



Article

Protective Effects of Ranolazine in a Rat Model of Ovarian Ischemia/Reperfusion Injury

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Abstract

Ovarian ischemia/reperfusion (I/R) injury is a major cause of tissue damage following the surgical detorsion of ovarian torsion and is characterized by excessive oxidative stress, inflammation, and progressive cellular injury. Although ranolazine has been reported to exert antioxidant and anti-inflammatory effects in various experimental models, its potential protective effects against ovarian I/R injury have not yet been investigated. This study aimed to evaluate whether pretreatment with ranolazine protects ovarian tissue against experimental I/R injury in rats. Twenty-four female Wistar albino rats were randomly assigned to four groups: a healthy group (HG), sham-operated group (SOG), ovarian ischemia/reperfusion (OIR) group, and ranolazine-treated ovarian ischemia/reperfusion (ROIR) group. Ovarian ischemia was induced by 1 h of torsion followed by 6 h of reperfusion. Ranolazine (50 mg/kg) was administered 1 h before the induction of ischemia as a preventive pretreatment. Oxidative stress markers (MDA, tGSH, SOD, and CAT), pro-inflammatory cytokines (TNF- α , IL-1 β , and IL-6), histopathological alterations, and the expression of COX-1, COX-2, IL-1 β , and IL-6 were evaluated by double immunofluorescence staining. Ovarian I/R significantly increased MDA and pro-inflammatory cytokine levels while markedly decreasing tGSH levels and SOD and CAT activities ($p < 0.001$). These biochemical alterations were accompanied by severe follicular degeneration, mononuclear cell infiltration, interstitial edema, decreased COX-1 immunoreactivity, and increased expression of COX-2, IL-1 β , and IL-6. Ranolazine treatment significantly attenuated oxidative stress and inflammatory responses, preserved the antioxidant defense system, ameliorated histopathological damage, restored COX-1 immunoreactivity, and inhibited the expression of COX-2, IL-1 β , and IL-6. This is the first experimental study evaluating ranolazine in ovarian I/R injury. These findings suggest that preventive pretreatment with ranolazine attenuates experimental ovarian I/R injury through antioxidant and anti-inflammatory mechanisms. Future studies are needed to determine whether similar protective effects



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can be achieved when ranolazine is administered after ovarian torsion or following surgical detorsion.

Keywords: ovarian ischemia/reperfusion; ranolazine; oxidative stress; inflammation; COX-1; COX-2; rat

1. Introduction

Ischemia is a pathological condition characterized by a reduction or complete interruption of blood flow to tissues, resulting in oxygen deprivation, cellular dysfunction, and ultimately necrosis. Therefore, the primary therapeutic intervention for ischemic tissue is the restoration of blood flow (reperfusion) [1]. However, re-establishing blood supply to an ischemic organ may paradoxically induce tissue damage that is even more severe than the oxidative and inflammatory injury caused by ischemia itself [2]. This phenomenon is known as I/R injury [3]. I/R-induced oxidative stress, together with the accompanying inflammatory response, contributes significantly to morbidity and mortality in affected patients [4]. The pathogenesis of I/R injury involves depletion of cellular energy stores, inhibition of the Sodium–Potassium Adenosine Triphosphatase (Na^+/K^+ -ATPase) Pump, and the consequent intracellular accumulation of Na^+ and calcium (Ca^{2+}) ions [1]. This process is initiated during ischemia by the shift to anaerobic metabolism and the depletion of intracellular ATP levels [5]. Moreover, the increase in intracellular Ca^{2+} during I/R activates phospholipase A_2 , leading to cyclooxygenase-2 (COX-2)-mediated conversion of arachidonic acid into pro-inflammatory prostaglandins and reactive oxygen species [6].

One of the most common causes of ovarian ischemia in clinical practice is ovarian torsion [1]. Ovarian torsion occurs when the ovary undergoes partial or complete rotation around its vascular pedicle, resulting in reduced or complete interruption of blood flow to the adnexa [2]. Accordingly, surgical detorsion to restore ovarian perfusion is the recommended treatment [3]. Ovarian torsion represents one of the most clinically relevant examples of ovarian I/R injury [7]. The ischemic phase is caused by torsion of the ovary around its vascular axis [8], whereas surgical detorsion represents the reperfusion phase of I/R injury [9]. Current evidence suggests that preservation of intracellular ATP levels and $\text{Na}^+/\text{Ca}^{2+}$ homeostasis, together with antioxidant and anti-inflammatory therapies, may be beneficial in attenuating ovarian I/R injury. These findings highlight the need to investigate potential pharmacological strategies capable of attenuating ovarian I/R injury.

Ranolazine, the agent investigated in the present study for its potential protective effects against ovarian I/R injury, is chemically designated as N-(2,6-dimethylphenyl)-4-(2-hydroxy-3-[2-methoxyphenoxy]propyl)-1-piperazineacetamide dihydrochloride [10]. Ranolazine is a piperazine derivative approved in the European Union for the treatment of chronic stable angina and possesses a unique mechanism of action [11]. Its pharmacological effects are primarily attributed to inhibition of the late sodium current, thereby reducing intracellular Na^+ accumulation and limiting Ca^{2+} influx through the $\text{Na}^+/\text{Ca}^{2+}$ exchanger [12]. Inhibition of $\text{Na}^+/\text{Ca}^{2+}$ exchange during inflammation has been shown to attenuate inflammatory responses both in vitro in human endothelial cells and in clinical studies involving humans [13]. Previous studies have demonstrated that ranolazine suppresses the increase in malondialdehyde (MDA), a marker of oxidative stress, while preventing reductions in antioxidant parameters, including total thiol content, catalase (CAT) activity, and total antioxidant capacity (TAC), thereby exerting cytoprotective effects [14]. In addition, ranolazine has been reported to improve impaired left ventricular function after the development of heart failure and to reduce the levels of pro-inflammatory mediators, including norepinephrine, tumor necrosis factor- α (TNF- α), interleukin-1 β (IL-1 β), and

interleukin-6 (IL-6) [15]. Furthermore, in a rabbit model of cardiac I/R injury, ranolazine effectively prevented ATP depletion and attenuated reperfusion-induced tissue damage [16]. Collectively, these findings suggest that ranolazine may also be beneficial in the treatment of ovarian I/R injury. To the best of our knowledge, this is the first experimental study evaluating ranolazine in ovarian ischemia/reperfusion injury. Our hypothesis was based on the established ability of ranolazine to preserve intracellular ionic homeostasis, reduce oxidative stress, attenuate inflammatory responses, and exert protective effects in previously reported models of ischemia/reperfusion injury. Therefore, we hypothesized that pretreatment with ranolazine may attenuate experimental ovarian ischemia/reperfusion injury. Accordingly, the aim of the present study was to evaluate the protective effects of ranolazine pretreatment in a rat model of ovarian ischemia/reperfusion injury.

2. Results

2.1. Biochemical Results

2.1.1. Ovarian Tissue MDA Levels

As shown in Figure 1A, ovarian tissue MDA levels were significantly higher in the OIR group than in both the HG and SOG ($p < 0.001$), indicating enhanced lipid peroxidation following ovarian I/R injury. In contrast, ranolazine treatment markedly reduced MDA levels in the ROIR group compared with the OIR group ($p < 0.001$), with values approaching those of the control groups (see Supplementary Table S1). No pronounced difference in MDA levels was observed between the HG and SOG groups ($p > 0.05$).

2.1.2. Ovarian Tissue tGSH Levels

As presented in Figure 1B, ovarian tissue tGSH levels were significantly decreased in the OIR group compared with the HG and SOG ($p < 0.001$). Administration of ranolazine significantly restored tGSH levels in the ROIR group relative to the OIR group ($p < 0.001$), bringing the values closer to those observed in the control groups (see Supplementary Table S1). No statistically pronounced difference was detected between the HG and SOG ($p > 0.05$).

2.1.3. Ovarian Tissue SOD and CAT Activities

As illustrated in Figure 1C,D, ovarian I/R markedly reduced SOD and CAT activities in the OIR group compared with the HG and SOG ($p < 0.001$). Ranolazine treatment significantly increased the activities of both antioxidant enzymes compared with the OIR group ($p < 0.001$), indicating substantial, but not complete, recovery of the antioxidant defense system. No statistically significant difference in SOD activity was observed between the HG and SOG ($p > 0.05$). In contrast, CAT activity differed significantly between the HG and SOG ($p < 0.05$), whereas no statistically significant difference was detected between the SOG and ROIR ($p > 0.05$). Complete one-way ANOVA and Tukey's HSD multiple-comparison results are provided in Supplementary Table S1.

2.1.4. Ovarian Tissue TNF- α , IL-1 β , and IL-6 Levels

As shown in Figure 2A–C, ovarian tissue levels of the pro-inflammatory cytokines TNF- α , IL-1 β , and IL-6 were significantly elevated in the OIR group compared with both the HG and SOG ($p < 0.001$). Treatment with ranolazine markedly reduced the concentrations of all three cytokines in the ROIR group relative to the OIR group ($p < 0.001$), with values approaching those observed in the control groups (see Supplementary Table S1). No statistically markedly elevated differences were detected between the HG and SOG for any of the cytokines ($p > 0.05$).

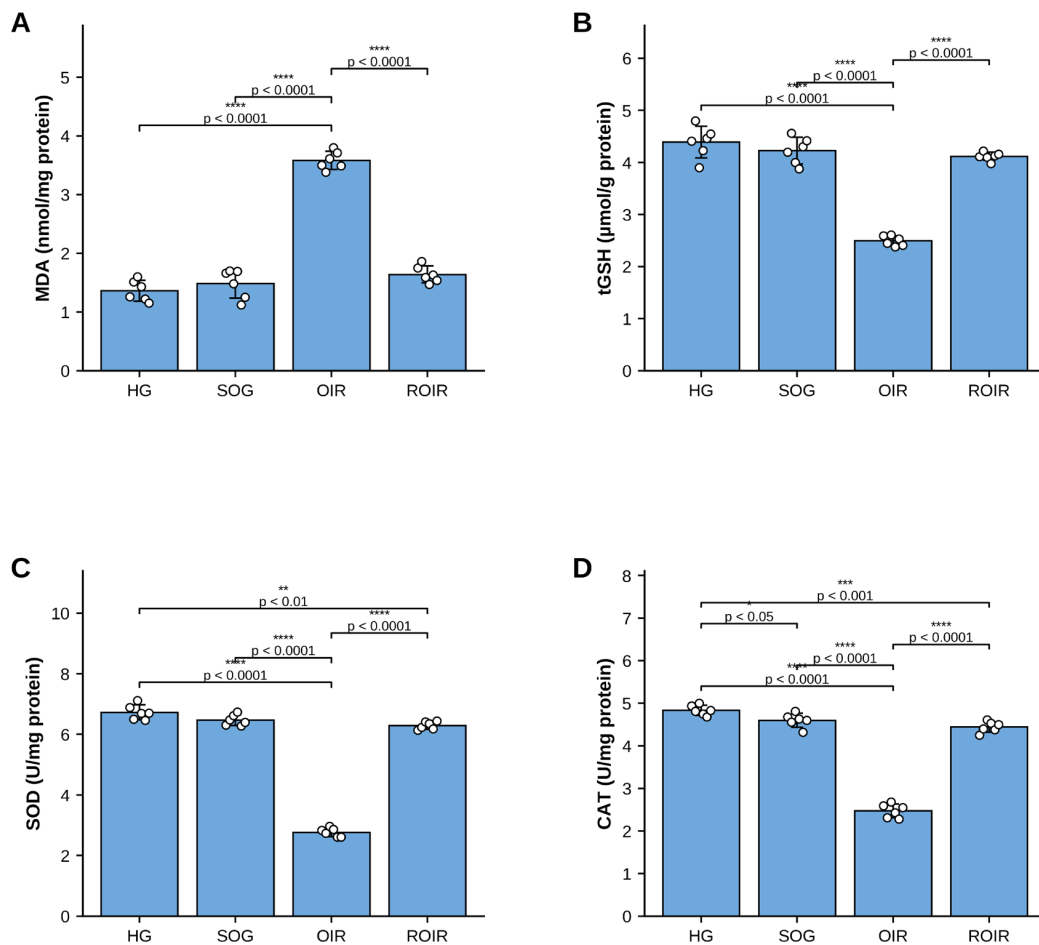


Figure 1. Effects of ranolazine on ovarian oxidative stress markers in rats subjected to ovarian I/R injury. **(A)** MDA, **(B)** tGSH, **(C)** SOD, and **(D)** CAT levels in ovarian tissue. Data are presented as mean $\pm$ SD ($n = 6$). Each dot represents one animal. Statistical significance is indicated as follows: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$. Abbreviations: HG, healthy group; SOG, sham-operated group; OIR, ovarian ischemia/reperfusion group; ROIR, ranolazine + ovarian ischemia/reperfusion group; MDA, malondialdehyde; tGSH, total glutathione; SOD, superoxide dismutase; CAT, catalase.

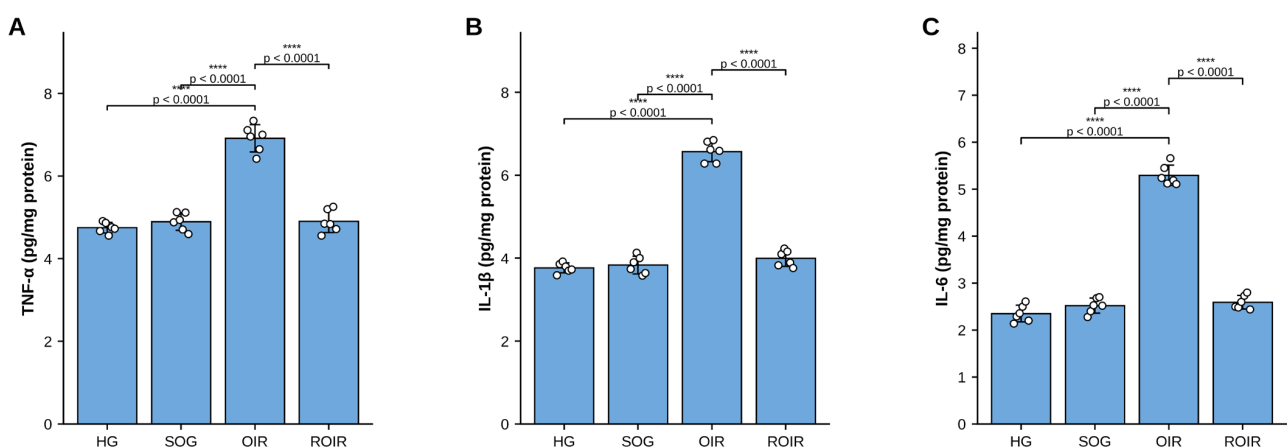


Figure 2. Effects of ranolazine on ovarian pro-inflammatory cytokine levels in rats subjected to ovarian I/R injury. **(A)** TNF-α, **(B)** IL-1β, and **(C)** IL-6 levels in ovarian tissue. Data are presented as mean $\pm$ SD ($n = 6$). Each dot represents one animal. Statistical significance is indicated as follows: **** $p < 0.0001$. Abbreviations: HG, healthy group; SOG, sham-operated group; OIR, ovarian ischemia/reperfusion group; ROIR, ranolazine + ovarian ischemia/reperfusion group; TNF-α, tumor necrosis factor-α; IL-1β, interleukin-1β; IL-6, interleukin-6.

2.2. Histopathological Findings

Histopathological examination revealed statistically significant differences among the experimental groups (Table 1). The HG and SOG exhibited normal ovarian histological architecture. Follicular degeneration, mononuclear cell infiltration in the parenchyma, and interstitial edema were observed in the experimental groups. These microscopic findings were severe in the OIR group, whereas in the ROIR group follicular degeneration and mononuclear cell infiltration were mild, and interstitial edema was not observed (Figure 3).

Table 1. Histopathological scores of ovarian tissue in the experimental groups (median [minimum–maximum]).

Groups (n = 6)	Follicular Degeneration	Mononuclear Cell Infiltration	Interstitial Edema
HG	0 (0–0)	0 (0–0)	0 (0–0)
SOG	0 (0–0)	0 (0–0)	0 (0–0)
OIR	3 (3–3) ^a	3 (2–3) ^a	3 (2–3) ^a
ROIR	1 (1–2) ^b	1 (1–2) ^b	0 (0–0) ^b

Values are presented as median (minimum–maximum). Histopathological scores were analyzed using the Kruskal–Wallis test followed by Dunn’s post hoc test. Different superscript letters indicate statistically significant differences between groups ($p < 0.05$). Abbreviations: HG, healthy group; SOG, sham-operated group; OIR, ovarian ischemia/reperfusion group; ROIR, ranolazine + ovarian ischemia/reperfusion group.

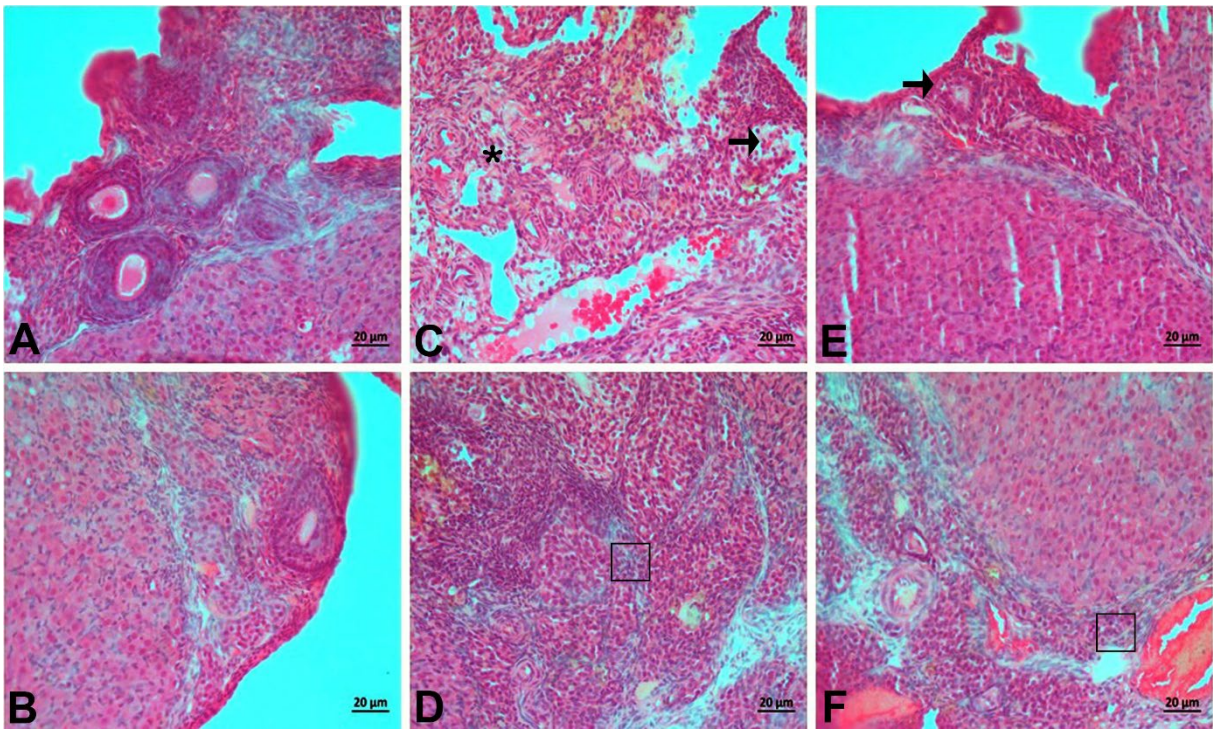


Figure 3. Representative histopathological images of ovarian tissue from the experimental groups. (A,B) The HG and SOG groups exhibited normal ovarian histological architecture. (C,D) The OIR group showed severe follicular degeneration (→), interstitial edema (*), and mononuclear cell infiltration (□). (E,F) The ROIR group exhibited mild follicular degeneration (→) and mononuclear cell infiltration (□), while interstitial edema was absent. Hematoxylin and eosin (H&E) staining. Abbreviations: HG, healthy group; SOG, sham-operated group; OIR, ovarian ischemia/reperfusion group; ROIR, ranolazine + ovarian ischemia/reperfusion group. Scale bar = 20 μ m.

2.3. Double Immunofluorescence Findings

Double immunofluorescence analysis of ovarian tissue revealed no appreciable COX-2, IL-1 β , or IL-6 immunopositivity in the HG and SOG, whereas COX-1 immunopositivity was strong in both groups. In contrast, COX-1 immunoreactivity was absent in the OIR group, while COX-2 and IL-1 β immunopositivities were moderate and IL-6 immunopositivity was strong. In the ROIR group, COX-2, IL-1 β , and IL-6 immunopositivities were all mild, accompanied by the reappearance of mild COX-1 immunopositivity (Table 2, Figures 4 and 5).

Table 2. Double immunofluorescence scores in ovarian tissue (median [minimum–maximum]).

Groups (n = 6)	COX-1	COX-2	IL-1 β	IL-6
HG	3 (2–3) ^a	0 (0–0)	0 (0–0)	0 (0–0)
SOG	3 (3–3) ^a	0 (0–0)	0 (0–0)	0 (0–0)
OIR	0 (0–0)	2 (1–2) ^a	2 (2–3) ^a	3 (3–3) ^a
ROIR	1 (1–2) ^b	1 (1–1) ^b	1 (1–2) ^b	1 (1–2) ^b

Values are presented as median (minimum–maximum). Immunofluorescence scores were analyzed using the Kruskal–Wallis test followed by Dunn’s multiple comparison test. Different superscript letters indicate statistically significant differences between groups ($p < 0.05$). Abbreviations: HG, healthy group; SOG, sham-operated group; OIR, ovarian ischemia/reperfusion group; ROIR, ranolazine + ovarian ischemia/reperfusion group.

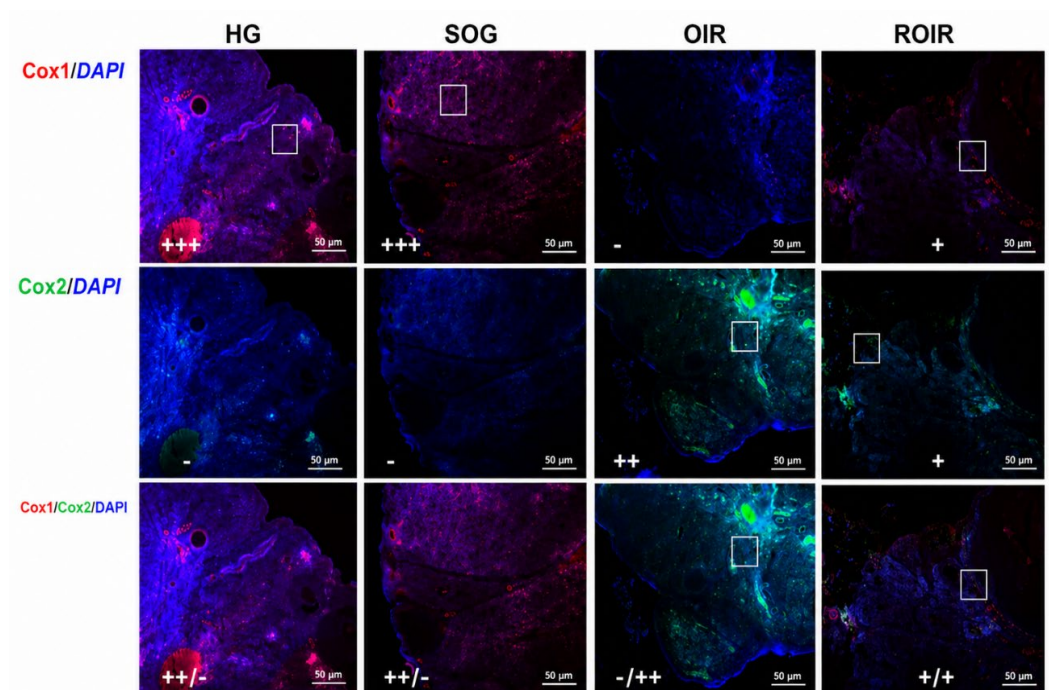


Figure 4. Representative double immunofluorescence images showing COX-1 and COX-2 expression in ovarian tissue. COX-1 immunopositivity was strong in the HG and SOG groups, absent in the OIR group, and mild in the ROIR group. In contrast, COX-2 immunopositivity was absent in the HG and SOG groups, moderate in the OIR group, and mild in the ROIR group. Scale bar = 50 μ m. (□, – (absent), + (mild), ++ (moderate), +++ (strong). Abbreviations: HG, healthy group; SOG, sham-operated group; OIR, ovarian ischemia/reperfusion group; ROIR, ranolazine + ovarian ischemia/reperfusion group.

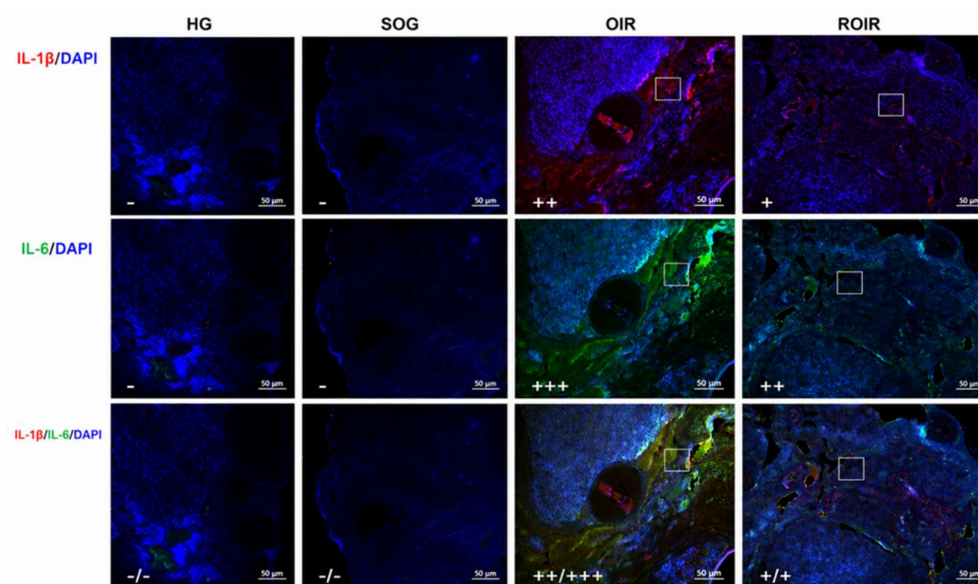


Figure 5. Representative double immunofluorescence images showing IL-1 β and IL-6 expression in ovarian tissue. IL-1 β immunopositivity was absent in the HG and SOG groups, moderate in the OIR group, and mild in the ROIR group. IL-6 immunopositivity was absent in the HG and SOG groups, strong in the OIR group, and mild in the ROIR group. Scale bar = 50 μ m. (□), – (absent), + (mild), ++ (moderate), +++ (strong). Abbreviations: HG, healthy group; SOG, sham-operated group; OIR, ovarian ischemia/reperfusion group; ROIR, ranolazine + ovarian ischemia/reperfusion group.

3. Discussion

In the present study, the protective effects of ranolazine against ovarian I/R injury in female rats were investigated using biochemical, histopathological, and double immunofluorescence analyses. Our biochemical findings demonstrated that I/R markedly increased oxidative stress and inflammatory markers in ovarian tissue, while markedly suppressing the antioxidant defense system. It is well established that excessive production of reactive oxygen species (ROS) during I/R initiates lipid peroxidation (LPO) of cellular membrane lipids, thereby contributing to the progression of cellular injury [17,18]. MDA, a major end product of LPO, is widely recognized as one of the most reliable biochemical indicators of oxidative damage [5,19]. In the present study, the marked elevation of ovarian MDA levels in the I/R group indicates the development of oxidative injury in ovarian tissue. This finding is consistent with previous experimental studies using ovarian I/R models, which have likewise reported pronounced increases in MDA levels [20]. The ability of ranolazine to significantly prevent the I/R-induced increase in ovarian MDA levels suggests that it suppresses ROS-mediated lipid peroxidation. Supporting our findings, a previous study demonstrated that ranolazine attenuated mitochondrial ROS generation during I/R and alleviated oxidative stress. The authors proposed that this protective effect may be associated with inhibition of the late Na⁺ current, leading to reduced intracellular Ca²⁺ overload and subsequent suppression of ROS production [21].

The protective effect of ranolazine against the adverse impact of I/R on ovarian tissue tGSH levels was also investigated. Our findings demonstrated that ranolazine preserved tGSH levels in ovarian tissue subjected to I/R, maintaining them at values close to those of the control group. Glutathione (GSH; γ -glutamyl-cysteinyl-glycine) is a cysteine-containing tripeptide that plays a crucial role in protecting lipids, proteins, and nucleic acids against oxidative damage [22]. During tissue I/R injury, suppression of the antioxidant defense system promotes ROS-mediated cellular damage [5]. The glutathione system constitutes one of the most important non-enzymatic antioxidant defense mechanisms in cells [19].

Therefore, a reduction in tissue tGSH levels indicates that the increased oxidative burden has depleted the endogenous antioxidant capacity [17,22].

Furthermore, the I/R process resulted in a pronounced reduction in the activities of the antioxidant enzymes SOD and CAT in ovarian tissue. Suppression of these enzymatic antioxidant defense mechanisms promotes the accumulation of toxic ROS, such as superoxide radicals and hydrogen peroxide, thereby exacerbating oxidative damage [23]. Consistent with these findings, the significant decreases in tissue tGSH levels as well as SOD and CAT activities observed in the I/R group indicate marked impairment of the antioxidant defense system in ovarian tissue. Previous experimental studies of ovarian I/R injury have likewise reported pronounced reductions in antioxidant parameters [20]. In the present study, the preservation of tGSH levels together with SOD and CAT activities following ranolazine treatment suggests that the drug exerts a protective effect on the antioxidant defense system.

Intracellular Ca^{2+} overload that develops during ischemia leads to mitochondrial dysfunction, ATP depletion, and increased ROS generation [17,23]. To the best of our knowledge, no previous studies have investigated the effects of ranolazine in an ovarian I/R injury model. In addition, a rabbit cardiac I/R model demonstrated that ranolazine effectively prevented ATP depletion and reduced reperfusion-induced injury [16]. Therefore, the antioxidant effects observed in the present study may be associated with the ability of ranolazine to preserve ATP levels and maintain intracellular ionic homeostasis.

The I/R procedure applied to the ovaries resulted in a marked increase in the levels of the pro-inflammatory cytokines TNF- α , IL-1 β , and IL-6. Taken together with previous reports, our findings indicate that inflammation, in addition to oxidative stress, is a major pathological event in organ and tissue I/R injury [24,25]. Following reperfusion, activated inflammatory cells stimulate the production of pro-inflammatory cytokines, including TNF- α , IL-1 β , and IL-6, thereby amplifying tissue injury. These cytokines increase vascular permeability, promote leukocyte infiltration, and further aggravate tissue injury. In the present study, the pronounced elevations in TNF- α , IL-1 β , and IL-6 levels observed in the I/R group indicate the development of a pronounced inflammatory response in ovarian tissue. Previous studies demonstrating that inflammation and oxidative stress progress concomitantly during ovarian torsion further support our findings [26].

In the present study, ranolazine treatment significantly reduced the levels of TNF- α , IL-1 β , and IL-6, representing one of the most notable findings of our study. Previous experimental studies have demonstrated that ranolazine suppresses the inflammatory response and reduces the production of pro-inflammatory cytokines by decreasing intracellular Na^+ levels [14]. Furthermore, a bidirectional relationship exists between ROS generation and inflammation, whereby excessive ROS production triggers inflammatory signaling, while activated inflammatory cells further enhance ROS generation [27,28]. Inhibition of $\text{Na}^+/\text{Ca}^{2+}$ exchange during inflammation has been shown to attenuate the inflammatory response both in vitro in human endothelial cells and in clinical studies involving humans [13]. Therefore, the ability of ranolazine to suppress both oxidative stress and inflammation in the present study supports its multifactorial protective effects against ovarian I/R injury.

The biochemical alterations observed in oxidative stress and inflammatory markers were consistent with the histopathological findings. The ovarian I/R (OIR) group exhibited marked follicular degeneration, interstitial edema, and mononuclear cell infiltration. In contrast, histopathological damage was substantially attenuated in the ranolazine-treated OIR (ROIR) group. Excessive ROS generation, lipid peroxidation, and activation of inflammatory cells during I/R contribute to characteristic histopathological alterations, including cellular degeneration, increased vascular permeability, interstitial edema, and inflamma-

tory cell infiltration [17,29]. Previous experimental studies using ovarian I/R models have similarly demonstrated that I/R induces follicular degeneration, stromal edema, and inflammatory cell infiltration in ovarian tissue, and that suppression of oxidative stress and inflammation significantly ameliorates these histopathological changes [20]. Collectively, these mechanisms may contribute to preserving cellular integrity, thereby attenuating the histopathological damage observed in the present study.

In the present study, immunofluorescence analysis revealed prominent COX-1 immunopositivity in the healthy (HG) and sham-operated (SOG) groups, whereas the expression of COX-2, IL-1 β , and IL-6 was minimal. COX-1 is constitutively expressed in many tissues under physiological conditions and plays a fundamental role in maintaining normal tissue homeostasis [30]. In contrast, the ovarian I/R (OIR) group exhibited a marked loss of COX-1 expression accompanied by pronounced increases in COX-2, IL-1 β , and particularly IL-6 immunoreactivity. Previous studies have shown that oxidative stress and inflammatory stimuli induced by I/R upregulate COX-2 expression, thereby contributing to the progression of tissue injury [27,30]. Accordingly, the reduction in COX-1 immunopositivity together with the increase in COX-2 expression observed in the OIR group provides immunofluorescence evidence of the inflammatory response induced in ovarian tissue. Consistent with our findings, previous experimental studies using ovarian I/R models have also reported decreased COX-1 activity and significantly increased COX-2 activity following I/R [31]. Parallel to the increase in COX-2 immunopositivity, IL-1 β and IL-6 immunoreactivities were also markedly elevated in the OIR group. IL-1 β is recognized as one of the principal cytokines responsible for initiating inflammatory cell activation, whereas IL-6 plays a pivotal role in sustaining the inflammatory response and promoting the progression of tissue injury [32]. In the ranolazine-treated group, COX-2, IL-1 β , and IL-6 immunopositivities were markedly attenuated, indicating that ranolazine effectively suppresses the inflammatory response. This finding is consistent with previous experimental and clinical studies demonstrating that ranolazine inhibits Na⁺/Ca²⁺ exchange, thereby reducing inflammatory responses and the production of pro-inflammatory cytokines [13]. Therefore, the decreased expression of COX-2, IL-1 β , and IL-6 observed at the immunofluorescence level in the present study likely reflects the tissue-level manifestation of the antioxidant and anti-inflammatory effects of ranolazine [13,14,21].

Overall, the present study represents a preventive pretreatment model in which ranolazine was administered before the induction of ischemia. Accordingly, our findings demonstrate only the protective effects of ranolazine under controlled experimental conditions and do not provide evidence regarding its efficacy when administered after the onset of ovarian torsion or following surgical detorsion. Future studies employing treatment protocols that more closely reflect clinical practice are essential to determine whether ranolazine confers similar protective effects when administered after the diagnosis of ovarian torsion or during the reperfusion period.

Although the molecular mechanisms of ranolazine were not directly investigated in the present study, previous experimental evidence suggests that inhibition of the late Na⁺ current reduces intracellular Ca²⁺ overload, preserves mitochondrial integrity, and suppresses oxidative stress and inflammatory responses [13,14,21]. Therefore, the antioxidant and anti-inflammatory effects observed in the present study may, at least in part, be explained by these previously proposed mechanisms.

This study has several limitations. First, no a priori sample size calculation was performed. The sample size was determined based on previous comparable experimental pharmacology studies while adhering to the principles of the 3Rs (Replacement, Reduction, and Refinement) to minimize animal use. Future studies should include formal power analyses to determine the optimal sample size. Second, the estrous cycle stage of the rats

was not determined before the experimental procedures. Because hormonal fluctuations associated with the estrous cycle may influence ovarian physiology and the response to ischemia/reperfusion injury, this factor should be addressed in future studies. Third, ranolazine was evaluated using only a single dose and a single treatment regimen; therefore, the effects of different doses and treatment durations were not investigated. Fourth, the present study evaluated only acute ovarian I/R injury, and the long-term protective effects of ranolazine were not assessed. Fifth, although biochemical, histopathological, and immunofluorescence analyses were comprehensively performed, the intracellular signaling pathways, mitochondrial function, and key molecular mechanisms underlying the protective effects of ranolazine, including the Nrf2/HO-1 pathway, NF- κ B signaling, and apoptosis-related pathways, were not investigated. In addition, the long-term effects of ranolazine on ovarian reserve, reproductive hormone levels, follicle count, ovulation, and fertility were not evaluated. The study did not include a healthy group treated with ranolazine alone. Therefore, the independent effects of ranolazine on ovarian oxidative stress, inflammatory markers, and histopathological parameters could not be evaluated. Another limitation of the present study is that ranolazine was administered before the induction of ischemia. Therefore, the study represents a preventive pretreatment model rather than a clinically applicable therapeutic protocol administered after the onset of ovarian torsion. Future studies are warranted to evaluate the efficacy of ranolazine when administered after the onset of torsion or following surgical detorsion. Finally, as this study was conducted in an experimental animal model, caution should be exercised when extrapolating the findings directly to clinical practice. Therefore, further comprehensive mechanistic studies are warranted to validate the therapeutic potential of ranolazine in ovarian I/R injury.

4. Materials and Methods

4.1. Animals

Twenty-four female Wistar albino rats (10–12 weeks old, weighing 250–262 g) were used in the present study. The animals were supplied by the Medical Experimental Application and Research Center of Erzincan Binali Yıldırım University. Prior to the experimental procedures, rats were acclimatized under standard laboratory conditions (22 ± 2 °C; 12-h light/12-h dark cycle) with unrestricted access to food and water. The study protocol was reviewed and approved by the Local Animal Ethics Committee (Approval date: 21 May 2026; Meeting No.: 2026/05).

4.2. Chemicals

Ranolazine (500 mg tablets; Menarini Pharmaceuticals, Istanbul, Türkiye) and ketamine (Pfizer Pharmaceuticals Co., Ltd., Istanbul, Türkiye) were used in the present study.

4.3. Animal Groups

The rats were randomly divided into four groups: a healthy group (HG), a sham-operated group (SOG), an ovarian ischemia/reperfusion (OIR) group, and a ranolazine-treated ovarian ischemia/reperfusion (ROIR) group.

4.4. Experimental Procedure

All surgical procedures were performed under sterile conditions in an appropriate experimental laboratory. Anesthesia was induced with ketamine (60 mg/kg, intraperitoneally [i.p.]), and anesthesia was maintained with intermittent sevoflurane inhalation as required. Adequate anesthesia was confirmed by the absence of pedal withdrawal and corneal reflexes [33]. Following anesthesia, the ovaries were exposed through a 2.0–2.5 cm

midline lower abdominal incision. In the SOG group, the ovaries were exposed without any additional intervention, and the abdominal incision was closed with surgical sutures. In the OIR and ROIR groups, the right ovary, together with the fallopian tube and its vascular pedicle, was rotated 360° clockwise to induce ovarian torsion. Ischemia was maintained for 1 h using atraumatic vascular clamps. At the end of the ischemic period, the clamps were removed, the ovaries were detorsioned, and reperfusion was allowed for 6 h. One hour before the induction of ischemia, rats in the ROIR group ($n = 6$) received ranolazine (50 mg/kg) orally by gavage as a preventive pretreatment [34]. Ranolazine tablets (500 mg; Menarini Pharmaceuticals) were crushed into a fine powder using a mortar and pestle. The required dose was calculated based on the ranolazine content of each tablet and the body weight of each rat. Immediately before administration, the powdered tablets were freshly suspended in distilled water. The suspension was administered orally by gavage at a dose of 50 mg/kg. Animals in the HG, SOG, and OIR groups ($n = 6$ each) received an equivalent volume of distilled water by the same route. At the end of the reperfusion period, all animals were euthanized under deep ketamine anesthesia (120 mg/kg, i.p.). Ovarian tissues were immediately excised after euthanasia. One ovary from each rat was used for biochemical analyses, including the determination of tissue concentrations of MDA, total glutathione (tGSH), TNF- α , IL-1 β , and IL-6, as well as the activities of total superoxide dismutase (SOD) and CAT. The contralateral ovary was fixed intact in 10% neutral buffered formalin and processed in its entirety for histopathological and double immunofluorescence analyses. The findings obtained from all experimental groups were statistically compared to evaluate the effects of ranolazine on ovarian I/R injury.

4.5. Biochemical Analyses

4.5.1. Sample Preparation

After the tissue samples were weighed, they were cut into small pieces, rapidly frozen in liquid nitrogen, and homogenized using a mortar and pestle. The homogenates were mixed with phosphate-buffered saline (PBS, pH 7.4) at a 1:10 (w/v) ratio and vortexed for 10 s. The samples were then centrifuged at $10,000 \times g$ for 20 min at 4 °C using a refrigerated centrifuge (Allegra X-30R, Beckman Coulter, Brea, CA, USA), and the resulting supernatants were collected for biochemical analyses. All supernatants were stored at −80 °C until biochemical analysis.

4.5.2. Determination of Ovarian Tissue MDA, tGSH, SOD, and CAT Levels

Tissue levels of MDA, GSH, and SOD were determined using rat-specific enzyme-linked immunosorbent assay (ELISA) kits (MDA, YLA0029Ra; GSH, YLA0121Ra; SOD, YLA0115Ra; YL Biont Co., Ltd., Shanghai, China) according to the manufacturer's instructions. CAT activity was determined using the method described by Goth [35], adapted for tissue homogenates. Briefly, ovarian tissues were homogenized in phosphate-buffered saline (PBS; 1:10, w/v) and centrifuged, and the resulting supernatants were used for the analysis. CAT activity was normalized to the total protein content of each tissue homogenate and expressed as U/g protein. Total protein concentrations were determined using the Bradford method [36], with bovine serum albumin as the standard. Absorbance was measured at 595 nm using a DU 730 UV/Vis spectrophotometer (Beckman Coulter Inc., Brea, CA, USA).

4.5.3. Determination of Ovarian Tissue TNF- α , IL-1 β and IL-6 Levels

The tissue concentrations of TNF- α (Rat TNF- α ELISA Kit, Catalog No. YLA0118Ra), IL-1 β (Rat IL-1 β ELISA Kit, Catalog No. YLA0030Ra), and IL-6 (Rat IL-6 ELISA Kit, Catalog No. YLA0031Ra) were measured using rat-specific ELISA kits supplied by YL Biont Co., Ltd. (Shanghai, China), according to the manufacturer's instructions.

4.6. Histopathological Analysis

Ovarian tissue samples were fixed in 10% neutral buffered formalin, routinely processed, and embedded in paraffin. Serial sections (5 µm thick) were obtained from each paraffin block, deparaffinized in xylene, and rehydrated through a graded ethanol series (100%, 90%, and 70%). The sections were subsequently stained with hematoxylin and eosin (H&E) for histopathological evaluation. Histopathological examination was performed under a light microscope. Follicular degeneration, mononuclear cell infiltration, and interstitial edema were considered as histopathological lesions. All tissue sections were independently analyzed by two blinded pathologists who were unaware of the experimental groups using a semiquantitative scoring system. The evaluation was performed at ×20 magnification by randomly selecting six different microscopic fields from each tissue section. The severity of each lesion was scored as follows: 0 = absent, 1 = mild, 2 = moderate, and 3 = severe.

4.7. Double Immunofluorescence Analysis

Paraffin-embedded ovarian tissue sections (5 µm) mounted on poly-L-lysine-coated slides were deparaffinized in xylene and rehydrated through a graded ethanol series. After washing with phosphate-buffered saline (PBS), antigen retrieval was performed by heating the sections in citrate buffer (pH 7.4) at 800 W for two cycles of 5 min. The sections were then washed twice with PBS for 10 min each and incubated in PBS containing 0.25% Triton X-100 and gelatin for 10 min to enhance tissue permeability. To minimize nonspecific antibody binding, the sections were blocked with 5% bovine serum albumin (BSA) for 1 h at room temperature. Subsequently, the sections were incubated overnight at 4 °C with the following primary antibody pairs diluted 1:200 in blocking solution: mouse monoclonal anti-COX-1 (Santa Cruz Biotechnology, Dallas, TX, USA (Cat. No. sc-19998)) together with rabbit polyclonal anti-COX-2 (Cat. No. BT-AP08027; Bioassay Technology Laboratory, Shanghai, China), or mouse monoclonal anti-IL-1β (Cat. No. sc-52012; Santa Cruz Biotechnology, Dallas, TX, USA) together with rabbit polyclonal anti-IL-6 (Affinity Biosciences, Cincinnati, OH, USA (Cat. No. DF6087)). Following PBS washes, the sections were incubated for 45 min with a secondary antibody cocktail containing anti-mouse Texas Red (Cat. No. 16853; AAT Bioquest, Pleasanton, CA, USA) and anti-rabbit FITC (Elabscience Biotechnology Co., Ltd., Wuhan, China (Cat. No. E-AB-1014)), each diluted 1:100 in 1% BSA. Thereafter, the sections were rinsed in 10 mM CuSO₄/50 mM NH₄Cl solution for 10 min and washed with distilled water. Cell nuclei were counterstained with 4',6-diamidino-2-phenylindole (DAPI). Fluorescent images were acquired using a Zeiss Axiolab 5 fluorescence microscope (Carl Zeiss Microscopy GmbH, Jena, Germany) equipped with an Axiocam 305 color camera (Carl Zeiss Microscopy GmbH, Jena, Germany) and Colibri 3 LED illumination system (Carl Zeiss Microscopy GmbH, Jena, Germany). Semiquantitative analysis was performed using ZEN Blue 3.1 software. Cyclooxygenase-1 (COX-1) and IL-1β immunoreactivity were visualized in the red fluorescence channel, whereas Cyclooxygenase-2 (COX-2) and IL-6 immunoreactivity were detected in the green fluorescence channel. Immunofluorescence staining intensity was scored as 0 (negative), 1 (mild), 2 (moderate), 3 (strong), and 4 (very strong).

4.8. Statistical Analysis

The primary endpoint of the study was the ovarian tissue MDA level. Secondary endpoints included ovarian tissue tGSH levels, SOD and CAT activities, TNF-α, IL-1β, and IL-6 concentrations, histopathological lesion scores (follicular degeneration, mononuclear cell infiltration, and interstitial edema), and semiquantitative immunofluorescence scores for COX-1, COX-2, IL-1β, and IL-6. The sample size (n = 6 per group) was determined

based on previous comparable experimental pharmacology studies demonstrating significant differences in the primary endpoint while adhering to the principles of the 3Rs (Replacement, Reduction, and Refinement) to minimize animal use. Biochemical data were expressed as mean $\pm$ standard deviation (SD). Data normality was assessed using the Shapiro–Wilk test, and homogeneity of variances was evaluated using Levene’s test before performing one-way analysis of variance (ANOVA). Differences among groups were analyzed using one-way ANOVA followed by Tukey’s honestly significant difference (HSD) post hoc test when the overall ANOVA was significant. Histopathological and double immunofluorescence scores were analyzed using the Kruskal–Wallis test followed by Dunn’s post hoc test. Effect sizes (η^2) and 95% confidence intervals for descriptive statistics are provided in the Supplementary Table S1. Individual raw data together with complete one-way ANOVA and Tukey’s HSD multiple-comparison results are also provided in the Supplementary Table S1. All statistical analyses were performed using IBM SPSS Statistics version 18.0 (IBM Corp., Armonk, NY, USA), and a p value < 0.05 was considered statistically significant.

5. Conclusions

This study demonstrated that ranolazine attenuated acute ovarian I/R injury in rats. Pretreatment with ranolazine significantly reduced oxidative stress and inflammatory responses while improving antioxidant status. These biochemical findings were supported by histopathological and immunofluorescence analyses, indicating acute tissue protection in this experimental model. However, the present findings are limited to acute biochemical and histopathological outcomes and do not permit conclusions regarding long-term ovarian function, fertility preservation, or clinical reproductive outcomes. Whether ranolazine provides therapeutic benefit when administered after the onset of ovarian torsion or following surgical detorsion remains to be determined in future studies.

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Abbreviations

The following abbreviations are used in this manuscript:

I/R	Ischemia/reperfusion
COX-1	Cyclooxygenase-1
COX-2	Cyclooxygenase-2
Na ⁺ /K ⁺ -ATPase	Sodium–Potassium Adenosine Triphosphatase
MDA	Malondialdehyde
tGSH	Total glutathione
SOD	Superoxide dismutase
CAT	Catalase
TNF- α	Tumor necrosis factor- α
IL-1 β	Interleukin-1 β
IL-6	Interleukin-6
ROS	Reactive oxygen species
LPO	Lipid peroxidation
ELISA	Enzyme-Linked Immunosorbent Assay
H&E	Hematoxylin and eosin

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