



# Effect of Anthocyanin on the Ovarian Damage and Oxidative Stress Markers in Adult Female Rat After Torsion/Detorsion

Afshin Teymoori<sup>1</sup>, Arash Khaki<sup>1\*</sup>, Ghazal Rahmanifar<sup>1</sup>, Arman Khatami<sup>1</sup>, Ali Rezaii<sup>1</sup>

## Abstract

**Objectives:** This experimental study aimed to explore the impact of anthocyanin on histopathological alterations and oxidative stress induced by ovarian torsion/detorsion in mature rats.

**Materials and Methods:** A total of 32 female Wistar rats were categorized into four groups: G1 (sham), G2 (ovarian torsion endured for 3 hours followed by detorsion), G3 (ovarian torsion persisted for 3 hours with prior administration of 100 mg/kg anthocyanin followed by detorsion), and G4 (received 100 mg/kg anthocyanin). After a reperfusion interval of 10 days, blood specimens were obtained for quantifying estrogen, testosterone, and oxidative stress markers in the plasma. Histomorphometric analysis was done to evaluate post-procedural tissue alterations.

**Results:** The histopathological observations revealed considerable degenerative modifications in the G2 group (subjected to torsion/detorsion) compared to the other groups ( $P < 0.001$ ). Estrogen, glutathione peroxidase (GPX), and superoxide dismutase (SOD) levels displayed notable reduction in the G2 group; however, these levels were reinstated in the anthocyanin-treated groups. The level of malondialdehyde (MDA), an indicator of oxidative stress, surged during the ischemic phase ( $P < 0.001$ ).

**Conclusions:** Administration of anthocyanin led to a decline in MDA levels across the other groups. These findings collectively suggest that anthocyanin supplementation holds the potential to shield ovarian tissue against oxidative stress and structural damage incurred as a consequence of torsion/detorsion events.

**Keywords:** Ovarian torsion, Tissue injury, Oxidative stress, Anthocyanin

## Introduction

Ovarian torsion is a medical emergency characterized by the rotation of the ovary and fallopian tube around their vascular and ligamentous structures. It is estimated that the prevalence of ovarian torsion among patients presenting with acute abdominal pain is approximately 3% (1). Torsion leads to impaired blood flow to the ovary, resulting in ischemia and potentially leading to ovarian necrosis. Detorsion, or the surgical intervention aimed at restoring blood flow, is performed as the initial step to preserve ovarian tissue and fertility. However, the process of detorsion, or reperfusion, can lead to the generation of reactive oxygen species (ROS), causing oxidative stress and subsequent damage to the ovarian tissue (1).

Oxidative stress refers to an imbalance between the production of ROS and the body's antioxidant defense system. In the context of ovarian torsion and detorsion, this process leads to an increase in ROS and free radicals, which in turn results in lipid peroxidation and a decrease in the activity of antioxidant enzymes. The resulting ischemia-reperfusion (I/R) injury and oxidative stress can have detrimental effects on the number of normal follicles and contribute to an increase in the presence of atretic bodies in the ovarian tissue (2-4). Previous studies have consistently demonstrated that ovarian torsion is a significant cause of

ovarian tissue damage and can lead to infertility. Although the process of detorsion or reperfusion is crucial to restore blood flow to the ovary, it can contribute to additional injury. The sudden reintroduction of blood flow during detorsion can trigger a cascade of events, including the generation of ROS, inflammation, and oxidative stress, which further contribute to tissue damage and impaired fertility. Therefore, while detorsion is necessary to salvage the ovary, it also poses a risk of exacerbating the existing injury and compromising reproductive outcomes.

Utilization of antioxidant compounds plays a significant role in the recovery process and effectively mitigates the detrimental effects of oxidative damage. Medicinal plants, with their natural products, offer a promising alternative to conventional drugs in combating oxidative damage. These plants are rich in bioactive compounds, such as antioxidants, which possess the ability to scavenge harmful free radicals and restore cellular homeostasis. It is possible to harness the beneficial properties of medicinal plants and enhance the body's antioxidant defense system by incorporating medicinal plants into therapeutic strategies. This approach holds great potential for attenuating oxidative damage and promoting overall health and well-being (2).

Anthocyanins, particularly cyanidin, are widely



## Key Messages

- ▶ Ovarian torsion/detorsion led to oxidative stress and tissue damage in the ovary of adult rats.
- ▶ Administration of anthocyanin improved ovarian tissue damage.

distributed in various plant tissues, including leaves, fruits, flowers, and roots. They are responsible for the vibrant colors observed in plants, such as cornflower blue and rose red. Delphinidin and pelargonidin are also common anthocyanins found in plants. Animal studies have demonstrated that anthocyanins are rapidly absorbed, with detectable levels appearing in the bloodstream within 6 to 20 minutes after ingestion. Peak plasma concentrations are typically reached between 16 to 60 minutes. The concentration of anthocyanins in the plasma varies, ranging from nanomolar to micromolar levels (5,6). Previous studies showed that anthocyanins have antioxidant and anti-inflammatory effects and can protect the tissue against oxidative damage (5-7).

This study aimed to investigate the impact of anthocyanins on ovarian tissue damage in a rat model of torsion/detorsion. We also assessed the potential of anthocyanins in mitigating the detrimental effects caused by torsion and subsequent detorsion of the ovary. We hypothesized that the antioxidant properties of anthocyanins help reduce oxidative stress and protect against tissue damage in the ovaries.

### Materials and Methods

A total of 32 female Wistar rats weighing 200-250 g were procured from the animal facility of Tabriz University of Medical Sciences and were accommodated under standard conditions within the animal housing facility. The animals had unrestricted access to both food and water. The sample size was selected as eight samples in each group based on a previous study (2). The rats were randomly allocated to four groups as follows:

- Group 1 (Sham): an incision measuring 2.5 cm in length was performed along the lower midline of the abdomen, subsequently sutured using 6/0 nylon (n=8).
- Group 2 (torsion/detorsion (TD) group): ovarian torsion was induced followed by detorsion after 3 hours. Half an hour prior to the detorsion procedure, the animals received a solution of normal saline (n=8).
- Group 3 (torsion/detorsion with anthocyanin (TDA) group): ovarian torsion was prompted, and detorsion was executed after 3 hours. All subjects in this group were subjected to a treatment regimen involving the administration of 100 mg/kg anthocyanin (obtained from Sigma-Aldrich, USA) through intraperitoneal injection, administered 30 minutes ahead of the detorsion process (n=8).

- Group 4 (anthocyanin): this group did not undergo the ovarian torsion/detorsion protocol. Instead, the rats in this group were solely administered 100 mg/kg anthocyanin via intraperitoneal injection (n=8).

### Surgical Procedure

For the surgical protocol, all specimens within the experimental cohorts were subjected to anesthesia using doses of 50 mg/kg ketamine and 10 mg/kg xylazine. A longitudinal incision, measuring 2.5 cm, was performed along the lower midline of the abdominal region. Subsequently, a minor incision was made within the peritoneal region, facilitating the exposure of the left uterine horns and associated adnexal structures. The left ovary was rotated 720 degrees in a clockwise manner around its ligament and vascular structures. To avert detorsion, the ovary was immobilized. The closure of the incision was accomplished utilizing 6/0 nylon sutures; this configuration was sustained for 3 hours. Approximately 30 minutes prior to the detorsion process, anthocyanin was introduced through intraperitoneal injection.

The ovarian rotation was reversed after an initial surgical procedure lasting for 3 hours, and the rats underwent a 10-day treatment period, referred to as the reperfusion period (2). At the culmination of the 10-day interval, all rats were subjected to anesthesia employing ketamine and xylazine. Blood specimens were extracted from the left ventricle of the heart to assess serum markers of oxidative stress and alterations in hormones. These blood samples were subjected to centrifugation at 3000 RPM for 5 minutes, followed by separation of the plasma into microtubes (500  $\mu$ L), which were subsequently preserved in a freezer at -80  $^{\circ}$ C until the experimental phase.

Concomitantly, ovarian tissue specimens were procured to examine histopathological changes. The acquired samples were immersed in a 10% formalin solution for 72 hours, subjected to dehydration, embedded within paraffin, and then sectioned to a thickness of 5  $\mu$ m using a microtome. The terminal step involved staining all sections utilizing the hematoxylin & eosin (H&E) technique (2).

### Histological Examination

For histological assessment, the ovarian tissue was fixed with 10% formalin. Then, the samples entered the tissue passage stage and were immersed in paraffin. After that, the paraffin sections were prepared from the tissue, and 5-micron slices were prepared from the samples using a microtome. Tissue slides were prepared and evaluated in a spiral manner from the cortex to the medulla of the ovarian tissue. The entire slide was examined to determine the number of preantral follicles, antral follicles, graafian follicles, as well as the presence of atretic bodies. To count the follicles, the area of the ovarian cortex was examined in a counterclockwise direction. These measurements were performed and compared among all the study groups (2).

### Evaluation of Biochemical Parameters

To measure the level of malondialdehyde (MDA), 0.2 cc of plasma was mixed with 3 cc of glacial acetic acid and 3 cc of thiobarbituric acid (TBA) solution containing 2% NaOH. The resulting mixture was placed on a shaker and then subjected to boiling water for 15 minutes. After cooling the solution, the absorbance was measured at a wavelength of 532 nm to quantify the level of MDA. The intensity of the pink color in the solution corresponded to the concentration of MDA (8).

### Measuring the Activity of SOD and GPX

The plasma levels of superoxide dismutase (SOD) and glutathione peroxidase (GPX) were assessed using the protocols provided by the Ransod and Randox kits, respectively. These kits are designed to measure the activity or concentration of SOD and GPX in the plasma samples. The specific steps and reagents outlined in the respective kit protocols were followed to accurately measure the levels of SOD and GPX in the samples.

### Measuring Testosterone and Estrogen Levels

The quantification of plasma testosterone and estrogen levels was achieved using an enzyme-linked immunosorbent assay (ELISA) kit, specifically the kit provided by the Demeditec Diagnostics, Germany, under the catalog number 4925-300A. This ELISA kit is systematically devised to detect and quantitate the concentrations of testosterone and estrogen present within the plasma samples. The fundamental mechanism of the assay encompasses the utilization of antibodies that are highly specific to testosterone and estrogen molecules. Through this binding interaction, a colorimetric reaction ensues, and subsequently, the absorbance of the samples is quantified at a wavelength of 405 nm. By juxtaposing the absorbance readings of the test samples against a pre-established standard curve, the concentrations of testosterone and estrogen within the plasma are accurately ascertained.

### Statistical Analysis

Statistical analysis was conducted using the IBM SPSS version 20 (IBM, USA). The normality of data was evaluated using the Kolmogorov-Smirnov test. The outcomes were expressed as mean  $\pm$  standard deviation (SD). To contrast the histopathological parameters and

oxidative stress measurements across the groups, a one-way analysis of variance (ANOVA) was initially executed, followed by Tukey's post hoc test. The criterion for statistical significance was established at  $P < 0.05$ , thereby indicating a noteworthy disparity among the groups.

## Results

### The Number of Follicles

The analysis of histopathological data disclosed notable findings. In the TD group, there was a significant reduction in the tally of preantral, antral, and graafian follicles compared to the sham group ( $P = 0.001$ ). Correspondingly, the TD group exhibited a markedly higher count of atretic bodies compared to the sham group ( $P = 0.001$ ). Furthermore, a statistically significant difference was observed between the TD group and the remaining therapeutic groups, specifically concerning the quantification of normal follicles and atretic bodies ( $P = 0.001$ ).

Significant changes were observed in the experimental groups treated with anthocyanin. Evidently, there was a significant increase in the quantification of preantral, antral, and graafian follicles compared to the TD group ( $P = 0.001$ ). Likewise, the therapeutic groups manifested a significant reduction in the number of atretic bodies compared to the TD group ( $P = 0.001$ ). Table 1 and Figure 1 show the comprehensive representation of these findings.

### The Level of Estrogen

There was a significant reduction in the estrogen levels in the TD group compared to the sham group ( $P = 0.001$ ). Interestingly, estrogen levels increased in the anthocyanin-treated group compared to the TD group ( $P = 0.001$ ). More precisely, in group 4, the estrogen levels exhibited an elevation compared to group 3; however, this increase was not statistically significant ( $P = 0.001$ ). Figure 2 depicts these results.

### The Level of Testosterone

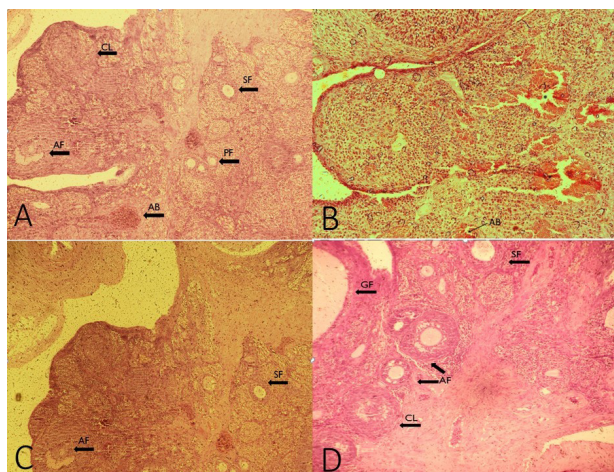
The serum testosterone level was significantly higher in the TD group compared to the sham group ( $P = 0.001$ ). Conversely, there was a significant reduction in the serum testosterone level in the experimental group treated with anthocyanin compared to the TD group ( $P = 0.001$ ). These findings are illustrated in Figure 3.

**Table 1.** The Count of Follicles in the Ovaries of Rats in the Study Groups

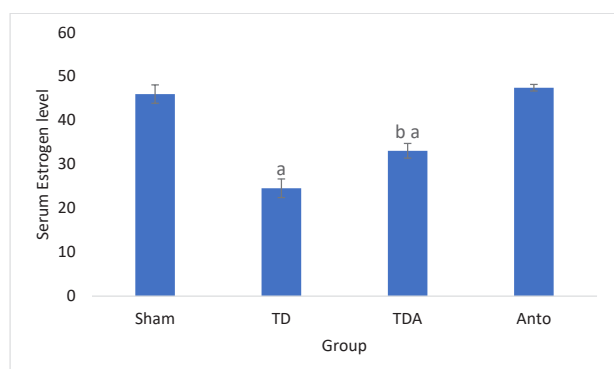
Group	Preantral Follicles	Antral Follicles	Graafian Follicles	Atretic Bodies	Corpus Luteum
Sham	6.8 $\pm$ 1.15	6.60 $\pm$ 0.75	5.25 $\pm$ 1.20	1.15 $\pm$ 0.74	5.3 $\pm$ 1.15
TD	1.5 $\pm$ 0.17 <sup>a</sup>	1.00 $\pm$ 0.75 <sup>a</sup>	0.50 $\pm$ 0.07 <sup>a</sup>	6.65 $\pm$ 0.92 <sup>a</sup>	0.50 $\pm$ 0.06 <sup>a</sup>
TDA	4.2 $\pm$ 0.25 <sup>a,b</sup>	3.6 $\pm$ 0.65 <sup>a,b</sup>	3.25 $\pm$ 0.43 <sup>a,b</sup>	1.2 $\pm$ 0.19 <sup>a,b</sup>	3.14 $\pm$ 0.29 <sup>a,b</sup>
Anthocyanin	7.50 $\pm$ 1.09 <sup>b</sup>	7.00 $\pm$ 0.81 <sup>b</sup>	6.2 $\pm$ 0.81 <sup>b</sup>	1.03 $\pm$ 0.50 <sup>b</sup>	5.60 $\pm$ 1.29 <sup>b</sup>

All data are displayed as mean  $\pm$  SD.

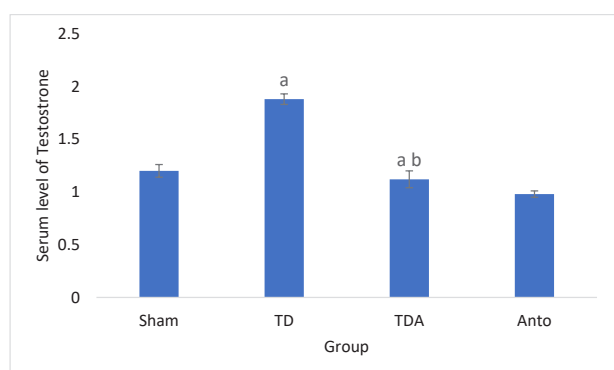
<sup>a</sup> shows a significant difference with sham and <sup>b</sup> shows a significant difference with TD.



**Figure 1.** Histological Findings. (A) Sham group; (B) Torsion/detorsion group; (C) Torsion/detorsion+ anthocyanin; (D) healthy rat received anthocyanin. AF: antral follicle; SF: secondary follicle; GF: graafian follicle; AB: atretic body; CL: corpus luteum.



**Figure 2.** The Estrogen Levels. <sup>a</sup> shows a significant difference with sham and <sup>b</sup> shows a significant difference with TD.



**Figure 3.** The Testosterone Levels. <sup>a</sup> shows a significant difference with sham and <sup>b</sup> shows a significant difference with TD.

### The Serum Antioxidant Enzymes

The serum concentration of SOD significantly decreased in the TD group compared to the sham group ( $p=0.001$ ). The animals which received anthocyanin experienced elevated serum SOD levels compared to the TD group ( $P=0.001$ ). In terms of the GPX level, the concentration significantly increased in the TD group compared to the

**Table 2.** Serum Oxidative Stress Markers of Rats in the Study Groups

Groups	SOD	GPX	MDA
Sham	1.65±0.85	225.69±16.50	0.97±0.06
TD	0.68±0.07 <sup>a</sup>	75.22±2.16 <sup>a</sup>	2.70±0.16 <sup>a</sup>
TDA	1.26±0.10 <sup>a,b</sup>	178.07±5.20 <sup>a,b</sup>	1.38±0.08 <sup>a,b</sup>
Anthocyanin	1.70±0.11 <sup>b</sup>	232.65±4.07 <sup>b</sup>	1.00±0.03 <sup>b</sup>

All data are displayed as mean ± SD.

<sup>a</sup> shows a significant difference with sham and <sup>b</sup> shows a significant difference with TD.

sham group ( $P=0.001$ ). Regarding the serum MDA levels, the TD group manifested an elevated level in contrast to the sham group. However, administration of anthocyanin effectively counteracted this elevation ( $P=0.001$ ). Table 2 shows a comprehensive overview of these findings.

### Discussion

Ovarian torsion/detorsion is a damaging condition that can affect female fertility. It can occur at any age and lead to tissue damage in the reproductive system. The process of torsion/detorsion or ischemia/reperfusion involves various mechanisms such as necrosis, reduced tissue oxygenation, overproduction of ROS, free radical activity, and disruption of microcirculation. When ovarian torsion occurs, blood vessels become blocked, resulting in decreased tissue perfusion and eventual necrosis of the ovarian tissue and follicles. Detorsion or reperfusion is performed to protect the ovarian tissue after ischemia. However, the reperfusion process leads to the overproduction of ROS, causing an imbalance between ROS and the antioxidant defense system. The ROS plays important roles in various physiological processes, including oocyte maturation, fertilization, embryo development, and pregnancy (2,9,10). The activation of ROS and free radicals contributes to histological damage in the ovaries. This includes degeneration of follicles, reduction in the count of normal follicles, and an increase in the number of atretic bodies. Additionally, these oxidative stress-related processes can lead to hemorrhagic disorders within the ovarian tissue (11).

The findings of this study support the notion that ovarian torsion/detorsion can cause degeneration of follicles and a decrease in the number of preantral, antral, and graafian follicles, as well as an increase in the number of atretic bodies. This may be attributed to the excessive production of ROS and the activity of free radicals after the detorsion procedure (reperfusion). These results are consistent with a previous study by Soltani et al, who reported that ovarian torsion for a duration of 3 hours followed by a reperfusion period of 10 days resulted in damage to the ovarian tissue, including a reduction in the number of follicles and an increase in the number of atretic bodies (2). In line with the findings of Shokri et al, their study demonstrated that torsion in the ovary resulted in a decrease in the count of ovarian follicles and



an increase in the number of atretic bodies. This further supports the notion that ovarian torsion causes damage to the ovarian tissue, leading to alterations in follicle counts and an increased presence of atretic bodies (10). Indeed, several studies have reported that torsion/detorsion in the testicles can result in tissue damage and oxidative stress. This condition can have detrimental effects on fertility by causing a reduction in fertility power and compromising the antioxidant defense system. The imbalance between ROS production and antioxidant capacity can lead to oxidative stress, which can further contribute to testicular damage and impaired fertility (12,13). Indeed, ovarian torsion/detorsion can disrupt the hormonal balance, leading to alterations in testosterone and estrogen levels (14). The degeneration of normal follicles and granulosa cells in the ovary can contribute to this hormonal imbalance. One of the consequences of follicular damage is a decrease in the activity of aromatase, an enzyme responsible for the conversion of testosterone (androgen) to estrogen (15). This reduction in aromatase activity can result in an increase in testosterone levels and a decrease in estrogen levels, as observed in the present study. These findings are consistent with previous research demonstrating that ovarian torsion can decrease aromatase expression and estrogen concentration, accompanied by an increase in testosterone levels.

Ovarian torsion/detorsion can induce oxidative stress, which is characterized by an imbalance between the production of ROS and the antioxidant defense system. The overgeneration of ROS and free radical activity during reperfusion can lead to lipid peroxidation, resulting in an increase in the levels of MDA, which is an end product of lipid peroxidation. Additionally, the activity of antioxidant enzymes such as SOD and GPX may be reduced due to the oxidative stress induced by ovarian torsion/detorsion. These findings are consistent with the results of our study because we observed an increase in MDA levels and a decrease in SOD and GPX levels. In a similar study, Erdal Türk et al. demonstrated that ovarian torsion could lead to oxidative stress (16). Several other studies have also shown that ovarian torsion can result in elevated levels of MDA and a reduction in the activity of antioxidant enzymes such as SOD and GPX. These findings highlight the involvement of oxidative stress in the pathophysiology of ovarian torsion/detorsion and its impact on the antioxidant defense system (2,10,11).

Numerous studies have indicated that natural products play a significant role in the management of oxidative stress and its associated complications. These natural compounds, derived from medicinal plants, herbs, and other sources, are known for their high antioxidant content and their ability to scavenge harmful ROS. By neutralizing the ROS, natural antioxidants help prevent oxidative damage to cells and tissues. Moreover, they can enhance the activity of endogenous antioxidant enzymes, such as SOD and GPX, thereby reinforcing the

body's defense against oxidative stress. The therapeutic potential of natural products in combating oxidative stress-related conditions, including cardiovascular diseases, neurodegenerative disorders, and inflammatory conditions, has been extensively investigated.

Studies have demonstrated the ability of natural antioxidants to mitigate oxidative stress, reduce inflammation, and protect cellular structures from damage. Anthocyanins, a group of pigments responsible for the vibrant colors of fruits and vegetables, exemplify natural products with potent antioxidant properties. Their capacity to scavenge free radicals, inhibit lipid peroxidation, and shield against oxidative stress-induced harm has been well-documented. Utilizing natural products, such as anthocyanins, in the management of oxidative stress may offer a promising approach to alleviate the complications associated with oxidative damage, including those observed in cases of ovarian torsion/detorsion. However, further research is warranted to elucidate the underlying mechanisms and determine the optimal dosages of natural products for effective oxidative stress management (2,9,10,17).

In our study, the utilization of anthocyanin demonstrated protective effects on ovarian tissue against damage induced by ROS production and free radical activity. The administration of anthocyanin in the experimental groups resulted in the reduction of MDA levels, indicating a decrease in lipid peroxidation, and an increase in SOD and GPX levels; this confirms the enhanced activity of antioxidant enzyme. These changes contribute to the preservation of ovarian tissue and restoration of hormone balance. These protective effects may be attributed to the antioxidant and anti-inflammatory properties of anthocyanin. Numerous studies have reported the use of anthocyanin and medicinal plants containing this compound as the main component of their extracts, highlighting its potent antioxidant properties and its ability to safeguard tissues against oxidative stress-induced damage (18,19).

#### Limitations of the Study

In this study, we did not investigate the expression of proteins, including the proteins related to tissue damage, due to financial deficiencies.

#### Conclusions

The results of this study indicated that ovarian torsion/detorsion can induce tissue damage, oxidative stress, and alterations in hormonal levels, characterized by reduced estrogen and elevated testosterone concentrations. However, the administration of anthocyanin, known for its antioxidant properties, mitigated these detrimental effects. Anthocyanin supplementation showed potential in ameliorating the observed tissue damage and oxidative stress caused by ovarian torsion/detorsion. It also helped to restore hormone levels.

**Authors' Contribution**

**Conceptualization:** Arash Khaki, Ghazal Rahmanifar, Afshin Teymoori, Arman Khatami, Ali Rezaei.

**Data curation:** Arash Khaki, Ghazal Rahmanifar, Afshin Teymoori, Arman Khatami, Ali Rezaei.

**Formal analysis:** Arash Khaki, Ghazal Rahmanifar, Afshin Teymoori, Arman Khatami, Ali Rezaei.

**Funding acquisition:** Arash Khaki, Ghazal Rahmanifar, Afshin Teymoori, Arman Khatami, Ali Rezaei.

**Investigation:** Arash Khaki, Ghazal Rahmanifar, Afshin Teymoori, Arman Khatami, Ali Rezaei.

**Methodology:** Arash Khaki, Ghazal Rahmanifar, Afshin Teymoori, Arman Khatami, Ali Rezaei.

**Project administration:** Arash Khaki.

**Resources:** Arash Khaki

**Software:** Ghazal Rahmanifar, Afshin Teymoori, Arman Khatami, Ali Rezaei.

**Supervision:** Arash Khaki.

**Validation:** Arash Khaki, Ghazal Rahmanifar, Afshin Teymoori, Arman Khatami, Ali Rezaei.

**Visualization:** Arash Khaki, Ghazal Rahmanifar, Afshin Teymoori, Arman Khatami, Ali Rezaei.

**Writing—original draft:** Arash Khaki, Ghazal Rahmanifar, Afshin Teymoori, Arman Khatami, Ali Rezaei.

**Writing—review & editing:** Arash Khaki, Ghazal Rahmanifar, Afshin Teymoori, Arman Khatami, Ali Rezaei.

**Conflict of Interests**

Authors declare that they have no conflict of interests.

**Ethical Issues**

This experimental research adhered to the ethical principles outlined by the Islamic Azad University of Tabriz, Tabriz, Iran (ethical code: IR.IAU.TABRIZ.REC.1401.243).

**Financial Support**

This study was supported by Women's Reproductive Health Research Center of Islamic Azad University of Tabriz, Tabriz, Iran.

**References**

- Özler A, Turgut A, Soydiñç HE, et al. The biochemical and histologic effects of adnexal torsion and early surgical intervention to unwind detorsion on ovarian reserve: an experimental study. *Reprod Sci.* 2013;20(11):1349-1355. doi:10.1177/1933719113485300
- Soltani M, Moghimian M, Abtahi H, Shokoohi M. The protective effect of *Matricaria chamomilla* extract on histological damage and oxidative stress induced by torsion/detorsion in adult rat ovary. *Int J Womens Health Reprod Sci.* 2017;5(3):187-192. doi:10.15296/ijwhr.2017.34
- Cadirci E, Oral A, Odabasoglu F, et al. Atorvastatin reduces tissue damage in rat ovaries subjected to torsion and detorsion: biochemical and histopathologic evaluation. *Naunyn Schmiedebergs Arch Pharmacol.* 2010;381(5):455-466. doi:10.1007/s00210-010-0504-y
- Rashed FK, Ghasemi B, Deldade Mogaddam H, Mesgari M. The effect of erythropoietin on ischemia/reperfusion injury after testicular torsion/detorsion: a randomized experimental study. *ISRN Urol.* 2013;2013:351309. doi:10.1155/2013/351309
- Wallace TC, Giusti MM. Anthocyanins. *Adv Nutr.* 2015;6(5):620-622. doi:10.3945/an.115.009233
- Castañeda-Ovando A, de Lourdes Pacheco-Hernández M, Páez-Hernández ME, Rodríguez JA, Galán-Vidal CA. Chemical studies of anthocyanins: a review. *Food Chem.* 2009;113(4):859-871. doi:10.1016/j.foodchem.2008.09.001
- Ghosh D, Konishi T. Anthocyanins and anthocyanin-rich extracts: role in diabetes and eye function. *Asia Pac J Clin Nutr.* 2007;16(2):200-208.
- Abtahi-Evari SH, Shokoohi M, Abbasi A, Rajabzade A, Shoorei H, Kalarestaghi H. Protective effect of *Galega officinalis* extract on streptozotocin-induced kidney damage and biochemical factor in diabetic rats. *Crescent J Med Biol Sci.* 2017;4(3):108-114.
- Shokoohi M, Soltani M, Abtahi-Eivary SH, et al. Effect of hydro-alcoholic extract of *Olea europaea* on apoptosis--related genes and oxidative stress in a rat model of torsion/detorsion--induced ovarian damage. *Asian Pac J Reprod.* 2019;8(4):148-156.
- Shokri F, Shokoohi M, Roudi Rasht Abadi A, Kalarestaghi H. The ameliorative effect of *Galega officinalis* extract on histological damages, oxidative stress induced by torsion-detorsion in adult rats' ovarian. *Int J Womens Health Reprod Sci.* 2019;7(1):119-123. doi:10.15296/ijwhr.2019.19
- Laganà AS, Sofo V, Salmeri FM, et al. Oxidative stress during ovarian torsion in pediatric and adolescent patients: changing the perspective of the disease. *Int J Fertil Steril.* 2016;9(4):416-423. doi:10.22074/ijfs.2015.4598
- Seker U, Eski F, Kurt S. [Investigation of the distribution and expression level of pro and anti-apoptotic Bax and Bcl-2 in ovarian follicles at different developmental stages]. *J Harran Univ Med Fac.* 2021;18(3):424-428. doi:10.35440/hutfd.995512
- Barghi B, Shokoohi M, Khaki AA, Khaki A, Moghimian M, Soltani M. Eugenol improves tissue damage and oxidative stress in adult female rats after ovarian torsion/detorsion. *J Obstet Gynaecol.* 2021;41(6):933-938. doi:10.1080/01443615.2020.1816938
- Khaje Roshanaee M, Abtahi-Eivary SH, Shokoohi M, Fani M, Mahmoudian A, Moghimian M. Protective effect of minocycline on Bax and Bcl-2 gene expression, histological damages and oxidative stress induced by ovarian torsion in adult rats. *Int J Fertil Steril.* 2022;16(1):30-35. doi:10.22074/ijfs.2021.522550.1069
- Romanski PA, Melamed A, Elias KM, Stanic AK, Anchan RM. Association between peak estradiol levels and ovarian torsion among symptomatic patients receiving gonadotropin treatment. *J Assist Reprod Genet.* 2017;34(5):627-631. doi:10.1007/s10815-017-0901-y
- Türk E, Karaca İ, Özcinar E, et al. The effect of hypothermia on adnexal torsion/detorsion injury in a rat ovary model. *J Pediatr Surg.* 2015;50(8):1378-1381. doi:10.1016/j.jpedsurg.2015.01.011
- Bayir Y, Karagoz Y, Karakus E, et al. *Nigella sativa* reduces tissue damage in rat ovaries subjected to torsion and detorsion: oxidative stress, proinflammatory response and histopathological evaluation. *Gynecol Obstet Invest.* 2012;74(1):41-49. doi:10.1159/000336295
- Rai V, Vajpayee P, Singh SN, Mehrotra S. Effect of chromium accumulation on photosynthetic pigments, oxidative stress defense system, nitrate reduction, proline level and eugenol content of *Ocimum tenuiflorum* L. *Plant Sci.* 2004;167(5):1159-1169. doi:10.1016/j.plantsci.2004.06.016
- Kaur G, Athar M, Alam MS. Eugenol precludes cutaneous chemical carcinogenesis in mouse by preventing oxidative stress and inflammation and by inducing apoptosis. *Mol Carcinog.* 2010;49(3):290-301. doi:10.1002/mc.20601