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# Research Article
# Effect of Sugammadex on Ischemia-Reperfusion-Induced Oxidative and Inflammatory Ovarian Damage in Rats: Biochemical and Histopathological Evaluation

[1]Berrin Goktug Kadioglu, [2]Bahadir Suleyman, [2]Renad Mammadov, [2]Bulent Yavuzer, [2]Durdu Altuner, [3]Gulce Naz Yazıcı , [4]Mine Gulaboglu and [2]Halis Suleyman

[1]Department of Obstetrics and Gynecology, University of Health Sciences, Erzurum Regional Training and Research Hospital, Erzurum 25240, Turkey
[2]Department of Pharmacology, Faculty of Medicine, Erzincan Binali Yildirim University, Erzincan 24100, Turkey
[3]Department of Histology and Embryology, Faculty of Medicine, Erzincan Binali Yildirim University, Erzincan 24100, Turkey
[4]Department of Biochemistry, Faculty of Pharmacy, Ataturk University, Erzurum 25240, Turkey

## Abstract

**Background and Objective:** Polymorphonuclear leukocytes (PMNLs) play an important role in the pathogenesis of oxidative ischemia-reperfusion (I/R) damage. There is information that sugammadex inhibits PMNL infiltration into organs and tissues undergoing I/R. Present study aimed to investigate the potential protective effect of sugammadex against I/R-induced ovarian damage in rats and to elucidate its mechanism of action. **Materials and Methods:** The 24 female albino Wistar-type rats were divided into four groups as follows: Sham operation (SG), ovarian I/R (IRG), sugammadex (4 mg/kg) +ovarian I/R (SIR-4) and sugammadex (8 mg/kg) +ovarian I/R (SIR-8). Sugammadex was administered intraperitoneally. The animals were subjected to 2 hrs of ischemia followed by 2 hrs of reperfusion. **Results:** Sugammadex significantly inhibited the increase in I/R-induced malondialdehyde and the decrease in total glutathione, superoxide dismutase and catalase levels in ovarian tissue at a dose of 8 mg/kg compared with the dose of 4 mg/kg. Moderate follicular degeneration, vascular congestion, necrosis, oedema and hemorrhage were observed in the sugammadex (4 mg/kg) group, which significantly reduced the increase in I/R-related PMNL infiltration in ovarian tissue. Only mild vascular congestion was observed in the ovarian tissue of the 8 mg/kg sugammadex group, which did not show PMNL infiltration. **Conclusion:** Histopathological findings show that sugammadex protects ovarian tissue from oxidative and inflammatory injury of the I/R by inhibiting PMNL infiltration. Results also suggest that an 8 mg/kg dose of sugammadex, which completely inhibits PMNL infiltration into ovarian tissue, may be more beneficial than the 4 mg/kg dose in the treatment of I/R-induced ovarian damage.

Key words: Ischemia-reperfusion damage, ovarian damage, oxidative and inflammatory, rats, sugammadex

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Corresponding Author: Berrin Goktug Kadioglu, Department of Obstetrics and Gynecology, University of Health Sciences, Erzurum Regional Training and Research Hospital, Erzurum 25240, Turkey



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

While ischemia is a decrease or complete cessation of blood flow and oxygen to the tissues for various reasons, reperfusion is a condition of re-blood supply and reoxygenation of tissues[1]. In the literature, this event is defined as ischemia-reperfusion (I/R). As it is known, since xanthine oxidase (XO) cannot metabolize hypoxanthine to xanthine in an oxygen-free environment, they accumulate excessively in ischemic tissue. Therefore, in reperfusion, the metabolism of hypoxanthine by XO is initiated and reactive oxygen radicals (ROS) are produced as an intermediate product[2]. Superoxide anion ($O_2^-$), hydroxyl radicals (•OH), Hydrogen Peroxide ($H_2O_2$), hypochloric acid and peroxynitrite composed of nitric oxide are the most examined types of ROSs today[3]. These ROSs, known as reperfusion mediators, increase the production of toxic products such as malondialdehyde (MDA) from lipids by peroxidation of cell membrane lipids (LPO)[4]. In addition to the increase of oxidants such as MDA in the I/R event, a decrease in endogenous antioxidant levels is also observed[5]. Moreover, polymorphonuclear leukocytes (PMNL) also play an important role in shaping reperfusion damage; the release of ROSs from these activated PMNLs forms the respiratory burst event[1]. Ovarian I/R damage in the clinic occurs after the applied detorsion procedure to restore blood supply to the torsioned ovaries[6]. As mentioned above, reperfusion of torsion-induced ischemia with detorsion causes aggravation of oxidative and inflammatory events in ovarian tissue. This information obtained from the literature indicates that antioxidant and anti-inflammatory treatment is important before and after detorsion of torsioned ovaries.

In present study, sugammadex, whose effect will try against ovarian I/R damage, is a modified gamma-cyclodextrin. Sugammadex is a drug that antagonizes the neuromuscular blockade induced by rocuronium and vecuronium[7]. For this reason, sugammadex is preferred to eliminate the rocuronium effect after surgery within a short time[8]. It has been reported that sugammadex significantly inhibits lymphocytic infiltration in the lung and edema and inflammatory cell infiltration due to the I/R procedure in the lower extremity muscle[9,10]. Also, in another study, it was reported that sugammadex protects brain tissue from I/R damage[11]. There was no information in the literature investigating the protective effect of sugammadex against I/R-induced ovarian damage and associated with its antioxidant activity. The aim of present study was to investigate the possible protective effect of sugammadex against I/R-induced ovarian damage in rats and to elucidate its mechanism of action.

## MATERIALS AND METHODS

**Study area:** The present study was carried out in Erzincan Binali Yildirim University Animal Experiments Laboratory between 02-01-2023 and 09-01-2023 in one week.

**Animals:** A total of 24 albino Wistar-type female rats with weights ranging between 255-277 g and 8-9 weeks old obtained from the Experimental Animal Research and Application Center of Erzincan Binali Yildirim University, were used in the experiment. The animals were randomly divided into 4 groups in such a way that their average body weights were similar. The animals were housed in groups (n = 6) in a suitable laboratory environment at room temperature of 22°C, 12 hrs in darkness and 12 hrs in light, humidity levels were 30-70%. The experimental animals were fed *ad libitum* with standard pellet chow (experimental animal chow; Bayramoglu A.Ş., Erzurum, Turkey) and tap water. All animal procedures were approved by Erzincan Binali Yildirim University Animal Experimentation Local Ethics Committee (Meeting date: 28-04-2022; Meeting no.: 2022/04 and Decision no.: 26) and all experiments performed according to the guidelines from Directive 2010/63/EU of the European Parliament (Approval Number 2016-24-199) as well as the ARRIVE guidelines[12].

**Chemicals:** Ketamine, were obtained from Pfizer Pharmaceuticals Ltd., Sti. (Turkey) and sugammadex (200 mg/2 mL injectable solution) was obtained from the Sanofi Pharmaceutical Industry (Turkey).

**Experimental groups:** Animals were divided into four groups: Group with sham operation on the ovaries (SG), group with I/R alone to ovaries (IRG), sugammadex (4 mg/kg) +ovarian I/R applied (SIR-4) and sugammadex (8 mg/kg) +ovarian I/R applied (SIR-8) groups.

**Anesthesia procedure:** Surgical procedures on the rats were carried out under sterile conditions by injecting 60 mg/kg of ketamine intraperitoneally (IP) and sniffing xylazine at appropriate intervals. After the injection of ketamine, the rats were kept waiting for the appearance of the appropriate period during which the surgical intervention would be performed. The period when the animals remain motionless in the supine position is considered a favorable period of anesthesia for surgical intervention[13].

**Surgical procedure:** The 1 hr before the I/R procedure was administered on rat ovaries, SIR-4 (n = 6) and SIR-8 (n = 6) rat

groups were injected sugammadex at doses of 4 and 8 mg/kg via IP route, respectively. The IRG (n = 6) and SG (n = 6) rat groups were also given the same volume of normal saline (0.9% NaCl) as solvent by the same route. During the anesthesia period, the ovaries were reached by opening the lower part of the abdomen 2-2.5 cm long vertically for all rat groups. Ovaries were closed without I/R in the SG group. Vascular clips were applied to the lower part of the right ovaries of SIR-4, SIR-8 and IRG group rats for 2 hrs ischaemia and 2 hrs reperfusion[14]. We use 2 hrs of ischaemia followed by 2 hrs of reperfusion because during this period, parameters that lead to oxidative and inflammatory ovarian damage are significantly enhanced[15]. The rats' right ovaries were removed immediately after 2 hrs of reperfusion. The removed ovarian tissues were examined biochemically and histopathologically. Biochemical and histopathological findings obtained from animals in the SIR-4, SIR-8 and SG groups were evaluated by comparing with the findings obtained from the IRG group.

### Biochemical analyzes

**Preparation of samples:** At this stage, 0.2 g of each ovarian tissue sample was weighed for biochemical analysis. The tissue samples were washed with cold (+4°C) 0.15 M potassium chloride (KCl). Tissue samples were homogenized in liquid nitrogen. They were then transferred to buffer solution (50mM, pH 7.4). Tissue homogenates were centrifuged at 5000 rpm for 20 min at +4°C and the supernatants were extracted for analysis of malondialdehyde (MDA), total glutathione (tGSH), superoxide dismutase (SOD) and catalase (CAT).

**Determination of MDA, GSH, SOD, CAT and protein:** Ovarian tissues MDA, GSH and SOD determination will be measured using commercially available Enzyme-Linked Immunosorbent Assay (ELISA) kits that have been prepared for use in laboratory animals (MDA: Cat. no. 10009055; tGSH: Cat. no. 703002; SOD: Cat. no. 706002; Cayman Chemical Company). The kit instructions were followed for each assay. The method proposed by Góth[16] will be performed to determine the CAT. Protein will be determined spectrophotometrically at 595 nm using the Bradford[17] method.

**Histopathological examination:** The tissue samples were embedded in a 10% formaldehyde solution for a period of 72 hrs. Then, the tissue samples were placed in cassettes and washed in tap water for 24 hrs. To remove water from the tissues, the samples were then subjected to a conventional alcohol treatment (70, 80, 90 and 100%). The tissues were processed through xylol and subsequently embedded in paraffin. The paraffin blocks were cut into four-to-five-micron sections and stained with Hematoxylin and Eosin (H&E). Using the DP2-SAL firmware program and a Light Microscope (Olympus Inc., Tokyo, Japan), sections were photographed and evaluated. One central and five peripheral areas were selected for semi-quantitative scoring from the serial sections. For each subject, the criteria for degeneration were scored in the selected areas. Damage to the ovarian tissue has been described as the presence of degeneration, vascular congestion, interstitial edema, hemorrhage and infiltration of PMNL. The score of each sample for each criterion was as follows: (0) Indicating no damage, (1) Mild damage, (2) Moderate damage and (3) Severe damage. On the other hand, primordial follicles, developing follicles, atretic follicles and corpus luteum were counted to understand the effects of I/R and sugammadex on ovarian follicles. A pathologist blinded to the experimental groups performed histopathologic evaluation and scoring.

**Statistical analysis:** The IBM SPSS Statistics for Windows program (IBM Corp., released in 2013, version 22.0., Armonk, New York) was used for all statistical analyses. The results were expressed as "Mean±Standard Error of the Mean" (Mean±SEM) for the biochemical and follicle count results. The Shapiro-Wilk test was used to determine the normality of the distribution of the continuous variables for the biochemical and follicle count results. As the data were normally distributed, One-way Analysis of Variance (ANOVA) was used to determine the significance of the differences between the groups. Afterwards, Levene's test was performed to determine whether the homogeneity of the variances was ensured. In order to determine the group that created the difference, Tukey's HSD (honestly significant difference) was used as a *post hoc* test if the homogeneity of variances was ensured and Games-Howell was used if not. In addition, the Kruskal-Wallis test, a non-parametric test, was performed on the histopathological data to determine the difference between the groups. The group that created the difference was also determined with the Mann-Whitney U test ($p<0.05$). Histopathological results were expressed as "median (minimum-maximum)". The statistical level of significance for all of the tests was considered to be 0.05.

## RESULTS

### Biochemical findings

**MDA analysis results of ovarian tissue:** Figure 1a and Table 1, MDA levels in ovarian tissues of rats subjected to the

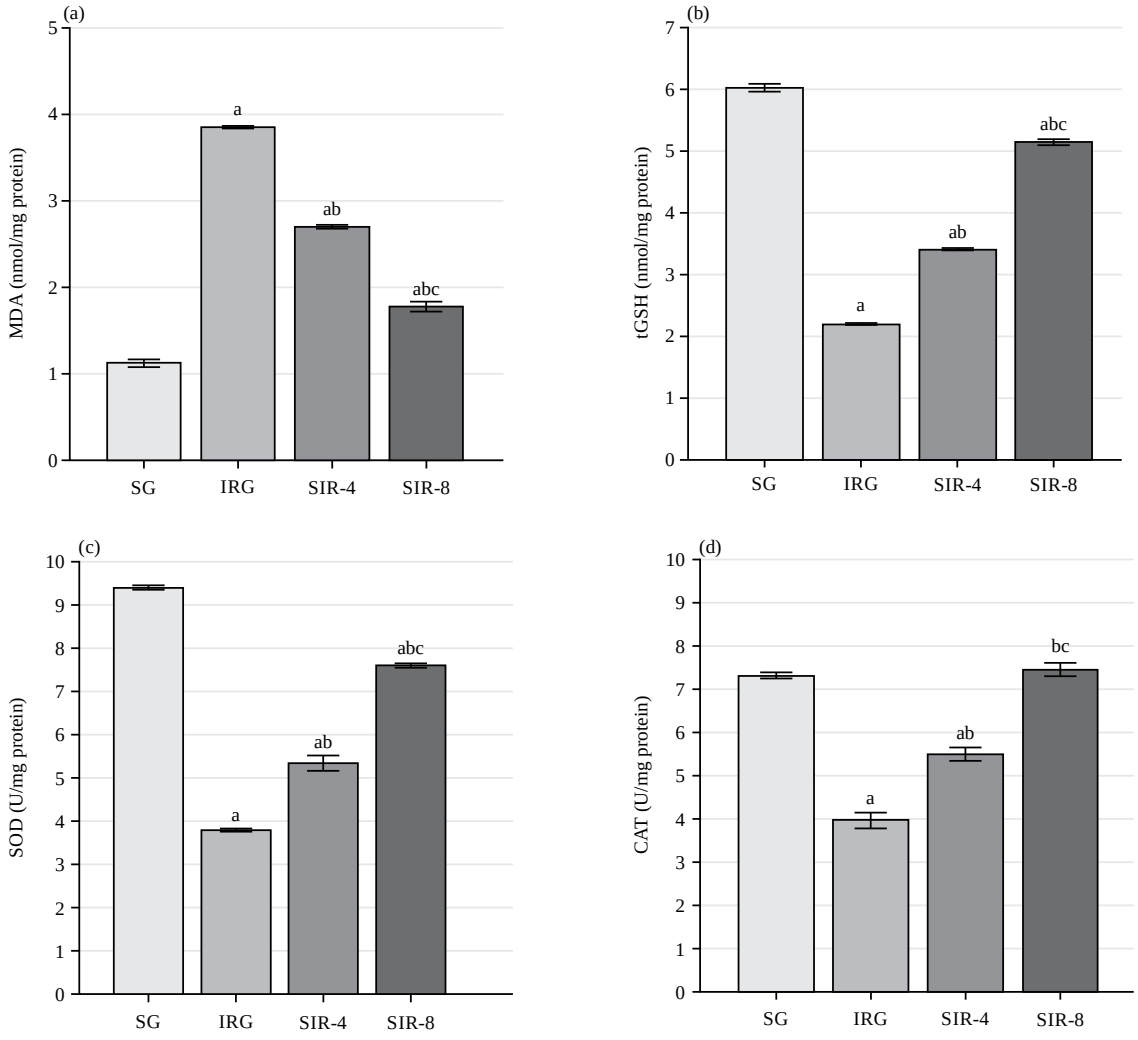


Fig. 1(a-d): Effects of ischemia-reperfusion and two different doses of sugammadex on, (a) MDA, (b) tGSH, (c) SOD and (d) CAT levels in rat ovarian tissue

Bars are Mean±SEM (standard error), [a]Means p<0.05 according to the SG, [b]Means p<0.001 according to the IRG and [c]Means p<0.001 according to the SIR-4. Statistical analyses were performed using One-way ANOVA followed by Games-Howell *post hoc* test for MDA and SOD, Tukey's honestly significant difference (HSD) *post hoc* test for tGSH and CAT, SG: Group with sham operation on the ovaries, IRG: Group with I/R alone to ovaries, SIR-4: Sugammadex (4 mg/kg) +ovarian I/R group, SIR-8: Sugammadex (8 mg/kg) +ovarian I/R group, MDA: Malondialdehyde, tGSH: Total Glutathione, SOD: Superoxide dismutase and CAT: Catalase

I/R procedure showed a significant increase compared to the sham-operated group (p<0.001). Sugammadex significantly suppressed the I/R-induced increase in ovarian tissue MDA levels at doses of 4 mg/kg (p<0.001) and 8 mg/kg (p<0.001). The group with the lowest MDA levels in ovarian tissue was the group receiving 8 mg/kg sugammadex.

**tGSH analysis results of ovarian tissue:** Figure 1b and Table 1, tGSH levels in the ovarian tissues of rats subjected to the I/R procedure showed a significant decrease compared to the sham-operated group (p<0.001). Sugammadex significantly prevented the I/R-induced decrease in ovarian tissue tGSH levels at doses of 4 mg/kg (p<0.001) and 8 mg/kg (p<0.001). The group with the highest tGSH level in the ovarian tissue was the group that received 8 mg/kg of sugammadex.

**SOD analysis results of ovarian tissue:** The SOD activity was found to be significantly lower in the ovarian tissue of the I/R group compared to the sham-operated group (p<0.001). The SOD activity was found to be statistically significantly

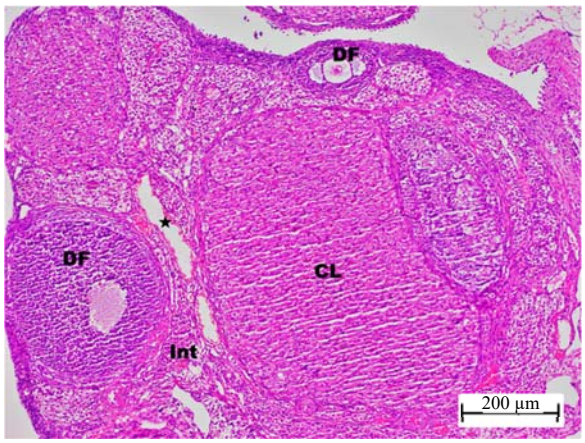


Fig. 2: Ovarian tissue stained with hematoxylin and eosin in the SG group
DF: Developing follicle, Int: Interstitial area, CL: Corpus luteum, ★Blood vessel, (H&E ×100) and SG: Group with sham operation on the ovaries

Table 1: Oxidant and antioxidant levels in rat ovarian tissue after ischaemia-reperfusion and two different doses of sugammadex

| | Biochemical variables (Mean±SEM) | | | |
|---|---|---|---|---|
| Group | MDA | tGSH | SOD | CAT |
| SG (n = 6) | 1.13±0.04 | 6.04±0.06 | 9.37±0.06 | 7.32±0.07 |
| IRG (n = 6) | 3.85±0.03 | 2.22±0.03 | 3.80±0.03 | 4.00±0.17 |
| SIR-4 (n = 6) | 2.71±0.03 | 3.44±0.03 | 5.34±0.17 | 5.51±0.14 |
| SIR-8 (n = 6) | 1.79±0.09 | 5.16±0.05 | 7.61±0.05 | 7.46±0.15 |
| | p-value | | | |
| Group comparisons | MDA** | tGSH* | SOD** | CAT* |
| SG vs. IRG | <0.001 | <0.001 | <0.001 | <0.001 |
| SG vs. SIR-4 | <0.001 | <0.001 | <0.001 | <0.001 |
| SG vs. SIR-8 | 0.002 | <0.001 | <0.001 | 0.898 |
| IRG vs. SIR-4 | <0.001 | <0.001 | <0.001 | <0.001 |
| IRG vs. SIR-8 | <0.001 | <0.001 | <0.001 | <0.001 |
| SIR-4 vs. SIR-8 | <0.001 | <0.001 | <0.001 | <0.001 |

*Statistical analyses were performed using the One-way ANOVA test followed by Tukey's HSD (honestly significant difference) test as *post hoc* **Statistical analyses were performed using the One-way ANOVA test followed by Games-Howell test as *post hoc*, SG: Group with sham operation on the ovaries, IRG: Group with I/R alone to ovaries, SIR-4: Sugammadex (4 mg/kg) +ovarian I/R group, SIR-8: Sugammadex (8 mg/kg) +ovarian I/R group, MDA: Malondialdehyde, tGSH: Total Glutathione, SOD: Superoxide dismutase and CAT: Catalase and SEM: Standard error of mean

higher in the 4 mg/kg (p<0.001) and 8 mg/kg (p<0.001) sugammadex treatment groups compared to the I/R group. The group with the highest SOD activity in the ovarian tissue was the group that received 8 mg/kg of sugammadex (Fig. 1c and Table 1).

**CAT analysis results of ovarian tissue:** Figure 1d and Table 1, CAT activity in the ovarian tissue of rats in the I/R group was significantly lower than in the sham-operated group (p<0.001). The CAT activity was statistically significantly higher in the 4 mg/kg (p<0.001) and 8 mg/kg (p<0.001) sugammadex treatment groups compared with the I/R group. The group with the highest level of CAT activity in the ovarian tissue was the group that received 8 mg/kg of sugammadex.

**Histopathological evaluation:** Figure 2 and Table 2, the normal appearance of the developing follicle, interstitial area, corpus luteum and blood vessel structures is observed in the ovarian tissue of the SG group. However, severe degeneration in the developing follicles, necrosis, congested blood vessels, intense edema in the interstitial area and hemorrhage in the intermediate connective tissue were observed in the ovaries of animals that had undergone the I/R procedure (Fig. 3 and Table 2). In addition, severe PMNL infiltration was observed in the IRG group (Fig. 4 and Table 2). In the ovaries of the SIR-4 group treated with 4 mg/kg sugammadex, moderate degeneration in developing follicles, necrosis, congested blood vessels, edema in the interstitial tissue, hemorrhage in the connective tissue and

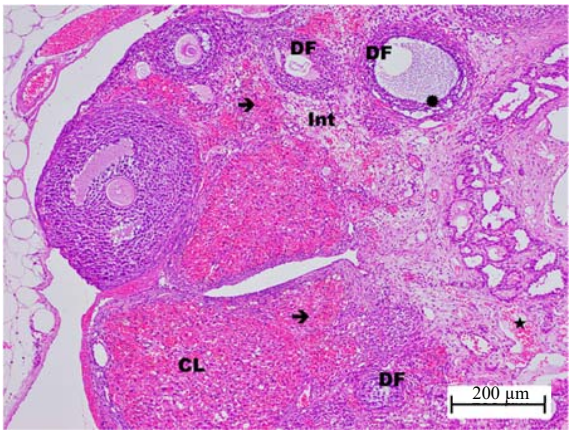


Fig. 3: Ovarian tissue stained with hematoxylin and eosin in the IRG group

DF: Intense degeneration in the developing follicles, ✸: Necrotized cell debris, CL: Corpus luteum, Int: Intense edema in the interstitial area, ➔: Hemorrhagic deposits in the intermediate connective tissue, ★: Densely congested blood vessel, (H&E ×100) and IRG: Group with I/R alone to ovaries

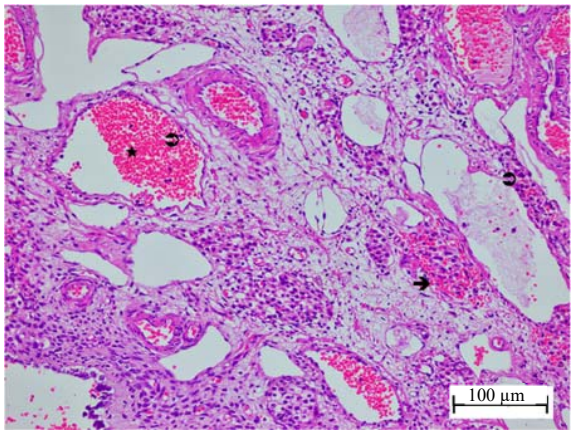


Fig. 4: Ovarian tissue stained with hematoxylin and eosin in the IRG group

At big magnification ➔: Hemorrhagic deposits in the intermediate connective tissue, ➲: Polymorphonuclear cell infiltration, ★: Densely congested blood vessel, (H&E ×200) and IRG: Group with I/R alone to ovaries

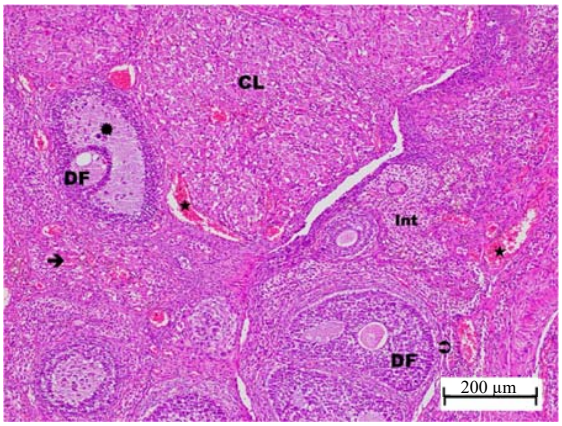


Fig. 5: Ovarian tissue stained with hematoxylin and eosin in the SIR-4 group

DF: Degenerated in some places developing follicle (developing follicle), Int: Moderate edema in the interstitial area (interstitial area), CL: Corpus luteum, ✸: Necrotized cell debris, ➔: Hemorrhagic deposits in the intermediate connective tissue, ★: Moderate graded congested blood vessels, ➲: Polymorphonuclear cell infiltration, (H&E ×100) and SIR-4: Sugammadex (4 mg/kg) +ovarian I/R group

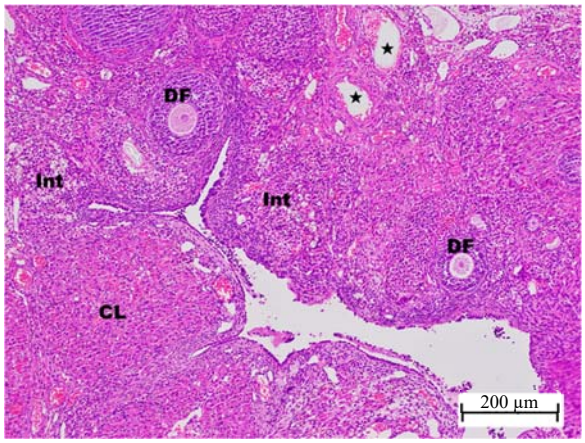


Fig. 6: Ovarian tissue stained with hematoxylin and eosin in the SIR-8 group
DF: Normally developing follicle (developing follicle), Int: Normally developing follicle (interstitial area), CL: Corpus luteum, ★: Slightly congested blood vessels in some places, (H&E ×100) and SIR-8: Sugammadex (8 mg/kg) +ovarian I/R group

Table 2: Histopathological evaluation results of ovarian tissue

| Group | Degeneration | Vascular congestion | Interstitial edema | Hemorrhage | PMNL infiltration |
|---|---|---|---|---|---|
| SG | 0 (0-0)[a] | 0 (0-0)[a] | 0 (0-0)[a] | 0 (0-0)[a] | 0 (0-0)[a] |
| IRG | 3 (2-3)[b] | 3 (2-3)[b] | 3 (2-3)[b] | 3 (2-3)[b] | 3 (2-3)[b] |
| SIR-4 | 2 (1-2)[c] | 2 (1-2)[c] | 2 (1-2)[c] | 1 (0-2)[c] | 1 (0-1)[c] |
| SIR-8 | 0 (0-1)[a] | 1 (0-2)[d] | 1 (0-1)[a] | 1 (0-1)[a] | 0 (0-0)[a] |

While groups containing the same letter in the same column are statistically similar (p>0.05), there is a statistical difference between groups containing different letters (p<0.05). Kruskal-Wallis test was used for the statistical analysis and the Mann-Whitney U test was used as a *post hoc* test. The results are presented as median (minimum-maximum), SG: Group with sham operation on the ovaries, IRG: Group with I/R alone to ovaries, SIR-4: Sugammadex (4 mg/kg) +ovarian I/R group, SIR-8: Sugammadex (8 mg/kg) +ovarian I/R group and PMNL: Polymorphonuclear leukocytes

Table 3: Follicle counts in ovarian tissues

| Group | Primordial follicle** | Developing follicle* | Atretic follicle* | Corpus luteum* |
|---|---|---|---|---|
| SG | 13.67±0.21[a] | 23.17±0.31[a] | 3.17±0.48[a] | 14.33±0.49[a] |
| IRG | 11.83±0.54[a] | 18.50±0.43[b] | 6.33±0.42[b] | 11.50±0.43[b] |
| SIR-4 | 12.83±0.54[a] | 20.33±0.33[c] | 4.67±0.21[a] | 13.00±0.37[ab] |
| SIR-8 | 13.50±0.34[a] | 22.67±0.33[a] | 3.33±0.42[a] | 14.00±0.37[a] |

While groups containing the same letter in the same column are statistically similar (p>0.05), there is a statistical difference between groups containing different letters (p<0.05). *Statistical analyses were performed using the One-way ANOVA test followed by Tukey's HSD (honestly significant difference) test as *post hoc*. **Statistical analyses were performed using the One-way ANOVA test followed by Games-Howell test as *post hoc*. Results are presented as Mean±Standard Error, SG: Group with sham operation on the ovaries, IRG: Group with I/R alone to ovaries, SIR-4: Sugammadex (4 mg/kg) +ovarian I/R group and SIR-8: Sugammadex (8 mg/kg) +ovarian I/R group

PMNL infiltration were observed (Fig. 5 and Table 2). No pathological findings were observed in the SIR-8 group, administered sugammadex at an 8 mg/kg dose, except in the normal structure developing follicles and slightly congested blood vessels (Fig. 6 and Table 2). Ovarian tissue follicle counts are shown in Table 3.

## DISCUSSION

In this study, the effect of sugammadex on I/R-induced ovarian damage was investigated biochemically and histopathologically. Current biochemical experimental results showed that sugammadex significantly suppressed the increase in MDA level caused by the I/R event in ovarian tissue. Furthermore, it was revealed that sugammadex prevented the decrease in non-enzymatic (tGSH) and enzymatic (SOD, CAT) antioxidants caused by I/R in ovaries. The reason why we measure the amount of MDA in evaluating I/R-related ovarian damage is that it is a toxic product of LPO and an important indicator of oxidative damage[4]. As it is known, it is the ROSs that initiate the LPO reaction and induce the production of MDA[3]. In the conducted studies, it was shown that ROSs interact with membrane lipids containing unsaturated fatty acids during the I/R period, causing an increase in the concentration of MDA[1]. In present study, the I/R procedure increased the MDA level in the ovarian tissue of rats.

In addition, in this I/R model, sugammadex suppressed the increase in MDA level caused by I/R at a dose of 8 mg/kg, better than at a 4 mg/kg dose.

It is known from the literature that the impaired redox balance is closely related to the ovarian I/R event[18]. Therefore, in current study, the effect of sugammadex on tGSH, SOD and CAT levels in ovarian tissue of rats that underwent I/R procedure was investigated. These parameters are important indicators of antioxidant capacity and are known to protect tissues against oxidative stress[19]. As can be understood from present experimental results, a significant decrease was observed in tGSH, SOD and CAT levels in parallel with the increasing MDA concentration following the I/R procedure. Çaltekin *et al.*[20] reported that the I/R process significantly reduces the levels of GSH and SOD in the ovarian tissue. At the same time, Ozlem *et al.*[21] have suggested that CAT enzyme activity decreases with the increase in MDA level caused by the ischemic process. In current study, following I/R, sugammadex inhibited the decrease of tGSH, SOD and CAT levels more significantly at an 8 mg/kg dose compared to a 4 mg/kg dose. There is no information about the effect of sugammadex on oxidative stress in the I/R ovarian tissue in the current literature. In addition, study data showing the protective effects of sugammadex on I/R damage in other tissues are highly controversial[11,22]. In an *in vivo* study investigating its effectiveness against cerebral I/R injury, no difference was observed between serum MDA and antioxidant values in sugammadex and I/R groups[11].

In current study, biochemical findings were supported by histopathological findings. In current study, histopathological findings have shown that severe degeneration, necrosis, interstitial edema, vascular congestion and hemorrhage developed in the ovarian follicles of the I/R group, where severe PMNL infiltration was detected. As is known, inflammatory reactions begin during reperfusion[23]. Reperfusion generates an inflammatory response characterized by the collection of active PMNLs and an increase in oxidative stress[1]. Also, the accumulation of leukocytes and PMNLs in the affected organs is part of the acute inflammatory response and is one of the features of I/R damage[24,25]. In present study, sugammadex (4 mg/kg), which significantly reduces the infiltration of PMNLs into ovarian tissue, significantly suppressed the severity of development of severe follicular degeneration, necrosis, vascular congestion, interstitial edema and hemorrhage caused by I/R damage. Current study findings were consistent with Alagöz *et al.*[10] study that sugammadex prevents tissue damage caused by I/R by inhibiting the formation of inflammatory cells in skeletal muscle reperfusion injury. Moreover, in the sugammadex (8 mg/kg) group, which completely prevented the infiltration of PMNLs into the ovarian tissue, no pathological findings except mildly congested blood vessels have been found. In the literature, it was been expressed that the main source of ROSs causing I/R damage is PMNL[26]. The PMNLs are ROSs' main source produced through the activity of the nicotinamide adenine dinucleotide phosphate oxidase complex[27].

Experimental results obtained in the present study suggested that sugammadex may be beneficial in the treatment of oxidative and inflammatory ovarian damage associated I/R. In addition, add to the growing body of research into the treatment of ovarian ischaemia caused by ovarian torsion and strategies for fertility preservation and highlight the importance of taking precautions against ischaemia-reperfusion injury that may occur in the ovaries. To elucidate the mechanism of protective action of sugammadex against I/R-induced ovarian damage, an investigation of its lower doses that do not inhibit PMNL infiltration is required in the future.

## CONCLUSION

The I/R process led to an increase in oxidants and a decrease in antioxidants in the ovarian tissue. Severe oxidative stress and histopathological damage were detected in the ovarian tissue of the I/R group, in which severe PMNL infiltration was detected. Moderate oxidative stress and histopathological findings were observed in the 4 mg/kg sugammadex group which moderate PMNL infiltration in ovarian tissue was observed. In the sugammadex group, also administered at a dose of 8 mg/kg, PMNL infiltration was not observed; in addition, oxidant/antioxidant levels and histopathological appearance of ovarian tissue were found close to the healthy group in the sugammadex group administered at a dose of 8 mg/kg. Current experimental results showed that sugammadex protects ovarian tissue from I/R's oxidative and inflammatory damage. It also points out that its protective effect is due to the inhibition of PMNL infiltration. From this information, it is understood that an 8 mg/kg sugammadex dose may be more beneficial than its 4 mg/kg dose in treating ovarian I/R damage.

## SIGNIFICANCE STATEMENT

Ovarian ischemia is a clinical emergency that occurs after ovarian torsion, if left untreated, can result in necrosis and ovariectomy. In this study, the potential protective effect of sugammadex against I/R-induced ovarian damage in rats was

investigated, its mechanism of action was tried to be elucidated. This study results provide critical information that I/R causes oxidative and inflammatory damage in ovaries, that sugammadex protects ovarian tissue from this damage by suppressing the increase in oxidant levels, the decrease in antioxidant levels and by inhibiting PMNL infiltration. It also suggests that the 8 mg/kg dose of sugammadex may be more beneficial than the 4 mg/kg dose in the treatment of I/R-induced ovarian injury by completely preventing PMNL infiltration into ovarian tissue.

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