

# Myricetin Decreases Ovarian and Lung Tissue Injury Induced by Ovarian Torsion-Detorsion: A Biochemical Study

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## ABSTRACT

**Objective:** This study was designed to examine the effects of myricetin (Myr) on ovarian and lung tissue in rats with induced torsion-detorsion (TD) of bilateral ovaries to determine the potential to reduce oxidative damage.

**Methods:** The study group comprised 32 female Wistar albino rats randomly allocated to 4 groups: sham, ovarian TD, Myr/25 (25 mg/kg dose of Myr+TD), and Myr/50 (50 mg/kg dose of Myr+TD).

**Results:** The total oxidant status (TOS), malondialdehyde (MDA) level, oxidative stress index (OSI), and myeloperoxidase (MPO) activity in both ovarian and lung tissues increased significantly in the ovarian TD group compared with the sham group, while the superoxide dismutase (SOD) and total antioxidant status (TAS) values decreased in the same group. In contrast, the SOD level increased, while MPO activity, the TOS, the OSI, and the MDA level decreased significantly in the Myr/25 and Myr/50 groups.

**Conclusion:** Myr demonstrated protection against lung and ovarian tissue injury in induced-TD experimental rats.

## INTRODUCTION

Ovarian torsion (OT) describes a condition when the ovary and its vascular peduncle become twisted around the suspensory ligament axis. This circumstance represents almost 3% of gynecological emergencies.<sup>[1]</sup> Vomiting, acute pelvic pain, and nausea are common symptoms.<sup>[2]</sup> It is particularly important and most frequently seen in women during their child-bearing years.<sup>[3]</sup> OT, also known as adnexal torsion, requires urgent gynecological surgery. Although it is most often observed in mature women, OT may also occur in prepubertal and postmenopausal women.<sup>[4]</sup> Various factors, including cytokines, reactive oxygen species (ROS), inflammation, and neutrophil activation, have a role in the pathogenesis of ischemia reper-

fusion (I/R) injury.<sup>[5]</sup> The reperfusion stage causes much more tissue damage than the ischemic phase.<sup>[6]</sup> Numerous experimental studies have investigated how to alleviate ovarian tissue damage due to adnexal torsion-detorsion (TD).<sup>[7,8]</sup> It has been established that I/R directly injures the primary organ. However, it has been reported by some researchers that I/R may also cause injury to a remote organ by initiating inflammatory and oxidative reactions in the tissues of secondary organs.<sup>[9,10]</sup> A literature review did not reveal any study of remote organ damage induced by ovarian TD. The current study represents a contribution to the literature resources related to this condition.

Myricetin (3, 3', 4', 5, 5', 7-hexahydroxyflavone, Myr) sources include tea, vegetables, berries, fruits, and nut.<sup>[11,12]</sup>

[15,16] Myr has been shown to have anti-inflammatory,[13,14] antioxidative,[14,15] analgesic, antitumor,[16,17] and antibacterial effects.[18]

The objective of this study was to analyze the effects of Myr on ovarian and lung tissues and determine the potential to reduce oxidative damage in an ovarian TD model.

## MATERIALS AND METHODS

### Experimental animals and ethics approval

The present study was approved by the Atatürk University Animal Research Ethics Committee on June 28, 2018 (no: 140). The experimental animals were acquired from the university Experimental Animal Application and Research Center. The rats were housed in polypropylene cages under accepted appropriate laboratory conditions of 12-hour light/darkness, 22±2°C temperature, and 55±5% humidity. Standard rat feed and drinking water were provided. No feed was supplied 12 hours before the experiment, but water was accessible.

### Groups and torsion-detorsion model

A total of 32 female Wistar albino rats, 220–240 g in weight, were randomly divided into 4 groups. In the sham group, anesthesia was administered, the abdominal region was shaved and cleaned, and an incision was created and closed without performing TD or administering any medication. In the ovarian torsion detorsion (OTD) group, following anesthesia, the rats were held in the dorsal horizontal position. Povidone-iodine was used as an antiseptic to disinfect the incision area. A median laparotomy incision 1–2 cm in size was performed. Both ovaries, the fallopian tubes, and the ovarian arteria and veins were spun clockwise about 720° and compressed with atraumatic microvascular clamps for 3 hours. Blood flow was reestablished to create the detorsion stage by releasing the clamps. The incision region was repaired using a 3/0 silk suture. After detorsion, the lung and ovary tissues were excised. In the Myr 25 mg/kg and Myr 50 mg/kg groups, Myr (Merck KGaA, Darmstadt, Germany) was administered to the subjects intraperitoneally (IP) at a dose of 25 or 50 mg/kg 30 minutes before detorsion. The Myr dose used was based on previous research.[18,23] The TD model was then performed. Anesthesia and euthanasia of 10 mg/kg IP xylazine hydrochloride (Rompun; Bayer AG, Leverkusen, Germany) and 60 mg/kg IP ketamine (Ketalar; Pfizer, Inc., NY, NY, USA) was applied as previously de-

scribed.[20] The lungs and ovaries were removed, washed, and kept frozen for analysis.

### Biochemical assessments

The total antioxidant status (TAS) and total oxidant status (TOS) were measured using Rel Assay Diagnostics kits, (Mega Tip San.Tic. Ltd. Sti., Gaziantep, Turkey). The oxidative stress index (OSI) was calculated using the formula  $OSI = [(TOS, \mu\text{mol H}_2\text{O}_2 \text{ equivalent L}) / (TAS, \text{mmol Trolox equivalent/L}) \times 10]$ . The superoxide dismutase (SOD) evaluation was based on the production of superoxide radicals generated by xanthine and the xanthine oxidase system, which acts with nitroblue tetrazolium to yield formazan dye.[21] The lipid peroxidation in ovarian and lung tissue was measured by assessing the malondialdehyde (MDA) level using the thiobarbituric acid test.[26] The activity of myeloperoxidase (MPO) in the ovarian and lung tissues were estimated according to methods described by Bradley et al.[23]

### Statistical analysis

All of the results were presented as mean±SEM. The results were analyzed using one-way analysis of variance and the Tukey test for pairwise comparisons of groups. Statistical significance was established at  $p < 0.05$ .

## RESULTS

### Biochemical results of the ovarian tissue

Evaluation of ovarian tissue TAS, TOS, and OSI findings revealed that the TAS values significantly decreased following OTD induction, while the TOS and OSI values increased, reflecting oxidative stress. However, when the groups that had received treatment with 25 and 50 mg/kg of Myr were compared with the OTD group, the TAS values increased and the TOS and OSI values decreased. This result was statistically significant ( $p < 0.05$ ; Table 1).

In the OTD group, the MDA level and MPO activity significantly increased compared with the sham group results, and SOD activity significantly decreased. These results were significantly different in both Myr treatment groups ( $p < 0.001$ ; Fig. 1a-c).

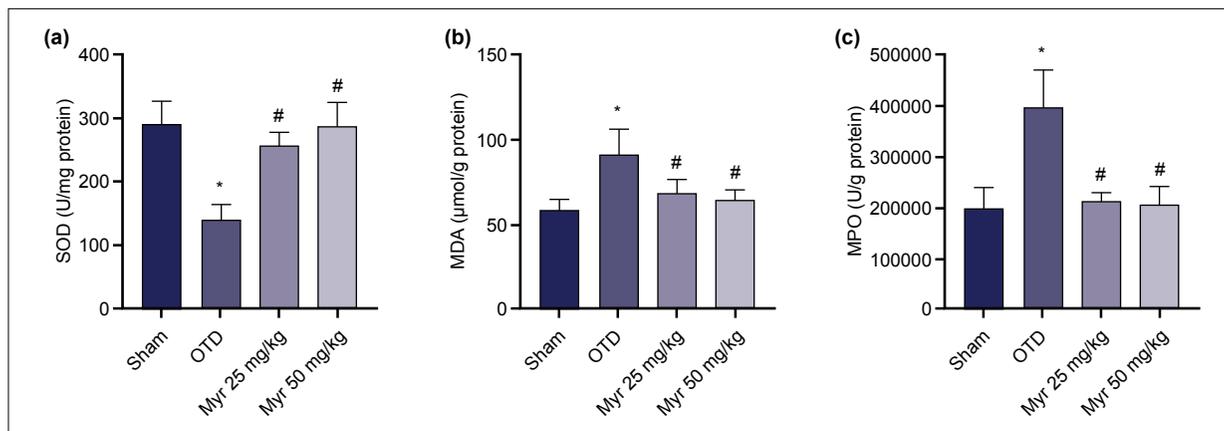
### Lung tissue biochemical results

All of the lung tissue data are presented as mean±SEM. Evaluation of the TAS, TOS, and OSI findings revealed that

**Table 1.** Results of total antioxidant status, total oxidant status, and oxidative stress index levels in the ovarian tissue of all groups

| Groups/Parameters                 | Sham      | OTD        | Myricetin 25 mg/kg | Myricetin 50 mg/kg |
|-----------------------------------|-----------|------------|--------------------|--------------------|
| Total antioxidant status (mmol/L) | 0.99±0.04 | 0.47±0.02* | 0.83±0.03#         | 0.96±0.03#         |
| Total oxidant status (μmol/L)     | 5.01±0.13 | 8.56±0.33* | 5.84±0.14#         | 5.15±0.22#         |
| Oxidative stress index            | 0.51±0.02 | 1.80±0.07* | 0.70±0.02#         | 0.54±0.03#         |

\* $P < 0.05$  compared to sham group. # $P < 0.05$  compared to OTD group. OTD: Ovarian torsion detorsion.



**Figure 1.** Comparison of ovarian tissue oxidative stress results by group: (a) superoxide dismutase (SOD), (b) malondialdehyde (MDA), and (c) myeloperoxidase (MPO). \*P<0.05 compared to sham group; #P<0.05 compared to ovarian torsion-detorsion (OTD) group. Myr: Myricetin.

the TAS values significantly decreased following OTD induction, while the TOS and OSI values increased and reflected oxidative stress in the lung tissue. Comparison of the groups with low and high doses of Myr treatment and the OTD group indicated that the TAS values increased and the TOS and OSI values decreased in the lung tissue of the Myr groups. This result was statistically significant ( $p<0.05$ ; Table 2).

The MDA level and MPO activity observed in the lung tissue were considerably greater in the OTD group compared with the sham group, and the SOD activity was significantly lower. The results were significantly different in the Myr groups ( $p<0.001$ , Fig. 2a-c).

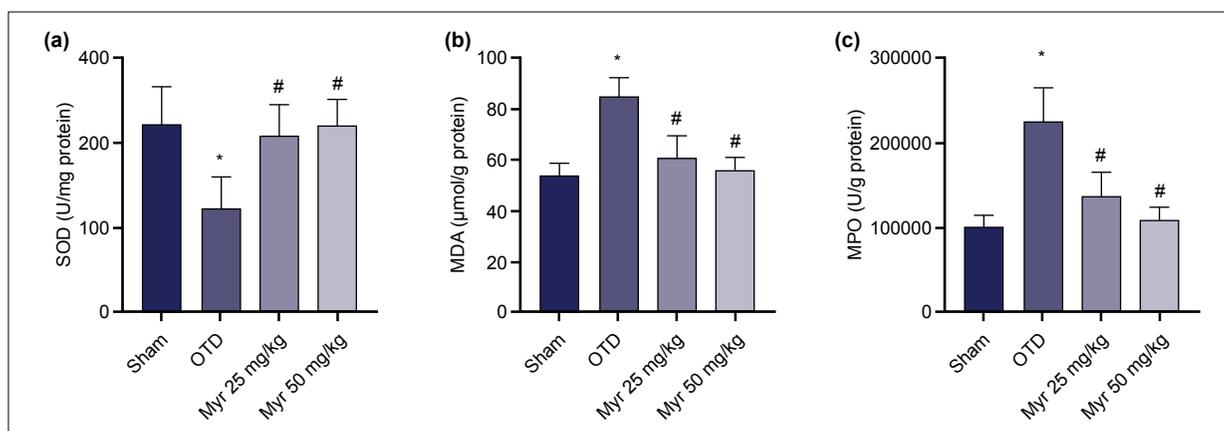
## DISCUSSION

Although women of all age ranges may experience OT, it is most common during the premenarchal or reproductive period.<sup>[24]</sup> OT occurs when the ovary rotates between the utero-ovarian and infundibulopelvic ligament.<sup>[3]</sup> Conservative treatment is important to protect fertility.<sup>[25]</sup> Ischemia can lead to hypoxic damage. Following the detorsion phase, reperfusion results in excessive ROS production.<sup>[1,26]</sup> When ischemic tissue is exposed to oxygen, a series of reactions take place and causes more damage than the ischemic stage.<sup>[27]</sup> I/R causes oxidant parameters to increase and antioxidant parameters to decrease in

**Table 2.** Results of total antioxidant status, total oxidant status, and oxidative stress index levels in the lung tissue of all groups

| Groups/Parameters                 | Sham      | OTD         | Myricetin 25 mg/kg | Myricetin 50 mg/kg |
|-----------------------------------|-----------|-------------|--------------------|--------------------|
| Total antioxidant status (mmol/L) | 1.03±0.07 | 0.73±0.04*  | 1.00±0.05#         | 1.03±0.05#         |
| Total oxidant status (µmol/L)     | 6.87±0.30 | 10.44±0.29* | 7.85±0.15#         | 7.34±0.29#         |
| Oxidative stress index            | 0.69±0.06 | 1.45±0.10*  | 0.79±0.04#         | 0.72±0.04#         |

\*P<0.05 compared to sham group. #P<0.05 compared to OTD group. OTD: Ovarian torsion detorsion.



**Figure 2.** Comparison of lung tissue oxidative stress results by group: (a) superoxide dismutase (SOD), (b) malondialdehyde (MDA), and (c) myeloperoxidase (MPO). \*P<0.05 compared to sham group. #P<0.05 compared to ovarian torsion-detorsion (OTD) group. Myr: Myricetin.

ovarian tissue, as well as liver, brain, intestine, and heart tissue.<sup>[28]</sup>

TOS and TAS reflect the antioxidation and oxidation balance. The TAS measurement demonstrates all antioxidant activity, while the TOS illustrates ROS.<sup>[29,30]</sup> In this study, the administration of different doses of Myr elevated TAS levels and reduced TOS values, which suggests that Myr has a protective effect against oxidative damage.

SOD catalyzes superoxide free radical conversion into molecular oxygen and superoxide free radicals. SOD and endogenous antioxidant enzymes defuse free radicals and protect tissues.<sup>[31]</sup> Myr has been shown to increase SOD and weaken lipopolysaccharide-induced cardiac damage.<sup>[32]</sup> Myr has also been shown to have a cardioprotective effect in diabetic cardiomyopathy.<sup>[33]</sup> Myr has been reported to increase SOD and catalase levels and have a mitigating effect on ochratoxin A-induced oxidative stress in the rat renal cortex.<sup>[34]</sup> The increase in TAS and SOD levels demonstrate that Myr acts as an antioxidant molecule.

ROS affects cell membrane lipids and results in elevated toxic materials, such as MDA.<sup>[35]</sup> MDA is an end product of lipid peroxidation and leads to cell damage through polymerization induction and the crosslinking of membrane components.<sup>[36]</sup> MDA is important for I/R-related oxidative injury evaluation.<sup>[37]</sup> Myr scavenges free oxygen radicals to protect against lipid peroxidation and has anti-inflammatory, anticarcinogenic, antihyperglycemic, and antiviral effects.<sup>[38]</sup> Myr has been reported to reduce the MDA level in an intestinal I/R injury model.<sup>[19]</sup> Myr has diverse immunoregulatory functions, including antioxidant,<sup>[39]</sup> and anti-inflammatory properties.<sup>[40]</sup> It has also been observed to have a protective effect against oxidative injury in neurodegenerative disorders.<sup>[41,42]</sup> Furthermore, it has been reported that Myr increased the level of antioxidant molecules and reduced the MDA level in a middle-cerebral artery occlusion rat model.<sup>[43]</sup> Our results were similar to those of other studies that have found that Myr reduced the level of MDA.

Macrophages and neutrophils produce MPO, which is a catalyst in the chlorine and hydrogen peroxide reaction to create hypochlorous acid. Hypochlorous acid plays a role in hydroxyl radical formation.<sup>[44]</sup> There are few studies investigating the relationship between Myr and MPO. As in the current study, Zhao et al.<sup>[45]</sup> noted that Myr decreased the MPO level in an ulcerative colitis model.

## CONCLUSION

This study analyzed oxidative stress in ovarian and lung tissue to investigate the potential protective effect of Myr against OTD-induced ovarian and lung injuries and found that oxidative stress was diminished with Myr treatment. Myr suppressed inflammation and oxidative stress pathways, which may provide a new molecule in the treatment of OTD. Additional studies are necessary to examine other protective mechanisms for OTD-related ovarian and lung tissue injuries.

## Ethics Committee Approval

Approved by the Atatürk University Animal Research Ethics Committee (June 28, 2018; No: 140).

## Peer-review

Internally peer-reviewed.

## Authorship Contributions

Concept: A.T., E.E.; Design: M.C.G., F.N.E.A.; Supervision: Y.E.T., M.C.G.; Fundings: Y.E.T., F.N.E.A.; Materials: A.T., E.E.; Data: F.N.E.A., M.C.G.; Analysis: A.T., E.E., F.N.E.A.; Literature search: Y.E.T., M.C.G.; Writing: Y.E.T., F.N.E.A., A.T.; Critical revision: A.T., Y.E.T.

## Conflict of Interest

None declared.

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## Mirisetin Over Torsiyonu Detorsiyon ile indüklenmiş Over ve Akciğer Hasarlarını Azaltır: Biyokimyasal Bir Çalışma

**Amaç:** Sıçanlarda oluşturulan iki taraflı over torsiyon distorsiyon modelinin neden olduğu over ve akciğer hasarı üzerine mirisetin'in olası yararlı etkilerinin araştırılması planlandı.

**Gereç ve Yöntem:** Otuz iki adet Wistar-Albino dişi sıçan rastgele dört gruba ayrıldı. Bu araştırmanın grupları, sham, over torsiyon detorsiyon, Myr/25 (25 mg/kg dozda mirisetin+torsiyon detorsiyon) ve Myr/50 (50 mg/kg dozda mirisetin+torsiyon detorsiyon) olarak tasarlandı.

**Bulgular:** TOS, MDA, OSI seviyeleri ve MPO aktivitesi, hem over hem de akciğer dokuları için, over torsiyon detorsiyon grubunda sham grubuna göre anlamlı olarak arttı. Bununla birlikte, over torsiyon detorsiyon grubunda SOD aktivitesi ve TAS değeri azaldı. Bunun aksine, Myr/25 ve Myr/50 gruplarında mirisetin tedavisi nedeniyle MPO aktivitesi, TOS, OSI ve MDA seviyeleri azalırken SOD aktivitesinin anlamlı olarak artmıştır.

**Sonuç:** Sonuç olarak, sıçanlarda over torsiyon detorsiyonunun neden olduğu over ve akciğer dokusu hasarına karşı korumada mirisetin uygulamasının etkili olduğu belirlendi.

**Anahtar Sözcükler:** Akciğer; mirisetin; over; over torsiyon detorsiyonu; sıçan.