

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 proanthocyanidin 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 proanthocyanidin 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: Proanthocyanidin, Fertility, Ischemia Reperfusion, Ovarian torsion.

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Introduction

Ovarian torsion is a surgical emergency that is frequently associated with a pre-existing 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 ische-

mia 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 there-

fore 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-

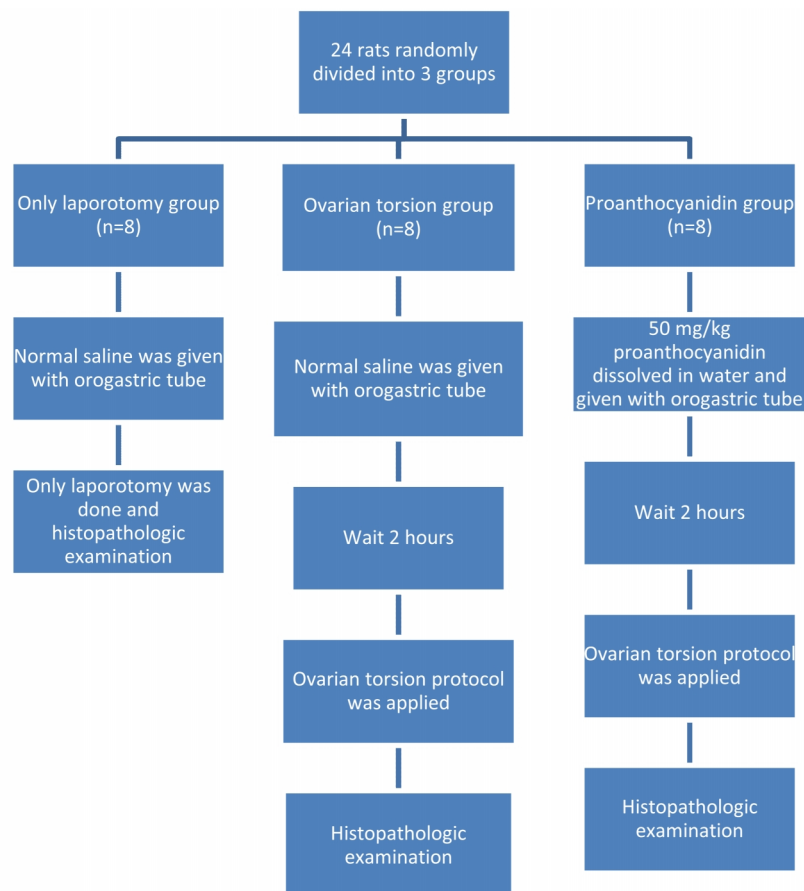


Fig. 1. Experimental model of the study

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 xylazine 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 counter-clockwise 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

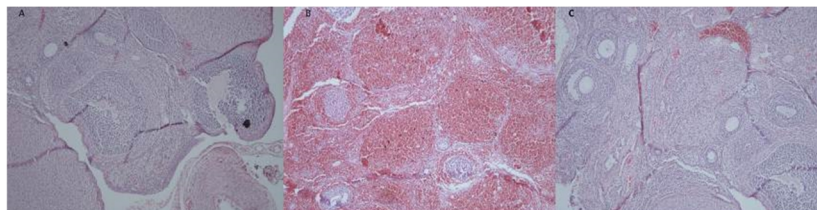


Fig. 2. Histopathologic examination of ovaries: A. Group 1, normal ovarian tissue (H&Ex40), B. Group 2, intense hemorrhage, congestion and inflammation (H&Ex40), C. Group 3, minimal hemorrhage and congestion (H&Ex40).

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. Dop-

pler 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 pro-cyanidins 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 et al. (32) showed that proanthocyanidins reduced oxidative damage in cardi-

omyocytes 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.

References

- Berger RL, Robbins G. Torsion of the normal ovary. *Am J Surg* 1961; 102:716–9.
- Meyer JS, Harman CM, Harty MP, Markowitz RI, Hubba AM, Bellah RD. Ovarian torsion: clinical and imaging presentation in children. *J Pediatr Surg* 1995;30:1433–6.
- Filho DW, Torres MA, Bordin AL, Crezcynski-Pasa TB, Boveris A. Spermatic cord torsion, reactive oxygen and nitrogen species and ischemia-reperfusion injury. *Mol Aspects Med*. 2004; 25(1-2):199-210.
- Oelsner G, Bider D, Goldenberg M, Admon D, Mashiach S. Long-term follow-up of the twisted ischemic adnexa managed by detorsion. *Fertil Steril* 1993; 60: 976–9.
- Sussman MS, Bulkley GB. Oxygen-derived free radicals in reperfusion injury. *Methods Enzymol* 1990; 186:711–23.
- Slater TF. Free-radical mechanisms in tissue injury. *Biochem J* 1984; 222:1–15.
- Halicı Z, Karaca M, Keles ON, Borekeci B, Odabasoglu F, Suleyman H, Cadirci E, Bayir Y, Unal B. Protective effects of amlodipine on ischemia–reperfusion injury of rat ovary: biochemical and histopathologic evaluation. *Fertil Steril* 2008; 90:2408–15.
- Mogilner JG, Lurie M, Coran AG, Nativ O, Shiloni E, Sukhotnik I. Effect of diclofenac on germ cell apoptosis following testicular ischemia–reperfusion injury in a rat. *Pediatr Surg Int* 2006; 22:99–105.
- Karaca M, Odabasoglu F, Kumtepe Y, Albayrak A, Cadirci E, Keles ON. Protective effects of erythropoietin on ischemia/reperfusion injury of rat ovary. *Eur J Obstet Gynecol Reprod Biol* 2009; 144:157–62.
- Yeung SY, Huang CS, Chan CP, Lin CP, Lin HN, Lee PH, et al. Antioxidant and pro-oxidant properties of chlorhexidine and its interaction with calcium hydroxide solutions. *Int Endod J*. 2007;40:837–44.[PubMed: 17877724]
- Mageshwaran T, Ebenezar AR, Madhanamadhubala M, Kavitha S, Mahalaxmi S. Counteraction of reactive oxygen species and determination of antibacterial efficacy of proanthocyanidin and lycopene when mixed with calcium hydroxide and chlorhexidine mixture: An in vitro comparative study. *J Conserv Dent*. 2012 Oct; 15(4): 337–41.
- Aron PM, Kennedy JA. Flavan-3-ols: Nature, occurrence and biological activity. *Mol Nutr Food Res*. 2008;52:79–104.[PubMed: 18081206].
- Yanarates O, Guven A, Sizlan A, Uysal B, Akgul O, Atim A, Ozcan A, Korkmaz A, Kurt E. Ameliorative effects of proanthocyanidin on renal ischemia/reperfusion injury. *Ren Fail*. 2008;30(9):931-8. doi: 10.1080/08860220802359410.
- Karaaslan O, Ulusoy MG, Kankaya Y, Tiftikcioglu YO, Kocer U, Kankaya D, Karaaslan GM, Tuncer S, Berktaş M. Protective effect of grape seed extract against ischaemia/reperfusion injury in a rat epigastric flap model. *J Plast Reconstr Aesthet Surg*. 2010 Apr;63(4):705-10. doi: 10.1016/j.bjps.2009.01.018. Epub 2009 Feb 25.
- Guler A, Sahin MA, Yucel O, Yokusoglu M, Gamsizkan M, Ozal E, Demirkilic U, Arslan M. Proanthocyanidin prevents myocardial ischemic injury in adult rats. *Med Sci Monit*. 2011 Nov;17(11):BR326-331.
- <http://comudam.comu.edu.tr/index>. Çanakkale Onsekiz Mart Üniversitesi, Experimental Research Application and Research Center.
- Bagchi D, Sen CK, Ray SD, Das DK, Bagchi M, Preuss HG, Vinson JA: Molecular mechanisms of cardioprotection by a novel grape seed proanthocyanidin extract. *Mutat Res*, 523-524, 87-97, 2003.
- Li WG, Zhang XY, Wu YJ, Tian X: Anti-inflammatory effect and mechanism of proanthocyanidins from grape seeds. *Acta Pharmacol Sin*, 22 (12): 1117-1120, 2001.
- Kara M, Daglioglu YK, Kuyucu Y, Tuli A, Tap O. The effect of edaravone on ischemia-reperfusion injury in rat ovary. *Eur J Obstet Gynecol*

col Reprod Biol. 2012 Jun;162(2):197-202. doi: 10.1016/j.ejogrb.2012.02.026. Epub 2012 Mar 22.

20. Taskin O, Birincioglu M, Aydin A, Buhur A, Burak F, Yilmaz I, Wheeler JM. The effects of twisted ischaemic adnexa managed by detorsion on ovarian viability and histology: an ischaemia-reperfusion rodent model. *Hum Reprod* 1998; 13:2823-27.

21. Spigland N, Ducharme JC, Yazbeck S. Adnexal torsion in children. *J Pediatr Surg* 1989; 24:974-6.

22. Van der Zee DC, Van Seumeren IGC, Bax KMA, Rövekamp MH, Pullter Gunne AJ. Laparoscopic approach to surgical management of ovarian cysts in the newborn. *J P Surg* 1995; 30: 42-43.

23. Lee EJ, Kwon HC, Joo HJ, Suh JH, Fleischer AC. Diagnosis of ovarian torsion with color Doppler sonography: depiction of twisted vascular pedicle. *J Ultrasound Med* 1998;17:83-9.

24. Graif M, Itzhak Y. Sonographic evaluation of ovarian torsion in childhood and adolescence. *AJR Am J Roentgenol* 1988;150:647-9.

25. Moravec WD, Angerman NS, Reale FR, Hajj SN. Torsion of the uterine adnexa: a clinicopathologic correlation. *Int J Gynaecol Obstet* 1980;18(1): 7-14.

26. Sak ME, Soydinc HE, Sak S, Evsen MS, Albalik U, Akdemir F, et al. The protective effect of curcumin on ischemia-reperfusion injury in rat ovary. *Int J Surg*. 2013 Jun 21. pii: S1743-9191(13)00168-4. doi: 10.1016/j.ijsu.2013.06.007. [Epub ahead of print]

27. Borekci B, Gundogdu C, B. Altunkaynak BZ, Calik M, Altunkaynak ME, Unal D, et al. The Protective Effect of Dehydroepiandrosterone on Ovarian Tissues After Torsion-Detorsion Injury: A Ste-

reological and Histopathological Study. *EAJM*, 2009;41:22-7.

28. Kolusarı A, Kamacı M, Zeteroğlu Ş, Altunay H, Şahin G. Rat Overinde İskemi Reperfüzyon Modeline Eritropoetin Koruyucu Etkisi. *Türkiye Klinikleri J Med Sci* 2010;30(4):1189-95.

29. Delabar JM, Nicole A, D'Auriol L, Jacob Y, Meunier-Rotival M, Galibert F, Sinet PM, Jérôme H: Cloning and sequencing of a rat CuZn superoxide dismutase cDNA. *Eur J Biochem*, 166 (1): 181-187, 1987.

30. Bagchi D, Garg A, Krohn RL, Bagchi M, Tran MX, Stohs SJ. Oxygen free radical scavenging abilities of vitamins C and E, and a grape seed proanthocyanidin extract in vitro. *Res Commun Mol Pathol Pharmacol* 2007, 95:179-89.

31. Rice-Evans CA, Miller NJ, Paganga G. Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radic Biol Med*. 1996;20(7):933-56. Erratum in: *Free Radic Biol Med* 1996;21(3):417.

32. Pataki T, Bak I, Kovacs P, Bagchi D, Das DK, Tosaki A. Grape seed proanthocyanidins improved cardiac recovery during reperfusion after ischemia in isolated rat hearts. *Am J Clin Nutr*, 75 (5): 894-899, 2002.

33. Vinson JA, Mandarano MA, Shuta DL, Bagchi M, Bagchi D. Beneficial effects of a novel IH636 grape seed proanthocyanidin extract and a niacin-bound chromium in a hamster atherosclerosis model. *Mol Cell Biochem*, 240 (1-2): 99-103, 2002.

34. Zhang XY, Bai DC, Wu YJ, Li WG, Liu NF: Proanthocyanidin from grape seeds enhances anti-tumor effect of doxorubicin both in vitro and in vivo. *Pharmazie*, 60 (7): 533-538, 2005.