ulcers in rats. Arch. Pharm. Res., 30: 1426-1434.
30. Xiang, M., Y. Lu, L. Xin, J. Gao and C. Shang *et al*., 2021. Role of oxidative stress in reperfusion following myocardial ischemia and its treatments. Oxid. Med. Cell. Longevity, Vol. 2021. 10.1155/2021/6614009.
31. Bozkurt, S., D.C. Arikan, E.B. Kurutas, H. Sayar, M. Okumus, A. Coskun and V. Bakan, 2012. Selenium has a protective effect on ischemia/reperfusion injury in a rat ovary model: Biochemical and histopathologic evaluation. J. Pediatr. Surg., 47: 1735-1741.
32. Klein, M.B., P.H. Chan and J. Chang, 2003. Protective effects of superoxide dismutase against ischemia-reperfusion injury: Development and application of a transgenic animal model. Plast. Reconstructive Surg., 111: 251-255.
33. Nishikawa, Y., S. Yamamoto, K. Ataka and K. Nakamura, 1991. The effect of superoxide dismutase and catalase on myocardial reperfusion injury in the isolated rat heart. Jpn. J. Surg., 21: 423-432.
34. Valavanidis, A., T. Vlachogianni and C. Fiotakis, 2009. 8-hydroxy-2'-deoxyguanosine (8-OHdG): A critical biomarker of oxidative stress and carcinogenesis. J. Environ. Sci. Health, Part C Environ. Carcinog. Ecotoxicol. Rev., 27: 120-139.
35. Feng, J., J. Wang, Y. Wang, X. Huang and T. Shao *et al*., 2022. Oxidative stress and lipid peroxidation: Prospective associations between ferroptosis and delayed wound healing in diabetic ulcers. Front. Cell Dev. Biol., Vol. 10. 10.3389/fcell.2022.898657.
36. Mittal, M., M.R. Siddiqui, K. Tran, S.P. Reddy and A.B. Malik, 2014. Reactive oxygen species in inflammation and tissue injury. Antioxid. Redox Signaling, 20: 1126-1167.
37. Hirano, T., 2021. IL-6 in inflammation, autoimmunity and cancer. Int. Immunol., 33: 127-148.
38. Lou, Z.Y., W.J. Zhang, J. Wang and C.Y. Yang, 2024. *In vitro* validation of the therapeutic potential of diallyl disulfide on benign prostatic hyperplasia: Effect on hyperproliferation, oxidative stress and inflammation. Int. J. Pharmacol., 20: 883-891.
39. Ibrahim, M.A., I.A. Albahlol, F.A. Wani, A.A.E. Tammam and M.T. Kelleni *et al*., 2021. Resveratrol protects against cisplatin-induced ovarian and uterine toxicity in female rats by attenuating oxidative stress, inflammation and apoptosis. Chem. Biol. Interact., Vol. 338. 10.1016/j.cbi.2021.109402.
40. Varon, J. and P.E. Marik, 2008. Perioperative hypertension management. Vasc. Health Risk Manage., 4: 615-627.
41. Browner, W.S., J. Li, D.T. Mangano, M. Hollenberg and J.F. Tubau *et al*., 1992. In-hospital and long-term mortality in male veterans following noncardiac surgery. JAMA, 268: 228-232.