ERCİYES ÜNİVERSİTESİ VETERİNER FAKÜLTESİ DERGİSİ
Journal of Faculty of Veterinary Medicine, Erciyes University
Araştırma Makalesi / Research Article
17(1), 57-62, 2020
DOI: 10.32707/ercivet.705004

Investigation of the Effects of Boric Acid on Preventing Lung Damage in the End of the Lower Extremity Ischemia Reperfusion in Rats

Cahit ÖZCAN[1], Aydın Şükrü BENGÜ*[2], H.Turan AKKOYUN[3], Aykut ULUCAN[4], Mahire BAYRAMOGLU AKKOYUN[5]

[1]Siirt University, Faculty of Veterinary Science, Department of Animal Nutrition and Nutritional dease, Siirt-TURKEY
[2]Bingöl University, Department of Medical Services and Technics, Vocational School of Health Services,Program of Medical Laboratory Techniques, Bingöl-TURKEY
[3]Siirt University, Faculty of Veterinary Science, Department of Physiology, Siirt-TURKEY
[4]Bingöl University, Department of Medical Services and Technics, Vocational School of Health Services,Program of Pathology Laboratory Techniques, Bingöl-TURKEY
[5]Siirt University, Faculty of Veterinary Science, Department of Biochemistry, Siirt-TURKEY

Corresponding author: Aydın Şükrü BENGÜ; E-mail: aydinbengu75@gmail.com; ORCID: 0000-0002-7635-4855
How to cite: Ozcan C, Bengü AS, Akkoyun HT, Ulucan A, Bayramoğlu Akkoyun M. Investigation of the Effects of Boric Acid on Preventing Lung Damage in the End of the Lower Extremity Ischemia Reperfusion in Rats. Erciyes Üniv Vet Fak Derg 2020;17(1): 57-62.

Summary: In this study, the biochemical and histopathological changes in the lungs due to lower extremity ischemia-reperfusion injury and protective effect of boric acid to prevent them were investigated. The rats were divided into three groups as Group 1 (Sham group, n=9), Group 2 (Ischemia-reperfusion group, I/R, n=9) and Group 3 (Ischemia-reperfusion, (I/R) + boric acid (BA), n=9). Anaesthesia was applied to the first group of rats without any ischemia-reperfusion process. Following a two-hour ischemia, two hours of reperfusion, with the help of left lower extremities tourniquet, were applied to the second group. To the third group, 200 mg/kg (i.p.) boric acid application was performed 10 minutes before the ischemia was initiated and two hours of ischemia and two hours of reperfusion application were carried out. Some antioxidant enzymes in lung tissue (SOD, GSH-Px, CAT) were analyzed. In addition the lung tissue was evaluated histopathologically. Boric acid application was found to significantly enhance the positive effect of anti-oxidant enzymes. Normal histological structure was better preserved, some of macrophages were non-activated, and a remarkable reduction of neutrophil infiltration was seen after treatment in boric acid given group.
Key words: Antioxidant enzyme, boric acid, histopathology, ischemia/reperfusion, lung damage

Sıçanlarda Alt Ekstremite İskemi Reperfüzyonu Sonucunda Gelişen Uzak Doku Akciğer Hasarının Önlenmesinde Borik Asidin Etkilerinin İncelenmesi

Özet: Bu çalışmada, alt ekstremite iskemi reperfüzyon hasarı sebebiyle akciğerlerde meydana gelen biyokimyasal ve histopatolojik değişiklikler ve bunlara karşı borik asidin koruyucu etkisi araştırıldı. Sıçanlar Grup 1 (Kontrol/sham grubu, n=9), Grup 2 (İskemi-reperfüzyon grubu, I/R, n=9) ve Grup 3 (İskemi-reperfüzyon, (I/R) + Borik asit (BA), n=9) olmak üzere üç gruba ayrıldı. İskemi-reperfüzyon prosesi olmayan ilk sıçan grubuna sadece anestezi uygulandı. İkinci gruba iki saatlik bir iskemi sonrası, sol alt ekstremite turnike yardımıyla iki saatlik reperfüzyon uygulandı. Üçüncü gruba, iskemi başlamadan 10 dakika önce 200 mg / kg (i.p.) borik asit uygulaması yapıldı, iki saatlik iskemi ve iki saatlik reperfüzyon uygulaması yapıldı. Akciğer dokusunda bazı antioksidan enzimlerin (SOD, GSH-Px, CAT) aktiviteleri analiz edildi. Ayrıca akciğer dokusu histopatolojik olarak değerlendirildi. Borik asit uygulamasının antioksidan enzimlerin pozitif etkisini önemli ölçüde güçlendirdiği tespit edildi. Borik asit verilen grupta tedaviden sonra normal histolojik yapının daha iyi korunduğu, bazı makrofajların aktive edilmediği ve nötrofil infiltrasyonun da belirgin bir azalmanın olduğu gözlemlendi.
Anahtar kelimeler: Akciğer hasarı, antioksidan enzim, borik asit, histopatoloji, iskemi/reperfüzyon

Introduction

Lower extremity ischemia reperfusion has significant out comes commonly observed in clinical case studies. After reperfusion followed by ischemia and the impact of inflammatory mediator and free-oxygen radicals, the development of a local and ischemia damage will start (Grace, 1994; Klausner et al., 1989a; Klausner et al., 1988). Despite ischemia is saved through re-start of blood flow, it leads to the development of multiple organ dysfunction and it is especially observed in heart, renal and lungs (Blaisdell, 2002). Lung tissue is the target organ being affected by the lower extremity ischemia reperfusion most and its aetiology is not fully known (Tekeli et al., 2001; Uysal, 2006). Boric acid (BA) occurs naturally in both sea and fresh water sat 1-5 ppm. Its concentrations in the soil are more than waters

Geliş Tarihi/Submission Date : 28.01.2020
Kabul Tarihi/Accepted Date : 17.03.2020

(Büyükgüzel et al., 2013). Boric acid is abundant in fruits, vegetables and legumes, so it can be obtained in the diet with the consumption of these (Devirian and Volpe, 2003). Found in nature at high concentrations, BA is widely used in the industry, agriculture, cosmetics and other small practices. It is considered that the supplemental use of BA in human and animal food has considerable effects on the physiological and metabolic systems. Acute oral toxicity of inorganic boron-containing compounds is stated as 2660-4000 for rats LD50 (g/kg) (Fail et al., 1998). In some studies it is stated that BA has effects on minerals (Ca and P), vitamin D, hormones (insulin, oestrogen, testosterone, T3,T4), energy substrates (triglyceride, glucose) and reactive oxygen species (Hunt, 1988; Meacham et al., 1994; Hunt, 1996; Armstrong et al., 2001; Eren et al., 2006; Turkez et al., 2007). Nevertheless, the biochemical mechanism of BA is not completely known. In this study, we examined two things biochemically and histopathologically; the lung tissue damage caused by lower extremity ischemia/ reperfusion and the role of boric acid in preventing this damage.

Material and Methods

Experimental animals

Our experimental studies were carried out after the experimental animals were approved by the Bingöl University Ethic Committee of Animal Research (decision no:2018/02-02/06). The animals were kept in cages in a controlled room at a fixed 20-22 ^{0}C temperature and a 12-hour shift of light and dark cycle (light 07:00-19.00, dark: 19:00-07:00). They were provided with water and standard food. The experiments started after the rats were rested in their cages for a week in order to provide their adaptation to the environment. Rats were fed with pellet feed obtained from the market and analysis contents given in Table 1.

Table 1. Nutrient composition of pellets

Dry matter	87.2%
Crude protein	23.0%
Crude fat	3.7%
Crude fiber	7.7%
Ash	5.8%
Sodium	0.6%
Mn	96 mg/kg
Fe	31 mg/kg
Zn	960 mg/kg
Co	0.60 mg/kg
S	0.30 mg/kg
I	2.28 mg/kg

The rats were fasted for 12 hours before the experiments, with free access to water. A total of 27 male *Wistar albino* rats weighing between 200 and 300 grams each were randomly divided into three groups. Group 1 (control/sham group, n: 9); these rats were only given anaesthesia. Group 2 (ischemia/ reperfusion, I/R, n: 9); Group 3 (ischemia reperfusion (I/R) + BA, n: 9) where 10 minutes before ischemia, a 200 mg/kg dosage of BA was applied intraperitoneally as described Başbuğ et al. (2015). All the rats were exposed to ischemia for two hours through the bilateral lower extremity tourniquet method. The loss of arterial flow was determined through the inability to detect Doppler signals through Doppler USG. Two hours later, the tourniquets was opened, and reperfusion was performed for two hours (Klausner et al., 1989b). The control of arterial flow was again checked by Doppler USG. At the end of the fourth hour, the rats were sacrificed through injection of a high dosage of 60 mg/kg (i.p.) ketamine hydrochloride (Ketalar, Parke-Davis) and 10 mg/kg Xylazine (i.p.). Their lung tissues were taken out through mediastinotomy, and biochemical and histopathological analyses (SOD, GSH-Px, CAT levels) of the tissues were carried out.

Biochemical assays

Superoxide dismutase activity (SOD) was performed according to the method Sun et al. (1988). Tissue glutathione peroxidase's activity (GSH-Px) Paglia et al. (1967) and Catalase activity (CAT) were performed according to the method Aebi (1984).

Anaesthesia, necropsy and histopathologic examination

The main materials of this study were 10-week-old 27 *Wistar albino* rats in 3 groups (n:9). Firstly, all rats were anesthetized with intraperitoneal injection of 10 mg/kg xylazine and 60 mg/kg ketamine, and euthanasia was applied by decapitation under deep anaesthesia (Tranquilli et al., 2013). For light microscopy, the lungs were inflated via the trachea with 10% phosphate-buffered formaldehyde after sacrificing the animal as described above (Fiette and Slaouni, 2011). The lungs were then removed and placed into 10% phosphate-buffered formaldehyde until ready for sectioning and treated as routine histopathological

protocol of dehydrated samples in ascending grades of ethanol (50%, 70%, 96% and 100%) cleared in xylene and then embedded in paraffin (Bancroft et al., 2008). The paraffin-embedded tissue samples were sectioned at 5 µm thicknesses by a rotary microtome (Leica, RM2125). The slides were stained with haematoxylin & eosin (H&E) for histopathological analyses (Fisher et al., 2008). Finally, the slides were examined and photographed by using a light microscope with an imaging system (Leica, DM2500/ DFC295) for detection of degenerative changes in the lung tissues.

Statistical analysis

The results are expressed as mean ± the standard error of the mean ($\overline{X}$ ± SEM). The comparisons between the groups were done with Kruskal Wallis and Benferroni corrected Mann Whitney U test. The statistical analysis was performed utilizing SPSS®, v23 statistical software (SPSS Inc. Chicago Illinois).

Results

When the enzyme activities of SOD, CAT and GSH-Px are compared to the control group, it is clear that, while the values decreased in the ischemia reperfusion group. According to the I/R group, the SOD, CAT, GSH-PX enzyme activities increased in the group which received boric acid treatment compared to the I/R group. Moreover, in comparison to the control group, a significant decrease in the GSH-Px enzyme activity level was observed in the group that received boric acid treatment (Table 2 and Figures 1,2 and 3). In the control (sham) group (Group 1), no significant histopathologic lesions were observed in the investigated lung tissues, and the alveolus, bronchioles and pneumocytes displayed a normal histological structure (Figure 4-A). In the group that received I/R (ischemia/reperfusion) treatment (Group 2) in the lung tissues, interstitial and perivascular oedema, neutrophil leukocyte accumulation, a small amount of fibrin deposits, and erythrocyte permeation in alveolus swelling and desquamation of bronchiolar and alveolar epithelial cells were determined (Figure 4-B). In addition to this, in the compared lung tissues of the boric acid treatment group (Group 3), the histopathological findings were seento be significantly decreased. Only limited swelling of bronchiolar and alveolar epithelial cells and remarkable reduction of neutrophil infiltration were observed after treatment inthe group that was given boric acid (Figure IV-C).

Table 2. Distribution of SOD, CAT and GSH-Px values based on the groups

Enzyme (Eu/mg)	Control (sham)	Group 1 (I/R)	Group 2 (I/R+BA)	P values
SOD	2828.68±2.45 ^{a}	2809.73±1.86 ^{b}	2815.64±2.83^{a}	***
CAT	34.96±1.87 ^{a}	17.07±1.50 ^{b}	32.31±1.79 ^{a}	***
GSH-Px	1.66±0.057 ^{a}	0.8513±0.073 ^{c}	1.301±0.046^{b}	***

***: P<0.001; a,b,c: different letters, significant differences between control (Sham), Group1(I/R), Group 2(I/R+BA) groups

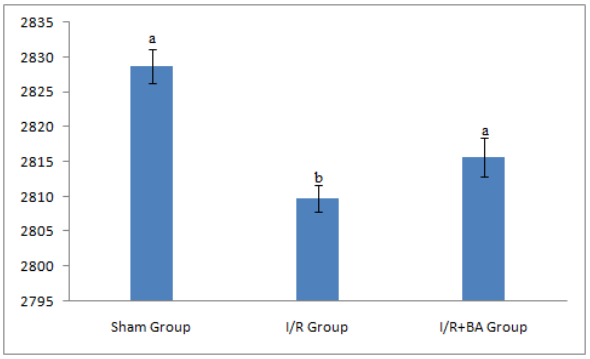

Figure 1. Super oxide dismutase levels in the lung tissues of the control (Sham), Group1(I/R), Group 2(I/R+BA) groups (SOD) (EU/g)

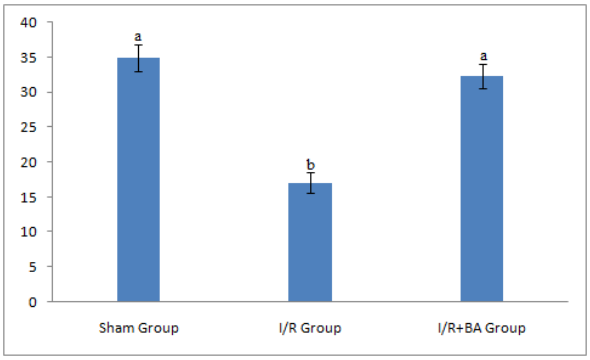

Figure 2. Catalase levels in the lung tissues of the control (Sham), Group1(I/R), Group 2(I/R+BA) groups (EU/g)

In this study, which evaluated the remote tissue damage that occurred in the lungs in connection with rat lower extremity ischemia reperfusion damage, antioxidant defence parameters such as SOD, CAT and GSH-Px enzyme activities were determined. The results ofthe SOD, CAT and GSH-Px values for each group are shown in Table 2.

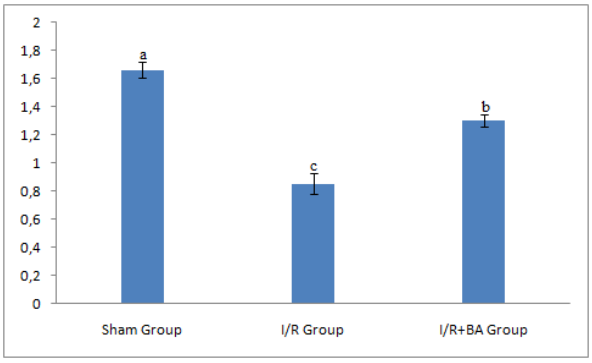

Figure 3. Glutation peroxidase levels in the lung tissue of the control (Sham), Group1(I/R), Group 2(I/R+BA) groups (EU/g)

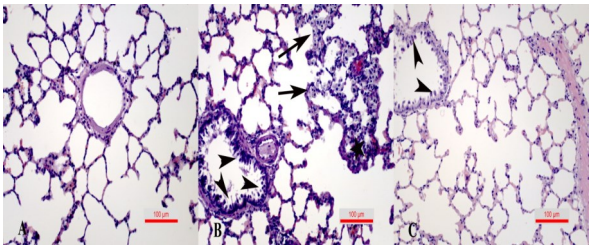

Figure 4. Histopathological Analyses of the lungs with H&E staining.(A): Normal histological structure of the lung tissue in the control group (Group 1). Swelling and desquamation of bronchiolar (arrow heads) and alveolar (arrows) epithelial cells, neutrophil leukocyte accumulation and erythrocyte permeation in alveolus (star) in the I-R group (Group 2).Limited swelling of bronchiolar and alveolar epithelial cells (arrow heads), in the I-R and boric acid treatment group (Group 3). All photographs were taken at 20x magnification by the same lens

Discussion and Conclusion

Türkez et al. (2007) reported that boron compounds (as boric acid) caused significant increases in antioxidant enzyme activities. However, the defence of boric acid against reactive oxygen species (ROS) has not been adequately described yet. The activities of antioxidant enzymes (SOD, CAT, GSH-Px) have important roles in cell defence (Tapiero et al., 2004). Superoxide dismutase has a central role against oxidative damage. This enzyme catalyzes the dismutation of superoxide to hydrogenperoxide and oxygen (Kakarla et al., 2005). Catalase activity protects lipids and proteins in erythrocyte membranesagainst peroxide radicals. Glutation peroxidase is associated with increases in the quantity of glutathione. Glutathione is a tripeptide that is very important for erythrocytes. It has a key role in antioxidant enzyme activities (Türkez et al. 2007). Hunt et al. (1996) stated that boron compounds have a protective effect on oxidative damage to human health by showing their effects on SOD, CAT and GSH. It is considered that the effects occur because they stimulate the immune system and lead to secretion of Vitamin D metabolism or insulin. It is stated that lung damage occurred in the reperfusion period after lower extremity ischemia in connection with free oxygen radicals. In the same study, it was also found that melatonin, an antioxidant compound, played a protective role against I/R damage. In this study, it was also determined that boric acid applications used against radicals which are caused by extremity ischemia led to a significant increase in the protective effect of antioxidant enzymes. In comparison to SOD and GSH-Px, the enzyme CAT, which is the main subject of this study, was also affected more positively. The late phase of reperfusion injury is known to be neutrophil-dependent (Uysal, 2006). Although there are important studies on the effects of remote organ ischemia-reperfusion cases that lead to lung injury (e.g., hind-limb, ischemia reperfusion) (Seekamp et al., 1993), much fewer studies have been conducted to focus on the lungs as the direct aim of ischemia and reperfusion injury. In this study, using an in vivo rat lung ischemia-reperfusion model, we characterized a model of injury in the lung during the early and delayed reperfusion periods by determining the protective effects of boric acid and its relationships in inflammatory injury. In similar studies, oedema was mentioned in the alveolar cells (Den-Hengst et al., 2010; Ferrari et al., 2015). Macrophages and neutrophils are determinants for the improvement of lung ischemia-reperfusion injury (Den-Hengst et al., 2010). Alveolar type II cells appear to be quite sensitive to the effects of ROS and RNS. These cells are responsible for the release of inflammatory mediators and cytokines (Sharmaet al., 2007). The light microscopy examinations observed considerable perivascular oedema in the lungs undergoing ischemia and 2 hours of reperfusion in comparison to the lungs of the sham-operated rats. At 2 hours of reperfusion, there were also significant interstitial and perivascular oedema, deposition of fibrin in the alveolar spaces, intra -alveolar haemorrhage and leukocyte accumulation. The normal histological structure was better preserved, some macrophages were non-activated, and a remarkable reduction of neutrophil infiltration was seen after treatment in the group that was given boric acid (Beckers et al., 2017).

Damage to the lung tissue of the rats exposed to ischemia-reperfusion was detected. This damage was observed as a decrease in enzyme activities and histopathological deterioration. After the application of boric acid, the increase in enzyme activity (SOD, CAT, GSH-Px) in lung tissue and histopathology were determined as normalization. According to these results, boric acid has therapeutic effects in lung damage after ischemia reperfusion.

References

Aebi H. Catalase in vitro assay methods. Methods Enzymol 1984; 105:121-6.

Armstrong TA, Spears JW, Lloyd KE. Inflammatory response growth and thyroid hormone concentrations are affected by long-term boron supplementation in gilts. J Anim Sci 2001; 79: 1549-56.

Bancroft J, Gamble M. Theory and Practice of Histological Techniques. Sixth Edition. New York: Elsevier Health Sciencesş, 2008; pp.15-25.

Başbuğ M, Yıldar M, Yaman İ, Özkan ÖF, Akşit H, Çavdar F, Derici H. Effects of boric acid in an experimental rat model of hepatic ischemia-reperfusion injury. Acta Medica Mediterranea 2015; 31: 1067-73.

Beckers PA, Gielis JF, Van Schil PE, Adriaensen D. Lung ischemia reperfusion injury: The therapeutic role of dipeptidyl peptidase 4 inhibition. Ann Transl Med 2017; 5(6): 129.

Blaisdell FW. The pathophysiology of skeletal muscle ıschemia and reperfusion syndrome: A review. Cardiovascular Surgery 2002; 10(6): 620-30.

Büyükgüzel E, Büyükgüzel K, Snela M, Erdem M, Radtke K, Ziemnicki K, Adamski Z. Effects of boric acid on antioxidant enzyme acitivity, lipid peroxidation and ultrastructure of mitgut and fat body of galeria mellonella. Cell Biology Toxicology 2013; 29:117-129.

Den Hengst WA, Gielis JF, Lin JY, Van Schil PE, De Windt LJ, Moens AL. Lung ischemia-reperfusion injury: A molecular and clinical view on a complex pathophysiological process. Am J Physiol Heart Circ Physiol 2010; 299(5): H1283-99.

Devirian TA,Volpe SL. The physiological effects of dietary boron. Crit Rev Food Sci Nutr 2003;43(2): 219-31.

Eren M, Uyanik F, Karabulut N. The Effects of Dietary Boron Supplementation on performance, carcass composition and serum lipids in Japanese quails. J Anim Vet Adv 2006; 5: 1105-8.

Fail PA, Chapin RE, Price CJ, Heindel JJ. General, reproductive, developmental, and endocrine toxicity of boronated compounds. Reproductive Toxicology 1998; 12: 1-18.

Ferrari RS, Andrade CF. Oxidative stress and lung ischemia-reperfusion injury. Oxid Med Cell Longev 2015; 590987.

Fiette L, Slaoui M. Necropsy and Sampling Procedures in Rodents, in Drug Safety Evaluation. Springer, 2011; pp. 39-67.

Fischer AH, Jacobson KA, Rose J, Zeller R. Hematoxylin and Eosin Staining of Tissue and Cell Sections. Cold Spring Harbor Protocols. 2008; (5): p. pdb. Prot 4986.

Grace PA. Ischaemia-reperfusion injury. BJS 1994; 81: 637-47.

Hunt CD. Boron homeostasis in the cholecalciferol-deficient Chick. P Nat Acad Sci USA 1988; 42: 60.

Hunt CD. Biochemical effects of physiological amounts of dietary boron. J Trace Elem Med Biol 1996; 9:185-213.

Kakarla P, Vadluri G, Reddy KS. Response of hepatic antioxidant system to exercise training in aging female rat. J. Exp. Zoolog. A Comp Exp Biol 2005; 303: 203-8.

Klausner JM, Paterson IS, Valeri R, Hechtman HB. Limb ıschemia induced increase in permeability is mediated by leucocytes and leukotriens. Ann Surg 1988; 208:755-60.

Klausner JM, Paterson IS, Kobzik LESTER, Valeri CR, Shepro DAVID, Hechtman HB. Leukotrienes but not complement mediate limb ischemia-induced lung ınjury. Ann Surg 1989a; 209(4): 462-70.

Klausner JM, Paterson IS, Kozbik L, Valeri R, Shepro D, Hechtman HB. Oxygen free radicals mediate ischemia induced lung injury. Surgery 1989b; 105:192-9.

Meacham SL, Taper LJ, Volpe SL. Effects of boron supplementation on bone mineral density and dietary, blood, and urinary calcium, phosphorus, magnesium, and boron in female athletes. Environ Health Perspect 1994; 102: 79-82.

Paglia DE, Valentina WN. Studies on the quantitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med 1967; (70): 158-69.

Seekamp A, Mulligan MS, Till GO, Ward PA. Requirements for neutrophil products and l-arginine in ischemia-reperfusion injury. Am J Pathol 1993; 142 (4): 1217-26.

Sharma AK, Fernandez LG, Awad AS, KronI L, Laubach VE. Pro inflammatory response of alveolar epithelial cells is enhanced by alveolar macrophage -produced tnf-α during pulmonary ischemia-reperfusion injury. Am J Physiol Lung Cell Mol Physiol 2007; 293(1): L105-13.

Sun Yi, Oberley LW, Ying Lİ. A simple method for clinical assay of superoxide dismutase. Clin Chem 1988; 3413:497-500.

Tapiero H, Townsend DM, Tew KD. The role of carotenoids in the prevention of human pathologies. Biomed. Pharmacother 2004; 58: 100-10.

Tekeli A, Akgün S, Civelek A, İşbir S, Ak K, Çobanoğlu A. Alt ekstremite iskemi reperfüzyonu sonucunda gelişen akciğer hasarının önlenmesinde farklı bir ajan: FK 506 (takrolimus). Turk Gogus Kalp Dama 2001; 9(4): 242-6.

Tranquilli WJ, Thurmon JC, Grimm KA. Lumb and Jones' Veterinary Anesthesia and Analgesia. 4th Edition. New York: John Wiley & Sons, 2013; pp. 765-85.

Turkez H, Geyikoglu F, Tatar A, Keles S, Ozkanc A. Effects of some boron compounds on peripheral human blood. Zeitschrift für Natur for schung 2007; 62:889-96.

Uysal A, Protective Effect of melatonin on lung ınjury caused by ıschemia-reperfusion of the lower extremities. Turk Gogus Kalp Dama 2006; 14(4): 308-14.