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Protective Effect of Aronia Melanocarpa Extract on Ischemia Reperfusion Damage in Rat Ovary

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ABSTRACT

Aronia melanocarpa fruit is one of the food sources containing the most anthocyanins, flavonoids, proanthocyanidins, flavanols, phenolic acids and flavonol polyphenols. Studies have shown that this fruit has antitumoral, antiviral, antibacterial, antidiabetic, anti-inflammatory, antioxidant and antimutagenic properties. In this study; the effects of Aronia melanocarpa extract (AME) on ovarian ischemia reperfusion injury in rats were investigated. For this purpose, Wistar albino female rats (n = 40) were used. Animals were divided into groups as control, ischemia-reperfusion (IR), Ary1 (low-dose AME), Ary2 (medium-dose AME) and Ary3 (high-dose AME). No substance was administered to the control group. Ovaries of rats in the IR group were subjected to 60 minutes of ischemia and 24 hours of reperfusion. Ary1, Ary2 and Ary3 groups were given AME daily via gavage one week before ischemia (2.5, 5 and 10 mL kg^{-1}, respectively). After the last dose of the extract was applied, bilateral ovarian ischemia (60 min) was created surgically in all treatment groups. Then, these groups were subjected to 24 hours of reperfusion. At the end of the experiment, blood and ovarian tissue were taken from the rats under anesthesia. Malondialdehyde (MDA), catalase (CAT) and super oxide dismutase (SOD) analyzes were performed on the tissue and serum samples taken. All doses of aronia extract did not have a positive effect on MDA and CAT levels ($p>0.05$). Moreover, the MDA level was higher in the Ary1 group than in the IR group ($p<0.05$). But the activity of the SOD enzyme increased in all treatment groups. Moreover, the difference was significant in Ary1 and Ary3 groups compared to the IR group ($p<0.05$). When ovarian tissue was evaluated histopathologically, tissue damage was observed to decrease in the treatment groups. Moreover, as the extract dose increased, it was determined that the ovary gained an appearance close to the control group ovaries. Immunohistochemically, Interleukin-1 beta, Interleukin-6 and Tumor necrosis factor alpha reactivity were severe in the IR group. A moderate immunoreactivity was detected in the Ary1 group and a mild immunoreactivity was detected in Ary2 and Ary3. As a result, it can be concluded that high-dose AME is a substance that can be used for protective purposes against ovarian ischemia-reperfusion damage.

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Key Words
Ischemia, reperfusion, aronia melanocarpa ektsract, rat

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INTRODUCTION

Ischemia is the cessation of flow to organs or tissues for various reasons. When blood circulation is interrupted, tissues cannot receive the substances they need and begin to fail to perform their functions. Many problems occur in tissues subjected to ischemia such as hypoxia, oxidative stress and lipid peroxidation. These problems will resolve spontaneously when the ischemia disappears. However, if ischemia continues and exceeds the critical period, irreversible damage to the tissue occurs. In this case, the organ becomes necrotic and parenchyma cells eventually die[1-3]. Tissue reperfusion is very important in reversing the damage caused by ischemia. However, as blood circulation restarts, molecular oxygen enters the cells and causes the release of free oxygen radicals. This event leads to more damage to the cell compared to ischemia. Different problems such as edema, cytoskeletal disorders and loss of permeability in the cell are due to reperfusion[4-5]. Immediately after ischemic tissue is reperfused, changes occur in the amount of reactive oxygen metabolites and nitric oxide. While the level of reactive oxygen metabolites increases, the amount of nitric oxide decreases[6]. This uncontrolled oxidation suppresses the cell's defense mechanisms and increases the release of inflammatory cytokines[7].

Ischemia-reperfusion injury occurs for different reasons in many organs such as heart, kidney, brain, testicle and ovary[8]. In the ovaries, it occurs when the organ rotates around its own axis. The cause of this pathology, also called adnexal torsion, is the anatomical structure of the organ's suspensory ligaments[9]. In addition, risk factors include large ovaries, presence of large masses such as tumors, pregnancy, ovulation induction and previous ovarian torsion[10-13]. Adnexal torsion usually occur with the symptom of abdominal pain. Diagnosis of the disease; It can be detected by visual examination techniques such as ultrasound, computerized tomography or magnetic resonance[12,14-15]. The definitive diagnosis is made through laparoscopy. Treatment is generally done surgically[16]. However, the response of the ovary to treatment may vary depending on the length of the ischemia period. For this reason, antioxidant drugs are used to eliminate tissue damage that may occur as a result of ischemia reperfusion[8,17-19].

Antioxidant substances are effective in stopping or minimizing the damage caused by adnexal torsion or detorsion. There are many studies on antioxidants to prevent ovarian ischemia reperfusion injury in the literature[20-24]. Aronia fruit (Aronia melanocarpa) attracts attention at this point[25]. Scientific studies conducted on Aronia suggest that antioxidants such as quercetin and chlorogenic acid found in the fruits of the plant may have a protective effect against tissue damage in the ovaries. There are no studies examining the effects of Aronia melanocarpa extract (AME) on ovarian IR damage[26]. We hypothesized that aronia extract could reduce oxidative stress and inflammation in the ovary. In this study, we aimed to determine the protective effects of aronia extract on ovarian ischemia reperfusion injury and to reveal its effectiveness in the treatment of adnexial torsion.

MATERIAL AND METHODS

Material: The material of the study consisted of 40 Wistar albino female rats, about 3 months old and weighing 250 g. AME was purchased from a local company ((Dr. Aronia®, Aronya Gıda, Türkiye)). The total phenolic content of the extract was measured by HPLC device at Karadeniz Teknik University. In the phenolic content profile; Protocatechuic acid was found in the amount of 229,8 μg mL^{-1}, Chlorogenic acid 149,48 μg mL^{-1}, Caffeic acid 245,2 μg mL^{-1}, Rutin 73,54 μg mL^{-1} and Coumaric acid 24,84 μg mL^{-1}.

Before starting the study, permission was obtained from Kahramanmaraş Sütçü İmam University, Faculty of Medicine, Animal Experiments Local Ethics Committee (16.6.2021, decision number: 02). This study was carried out at Kahramanmaraş Sütçü İmam University, Medical Experimental Application and Research Center. For adaptation purposes, the rats were placed in cages one week before the experiment. Rats were housed in transparent cages made of polycarbonate material. Feed and water were given ad libitum. Sawdust was used as a substrate. Animals were kept at room temperature (22±2°C) with 12 hrs of light and 12 hours of darkness daily.

Methods: Rats were randomly divided into 5 groups. Each group consisted of 8 animals:

- **Control group (n = 8):** The animals were anesthetized without any drugs. They were sacrificed and then their blood and ovarian tissues were taken carefully
- **Ischemia-reperfusion (IR) group (n = 8):** The anesthetized animals were operated and ischemia was created in the ovaries. Following 60 minutes of ischemia, blood flow was begun again and reperfusion was allowed for 24 hrs. Subsequently, blood and tissue samples were collected from the rats
- **Ary1 group (n = 8):** Sixty minutes of ischemia and 24 hrs of reperfusion were applied to the ovary. The extract was given by gavage at a daily dose of 2, 5 mL kg^{-1} for 7 days, starting one week before the ischemia
- **Ary2 group (n = 8):** In this group, 60 min of ischemia and 24 hrs of reperfusion were applied and the extract (5 mL kg^{-1}) was given by gavage for 7 days, starting one week before the ischemia

- **Ary3 group (n = 8):** In the last group, 60 min of ischemia and 24 hrs of reperfusion were applied and the extract at a dose of 10 mL kg^{-1} was given by gavage for 7 days, starting one week before the ischemia

Previous studies were taken as basis in determining the above doses and application times[27-29].

Laparotomy and IR experiments were performed under general anesthesia. In all groups, anesthesia was provided using the xylazine (5 mg kg^{-1}) and ketamine (60 mg kg^{-1}) protocol before the surgery. Dorsal laparotomy was performed in rats. Bilateral ischemia was created by applying a clamp to the vessels of the ovary from the incised area. The exposed area was covered with sterile gauze soaked in physiological saline[30]. At the end of the experiment period, samples were taken from the anesthetized rats and they were sacrificed by exsanguinations. Ovarian tissue and serum samples were stored at -20°C until analysis. Catalase (CAT), superoxide dismutase (SOD) and malondialdehyde (MDA) levels were determined in blood and ovarian tissue samples taken from animals. Ovarian tissue samples were placed in 10% formol solution and stored at +4°C for histopathological evaluation.

Biochemical analyses: Each sample was weighed as 1 g on a precision balance and placed in 2 mL Eppendorf bottles. 1 mL PBS was added to the samples. The samples were disintegrated in the homogenizer (Next Advance, Inc., Averill Park, NY, USA) for 3 min using Next level brand zirconium oxide beads. Then, the homogenates were centrifuged at 5000xg for 5 min. The supernatant was taken into 1.5 mL Eppendorfs to be used in tests.

CAT measurement: CAT measurement in serum and tissue was performed with a USCN brand Rat CAT ELISA measurement kit (Catalogue No: SEC418Ra, Wuhan USCN Business Co., Ltd., Wuhan, China). Absorbance reading was made on Chromate 4300 brand elisa reader device (Awareness Technology, Inc. Martin Hwy. Palm City, USA). The measurement was made at 450 nm. Data were calculated using linear regression analysis. Results were given as ng mL^{-1}.

SOD measurement: SOD measurement in serum and tissue was performed with the USCN brand Rat SOD ELISA measurement kit (Catalogue No: SES134Ra, Wuhan USCN Business Co., Ltd., Wuhan, China). Absorbance reading was made on Chromate 4300 brand elisa reader device. The measurement was made at 450 nm. Data were calculated using linear regression analysis. Results were given as ng mL^{-1}.

MDA measurement: MDA measurement in serum and tissue was made with a USCN brand Rat MDA ELISA measurement kit (Catalogue No: CEA597Ge, Wuhan USCN Business Co., Ltd., Wuhan, China). Absorbance reading was made on Chromate 4300 brand elisa reader device. The measurement was made at 450 nm. Data were calculated using 4-parameter logistic regression analysis. Results were given as ng mL^{-1}.

Histopathological analyses: The fixed tissue samples were washed in running water overnight to remove formalin. Then, the samples were monitored for pathological tissue and passed through graded alcohol (50, 75, 96 and 100%) and xylol series. Then it was blocked in paraffin. 5 µm thick sections were made from the prepared blocks with RM 2125 RT. The first three sections and every tenth section were taken on slides. The prepared slides were passed through alcohol and xylol series and stained with hematoxylin and eosin (HE). All samples were examined under a high-resolution light microscope (Olympus DP-73 camera, Olympus BX53-DIC microscope; Tokyo, Japan). All detected changes were noted and graded according to the presence and status of the finding (0: absent, 1: mild, 2: moderate, 3: severe)[31].

Immunohistochemical analyses: Five micron thick sections were cut from previously prepared paraffin blocks onto Poly-L-lysine coated glass slides. Then, routine deparaffinization and rehydration methods were applied. Then, it was stained according to the streptavidin-biotin-peroxidase complex (ABC) technique (Zymed, Histostain Plus Kit, California, USA). For antigen retrieval in the tissues, microwave heat treatment was applied with citrate buffer (pH 6.0 and 20 min). Endogenous peroxidase activation was blocked with 0.3% hydrogen peroxide in methanol in 0.01 M PBS for 10 min. Protein blocking was performed by incubating the tissues with 5% normal goat serum for 30 min before the primary antibody. The sections were labeled with TNF alpha (1:50, ab6671; Abcam, Cambridge, USA), IL-1β (1:200, sc-52012, Santa Cruz Biotechnology, Inc. Texas, U.S.A.) and IL-6 (10 µg mL^{-1}, RPA079Ga01-Recombinant Interleukin 6; Cloud-Clone Corp. (CCC, USA)) primary antibodies by incubating for 60 min at room temperature. Tissues were incubated in rabbit anti-mouse biotinylated secondary antibody for 30 min and then incubated with streptavidin-peroxidase conjugate for 30 min. In order to make the reaction visible, diaminobenzidine (DAB, Dako/Denmark) chromogen was added to the sections and left for 10 min. Finally, the sections were stained with hematoxylin for 1-2 min for backstaining. Then it was washed in running water. Tissues were passed through a graded alcohol and xylol series and glued with Entellan. The entire staining procedure was carried out at 24°C in humid chambers. Phosphate buffered saline (PBS) solution was used to wash the sections during the staining stages. Immunopositive cells identified for each sample under a high-resolution microscope (Olympus DP 73 camera, Olympus BX53-

DIC microscope; Tokyo, Japan) at magnifications of 40 and 100 were counted, recorded and scored. Staining indices were calculated as follows. Negative: 0 (<1% positive); mild: 1 (1-25% positive); moderate: 2 (>25-75% positive); and severe: 3 (>75% positive)[31].

Statistical analyses: The obtained values were entered into the Excel file in accordance with the table format. Measurements were made using the SPSS program (SPSS 20.0). Kruskal-Wallis test was used for comparison between groups and Mann-Whitney U was used to determine the groups that made the difference.

RESULTS

Forty rats were used in the study. However, one animal in the IR group died of an unknown cause just before the operation. Therefore, samples from 7 animals were evaluated.

Biochemical results: To compare the antioxidant and oxidant levels in the blood, SOD, CAT and MDA levels were determined in the serum (Table 1). When the enzyme levels in the blood of the groups were compared with the Kruskal-wallis test, it was determined that the difference between the groups was statistically significant ($p<0.05$). Compared to the IR group, the MDA level was significantly higher in the Ary1 group ($p<0.05$). Again, compared to the IR group, CAT level was lower in the Ary2 ($p<0.001$) and Ary3 groups ($p<0.01$). SOD level was also significantly higher in the Ary1 ($p<0.01$) and Ary3 groups ($p<0.05$) compared to the IR group.

To compare the antioxidant and oxidant levels in ovarian tissue, SOD, CAT and MDA levels were determined in tissue homogenates (Table 2). When the enzyme levels of the groups were compared with the Kruskal-wallis test, it was determined that the difference between the groups was statistically insignificant ($p>0.05$).

Parameters of the groups are given as mean ± std error. P-value was accepted as 0.05.

Histopathological results: Edema, dilatation, congestion, hemorrhage, necrosis, degeneration and polymorphonuclear (PMN) cell infiltration were determined histopathologically in the experimental groups (Table 3-7).

The photographs below show light microscope images of sections of ovarian tissue. It was observed that the ovaries had a normal structure in the control group (Fig. 1A). The ovaries were severe necrotic and degenerative changes in the IR group (Fig. 1B).

Table 1: Serum MDA, CAT and SOD levels (ng mL^{-1}) of rats in the experimental groups

Groups	n	MDA	CAT	SOD
Control	8	425.6±37.29	2.62±0.43*	1969±0.11*
IR	7	508.7±35.22	4.81±0.34	1493±0.05
Ary1	8	676.5±33.02*	3.75±0.70	1819±0.06*
Ary2	8	604.9±46.20	1.68±0.23*	1688±0.18
Ary3	8	583.2±84.87	1.87±0.40*	1919±0.15*

Parameters of the groups are given as Mean±SD, *Means in the same column are significantly different from the IR group ($p<0.05$)

Table 2: Ovarian MDA, CAT and SOD levels (ng mL^{-1}) of rats in the experimental groups

Groups	n	MDA	CAT	SOD
Control	8	655.2±55.37	16.24±0.77	161.24±6.95
IR	7	720.1±66.35	16.81±1.04	161.9±5.71
Ary1	8	749.5±84.18	14.79±1.41	176.1±13.45
Ary2	8	677.3±39.53	15.06±1.23	169.5±9.52
Ary3	8	553.4±55.66	16.08±1.12	175.3±10.59
p-value		-	-	-

Table 3: Distribution of histopathological lesions in the ovaries of control group rats

Score	Edema	Dilatation	Congestion	Hemorrhage	Necrosis	Degeneration	PMN
0	7	8	7	8	8	8	8
1	1	0	1	0	0	0	0
2	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0

The numbers in the columns show the distribution of animals according to the score

Table 4: Distribution of histopathological lesions in the ovaries of IR group rats

Score	Edema	Dilatation	Congestion	Hemorrhage	Necrosis	Degeneration	PMN
0	0	1	0	0	1	1	1
1	1	0	1	1	0	0	0
2	1	5	0	0	3	0	6
3	5	1	6	6	3	6	0

The numbers in the columns show the distribution of animals according to the score

Table 5: Distribution of histopathological lesions in the ovaries of Ary1 group rats

Score	Edema	Dilatation	Congestion	Hemorrhage	Necrosis	Degeneration	PMN
0	0	1	0	0	2	1	2
1	2	1	1	1	0	1	0
2	4	6	5	6	6	5	6
3	2	0	2	1	0	1	0

The numbers in the columns show the distribution of animals according to the score

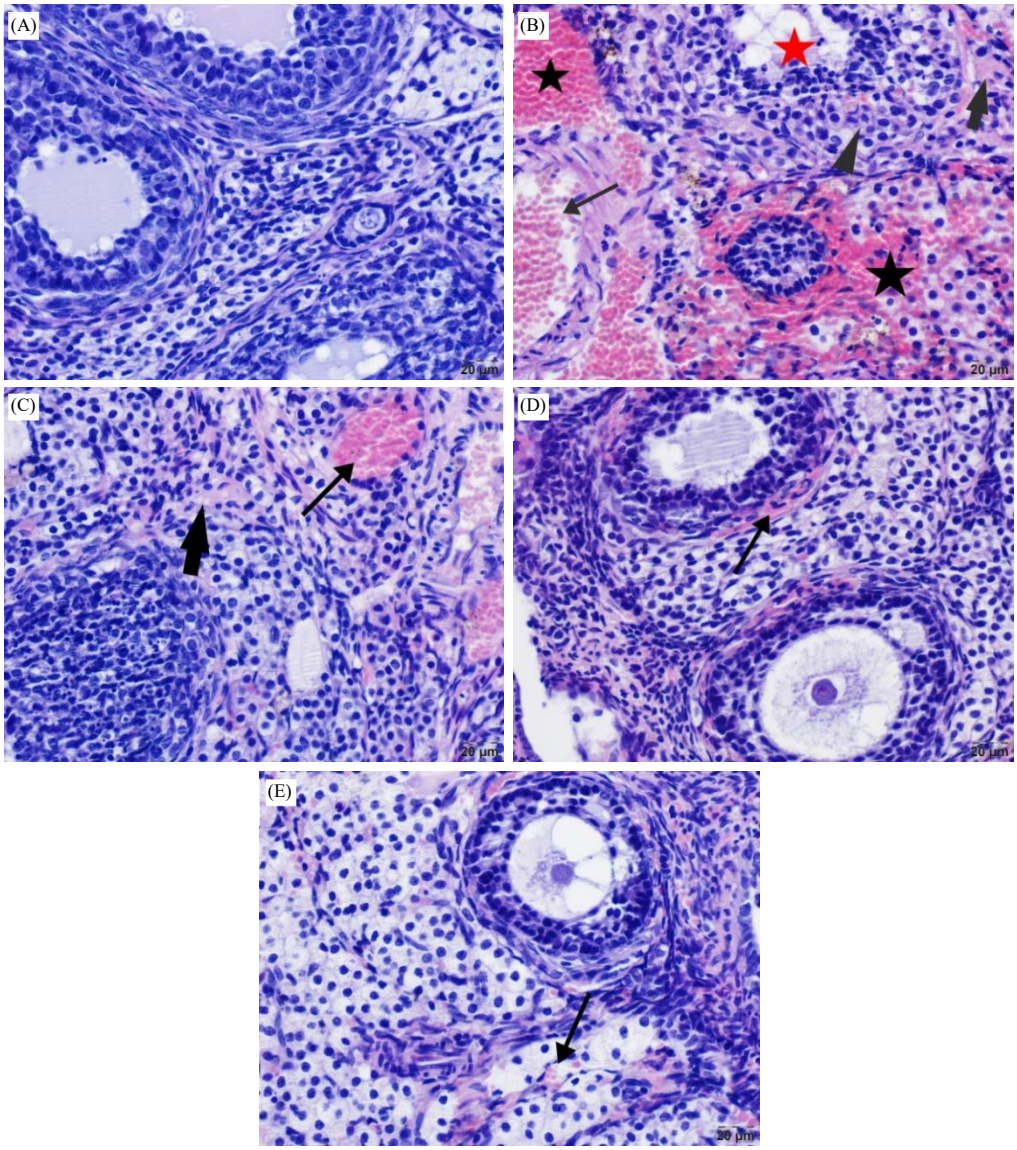

Fig. 1(A-E): Histopathological examination of rat ovaries, (A) Normal ovarian tissue of rats in the control group, (B) Ovarian tissue of rats in the IR group (Black star: severe bleeding, Red star: degenerative follicle, Thick arrow: severe edema, Thin arrow: severe congestion, Arrowhead: degenerative cells), (C) Ovarian tissue of rats in the Ary1 group (Thick arrow: edema, Thin arrow: congestion), (D) Ovarian tissue of rats in the Ary2 group (Arrow: congestion) and (E) Ovarian tissue of rats in the Ary3 group (Arrow: congestion) (hematoxylene and eosin)

Table 6: Distribution of histopathological lesions in the ovaries of Ary2 group rats

Score	Edema	Dilatation	Congestion	Hemorrhage	Necrosis	Degeneration	PMN
0	1	6	1	4	7	7	7
1	6	2	6	3	0	0	1
2	1	0	1	1	1	1	0
3	0	0	0	0	0	0	0

The numbers in the columns show the distribution of animals according to the score

Very mild edema and congestion were detected in the groups given AME (Fig. 1C-E).

Immunohistochemical results: Proinflammatory cytokines, an important marker of inflammation, were observed to be immunonegative in the control group ovaries (Fig. 2, Table 8). In the IR group, these cytokines were found to be strongly positive (Fig. 3, Table 8). A moderate immunopositivity was detected in those given low doses of aronia extract (Fig. 4) and mild immunopositivity was detected in those given medium and high doses (Fig. 5 and 6, Table 9).

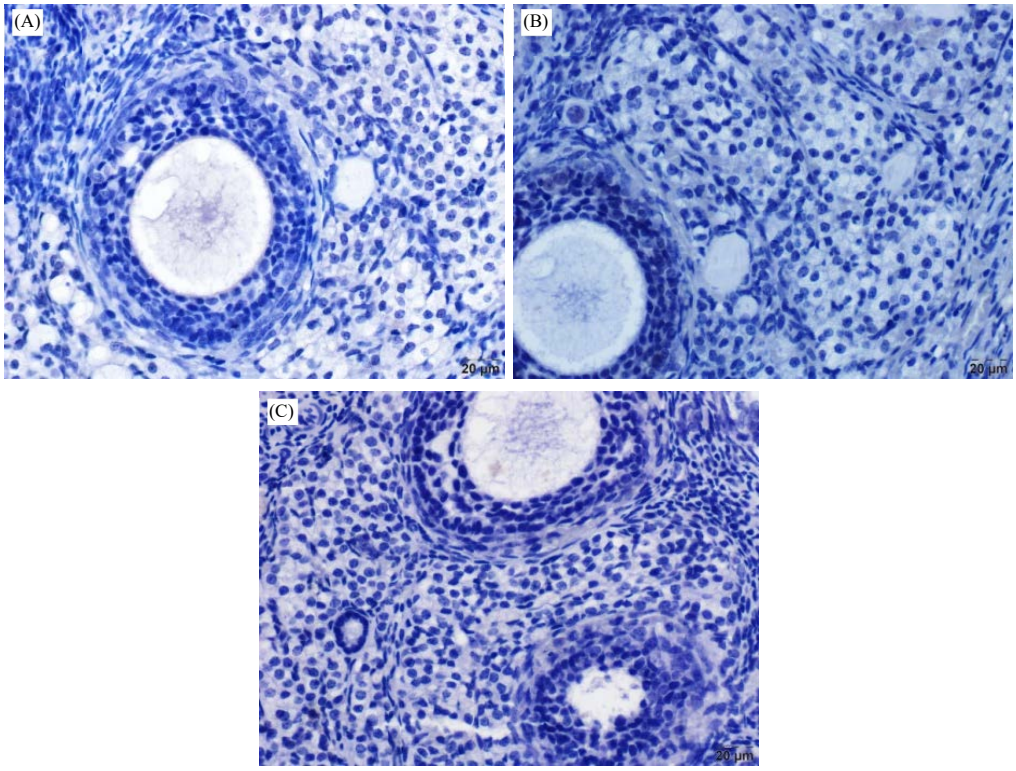

Fig. 2(A-C): IL-1β (A), IL-6 (B) and TNF-alpha (C) immunonegativity in control animals (AEC, DAB chromogen)

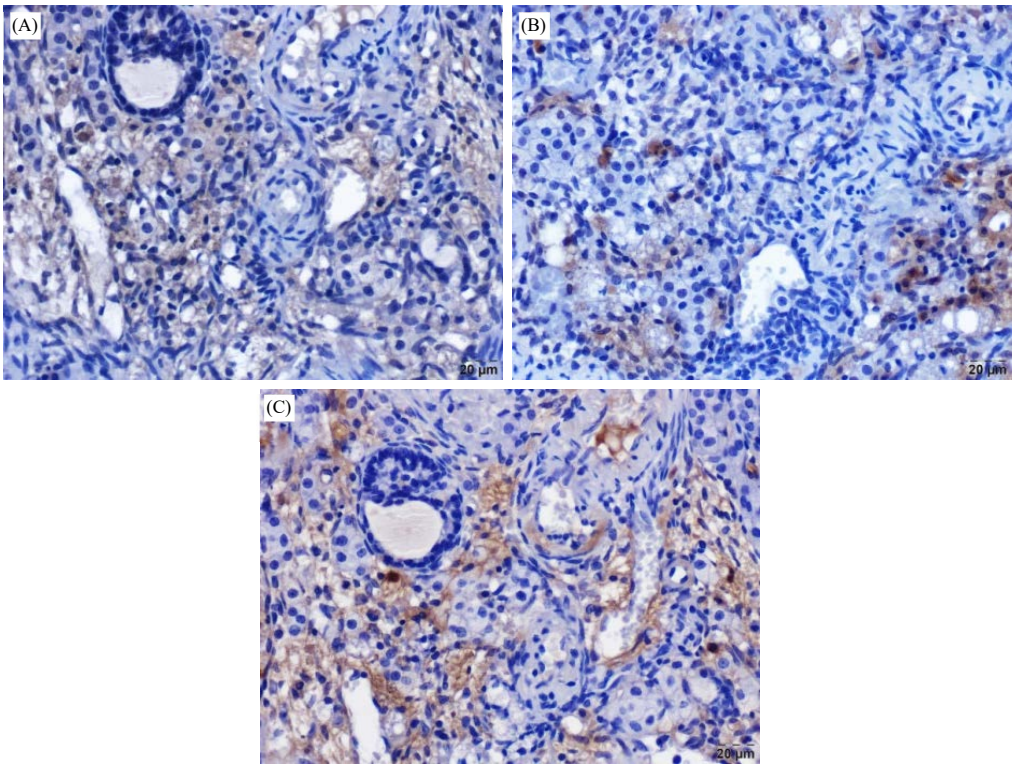

Fig. 3(A-C): IL-1β (A), IL-6 (B) and TNF-alpha (C) varying from moderate to severe immunopositive in the ovary of in IR group (AEC, DAB chromogen)

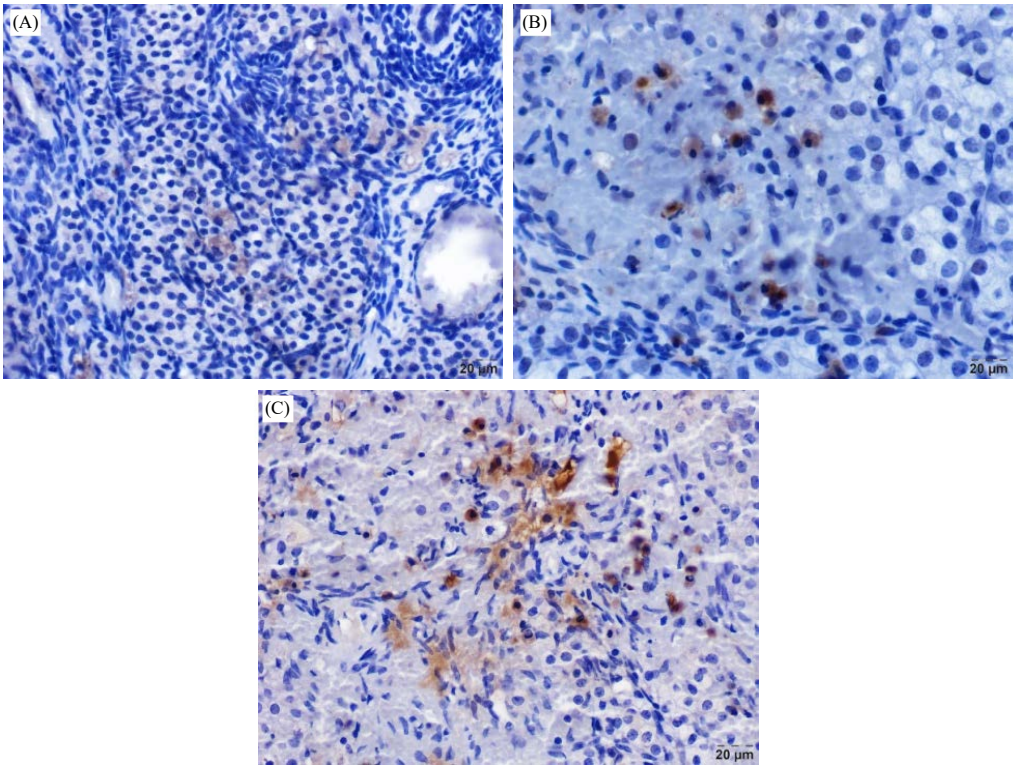

Fig. 4(A-C): IL-1β (A), IL-6 (B) and TNF-alpha (C) moderate immunopositive in the ovary of Ary1 group (AEC, DAB chromogen)

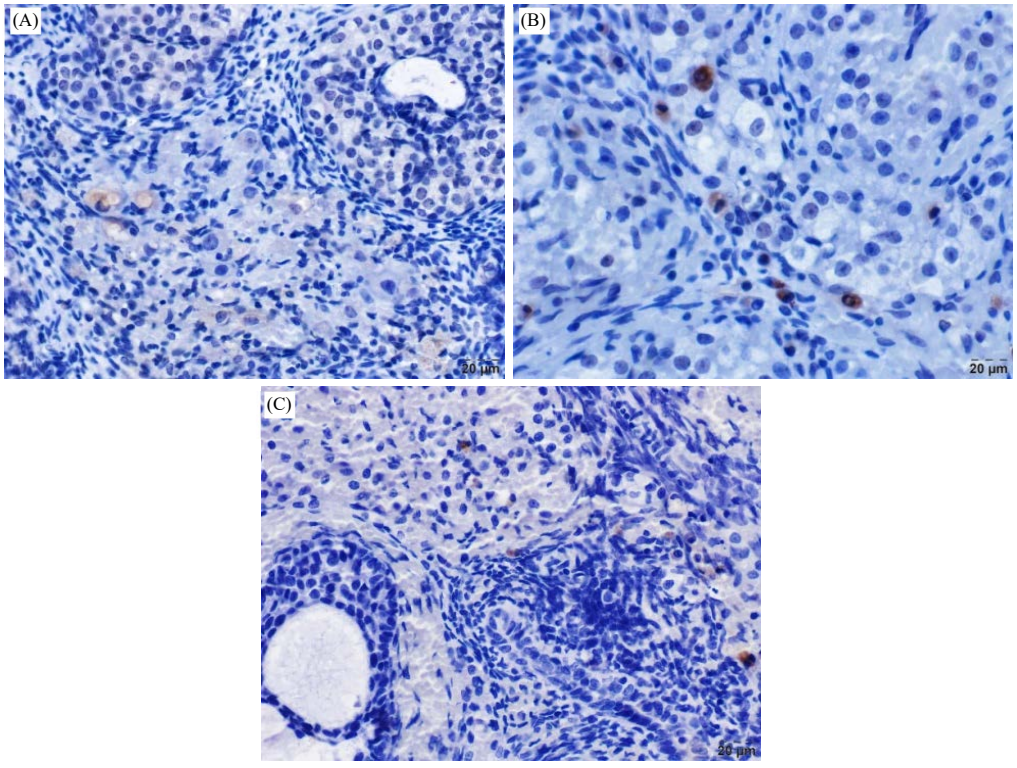

Fig. 5(A-C): IL-1β (A), IL-6 (B) and TNF-alpha (C) mildly immunopositive in the ovary of Ary2 group (AEC, DAB chromogen)

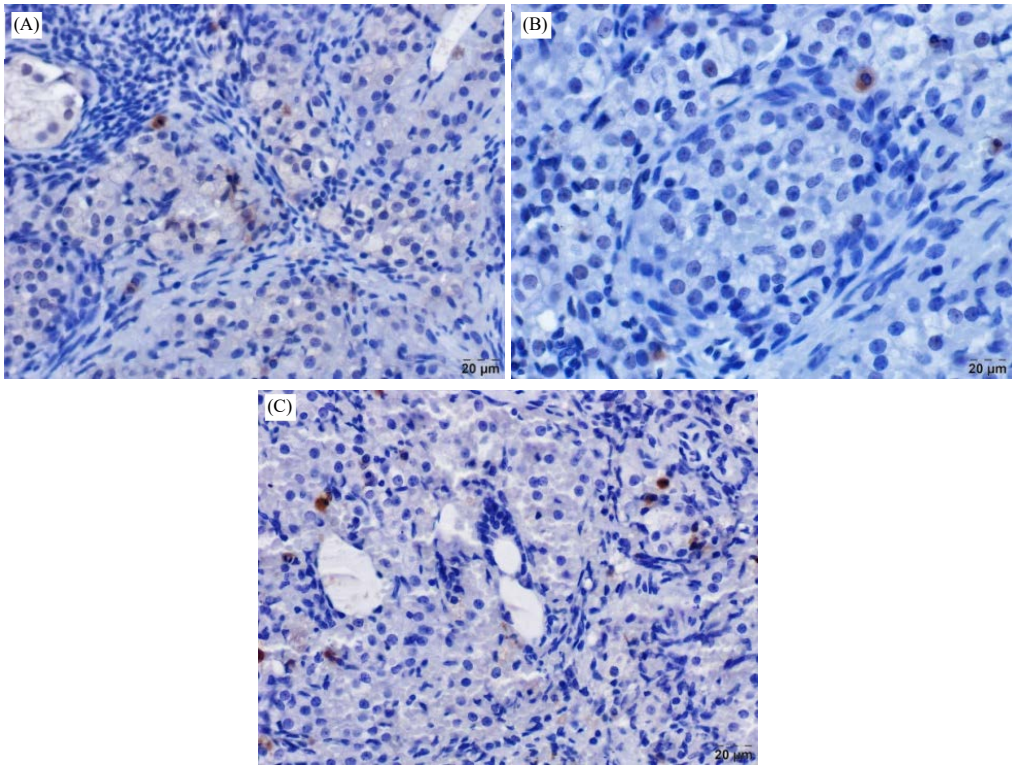

Fig. 6(A-C): IL-1β (A), IL-6 (B) and TNF-alpha (C) mildly immunopositive in the ovary of Ary3 group (AEC, DAB chromogen)

Table 7: Distribution of histopathological lesions in the ovaries of Ary3 group rats

Score	Edema	Dilatation	Congestion	Hemorrhage	Necrosis	Degeneration	PMN
0	1	5	3	6	5	7	4
1	6	3	3	2	3	1	4
2	1	0	2	0	0	0	0
3	0	0	0	0	0	0	0

The numbers in the columns show the distribution of animals according to the score

Table 8: IL-1β, IL-6 and TNF alpha immunoreactivity of rats in the control and IR groups

	Control			IR		
Score	IL-1ß	IL-6	TNF alpha	IL-1ß	IL-6	TNF alpha
0	8	8	8	0	1	0
1	0	0	0	1	0	1
2	0	0	0	2	2	0
3	0	0	0	4	4	6

The numbers in the columns show the distribution of animals according to the score

Table 9: IL-1β, IL-6 and TNF alpha immunoreactivity of rats in treatment groups

	Ary1			Ary2			Ary3		
Score	IL-1β	IL-6	TNF alpha	IL-1β	IL-6	TNF alpha	IL-1β	IL-6	TNF alpha
0	0	0	0	1	4	2	3	4	2
1	2	1	1	5	3	5	4	3	5
2	4	3	4	2	1	1	1	1	1
3	2	4	3	0	0	0	0	0	0

The numbers in the columns show the distribution of animals according to the score

DISCUSSION

Phytotherapy is a treatment method that has been used all over the world for a long time. This method, which is included in traditional medicine, is a natural treatment that allows the body to heal by taking advantage of the therapeutic effects of the medicinal substances found in plants[32]. Phytotherapeutic substances can be obtained from many plants. In this context, the number of studies is increasing on Aronia melanocarpa[33-38]. However, there are only two studies investigating the effects of its on IR damage. One of these studies was conducted to research its effects on renal and the other on intestinal ischemia[39-40]. To the best of our knowledge, there are no studies evaluating the effect of Aronia melanocarpa on ovarian IR damage.

In this study, IR damage caused severe degeneration and necrosis in the ovary (Fig. 1). At the same time, edema, dilatation, congestion, hemorrhage and PMN infiltration occurred (Table 4). In addition, follicular degeneration was also detected (Fig. 1b). In the treatment groups, 3 different doses of aronia extract showed a protective effect. As the dose of the extract increased, the damage to the ovarian tissue gradually decreased. These findings coincide with the literature. When Li *et al*.[40], compared the experimental groups histopathologically in their study in which they created renal IR, they reported that tubular damage was severe in the IR group and the tubules were better in those given aronia-derived anthocyanin.

Lipids are important substances that form the structure of the cell membrane[41]. When they exposed to oxidation, MDA occurs. This metabolite is used to detect lipid peroxidation[42]. In the present study, we determined MDA levels in serum and ovarian tissue to demonstrate oxidative damage. In both samples, MDA was higher in rats with IR compared to the control group. However, this increase was statistically insignificant ($p>0.05$). Notably, serum MDA level was higher in the treatment groups and the difference was statistically significant in the Ary1 group than IR group ($p<0.05$). Our results were in agreement with the previous IR study[39]. Researchers created intestinal IR in rats given aronia and investigated the level of MDA in the intestines. As a result, they reported that the MDA level was higher in the aronia-administered group compared to the IR and sham group in their analysis of colon tissue. Researchers have reported that this plant, which has antioxidant components, does not show antioxidant activity in mice. In this study, when all groups were evaluated, it was determined that the IR group had the highest values in terms of serum and ovarian CAT levels. Serum CAT level was significantly higher in the IR group compared to the control group ($p<0.05$). When compared with the treatment groups, the CAT level was significantly different at IR group than Ary2 and Ary3 groups ($p<0.05$). When we look at the literature, these findings are meaningful. Çığşar *et al*.[43] tested osagenin, a herbal substance, on the ovarian IR model in their study. Researchers found the CAT level of the IR group to be higher than the control and treatment groups. They explained this situation by the activation of endogenous antioxidant mechanisms in rats with IR. In our study, unlike CAT, SOD levels were higher in all treatment groups compared to the IR group. There was also a significant increase in serum SOD levels in the Ary1 and Ary3 groups ($p<0.05$). It appears that this increase is due to the use of AME. In other words, while this extract did not have a positive effect on MDA and CAT levels, it increased the activity of the SOD enzyme. Our findings are generally parallel to the above studies[39,43]. In this study, serum MDA level was found to be high while CAT level was found to be lower in the groups treated with aronia extract. This can be interpreted as aronia extract showing its effect not through antioxidants but by preventing inflammation.

Inflammation is one of the events that cause tissue damage. During this process, many inflammatory cells arrive at the injured tissue. Abundant amounts of cytokines are secreted from both tissue and inflammatory cells[44]. Of these, especially IL-1β, IL-6 and TNF alpha are important cytokines. These molecules, also known as pro-inflammatory cytokines, are used as inflammation markers[45]. These cytokines are also secreted in IR injury[46]. Previous studies have proven the anti-inflammatory effects of aronia melanocarpa[40,47]. In a study conducted on male mice, renal IR damage was created and aronia derived anthocyanins were administered to the animals[40]. The researchers reported that TNF alpha, IL-1β, IL-6 and MCP-1 levels decreased in both serum and kidney in mice treated with anthocyanin compared to the IR group. Our findings are parallel with those of Li *et al*.[40]. The decrease in the immunoreactivity of proinflammatory cytokines also proves this situation (Table 8-9). In this study, we observed that tissue damage occurred less in the ovaries of rats treated with aronia extract (Fig. 4-6). These findings lead to the conclusion that aronia extract exerts its effect through anti-inflammatory mechanisms.

In our study, we hypothesized that aronia melanocarpa could treat ovarian IR damage. As a result of the study, we obtained histopathological results supporting this situation (Table 3-7). Aronia application reduced PMNs in the injured ovary. Thus, the release of proinflammatory cytokines decreased and an anti-inflammatory effect emerged. In groups Ary1, 2 and 3, the ovarian tissue was similar to the tissue of control group rats (Fig. 1, 4-6). Histopathology, biochemistry and immunohistochemistry results show that aronia acts dose-dependently. Serum and tissue MDA levels varied depending on the dose. As the dose increased, MDA levels gradually decreased, although not significantly (Table 1 and 2). We can see the same situation when we look at immunohistochemical scoring (Table 8-9). These findings are consistent with clinical improvement.

In conclusion, this study found that AME had a significant protective effect against IR damage in the ovaries of female rats. The fact that IL-1β, IL-6 and

TNF-alpha immune reactivity was less in the ovaries of rats treated with high-dose aronia has been shown that this extract protects the ovarian tissue. Considering the results of this study, it was concluded that aronia melanocarpa extract at a dose of 10 mL kg^{-1} could be used against IR damage in female rats.

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