



Protective effects of glutathione administration on ischemia-reperfusion injury in rat ovaries

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Abstract

We aimed to examine the biochemical and histopathological potential beneficial effects of glutathione administration on the ovarian ischemia/reperfusion injury (IRI) model. Thirty Wistar Albino female rats were used in this experimental study and were divided into five groups. Group 1 (sham) underwent observational laparotomy. Group 2 (torsion) had their left ovaries torsioned. Group 3 (torsion + detorsion) was detorsioned after torsion. Groups 4 and 5 received the same procedure as group 3. 0,2 ml glutathione was applied to the left ovaries of group 4 (torsion + detorsion + intraovarian glutathione injection) after detorsion. Group 5 (torsion + detorsion + intraperitoneal glutathione injection) was administered 1 ml glutathione intraperitoneally five times. Fifteenth-day blood samples were taken to examine total antioxidant status, total oxidant status, oxidative stress index, anti-mullerian hormone (AMH), and malondialdehyde (MDA) values. Besides, the left ovaries were resected for histopathological examination. Total antioxidant status was significantly higher in the intraperitoneal injection group ($p < 0.05$). The AMH values of the sham and intraovarian groups were similar ($p > 0.05$). MDA value did not differ significantly between the sham, intraovarian, and intraperitoneal injection groups ($p > 0.05$). In histopathological examination, no significant benefit of glutathione application on follicle numbers was shown. The main limitations of our study were the relatively small size of our series, the absence of serial blood measurement, the absence of a group in which intraovarian and intraperitoneal injections were administered together, and the absence of a sham + drug group. Glutathione administration reduces the detrimental effects of ovarian IRI.

Keywords: anti-mullerian hormone, glutathione, ischemia reperfusion injury, ovarian torsion

1. Introduction

Ovarian torsion is a gynecological emergency that causes reduction or cessation of blood flow due to the partial or complete rotation of the ovaries around their ligament supports. Even though ovarian torsion is most common in women between 20 and 30, it can be seen at any age, from prepubertal to postmenopausal period (1,2).

The venous return becomes interrupted since venous pressure is lower after ovarian torsion. Edema develops in the ovarian tissue due to the continuation of the arterial blood flow. This edema causes ischemic damage by slowing down and stopping the blood flow (3). Early diagnosis and appropriate treatment should be made quickly to prevent possible ovarian necrosis, decrease in fertility, and life-threatening complications. In the case of ovarian torsion, the first therapeutic option is surgical detorsion as soon as possible because delay in diagnosis and treatment is associated with decreased ovarian reserve and infertility (4).

Abundant oxygen uptake occurs after ovarian detorsion, and the resulting free oxygen radicals increase tissue damage.

Following detorsion, the return of blood flow increases, and accordingly, the production of toxic reactive oxygen species (ROS) increases (5,6). By providing oxygen during reperfusion following detorsion, xanthine oxidase converts the accumulated hypoxanthine to xanthine. Thus, an excessive amount of free oxygen radicals is formed, which causes a deterioration in the antioxidant mechanism of the body (7,8). Overproduction of ROS causes a significant increase in lipid peroxidation, creating malondialdehyde (MDA), which can destroy the human body's antioxidant defense systems (9,10).

Glutathione is a tripeptide (cysteine, glycine, and glutamic acid) molecule found in high concentrations in tissues. It plays an essential role in pathophysiological processes such as reducing oxidative stress, increasing metabolic detoxification, and regulating immune system functions (11,12).

Many studies have investigated the effects of glutathione on female fertility. For example, while intracellular glutathione levels were found to be higher in young women, glutathione levels were found to be low in cases of premature ovarian

insufficiency and ovarian cancer (13,14). In addition, glutathione protects follicles from damage caused by oxidative stress during folliculogenesis. Thus, oocytes with high intracellular glutathione levels produce healthier and stronger embryos (15).

In experimental animal models, the protective effect of glutathione administration has been shown in liver damage, liver transplantation, neurotoxicity, and testis torsion situations (16-19). Although many studies demonstrate the protective effect of glutathione administration against ischemia-reperfusion injury (IRI) in various tissues, no studies have been performed on ovarian IRI.

Glutathione is the cell's primary antioxidant and can reduce oxidative stress by preventing the formation of harmful free radicals in the reproductive system (20). Based on the known positive effects of glutathione on the ovaries and follicles, we hypothesized that glutathione administration could have histopathologically and biochemically protective effects in a rat model ovarian IRI.

2. Materials and Methods

This study was approved by the Institutional Animal Use and Care Committee and performed in accordance with the Helsinki Declaration of World Medical Association recommendations on animal studies. The rats included in the study were female Wistar Albino type, 12 weeks and weighing 187-220 grams. Rats were placed in individual cages and fed ad libitum in an environment at a 20-22 ° temperature, 50%-55% humidity, and 12-hour light/dark cycles. Before the study, the rats were given ten days to get used to the environment. The rats were randomly divided into five groups. All surgical interventions were performed under general anesthesia and sterile conditions, with a midline (2.0 cm) incision in the lower abdomen of the rat.

2.1. Group I (Sham group, n: 6)

The ovaries and tubes were imaged. After the evaluation, the incision was closed with 4.0 polyglactin 910 sutures.

2.2. Group II (Torsion group, n: 6)

Adnexal torsion was performed by fixing the left adnexa containing the ovarian vessels and tubes to the abdominal wall by rotating it in a clockwise direction by 720 °. After the procedure, the incision was closed.

2.3. Group III (Torsion - Detorsion Group, n: 6)

After the torsion process, 3 hours were spent waiting to complete the ischemic process, and laparotomy was performed again. A Detorsion procedure was applied to the left ovary. The incision was closed.

2.4. Group IV (Torsion - Detorsion + Intraovarian Glutathione Injection Group, n: 6)

After the torsion and detorsion procedure, 0.2 ml of glutathione (Tationil 600 mg / 4 ml) was applied to the left ovarian tissue, and the abdominal wall was closed.

2.5. Group V (Torsion - Detorsion + Intraperitoneal Glutathione Injection Group, n: 6)

After the torsion and detorsion procedure, five times intraperitoneal glutathione (Tationil 600 mg / 4 ml) application was performed (on the day of the operation, on the 3rd day after the operation, on the 6th day, on the 9th day, and the 12th day). In each administration, 0.2 ml glutathione was injected intraperitoneally.

The rats were taken into cages in all groups for 15 days following the first laparotomy. At the end of the fifteenth day, following anesthesia, intracardiac blood samples were taken from all rats, and their left ovaries were resected. Euthanasia was applied after the procedure. The rats were anesthetized with xylazine hydrochloride (Rompun, Bayer, Germany) and ketamine hydrochloride (Ketalar, Eczacıbası, Turkey).

2.6. Histopathologic Evaluation

Ovarian samples were fixed in 10% formalin for 48 h, dehydrated in ethanol series, cleaned, and embedded in paraffin. The paraffin blocks were sectioned at a thickness of 5 mm using a sliding microtome (Leica RM2125RTS Nussloch Germany). Sections were stained with eosin and haematoxylin and analyzed using a light microscope (Nikon Eclipse E600 microscope) by an experienced pathologist. Hemorrhage, edema, and inflammation (neutrophil infiltration) were scored from 0 to +3 for ovarian injury as follows: None, Mild, Moderate, Severe

Follicles were counted in the largest section of the ovary to evaluate the ovarian reserve. Total scores were calculated. Follicles were defined as primordial follicle, primary follicle, preantral (secondary) follicle, and antral (tertiary) follicle (21):

2.7. Biochemical Evaluation

Blood samples were centrifuged at 3000 g for 10 minutes to obtain serum. Serum samples were stored at -80°C until measurement. In the blood samples, AMH (Elabscience, USA), Inhibin B (Elabscience, USA), Malondialdehyde (Bioassay Technology Laboratory, China), Total Antioxidant Status (TAS) (Bioassay Technology Laboratory, China) and Total Oxidant Status (TOS) (Bioassay Technology Laboratory, China) were measured by Enzyme-linked Immunosorbent Assay. Readings were done by a microplate reader (Biotek Synergy Reader).

2.8. Statistical Analysis

Mean, standard deviation, median, minimum, maximum value frequency, and percentage were used for descriptive statistics. The distribution of variables was checked with the Kolmogorov-Smirnov test. Kruskal-Wallis and Mann-Whitney U tests were used to compare quantitative data. SPSS 27.0 was used for statistical analysis.

3. Results

The weights of the rats in the groups were found to be similar ($p>0.05$). TOS value in the detorsion group, intraovarian injection group, and intraperitoneal injection group was significantly higher than the torsion group ($p<0.05$). TOS value

did not differ significantly between the detorsion group, intraovarian injection group, and intraperitoneal injection group (p>0.05). The TAS value of the intraperitoneal injection group was significantly higher than the sham group, torsion group, and detorsion group (p<0.05) (Table 1). The oxidative stress index (OSI) value of the detorsion group was significantly higher (p<0.05) than the other groups.

MDA values were similar (p>0.05) between the sham group, intraovarian injection group, and intraperitoneal injection groups (Table 1). AMH values of the torsion, detorsion, and intraperitoneal injection groups were significantly lower than the intraovarian and sham groups (p<0.05). AMH values were similar (p>0.05) between the sham and intraovarian groups (Table 1).

Table 1. Comparison of biochemical and histopathologic features between groups

| | | Control Group ¹ | Torsion Group ² | Δ ³ | Δ ⁴ | Δ ⁵ | p | 1,2 | 1,3 | 1,4 | 1,5 | 2,3 | 2,4 | 2,5 | 3,4 | 3,5 | 4,5 |
|---------------------------|----------|----------------------------|----------------------------|---------------------|---------------------|-------------------|-------------------|------|------|------|------|------|------|------|------|------|------|
| Weight | Mean±s.d | 204.3±10.2 | 00.8±8.4 | 02.3±11.7 | 203.0±7.9 | 202.5±8.4 | 0.97 ^k | 0.52 | 0.63 | 0.93 | 0.74 | 0.93 | 0.62 | 0.63 | 0.87 | 0.87 | 0.93 |
| | Median | 206.0 | 200.5 | 202.5 | 202.5 | 200.0 | 5 | | | | | | | | | | |
| TOS | Mean±s.d | 7.01±0.39 | 7.75±0.39 | 8.42±0.61 | 8.58±0.9 | 9.00±0.9 | 0.00 ^k | 0.01 | 0.00 | 0.00 | 0.00 | 0.04 | 0.04 | 0.00 | 1.00 | 0.22 | 0.26 |
| | Median | 6.97 ²³⁴⁵ | 7.79 ³⁴⁵ | 8.40 | 8.20 | 8.80 | 1 | 6 | 6 | 4 | 4 | 5 | 8 | 5 | 0 | 9 | 1 |
| TAS | Mean±s.d | 2.64±0.38 | 2.39±0.40 | 1.94±0.46 | 3.46±1.38 | 3.48±0.44 | 0.00 ^k | 0.19 | 0.03 | 0.26 | 0.01 | 0.06 | 0.07 | 0.01 | 0.01 | 0.00 | 0.52 |
| | Median | 2.61 ⁵ | 2.41 ⁵ | 1.74 ¹⁴⁵ | 3.03 | 3.62 | 3 | 9 | 7 | 1 | 0 | 5 | 8 | 0 | 6 | 6 | 2 |
| OSI | Mean±s.d | 2.71±0.46 | 3.33±0.61 | 4.49±0.82 | 2.80±1.03 | 2.63±0.46 | 0.01 ^k | 0.10 | 0.00 | 0.87 | 0.87 | 0.02 | 0.42 | 0.05 | 0.02 | 0.00 | 0.52 |
| | Median | 2.73 ³ | 3.28 ³ | 4.65 | 3.03 ³ | 2.41 ³ | 0 | 9 | 6 | 3 | 3 | 5 | 3 | 2 | 5 | 6 | 2 |
| MDA | Mean±s.d | 0.68±0.07 | 0.88±0.13 | 1.17±0.26 | 0.68±0.09 | 0.76±0.09 | 0.00 ^k | 0.00 | 0.00 | 0.93 | 0.07 | 0.04 | 0.01 | 0.14 | 0.00 | 0.00 | 0.17 |
| | Median | 0.67 ²³ | 0.87 ³ | 1.15 | 0.68 ²³ | 0.79 ³ | 1 | 6 | 4 | 6 | 8 | 5 | 0 | 9 | 4 | 6 | 3 |
| AMH | Mean±s.d | 3.89±0.65 | 2.02±0.75 | 2.79±0.22 | 3.72±0.66 | 2.89±0.79 | 0.00 ^k | 0.00 | 0.00 | 0.87 | 0.03 | 0.06 | 0.00 | 0.05 | 0.01 | 1.00 | 0.04 |
| | Median | 3.63 ²³⁵ | 2.14 | 2.74 | 3.91 ²³⁵ | 2.76 | 1 | 4 | 4 | 3 | 7 | 5 | 4 | 5 | 0 | 0 | 8 |
| Hemorrhage Score | Mean±s.d | 0.00±0.00 | 3.00±0.00 | 1.83±0.75 | 1.33±0.52 | 1.67±0.82 | 0.00 ^k | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.21 | 0.66 | 0.46 |
| | Median | 0.00 ²³⁴⁵ | 3.00 | 2.00 ² | 1.00 ² | 1.50 ² | 0 | 1 | 2 | 2 | 2 | 7 | 2 | 7 | 2 | 5 | 5 |
| Edema Score | Mean±s.d | 0.00±0.00 | 3.00±0.00 | 1.83±0.75 | 1.33±0.52 | 2.00±1.10 | 0.00 ^k | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.02 | 0.21 | 0.54 | 0.12 |
| | Median | 0.00 ²³⁴⁵ | 3.00 | 2.00 ² | 1.00 ² | 2.00 ² | 0 | 1 | 2 | 2 | 7 | 7 | 2 | 1 | 2 | 6 | 7 |
| Inflammation Score | Mean±s.d | 0.00±0.00 | 3.00±0.00 | 2.00±0.89 | 1.33±0.52 | 1.33±0.82 | 0.00 ^k | 0.00 | 0.00 | 0.00 | 0.00 | 0.02 | 0.00 | 0.00 | 0.16 | 0.14 | 0.67 |
| | Median | 0.00 ²³⁴⁵ | 3.00 | 2.00 ² | 1.00 ² | 1.00 ² | 0 | 1 | 2 | 2 | 1 | 2 | 2 | 5 | 2 | 9 | 3 |
| Primordial Follicle Count | Mean±s.d | 6.33±1.03 | 0.17±0.41 | 4.17±1.17 | 3.00±1.10 | 2.50±1.05 | 0.00 ^k | 0.00 | 0.01 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.12 | 0.03 | 0.35 |
| | Median | 6.00 ²³⁴⁵ | 0.00 | 4.00 ²⁵ | 3.00 ² | 2.50 ² | 0 | 3 | 4 | 3 | 4 | 3 | 3 | 3 | 8 | 2 | 6 |
| Primary Follicle Count | Mean±s.d | 7.00±2.37 | 1.00±0.63 | 4.33±1.03 | 3.50±1.05 | 2.67±0.52 | 0.00 ^k | 0.00 | 0.04 | 0.01 | 0.00 | 0.00 | 0.00 | 0.00 | 0.20 | 0.00 | 0.12 |
| | Median | 7.00 ²³⁴⁵ | 1.00 | 4.00 ²⁵ | 3.50 ² | 3.00 ² | 0 | 3 | 1 | 2 | 3 | 3 | 4 | 4 | 9 | 7 | 0 |
| Preantral Follicle Count | Mean±s.d | 6.83±1.83 | 1.50±0.55 | 4.00±0.63 | 2.67±1.03 | 2.67±1.03 | 0.00 ^k | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.04 | 0.04 | 0.02 | 0.02 | 1.00 |
| | Median | 6.50 ²³⁴⁵ | 1.50 | 4.00 ²⁴⁵ | 3.00 ² | 3.00 ² | 0 | 3 | 5 | 4 | 4 | 3 | 5 | 5 | 2 | 2 | 0 |
| Antral Follicle Count | Mean±s.d | 5.50±1.38 | 1.50±0.55 | 3.83±1.47 | 3.83±1.17 | 3.17±1.17 | 0.00 ^k | 0.00 | 0.04 | 0.04 | 0.01 | 0.00 | 0.00 | 0.01 | 0.93 | 0.41 | 0.32 |
| | Median | 5.00 ²³⁴⁵ | 1.50 | 3.50 ² | 4.00 ² | 3.00 ² | 2 | 3 | 5 | 5 | 4 | 7 | 7 | 2 | 5 | 0 | 3 |

^k Kruskal-wallis test (Mann-whitney u test) ¹ Difference with Control Group ² Difference with Torsiyon Group ³ Difference with Intraperitoneal Group

⁴ Difference with Intraovaryen Group ⁵ Difference with Detorsiyon Group

Hemorrhage, edema, and inflammation scores were significantly higher (p<0.05) in the torsion group, detorsion group, intraovarian injection group, and intraperitoneal injection group, respectively, compared to the sham group. The scores in the torsion group were significantly higher (p<0.05) than the detorsion group, intraovarian injection group, and intraperitoneal injection group (Fig. 1).

While the number of primordial and primary follicle numbers were significantly lower (p<0.05) in the intraperitoneal injection group than in the detorsion group, there was no significant difference (p>0.05) between the detorsion group and intraovarian injection groups. The preantral follicle count was significantly lower than the detorsion group in the intraperitoneal injection group and intraovarian injection group (p<0.05). Follicle numbers were not significantly different between the detorsion group,

intraovarian injection group, and intraperitoneal injection groups (p>0.05) (Table 1).

4. Discussion

We evaluated the difference between biochemical markers TOS, TAS, OSI, AMH, and MDA values as a primary outcome. In addition, we compared the change in follicle numbers by making a histopathological evaluation as a secondary outcome.

TAS was measured to determine the general antioxidant status, and TOS was measured to determine the oxidant status (22,23). OSI is calculated as the ratio of TOS to TAS. OSI is considered an indicator of oxidative stress in the tissue (24). We found that glutathione administration increased the TAS value, especially with intraperitoneal administration. It was

found that OSI value increased mostly in the detorsion group. With the application of glutathione, we could keep OSI at a lower level than detorsion. Thus, similar to the publications showing that glutathione reduces oxidative stress damage in the female reproductive system, we revealed that glutathione protects the ovaries from the effects of free oxygen radicals in cases of ovarian IRI (20, 25).

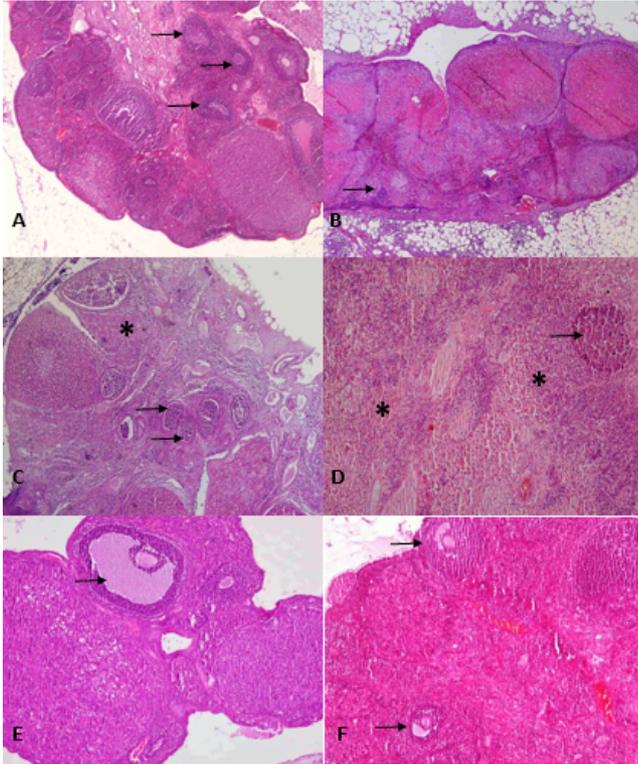


Fig. 1 - Histopathologic Evaluation

- A. Sham group with a normal appearance of ovary (4X magnification)
 B. Torsion and detorsion group with increased inflammation, hemorrhage and edema (4X magnification)
 C. Torsion group with similar morphologic features to the B.
 D. Intense polymorphonuclear cell infiltration and intercellular edema are seen in torsion group. Note the separation and degenerative changes in follicles (20X magnification)
 E. Torsion, detorsion and intraovarian glutathione injection group with decreased inflammation, hemorrhage and edema (10X magnification)
 F. Torsion, detorsion and intraperitoneal glutathione injection group has similar morphology.
 Ovarian follicles are marked with black arrows, dense inflammation is marked with *

The most harmful effect of free oxygen radicals in the cell is lipid peroxidation, and its end product is MDA. Lipid peroxidation causes loss of cell membrane integrity, increased permeability to ions, and cell damage. Therefore, inhibiting or reducing the lipid peroxidation process may help prevent tissue damage (26, 27). Our study found that MDA levels increased in torsion and torsion-detorsion groups compared to the sham group. This result supports that ischemia and IRI increased tissue damage. We also noticed that the MDA value did not differ significantly between the sham group and intraperitoneal, intraovarian injection groups ($p>0.05$). Thus, we showed that intraperitoneal and also intraovarian

administration of glutathione has a reducing effect on tissue damage by inhibiting lipid peroxidation.

Anti-mullerian hormone (AMH) is produced by granulosa cells. It is used as an indicator of ovarian follicle reserve and can be measured in serum. Decreased AMH values are generally associated with decreased ovarian reserve (28). AMH value was significantly higher in the intraovarian injection group than in the torsion group, detorsion group, and intraperitoneal injection group. AMH values did not differ significantly ($p>0.05$) between sham and intraovarian injection groups. We determined the protective effect of ovarian reserve based on the positive effect of intraovarian injection of glutathione on AMH value.

While primordial and primary follicle numbers were not significantly different between the detorsion group and intraovarian injection group ($p>0.05$), they were significantly lower in the intraperitoneal injection group ($p<0.05$). Preantral follicle number was significantly lower in the intraperitoneal injection group and intraovarian injection group than in the detorsion group ($p<0.05$). Antral follicle numbers were similar between the detorsion, intraovarian injection, and intraperitoneal injection groups ($p>0.05$). Some researchers stated that follicle numbers might not give accurate results as an indicator of ovarian reserve in such studies. They argued that it is not a definitive indicator of the viability of damaged follicles that are not included in the count because they are damaged (29). We attributed the difference in terms of follicle numbers in our study to this reason.

The main limitation of our study was the relatively small size of our series and the absence of serial blood measurement in this experimental model. In addition, we identified the absence of a group in which intraovarian and intraperitoneal injections were administered together and the absence of a sham + drug group as another limitation of our study.

The results of the study showed that exogenous glutathione administration might be effective in preventing tissue damage, oxidative stress, and loss of ovarian reserve caused by IRI in rat ovaries. In order to provide the most effective therapeutic benefit in clinical practice, further studies are needed to define dosage setting, administration method, and frequency of administration.

Conflict of interest

None for each author

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Authors' contributions

Concept: A.G., B.O.I., Design: A.G., B.O.I., Data Collection

or Processing: A.G., E.D.T., K.A., Analysis or Interpretation: A.G., B.O.I., A.G.S., M.E.S., Literature Search: A.G., Writing: A.G., K.A.

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