











Protective effects of passiflora incarnata on ovarian ischemia/reperfusion damage in rats with ovarian torsion

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ABSTRACT

BACKGROUND: This study aimed to investigate whether *Passiflora Incarnata* (PI) has a protective effect against ischemia-reperfusion (IR)-induced oxidative and inflammatory ovarian damage.

METHODS: The effects of PI on ovarian ischemia-reperfusion injury were investigated in female Wistar albino rats. The animals were randomly divided into three groups: Group 1 (sham), Group 2 (IR), and Group 3 (IR+PI).

RESULTS: The mean levels of Malondialdehyde (MDA), Myeloperoxidase (MPO), and Total Oxidant Status (TOS) were higher in the IR group ($p=0.025$, $p<0.001$, and $p=0.016$, respectively). The Total Antioxidant Status (TAS) levels were lower in the IR group ($p=0.005$). Immunostaining revealed significant differences across the groups for Tumor necrosis factor-alpha (TNF- α): 13.84%, 49.51%, and 22.51% for Groups 1, 2, and 3, respectively ($p<0.01$). Bax: 10.53%, 46.74%, and 26.46% for Groups 1, 2, and 3, respectively ($p<0.01$). Annexin V: 12.24%, 44.86%, and 23.28% for Groups 1, 2, and 3, respectively ($p<0.01$). The mean scores for hemorrhage, inflammation, follicular degeneration, and congestion showed significant variations among the groups, all registering $p<0.001$.

CONCLUSION: *Passiflora Incarnata* exhibited antioxidant, anti-inflammatory, and anti-apoptotic properties, promoting cell survival, histologically protecting ovarian tissue, and ameliorating IR injury by reducing oxidative stress.

Keywords: Ischemia reperfusion; *passiflora incarnata*; ovarian torsion; ovarian damage; oogenesis.

INTRODUCTION

Ovarian torsion is a sudden and serious condition often found in women of reproductive age, leading to a reduction in ovarian reserve.^[1] This diagnosis accounts for about 2.5% to 7.4% of cases involving acute abdominal pain.^[2] The underlying mechanism of this disease involves the cessation of arterial

and venous blood flow due to the twisting of the ovarian tissue on its stem. Typical symptoms include abrupt pelvic pain not alleviated by painkillers, increased white blood cell count, vomiting, fever, and nausea.^[3] Key ultrasound indicators are the presence of a more central and enlarged ovary and reduced blood flow, which are diagnostic of ovarian torsion.^[4] The occurrence of ovarian torsion is often linked to benign

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ovarian tumors and cysts, specifically dermoid cysts.^[5,6]

Passiflora incarnata L., (PI), commonly known as the passionflower, is a perennial plant that can grow up to 10 meters tall and produces egg-shaped edible fruits. These fruits are low in calories (41-53 kcal/100 g) and rich in vitamins such as A, C, B1, and B2, as well as essential minerals like calcium, phosphorus, and iron. Originally native to South America, Australia, and Southeast Asia, the plant is now cultivated for medicinal purposes. PI is particularly noted within the *Passiflora* genus^[7] for its well-documented therapeutic effects. Different components of the plant, including its aerial parts, flowers, and fruits, are used in medicine due to their anti-worm, antispasmodic, and anxiolytic properties. The passionflower is utilized to treat a wide array of ailments, from burns and diarrhea to menstrual pain, neurotic conditions, and insomnia.^[6,7] A study by Soumya et al.^[8] first revealed that extracts of passionflower juice might help reduce heart attack damage, partly by inhibiting oxidative stress. Moreover, it has been found effective in managing morphine addiction and might be beneficial for conditions like convulsions or neuralgia.^[7] The plant contains various compounds, such as alkaloids, phenolic substances, flavonoids, and cyanogenic glycosides, with flavonoids (including apigenin, luteolin, quercetin, kaempferol) and flavonoid glycosides (such as vitexin, isovitexin, orientin, isoorientin) being the most prevalent.^[7,8] It is especially rich in isovitexin.^[7,8]

To date, no research has been conducted on the protective effect of PI in ovarian ischemia-reperfusion. This study investigated whether PI had a protective effect against IR-induced oxidative and inflammatory ovarian damage. We hypothesized that PI might have the potential to prevent and treat the injury caused by ischemia-reperfusion (IR) in the ovaries, specifically in the context of ovarian torsion in rats.

MATERIALS AND METHODS

This study investigated the effects of *Passiflora incarnata* (PI) on ovarian ischemia-reperfusion injury in female Wistar albino rats. In accordance with institutional guidelines and the principles set forth by the National Research Council for the Care and Use of Laboratory Animals, 21 female rats were selected for the study. Each rat was 12 weeks old and averaged 230 grams in weight. Ethical approval for the experiment was obtained from the Dicle University Animal Studies Ethical Committee (approval number: 2021/10, date: 24.02.2021). Our study was conducted with the support of the Dicle University scientific research project (No: TIP.21.024).

The rats were housed in an environment maintained at a temperature of 20-23°C with a 12-hour light/dark cycle. They were nourished with standard pellets and water, provided *ad libitum*. The animals were randomly divided into three groups: Group 1 (sham), Group 2 (IR), and Group 3 (IR+PI). In Group 1, all surgical steps except for ovarian torsion-detorsion were performed to establish baseline measurements. The ovary was exposed through a midline abdominal incision and then

repositioned in its normal place, without any torsion applied. Group 2 underwent ovarian torsion and detorsion without any medication, while Group 3 received 500 mg/kg/day of PI orally for five days before ischemia/reperfusion (I/R), diluted in a 0.9% saline solution immediately before administration. In Groups 2 and 3, the ovary was subjected to ischemia-reperfusion injury by extracting it through a midline abdominal incision, twisting it 720 degrees clockwise, and securing it for two hours using 5.0 prolene suture. This procedure aimed to create a controlled model for studying the effects of ischemia followed by reperfusion. After the ischemia period, the ovary was untwisted and left *in situ* for four hours to assess reperfusion injury. An oophorectomy was then performed to collect the ovarian tissue for histological analysis, and blood samples were obtained by cardiac puncture for biochemical assessments. All procedures were conducted under appropriate anesthesia and in sterile conditions. For anesthesia, the rats were injected intraperitoneally with a combination of xylazine hydrochloride (Rompun 2%, Bayer, Turkey) and ketamine hydrochloride (Alfamine 10%, Ege Vet, Turkey), at dosages of 10 mg/kg and 50 mg/kg, respectively. Xylazine hydrochloride served as a sedative and muscle relaxant, while ketamine hydrochloride was used for its dissociative anesthetic properties.

Biochemical Evaluation

After obtaining blood samples through cardiac puncture, they were immediately transported to the biochemistry laboratory, kept chilled on ice. The samples were centrifuged at 4,000 revolutions per minute for 5 minutes to separate the serum. Subsequent analyses were conducted to determine levels of Total Antioxidant Status (TAS), Total Oxidant Status (TOS), Malondialdehyde (MDA), Glutathione (GSH), and Myeloperoxidase (MPO). An Abbott Architect CI6000 autoanalyzer was used to measure TAS and TOS levels, utilizing commercial kits provided by Rel Assay Diagnostics, Gaziantep, Turkey, along with automated colorimetric techniques developed by Erel et al.^[9,10] TAS results were reported in micromolar Trolox equivalent per liter, and TOS results were expressed in micromolar hydrogen peroxide equivalent per liter. MDA content was evaluated using a spectrophotometric method, based on the color change that occurs when thiobarbituric acid reacts with MDA, as previously described.^[11] Similarly, MPO activity was assessed spectrophotometrically, as referenced earlier. The method suggested by Paglia et al.^[12] was used to measure glutathione peroxidase (GSH-Px) activity, monitoring the enzyme's ability to catalyze the conversion of reduced glutathione to its oxidized form (GSSG) in the presence of hydrogen peroxide.

Immunohistochemical Examination

Sections of the ovary were first deparaffinized, then rehydrated through a series of graded alcohols, and rinsed with distilled water. To inhibit endogenous peroxidase activity, a 3% hydrogen peroxide (H₂O₂) solution was applied to the

slides. After rinsing in PBS, the sections were treated with antibodies against blood-brain barrier (Biolegend, catalog no: 836804), Annexin V (Boster Biology Tech., catalog no: 0902012, 1/100 dilution), and TNF- α and Bax (Biorbyt, catalog no: 15970 and 17069, 1/100 dilution), incubating them overnight at +4°C. The sections were then biotinylated and incubated with a streptavidin peroxidase solution (Thermo Fisher, US) for 15 minutes. Following a wash in PBS, diaminobenzidine (DAB) was used as a chromogen to detect color changes. The reactions were stopped with PBS, and the sections were subsequently counterstained with hematoxylin. The prepared slides were examined and imaged using a Zeiss Imager A2 light microscope, and all images were processed and quantified using ImageJ software. The staining intensity related to protein expression was assessed with ImageJ software (version 1.53, available at <http://imagej.nih.gov/ij/>), using the method developed by Crowe et al.^[13] Each sample's ovarian histopathological scores were determined by examining ten fields using the method defined by Kalyoncu et al.,^[14] with scores ranging from 0 (none) to 3 (severe). Two experts, blinded to the sample details, performed the histological scoring.

Statistical Analysis

The data were analyzed using IBM SPSS 25.0 software (IBM, Armonk, New York, US). The Shapiro-Wilk and Kolmogorov-Smirnov tests were employed to determine the distribution of the data. When the data followed a normal distribution, means and standard deviations were calculated, and the ANOVA test was utilized for analysis. In cases of non-normal

distribution, medians and interquartile ranges (IQR) were presented. For comparisons involving more than two groups, the non-parametric Kruskal-Wallis test was chosen, and the post-hoc Dunn test was applied due to the small sample sizes in the groups. A p-value of less than 0.05 was considered statistically significant.

RESULTS

The mean levels of Malondialdehyde (MDA) differed significantly among the groups ($p=0.025$), with Group 2 exhibiting the highest levels (1.467 ± 0.44) compared to Group 1 (0.871 ± 0.07) and Group 3 (0.933 ± 0.23). A marked disparity in Myeloperoxidase (MPO) levels was observed, with Group 2 registering the highest value (15.867 ± 2.83), followed by Group 3 (11.572 ± 1.57) and Group 1 (9.198 ± 1.58) ($p<0.001$). No statistically significant difference was detected in Glutathione (GSH) levels across the groups ($p=0.346$). Group 2 exhibited the lowest Total Antioxidant Status (TAS) (0.65 ± 0.21), significantly different from Group 1 (1.281 ± 0.47) and Group 3 (1.116 ± 0.27) ($p=0.005$). A significant difference in Total Oxidant Status (TOS) levels was noted ($p=0.016$), with Group 2 recording the highest levels (213.6 ± 97.3). Immunostaining revealed significant variations across the groups for Tumor Necrosis Factor-alpha (TNF- α): 13.84%, 49.51%, and 22.51% for Groups 1, 2, and 3, respectively ($p<0.01$). Bax: 10.53%, 46.74%, and 26.46% for Groups 1, 2, and 3, respectively ($p<0.01$). Annexin V: 12.24%, 44.86%, and 23.28% for Groups 1, 2, and 3, respectively ($p<0.01$). The mean scores for hemorrhage, inflammation, follicular degeneration, and congestion showed marked variations among the groups, all

Table 1. Biochemical and immunohistopathological parameters of all groups

	Group 1	Group 2	Group 3	p-value
Blood				
MDA	0.871 \pm 0.07	1.467 \pm 0.44	0.933 \pm 0.23	0.025 ^{*§}
MPO	9.198 \pm 1.58	15.867 \pm 2.83	11.572 \pm 1.57	<0.001 ^{*§}
GSH	133.9 \pm 20.77	138.90 \pm 19.89	157.99 \pm 34.88	0.346
TAS	1.281 \pm 0.47	0.65 \pm 0.21	1.116 \pm 0.27	0.005 ^{*§}
TOS	60.8 \pm 49.5	213.6 \pm 97.3	86.6 \pm 25.8	0.016 ^{*§}
Tissue				
Tnf- α immunostaining*	13.84%	49.51%	22.51%	<0.01 ^{*§}
Bax immunostaining*	10.53%	46.74%	26.46%	<0.01 ^{*§}
Annexin V immunostaining*	12.24%	44.86%	23.28%	<0.01 ^{*§}
Histopathological scoring				
Hemorrhage	0.00 \pm 0.00	2.90 \pm 0.27	1.77 \pm 0.30	<0.001 ^{*§}
Inflammation	0.20 \pm 0.12	2.01 \pm 0.20	1.40 \pm 0.52	<0.001 ^{*§}
Follicular degeneration	0.11 \pm 0.10	2.33 \pm 1.11	1.28 \pm 0.59	<0.001 ^{*§}
Congestion	0.21 \pm 0.18	2.94 \pm 0.85	1.98 \pm 0.98	<0.001 ^{*§}

*Meaningful between group 1 and 2; §Meaningful between group 2 and 3.

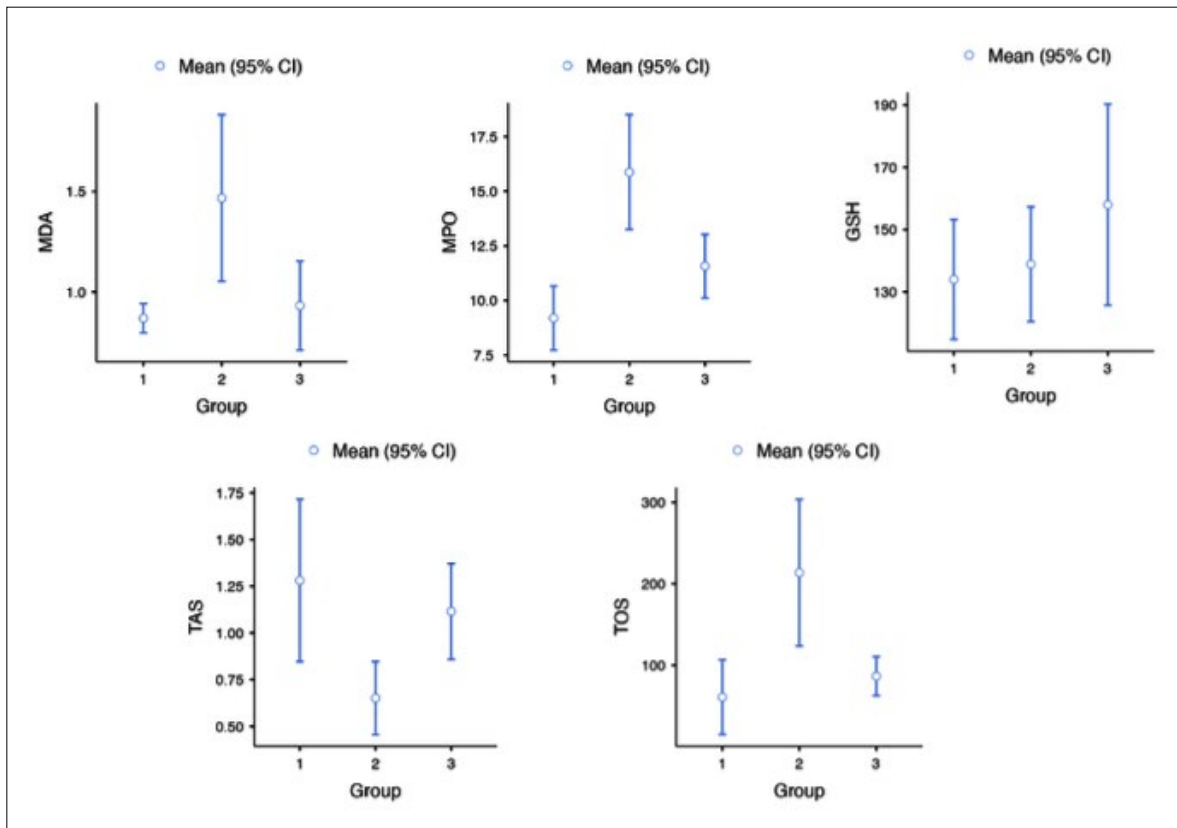


Figure 1. Comparison blood results.

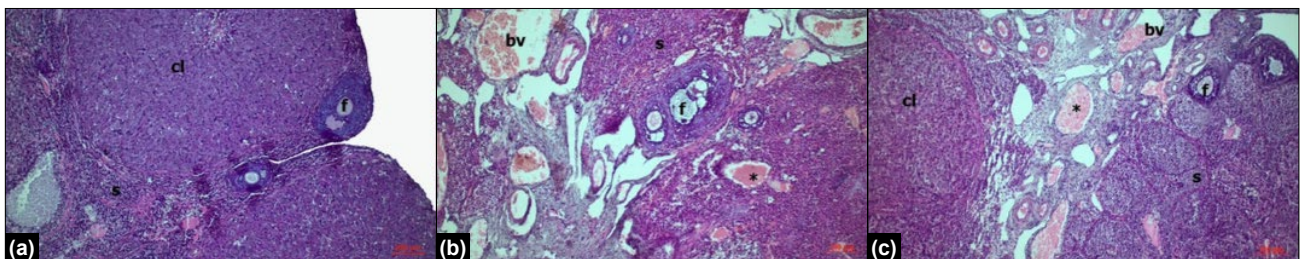


Figure 2. Hematoxyline eosin staining (a) sham group, (b) IR group; (c) IR+PI group. f: follicles, s: stroma, bv: blood vessel, cl: corpus luteum., asterisk: leukocyte, Scale bar: 100 μ m, magnification: 10x.

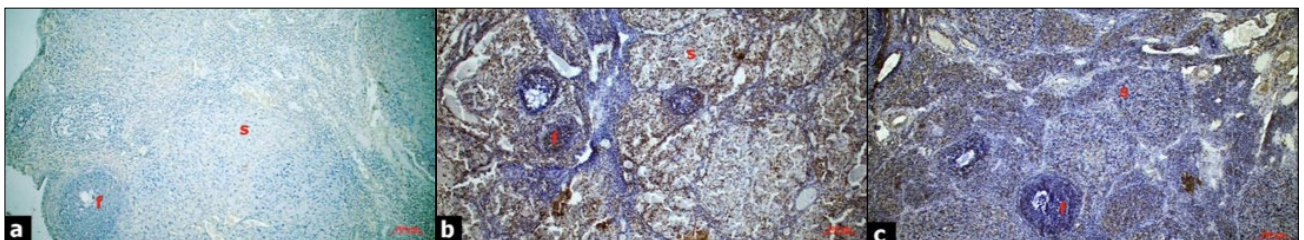


Figure 3. Tnf- α immunostaining. (a) sham group, (b) IR group; (c) IR+PI group. f: follicles, s: stroma, bv: blood vessel, cl: corpus luteum. Scale bar: 100 μ m, magnification: 10x.

registering $p < 0.001$. Group 2 consistently presented higher values for each category compared to Groups 1 and 3 (Table I and Figure 1).

Hematoxylin and eosin staining of ovarian tissues is depicted

in Figure 2. In sections from the Sham group, normal ovarian tissue structure was observed. The ovarian stroma and corpus luteum appeared normal, with ongoing folliculogenesis. No pathology was detected. In the IR group, degeneration of follicles, increased inflammation, vascular dilation and congest-

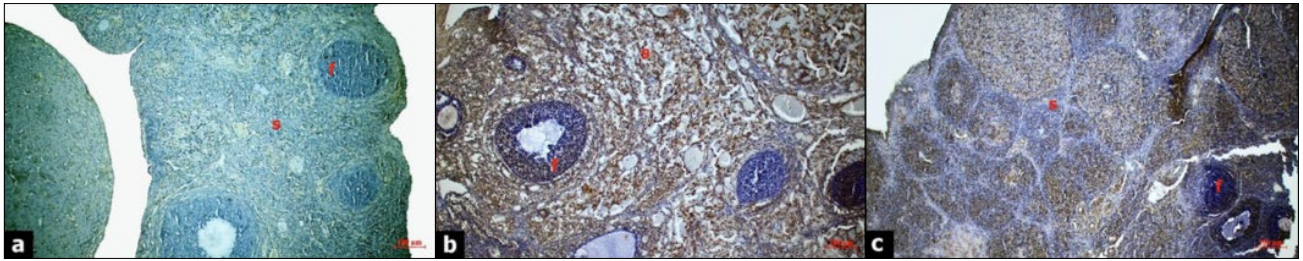


Figure 4. Bax immunostaining. (a) sham group, (b) IR group; (c) IR+PI group. f: follicles, s: stroma, bv: blood vessel, cl: corpus luteum. Scale bar: 100 μ m, magnification: 10x.



Figure 5. Annexin V immunostaining. (a) sham group, (b) IR group; (c) IR+PI group. f: follicles, s: stroma, bv: blood vessel, cl: corpus luteum. Scale bar: 100 μ m, magnification: 10x.

tion, and leukocyte infiltration were observed, compromising the integrity of the ovarian tissue. In the group treated with IR + Passiflora, a reduction in the number of degenerated follicles was noted. There was a decrease in leukocyte infiltration, partial continuation of congestion and dilation in vascular structures, and a reduction in inflammation in the ovarian stroma. Overall, PI treatment was observed to have a protective effect on the integrity of ovarian tissue following IR.

TNF- α immunoreactivity in ovarian tissues is shown in Figure 3. In the Sham group, TNF- α expression in the follicles, ovarian stroma, and corpus luteum was generally negative. In the IR group, TNF- α expression was intensely observed in the granulosa cells of the follicles and particularly in the connective tissue cells of the ovarian stroma. A decrease in TNF- α immune reaction was observed following PI treatment. TNF- α immune reactivity was mostly negative in the ovarian follicle and stroma. It can be inferred that Passiflora suppresses the expression of TNF- α cytokine and inhibits the inflammation pathway due to its anti-inflammatory effect against IR damage.

Bax immunoreactivity in ovarian tissues is shown in Figure 4. In the Sham group, negative Bax expression was observed in the granulosa cells, stromal cells, and corpus luteum. IR damage activated the apoptotic pathway, increasing Bax expression. Bax immune reaction showed an increase in the granulosa cells of the follicles and in the stromal cells. Due to Passiflora's anti-apoptotic effect, pro-apoptotic Bax expression decreased, supporting cell survival. The reduction in Bax immune reactivity in ovarian follicles and the stromal area demonstrated that PI treatment promoted the survival of ovarian cells by inhibiting the apoptotic pathway, thus exerting a protective effect on the ovary.

Annexin V immunoreactivity in ovarian tissues is shown in Figure 5. In the Sham group, Annexin V immune reactivity was predominantly negative. Annexin V reaction was weak in the granulosa cells, stromal cells, and corpus luteum. In the IR group, the apoptotic pathway was activated as a result of IR, and Annexin V expression was intensely observed in the granulosa and stromal cells of the ovary. Due to PI's anti-apoptotic effect, a significant decrease occurred in the immune reaction of Annexin V, an apoptotic cell marker. Annexin V expression showed a reduction in the follicles and ovarian stroma compared to the IR group. PI treatment promoted the survival of granulosa cells by inhibiting the apoptotic pathway, thereby exerting a protective effect on the ovarian tissue.

DISCUSSION

In this research, we explored the impact of Passiflora incarnata (PI) on the treatment of ovarian torsion using a rat model for ischemia-reperfusion (I/R) injury. To our knowledge, this study is the first to demonstrate that PI treatment can protect the ovaries from I/R damage.

Currently, laparoscopic detorsion is the preferred method for treating ovarian torsion to preserve ovarian integrity and fertility. However, oophorectomy may be necessary in cases of significant necrosis.^[15] Conversely, preserving ovarian circulation post-detorsion can exacerbate tissue damage, leading to I/R injury characterized by oxidative stress (OS).^[16,17] During detorsion, tissues are exposed to an excess of molecular oxygen, resulting in the overproduction of reactive oxygen species (ROS) such as superoxide radicals (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radicals (OH), and reactive nitrogen species (RNS). This surplus of ROS and RNS, along with their

toxic byproducts, leads to DNA and lipid peroxidation within cell and mitochondrial membranes, causing cellular death.^[18] These events are crucial in various physiological processes, including the menstrual cycle, ovulation, embryo implantation, placental development, and menopause.^[19] To counteract the potential dangers of ROS, the human body possesses complex enzymatic and non-enzymatic antioxidant defenses.^[18] Additionally, the external application of antioxidants might be beneficial. Although there has been significant research on the benefits of anti-inflammatory and antioxidant agents in preventing I/R damage post-ovarian detorsion, primarily in rat models, clear and universally accepted evidence for routine clinical application is still lacking.^[18]

In this context, numerous experimental studies have been conducted, revealing that certain molecules protect the ovary against I/R damage. Kula et al.^[20] discovered that *Allium cepa* has a strong protective effect on cellular tissue, reducing I/R damage to ovarian tissue from a histological perspective. However, our research indicates that *A. cepa* does not influence the mediators related to fibrosis in the rat ovary. In another study, Osmanlioğlu et al.^[21] reported that pretreatment with Artemisinin (ARS) can lessen ovarian I/R damage. The beneficial effects observed with ARS appear to be linked to the inhibition of oxidative stress and cell death, though further research is needed to explore other potential mechanisms. Uzel et al.^[22] concluded that tocilizumab could be a new treatment alternative for ischemia-reperfusion injury resulting from ovarian torsion, but more comprehensive experimental and clinical studies are required to determine the appropriate dosage and to evaluate the benefits versus risks.

On the other hand, *Passiflora incarnata* (PI) has been shown to reduce I/R damage in many experimental studies. The study by Soumya et al. was the first to reveal that juice extracted from passionflower could alleviate myocardial infarction, partly by inhibiting oxidative stress.^[8] Amini et al.^[23] demonstrated that PI might reduce behaviors resembling autism in an animal disease model, suggesting that the extract could act as both a neuroprotective agent and a promising source of antioxidants. Administering PI juice (2 ml/kg) as a pre-treatment for 28 days, followed by isoproterenol treatment, was found to have protective qualities against isoproterenol-triggered myocardial infarction in rats.^[8] Another study concluded that the *Passiflora* species, through their derivatives and flavonoids like quercetin, apigenin, and vitexin, could potentially serve as a strong basis for anti-inflammatory and antioxidant therapies.^[24] These could play a crucial role in preventing and managing various diseases characterized by complex inflammatory responses.

However, our study is the first experimental investigation into the positive effects of *Passiflora incarnata* (PI) on ovarian ischemia-reperfusion (IR). Our findings reveal significant variations in Malondialdehyde (MDA), Myeloperoxidase (MPO), Total Antioxidant Status (TAS), Total Oxidant Status (TOS) levels, and immunostaining results across the three groups.

Group 2 consistently exhibited higher or more adverse values in these categories. No significant difference was detected in Glutathione (GSH) levels. Hematoxylin and eosin staining showed normal ovarian tissue in the Sham group but degeneration in the IR group. Treatment with PI demonstrated a protective effect on ovarian tissue, reducing degeneration, inflammation, and other negative effects caused by IR.

CONCLUSION

Passiflora incarnata possesses numerous biological activities. In our study, we observed that inflammation and cell deaths (apoptosis) increased following ovarian IR injury. We believe that, through its antioxidant, anti-inflammatory, and anti-apoptotic properties, *Passiflora* promoted cell survival, histologically protected the ovarian tissue, and improved IR injury by reducing oxidative stress.

Ethics Committee Approval: This study was approved by the Dicle University Animal Studies Ethics Committee (Date: 24.02.2021, Decision No: 2021/10).

Peer-review: Externally peer-reviewed.

Authorship Contributions: Concept: S.A., F.A.; Design: S.A., E.A.G; Supervision: E.B.; Resource: S.A., M.A.; Materials: M.A.K.; Data collection and/or processing: S.M.Ö.O.; Analysis and/or interpretation: İ.K.; Literature search: M.A.K.; Writing: M.A.; Critical review: M.H.O., E.G.Ö.

Conflict of Interest: None declared.

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DENEYSSEL ÇALIŞMA - ÖZ

Over torsiyonu olan sıçanlarda Passiflora incarnata'nın overin iskemi/reperfüzyon hasarı üzerinde koruyucu etkileri

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AMAÇ: Bu çalışmada, Passiflora incarnata'nın (PI) iskemi reperfüzyon (IR) kaynaklı oksidatif ve enflamatuvar over hasarına karşı koruyucu etkisinin olup olmadığının araştırılması amaçlandı.

GEREÇ VE YÖNTEM: Bu çalışmada, dişi Wistar albino sıçanlarda PI'nin over iskemi-reperfüzyon hasarı üzerindeki etkileri araştırıldı. Sıçanlar rastgele üç ayrı gruba ayrıldı: Grup 1 (sham), Grup 2 (IR:) ve Grup 3 (IR+PI).

BULGULAR: Ortalama malondialdehit (MDA), miyeloperoksidaz (MPO) ve total oksidant status (TOS) düzeyleri IR grubunda daha yüksekti (sırasıyla, $p=0.025$, $p<0.001$ ve $p=0.016$). Total antioksidant status (TAS) düzeyleri IR grubunda daha düşüktü ($p=0.005$). İmmün boyama, gruplar arasında anlamlı fark vardı. Tümör nekroz faktör-alfa (TNF- α): Grup 1, 2 ve 3 için sırasıyla %13.84, %49.51 ve %22.51 ($p<0.01$). Bax: Grup 1, 2 ve 3 için sırasıyla %10.53, %46.74 ve %26.46 ($p<0.01$). Aneksin V: Grup 1, 2 ve 3 için sırasıyla %12.24, %44.86 ve %23.28 ($p<0.01$). Kanama, enflamasyon, foliküler dejenerasyon ve konjesyona ilişkin ortalama skorlar gruplar arasında belirgin farklılıklar vardı ve tümü $p<0.001$ olarak kaydedildi.

SONUÇ: Passiflora incarnata, antioksidan, antiinflamatuvar ve antiapoptotik biyolojik aktiviteleri sayesinde hücre hayatta kalmasını destekledi, over dokusunu histolojik olarak korudu ve oksidatif stresi azaltarak IR hasarını iyileştirdi.

Anahtar sözcükler: Passiflora incarnata; iskemi reperfüzyon; over torsiyon; over hasarı; oogenez.

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