

# The Protective Role of Syringic Acid on Ovarian Injury Created by Ischemia Reperfusion

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## Abstract

**Introduction:** Syringic acid (SA), a natural phenolic substance, possesses anti-inflammatory and antioxidant properties. We investigated the likely preventive effects of SA on organ damage to the reproductive system caused by bilateral ovarian ischemia-reperfusion (I/R).

**Materials and Methods:** 32 female Sprague-Dawley rats in total were used; they were split into four groups: a sham group for control (group I), I/R group (group II), I/R group given 10 mg/kg SA (group III), and an I/R group given 50 mg/kg SA (group IV). Group I underwent only an abdominal incision without I/R. In groups II, III, and IV, I/R procedures were conducted for 3 hours. SA was given intraperitoneally to groups III and IV for 30 minutes before reperfusion at 10 mg/kg and 50 mg/kg, respectively. The ovarian tissues were surgically removed for analysis.

**Results:** TNF- $\alpha$ , MDA, IL-1 $\beta$ , MPO, TOS and OSI levels in group II were found to be significantly higher than in group I. Additionally, a decrease was detected in TAS and SOD values in group II. In contrast, SA administration led to significant increases in the level of antioxidant enzymes in both groups III and IV. Also, in the SA-given groups, significant decreases were detected in OSI and TOS levels, along with a decrease in MPO, MDA, TNF- $\alpha$ , and IL-1 $\beta$  levels.

**Conclusion:** It has been demonstrated that SA has a protective effect against organ damage caused by bilateral ovarian I/R in the reproductive system. This is the first study to show that SA can reduce ovarian damage due to I/R injury.

**Key words:** Ischemia reperfusion; syringic acid; ovary; oxidative stress.

## Introduction

Ovarian torsion is a condition that occurs in approximately 2.7% of gynecological emergencies (1). It involves the twisting of the ovaries within the adnexal region, which leads to a disruption in blood circulation. If this torsion persists beyond the ovary's tolerance, it results in an ischemic period due to venous congestion and the impairment of arterial blood supply (2). However, the process of detorsion, while relieving the immediate ischemic condition in the ovaries, can also lead to oxidative injury. The return of blood flow may produce the release of reactive oxygen species (ROS), which in turn may cause tissue damage and inflammation. This phenomenon is termed an ischemia-reperfusion (I/R) injury (3). The I/R injury is characterized by oxidative stress.

ROS and toxic products, which may represent oxidative stress, cause tissue damage through DNA damage and lipid peroxidation in membranes. After I/R injury, there is an increase in lipid peroxidation mediators such as malondialdehyde (MDA), a decrease in the amount of antioxidant enzymes such as Superoxide dismutase (SOD), and an increase in inflammation markers such as myeloperoxidase (MPO) (4). Oxidants and antioxidants in cells can determine the level of tissue damage. Total oxidant status (TOS) is ascertained the overall oxidation status. Total antioxidant status (TAS) is used to assess the general antioxidative status. The ratio of TOS to TAS, oxidative stress index (OSI), is regarded as a more precise index of oxidative

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stress on the tissue (5). Activation of nuclear factor kappa beta (NF- $\kappa$  $\beta$ ) and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) signaling pathway leads to inflammation in I/R injury (6). This signaling pathway, which is necessary to control inflammation, can cause tissue damage when overactive (7). Therefore, preventing I/R injury is important for controlling the damage. Syringic acid (SA), a naturally occurring phenolic compound, is generated from plants and is also known as 4-hydroxy-3,5-dimethoxybenzoic acid (8). It has a wide range of pharmaceutical applications such as antioxidant (9), and anti-inflammatory features (10). Also, SA has antioxidant, anti-osteoporotic and anti-inflammatory properties (11). As we know, there are no study on the effects of SA on ovarian I/R in the literature, In the present study, we assessed the impact of SA on reproductive organ damage brought on by bilateral ovarian I/R.

## Materials and Methods

**Laboratory conditions:** All rats were housed in the laboratory with 12-hour light/12-hour dark cycle, 55% humidity, and an average temperature of 21°C, ad libitum. However, all rats were subjected to a 12-hour fasting period before the experimental process. **Groups and Bilateral Ovarian Ischemia/Reperfusion Model:** 32 female Sprague Dawley rats with weights ranging from 230 to 250 grams were divided into 4 groups. In group I (sham control), an incision was made in the abdominal area under anesthesia, but neither the I/R model nor medication was applied, and the incision was closed. In group II (ischemia-reperfusion), a 1-2 cm incision was made via median laparotomy. The ovarian and fallopian tube veins were rotated 360 degrees in a clockwise direction, and microvascular, atraumatic clamps were used to block these structures for 3 hours. Afterward, blood circulation was restored by releasing the clamps during the reperfusion stage. The incision site was sealed. Following reperfusion, the ovarian tissues were excised. In group III (I/R+10 mg/kg syringic acid) and group IV (I/R+50 mg/kg syringic acid), syringic acid (S6881, Sigma Aldrich Co) was given intraperitoneal into rats at a dose of 10 mg/kg (12) and 50 mg/kg (13) 30 minutes before reperfusion, followed by the implementation of the I/R model. Ketamine/xylazine was used for the anesthesia process. The collected ovarian tissues were frozen for future biochemical studies. **Biochemical Analysis of Ovarian Tissues:** All biochemical measurements and analyses were performed on homogenized tissues. In the ovary

samples, the level of MDA (abcam, ab118970) was measured to assess lipid peroxidation status. The values were expressed as  $\mu$ mol/g. SOD activity (Elabscience, E-EL-R1425) was calculated as U/mg protein. MPO (abcam, ab285308) was evaluated as U/g protein. Using Rel Assay Diagnostics' commercially available assays, TOS (RL0024) and TAS (RL0017) were assessed. Both TOS and TAS values were expressed in nmol/L. The Oxidative Stress Index (OSI) was determined using the TOS to TAS ratio. The OSI level was calculated using the formula  $OSI = [(TOS, \text{mol H}_2\text{O}_2 \text{ equivalent/L}) / (TAS, \text{mmol Trolox equivalent/L})]$ . The levels of TNF- $\alpha$  (E-EL-R2856) and IL-1 $\beta$  (E-EL-R0012) were measured by using kits from Elabscience Company, Wuhan, China.

**Ethical consent:** This experiment was conducted at the Experimental Animal Research and Application Center Atatürk University. The current work was approved (Date 28.03.2019, Number 79) by the Local Ethics Committee at Atatürk University.

**Statistical analysis:** All the data were analyzed using the SPSS 21 program. Descriptive statistics were presented as mean  $\pm$  SD. A 0.05 p-value was chosen as the significant level. To assess the assumption of normality, the Shapiro-Wilk test was utilized. The one-way ANOVA test was chosen when the data had a normal distribution, and the Tukey test for post-hoc paired comparisons was then used. The Kruskal-Wallis test was used for parameters that did not follow a normal distribution. When significance was detected, the Mann-Whitney U test was applied for further analysis.

## Results

The TAS, OSI, and TOS levels in ovarian tissue are summarized in Table 1, along with the comparative analysis among all the groups. In the comparison between groups I and II, there was a significant decrease in TAS values and an increase in OSI and TOS within group. TOS and OSI levels in Group IV were found to be significantly lower than those in Group III ( $p < 0.005$ ). A significant difference was detected in terms of TAS, TOS and OSI levels between groups III and IV, where SA was given. Compared to Group III, the TAS level increased significantly and the TOS level decreased significantly in Group IV. Table II presents the levels of SOD, MPO, and MDA, while Figure I displays the values for IL-1 $\beta$  and TNF- $\alpha$ . When comparing the SOD, MPO, MDA, IL-1 $\beta$ , and TNF- $\alpha$  values between groups I and II, a significant decrease in SOD and a significant

increase in the other parameters were detected in the group subjected to I/R (Group II). It showed

significant differences between Groups III and IV

**Table 1:** Descriptive statistics and comparison results of TAS, TOS and OSI levels between groups

| Experimental Groups (n=8)            | TAS<br>(mmol Trolox equivalent/L)<br>Mean ± SD | TOS<br>(µmol H <sub>2</sub> O <sub>2</sub> equivalent/L)<br>Mean ± SD | OSI         |
|--------------------------------------|--|---|-------------|
| Group I (1)                          | 0.793±0.062                                    | 4.780±0.559   | 0.607±0.101 |
| Group II (2)                         | 0.307±0.034                                    | 9.634±0.765   | 3.157±0.343 |
| Group III (3)                        | 0.637±0.061                                    | 5.832±0.706   | 0.923±0.147 |
| Group IV (4)                         | 0.775±0.083                                    | 5.356±0.483   | 0.699±0.111 |
| p value                              | 0.001 (1-2)                                    | 0.001 (1-2)   | 0.001 (1-2) |
| (Significant intergroup comparisons) | 0.001 (1-3)                                    | 0.005 (1-3)   | 0.001 (1-3) |
|                                      | 0.001 (2-3)                                    | 0.045 (1-4)   | 0.001 (2-3) |
|                                      | 0.001 (2-4)                                    | 0.001 (2-3)   | 0.001 (2-4) |
|                                      | 0.002 (3-4)                                    | 0.001 (2-4)   | 0.004 (3-4) |

**TAS:** Total Antioxidant Status; **TOS:** Total Oxidant Status; **OSI:** Oxidative Stress Index; **SD:** Standard Deviation

**Table 2:** Descriptive statistics and comparison results of oxidative markers (SOD, MPO, and MDA) levels between experimental groups

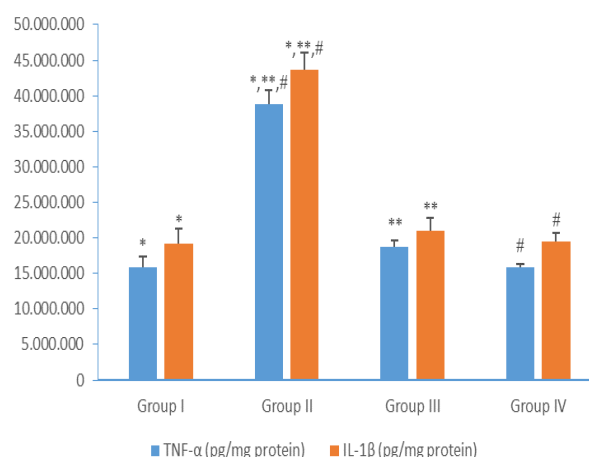
| Experimental Groups (n=8)                    | SOD<br>(U/mg protein)<br>Mean ± SD | MPO<br>(U/g protein)<br>Mean ± SD | MDA<br>(µmol/g tissue)<br>Mean ± SD |
|--|------------------------------------|-----------------------------------|-------------------------------------|
| Group I (1)                                  | 355.928±45.761                     | 250403.099±40958.499              | 67.584±3.062                        |
| Group II (2)                                 | 171.319±17.566                     | 554429.869±87447.078              | 124.255±13.329                      |
| Group III (3)                                | 310.280±19.680                     | 308350.176±31820.479              | 82.512±7.034                        |
| Group IV (4)                                 | 358.147±33.649                     | 253921.912±20690.045              | 70.032±6.354                        |
| p value (Significant intergroup comparisons) | 0.001 (1-2)                        | 0.001 (1-2)                       | 0.001 (1-2)                         |
|  | 0.021 (1-3)                        | 0.007 (1-3)                       | 0.001 (1-3)                         |
|  | 0.001 (2-3)                        | 0.001 (2-3)                       | 0.001 (2-3)                         |
|  | 0.001 (2-4)                        | 0.001 (2-4)                       | 0.001 (2-4)                         |
|  | 0.004 (3-4)                        | 0.001 (3-4)                       | 0.002 (3-4)                         |

**SOD:** superoxide dismutase; **MPO:** myeloperoxidase; **MDA:** malondialdehyde level. Mean ± SD

in all parameters (SOD, MPO, MDA, and TNF-α) except IL-1β. No statistical difference was detected between Groups I and IV in terms of SOD, MPO, MDA, TNF-α, and IL-1β. When both group III and group IV were evaluated with group I in terms of SOD, MPO, MDA, TNF-α, and IL-1β parameters, a significant difference was detected between the groups in all parameters.

## Discussion

Ovarian torsion is one of the most important gynecological emergencies to be treated. Many factors such as ovarian cysts, ovarian stimulation and pregnancy can cause ovarian torsion. Clinical findings are nonspecific and delays in treatment may lead to severe undesirable clinical consequences. Untreated torsion can lead to



**Figure 1:** Comparisons of TNF-α and IL-1β between experimental groups.

necrosis (14), which can directly affect fertility. The urgent management of ovarian torsion is crucial, with laparoscopic detorsion being the favored and efficient therapeutic choice due to its established safety and capability in preserving ovarian (15). Detorsion via reperfusion protects the ovary from damage caused by ischemia. Nevertheless, throughout this reperfusion phase, additional tissue injury persists due to neutrophil infiltration and reactive oxygen species stemming from oxygen (15). Some oxidation processes, such as lipid peroxidation, result in the production of toxic molecules in damaged tissue. To prevent this damage after reperfusion, it is extremely important to investigate anti-inflammatory, antioxidant, and free radical scavenging molecules. For this reason, the possible therapeutic effects of known or new molecules in preventing or protecting I/R injury are being investigated (16-19). Our study also includes a protective approach after I/R injury. SA is a naturally occurring phenolic acid and has important cellular roles. There are many studies showings that SA has antioxidant, anti-inflammatory hepatoprotective, neuroprotective, renoprotective effects (10, 20-23). Tokmak et al. (2017) showed that SA reduced axonal degeneration and oxidative stress in the rat sciatic nerve after I/R injury (24). SA reduced TOS and OSI and increased TAS by preventing oxidative stress in L-arginine-induced acute pancreatitis (24). It has shown that SA can alleviate myocardial I/R injury through by inhibiting apoptosis (13) Also, SA protects from isoproterenol-induced cardiotoxicity by decreasing lipid peroxidation and inflammatory cytokines (26). SA has protective properties from I/R injury, as seen in many studies. It generally achieves this through antioxidant and anti-apoptotic pathways (13, 24). Liu et al. (2020) reported that syringic acid showed an anti-apoptotic effect through the PI3K/Akt/GSK-3b signaling pathway in the damage occurring after myocardial I/R injury (13). It is stated that SA mitigates ovarian damage by modulating endoplasmic reticulum stress, inflammation and Nrf2 pathway in ovarian damage caused by cisplatin (27). These results showed that SA has multiple cellular protective and preventive roles against I/R injury. In this study, the antioxidant and anti-inflammatory effects of SA were demonstrated in the ovarian I/R model. In the I/R group, TAS and SOD decreased while MPO, MDA, TOS, TNF- $\alpha$ , OSI, and IL-1 $\beta$  increased. In the groups where SA was given, it was determined that TAS capacity increased depending on the dose of SA, whereas other markers such as MPO, MDA, TOS, TNF- $\alpha$ ,

OSI, and IL-1 $\beta$  values decreased. These results showed that SA clearly reduced oxidative stress and inflammation resulting from ovarian I/R. Thus, the results in this study were found to be compatible with the antioxidant and anti-inflammatory properties of SA reported in the literature. SA may exert its antioxidant effect by regulating ER stress and mitochondrial metabolism resulting from I/R damage. In this way, both the formation of reactive oxygen species and errors in protein folding are prevented. Because SA has a strong and general antioxidant effect, the pathways mentioned are candidate intracellular pathways that are likely to be regulated.

**Study limitaitons:** To definitively establish the anti-oxidant efficiency of syringic acid, clinical studies involving humans are needed.

## Conclusions

Our study demonstrated that SA reduced ovarian injury in an I/R rat model through its antioxidant and anti-inflammatory effects. This is the first study to show that syringe acid can reduce ovarian damage due to I/R injury. However, future studies may be useful in elucidating the protective role of SA against I/R-induced ovarian damage and the molecular mechanisms.

**Ethic consent:** This study was approved (Date 28.03.2019, Number 79) by the Experimental Animals Local Ethics Committee at Atatürk University.

**Conflict of interest:** The authors of this study declare that they have no conflicts of interest to disclose

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