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Research Article

Investigation of the Protective Effect of Terebinth (*Pistacia terebinthus* L.) against Ovarian Ischemia-Reperfusion Damage in Rat

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Abstract

The aim of this study is to investigate the therapeutic and protective effects of *Pistacia terebinthus* L. (terebinth) oil treatment on experimental ovarian ischemia and reperfusion injury in rats. In the study, 32 Sprague-Dawley female rats; They were divided into 4 groups: control, ischemia, ischemia/reperfusion (I/R), ischemia/reperfusion + terebinth oil (I/R + terebinth oil), 8 in each group. Terebinth oil was applied via oral gavage at a daily dose of 2 ml/kg for 4 weeks. In histopathological examination of ovarian sections, the control group showed a regular histological appearance. In the ischemia and I/R groups, degenerations in the epithelium, inflammatory cell infiltration in the lamina propria region, and bleeding due to thrombosis in the enlarged vessels were observed. It was determined that terebinth oil protected the epithelial tissue after ovarian I/R injury, reduced inflammation in the connective tissue, and brought the artery-vein contours to a regular appearance. In the ovarian sections where immunohistochemistry analysis was performed, it was observed that radixin protein expression showed a positive reaction in the granulosa cells and a negative reaction in the stromal areas in the terebinth oil applied group compared to the I/R group. As a result, it was revealed that terebinth oil has a healing and protective effect in the treatment of ovarian ischemia/reperfusion injury.

Keywords: Terebinth (*Pistacia terebinthus* L), Ovary, Ischemia, Reperfusion

INTRODUCTION

Adnexal torsion, the fifth most common gynecological emergency, is an important health problem in women worldwide due to delayed diagnosis or misdiagnosis [1,2,3]. Although it is seen in women of all ages; It is especially common in women of reproductive age [4,5]. This case, which requires urgent surgical intervention to preserve ovarian functions, is caused by partial or complete rotation of the ovary and fallopian tube in the axis formed between the infundibulopelvic ligament and the utero-ovarian ligament [6,5]. Adnexal torsion is more common on the right side because the sigmoid colon on the left supports the left ovary and the right infundibulopelvic ligament is longer [6,7]. The main risk factors that cause adnexal torsion include adnexal mass and ovarian cysts (those that are benign and >5 cm in diameter) [4,7]. It is thought that this situation is due to the increase in adnexal tissue volume and weight. Malignant tumors are less likely to cause torsion due to their adhesion properties to neighboring tissues [8]. Torsion causes obstruction of venous blood flow and lymphatic flow, causing edema and obstruction in the ovaries. The resulting edema further reduces the decreased arterial blood flow, leading to ischemic changes that cause irreversible damage to the ovaries. Ovaries that become necrotic as a result of ischemia lose their functions, and failure to treat them negatively affects fertility [1,8-10]. Detorsion performed to restore arterial blood flow causes ischemia-reperfusion injury and leads to other complications in the ovaries. Reperfusion causes disruption of cellular integrity in tissues, increase in reactive oxygen species, and apoptosis [7]. Acute abdominal pain, nausea, vomiting and fever resulting from disruption of vascular flow are among the typical clinical findings of adenxial torsion [2]. Medicinal plants are used quite frequently to prevent complications arising from ischemic damage [11-13]. The effect of terebinth (*Pistacia terebinthus* L.) oil on adnexal torsion is unknown. Therefore, this study was designed to investigate the therapeutic and protective effect of terebinth oil application on experimental ovarian ischemia reperfusion injury in rats. *Pistacia terebinthus* L. (Anacardiaceae), which grows in different regions in Turkey and the world, is a plant rich in flavonoids, carotenoids, linoleic acid, oleic acid and phenolic compounds. Its antimicrobial, antioxidant and anti-inflammatory effects have made it a widely used plant preferred in many diseases. [14-16]. It has been reported that terebinth oil is effective in diseases such as wound healing [17], diabetes mellitus [18], and cancer [19].

MATERIAL AND METHODS

Animals were obtained from Dicle University DÜSAM. Ethics committee approval certificate with code 240638 was received by Dicle University Animal Experiments Local Ethics Committee Presidency (DÜHADEK) on 28/02/2022. Terebinth oil, developed in cooperation with ZADE VITAL and EGE University ARGEFAR, was used in the study. 32 adult Sprague-Dawley female rats, each weighing approximately 250-300 g (±10 g), were randomly divided into four groups, with an equal number of 8 animals in each group. All rats were kept in rooms with controlled humidity (40-60%), temperature (21-24 °C) and light (12-hour light/dark cycle). Water and feed were given ad libitum. Animals were anesthetized with Xylasine (5 mg/kg) (Rompun 2%, Bayer, Germany) and Ketamine hydrochloride (60 mg/kg) (Ketalar, Pfizer, New York, USA) before the operation was performed.

Control group: No treatment was applied to the animals. At the end of the experiment, the animals were sacrificed under general anesthesia and their ovarian tissues were removed.

Ischemia group: Under general anesthesia, the abdomen of the animals was opened with a 2 cm lower midline incision. Ovarian adnexias were exposed. It was rotated 360° clockwise and the ovaries were fixed to the abdominal wall. The ischemia procedure was applied for 2 hours. Then, before the ovaries were restored, the animals were sacrificed under anesthesia and their ovarian tissues were removed.

Ischemia/reperfusion (I/R): Under general anesthesia, the abdomen of the animals was opened with a 2 cm lower midline incision. The ovarian adnexa were exposed, rotated 360° clockwise, and the ovaries were fixed to the abdominal wall. After torsion was applied for 2 hours, the ovaries were brought to their normal positions and placed in their anatomical location. The ovary was reperfused by detorsion for 2 hours. At the end of the experiment, the animals were sacrificed under anesthesia and their ovarian tissues were removed.

Ischemia/reperfusion + Terebinth oil (I/R+Terebinth oil): Under general anesthesia, the abdomen of the animals was opened with a 2 cm lower midline incision. Ovarian adnexias were exposed. It was rotated 360° clockwise and the ovaries were fixed to the abdominal wall. After torsion was applied for 2 hours, the ovaries were brought to their normal positions and placed in their anatomical location. The ovary was reperfused by detorsion for 2 hours. Then, 2 ml/kg terebinth oil was given orally to the rats every day for 4 weeks. At the end of the 4th week, the animals were sacrificed under anesthesia and their ovarian tissues were removed.

Ovarian tissues were fixed in 10% formaldehyde for 48 hours. Then, the tissue was subjected to routine paraffin wax tissue processing. 5 micron sections were taken from paraffin blocks with a microtome. To examine the histopathology of the sections, the expression of radixin protein on ovarian tissues was examined by Hematoxylin Eosin Staining and Immunohistochemical analysis.

RESULTS

Histopathology

In the ovarian section of the control group, the nuclei of germinal epithelial cells were observed to be rich in chromatin and dense. A large enlarged graafian follicle transformed into a corpus luteum was observed. Dilated blood vessels and, in some places, small groups of small inflammatory cells and collagen arrangement were observed parallel to each other and normally around the blood vessels (Figure 1). In the ovarian section of the ischemia group, apoptotic changes and occasional degenerations were observed in the germinal epithelial region. Pictatic nuclei shrinking towards the lumen were observed. Small and degenerative nuclei were detected in the cells in the corpus luteum region, and inflammatory cell infiltrates were detected in the lamina propria region. Additionally, dilated blood vessels, thrombosed blood vessels, and widespread inflammatory cell infiltrates around the vessels were observed (Figure 2). In the ovarian section of the I/R group, hyperplasia was observed in the germinal epithelium, while an increase in the underlying basement membrane thickness was observed. Bleeding due to thrombosis was observed in the vessels expanding towards the stromal region, and although no oocyte II cells were seen in the developed graafian follicles, they were observed to have dense fluid content (Figure 3). In the ovarian section of the I/R and terebinth groups, mild hyperplasia was observed in the germinal epithelium, while the epithelial structure was partially preserved. However, one of the most important effects is that the graafian follicle in the lower parts has a smooth structure, inflammation in the connective tissue in between decreases, and artery-vein contours are regular. In addition, it was determined that the follicular fluid was dense and widespread in a developed graafian follicle and the follicular structures were regular (Figure 4).

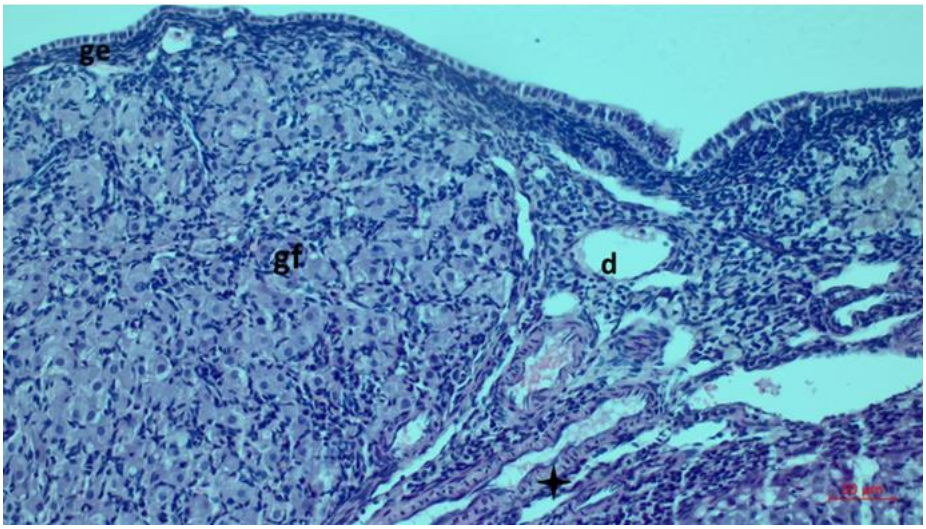

Figure 1: Control group ovary section.
ge: germinal epithelium, gf: Graaf follicle, d: dilated blood vessel and star: collagen arrangement. Hematoxylin Eosin, Bar: 50 µm

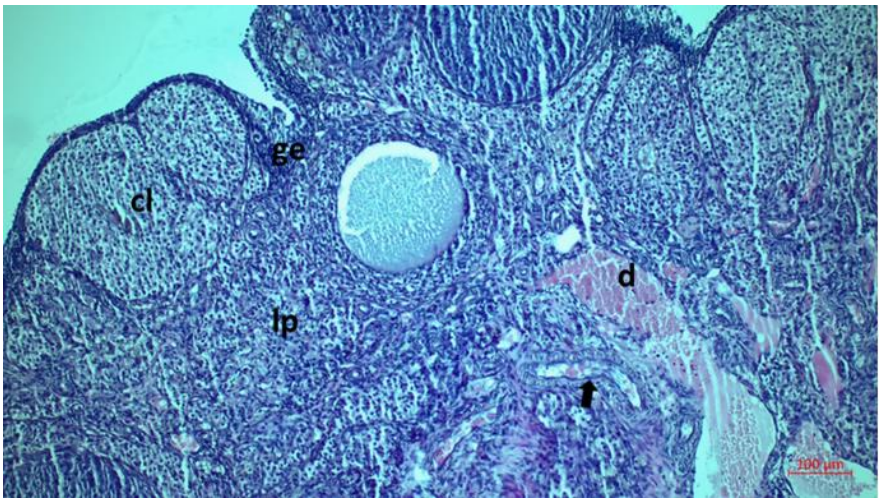

Figure 2: Ovary section in the ischemia group.
ge: germinal epithelium, cl: corpus luteum, lp: lamina propria, d: dilated blood vessel, black arrow: inflammatory cells. Hematoxylin Eosin, Bar: 100 µm

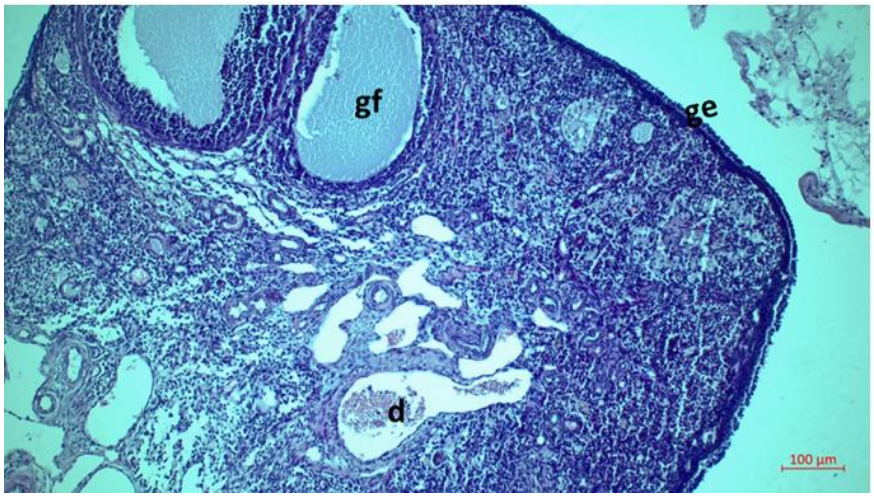

Figure 3: I/R group ovarian section.
ge: germinal epithelium, d: dilated blood vessel, gf: graaf follicle. Hematoxylin Eosin, Bar: 100 µm

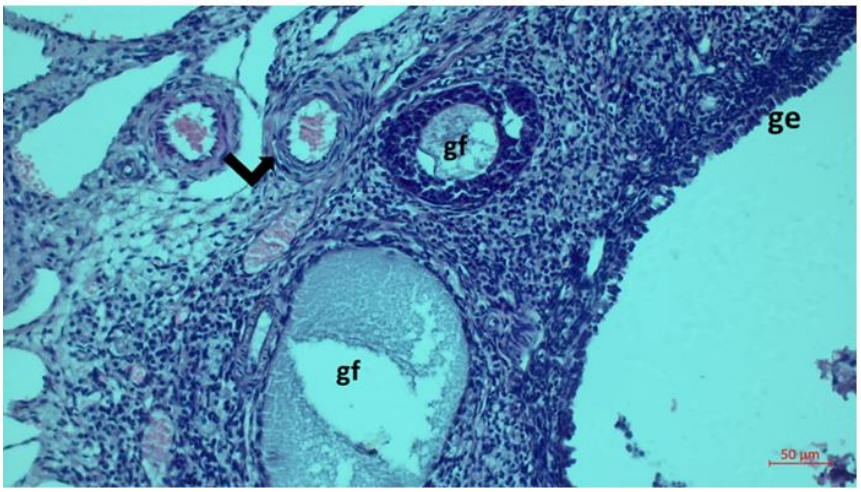

Figure 4: Ovary section of the I/R + Terebinth group.
ge: germinal epithelium, gf: Graaf follicle, double black arrow: artery-vein contours. Hematoxylin Eosin, Bar: 50 µm

İmmunohistochemistry

In the ovarian section of the control group, negative radixin expression was observed in the basement membrane, outer parts of the vessels, areas where granulosa cells are located, and connective tissue cells. Mild radixin expression was found in the graaf follicle cells of the corpus luteum. However, considering that radixin is a molecule that exhibits a particularly stimulating pro-inflammatory reaction, it was also determined that it continued to exert a moderate stimulatory effect in some cells (Figure 5). In the ovarian section of the ischemia group, it was observed that radixin expression showed a positive reaction in the nuclear structures of the epithelial cells, partially in the inflammatory cells in the parenchymal region, and in some granulosa cells close to the lumen. Additionally, dispersion was detected in the theca follicle externa region. It was determined that radixin

expression showed a positive reaction in some cells in this region (Figure 6). In the ovarian section of the I/R group, the density of radixin was observed to be positive in the oocyte II nucleus of a developed graafian follicle, in the membrane pellucida region, in some of the granulosa cells, in the parenchyma region and around the dilated vessel (Figure 7). Moderate radixin expression was observed in the epithelial layer in the ovarian section of the I/R + Terebinth group. Partially positive radixin expression was observed in the nuclei of granulosa cells. Negative radixin reaction was detected in the stromal region (Figure 8).

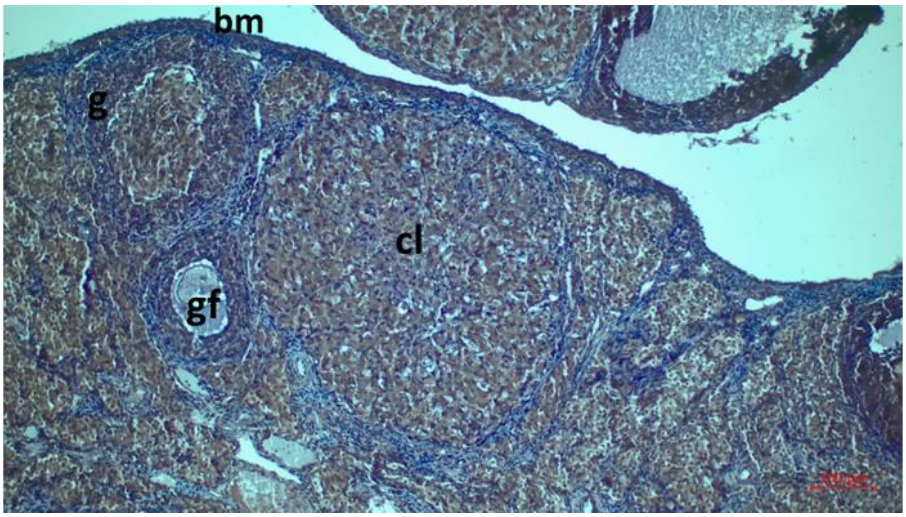

Figure 5: Radixin control group ovarian section.
bm: basement membrane, gf: graaf follicle, cl: corpus luteum, g: granulosa cells. Radixin immunostaining, Bar: 100 µm

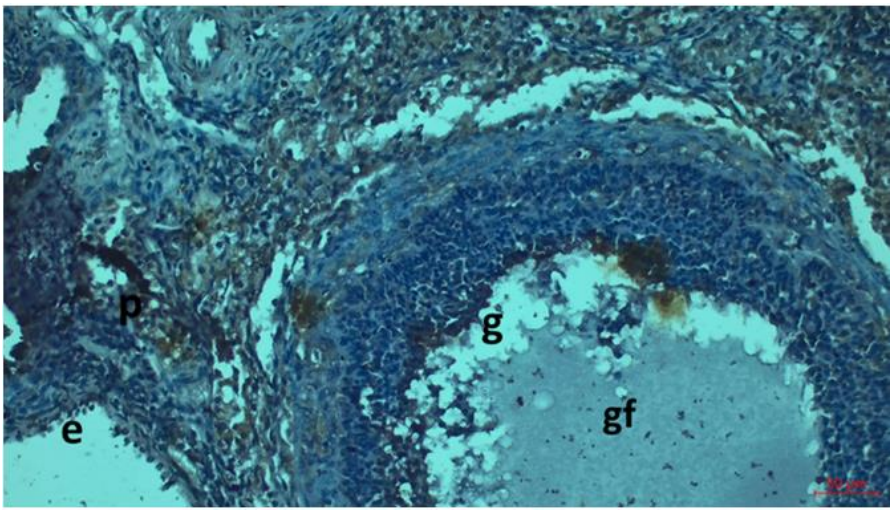

Figure 6: Ovary section of the ischemia group of the radixin.
e: epithelial cell, p: parenchyma, g: granulosa cells, gf: graafian follicle. Radixin immunostaining, Bar: 50 µm

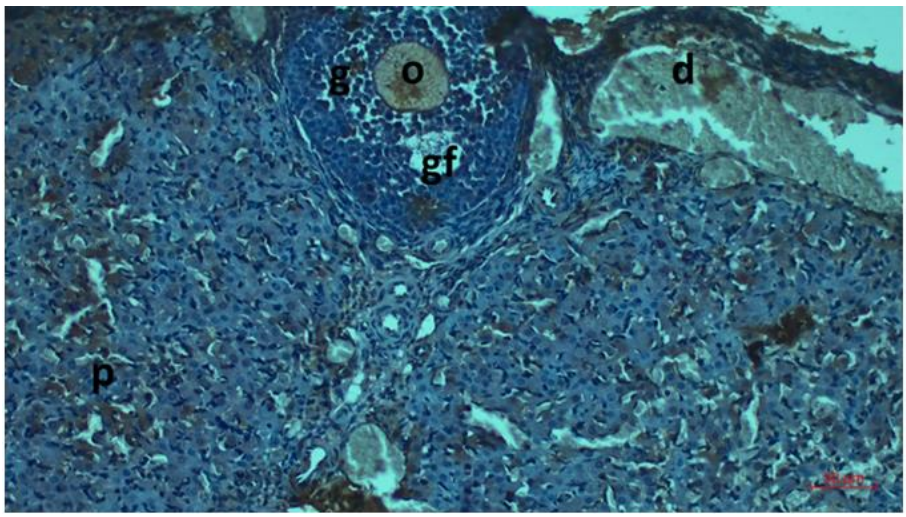

Figure 7: Ovary section of the radixin I/R group.
g: granulosa cells, gf: graaf follicle. p: parenchyma, o: oocyte II, d: dilated blood vessel. Radixin immunostaining, Bar: 50 µm

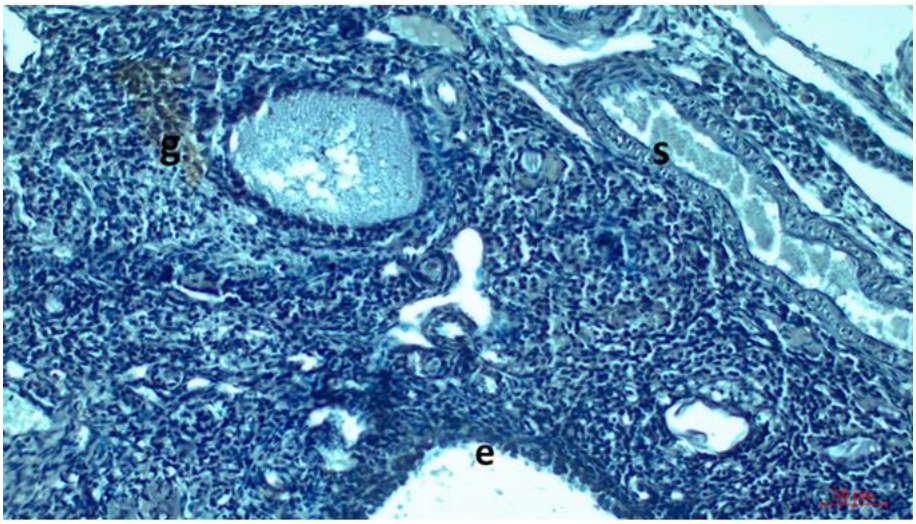

Figure 8: Ovary section of the radixin I/R+ Terebinth group.
e: epithelial cell, g: granulosa cells, s: stromal area. Radixin immunostaining, Bar: 50 µm

DISCUSSION

Adnexal torsion is characterized by the twisting or rotation of the ovary around its supporting ligaments. Thus, it causes occlusion of the ovarian artery and vein and results in ovarian ischemia [20]. Early diagnosis and intervention is very important. Adnexal torsion has serious consequences for women who plan to have more children, as it causes serious ovarian reserve complications. Therefore, it can be treated with adnexectomy or detorsion of the ovary [21]. Ischemia is a serious reversible or irreversible condition that occurs as a result of the interruption of blood flow (arterial and venal blood) feeding the organ or tissue and develops depending on its duration [22]. Reperfusion is; It refers to the resumption of blood flow to the ischemic tissue or organ as a result of medications or surgical interventions [23]. It is very important to ensure reperfusion after ischemia without delay. However, reoxygenation of the tissue after reperfusion may paradoxically cause reperfusion injury, and this condition is more pathological than ischemic injury [24]. Terebinth plant has different biological activities and has been reported to have high antioxidant, antimicrobial, anti-inflammatory and cytotoxic properties due to the richness of secondary compounds in its fruits and resins [14]. It is also used in traditional Turkish folk medicine to treat peptic ulcer, asthma, gastralgia, rheumatism, sunstroke, cough (externally), skin inflammation, intestinal looseness and throat inflammation [25]. Uyar et al. showed that meningicin had a protective effect on liver, kidney and pancreatic tissue damage in diabetic rats due to its antidiabetic effect [18]. Another study revealed that topical applications of terebinth oil had beneficial effects on wound healing [26].

In the study by Zhang et al., it was determined that the mRNA level of radixin in oocytes was approximately 700 times higher than that in ovarian somatic cells and other tissues. Additionally, immunofluorescence signals of radixin have been demonstrated to be present in both the membrane and cytoplasm of oocytes at all stages of folliculogenesis, including quiescent primordial follicles [27]. In another study, the role of ERM and EWI-2 and EWI-F proteins in gamete fertilization was investigated. Individual inhibition of the expression of each protein (Ezrin, Radixin, Moesin, EWI-2, and EWI-F) showed no effect on fertilization, but combined inhibition of EWI-2/EWI-F or the three ERMs resulted in a significant reduction in the fertilization index. It has been found to trigger [28]. Wakayama and colleagues demonstrated that radixin binds to both actin and tubulin as a result of immunoprecipitation in mouse testis. It has been suggested that radixin immunoreactivity is observed in the apical cytoplasm of Sertoli cells from the 1st to the 2nd week after birth and that radixin plays a role in the maturation of Sertoli cells [29]. In a different study, it was stated that radixin was distributed along both the sinusoidal (basal) and canalicular (apical) membranes in hepatocytes and its highest concentrations were seen in the canalicular membrane [30]. As a result of the I/R we performed in the rat ovary, radixin expression was; It was observed that there was a significant positive reaction in the nuclear structures that broke away from the epithelial cells, in some granulosa cells that fell off close to the lumen, and in the inflammatory cells around the vessels in the parenchymal region and stromal area. In addition, it was observed that the apoptotic process, which developed due to inflammation in the tissue after ischemia, continued. As a result of I/R + terebinth application; While moderate positive radixin expression was observed in some of the epithelial cells and occasionally in the nuclei of granulosa cells, negative radixin expression was detected in the stromal area. However, as a result of the study, it was noteworthy that the nuclei of the granulosa cells shrank, while the radixin reaction was negative.

CONCLUSION

As a result, it was observed that terebinth oil provided healing activity by reducing the negative effects resulting from ovarian ischemia/reperfusion injury. It can be thought that the reason for this situation is that the oil obtained from the plant has anti-inflammatory and antioxidant properties.

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