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Protective role of Proanthocyanidin in experimental ovarian torsion

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Abstract

Background: Proanthocyanidin is a potent bioactive antioxidant naturally occurring in grape seed and acts as reactive oxygen species (ROS) scavenger. The aim of this study was to investigate the effects of proanthocyanidinin in experimental ovarian torsion injury.

Methods: Twenty four rats were randomly divided into three groups (n=8). Group 1: the laparotomy group, group 2: ovarian torsion group, and group 3: intervention group administered proanthocyanidinin of 50 mg/kg before bilateral ovarian ischemia and reperfusion. Histologic examination and scoring was done at the end of the experiment. Statistical analyses were performed using the SPSS v. 19.

Results: Ovarian histopathologic findings of all three groups were significantly different in terms of hemorrhage ($p<0.001$), edema ($p=0.001$) and vascular dilatation ($p< 0.001$). Pathologic changes induced by I/R were reduced in ovaries of rats administered proanthocyanidin, in particular, hemorrhage, edema and vascular dilatation.

Conclusion: Proanthocyanidin, known as free radical scavenger and antioxidant, is protective against tissue damage induced by ischemia and/or ischemia/reperfusion in rat ovaries.

Keywords: Proanthocyanidinin, Fertility, Ischemia Reperfusion, Ovarian torsion.

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Introduction

Ovarian torsion is a surgical emergency that is frequently associated with a preexisting ovarian mass. Compared to women, it more commonly occurs in young and adolescent girls (1, 2).

Urgent intervention is required to preserve fertility and salvage the twisted ovary. The primary pathophysiology is ischemia followed by reperfusion, so that ovarian torsion is one of the ischemia/reperfusion (I/R) injuries (3, 4). As a result of I/R, reactive oxygen species (ROS) are released in tissues (5). Another considered pathogenesis is the migration and activation of neutrophils releasing ROS during the reperfusion phase of tissue injury (6). Therefore various agents capable of scavenging free radicals have been used to

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protect against such I/R injury in tissues (7-9)

To counteract ROS formation, ROS scavengers/antioxidants are of prime importance to prevent and control human diseases. Antioxidants are necessary for the destruction of these free radicals, by reacting with oxygen and thereby preventing the harmful effects caused by oxygen radicals (10). Proanthocyanidin is a potent bioactive antioxidant naturally occurring in grape seed and acts as ROS scavenger (11). Proanthocyanidins are found at high concentrations in grapes. They have antibacterial and anti-allergic properties and inhibit platelet aggregation and capillary permeability; these effects contribute to potent antioxidant ability (12). Its protective effects on I/R injury of renal, gastric and cardiac cells have been shown by previous studies (13-15).

To our knowledge, there is no previous information about the effects of proanthocyanidins on ovarian I/R injury. We therefore decided to perform an experimental study to evaluate the effects of proanthocyanidins on experimental I/R injury in the rat ovaries.

Methods

Twenty four Sprague-Dawley rats randomly divided in three groups, weighing 200-250 g were studied. During the study, all rats were housed in special cages and with appropriate feeding conditions at Çanakkale Onsekiz Mart University Experimental Research Center (16). Animal care and all procedures were approved by the Animal Care Committee (30.05.2013/2013-05-01/Gündüz B) of Çanakkale Onsekiz Mart University.

Experimental model

The twenty-four rats were divided equally into three groups (n=8). Group I: the laparotomy group, group II: ovarian torsion group, and group III: intervention group. Fifty mg / kg grape seed extract (proantho-

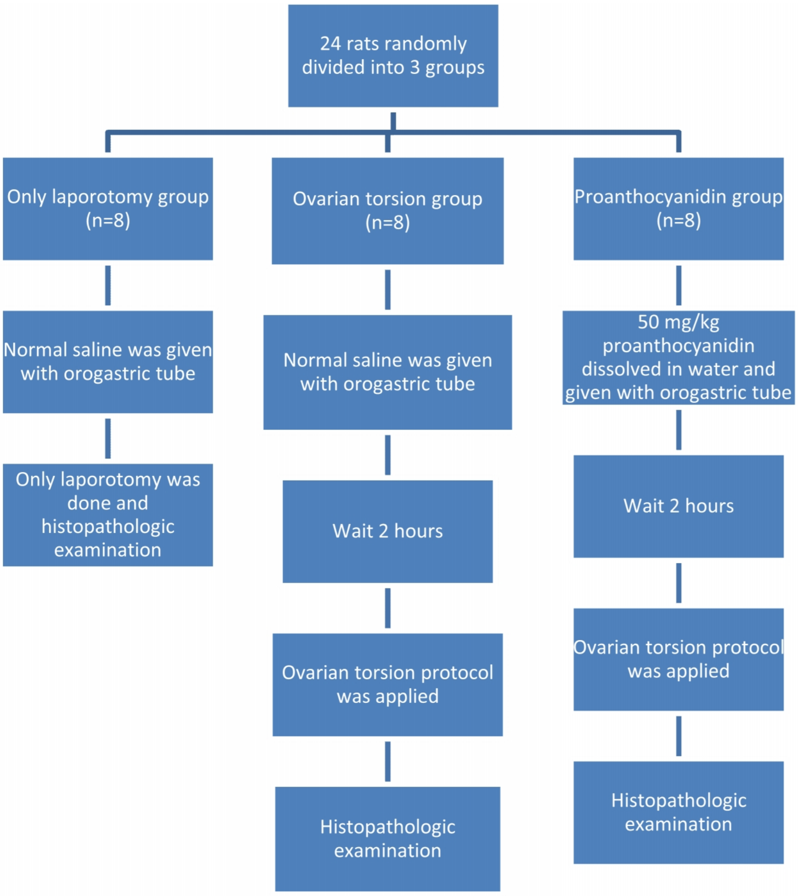

Fig. 1. Experimental model of the study

cyanidin) dissolved in water was administered by orogastric tube in the intervention group and the same amount of normal saline was given to other groups by orogastric tube at the same time (Fig. 1). All rats were anesthetized with intramuscular 50 mg/kg ketamine hydrochloride (Ketalar R, Eczacıbası, Istanbul, Turkey) and 10 mg/kg xylasine hydrochloride (10mg/kg, Rompun, Bayer, Istanbul, Turkey). A midline 2.5 cm longitudinal incision was performed in the lower abdominal region and adnexa were located. In experimental groups, right adnexa was rotated by 180° in a counterclockwise direction and the twisted adnexa was fixed to the anterior abdominal wall by 4/0 silk suture and the anterior wall was sutured in two layers with 3/0 silk. In the control group, the adnexa were palpated and left in their own anatomical position without rotation.

Group I underwent only laparotomy. In groups II and III, 3 hours ischemia was induced by using atraumatic vascular clips just below the ovaries and then bilateral ovarian I/R protocol was applied for 3 hours. After that, bilateral ovaries were surgically removed for histologic examination.

In group III, 50mg/kg/dose proanthocyanidin without food only as a solution dissolved in water was administered by orogastric tube before 2 h of ischemia. It was used between doses of 10-100 mg/kg in the literature, in these dose ranges it was shown to reduce MDA content, inhibit NOS activity and lower the content of NO, IL-1 beta, TNF-alpha (11, 13, 17, 18).

Grape seed extract used in this study was provided from NEFA Health Food Drug and Cosmetics Industry Trade A.Ş. Perpa Trade Center, second floor, B Blok. No: 1586 Okmeydani – Istanbul.

Histopathologic examination

Ovaries were fixed in 10% formalin solution. Tissues were dehydrated and embedded in paraffin. Five μm thickness ovarian tissue pieces were stained with hematoxylin and eosin. The sections were examined and photographed with a light microscope. A pathologist blind to the study groups examined and scored the samples. Congestion, hemorrhage, leukocyte infiltration, follicular degeneration, and interstitial edema were scored from 0 to 3 according to the injury severity, where 0 represented no pathologic findings and 1, 2 and 3 represented pathologic findings of less than 33%, 33% to 66%, and more than 66% of the ovarian section, respectively (19).

Statistical analysis

Statistical analyses were performed using SPSS v. 19. Normality was checked using Kolmogorov–Smirnov test. Tissue damage scores were compared by nonparametric analysis, and statistical significance was determined by Kruskal–Wallis test. Mann-Whitney U test was used for two group comparisons. $p < 0.05$ was considered significant.

Results

All measurements were evaluated with Kolmogorov-Smirnov test and were found to be non-normally distributed ($p=0.012$). By comparing the ovarian histopathologic results of all three groups, significant statistical differences were found in terms of

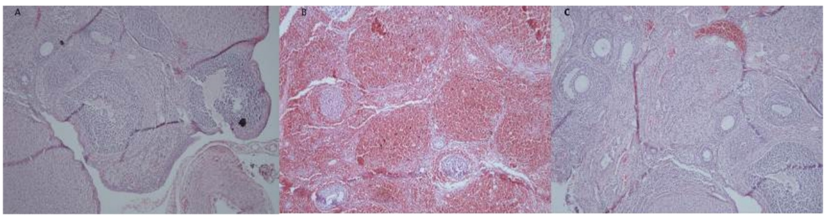

Fig. 2. Histopathologic examination of ovaries: A. Group 1, normal ovarian tissue (H&Ex40), B. Group 2, intense hemorrhage, congestion and inflammation (H&Ex40), C. Group 3, minimal hemorrhage and congestion (H&Ex40).

Table 1. Histopathologic examinations of rats' ovaries

	Group 1 Median (min-max)	Group 2 Median (min-max)	Group 3 Median (min-max)	p*
Hemorrhage	1 (0-1)	3 (2-3)	2 (1-3)**	<0.001
Edema	1 (1-2)	3 (2-3)	2 (2-3)**	0.001
Vascular dilatation	1 (0-1)	3 (2-3)	2 (2-3)**	<0.001
Leukocyte infiltration	0(0-1)	2 (1-2)	2 (1-2)	0.004
Follicular atresia	0 (0-1)	1 (0-2)	0 (0-2)	0.175
Total score	4 (2-4)	12 (9-13)	9 (7-10)**	<0.001

*Kruskal-Wallis test, **Mann Whitney U test between group 2 and 3; p<0.05

hemorrhage (p< 0.001), edema (p=0.001) and vascular dilatation (p< 0.001) (Table 1). Comparing the sham group with the treatment group, we found statistical differences in terms of hemorrhage (p= 0.032), edema (p= 0.037) and vascular dilatation (p= 0.037).

Pathologic changes induced by I/R were reduced in ovaries of rats administered proanthocyanidin (Fig. 2), in particular, hemorrhage, edema and vascular dilatation. Total tissue damage scores were significantly different among groups (p< 0.001). Group 3 had significantly higher histologic scores compared with group 1 and group 2 (Mann-Whitney U test, p= 0.001 and 0.022, respectively).

Discussion

Proanthocyanidins are strong antioxidant, vasodilator, antithrombotic, anti-inflammatory and immunostimulant oligomeric flavonoids which are present in large quantities in grape seeds with known protective effect on hepatic, renal and myocardial ischemia. In the current study, it was shown that they may also be protective against I/R damage in ovarian torsion.

Preoperative exact diagnosis of ovarian torsion is very rare. It may be too late to protect the ovary surgically, due to delay in diagnosis and treatment (20-22). Ovarian torsion is the rotation of ovary or adnexa on its own vascular peduncle and axis to such a degree as to occlude the arterial, venous or lymphatic drainage. As a result massive parenchymal congestion, infarction and finally hemorrhagic necrosis may occur (23, 24). Abdominal pain and ovarian mass on ultrasonography are the hallmarks of the clinical diagnosis of ovarian torsion. Doppler sonography has been suggested to detect torsion but visualization of the torsed ovary by laparoscopy or laparotomy is necessary for accurate diagnosis (25).

In surgical treatment, there is no consensus about conservative management or radical adnexectomy. Thus, there is a need for an agent to stop or return ischemia in patients planning to have conservative management. Some substances such as nigella extract, erythropoietin and dehydroepiandrosterone have been used to prevent ischemia in ovarian torsion (26-28). In our study, grape seed extract was given orally and significant improvement was seen in the treatment group compared to control group.

In a healthy body, there is a balance between cellular antioxidant enzymes, antioxidant substances and free radicals. During the I/R period, free oxygen radicals can be harmful to cell membranes and intracellular substances (6, 29). Due to the huge amount of free radicals released in the reperfusion phase and as pathologic changes depend on the amount of these radicals, free oxygen radical scavengers have been investigated in many studies. Polyphenols such as procyanidins and proanthocyanidins are powerful free radical scavengers found in grape seed extract in large amounts (30). Improvement of the intervention group in our study supports the view that grape seed extract may be a powerful free radical scavenger. Furthermore, proanthocyanidin is vasodilatory, anti-carcinogenic, anti-allergic, anti-inflammatory, anti-bacterial, cardioprotective, immunostimulant, antiviral and has estrogenic activity (31). Pataki at al. (32) showed that proanthocyanidins reduced oxidative damage in cardiomyocytes and limited the infarct size in I/R damage. Some investigators reported that proanthocyanidin is useful in atherosclerosis, gastric ulcers, diabetic oxidative damage and also to potentiate the effect of chemotherapeutic drugs (17, 33, 34). Lastly, pharmacologic and therapeutic effects of grape seed extract on reduction of apoptotic cell death has also been reported (18).

In this study rats treated with proanthocyanidin showed significant recovery compared to the control group in terms of hemorrhage, vascular dilatation and edema. Indeed, there was improvement in leukocyte infiltration and follicular degeneration, though not statistically significant. We consider that the failure in these two parameters may depend on the dose and the duration.

Conclusion

Proanthocyanidin, known as a free radical scavenger and antioxidant, may be used in the protection against ovarian I/R injury. Further experimental and clinical investigations to regulate the dose and duration of treatment will complement this study.

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