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ORIGINAL ARTICLE
ORİJİNAL ARAŞTIRMA

The Effect of Rosmarinic Acid Against Ovarian and Lung Injuries Induced by Ovarian Torsion Detorsion in Rats

Over Torsiyon Detorsiyon Kaynaklı Over ve Akciğer Hasarına Karşı Rosmarinik Asidin Etkisi

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ABSTRACT

Aim: Here, we purposed to find out the effects of two different doses of Rosmarinic acid (RA) against ovarian and lung injury caused by ovarian ischemia-reperfusion.

Material and Method: We planned the groups as sham, ovarian torsion detorsion (O/TD; 3hours torsion/3hours detorsion), RA 40 mg/kg (40 mg/kg RA+O/TD), and RA 80 mg/kg (80 mg/kg RA+O/TD) groups. Following the experimental procedure, we sacrificed the rats and then, collected the lung and ovarian tissues for biochemical evaluations.

Result: Total oxidant status (TOS), myeloperoxidase (MPO) activity, malondialdehyde (MDA) levels, and oxidative stress index (OSI) were elevated in the O/TD group compared to the sham group. These parameters declined due to low and high doses of RA administration. Total antioxidant status (TAS) level and superoxide dismutase (SOD) activity diminished in the O/TD group while increasing in RA treatment groups. However, the high dose of RA treatment group enhanced the antioxidant activity further and reduced the oxidant parameters compared to the low dose RA treatment group.

Conclusion: In this study, RA treatment reduced O/TD-induced ovarian and lung injuries in the experimental animals.

Keywords: Ovary, rat, rosmarinic acid, torsion detorsion

ÖZ

Amaç: Bu çalışmada over torsiyon detorsiyonunun neden olduğu over ve akciğer hasarına karşı iki farklı Rosmarinic asit (RA) dozunun etkilerini bulmayı amaçladık.

Gereç ve Yöntem: Grupları sham, over torsiyon detorsiyon (O/TD; 3 saat torsiyon/3 saat detorsiyon), RA 40 mg/kg (40 mg/kg RA+O/TD) ve RA 80 mg/kg (80 mg/kg RA+O/TD) olarak planladık. Deneyin ardından sıçanları sakrifiye edip biyokimyasal değerlendirmeler için akciğer ve over dokularını aldık.

Bulgular: Total oksidan durum (TOS), myeloperoksidaz (MPO) aktivitesi, malondialdehit (MDA) seviyeleri ve oksidatif stres indeksi (OSI), O/TD grubunda sham gruba kıyasla yükselmişti. Bu parametreler, düşük ve yüksek doz RA uygulaması sonucunda azalma gösterdi. Total antioksidan durum (TAS) düzeyi ve süperoksit dismutaz (SOD) aktivitesi, RA tedavi gruplarında artarken O/TD grubunda azaldı. Ancak, yüksek doz RA tedavi grubu, düşük doz RA tedavi grubuna kıyasla antioksidan aktiviteyi daha da arttırıp oksidan parametreleri azaltmıştır.

Sonuç: Mevcut çalışmada RA tedavisi sonucu deney hayvanlarında O/TD'nin neden olduğu over ve akciğer hasarı azalmıştır.

Anahtar Kelimeler: Over, rat, rosmarinik asit, torsiyon detorsiyon

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INTRODUCTION

Different conditions such as a prolonged mesovarium and adnexal venous obstruction may cause ovarian torsion and occlusion of ovarian vessels. This condition leads to a critical decrease in blood flow to the tissues resulting in permanent injury (1). Thereby, ovarian torsion should be diagnosed and treated immediately to maintain ovarian function and fertility (2). Ovarian torsion composes nearly 3% of acute abdominal pain cases applying to emergency department (3). Ovarian torsion may be observed for all age groups in women, but mostly between the ages of 29 to 34 (4), which makes it a serious health condition in terms of fertility. Besides torsion and ischemia, detorsion also causes tissue damage during reperfusion through the overproduction of reactive oxygen species (ROS) (5). ROS contributes to ischemic injury at the cellular level during reperfusion (6).

High ROS levels and leukocyte deposition are observed at the reperfusion stage. Ovarian injury develops unless the intracellular antioxidants prevent ROS (7). Oxidative stress occurs when the oxidant mechanisms (ROS, free radical generation, etc.) overcome the antioxidant systems (8). During the reperfusion stage neutrophil recruitment induces ROS release and thus, plays a key role in tissue injury (9). Activated neutrophils release the myeloperoxidase (MPO) enzyme, which contributes to forming ischemia and reperfusion (10). ROS and malondialdehyde (MDA) accumulation and decreased superoxide dismutase (SOD) levels lead to oxidative stress injury (11).

Rosmarinic acid (RA) has antioxidant, anti-angiogenic, and anti-inflammatory functions (12). Fonteles et al. found that RA demonstrates anti-inflammatory features in ischemic mice. (13). It has been shown that RA protects the ischemic liver and cardiovascular systems through anti-inflammatory and antioxidant functions (14, 15).

Various agents have been examined against ovarian torsion detorsion (O/TD) in previous studies (16). Here, we searched the potential beneficial effects of RA on ovarian and lung tissues in an O/TD model.

MATERIAL AND METHOD

Experimental Animals and Ethical Approval

The current search was confirmed by Atatürk University Local Ethics Council of Animal Experiments (protocol number: 28.06.2018/141). Animal procurement and experimental procedure were carried out at Medical Experimental Application and Research Center of Atatürk University. Rats were put in standard rat cages with regular laboratory conditions. They were fed with regular rat feed and supplied tap water. Feeding was prohibited 12 hours before the experiment, but the water was allowed. to drink.

Groups and Torsion/Detorsion Model

32 Sprague Dawley female rats were weighted (240-250 g). Four groups were created (n=8) randomly as sham, O/TD (3hours torsion/3hours detorsion), RA 40 mg/kg (40 mg/kg RA+O/TD), and RA 80 mg/kg (80 mg/kg RA+O/TD) groups. The animals were immobilized in the supine position and then, the abdominal regions were shaved and disinfected. 10% povidone-iodine was preferred for disinfection. 10 mg/kg intraperitoneal (i.p.) xylazine hydrochloride and 60 mg/kg i.p. ketamine were used for anesthesia during the procedures (17, 18).

A 1-2 cm sized median laparotomic incision was established in the sham group, but no T/D model or medication was performed. The incision was repaired via silk 3/0 suture. In the O/TD group, following the incision, ovaries, ovarian vessels, and fallopian tubes were spun 360 degrees clockwise. They were fixed for 3 hours with atraumatic microvascular clamps, and thus, bilateral torsion was created. In the detorsion period, blood circulation was available for 3 hours by removing the clamps, and the incision was sutured. The O/TD model was preferred from previous studies (16, 19, 20). In low dose and high dose RA treatment groups, following the torsion phase, RA was applied to the rats i.p. at the doses of 40 mg/kg and 80 mg/kg just before detorsion, respectively. Then, the detorsion stage was carried out. The RA doses were based on a previous study (21).

Following the experiment, a high dose of anesthesia was performed for the sacrifice of the rats. The ovarian and lung tissues were removed. They were cleaned by washing and maintained frozen until the biochemical analysis.

Biochemical Analysis

Various parameters were examined in lung and ovarian tissue samples. MDA levels (µmol/g protein) were measured due to the methods explained by Ohkawa et al. (22) to determine the lipid peroxidation status. SOD (U/mg protein) and MPO (U/g protein) activities were evaluated as defined by Sun et al. (23) and Bradley et al. (24), respectively. TAS and TOS levels were gauged through commercially available kits (Rel Assay Diagnostics). OSI is the ratio of TOS to TAS (25), and is measured for the oxidative stress evaluation.

Statistical analysis

We analyzed the data using One-way ANOVA and demonstrated as Mean±Standard Error of Mean (SEM) through SPSS software. We used the Tukey test for the group pairwise comparisons. We admitted the differences as significant if $p<0.05$.

RESULTS

TAS, TOS, and OSI values of ovarian and lung tissues were shown in **Figures 1** and **2**, respectively. A significant raise occurred in the O/TD group compared to the sham group for the TOS and OSI levels, while the TAS value was diminished. Besides, the TAS value elevated significantly while TOS and OSI parameters declined in high and low dose RA groups compared to the O/TD group.

Results of MDA, SOD, and MPO activities in ovarian and lung tissues are presented in **Figure 3** and **Figure 4**, respectively. When the O/TD group was compared to the sham group, MPO activity and MDA levels were increased significantly, but SOD activity was decreased. Besides, when the RA treatment groups were compared to the O/TD group, MPO activity and MDA levels declined, but SOD activity was raised.

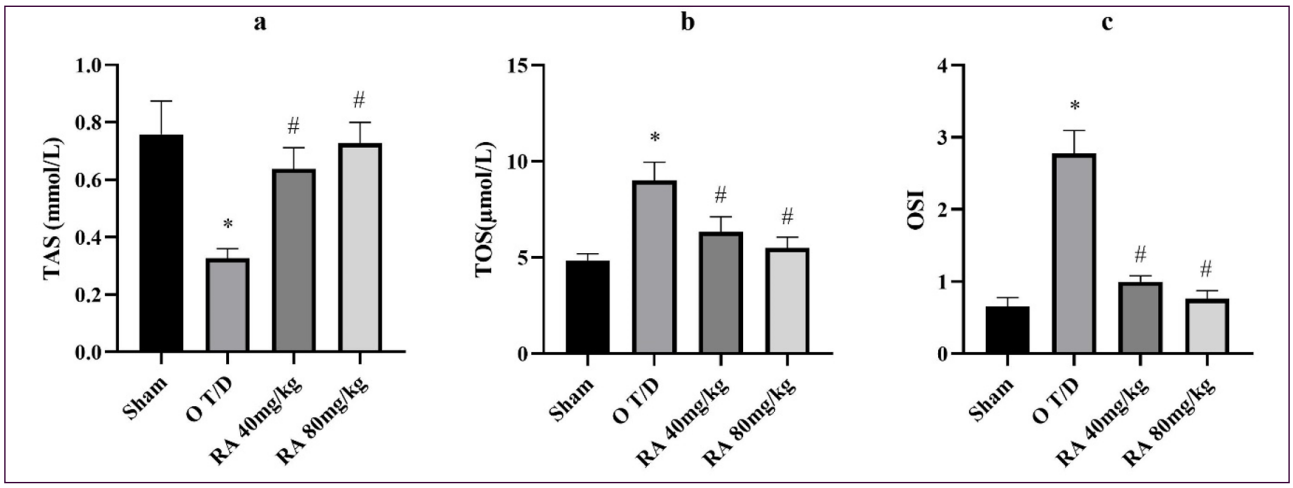

Figure 1. (a) TAS, (b) TOS, and (c) OSI values of ovarian tissue. *p<0.05 compared to sham group. #p<0.05 compared to O/TD group

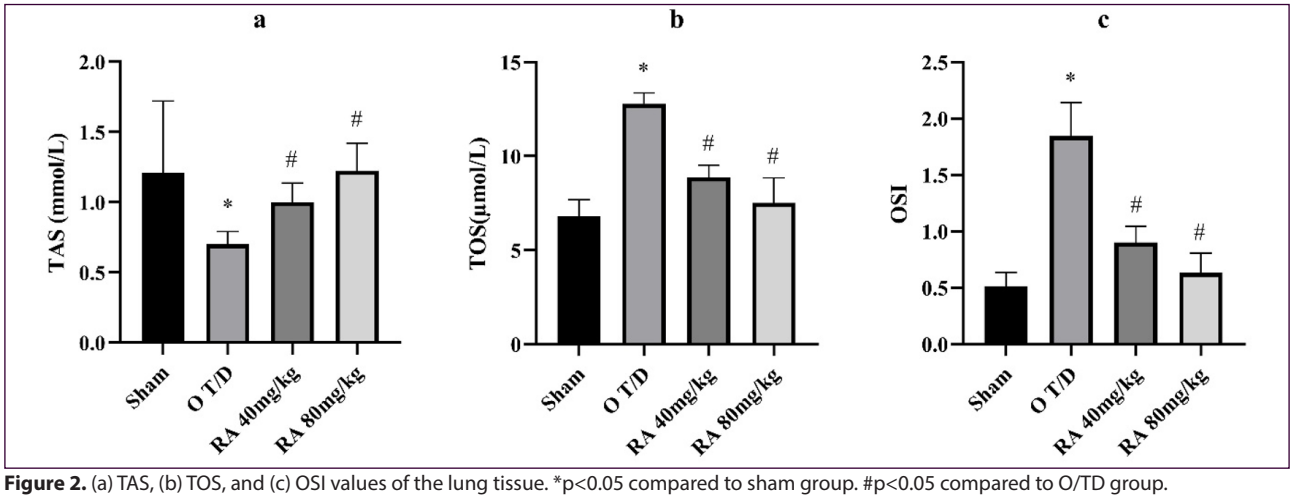

Figure 2. (a) TAS, (b) TOS, and (c) OSI values of the lung tissue. *p<0.05 compared to sham group. #p<0.05 compared to O/TD group.

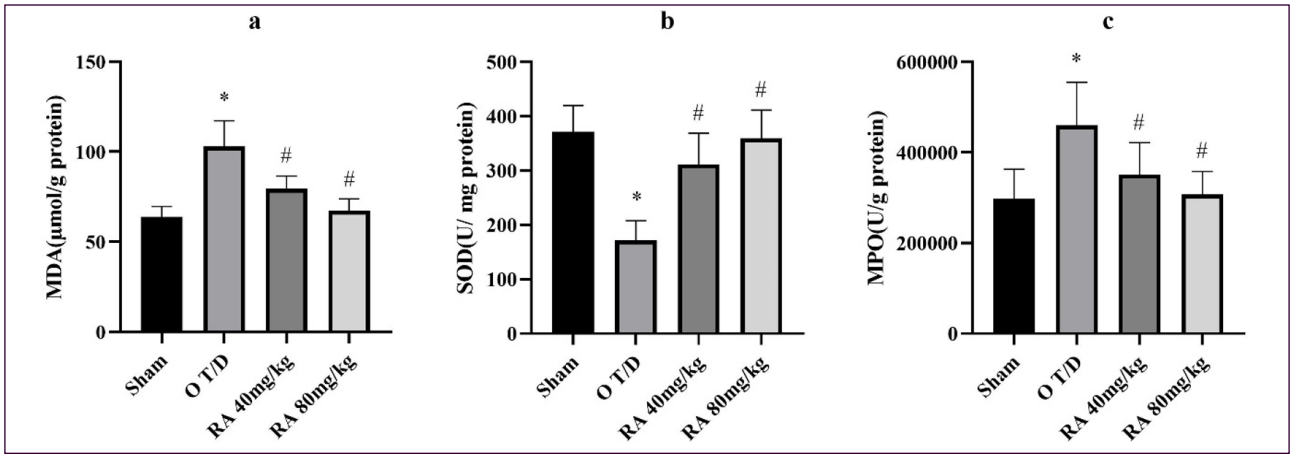

Figure 3. (a) MDA, (b) SOD, and (c) MPO values of ovarian tissue. *p<0.05 compared to sham group. #p<0.05 compared to O/TD group.

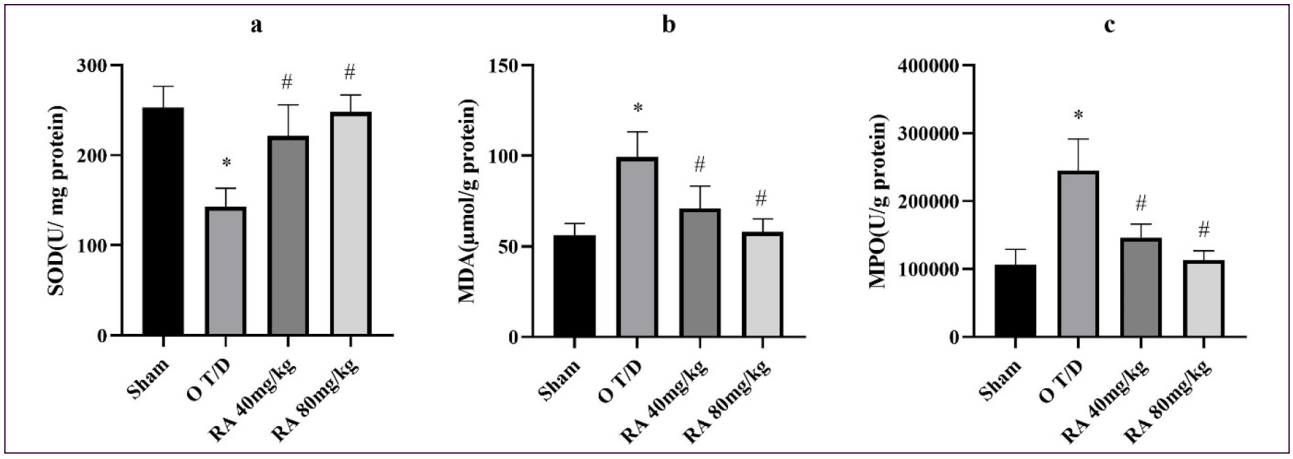

Figure 4. (a) SOD, (b) MDA, and (c) MPO values of the lung tissue. *p<0.05 compared to sham group. #p<0.05 compared to O/TD group.

DISCUSSION

Ovarian torsion affects all women of different ages. It is the rotation of the ovaries around utero-ovarian and infundibulopelvic ligaments (26). Once the ovarian torsion is diagnosed, detorsion of the twisted adnexa is vital to prevent infertility (2, 27). This O/TD process is named ischemia-reperfusion (I/R) injury (28). Ischemia leads to hypoxic injury. During the detorsion phase, even though blood flow may alleviate the injury, reperfusion stimulates the overexpression of reactive oxygen species (ROS) (29, 30). ROS enhances oxidant molecule production and decreases antioxidant levels, including SOD (31). SOD protects against the undesirable effects of ROS, and it is a part of TAS (32).

OSI is a sensitive rate for the oxidative stress assessment (33). The measurement of TAS and TOS levels preferred for the evaluation of I/R injuries (34). High ROS levels and decreased antioxidant activity negatively affect the oxidative–antioxidative balance in favor of oxidative stress (30). ROS enhances MDA production. MDA is a toxic lipid peroxidation product and may alter the membrane structure and cell functions (10). Therefore, it is an indicator of the stress levels both in vitro and in vivo (35). MPO is an enzyme located in neutrophils and is a marker of neutrophil infiltration (36). MPO activity increases during I/R-induced ovarian injury (37).

RA is a phenolic compound (38) with various pharmacological properties, including anti-inflammatory (39), anticancer, and antioxidant activities (40). RA alleviated renal I/R injury with its anti-inflammatory and antioxidant effects in a previous rat study (41). Another study showed that RA protects against cerebral ischemia in diabetic rats with its anti-inflammatory properties (42). RA treatment has been reported to prevent sepsis-induced oxidative damage by raising the SOD levels while diminishing the MDA values in rats. (43). It has been shown that RA increases antioxidant enzyme activity (SOD, etc.) and decreases MDA levels in renal and liver tissues of elderly mice (44).

RA administration performed a renoprotective effect against gentamicin-induced renal cortical oxidative stress in rats by increasing SOD levels and decreasing MDA values (45). Previous research has established that RA reduces spinal cord damage by reducing ROS and lipid peroxidation while increasing antioxidant parameters (46). In a rat model, RA administration alleviated O/TD-related damage in ovarian tissues (47) in harmony with our results. In addition, we also examined the lung tissues and here, we investigated RA to find out the possible protective effects against O/TD in both ovarian and lung tissues.

Understanding the injury pathways of O/TD is vital for new treatment methods. O/TD studies represented that the suppression of oxidative stress might contribute to the treatment. Here, oxidative stress parameters were suppressed, and antioxidant activity enhanced by RA administration, which encourages hope in the treatment of O/TD.

CONCLUSION

In this study, RA treatment reduced O/TD-induced ovarian and lung injuries in the experimental animals. Further research are necessary to find out the possible preventive mechanisms against ovarian and lung injuries induced by O/TD.

ETHICAL DECLARATIONS

Ethics Committee Approval: The current search was confirmed by Atatürk University Local Ethics Council of Animal Experiments (protocol number: 28.06.2018/141).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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