Clinical and Experimental Obstetrics & Gynecology

Original Research

Protective effects of apocynin and melatonin on ovarian ischemia/reperfusion injury in rats

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Objective: This study aims to determine the protective effects of apocynin, a NADPH oxidase inhibitor, and melatonin, an endogenous anti-oxidant, in an animal model of ovarian ischemia/reperfusion (I/R) injury. *Materials/Methods:* Thirty-five female rats were randomly divided into five groups, namely group I (sham), group II (I/R), group III (I/R + 10 mg/kg apocynin), group IV (I/R + 20 mg/kg apocynin), and group V (I/R + 10 mg/kg melatonin). Ovarian tissue and serum superoxide dismutase (SOD) and catalase (CAT) activities and malondialdehyde (MDA) and protein carbonyl (PC) levels were measured. Ovarian histopathology was examined and Bax, caspase 3, and iNOS immunoreactivities were evaluated. *Results:* Preoperative apocynin and melatonin significantly increased SOD and CAT activities ($P < 0.05$, $P < 0.05$, $P < 0.01$, $P < 0.01$, $P < 0.01$, $P < 0.05$, $P < 0.05$, and $P < 0.01$, respectively, for both apocynin and melatonin). In addition, preoperative apocynin and melatonin significantly decreased the ovarian I/R injury score ($P < 0.01$ for both). Bax, caspase 3, and iNOS immunoreactivities were significantly lower in the I/R + 10 mg/kg apocynin and I/R + 10 mg/kg melatonin groups than in the I/R group ($P < 0.01$, $P < 0.01$, $P < 0.01$, $P < 0.01$, $P < 0.05$, and $P < 0.01$, respectively). *Conclusions:* Apocynin and melatonin are powerful anti-oxidant agents with considerable bioavailability and safety. Preoperative apocynin and melatonin administration might protect ovarian tissue from I/R injury after surgical adnexal detorsion.

Keywords

Apocynin; Ischemia; Melatonin; Ovary; Reperfusion injury

1. Introduction

Ovarian torsion is a gynecological emergency that mostly affects adolescents and young women [1]. The rotation of an ovary around its own axis leads to biochemical and histological alterations that ultimately result in ovarian dysfunction [2]. Ovarian torsion impairs blood flow to tissues and causes ovarian ischemia. Ischemia eventually triggers the degradation of adenosine triphosphate and increases hypoxanthine. After ovarian torsion is reversed, tissues begin to receive blood flow again, and hypoxanthine is converted into superoxide anions, hydroxyl radicals, and peroxynitrite within an oxygenated environment. The phase related to torsion/detorsion damage corresponds to ovarian ischemia/reperfusion (I/R) injury [3, 4]. Experimental studies have attempted to prevent oxidative damage in ovaries by using a number of anti-oxidant agents [5, 6]. Additionally, various treatments, such as pre-treatment with losartan, granulocyte colony-stimulating factor, and methylene blue, have also been found to be useful [7–9].

Apocynin (4-hydroxy-3methoxy-acetophenone) is naturally found in the roots of *Apocynum cannabinum* (Canadian hemp) and *Picrorhiza kurroa* (Scrophulariaceae). This molecule inhibits NADPH oxidase, which catalyzes the synthesis of superoxide anion from oxygen [10]. It has been reported that apocynin suppresses apoptosis by decreasing myeloperoxidase activity and supporting anti-oxidant defense mechanisms in animal models of cerebral artery occlusion [11], renal I/R injury [12], and testicular torsion/detorsion damage [13].

Melatonin (N-acetyl-5-methyl-tryptamine), which is secreted by the pineal gland, is one of the most powerful anti-oxidant agents. Melatonin scavenges hydroxyl radicals, peroxyl radicals, singlet oxygen molecules, peroxynitrite anions, and superoxide anions and inhibits anti-oxidant enzymes, including glucose-6-phosphate dehydrogenase, glutathione peroxidase, and superoxide dismutase (SOD) [14, 15]. Melatonin has also been reported to exert anti-oxidant effects in cardiac I/R injury [14, 16] and testicular torsion/detorsion damage [15–18].

This study aims to determine the protective effects of apocynin and melatonin in an animal model of ovarian I/R injury.

2. Materials and methods

2.1 Experimental procedure

This study was approved by the local ethics committee (2015 HADYEK-29). Thirty-five adult female Wistar albino rats (body weight, 200-250 g) were randomly divided into five groups, namely group I (control, $n = 7$), group II (I/R, $n = 7$), group III (I/R + APC1, $n = 7$), group IV (I/R + APC2), and group V (I/R + MEL, $n = 7$).

I/R injury was not induced in group I rats. Rats underwent a sham operation and received 1 mL of 1% ethanol. I/R injury was induced in group II rats, followed by the administration of 1 mL of normal saline. I/R injury was induced in group III rats, followed by the intraperitoneal injection of 10 mg/kg apocynin 30 minutes before reperfusion. I/R injury was induced in group IV rats, followed by the intraperitoneal injection of 20 mg/kg apocynin before reperfusion. Apocynin was dissolved in normal saline (Sigma Chemical Co., St. Louis, MO, USA) [19, 20]. I/R injury was induced in group V rats, followed by the intraperitoneal injection of 10 mg/kg melatonin 30 minutes before reperfusion. Melatonin was dissolved in 1% ethanol (Sigma Chemical Co.) [21, 22].

The rat ovarian I/R model was established as previously described by Ozsoy *et al.* [23]. Rats were starved 12 hours before laparotomy and anesthetized with 50 mg/kg ketamine hydrochloride (Ketalar®, Eczacıbası, Istanbul, Turkey) and 10 mg/kg xylazine hydrochloride (Rompun®, Bayer, Leverkusen, Germany). A midline incision was made in the lower abdomen. Group I rats underwent a sham operation. For the remaining groups, the left adnexa was clockwise-rotated approximately 720 degrees along with ovarian and tubal vessels. The twisted adnexa was fixed to the abdominal wall with 4/0 silk sutures. The ovary was counter-rotated to the normal position after 3 hours of torsion, and I/R injury was induced at this time.

2.2 Sample collection

Blood samples were obtained by direct cardiac puncture under anesthesia and stored at -80 °C until biochemical analysis. Left ovaries were removed surgically. One ovary from each rat was stored at -80 °C until biochemical analysis, and the other ovary was fixed in 10% formalin solution for 24 hours for histopathological analysis.

2.3 Biochemical analysis

Oxidative stress in ovarian tissue homogenates was determined by measuring the activities of SOD and CAT, as well as the levels of MDA and PC, respectively. Analyses of all samples were performed at ambient temperature. All tissues were rinsed with ice-cold isotonic saline solution and homogenized by using a homogenizer (IKA Ultra-Turrax t 25 Basic, Stanfen, Germany). After filtration and centrifugation of the homogenates, supernatants were used to determine enzymatic activities. SOD activity was specified by inhibiting the nitroblue tetrazolium (NBT) reduction, and one unit of SOD was defined as the amount of enzyme causing 50% inhibition of the NBT reduction rate [24]. Catalase (CAT) activity was specified by determining the rate constant of the H_2O_2 decomposition rate at 240 nm. Both SOD and CAT activities were expressed as units per gram protein (U/g protein). The MDA level was measured by using a method that was based on the reaction of MDA with thiobarbituric acid (TBA) at 90-100 °C [25]. The protein carbonyl (PC) content was measured spectrophotometrically (GBC Cintra 10 E UV/Visible Spectrophotometer, Melbourne, Australia) by reacting the carbonyl group with 2,4-dinitrophenylhydrazine to form 2,4-dinitrophenylhydrazine. Both MDA and PC levels were expressed as nanomoles per gram protein (nmol/g protein).

2.4 Histopathological examination

After fixation in 10% formalin solution, ovarian tissues were washed, dehydrated, and embedded in paraffin wax. Paraffin blocks were cut to generate 5-μm-thick sections by using a Leica RM2125RT microtome (Leica Biosystems, Nussloch, Germany) and stained with hematoxylin and eosin (H&E). Stained sections were examined under a Zeiss Axio Lab A1 light microscope (Carl Zeiss, Oberkochen, Germany).

Histological changes were evaluated according to their severity: score 0 indicated an absence of pathological findings, and scores 1, 2, and 3 indicated an involvement of $<$ 33%, 33-66%, and $>$ 66%, respectively [26]. Total tissue damage scores were calculated using the sum of scores for follicular degeneration, hemorrhage, vascular congestion, edema, and inflammatory cell infiltration.

2.5 Immunohistochemistry

Immunohistochemistry was performed as previously described [27]. Briefly, 5-μm-thick serial sections were collected on poly-L-lysine coated slides (Sigma-Aldrich Co.) and incubated overnight at 56 °C. After deparaffinization in xylene, sections were rehydrated through a graded ethanol series. Sections were microwave-treated in 10 mM citrate buffer at pH 6.0 twice for 5 min each time and allowed to cool for 20 min. Sections were rinsed three times in phosphate-buffered saline (PBS). Endogenous peroxidase activity was blocked by immersion in 3% hydrogen peroxide in PBS for 20 min, and sections were washed three times in PBS. Sections were treated with blocking serum (Ultra V Block, ScyTek Laboratories, UT, USA) for 10 min and incubated overnight at 4 °C in a humidified chamber with primary antibodies, namely rabbit polyclonal active-caspase 3 (ab13847, Abcam, Cambridge, UK), rabbit polyclonal anti-iOS (ab15323, Abcam), and rabbit polyclonal anti-Bax (ab7977, Abcam). Sections were washed in PBS at room temperature and incubated with biotinylated polyvalent antibodies (ScyTek Laboratories), followed by peroxidase-labeled streptavidin (ScyTek Laboratories). Immunohistochemical analysis was carried out according to the manufacturer's instructions (ScyTek Laboratories) by using a horseradish peroxidase-labeled streptavidin-biotin kit (SensiTek HRP). The development of bound peroxidase was ensured by 3-amino-9-ethylcarbazole (ScyTek Laboratories). Sections were counterstained with Mayer's hematoxylin (ScyTek Laboratories) and mounted in Permount

Table 1. Tissue SOD, CAT, MDA, and PC levels in each group

	SOD	CAT	MDA	PC
Control	50.61 ± 3.37	0.39 ± 0.11	1.79 ± 0.71	768 ± 230
I/R	25.06 ± 8.33*	0.20 ± 0.05‡	3.84 ± 1.21$^{\#}$	1885 ± 852$^{\#}$
IR + APC1	35.50 ± 2.93$^{\#,†}$	0.27 ± 0.12	2.97 ± 0.81‡	694 ± 307†
IR + APC2	33.46 ± 5.62$^{\#,†}$	0.33 ± 0.13$^{¥}$	2.62 ± 0.89$^{¥}$	576 ± 209†
IR + MEL	36.01 ± 4.45$^{\#,†}$	0.36 ± 0.09$^{¥}$	2.52 ± 0.43$^{¥}$	652 ± 156†

*$P < 0.001$ compared with control; $^{\#}P < 0.01$compared with control; $^{†}P < 0.01$ compared with I/R; $^{‡}P < 0.05$ compared with control; $^{¥}P < 0.05$ compared with I/R. SOD: superoxide dismutase; CAT: catalase; MDA: malondialdehyde; PC: protein carbonyl.

Table 2. Serum SOD, CAT, MDA, and PC levels in each group

	SOD	CAT	MDA	PC
Control	6.26 ± 0.60	0.10 ± 0.04	0.77 ± 0.09	160 ± 43
I/R	4.39 ± 1.50$^{\#}$	0.05 ± 0.02$^{\#}$	1.14 ± 0.15*	461 ± 97*
IR + APC1	5.27 ± 1.95	0.09 ± 0.04†	0.67 ± 0.13‡	223 ± 48$^{\#,‡}$
IR + APC2	6.83 ± 2.37†	0.12 ± 0.05†	0.78 ± 0.27‡	219 ± 36$^{\#,‡}$
IR + MEL	6.64 ± 2.13†	0.12 ± 0.03†	0.66 ± 0.12‡	205 ± 44‡

$^{\#}P < 0.05$ compared with control; $^{†}P < 0.05$ compared with I/R; *$P < 0.001$ compared with control; $^{‡}P < 0.01$ compared with I/R. SOD: superoxide dismutase; CAT: catalase; MDA: malondialdehyde; PC: protein carbonyl; I/R: ischemia/reperfusion; APC: apocynin; MEL: melatonin.

medium (Fisher Chemicals, Springfield, NJ, USA). Normal rabbit serum at a concentration identical to that of the primary antibody served as the control. Photomicrographs were taken with a Zeiss Axio Lab A1 light microscope (Carl Zeiss, Oberkochen, Germany).

Evaluation of the immunohistochemical labeling was performed using H-score analysis as previously described [27]. Semi-quantitative assessments of immunoreactivity for caspase 3, Bax, and iNOS were classified as follows: 0 (no staining), 1+ (weak but detectable staining), 2+ (moderate or distinct staining), and 3+ (intense staining). H-scores for each tissue were calculated by using the formula: H-score = $\sum$ Pi (i+ l). In this formula, 'i' indicated the intensity score, and 'Pi' indicated the percentage of corresponding cells. The percentage of cells stained in each intensity category was calculated and then multiplied by the weighted intensity of staining. The H-score value was calculated using five different areas for each section at 400× magnification, and values of each of the two sections were averaged for each animal. Two investigators, who were blinded to the type and source of the tissues, determined the percentage of cells without staining and with 1+, 2+ and 3+ staining. The combined average score of both observers was used.

2.6 Statistical analysis

Collected data were analyzed by using Statistical Package for Social Sciences version 20.0 (SPSS IBM, Armonk, NY, USA). Results were expressed as means ± standard deviation (SD). Differences among five groups were analyzed by one-way analysis of variance (ANOVA), and Tukey test was used for post-hoc analysis. Two-tailed *P*-values less than 0.05 were considered to be statistically significant.

3. Results

3.1 Biochemical results

Ovarian tissue and serum SOD, CAT, MDA, and PC activities in all groups are given in Table 1,2. Tissue and serum SOD and CAT activities were decreased in the I/R group compared with the control group. Compared with the I/R group, melatonin and apocynin treatment significantly increased SOD and CAT activities, but this increase was more prominent in I/R + MEL and I/R + APC2 groups compared with the I/R + APC1 group. Tissue and serum MDA and PC levels were increased in the I/R group compared with control and treatment groups. Additionally, melatonin and apocynin treatment decreased MDA and PC levels closer to the control group, but this decrease was not applicable to the serum PCA level, which was significantly higher in I/R + APC1 and I/R + APC2 groups compared with the control group.

3.2 Histopathological examination

Microscopic images of histopathological findings in all groups are presented in Fig. 1. Ovaries of rats in the control group had a normal structure. However, in the I/R group, severe histopathological changes were observed. Follicular degeneration, inflammatory cell infiltration, interstitial edema, massive hemorrhage, and diffuse congestion were noted in the ovaries of rats with I/R injury. Melatonin and apocynin reversed the cellular defects in follicles and reduced hemorrhage and congestion.

Histopathological findings in ovaries are given in Table 3. Tissue injury scores demonstrating follicular degeneration, inflammatory cell infiltration, edema, hemorrhage, and vascular congestion were significantly higher in the I/R group compared with the control group ($P < 0.001$). Compared with the I/R group, melatonin and apocynin administration significantly decreased the degree of ovarian injury ($P < 0.01$), but this decrease was more prominent in the melatonin group.

3.3 Immunohistochemistry

Bax, caspase 3, and iNOS were immunohistochemically stained in ovarian tissues (Figs. 2-4). H-score analysis revealed that bax, caspase 3, and iNOS immunoreactivities were significantly increased in the I/R group compared with the control group ($P < 0.001$ for all). Compared with the I/R group, bax immunoreactivity was significantly decreased in I/R + APC1, I/R + APC2, and I/R + MEL groups ($P < 0.01$ for all). Caspase 3 immunoreactivity was significantly lower in I/R + APC1, I/R + APC2, and I/R + MEL groups than in the I/R group ($P < 0.05$, $P < 0.01$ and $P < 0.01$, respectively). iNOS immunoreactivity was significantly decreased in I/R + APC2 and I/R + MEL groups compared to the I/R group ($P < 0.05$ and $P < 0.01$, respectively) (Table 4).

4. Discussion

Adnexal torsion may cause permanent damage in ovaries and defects in folliculogenesis, which may eventually result

Table 3. Histological injury scores of ovarian tissues in each group

	Control	I/R	I/R + APC1	IR + APC2	IR + MEL
Follicular degeneration	0	1.3	0.8	0.6	0.5
Inflammatory cell infiltration	0.2	1.1	0.9	0.75	0.5
Edema	0.25	1.5	0.75	0.6	0.8
Hemorrhage	0.2	1.9	0.9	1.3	1.1
Vascular congestion	0.3	2.1	1.4	1.2	1.2
Tissue injury score	0.95	7.9*	$4.75^{\#,\dagger}$	$4.45^{\#,\dagger}$	$4.1^{\#,\dagger}$

*$P < 0.001$ compared with control; #$P < 0.01$ compared with control; †$P < 0.01$ compared with I/R. I/R: ischemia/reperfusion; APC: apocynin; MEL: melatonin.

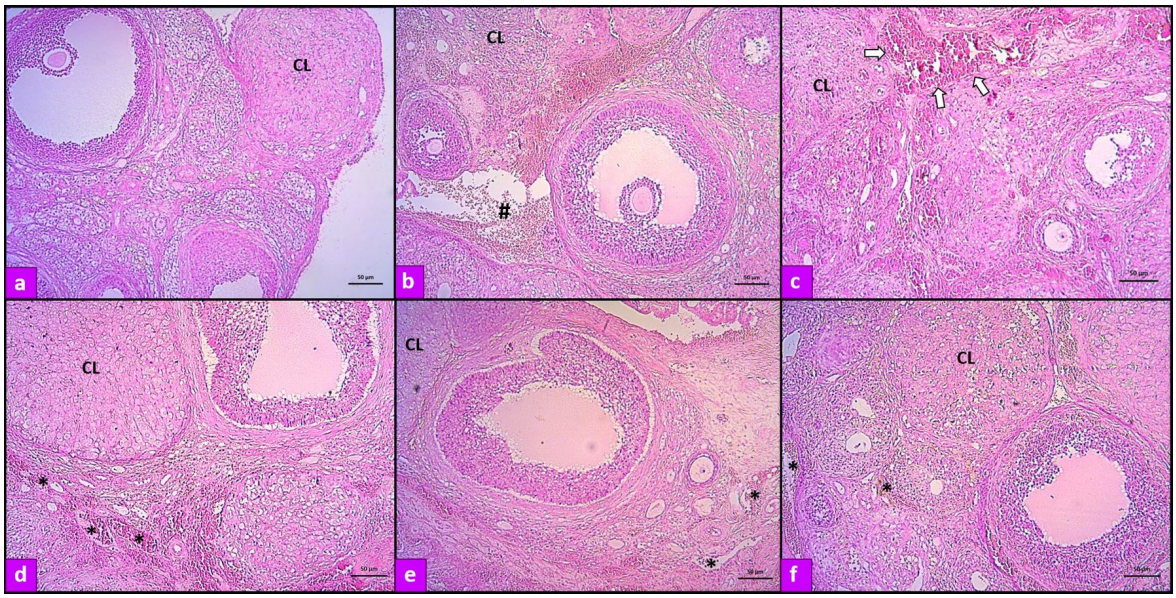

Fig. 1. Photomicrographs of ovarian sections demonstrated by hematoxylin and eosin staining. (a) Normal tissue; (b, c) Ovarian tissue from rats with I/R injury. The number symbol [#] and arrow indicate hemorrhage and vascular congestion, respectively; (d) Ovarian tissue from rats with I/R injury and treatment with 10 mg/kg apocynin; (e) Ovarian tissue from rats with I/R injury and treatment with 20 mg/kg apocynin; (f) Ovarian tissue from rats with I/R injury and treatment with melatonin. The asterisks [*] indicate that apocynin and melatonin treatment reduces vascular congestion. (10× objective).

Table 4. H-score values of bax, caspase-3, and iNOS immunoreactivity

	Control	I/R	I/R + APC1	I/R + APC2	I/R + MEL
Bax	12 ± 2	85 ± 16*	$56 \pm 8^{\#,\S}$	$45 \pm 9^{\#,\S}$	$47 \pm 11^{\#,\S}$
Caspase-3	15 ± 3	132 ± 21*	$87 \pm 12^{\#,¥}$	$73 \pm 15^{\#,\S}$	$62 \pm 10^{\#,\S}$
iNOS	10 ± 2	74 ± 11*	$60 \pm 13^{\#}$	$42 \pm 10^{\#,\S}$	$36 \pm 7^{\ddagger,\S}$

*$P < 0.001$ compared with control; #$P < 0.01$ compared with control; §$P < 0.01$ compared with I/R; ¥$P < 0.05$ compared with I/R; ‡$P < 0.05$ compared with control. I/R: ischemia/reperfusion; APC: apocynin; MEL: melatonin; iNOS: inducible nitric oxide synthase.

in female infertility. The mechanism of injury is usually attributed to the generation of reactive oxygen species (ROS) during both ischemia and reperfusion. Thus, it would be prudent to sustain anti-oxidative defense mechanisms by using anti-oxidant agents so that ovaries can overcome ROS-mediated I/R injury after adnexal detorsion is achieved [1–4].

This study was successful in establishing an animal model of ovarian I/R injury as evidenced by increasing MDA and PC levels and decreasing SOD and CAT activities in both ovarian tissue and serum. MDA is an index of lipid peroxidation by which ROS-induced oxidative damage is monitored within tissues. On the other hand, PC is a product of protein denaturation that appears as a result of I/R injury [25]. Both SOD and CAT are anti-oxidant enzymes participating in defense mechanisms in tissues. SOD catalyzes the transformation of superoxide into $H_2O_2$and O_2, which is the first step of antioxidant defense, whereas CAT transforms H_2O_2 into O_2 and H_2O [24].

To date, various pharmaceuticals and chemicals have been experimentally used to eliminate the hazards of torsion related I/R injury in ovaries [3, 4]. A thorough review of the literature has yielded no studies on the protective effects of apocynin and melatonin on ovarian I/R injury and, to the best of our knowledge, this is the first study to investigate the possible benefits of preoperative apocynin and melatonin treatment on I/R injury associated with surgical correction of adnexal torsion.

Apocynin inhibits the generation of ROS by blocking NADPH oxidase. This enzyme is considered to be the ma-

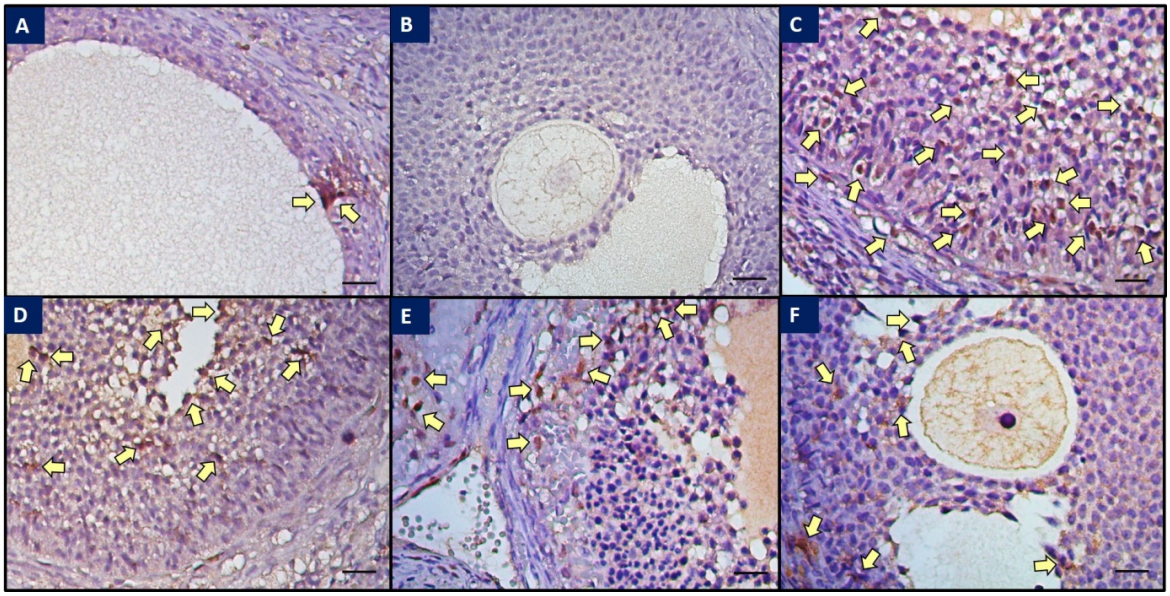

Fig. 2. Immunohistochemical staining of caspase-3 in ovaries of all groups. (a) Weak caspase-3 immunoreactivity was observed in the control group; (b) Control tissue, no immunohistochemical staining was observed; (c) Strong caspase-3 immunoreactivity was observed in the I/R group; (d) Immunoreactivity was decreased in the I/R + APC1; (e) I/R + APC2, and (f) I/R + MEL groups. The arrows indicate caspase-3 immunopositive granulosa cells and stromal cells in secondary follicles (40× objective).

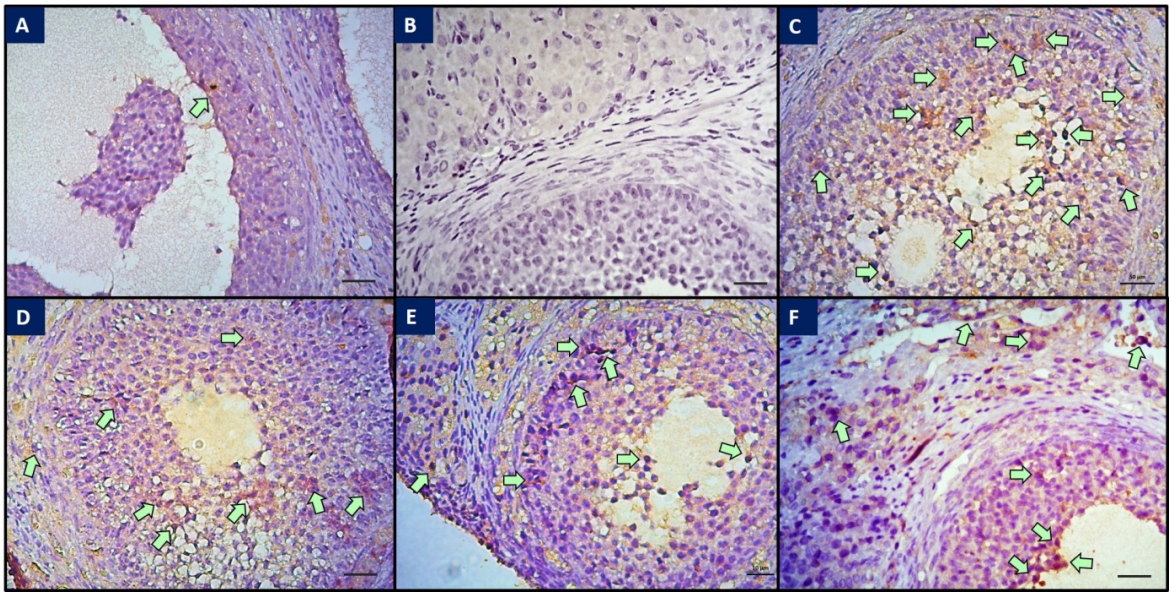

Fig. 3. Immunohistochemical staining of Bax in ovaries of all groups. (a) Weak Bax immunoreactivity was observed in the control group; (b) Control tissue, no immunohistochemical staining was observed; (c) Strong Bax immunoreactivity was observed in the I/R group; (d) Immunoreactivity was decreased in the I/R + APC1; (e) I/R + APC2, and (f) I/R + MEL groups. The arrows indicate Bax immunopositive stromal cells and granulosa cells in secondary follicles (40× objective).

jor source of ROS-mediated I/R injury, as activated NADPH oxidase helps to transport electrons to oxygen and subsequently produce ROS molecules. Moreover, apocynin stimulates γ-glutamylcysteine synthase, which synthesizes glutathione. Therefore, apocynin acts as an important antioxidant that protects tissues from torsion/detorsion damage [7, 28].

Both low (20 mg/kg) and high (50-100 mg/kg) doses of apocynin have been used to suppress oxidative stress in renal, cerebral, and endothelial tissues [11–13]. In another study, diabetes-induced testicular damage could be effectively treated by apocynin administered at a dose of 16 mg/kg for four weeks. Low dose apocynin treatment was able to reverse all histopathological changes caused by I/R injury in testes [29]. On the other hand, a high dose of apocynin was significantly more effective in restoring biochemical and histologi-

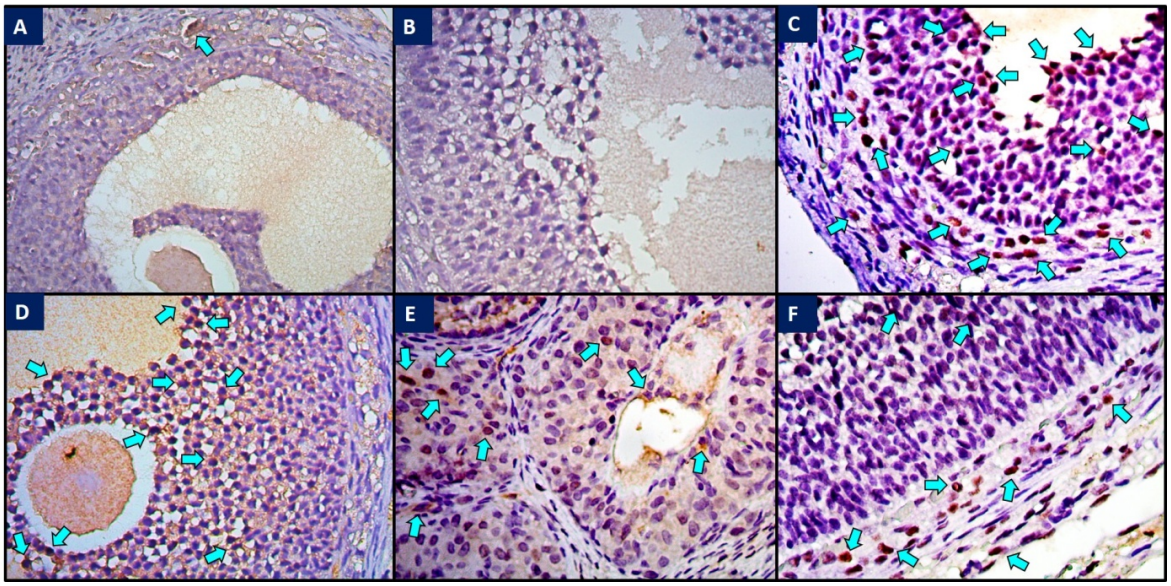

Fig. 4. Immunohistochemical staining of iNOS in ovaries of all groups. (a) Weak iNOS immunoreactivity was observed in the control group; (b) Control tissue, no immunohistochemical staining was observed; (c) Strong iNOS immunoreactivity was observed in the I/R group. The arrows indicate iNOS immunopositive granulosa cells in secondary follicles; (d) Immunoreactivity was decreased in the I/R + APC1; (e) I/R + APC2, and (f) I/R + MEL groups. The arrows indicate iNOS immunopositive granulosa cells and stromal cells in secondary follicles (40× objective).

cal parameters to normal levels in testicular tissue. Both low and high doses of apocynin could trigger the spermatogenic process, which was disrupted by I/R injury [30]. In this study, two doses (10 and 20 mg/kg) of apocynin were used, and we found that these regimens were similar in terms of efficacy. Moreover, both apocynin doses significantly improved biochemical, histopathological, and immunohistochemical parameters of oxidative damage in ovaries that underwent I/R injury.

Melatonin, as a major neuroregulatory hormone, is mainly secreted from the pineal gland within the circadian rhythm. Because of its lipophilic and hydrophilic properties, melatonin can easily pass through all biological membranes, enter cells, and distribute to subcellular compartments. In this way, melatonin protects cells from oxidative damage in both lipid and aqueous environments. Furthermore, melatonin impairs leukocyte migration, inhibits leukocyte adhesion to endothelial cells, and improves blood flow in oxidative processes [21, 22].

An animal study reported that the administration of melatonin caused a significant decrease in lipid peroxidation content and a significant improvement in SOD and CAT activities in testes that underwent torsion-related I/R injury. On the contrary, changes in enzyme activities of the contralateral testis were insignificant [31]. A similar Iranian study indicated that either melatonin or its combination with metformin restored SOD activity, as well as MDA and myeloperoxidase levels in rat testes that had been exposed to I/R injury. No significant difference was found between the administration of melatonin or melatonin and another compound. The beneficial effects of melatonin were also demonstrated by means of histopathological recovery [32]. As for the present study, melatonin could reverse I/R injury in terms of biochemical, histopathological, and immunohistochemical alterations in ovaries.

In conclusion, conservative detorsion surgery may not provide adequate protection to ovaries. Our results suggest that preoperative apocynin and melatonin administration can be used to protect ovaries from ROS-mediated I/R injury after adnexal detorsion is performed surgically. Both apocynin and melatonin are powerful as therapeutic anti-oxidant agents with considerable bioavailability and safety. In clinical practice, the application of apocynin and melatonin to treat I/R injury as an alternative therapeutic approach may help to rescue ovaries from subsequent subfertility after adnexal detorsion. However, these findings should be interpreted carefully as their power is limited by a relatively small study cohort and a lack of longitudinal data on fertility. The clinical availability of these compounds merits application in I/R injury studies following adnexal torsion. Further experimental animal and clinical studies are warranted to clarify the protective effects and to develop shared protocols for the clinical use (dosage, duration, method of administration) of apocynin and melatonin treatment in ovarian torsion-related I/R injury before conservative surgery.

Author contributions

CKİ, MU contributed to the concept and designed of the study; CKİ, MU, AA, VU contributed to data collection and /or processing; CKİ, MU, MKP, AA analyzed and /or interpreted data; CKİ, MU, ÖKC, MKP contributed to the literature review and writing of the manuscript and all authors read and approved the final manuscript.

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Conflict of interest

All authors declare that they have no conflict of interests. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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