Potential therapeutic effect of pomegranate seed oil on ovarian ischemia/reperfusion injury in rats

Muhammed Yayla 1*, Damla Cetin 1, Yasemen Adali 2, Pinar Aksu Kilicle 3, Erdem Toktay 4

1Department of Pharmacology, Faculty of Medicine, Kafkas University, Kars, Turkey
2Department of Pathology, Highlighted sentences should be changed as: Canakkale Onsekiz Mart University Faculty of Medicine, 17100 Canakkale/Turkey
3Department of Biology, Canakkale Onsekiz Mart University Faculty of Medicine, 17100 Canakkale/Turkey
4Department of Histology and Embryology, Atatürk University, Faculty of Medicine, 25240 Erzurum/Turkey

Abstract: The aim of this study is to determine the therapeutic effects of pomegranate seed oil, which is a powerful antioxidant and anti-inflammatory agent, on ovarian-ischemia and reperfusion injury in rats.

Keywords: Ischemia/Reperfusion, Oxidative stress, Ovary, Pomegranate seed oil, Punicaeae, Rats

Introduction

Development of ischemia in the vessels supplying the ovary is serious health problems that cause especially young girls to be sterile and lead to psychosocial disorders in the world (1). Ovarian cysts, pregnancy, polycystic ovary syndrome and transient or permanent obstruction of the ovarian artery may cause ischemia. However, the most common pathologic condition that eventuate as ovarian ischemia is ovarian torsion (1). If timely and adequate surgical and medical intervention done, fertility can be maintained by avoiding tissue damage (2). During ovarian ischemia, the energy production ceases in the absence of oxygen and resulting a series reactions with oxidative stress (3). Increased oxidative stress cause tissue damage which can be end up with necrosis or apoptosis (4, 5).

In the treatment of ischemia, it is aimed to reperfusion which can be identified as providing blood supply again (3). However, studies showed that injury is exacerbated during reperfusion (3, 6) because of increasing free oxygen radicals, endothelial damage and inflammation (3, 6, 7). Experimental and clinical studies carried out to overcome this damage have shown that agents with antioxidant activity can prevent oxidative stress-induced damage (8). Although there are many antioxidant agents in the literature, there is no effective treatment on the ovarian ischemia and reperfusion injury. Therefore, the development and studying of antioxidant and anti-inflammator substances is also important for the future science.

Pomegranate (Punica granatum), an important member of the Punicaeae family, is known a fruit with many features since ancient times (9). Pomegranate consists of 3 parts; seed (3% of the weight), water (30% of the weight) and peel (9). In recent years significant progress has been made in the identification of the chemical components of pomegranate and their pharmacological effects (10). Pomegranate seeds are rich in sugar, unsaturated- polyunsaturated fatty acids, vitamins, polysaccharides, polyphenols and minerals (11). In particular, pomegranate seed oil contains high levels of phenolic compounds which is punicic acid, punicalagins (PNG), as well as important fatty acids such as linoleic acid, gallic acid and elagic acid (12, 13).

The high amount of punicic acid and PNG in pomegranate seed oil provides many beneficial biological effects such as anti-inflammatory, antioxidant, anti-apoptotic, anticancer and so on (14, 15). Other fatty acids found in the seed oil also have strong antioxidant and anti-inflammatory effects. Therefore, all these conditions suggest that the pomegranate seed oil may have serious therapeutic effects. Many experimental
Material and Methods

A total of 56 female albino Wistar rats were used in the experiments. Each rat weighed 200–250 g, and all were obtained from Ataturk University’s Experimental Animal Laboratory at the Medicinal and Experimental Application and Research Centre. The animal experiments and procedures were performed in accordance with national guidelines for the use and care of laboratory animals and approved by Kafkas University’s Local Animal Care Committee (28.01.2016-2016/19). The rat was housed in standard plastic cages on sawdust bedding in an air-conditioned room at 22± 1°C. Standard rat food and tap water were given ad libitum.

Chemicals

All of the chemicals used in our laboratory experiments were purchased from Sigma Chemical Co (Munich, Germany). Punica granatum spp., known as Hicaz in our country, was purchased from city of Mersin (obtained year: 2016). Thiopental sodium was obtained from IE Ulagay AS (Istanbul, Turkey).

Preparation of pomegranate seed oil extract

Essential oils of the plants were obtained on a Clevenger (Wisd-Wise Therm) device by water vapor distillation. For this purpose, the fruits were dried and then the seeds were separated. One hundred and sixty g of the plant was pulverized in the shredder. The sample was placed in a glass balloon and 1600 ml of distilled water was added to it, then placed in a Clevenger apparatus and the apparatus was operated. After the evaporation started, it was left to stand for 3 hr. During this time, the hydrosol accumulated in the Clevengerin collection tube was taken in a sterile separate bottle. After the taken last hydrosol accumulated in the Clevenger apparatus, the apparatus was operated. After the evaporation started, it was left to stand for 3 hr. During this time, the hydrosol accumulated in the Clevengerin collection tube was taken in a sterile separate bottle. After the taken last hydrosol accumulated in the collection tube, the remaining volatile oil was stored in the dark bottles in the refrigerator at + 4°C until used in the experiment.

Analysis of ingredients in pomegranate seed oil extract by GC/MS

Ingredients and fatty acids were detected in extract and quantitative determinations were made on the Agilent GC/MS (Germany).

The extract was dissolved in methanol to a concentration of 1 mg/ml and a stock solution was obtained and centrifuged at 10000 rpm for 5 min. Workup solutions were prepared by diluting the supernatant with phosphate buffer (pH = 2.5, 0.025 M). The working solutions were injected into the system after passing through the injection filter. Each injection was repeated three times (Table 1).

Surgical technique and PSO administration

Animals were anesthetized via intra-peritoneal (IP) injections of 25 mg/kg thiopental sodium. A longitudinal incision (2.5 cm) was created in the midline area of the lower abdomen. A small peritoneal incision was made, and the uterine horns and adnexa were located. Bilateral ovarian ischemia was induced by applying vascular clips below the ovaries. Three hours after ischemia, reperfusion was performed and then 3 hr after the rats’ ovaries were collected (16, 17). PSO was used in 0.32 and 0.64 ml/kg doses (IP injection). PSO administration to the treatment groups was performed 30 minutes before ischemia or reperfusion application as described below.

| Group | Description | Ovarian ischemia or reperfusion dose |
|-------|-------------|------------------------------------|
| 1     | Sham operation | 0.32 ml/kg PSO |
| 2     | Ischemia for 3 hr | 0.64 ml/kg PSO |
| 3     | Ischemia for 3 hr | 0.32 ml/kg PSO |
| 4     | Ischemia for 3 hr | 0.64 ml/kg PSO |
| 5     | Ischemia for 3 hr | 0.32 ml/kg PSO |
| 6     | Ischemia for 3 hr | 0.64 ml/kg PSO |
| 7     | Ischemia for 3 hr | 0.32 ml/kg PSO |

Biochemical analyses

Rat tissues were kept at -86°C. 100 mg of tissue from each rat was firstly perfused with PBS/heparin. All tissue samples from each rat were ground in liquid nitrogen using a Tissuelyser II grinding jar set (Qiagen, Hilden, Germany). Then they were centrifuged according to the manufacturer’s instructions. Subsequently, SOD activity, and MDA level, GSH level, NOX1 activity and TNF-α levels from each supernatant were measured in duplicate with highly sensitive kits (Cayman-706002, 10009055, 703002 (USA), and Sunredbio-201611, 201-705 (China), respectively), specifically designed for rat tissue, according to the manufacturer’s instructions. The protein concentrations were determined by the Lowry method using commercial protein standards (Sigma Aldrich, Total protein kit-TP0300-1KT(USA)). All the data was presented as the mean ± standard deviation (S.D.) results based on per mg of protein.

Table 1. Amounts of substance in 1.00-g extract of pomegranate seed oil

| Substance | Amounts (Relative, %) |
|-----------|-----------------------|
| Palmitic Acid (16:0) | 0.9 |
| Stearic Acid (18:0) | 0.8 |
| Oleic Acid (18:1) | 2.1 |
| Linoleic Acid (18:2) | 2.6 |
| Arachidic Acid (20:0) | 0.3 |
| Punicic Acid (18:3) | 45.1 |
| Punicic Acid isomer (18:3) | 14.8 |
| Punicic Acid isomer (18:3) | 14.8 |
| Punicic Acid isomer (18:3) | 15.0 |

% flame ionization detector (FID)

*Punicic Acid: 92, 11E, 13z octatrienoic acid

Determinations were performed in triplicate and results are presented as the mean ± standard deviation (S.D.) results based on per mg of protein.
Statistical analyses

IBM SPSS statistical software Version 20.0 was used for the biochemical analysis. The results are presented as the means ± standard deviation (SD). Between-group comparisons for biochemical and molecular analyses were performed with one-way ANOVA and Duncan’s multiple comparison tests. Significance was accepted at \( p < 0.05 \). Means in the same column with the same letter are not significantly different; means in the same column with different letters indicate significant differences between the groups according to the Duncan test.

Results

Oxidative stress marker

Tissue MDA levels

In our study, MDA, an important marker of oxidative stress-related tissue damage, was measured by ELISA in all groups (Figure 1). MDA levels in ischemia and I/R groups were found to be significantly increased compared to healthy group \( (P=0.011, P=0.001, \text{respectively}) \). Reduced MDA levels observed in low dose treated PSO groups while no significant MDA level reduction observed in high dose treated groups \( (P=0.023, P=0.861, \text{respectively}) \). It is thought here that PSO may have therapeutic benefits up to certain doses.

Tissue NOX1 levels

In our study, we assessed the level of NOX1, which has an important role in the formation of oxidative damage and whose expression has also been detected in oocyte cells (Figure 2). There was a significant increase in NOX1 level in the ischemia and I/R groups compared to the control group \( (P=0.044, P=0.001, \text{respectively}) \). It has been observed that PSO administration, especially in the 1st dose, prevented both ischemia and reperfusion injury and regresses the NOX1 levels \( (P=0.028, P=0.013, \text{respectively}) \). It is thought that increased NOX1 due to damage and decreased NOX1 due to treatment may have significant effects on the development of ovarian ischemia and reperfusion injury.

Antioxidant markers

Tissue SOD activity and glutathione levels

SOD and GSH are major defense mechanisms that protect our cells against oxidants. In situations such as ischemia, the reduction of antioxidant defense system in our tissues leads to the development and increases in damage. In our study, antioxidants decreased significantly in ischemia and I/R groups compared to healthy group (Figure 3-4) \( (P=0.001, P=0.003, \text{respectively}) \). PSO first dose application increased antioxidant defense systems in both ischemia and I/R groups \( (P=0.001, P=0.015, P=0.015, P=0.037, \text{respectively}) \). However, the second dose of PSO did not show any protective effect on antioxidant defense system.

Inflammatory markers

TNF-α tissue levels and mRNA expressions

We assessed TNF-α level, a pro-inflammatory cytokine that contribute to the development of ischemia-reperfusion injury, both molecular and
biochemically in our study (Figure 5-6). Tissue TNF-α expression increased 3-4 fold in ischemia and reperfusion groups compared to control \((P=0.043, P=0.02, \text{ respectively})\). With PSO application, TNF-α expression was significantly down regulated, especially in the 1st dose \((P=0.001)\). Biochemical findings of TNF-α are also parallel to TNF-α mRNA expression. While there was no significant difference between the ischemia and I/R groups of the tissue TNF-α level at the end of the ELISA measurement \((P=0.318)\), TNF-α expression was more exaggerated in the I/R group than in the ischemia group \((P=0.02)\). As it is determined here, increasing in the gene level of the inflammatory cytokines cannot be completely transformed into an active product.

**Pathological results**

Pathological examinations of ovary tissues were performed in H&E staining study (Table 2, Figure 7-8). Necrotic cells and neutrophil infiltration were not found in any group. In the ischemia and I/R groups, hemorrhagic foci were severe, but in the PSO group, especially in the first dose, the pathological changes were mild. While the capillary permeability increased in the ischemia and reperfusion groups, PSO application revealed to decrease capillary permeability. Moderate apoptotic cell foci were detected in I/R group (Figure 7) which suggests the severity of the damage after reperfusion. However, no apoptotic cell foci were seen in the other groups.

| Table 2. Pathological scoring |
|-----------------------------|
| Groups          | Hemorrhage | Apoptotic cell | Increased capillary permeability |
| Healthy         | 0          | 0              | 0                               |
| Ischemia        | 3          | 0              | 2                               |
| I/R             | 2          | 2              | 3                               |
| I+PSO1          | 1          | 0              | 1                               |
| I+PSO2          | 2          | 0              | 2                               |
| I+R+PSO1        | 1          | 0              | 1                               |
| I+R+PSO2        | 2.5        | 0              | 2                               |

0: none, 1: mild, 2: moderate, 3: severe, I: ischemia I/R: ischemia and reperfusion, PSO: pomegranate seed oil
Yayla et al. Pomegranate on ovarian ischemia in rats

Yayla et al. Pomegranate on ovarian ischemia in rats via biochemical, molecular and pathological analyses.

Discussion

We have shown the potential therapeutic effects of pomegranate oil in experimental ovarian I/R injury in rats via biochemical, molecular and pathological analyses.

Pomegranate is one of the most consumed fruit since ancient times for strong antioxidant and anti-inflammatory activity. Many pharmacological effects of fruit juice, peel and seed extracts of the pomegranate have been shown. In particular, pomegranate seed oil (PSO) is enriched punicic acids and punicalagins. It also contains many fatty acids (especially linoleic acid, gallic acid and elagic acid). Punicic acid, which is abundant in pomegranate seed, has common pharmacological properties. Previous studies demonstrated the protective effect of PSO in different experimental I/R models and PSO showed these effects by reducing oxidative stress, increasing antioxidants and preventing-limiting inflammation (20, 21).

Oxidative stress is the primary cause of ovarian ischemia and reperfusion damage. Disruptions of energy metabolism in the cells undergo stress, oxidant agents are started to be produced (20-22). MDA is the final degradation product of membrane proteins during oxidative stress. In experimental studies, MDA, an indicator of oxidative damage, has measured as a gold standard. In our study, oxidative stress significantly increased in ischemia and I/R groups, while PSO administration significantly reduced the MDA level. Previous studies demonstrated that PSO administration significantly decreased oxidative stress and MDA levels (23). In this respect, PSO exerted antioxidant effect against ovarian injury during ischemia and I/R.

During ischemia and reperfusion changing in the structure and function of many enzymes also contributes to aggravation of oxidative stress and inflammation. The most important of these are xanthine oxidase (XO) and NADPH oxidase (NOX) (24, 25). XO cause damage in acute phase of ischemia and reperfusion injury. NOX is involved in the late phase of I/R (3). In our study, 3-hr ischemia and 3-hr reperfusion model (a total 6-hr I/R model) were established. In the late phase of reperfusion, NOX increase oxidative damage and stimulates pro-inflammatory cytokines such as TNF-α. NOX is normally synthesized from phagocytic cells (26). Recently, it has been shown NOX expressed and synthesized in many tissues and cells and its subtypes 1 to 5. NOX1 has been shown to be present in ovaries and oocytes in experimental animals (27). NOX1 is mainly derived from phagocytic cells (26). Unlike NOX2, NOX1 directly stimulates TNF-α expression (28). This suggests that NOX1 also may have important effects on acute inflammatory response in I/R injury. NOX1 also induces apoptosis of cells during I/R (29).

In our study, NOX1 level was found to be at a high level in the ischemia and I/R group and parallel to this, cells were shown to apoptosis after reperfusion. TNF-α level significantly increased in parallel with the level of NOX1 (28). TNF-α has an important role of developing inflammation and triggering apoptosis of cells. Ferreira et al. demonstrated that PSO has a strong anti-inflammatory and anti-nociceptive activity and it is proposed PSO may be used as alternatives for NSAIDs (30, 31). In another study, PSO and punicic acid exert anti-inflammatory effects on colon inflammation in rats. Current study, PSO and punicic acid inhibited TNF-α induced priming of NADPH oxidase by targeting the p38MAPKinase/Ser345-p47phox-axis (32). This respect, current study demonstrated that the increased expression of TNF-α parallel with NOX1 caused both inflammation and the onset of apoptosis in the late phase of reperfusion. First dose of PSO administration significantly prevented irreversible damage related to improved NOX1 and TNF-α level.

Figure 7. Sham: A) 100x, H-E staining results of Sham group. White arrow: corpus luteum, yellow: mild vascular congestion, green: ovarian follicles. Ischemia: A) 40x, H-E staining results of ischemia. White arrow: severe hemorrhage B) 100x, White arrow: severe hemorrhage, yellow: corpus luteum, green: ovarian follicles. Ischemia and Reperfusion: A) 40x, H-E staining results of I/R groups. White arrow: severe hemorrhage, yellow: corpus luteum, green: ovarian follicles. B) 100x, White arrow: vascular congestion, yellow: hemorrhagic corpus luteum C) 200x, H-E staining results of I/R groups. white arrow: apoptotic cells.

Figure 8. 40x, H-E staining results of PSO1+ischemia groups. white arrow: vascular congestion. 100x, H-E staining results of PSO2+ischemia groups. White arrow: vascular congestion, yellow: ovarian follicle. 40x, H-E staining results of PSO1+I/R groups. white arrow: mild hemorrhagic and congestion, yellow: corpus luteum, green: primary ovarian follicle. 100x, H-E staining results of PSO2+I/R groups. moderate hemorrhagic and congestion, yellow: corpus luteum, green: primary ovarian follicle; PSO: Pomegranate seed oil.
There are many mechanisms in our body to prevent I/R injury. Antioxidants are responsible for the scavenging of the oxidants. SOD is known one of the most important antioxidant and GSH, another important antioxidant, allows free radicals and toxins to be removed by glucronyl conjugation. SOD and GSH protect our cells against the cytotoxic effects of free radicals. A reduction in SOD activity and GSH levels in ischemia and reperfusion may be due to the overproduction of superoxide radical anions (33-35).

In our study, PSO application significantly improved antioxidant activity in ischemia and I/R groups. However, PSO has shown very low pharmacological activity at high doses. This suggests that natural products may have therapeutic efficacy in certain dose ranges, it is clear that excessively consumed pomegranate will not have a therapeutic benefit.

We finally assessed the pathology of over-tissues to support all of these findings. Severe hemorrhage and increased capillary permeability were seen in the ischemia and reperfusion groups. Increased capillary permeability lead to exacerbation of inflammation during ischemia. This supporting multiple mechanisms have role in ischemia and reperfusion injury. It was also seen that in I/R group, damage progressed and apoptosis occurred. In previously, it is demonstrated that anti-apoptotic effect of PSO on brain hypoxic ischemia through inhibition of caspase 3 (36, 37). In our study, PSO administration has been able to protect the cells especially in the first dose by showing anti-apoptotic effect. If there is no operation and medical treatment during I/R, the damage is irreversible and the person will be sterile.

Conclusion
PSO has shown protective effect on ovarian ischemia and reperfusion injury by decreasing oxidative stress, improving TNF-α and NOX1 levels, increasing antioxidant defense system and preventing development of apoptosis. It has been shown in our study that PSO may be a potential therapeutic agent in the ovarian-I/R injury. However, our study should be supported by more detailed experimental studies and clinical trials. Also, the effects of Punicalagins and other ingredients of PSO should be demonstrated directly on ovarian I/R injury.

Acknowledgment
This work was supported by Kafkas University Scientific Research Project (grant number 2016TS32). A part of this study was presented at international congress by 2nd International Science Symposium (Science Festival) Tiflis, Georgia, 2017. There is no conflict interest and financial disclosure for all author.

References
1. Hibbard LT. Adnexal torsion. Am J Obstet Gynecol 1985; 152:456-461.
2. Hasilakos D, Papakonstantinou K, Kontoravdis A, Gogas L, Aravantinos L, Vitoratos N. Adnexal torsion during pregnancy: report of four cases and review of the literature. J Obstet Gynaecol Res 2008; 34:683-687.
3. Kalogeris T, Baines CP, Krenz M, Korthuis RJ. Cell biology of ischemia/reperfusion injury. Int Rev Cell Mol Biol 2012; 298:229-317.
4. Kroemer G, Galluzzi L, Brenner C. Mitochondrial membrane permeabilization in cell death. Physiol Rev 2007; 87:99-163.
5. Broughton BR, Reuten D, Sobey CG. Apoptotic mechanisms after cerebral ischemia. Stroke 2009; 40:e331-339.
6. Cerqueira NF, Husni CA, Yoshida WB. Pathophysiology of mesenteric ischemia/reperfusion: a review. Acta Cir Bras 2005; 20:336-343.
7. Piper HM, Meuter K, Schafer C. Cellular mechanisms of ischemia-reperfusion injury. Ann Thorac Surg 2003; 75:S644-648.
8. Perrelli MG, Pagliaro P, Penna C. Ischemia/reperfusion injury and cardioprotective mechanisms: Role of mitochondria and reactive oxygen species. World J Cardiol 2011; 3:186-200.
9. Bedriniam Yilmaz, Usta. C. Therapeutic effects of punica granatum. Türk Aile Hek Derg 2010; 14:146-153.
10. Lansky EP, Newman RA. Punica granatum (pomegranate) and its potential for prevention and treatment of inflammation and cancer. J Ethnopharmacol 2007; 109:177-206.
11. Afac F, Saleem M, Krueger CG, Reed JD, Mukhtar H. Anthocyanin- and hydrolyzable tannin-rich pomegranate fruit extract modulates MAPK and NF-kappaB pathways and inhibits skin tumorigenesis in CD-1 mice. Int J Cancer 2005; 113:424-433.
12. Gil MI, Tomas-Barberan FA, Hess-Pierce B, Holcroft DM, Kader AA. Antioxidant activity of pomegranate juice and its relationship with phenolic composition and processing. J Agric Food Chem 2000; 48:4581-4589.
13. Xu J, Guo CJ, Yang J, Wei JY, Li YF, Pang W, et al. [Intervention of antioxidant system function of aged rats by giving fruit juices with different antioxidant capacities]. Zhonghua Yu Fang Yi Xue Za Zhi 2005; 39:80-83.
14. Aviram M, Rosenblat M. Pomegranate Protection against Cardiovascular Diseases. Evid Based Complement Alternat Med 2012; 2012:382763.
15. Villadomiu M, Hontecillas R, Lu P, Bassaganya-Riera J. Preventive and prophylactic mechanisms of action of pomegranate bioactive constituents. Evid Based Complement Alternat Med 2013; 2013:799764.
16. Alsak Karamese S, Toktay E, Unal D, Selli J, Karamece M, Malkoc I. The protective effects of beta-carotene against ischemia/reperfusion injury in rat ovarian tissue. Acta Histochem 2015; 117:790-797.
17. Bayir Y, Cadirci E, Polat B, Kilic Baygutalp N, Albayrak A, Karakus E, et al. Alsikiren - a promising strategy for ovarian ischemia/reperfusion injury protection in rats via RAAS. Gynecol Endocrinol 2016; 32:675-683.
18. Tatar A, Yayla M, Kose D, Halici Z, Yoruk O, Polat E. The role of endothelin-1 and endothelin receptor antagonists in allergic rhinitis inflammation: ovalbumin-induced rat model. Rhinology 2016; 54:266-272.
19. Palabyik SS, Karakus E, Alpinar E, Halici Z, Bayir Y, Yayla M, et al. The role of urotensin receptors in the paracetamol-induced hepatotoxicity model in mice: ameliorative potential of urotensin II antagonist. Basic Clin Pharmacol Toxicol 2016; 118:150-159.
20. Hashem HE, Abd El-Haleem MR, Amer MG, BorI A. Pomegranate protective effect on experimental ischemia/reperfusion retinal injury in rats (histological and biochemical study). Ultrastruct Pathol 2017; 41:346-357.
21. Ahmed MA, El Morsy EM, Ahmed AA. Pomegranate extract protects against cerebral ischemia/reperfusion injury and preserves brain DNA integrity in rats. Life Sci 2014; 110:61-69.
22. Sancaktutar AA, Bodakci MN, Hatipoglu NK, Soylemez H, Basarli K, Turlcu G. The protective effects of pomegranate extracts against renal ischemia-reperfusion injury in male rats. Urol Ann 2014; 6:46-50.
23. Boroushaki MT, Asadpour E, Sadeghnia HR, Dolati K. Effect of pomegranate seed oil against gentamicin-induced nephrotoxicity in rat. J Food Sci Technol 2014; 51:3510-3514.
24. Dorweiler B, Pruefer D, Andrasi TB, Maksan SM, Schmiedt W, Neufang A, et al. Ischemia-Reperfusion Injury: Pathophysiology and Clinical Implications. Eur J Trauma Emerg Surg 2007; 33:600-612.
25. Collard CD, Gelman S. Pathophysiology, clinical manifestations, and prevention of ischemia-reperfusion injury. Anesthesiology 2001; 94:1133-1138.
26. Bedard K, Krause KH. The NOX family of ROS-generating NADPH oxidases: physiology and pathophysiology. Physiol Rev 2007; 87:245-313.
27. Maru Y, Nishino T, Kakinuma K. Expression of Nox genes in rat organs, mouse oocytes, and sea urchin eggs. DNA Seq 2005; 16:83-88.
28. Kim YS, Morgan MJ, Cholsi S, Liu ZG. TNF-induced activation of the Nox1 NADPH oxidase and its role in the induction of necrotic cell death. Mol Cell 2007; 26:675-687.
29. Kahles T, Kohnen A, Heumueller S, Rappert A, Bechmann I, Liebner S, et al. NADPH oxidase Nox1 contributes to ischemic injury in experimental stroke in mice. Neurobiol Dis 2010; 40:185-192.
30. Ferreira LM, Sari MHM, Cervi VF, Gehrcke M, Barbieri AV, Zborowski VA, et al. Pomegranate seed oil nanoemulsions improve the photostability and in vivo antinociceptive effect of a non-steroidal anti-inflammatory drug. Colloids Surf B Biointerfaces 2016; 144:214-221.
31. Costantini S, Rusolo F, De Vito V, Moccia S, Picariello G, Capone F, et al. Potential anti-inflammatory effects of the hydrophilic fraction of pomegranate (Punica granatum L.) seed oil on breast cancer cell lines. Molecules 2014; 19:8644-8660.
32. Boussetta T, Raad H, Letteron P, Gougerot-Pocidalo MA, Marie JC, Driss F, et al. Punicic acid a conjugated linolenic acid inhibits TNFalpha-induced neutrophil hyperactivation and protects from experimental colon inflammation in rats. PLoS One 2009; 4:e6458.
33. Polat B, Albayrak A, Halici Z, Karakuş E, Bayir Y, Demirci E, et al. The effect of levsimendan in rat mesenteric ischemia/reperfusion injury. J Invest Surg 2013; 26:325-333.
34. Albayrak Y, Halici Z, Odabasoglu E, Unal D, Keles ON, Malkoc I, et al. The effects of testosterone on intestinal ischemia/reperfusion in rats. J Invest Surg 2011; 24:283-291.
35. Vehbi Yavuz Tokgoz, Mehmet Sipahi, Ozlem Keskin, Gulanmke Findik Guvendi, Takir S. Protective effects of vitamin D on ischemia-reperfusion injury of the ovary in a rat model. Iran J Basic Med Sci 2017; 21:593-599.
36. West T, Atzeva M, Holtzman DM. Pomegranate polyphenols and resveratrol protect the neonatal brain against hypoxic-ischemic injury. Dev Neurosci 2007; 29:363-372.
37. Shaban NZ, El-Kersh MA, El-Rashidy FH, Habashy N. Protective role of Punica granatum (pomegranate) peel and seed oil extracts on diethylnitrosamine and phenobarbital-induced hepatic injury in male rats. Food Chem 2013; 141:1587-1596.