ARAŞTIRMA MAKALESİ / *RESEARCH ARTICLE*

Protective Effects of D-Carvone on Ovarian Ischemia-Reperfusion Injury: An Experimental Study

D-Carvone'un Over İskemi-Reperfüzyon Hasarı Üzerindeki Protektif Etkileri: Deneysel Çalışma

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ABSTRACT

Aim: *The aim of this study was to measure the protective efficacy of D-carvone against ovarian ischemia-reperfusion (OIR) injury by evaluating apoptosis, inflammation, and oxidative stress.*

Material and Methods: *24 Wistar albino female rats were randomly divided into three groups with eight animals in each group. In the sham group, a lower abdominal incision was made. In the ovarian ischemia-reperfusion (OIR) group, 3 hours of ischemia followed by 3 hours of reperfusion were applied. In the OIR+D-carvone group, 20 mg/kg D-carvone was administered intraperitoneally for 14 days. On day 15, after 3 hours of ovarian ischemia, 3 hours of reperfusion were performed by administering the last dose of D-carvone just before reperfusion. ELISA and immunohistological analyses were performed on the ovarian tissues.*

Results: *TNF-α, IL-1β, IL-6, malondialdehyde (MDA), myeloperoxidase (MPO), total oxidative level (TOS), oxidative stress index (OSI), and ischemia-modified albumin (IMA) levels, which were higher in the OIR group compared to the sham group, decreased significantly in the OIR+D-Carvone group. IL-10, total antioxidant level (TAS), glutathione (GSH), and catalase (CAT) levels were significantly higher in the D-carvone-treated group compared to the OIR group and lower in the OIR group compared to the sham group. Ischemia-modified albumin levels of the OIR+D-carvone group were significantly lower compared to the OIR group. 8-OHdG and NFκB, which rise with ischemia reperfusion (IR) injury, were reduced considerably in the treatment group. Caspase 3 immunopositivity is significantly lower in the OIR+D-carvone group compared to OIR.*

Conclusion: *In the present study, D-carvone showed anti-inflammatory and antioxidant effects against OIR injury, and protective activity was determined. These results suggested that D-carvone may be a potential agent for OIR injury.*

Key words: *ovary ischemia reperfusion; oxidative stress; inflammation; apoptosis*

ÖZET

Amaç: *Bu çalışmanın amacı, apoptozis, enflamasyon ve oksidatif stresi değerlendirerek D-Carvone'un over iskemi-reperfüzyon (OIR) hasarına karşı koruyucu etkinliğini ölçmektir.*

Gereç ve Yöntem: *24 Wistar albino dişi sıçan, her grupta sekiz hayvan olacak şekilde rastgele üç gruba ayrıldı. Sham grubunda, alt karın kesisi yapıldı. Over iskemi-reperfüzyon grubunda, üç saat iskemi ve ardından üç saat reperfüzyon uygulandı. OIR+D-carvone grubunda, 14 gün boyunca 20 mg/kg D-carvone intraperitoneal olarak uygulandı. 15. günde, üç saat over iskemisinden sonra, reperfüzyondan hemen önce son D-carvone dozu uygulanarak üç saat reperfüzyon gerçekleştirildi. Over dokularında ELISA ve immünohistolojik analizler yapıldı.*

Bulgular: *OIR grubunda sham grubuna göre daha yüksek olan TNF-α, IL-1β, IL-6, MDA (malondialdehyde), MPO (myeloperoxidase), TOS (total oxidative level), OSI (oxidative stress index) ve iskemik modifiye albumin (IMA) düzeyleri, OIR+D-carvone grubunda önemli ölçüde azaldı. IL-10, TAS (total antioxidant level), glutatyon (GSH) ve katalaz (CAT) düzeyleri, D-carvone ile tedavi edilen grupta OIR grubuna göre önemli ölçüde daha yüksek ve OIR grubunda sham grubuna göre daha düşüktü. OIR+D-carvone grubunun IMA düzeyleri, OIR grubuna göre önemli ölçüde düşüktü. İskemi-reperfüzyon (IR) hasarıyla birlikte artan 8-OHdG ve NFκB, tedavi grubunda önemli ölçüde azaldı. Kaspaz 3 immünopozitifliği, OIR+D-carvone grubunda OIR'ye göre önemli ölçüde daha düşüktür.*

Sonuç: *Bu çalışmada, D-carvone, OIR hasarına karşı antienflamatuvar ve antioksidan etkiler gösterdi ve koruyucu aktivite belirlendi. Bu sonuçlar D-carvone'un OIR hasarı için potansiyel bir ajan olabileceğini düşündürmektedir.*

Anahtar kelimeler: *over iskemi reperfüzyon; oksidatif stres; enflamasyon; apoptozis*

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Introduction

Ovarian torsion is a gynecologic emergency that can be seen in women of all age groups. It causes severe pain, and if not diagnosed and treated early, it may cause necrosis, hemorrhage, and ovarian loss, leading to infertility. It is also an important cause of acute abdomen, which may lead to mortality in cases with late diagnosis and complications such as thromboembolism and peritonitis. The exact etiology is uncertain. It is frequently seen in dermoid and serous cystadenomas[1–3].

The partial or complete rotation of ligaments and structures supporting the adnexa results in ischemia, leading to a disruption in oxidative phosphorylation in the tissue, a decrease in ATP production, and an inability to fulfill energy-dependent functions, ultimately causing impairment of cellular functions. The disturbance in intracellular osmolarity leads to cellular swelling, while the activation of cytotoxic enzymes causes damage to membranes and organelles. Furthermore, these cellular changes contribute to an increase in pro-inflammatory cytokines and oxidative stress, further exacerbating cellular damage. The repair of damage to membranes and organelles caused by ischemia, along with the reperfusion required for the clearance of toxic metabolites, can paradoxically lead to more severe injuries by further increasing oxidative stress and inflammation than those originally caused by ischemia. In general, the prognosis of ovarian torsion is contingent upon the duration of ischemia and the degree of ischemia-reperfusion (IR) injury that ensues. Despite numerous studies, early diagnosis and treatment remain the only known methods to prevent serious complications such as ovarian damage and potential infertility. However, the variability of clinical presentations and the potential for confusion with other acute abdominal pain conditions often impede the timely diagnosis of early ovarian torsion. Therefore, there is a significant need for therapeutic interventions to prevent the development of complications associated with ovarian torsion[4,5].

D-carvone is a molecule found in the volatile oil of seeds consumed in the diet, particularly cumin, which possesses antitumor, antiproliferative, and antihypertensive properties[6,7]. Studies have demonstrated that it slows down tumor development in the colon, reduces glutathione levels in rat liver cells, and increases lactate dehydrogenase levels[8–10].

This study aimed to measure the protective efficacy of D-carvone against ovarian ischemia-reperfusion (OIR) by evaluating apoptosis, inflammation, and oxidative stress.

Materials and methods

Ethics Committee Approval and Animals

The Ataturk University Experimental Animal Ethics Committee approved the investigation under decision number 2022/7–135. In the study, 24 Wistar-Albino female rats weighing between 200 and 250 g and aged 12 to 16 weeks participated in the Ataturk University Experimental Animals Research and Application Center. Tap water was provided ad libitum the night before the experiment, and the rats were fasted overnight with no access to food.

Study Design and Reagents

Twenty-four Wistar albino rats were randomly divided into three groups with eight animals in each: Sham, ovarian ischemia-reperfusion (OIR), and treatment (OIR+DCAR) groups. The animals in the sham group had their lower abdomens incised 1–2 cm. The location of the wound was closed without the need for surgery. The ovarian tissues in the ischemia-reperfusion (OIR) group were clamped with a clamp to induce ischemia following a 1–2 cm abdominal incision. The ischemia period was determined as 3 hours. At the end of this period, the clamp was removed, and 3-hour reperfusion was started. During the reperfusion period, the incision site was closed with a 3–0 silk suture. After 3 hours of reperfusion, the ovarian tissues of the rats were taken and sacrificed. 20 mg/kg D-carvone was administered intraperitoneally to the rats in the treatment group (OIR+DCAR) every day for 15 days. On the 16th day, a 1–2 cm incision was made as in the IR group, and 3 hours of ischemia were applied. The last dose of D-carvone (20 mg/kg) was administered intraperitoneally 15 minutes before reperfusion, and then 3 hours of reperfusion were provided. Rats under anesthesia were killed following surgery by having their hearts blood extracted, and all of the rats' ovarian tissues were gathered for histological, immunohistochemical, and biochemical examinations (Fig. 1).

Tissue Homogenization and Biochemical Analysis

To conduct biochemical assays, tissues were mixed with phosphate buffer to make a 10% homogenate, which was then homogenized by centrifugation at 12000 rpm for one to two minutes on ice (IKA, Germany). To separate the supernatant, homogenized tissue samples were centrifuged for 30 minutes at +4°C at 5000 rpm. Rat-specific ELISA kits were used to measure the

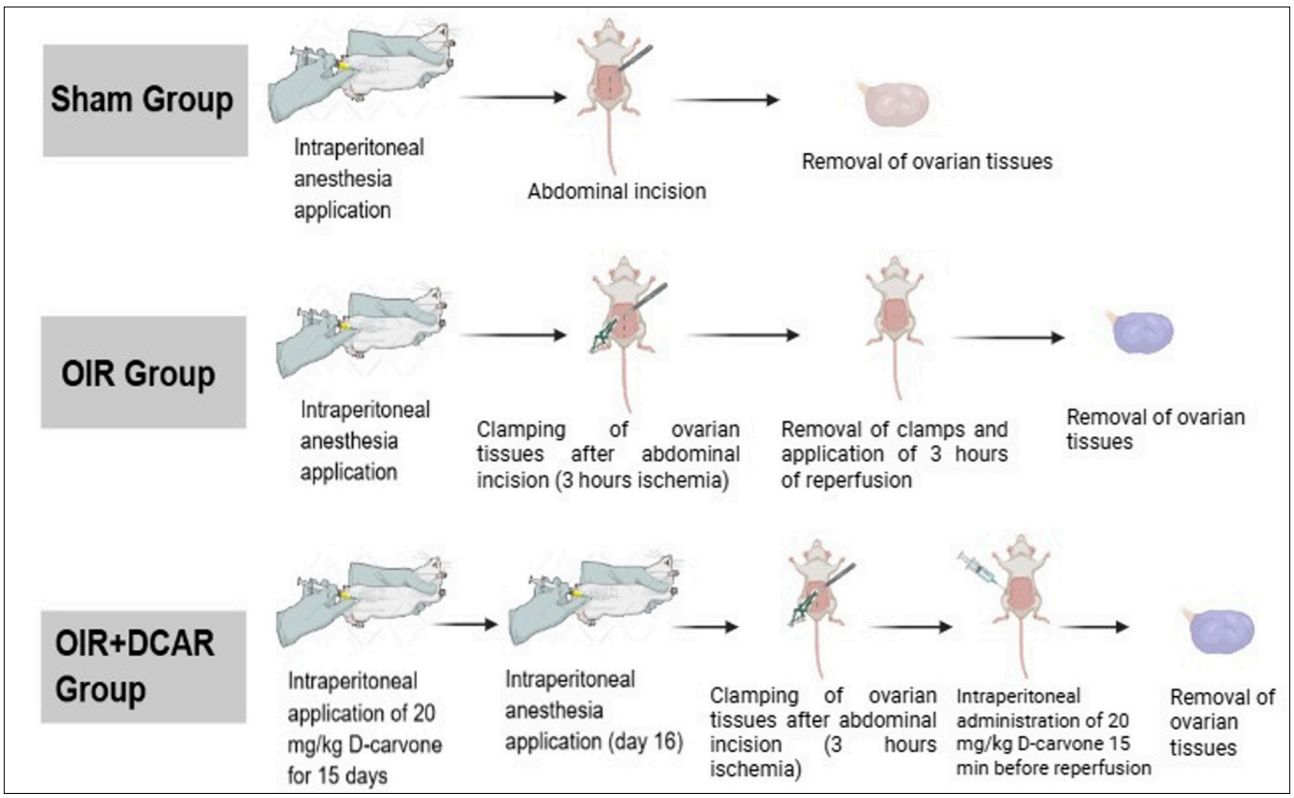

Figure 1. *Experimental protocol scheme.*

levels of IL-1β (Cat No: E-EL-R0012, Elabscience), IL-6 (Cat No: E-EL-R0015, Elabscience), IL-10 (Cat No: E-EL-R0016, Elabscience), TNF-α (Cat No: E-EL-R0019, Elabscience), and IMA (Cat No: EA0053Ra, BT LAB), from supernatants in the biochemical evaluation of the groups. The measurements were performed according to the guidelines provided by the kits. Ovarian tissue levels of IL-1β, IL-6, TNFα, and IL-10 were reported as pg/mg protein, while IMA concentrations were reported as U/mg protein. Additionally, the resulting supernatants were utilized for subsequent determinations. The chemicals and enzymes required for measuring CAT, MDA, GSH, and MPO were sourced from Sigma (St. Louis, USA). All reagents employed were of analytical grade.

Oxidative Stress and Antioxidant Parameters

Catalase activity was assessed following the procedure outlined by Aebi (1974). Protein kg^{-1} is used to express the results. Every sample was examined twice. The Ohkawa et al. method was used to quantify MDA levels, which were identified as an indicator of lipid peroxidation. The results are shown as μmol/g protein. The Ellman method was used to measure glutathione (GSH) levels, and the results are shown as μmol/mg protein. Using the methodology outlined by Hillegas et al., myeloperoxidase (MPO) activity was determined as U/mg protein. Using the proper kits, the values of the total antioxidant level (TAS) and total oxidative level (TOS) were determined and expressed in mmol/L and μmol/L, respectively. The TOS/TAS ratio was used to calculate the oxidative stress index (OSI).

The measurement of tissue protein was performed spectrophotometrically following the procedure outlined by Lowry et al. (1951). All samples were assayed in duplicate.

Histopathological Examinations

For 72 hours, ovarian tissues removed for histopathological analysis were fixed in 10% formalin buffer. Following fixation, ovarian tissues were rinsed with running tap water to get away from formaldehyde before being left in various ethanol solutions for 45 minutes each to dehydrate them. After two xylene changes, the tissues were embedded in paraffin and prepared for sectioning. Tissue sections were taken at 5-μ-thick and stained with hematoxylin and eosin (H&E) for histopathological analysis. The sections were examined and photographed using a TAPTEK digital camera and an Olympus BX51 microscope. Degeneration, hemorrhage, and edema were analyzed in stained sections.

Immunohistochemical Staining

After having been cut to a thickness of 5 μ, tissue sections were put on adhesive slides. Following deparaffinization and rehydration, endogenous peroxidase activity was inhibited for 10 minutes by using 3% H_2O_2. Sections were boiled in Tris-EDTA buffer solution for 10 minutes to retrieve antigens from the tissues. Anti-8-OHdG (1: 100, sc-66036, Santa Cruz, California, USA), anti-caspase 3 (1: 100, sc-65497, Santa Cruz, California, USA), and anti-nuclear factor-kappa ß (NF-κß) (1: 100, sc-8414, Santa Cruz, California, USA) were dropped onto tissue sections and incubated overnight at 4°C. After waiting for biotinylated secondary antibody for half an hour, sections were rinsed in PBS for five minutes. In the next step, streptavidin-conjugated peroxidase was used for half an hour. Following a 5-minute administration of 3.3-diaminobenzidine, the sections were counterstained with hematoxylin to assess immunopositivity once the brown color had developed. Using a TAPTEK digital camera and an Olympus BX51 microscope, the sections were examined and photographed. 8-OHdG, NFkB, and caspase 3 immunopositivity were scored as follows: none (−), mild (+), moderate (++), and intense (+++).

Statistical Analysis

For the analysis, the data's mean ± standard error was computed. The Mann-Whitney U test was used to determine which group the difference originated from, and the Kruskal-Wallis test was used to examine the differences between the groups.

Results

Biochemical Results

Impacts on inflammation

Levels of TNF-α, IL-1β, and IL-6 were considerably higher in the OIR group than in the sham group and significantly lower in the D-carvone-treated group than in the I/R group. IL-10 levels, however, were much lower in the OIR group than in the sham group and substantially higher in the D-carvone-treated group than in the OIR group (Table 1, Fig. 2).

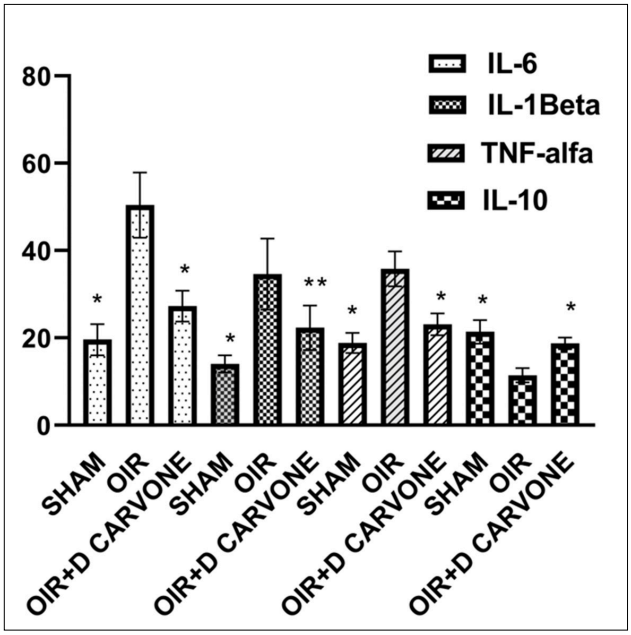

Figure 2. *Quantitative comparison of inflammatory (IL-6, IL-1β, TNF-α) and anti-inflammatory (IL-10) cytokine levels across study groups. Cytokine concentrations (pg/mg protein) were significantly elevated in the OIR group compared to the sham group (p <0.001), indicating an inflammatory response. Treatment with D-carvone partially attenuated these changes, with statistically significant reductions observed for IL-6, IL-1β, and TNF-α, and an increase in IL-10 (p=0.005, p <0.001). Data are presented as mean ± SD.*

Impact on Oxidant-Antioxidant System

Oxidative stress markers, MDA, MPO, TOS, OSI, and IMA levels were higher in the 0IR group than in the sham group. In the D-carvone-treated group, the levels of these markers were significantly lower than in the OIR group, and they were close to the levels of the sham group.

Table 1. *Comparison of inflammatory and anti-inflammatory cytokine levels of the groups*

	IL-6 (pg/mg protein)	IL-1b (pg/mg protein)	TNF-a (pg/mg protein)	IL-10 (pg/mg protein)
SHAM (Group I)	19.61±3.56	14.06±1.98	18.84±2.29	21.40±2.68
OIR (Group II)	50.43±7.49	34.62±8.14	35.86±4.00	11.44±1.64
OIR+D CARVONE (Group III)	27.31±3.56	22.36±5.11	23.13±2.53	18.78±1.36
I vs II	P <0.001	P <0.001	P <0.001	P <0.001
I vs III	P=0.054	P=0.056	P=0.066	P=0.086
II vs III	P <0.001	P=0.005	P <0.001	P <0.001

Values are given as mean ± standard deviation. OIR: ovarian ischemia-reperfusion.

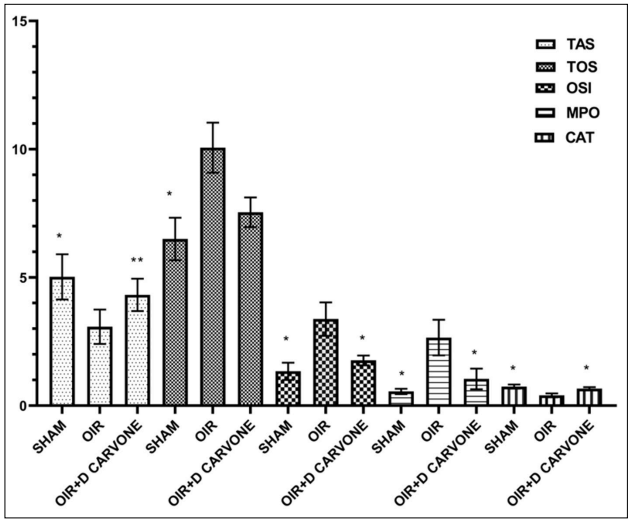

Figure 3. *Oxidative stress and antioxidant capacity markers among the study groups. Levels of TAS (mmol/L), TOS (μmol/L), and OSI were evaluated, revealing a significant increase in oxidative burden in the OIR group (p <0.001) and a significant amelioration in the D-carvone-treated group (p=0.027, p <0.001). These findings demonstrate the antioxidant potential of D-carvone. Values are shown as mean ± SD.*

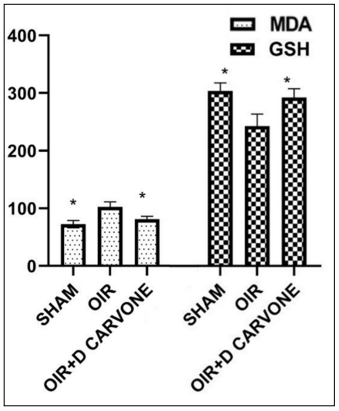

Figure 4. *Comparison of glutathione (GSH) and malondialdehyde (MDA) levels in ovarian tissues. Glutathione (μmol/mg protein) levels significantly decreased while MDA (μmol/g protein), a lipid peroxidation marker, significantly increased in the OIR group compared to controls (p <0.001). D-carvone treatment reversed these changes to a statistically significant extent (p=0.002, p <0.001), indicating reduced oxidative damage*

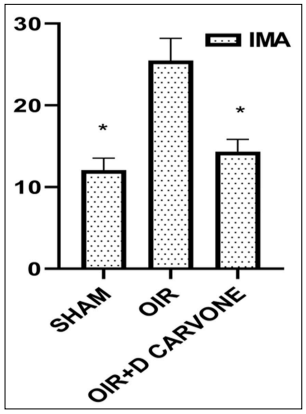

Figure 5. *Levels of ischemia-modified albumin (IMA) in ovarian tissues of experimental groups. IMA (U/mg protein) was markedly elevated in the OIR group (p <0.001), consistent with oxidative stress and tissue damage. D-carvone administration resulted in a significant reduction in IMA levels, supporting its protective role.*

Table 2. *Comparison of oxidant-antioxidant levels*

	TAS (mmol/L)	TOS (μmol/L)	OSI	GSH (μmol/mg protein)	CAT (k/g protein)	MDA (μmol/g protein)	MPO U/mg protein)	IMA (U/mg protein)
SHAM (Group I)	5.02±0.88	6.5±0.83	1.34±0.34	303.73±14.25	0.74±0.08	72.54±6.22	0.56±0.10	12.09±1.44
OIR (Group II)	3.08±0.67	10.06±0.98	3.38±0.65	242.69±21.07	0.40±0.07	102.48±8.58	2.66±0.69	25.49±2.71
OIR+D CARVONE (Group III)	4.32±0.63	7.54±0.57	1.77±0.19	292.52±15.20	0.66±0.06	81.02±5.03	1.04±0.40	14.33±1.50
I vs II	P <0.001	P <0.001	P <0.001	P <0.001	P <0.001	P <0.001	P <0.001	P <0.001
I vs III	P=0.254	P=0.099	P=0.244	P=0.509	P=0.170	P=0.110	P=0.199	P=0.155
II vs III	P=0.027	P <0.001	P <0.001	P <0.001	P <0.001	P <0.001	P <0.001	P <0.001

Values are given as mean ± standard deviation. OIR: ovarian ischemia-reperfusion; TAS: total antioxidant level; TOS: total oxidative level; OSI: oxidative stress index; GSH: glutathione; CAT: catalase; MDA; malondialdehyde; MPO: myeloperoxidase; IMA: ischemia-modified albumin.

The OIR group had lower levels of antioxidant biomarkers, including TAS, GSH, and CAT, compared to the sham group. The levels of these markers were nearly identical to those of the sham group and much higher in the D-carvone-treated group than in the OIR group (Table 2, Fig. 3–5).

Effect on IMA

The D-carvone+OIR group IMA levels were substantially lower than those of the OIR group, whereas the OIR group's IMA levels were significantly higher than those of the sham group (Table 2)

Microscopic Examination

Microscopic examination revealed numerous follicles of different stages and sizes in the ovary in the sham group. The corpus luteum structure consisted of lutein cells with normal histology. In the OIR group, the normal histological structure was seen to be disrupted. Numerous disrupted degenerated follicles were seen along with severe hemorrhage, vascular congestion, and edema. Corpus luteum cells were degenerated and lost their acidophilic properties. Vascular congestion and hemorrhage were reduced in the group treated with D-carvone and OIR. The follicular structures had a normal histological appearance. There were mild degenerative changes in the lutein cells (Fig. 6).

Immunohistochemistry Results

The ovarian tissue in the sham group showed a negative immune reaction to 8-OHdG immunostaining. In contrast, the corpus luteum's lutein cells in the OIR group showed strong cytoplasmic 8-OHdG expression

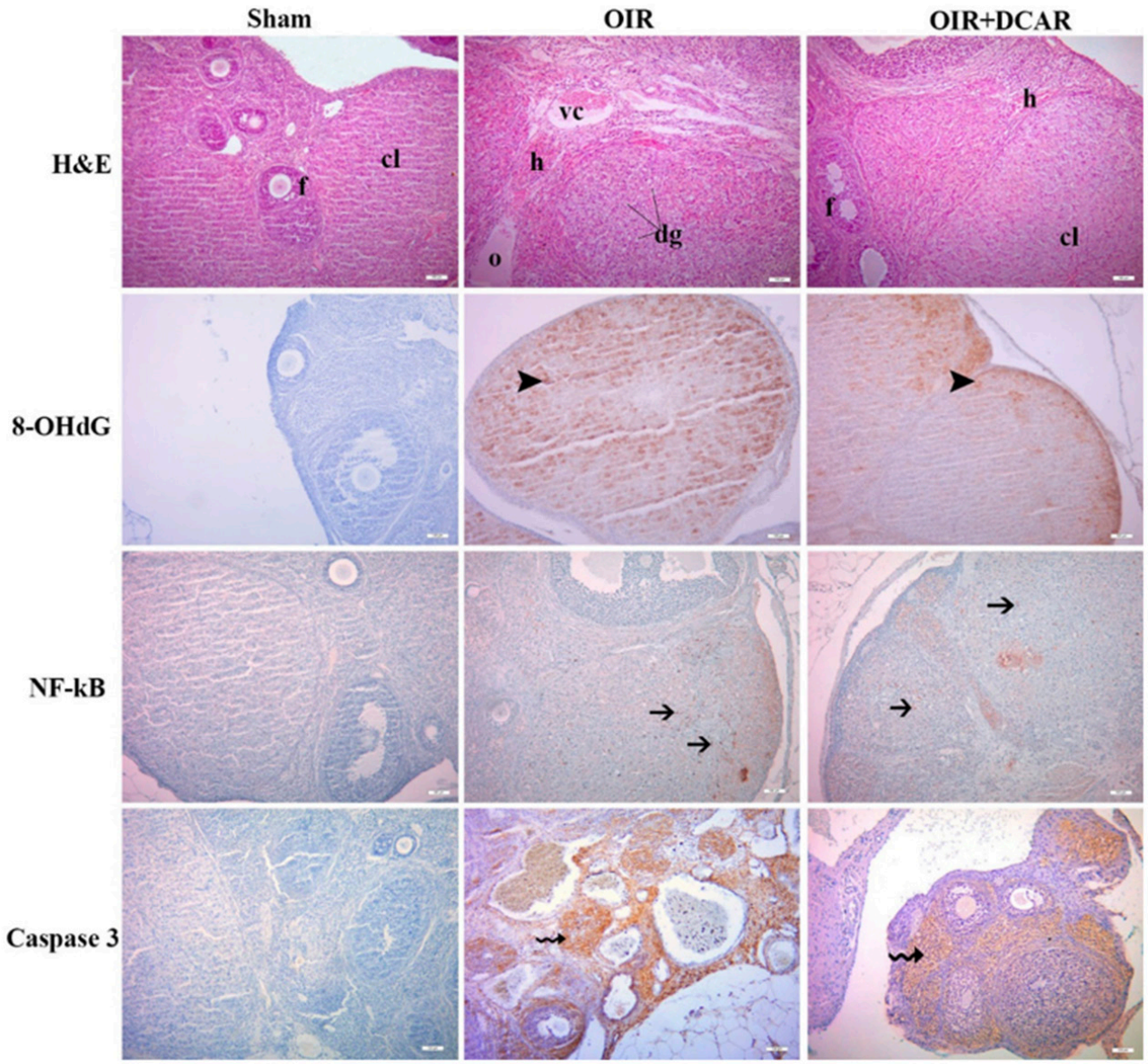

Figure 6. *Photomicrographs of H&E sections of ovaries of rats in the sham group show normal ovarian follicles (f) and corpus luteum (cl) structure. Ovaries of rats treated with OIR show hemorrhage (h), vascular congestion (vc), edema (o), and degeneration of lutein cells in the corpus luteum (dg). Ovaries of rats treated with OIR+DCar show mild hemorrhage (h) and normal follicular (f) and corpus luteum (cl) histology. Ovaries of rats in the sham group show negative expression of 8-OHdG (arrowhead), NFkB (arrow), and caspase 3 (zigzag arrow). Ovarian tissues of rats in the OIR group show intense expression of 8-OHdG (arrowhead) and caspase 3 (zigzag arrow) and moderate expression of NFkB (arrow). Ovarian tissues of rats in the OIR+Dcar group show moderate expression of 8-OHdG (arrowhead) and caspase 3 (zigzag arrow) and mild expression of NFkB (arrow).*

($p<0.001$). 8-OHdG expression in the corpus luteum's lutein cells decreased and manifested cytoplasmic expression in the group that received D-carvone treatment (Fig. 6–7; $p<0.05$).

Regarding anti-NFkB immunostaining, ovarian tissue in the sham group showed negative immunostaining. However, in the OIR group, strong immunoexpression of inflammatory cells in the corpus luteum was frequently observed ($p<0.05$). In the D-carvone-treated group, less cytoplasmic expression of inflammatory cells in the corpus luteum was noted (Fig. 6–7, $p<0.05$).

When caspase 3 immunopositivity was evaluated, ovarian tissue in the sham group showed negative immunostaining. However, in the OIR group, a strong immunoexpression was seen, especially in the stroma around the follicles ($P <0.001$). In the D-carvone-treated group, this positivity in the stroma was reduced, and negative immunoexpression was observed in corpus luteum cells (Fig. 6–7, $p<0.05$).

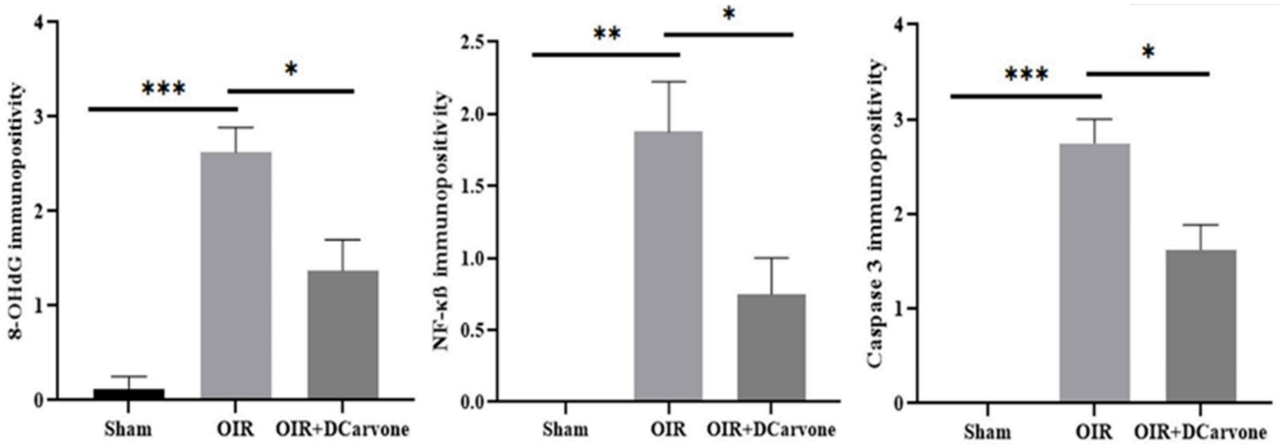

Figure 7. *Semi-quantitative immunohistochemistry analysis of 8-OHdG, NF-kB, and caspase 3 expressions in the ovary. Values are means ± SEM. P*** <0.001, P** <0.05 vs. control group, and P* <0.05 vs. OIR administered group.*

Discussion

Ovarian torsion is the most common gynaecological emergency in women of all age groups, most commonly in women aged 29–34 years. Ovarian torsion, which is characterised by complete or partial rotation of the ovary around its ligaments and impaired tissue perfusion, accounts for 2.7% of all surgical emergencies. Compression of vascular structures by rotation around the infundibulopelvic and utero-ovarian ligaments may cause further deterioration of arterial perfusion and deepening of ischaemia and necrosis by suppressing venous and lymphatic return[4,11]. One of the typical symptoms, abdominal or pelvic pain, can be confused with various pathologies, leading to delays in diagnosis and consequently resulting in several significant complications, particularly infertility[12]. The sole treatment for ovarian torsion is detorsion, which restores blood flow and alleviates ischemic damage, thus preventing necrosis. However, the reperfusion that follows introduces oxidants and pro-inflammatory molecules to the ovarian tissue, leading to secondary damage characterized by necrosis, apoptosis, autophagy, and necroptosis. Despite numerous studies conducted, no effective agents have been identified to protect against ischemia-reperfusion injury caused by ovarian torsion[13,14]. The present study is an original study in which the protective effects of D-carvone against OIR injury were evaluated for the first time. The treatment with D-carvone significantly reduced the levels of oxidative stress markers, including MDA, MPO, TOS, OSI, and IMA, while concurrently increasing the levels of antioxidants such as TAS, GSH, and CAT, thereby mitigating oxidative stress. The most prevalent protein in plasma, albumin, is distinguished by acidosis, a rise in oxidative stress. Albumin's N-terminus is harmed by ischemia circumstances, which prevents it from binding metals and enables an albumin cobalt-binding test to quantify it. Ischemia-modified albumin (IMA) has been associated with the detection of acute ischemia before necrosis because its blood levels increase minutes after the ischemia begins and return to normal within 6–12 hours[15,16]. In this study, IMA levels in the D-carvone+OIR group were significantly higher than those in the IR group. The decrease in IMA levels in the treatment group suggests that D-carvone decreases ischaemia-induced necrosis and oxidative stress. Furthermore, it has been demonstrated that the substance exhibits a protective effect against OIR by reducing the levels of pro-inflammatory cytokines, such as TNF-α, IL-1β, and IL-6, while simultaneously increasing the levels of the anti-inflammatory cytokine IL-10. This indicates its potential anti-inflammatory efficacy.

In addition, immunohistochemical analyses have revealed that in cases of OIR, the vascular congestion, edema, and degeneration within the corpus luteum were restricted to mild hemorrhage following D-carvone treatment. Notably, the expression levels of 8-hydroxydeoxyguanosine (OHdG), a marker indicative of oxidative stress-induced DNA damage, along with nuclear factor kappa B (NFkB) expression, which signifies inflammation, and caspase-3 expression, which is associated with apoptosis, were found to be significantly reduced following D-carvone administration.

The results of the current research demonstrated that D-carvone's protective effects, which showed

anti-inflammatory, antiapoptotic, and antioxidant properties against OIR injury.

D-carvone is a monoterpene that exists in the essential oils of several natural plants[17]. Recent studies showed that D-carvone has antiproliferative, antitumour[9,10,18]. Additionally, D-carvone has antioxidant, anti-inflammatory, antiapoptotic, and antifibrotic effects[19,20].

In a study investigating hepatic IR injury in rats, it was found that D-carvone exhibited protective effects on the liver by significantly reducing the levels of NFκB. It was determined that D-carvone decreased the levels of caspases 1, caspase 3, and caspase 9, while concurrently increasing the B-cell lymphoma 2 (Bcl-2) levels. These results suggest that D-carvone possesses both anti-inflammatory and anti-apoptotic properties, thereby contributing to its hepatoprotective efficacy. Furthermore, it has been demonstrated that D-carvone enhances the activities of antioxidant enzymes such as SOD, catalase, glutathione peroxidase (GSH-Px), and glutathione reductase (GSH-R). It also reduces the levels of MDA, a marker of lipid peroxidation, while decreasing levels of MPO, IL-1b, IL-6, and TNFa, while increasing the level of IL-10. These results indicate a similar anti-inflammatory and antioxidant efficacy as observed in our study[21].

In a study conducted on rats with cerebral IR injury, it was observed that the levels of SOD, Mn SOD and Cu/Zn SOD, were significantly reduced following treatment with D-carvone. Furthermore, D-carvone markedly enhanced the activities of CAT, GSH-Px, and GSH. Notably, treatment with D-carvone led to a significant decrease in MDA levels in brain tissues when compared to the control group. These results indicate that D-carvone has the potential to improve cerebral activity by enhancing antioxidant defense. Lower levels of interleukins IL-6, IL-4, and IL-10, as well as pro-inflammatory cytokines like IL-1β and TNF-α, were also linked to the therapy. Moreover, the expressions of NLRP3, caspase-1, TNF-α, apoptotic speck protein containing a CARD (ASC), and IL-1β were down-regulated in the cerebral I/R rats following D-carvone treatment. Previous study demonstrates protective effects on brain tissue against IR injury, showing antioxidant, anti-inflammatory, and anti-apoptotic activities similar to previous studies. Notably, in our study, treatment with d-carvone increased IL-10 levels, whereas in the cerebral IR experiments, the levels of IL-10 decreased following d-carvone administration. This discrepancy may arise from the findings in the study by Dai et al., where the anti-inflammatory effects were achieved without a corresponding increase in IL-10 levels, suggesting that the suppression of pro-inflammatory cytokines by D-carvone may prevent inflammation effectively[22].

Conclusion

The present study provides novel evidence that D-carvone exerts significant protective effects against ovarian ischemia-reperfusion injury through multiple mechanisms. D-carvone administration effectively attenuated oxidative stress by reducing lipid peroxidation products MDA, MPO, TOS, OSI, and IMA levels, while enhancing antioxidant defense systems, as reflected by elevated TAS, GSH, and CAT levels. Moreover, D-carvone demonstrated a potent anti-inflammatory profile, evidenced by the suppression of proinflammatory cytokines TNF-α, IL-1β, IL-6, and upregulation of the anti-inflammatory cytokine IL-10. Histopathological and immunohistochemical evaluations corroborated these findings, showing reduced structural tissue injury and decreased expression of 8-OHdG, NFκB, and caspase-3, indicating diminished oxidative DNA damage, inflammatory activation, and apoptotic processes. Collectively, these results suggest that D-carvone confers multi-faceted protection against OIR injury through its antioxidant, anti-inflammatory, and anti-apoptotic properties. Further mechanistic studies and translational research are warranted to validate these findings and explore the potential clinical applicability of D-carvone as a therapeutic adjunct in the management of ovarian torsion and other ischemia-reperfusion-related pathologies.

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