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The protective effect of propolis on rat ovary against ischemia-reperfusion injury: Immunohistochemical, biochemical and histopathological evaluations

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1. Introduction

In women, ovarian torsion is a major cause of severe morbidity. Early diagnosis and treatment are necessary to preserve the function of the ovaries and to maintain fertility [1]. The most common symptom of ovarian torsion (adnexal torsion or turbo-ovarian torsion) is acute abdominal pain [2]. Adnexal torsion leading to ischemia is most common in sexually mature women [3]. Investigations have showed that it may also occur in pre-pubertal, post-menopausal and pregnant women [4]. Ovarian torsion is an emergency condition and the surgical intervention is an effective method to prevent ovarial ischemic stroke [5]. However, this surgical procedure may cause vascular ovarian damage. Ovary injury begins with hypoxia-ischemia and this is associated with over generation of reactive oxygen species (ROS) [6].

Propolis is a natural product collected by bees from the poplar and conifer trees. Bees use propolis as an antibiotic against foreign organisms and also to repair the cracks of their hives [7]. It has vast majority of biological activities such as anti-inflammatory, anti-fungal, antioxidant, and immune-stimulating activity [8]. Most of these effects have been related to the remarkable in vitro antioxidant activity and free radical scavenging ability of propolis [9]. The major components of propolis are polyphenolics including aldehydes, caffeic acid, and caffeic acid phenethyl ester which plays a critical role in neurological disorders [10], cardiovascular diseases [11], pathophysiology of cancer [12], and diabetes [13]. But effects of propolis on ovarian ischemia-reperfusion injury (I/RI) were never studied.

Propolis enabled to enrich regulatory T (Treg) cells to exert anti-inflammatory effects [14]. There is also evidence, indicating that propolis inhibited apoptosis by preventing phosphorylation of proapoptotic proteins [15]. By considering these investigations propolis can probably have a protective role against ovary I/RI. Thus, here for the first time, the protective effects of propolis on ovary torsion-detorsion injury were evaluated in a rat model. As far as we know, malondialdehyde (MDA) is the final product of lipid peroxidation and is frequently used to define oxidative stress; superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH) represent antioxidant potential; caspase-3 reveals apoptotis; tumour necrosis factor-alpha (TNF-α), interleukin-6 (IL-6) and myeloperoxidase (MPO) show inflammatory response, and 8-hydroxy-2'-deoxyguanosine (8-OHdG) is a significant marker of oxidative DNA damage. Therefore, in this study we evaluated lipid peroxidation (LPO), antioxidant status, proapototic protein, DNA damage, and markers of inflammation. We also histologically evaluated ovarian cell damages by using hematoxylin and eosin (H&E) staining methods.

2. Materials and methods

2.1. Experimental animals

Adult Sprague-Dawley rats (n = 28), weighing 250–300 g, were purchased from Atatürk University Experimental Research Center (Erzurum/Turkey). The rats were kept under standard laboratory conditions, maintained in temperature-(22 ± 2 °C) and humidity- controlled rooms on a 12-h/12-h light/dark cycle, and had free access to food and water. Experiments were performed according to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication No. 85-23, revised 1996). All experimental procedures in this study were approved by the Atatürk University Local Ethics Committee for Animal Experiments (No. 129, 11.07.2016).

2.2. Ovarian I/R procedure

I/R injuries were created as described previously [16]. Briefly, each rat was anesthetized via intraperitoneal injection of a combination of

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ketamine (75 mg/kg, i.p) and xylazine (10 mg/kg, i.p.). And then ovaries were visualized by a 2–2.5 cm incision in the lower abdomen under anesthesia. Vascular clips were applied on the lower part of the ovaries of the rats in I/R and treatment groups. Then, 3 h of ischemia and following 3 h of reperfusion were created.

2.3. Propolis preparation and experimental design

The water-soluble propolis extract was purchased from Aksuvital Natural Products Food Industry Trade Inc. (İstanbul, Turkey). Then, the propolis extract dissolved in 100 ml distilled water and diluted at 200 mg/kg concentrations. The solution was given to rats as 1 cc by gavage 1 h before ischemia onset. The rats were randomly divided into 4 groups (n = 7 per group): I) Control group (a healthy control group scheduled for a sham operation), II) I/R group, III) Propolis group (200 mg/kg propolis alone treatment group), IV) I/R + Propolis group (protected by previous administrations of 200 mg/kg propolis). The dose was selected according to the literature data [17] and our preliminary studies.

After above processes, rats were euthanized and their ovaries were removed for all of the assays.

2.4. Measurement of biochemical parameters

Left ovarian tissues were homogenized, and the supernatants were used to determine the antioxidant enzyme profile, oxidative status and cytokine levels. The levels of superoxide SOD, CAT, GSH, LPO, MPO and IL-6 in the rat ovarian tissue were measured with the Clinical Automatic Biochemistry Analyzer 7600 (Hitachi, Japan) employing enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Minneapolis, MN) according to the manufacturer's instructions. All experiments were performed with triplicate samples and repeated three times.

2.5. Immunohistochemical assessments

Caspase-3, TNF-α, and 8-OHdG were detected by specific monoclonal antibodies. From paraffin-embedded each ovarian tissue were sliced at a thickness of 4 μm, and the sections were deparaffinized. After Diaminobenzidine (DAB) was applied as chromogen, slides were counterstained with hematoxylin, dehydrated, and covered by coverslips. Caspase-3 immunostaining was performed using polyclonal rabbit-anti-human (rabbit, 1:1000, Cell Signaling Technology, Beverly, MA, USA). The expression of TNF-α was determined by goat moloclonal anti- TNF-α (1:300 dilution; Sigma, USA). Immunohistochemical staining of 8-OHdG was performed using anti–8-hydroxydeoxyguanosine (8-OHdG) antibody (Santa Cruz; 1:2500 dilution) with a Novolink Polymer Detection kit (Leica Microsystems Pte Ltd, Taipei, Taiwan), following the manufacturer's instructions. The pathologists continuously observed at least 10 high-power fields (×200) for each slice, counted the number of positive cells in each high-power field, and calculated the average number of positive cells to reflect the intensity of positive expression. The sections were evaluated as none (−), mild (+), moderate (++) and severe (+++) according to their immunity positivity [18].

2.6. Histopathological examinations and assessments

The ovarian tissues were fixed in 10% neutral buffered formalin overnight, dehydrated, embedded in paraffin and sectioned at 5 μm. For histological analysis, sections were stained with H&E and analyzed using a light microscope (Leica DM 1000, Germany). Ovarian damage, including congestion, haemorrhage, edema, cell degeneration, necrosis, and Neutrophil infiltration were scored histologically using a graduated scale- none (−), mild (+), moderate (++) and severe (+++) [18]. Evaluation was performed by a pathologist who was blind to the study groups.

2.7. Statistical analysis

The differences in variance were analysed statistically using a one−way analysis of variance (ANOVA) test by Graphpad prism 5.0 statistics software (GraphPad, La Jolla, CA, USA). Tukey's test was used as a post hoc. $p < 0.05$ was considered as statistically significant. The superscripts of * and # were used to compare the control and I/R groups with other studied groups. Thenon-parametric Kruskal-Wallis test was used to analyze variations among data obtained using the semi-quantitative method at histopathological examination. Analyses between two groups were performed using the Mann-Whitney *U* test. $p < 0.05$ was regarded as statistically significant.

3. Results

3.1. Effect of Propolis on antioxidant enzyme profile in ovarian I/R

The activities of antioxidant enzymes SOD, CAT, GSH and the levels of LPO, MPO and IL-6 in ovary of the control and experimental groups were shown in Fig. 1. Ovarian I/R caused increases in the LPO, MPO and IL-6 levels and decreases in the SOD, CAT, and GSH activities in rats ($p < 0.001$). The rats pretreated with propolis had significant decreases in the LPO, MPO and IL-6 levels and significant increases in the SOD, CAT, and GSH activities in ovary compared with the I/R group ($p < 0.001$). Pretreatment with propolis also markedly prevented I/R-induced elevation of LPO, MPO and IL-6 level in ovary.

3.2. Effect of Propolis on 8-OHdG, TNF-α, and Caspase-3 immunoreactivity in ovarian I/R

8-OHdG formation was markedly increased in the I/R group ovary compared with the control group (Fig. 2). Pretreatment with propolis effectively attenuated expression of 8-OHdG in the ovary with I/R (Fig. 2D and Table 1). Furthermore, there was no immunoreactive Caspase-3 and TNF-α in ovary of control and propolis groups, but they became present in I/R group (respectively, Figs. 3B and 4 B). Nevertheless, ovary in I/R + Propolis group showed mild immunoreactivity for Caspase-3, and TNF-α (respectively, Figs. 3D and 4 D, and Table 2).

3.3. Histopathological effects of Propolis in ovarian I/R

As shown in the Fig. 5, the ovary of the control and Propolis group rats revealed normal ovarian structure. H&E staining showed that vascular congestion, haemorrhage, edema, cell degeneration, necrosis, and neutrophil infiltration in I/R group. In contrast, Popolis pretreatment markedly ameliorated the ovarian damage induced by I/R ($p < 0.05$). Also, there was no haemorrhage and necrosis in I/R + Propolis group.

4. Discussion

I/RI caused by ovarian torsion-detorsion must be diagnosed and treated as much early as possible [19]. To date, the treatment strategy for protecting the ovarian following torsion/detorsion is surgical; hence, exploring additional options is necessary [20]. On the other hand, the current practice for treating ovarian damage is based on unproven, mainly reactive drug interventions [21]. Thus, the present study investigated whether propolis is useful or not in the prevention of ovarian damage in I/R conditions in rat ovaries and it was found to have beneficial effects.

The process of I/R is multifactorial and there are several mechanisms involved in the pathogenesis [22]. Accumulating evidence has strongly implicated that the precise mechanism for ischemia/induced ovarian damage may involve oxidative stress [23]. We showed that this complication in ovarian exhibited significantly aggravated oxidative stress, inflammatory responses, and apoptosis related the cell death

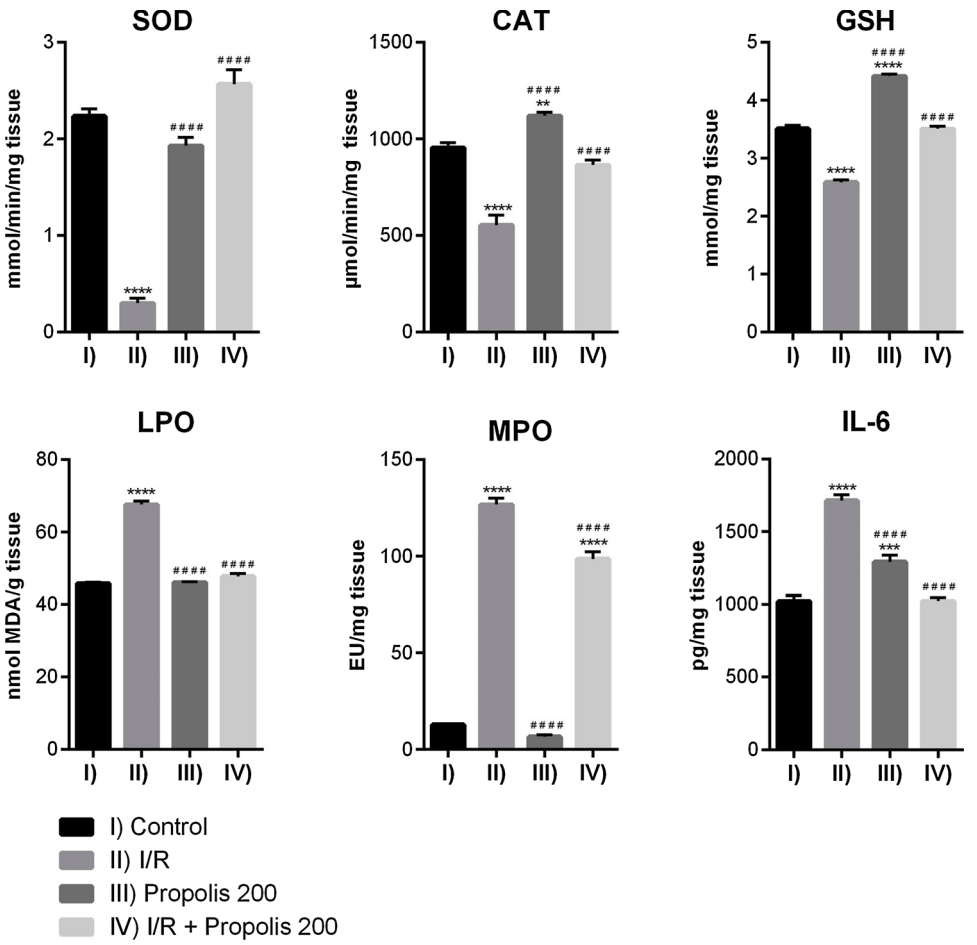

Fig. 1. The effects of Propolis on ovary SOD, CAT, GSH, LPO, MPO, and IL-6 levels after I/R. Data are presented as mean ± Standard Error of Mean (SEM) (n = 7). * denotes significant differences between other studied groups and control (*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, ****: $p < 0.0001$), # denotes significant differences between other studied groups and I/R group (#: $p < 0.05$, ##: $p < 0.01$, ###: $p < .0.001$, ####: $p < 0.0001$) by Tukey's multiple range tests. Abbreviation used: I/R: Ischaemia/Reperfusion.

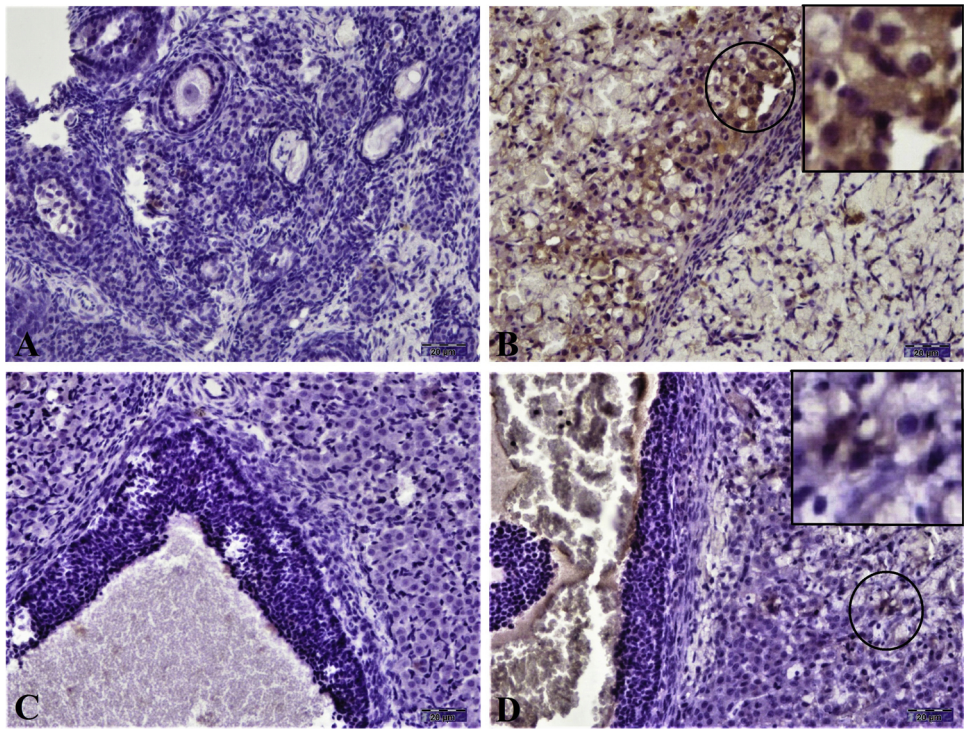

Fig. 2. 8-OHdG expression in the rat ovary. A) Control group: 8-OHdG negative B) I/R group; severe 8-OHdG expression was detected in ovarian tissue (inside the circle), C) Propolis group: 8-OHdG negative D) I/R + Propolis group: mild 8-OHdG expression was detected in ovarian tissue (inside the circle) (Immunohistochemistry (IHC), Bar: 20 µm). Abbreviation used: I/R: Ischaemia/Reperfusion.

Table 1
Immunohistochemical findings and their scores in ovarian tissue.

Groups	8-OhDG	Tnf-α	Caspase-3
Control	–	–	–
I/R	+ + +	+ + +	+ + +
Propolis	–	–	–
I/R + Propolis	+	+	+

I/R: Ischaemia/Reperfusion.
According to immunohistochemical findings: – none (−), mild (+), moderate (+ +) and severe (+ + +).

compared with control rats. We further showed that I/RI increased the levels of 8-OHdG and LPO, suggesting that oxidative stress induced during I/R caused oxidative damage in cellular DNA, protein, and lipids in ovarian cells.

Excessive intracellular ROS generation is thought to trigger extensive mitochondrial oxidative damage [24]. Tissue cells undergoing ischemic insult produce excess amounts of ROS, which attack mitochondrial DNA (mtDNA) due to its special structural characteristics, resulting in aggregation of 8-OHdG, a sensitive marker of oxidative DNA damage, and excessive intracellular ROS generation is thought to trigger extensive mitochondrial oxidative damage [25]. The cells undergoing ischemic insult produce excess amounts of ROS, which attack mtDNA due to its special structural characteristics, resulting in aggregation of 8-OHdG, a sensitive marker of oxidative DNA damage, and mitochondria dysfunction [26]. Hence, it will be interesting to explore whether propolis plays a role in alleviating mitochondrial oxidative damage after ovarian ischemia in rats. In the present study, we found that ovarian ischemia in rat model significantly increased mitochondrial 8-OHdG and ROS generation. However, propolis markedly reduced the content of mitochondrial 8-OHdG and ROS in in vivo model, suggesting that mitochondrial oxidative stress generation may be involved in propolis-induced protection in ischemic ovaries. Turkez et al. [27] evaluated the effects of treatment with propolis on aluminium-induced micronucleated hepatocytes and oxidative stress in rat liver. In another study, Tohamy et al. [28] evaluated the effects of treatment with propolis against on cisplatin-induced hepatic, renal, testicular genotoxicity by the bone marrow chromosomal aberration assay in male albino mice. So, it is clear from these observations that propolis may act not only as direct antioxidant, inactivating free radicals, but also as an attractive tool for prevention of DNA-instability.

Recent studies suggest that inflammatory mediators released by macrophages contribute to tissue damages in experimental models of I/RI [29]. Our biochemical and immunohistochemical results demonstrated that this process induced the increases in levels of pro-inflammatory cytokines such as TNF-α and IL-6 in ovarian, and this increase was not observed in groups with propolis. A possible mechanism influenced by ischemia involves TNF-α, which is considered to be a key regulator of inflammation and apoptosis after ischemic ovarian injury [30]. Therefore, down-regulation of TNF-α might be another possibility for activation free radical scavengers of under our present experimental conditions. In our study, the changes observed with propolis were remarkable in comparison with those observed with I/R group. Since anti-inflammatory property of propolis remains unclear, it has received much attention in recent years for inflammatory disorders [31]. The treatment with propolis impaired the inflammatory response in vivo, as indicated by inhibition of proinflammatory mediators. Our histological observations also supported this conclusion. Treatment with propolis also reduced ovarian neutrophil recruitment, which was reflected by decreased inflammatory cell infiltration, MPO levels in the ovarian tissue and lower white cell counts in stroma. Thus, the present findings emphasize that ovarian acute inflammatory mediators are associated with each other, as evidenced by serum levels of neutrophilic enzyme. It has shown that activated neutrophils secrete enzymes (e.g., MPO, proteases, and elastase) and liberate oxygen radicals [32,33]. It is suggested that oxidative stress and inflammation cooperate in the development of ovarian damage [34]. Our results showed that propolis protected ovarian against I/RI by suppressing TNF-α, IL-6 activation and oxidative stress. More importantly, the levels of cytokines consistently correlated with the generation of ROS and markers of damage. Previous studies have suggested that ROS derived from polymorphonuclear neutrophils and endothelial cells are involved in the pathogenesis of ovarian injury [35]. Since the source of ROS could be neutrophils sequestered in systemic organs as a result of the inflammatory reaction, it is possible agents which can inhibit the activation and

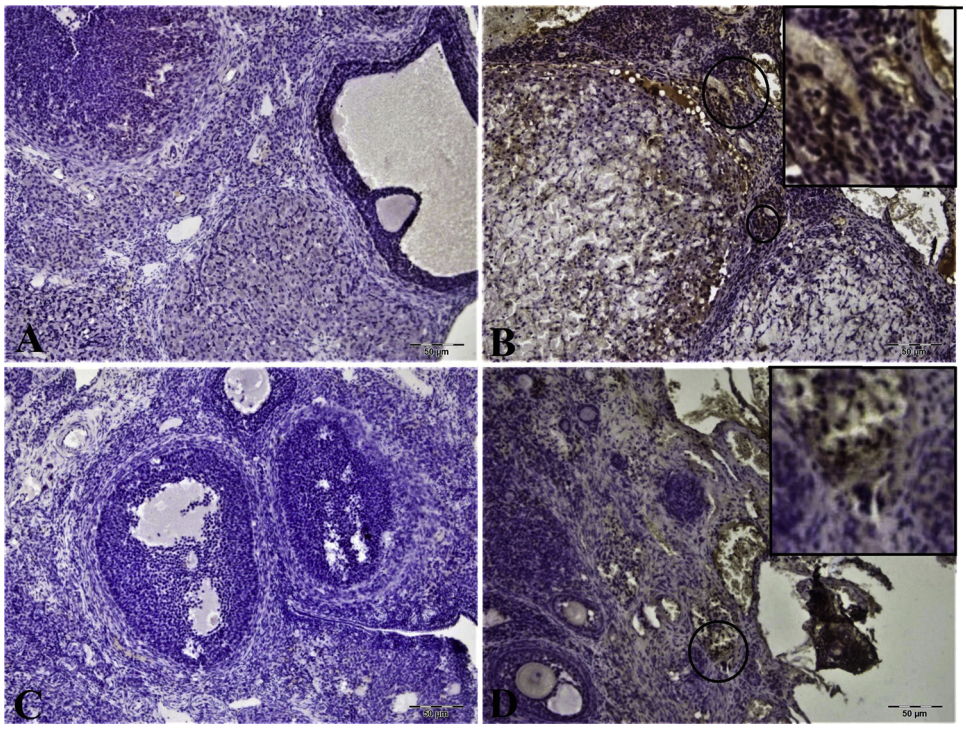

Fig. 3. Tnf-α expression in the rat ovary. A) Control group: Tnf-α negative B) I/R group; severe Tnf-α expression was detected in ovarian tissue (inside the circle), C) Propolis group: Tnf-α negative D) I/R + Propolis group: mild Tnf-α expression was detected in ovarian tissue (inside the circle) (IHC, Bar: 20 μm). Abbreviation used: I/R: Ischaemia/Reperfusion.

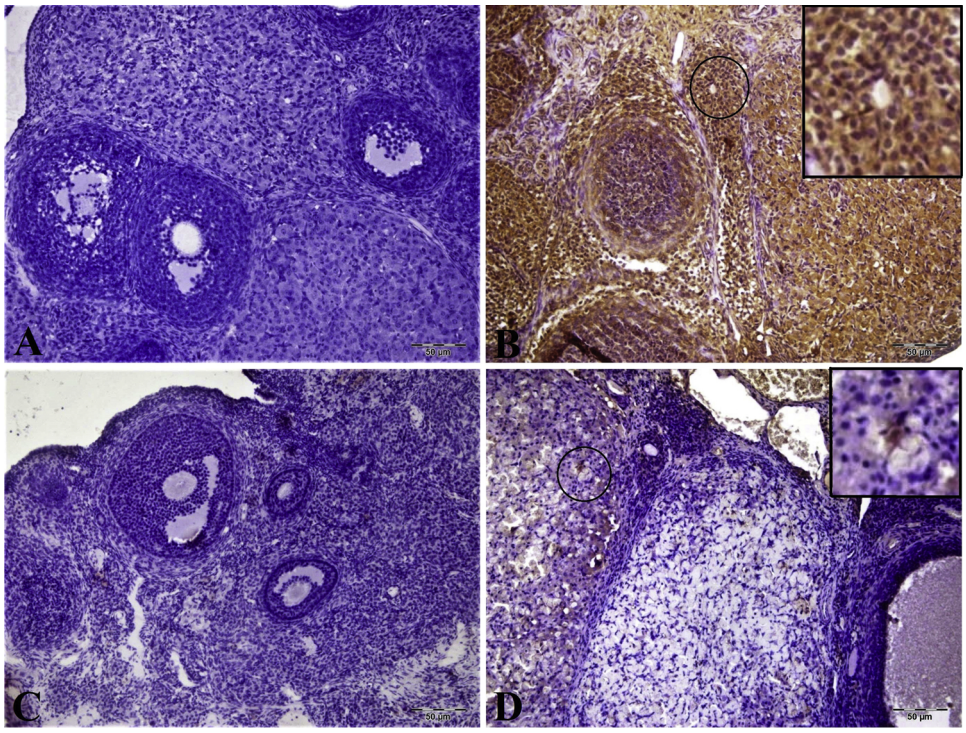

Fig. 4. Caspase-3 expression in the rat ovary. A) Control group: Caspase-3 negative B) I/R group; severe Caspase-3 expression was detected in ovarian tissue (inside the circle), C) Propolis group: Caspase-3 negative D) I/R + Propolis group: mild Caspase-3 expression was detected in ovarian tissue (inside the circle) (IHC, Bar: 20 μm). Abbreviation used: I/R: Ischaemia/Reperfusion.

adherence of neutrophils might exert protective effects against oxidant tissue injury [36]. Thus, antioxidants appear to be an important component of therapeutic agents for treating ovarian I/RI. There have been many reports indicating that various types of antioxidants such as curcumin, 2-aminoethoxydiphenyl borate, and dimethylsulfoxide can attenuate ovarian I/RI [37–39]. We observed that propolis significantly ameliorated the disturbances in the levels of GSH induced by I/R in ovarian; in line with our findings, several studies consistently showed the antioxidant properties of propolis. Jasprica et al. [40] showed that propolis reduces the disturbances of the redox status in red blood cells of rats. A study suggested that propolis may be effective in decreasing of methoxychlor-induced ovarian toxicity in rat [41]. In another study, Kwon et al. [42] observed that propolis was able to reduce the alterations on the antioxidant enzymes SOD, CAT, and GPx and on the levels of GSH in skeletal muscle and liver of rat.

In the current study, ovary I/RI caused significant increases in the LPO and caspase-3 levels in ovarian tissue. During I/RI, the most important mediator which is released in this process, and contributes as biomarkers of oxidative stress, is MDA [43]. MDA is a stable end product of LPO and is also a significant indicator of ROS-dependent tissue damage [44]. Our results clearly indicate that pre-treatment with orally administered propolis significantly attenuated ovarian I/RI in rat. Notably, the protective effects of propolis on ovarian I/R injury could be due to the effective scavenging of TNF-α-induced ROS and the decrease in LPO production, and thereby has promising anti-inflammatory potencies. The current literature showed that caspase-3, the “executor” protease that is the key machinery of cellular death and functions at the terminal stages of apoptosis [45]. Currently, caspase inhibitor drugs are in clinical trials for liver reperfusion injury with promising results [46]. In our study, the rats treated with propolis had a significant decrease in caspase-3 level in ovarian compared with I/R group. Our results demonstrated that TNF-α trigger the apoptosis pathway by up-regulating the level of caspase-3 in ischemia/related ovarian damage. In essence, we believe our findings indicate TNF-α and caspase-3 as a potential therapeutic target of I/R patients.

I/R-induced ovarian damage was also supported by the histopathological changes observed in the ovary tissue, which revealed neutrophil infiltration, congestion, haemorrhage, necrosis, apoptosis, and cell degenerations. Conversely, propolis pretreatment markedly ameliorated the ovarian damage induced by I/R. We observed lower histopathological damage scores in propolis group than in other I/R groups. Zhu et al. [47] have endorsed the inhibitory influence of propolis on histological lesions in diabetic nephropathy. Furthermore, a previous study demonstrated that propolis reduced hepatotoxicity in a mice model of diabetes [48]. In accordance with the above mentioned studies, our current investigation showed that propolis improved I/R-induced histopathological changes in ovarian tissue.

In conclusion, the above findings demonstrate that propolis exerted a protective effect against ovarian I/RI in rats, through the inhibition of oxidative stress, the suppression of inflammatory processes, down-regulation of 8-OHdG formations and the inhibition of ovarian cell apoptosis.

Table 2
Histopathological findings and their scores in ovarian tissue.

Groups	Congestion	Hemorrhage	Edema	Cell degeneration	Cell Necrosis	Neutrophil infiltration
Control	–	–	–	–	–	–
I/R	+++	+++	+++	+++	+++	+++
Propolis	–	–	–	–	–	–
I/R + Propolis	++	–	++	+	–	+

I/R: Ischaemia/Reperfusion.
According to immunohistochemical findings: – none (−), mild (+), moderate (++) and severe (+++).

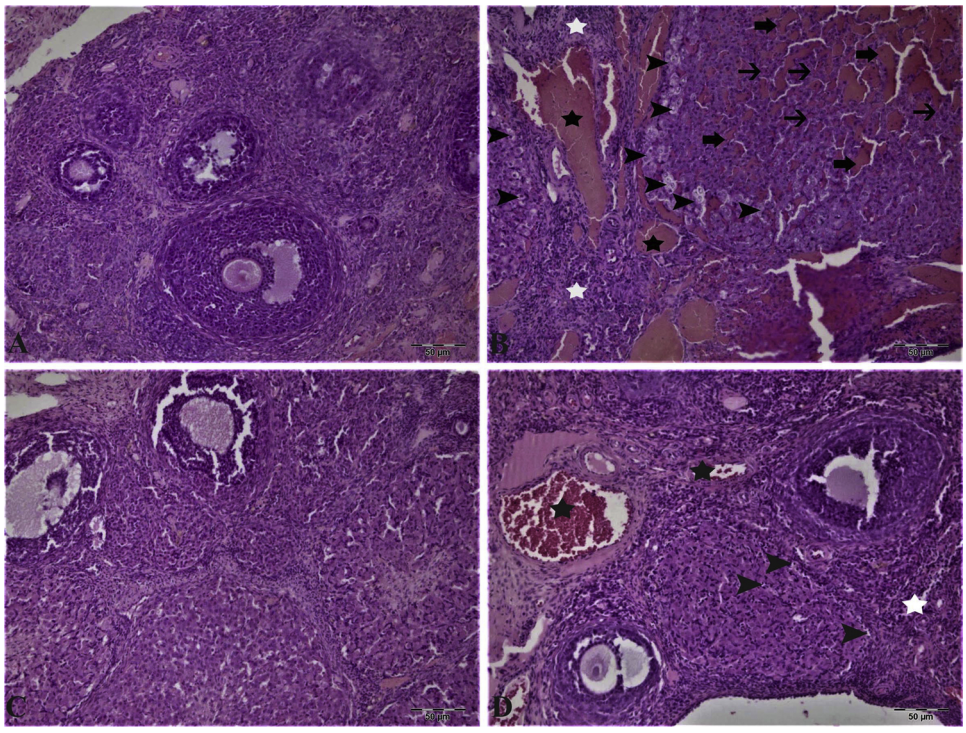

Fig. 5. Histopathological examination of rat ovary. A) Control group with normal ovary histology, (B): I/R group; Arrow heads: Degenerated parenchyma cell, Thick arrows: Hemorrhage, Thin arrows: Necrosis, Black asterisk: Congestion, White asterisk: Neutrophil infiltration (C): Propolis alone treatment group with normal ovary histology, (D): I/R group protected by previous administrations of propolis, (H & E staining, Bar: 20 µm). Abbreviation used: I/R: Ischaemia/Reperfusion.

Conflict of interests

No potential conflict of interest was reported by the authors. This work was supported by the Bilimsel Araştırma Projeleri (BAP) from Atatürk university [grant number 2017/6014].

References

[1] J.M. Rotoli, Abdominal pain in the post-menopausal female: is ovarian torsion in the differential? J. Emerg. Med. 52 (2017) 749–752.
[2] Z. Sidiropoulou, A. Setúbal, Acute abdomen in pregnancy due to isolated fallopian tube torsion: the laparoscopic treatment of a rare case, World J. Clin. Cases 2 (2014) 724.
[3] B.W. Rackow, P. Patrizio, Successful pregnancy complicated by early and late adnexal torsion after in vitro fertilization, Fertil. Steril. 87 (2007) 697–e9.
[4] J. Bouquet de Joliniere, J.B. Dubuisson, F. Khomsi, A. Fadhlaoui, G. Grant, N.B. Ali, A. Major, A. Feki, Laparoscopic adnexectomy for ovarian torsion during late pregnancy: case report of a non-conservative treatment and literature analysis, Front. Surg. 4 (2017) 50–54.
[5] H. Jeon, A. Ryu, H.G. Seo, S.H. Jang, Ovarian torsion of mixed epithelial tumor misdiagnosed as a malignancy in postmenopausal woman: a case report, Medicine 96 (2017) e8207.
[6] G. Ssi-Yan-Kai, A.L. Rivain, C. Trichot, M.C. Morcelet, S. Prevot, X. Deffieux, J. De Laveaucoupet, What every radiologist should know about adnexal torsion, Emerg. Radiol. 25 (2018) 51–59.
[7] S. El-Guendouz, N. Al-Waili, S. Aazza, Y. Elamine, S. Zizi, T. Al-Waili, A. Al-Waili, B. Lyoussi, Antioxidant and diuretic activity of co-administration of Capparis spinosa honey and propolis in comparison to furosemide, Asian Pac. J. Trop. Med. 10 (2017) 974–980.
[8] S.G. Park, D.Y. Lee, S.K. Seo, S.W. Lee, S.K. Kim, W.K. Jung, M.S. Kang, Y.H. Choi, S.S. Yea, I. Choi, I.W. Choi, Evaluation of anti-allergic properties of caffeic acid phenethyl ester in a murine model of systemic anaphylaxis, Toxicol. Appl. Pharmacol. 226 (2008) 22–29.
[9] J.M. Sforcin, Biological properties and therapeutic applications of propolis, Phytother. Res. 30 (2016) 894–905.
[10] R.A. Barbosa, T.L.G.M. Nunes, T.L.G.M. Nunes, A.O.D. Paixão, R.B. Neto, S. Moura, R.L. Albuquerque Junior, E.A. Cândido, F.F. Padilha, L.J. Quintans-Júnior, M.Z. Gomes, Hydroalcoholic extract of red propolis promotes functional recovery and axon repair after sciatic nerve injury in rats, Pharm. Biol. 54 (2016) 993–1004.
[11] C.C. Chen, C.Y. Kuo, R.F. Chen, Role of CAPE on cardiomyocyte protection via connexin 43 regulation under hypoxia, Int. J. Med. Sci. 13 (2016) 754–758.
[12] A. Kabała-Dzik, A. Rzepecka-Stojko, R.Ż. Kubina, Jastrzębska-Stojko, R. Stojko, R.D. Wojtyczka, J. Stojko, Migration rate inhibition of breast cancer cells treated by caffeic acid and caffeic acid phenethyl ester: an in vitro comparison study, Nutrients 9 (2017) 1144.
[13] J. Nie, Y. Chang, Y. Li, Y. Zhou, J. Qin, Z. Sun, H. Li, Caffeic acid phenethyl ester (Propolis extract) ameliorates insulin resistance by inhibiting JNK and NF-κB inflammatory pathways in diabetic mice and HepG2 cell models, J. Agric. Food Chem. 65 (2017) 9041–9053.
[14] K.W. Cheung, D.M.Y. Sze, W.K. Chan, R.X. Deng, W. Tu, G.C.F. Chan, Brazilian green propolis and its constituent, Artepillin C inhibits allogeneic activated human CD4 T cells expansion and activation, J. Ethnopharmacol. 138 (2011) 463–471.
[15] H. Tang, X. Yao, C. Yao, X. Zhao, H. Zuo, Z. Li, Anti-colon cancer effect of caffeic acid p-nitro-phenethyl ester in vitro and in vivo and detection of its metabolites, Sci. Rep. 7 (2017) 7599.
[16] T. Behroozi-Lak, M. Ebrahimpour, L. Zarei, M. Pourjabali, N. Farhad, H. Mohaddesi, Systemic administration of curcumin nanoparticles protects ischemia-reperfusion injury in ovaries: an animal model study, Revista da Associação Médica Brasileira 64 (2018) 22–31.
[17] A. Gheisari, S. Shahrvand, N. Landy, Effect of ethanolic extract of propolis as an alternative to antibiotics as a growth promoter on broiler performance, serum biochemistry, and immune responses, Vet. World 10 (2017) 249–254.
[18] S. Yıldırım, Investigation by histopathological and TUNEL method of the protective effect of Resveratrol on carbon tetrachloride induced liver toxicity in rats, Ataturk Univ. J. Vet. Sci. 12 (2017) 178–186.
[19] R. Dasgupta, E. Renaud, A.B. Goldin, R. Baird, D.B. Cameron, M.A. Arnold, K.A. Diefenbach, A. Gosain, J. Grabowski, Y.S. Guner, T. Jancelewicz, A. Kawaguchi, D.R. Lal, T.A. Oyetunji, R.L. Ricca, J. Shelton, S. Somme, R.F. Williams, C.D. Downard, Ovarian torsion in pediatric and adolescent patients: a systematic review, J. Pediatr. Surg. 53 (2017) 1387–1391.
[20] X. Liu, H. Chen, B. Zhan, B. Xing, J. Zhou, H. Zhu, Z. Chen, Attenuation of reperfusion injury by renal ischemic postconditioning: the role of NO, Biochem. Biophys. Res. Commun. 359 (2007) 628–634.
[21] C. Huang, M.K. Hong, D.C. Ding, A review of ovary torsion, Tzu-Chi Med. J. 29 (2017) 143–147.
[22] F. Zhang, N. Ru, Z.H. Shang, J.F. Chen, C. Yan, Y. Li, J. Liang, Daidzein ameliorates spinal cord ischemia/reperfusion injury-induced neurological function deficits in Sprague-Dawley rats through PI3K/Akt signaling pathway, Exp. Ther. Med. 14 (2017) 4878–4886.
[23] M. Cai, Y. Ma, W. Zhang, S. Wang, Y. Wang, L. Tian, Z. Peng, H. Wang, T. Qingrong, Apigenin-7-O-β-D-(-6''-p-coumaroyl)-glucopyranoside treatment elicits neuroprotective effect against experimental ischemic stroke, Int. J. Biol. Sci. 12 (2016) 42–52.
[24] C. Dai, G.D. Ciccotosto, R. Cappai, Y. Wang, S. Tang, D. Hoyer, E.K. Schneider, T. Velkov, X. Xiao, Rapamycin confers neuroprotection against colistin-induced oxidative stress, mitochondria dysfunction, and apoptosis through the activation of autophagy and mTOR/akt/CREB signaling pathways, ACS Chem. Neurosci. 9 (2017) 824–837.
[25] Q. Tan, X. Yan, L. Song, H. Yi, P. Li, G. Sun, D. Yu, L. Li, Z. Zeng, Z. Guo, Induction of mitochondrial dysfunction and oxidative damage by antibiotic drug doxycycline enhances the responsiveness of glioblastoma to chemotherapy, Med. Sci. Monit. 23 (2017) 4117–4125.
[26] Y. Pan, N. Wang, P. Xia, E. Wang, Q. Guo, Z. Ye, Inhibition of Rac1 ameliorates neuronal oxidative stress damage via reducing Bcl-2/Rac1 complex formation in mitochondria through PI3K/Akt/mTOR pathway, Exp. Neurol. 300 (2018) 149–166.

[27] H. Türkez, M.I. Yousef, F. Geyikoglu, Propolis prevents aluminium-induced genetic and hepatic damages in rat liver, Food Chem. Toxicol. 48 (2010) 2741–2746.
[28] A.A. Tohamy, E.M. Abdella, R.R. Ahmed, Y.K. Ahmed, Assessment of anti-mutagenic, anti-histopathologic and antioxidant capacities of Egyptian bee pollen and propolis extracts, Cytotechnology 66 (2014) 283–297.
[29] Y. Liu, B. Shi, Y. Li, H. Zhang, Protective effect of luteolin against renal ischemia/reperfusion injury via modulation of pro-inflammatory cytokines, oxidative stress and apoptosis for possible benefit in kidney transplant, Med. Sci. Monit. 23 (2017) 5720–5727.
[30] A.M. Fouda, A.R. Youssef, O. Sharaf Eldin, Comparative study of amlodipine vs. Cilnidipine for the prevention of hepatic ischemia-reperfusion injury in rat model, Fundam. Clin. Pharmacol. 32 (2018) 163–173.
[31] L. Chen, Y. Jin, H. Chen, C. Sun, W. Fu, L. Zheng, M. Lu, P. Chen, G. Chen, Y. Zhang, Z. Liu, Y. Wang, Z. Song, G. Liang, Discovery of caffeic acid phenethyl ester derivatives as novel myeloid differentiation protein 2 inhibitors for treatment of acute lung injury, Eur. J. Med. Chem. 143 (2018) 361–375.
[32] M.B. Grisham, L.A. Hernandez, D.N. Granger, Xanthine oxidase and neutrophil infiltration in intestinal ischemia, Am. J. Physiol. 251 (1986) G567–G574.
[33] R.J. Reiter, Oxygen radical detoxification processes during aging: the functional importance of melatonin, Aging Clin. Exp. Res. 7 (1995) 340–351.
[34] S.A. Bukhari, K. Zafar, M.I. Rajoka, Z. Ibrahim, S. Javed, R. Sadiq, Oxidative stress-induced DNA damage and homocysteine accumulation may beinvolved in ovarian cancer progression in both young and old patients, Turk. J. Med. Sci. 46 (2016) 583–589.
[35] A.R. Buz'Zard, B.H. Lau, Pycnogenol® reduces talc-induced neoplastic transformation in human ovarian cell cultures, Phytother. Res. 21 (2007) 579–586.
[36] R.J. Reiter, D.X. Tan, L.C. Manchester, W. Qi, Biochemical reactivity of melatonin with reactive oxygen and nitrogen species, Cell Biochem. Biophys. 34 (2001) 237–256.
[37] Y. Ergun, A. Koc, K. Dolapcioglu, Y. Akaydin, G. Dogruer, T. Kontas, T. Kozlu, E. Aslan, The protective effect of erythropoietin and dimethylsulfoxide on ischemia-reperfusion injury in rat ovary, Eur. J. Obstet. Gynecol. Reprod. Biol. 152 (2010) 186–190.
[38] M.E. Sak, H.E. Soydinc, S. Sak, M.S. Evsen, U. Alabalik, F. Akdemir, T. Gul, The protective effect of curcumin on ischemia-reperfusion injury in rat ovary, Int. J. Surg. 11 (2013) 967–970.
[39] M.I. Taskin, A.A. Hismiogullari, A. Yay, E. Adali, A.C. Gungor, G.O. Korkmaz, U. Inceboz, Effect of 2-aminoethoxydiphenyl borate on ischemia-reperfusion injury in a rat ovary model, Eur. J. Obstet. Gynecol. Reprod. Biol. 178 (2014) 74–79.
[40] I. Jasprica, A. Mornar, Ž. Debeljak, A. Smolčić-Bubalo, M. Medić-Šarić, L. Mayer, Z. Romić, K. Bućan, T. Balog, S. Sobocanec, V. Šverko, In vivo study of propolis supplementation effects on antioxidative status and red blood cells, J. Ethnopharmacol. 110 (2007) 548–554.
[41] E.E. El-Sharkawy, A.O. Kames, S.M. Sayed, N.A. Nisr, N.M. Wahba, W.M. Elsherif, A.M. Nafady, M.M. Abdel-Hafeez, A.A. Aamer, The ameliorative effect of propolis against methoxychlor induced ovarian toxicity in rat, Exp. Toxicol. Pathol. 66 (2014) 415–421.
[42] T.D. Kwon, M.W. Lee, K.H. Kim, The effect of exercise training and water extract from propolis intake on the antioxidant enzymes activity of skeletal muscle and liver in rat, J. Exerc. Nutr. Biochem. 18 (2014) 9–17.
[43] Y. Wang, S. Ma, Q. Wang, W. Hu, D. Wang, X. Li, Y. Wang, S. Ma, Q. Wang, W. Hu, D. Wang, X. Li, T. Su, X. Qin, X. Zhang, K. Ma, J. Chen, L. Xiong, F. Cao, J. Chen, Effects of cannabinoid receptor type 2 on endogenous myocardial regeneration by activating cardiac progenitor cells in mouse infarcted heart, Sci. China Life Sci. 57 (2014) 201–208.
[44] Q. Xu, Y. Zhou, R. Zhang, Z. Sun, L.F. Cheng, Antiarthritic activity of Qi-Wu rheumatism granule (a Chinese herbal compound) on complete freund's adjuvant-induced arthritis in rats, Evid. Complement. Alternat. Med. 2017 (2017) 1–13.
[45] P. Mao, L. Smith, W. Xie, M. Wang, Dying endothelial cells stimulate proliferation of malignant glioma cells via a caspase 3-mediated pathway, Oncol. Lett. 5 (2013) 1615–1620.
[46] V. Ratziu, M.Y. Sheikh, A.J. Sanyal, J.K. Lim, H. Conjeevaram, N. Chalasani, M. Abdelmalek, A. Bakken, C. Renou, M. Palmer, R.A. Levine, B.R. Bhandari, M. Cornpropst, W. Liang, B. King, E. Mondou, F.S. Rousseau, J. McHutchison, M. Chojkier, R.A. Levine, A phase 2, randomized, double-blind, placebo-controlled study of GS-9450 in subjects with nonalcoholic steatohepatitis, Hepatology 55 (2012) 419–428.
[47] W. Zhu, Y.H. Li, M.L. Chen, F.L. Hu, Protective effects of Chinese and Brazilian propolis treatment against hepatorenal lesion in diabetic rats, Hum. Exp. Toxicol. 30 (2011) 1246–1255.
[48] N. Oršolić, D. Sirovina, G. Gajski, V. Garaj-Vrhovac, M.J. Jembrek, I. Kosalec, Assessment of DNA damage and lipid peroxidation in diabetic mice: effects of propolis and epigallocatechin gallate (EGCG), Mutat. Res. 757 (2013) 36–44.