

Borate reduces experimental supra-celiac aortic clamping-induced oxidative stress in lung and kidney, but fails to prevent organ damage

Borat akciğer ve böbrekte deneysel supraçölyak aort klemplenmesine bağlı oksidatif stresi azaltır, ancak organ hasarını önlemekte yetersizdir

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ABSTRACT

Background: This study aims to investigate the effects of 2-aminoethoxydiphenyl borate (2-APB) on aortic clamping-induced lung and kidney tissue oxidation, tissue inflammation, and histological damage in a rat model.

Methods: A total of 28 adult female Wistar albino rats were randomly allocated to four equal groups: Control group, ischemia-reperfusion group, dimethyl sulfoxide group, and 2-APB group. Animals in the control group underwent median laparotomy. In the remaining groups, supra-celiac aorta was clamped for 45 min and, then, reperfusion was constituted for 60 min. The 2-APB (2 mg/kg) was administered before clamping. The remaining groups received saline (ischemia-reperfusion group) or dimethyl sulfoxide (dimethyl sulfoxide group). Kidney and lung tissue samples were harvested at the end of reperfusion.

Results: Aortic occlusion caused increased tissue total oxidant status and reduced total antioxidant status and glutathione levels in the ischemia-reperfusion and dimethyl sulfoxide groups. Tissue interleukin-1 beta and tumor necrosis factor-alpha levels, nuclear factor kappa beta activation, and histological damage severity scores were also higher in these groups. The 2-APB treatment eliminated the increase in total oxidant status and the decrease in total antioxidant status and glutathione levels. It also caused a decrease in the interleukin-1 beta levels, although it did not significantly alter the tumor necrosis factor-alpha levels, nuclear factor kappa beta immunoreactivity, and histological damage scores.

Conclusion: Borate exerted a beneficial antioxidant effect as evidenced by reduced oxidative stress; however, it did not inhibit nuclear factor kappa beta activation and prevent histological damage in supra-celiac aortic clamping-induced kidney and lung injury in rats.

Keywords: Cytokines, borate, nuclear factor kappa beta, oxidative stress, reperfusion injury.

ÖZ

Amaç: Bu çalışmada sıçan modelinde 2-aminoetoksifenil boratın (2-APB) aortik klemplenmeye bağlı akciğer ve böbrek dokusu oksidasyonu, doku enflamasyonu ve histolojik hasar üzerindeki etkileri araştırıldı.

Çalışma planı: Toplam 28 erişkin dişi Wistar albino sıçan, rastgele dört eşit gruba ayrıldı: Kontrol grubu, iskemi-reperfüzyon grubu, dimetil sülfoksit grubu ve 2-APB grubu. Kontrol grubundaki hayvanlara medyan laparotomi yapıldı. Kalan gruplarda supraçölyak aort 45 dk. süreyle klemplendi ve ardından 60 dk. reperfüzyon oluşturuldu. Klemplemeden önce 2-APB (2 mg/kg) uygulandı. Diğer gruplarda hayvanlara salin (iskemi-reperfüzyon grubu) veya dimetil sülfoksit (dimetil sülfoksit grubu) verildi. Reperfüzyon sonunda böbrek ve akciğer doku örnekleri alındı.

Bulgular: Aortun klemplenmesi, iskemi-reperfüzyon ve dimetil sülfoksit gruplarında dokudaki toplam oksidan statüsünün artmasına ve total antioksidan statüsünün ve glutatyon düzeylerinin azalmasına neden oldu. Doku interlökin-1 beta ve tümör nekroz faktör-alfa düzeyleri, nükleer faktör kappa beta aktivasyonu ve histolojik hasar şiddeti skorları da bu gruplarda daha yüksek bulundu. 2-APB tedavisi, toplam oksidan statüsündeki artışı ve total antioksidan statüsü ve glutatyon düzeylerindeki düşüşü ortadan kaldırdı. Ayrıca, interlökin-1 beta düzeylerinde anlamlı azalmaya neden oldu; ancak tümör nekroz faktör-alfa düzeylerini, nükleer faktör kappa beta aktivasyonunu ve histolojik hasar skorlarını önemli ölçüde değiştirmede.

Sonuç: Borat, sıçanlarda supraçölyak aortik klemplenmeye bağlı böbrek ve akciğer hasarında oksidatif stresi azaltarak faydalı bir antioksidan etki göstermiştir; ancak, nükleer faktör kappa beta aktivasyonunu inhibe etmemiş ve histolojik hasarı önleyememiştir.

Anahtar sözcükler: Sitokinler, borat, nükleer faktör kappa beta, oksidatif stres, reperfüzyon hasarı.

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Reperfusion following ischemia triggers tissue damage, which is a leading cause of organ failure in various treatment modalities, such as vascular surgical procedures, endoaortic occlusion for traumatic bleeding, or organ transplantation. This phenomenon is commonly known as ischemia-reperfusion (IR) injury. The reactive oxygen species (ROS), which can cause direct damage to lipids in cell membranes and also to the intracellular structures such as nucleic acids and mitochondria, are the major mediators of these harmful effects.^[1] The ROS can induce the production of proinflammatory cytokines that trigger acute tissue inflammation and injury in the ischemic and remote organs through activating various molecules, including the intracellular signaling molecule nuclear factor kappa beta (NF- κ B).^[2]

Accumulation of calcium ions (Ca²⁺) in the cell is an essential factor in the occurrence of IR injury. The rapid decrease of intracellular pH causes changes in transmembrane ion transportation and the resultant increase in intracellular sodium ions (Na⁺). In the reperfusion period, prompt recovery of intracellular pH through the delivery of oxygen to the affected cell causes the reversal of the Na⁺/Ca²⁺ pump function and further increases the intracellular Ca²⁺ content for removal of excess Na⁺.^[3] In response to Ca²⁺ overload, mitochondrial permeability transition (mPT) channels located in the inner mitochondrial membrane are opened, thereby, triggering a drastic increment in the ROS production.^[4]

Boron is a trace element that can potentially affect many metabolic processes and physiological systems. It is usually found in the form of borate by binding to inorganic salts in nature.^[5] Accumulating evidence regarding the therapeutic use of boron in humans shows that it may have a role in the treatment of various diseases including osteoarthritis and hormonal disorders.^[6] Furthermore, it has been suggested that intake of boron-containing compounds may help to maintain cardiovascular health by modifying inflammatory processes, leading to atherosclerosis in humans.^[7] 2-Aminoethoxydiphenyl borate (2-APB) is a molecule produced by esterifying diphenylboronic acid in ethanol with aminoethanol.^[8] It is classified as a pharmacological calcium channel blocker, as it can reduce calcium release mediated by inositol (1,4,5)-trisphosphate receptors in plasma membranes.^[9] 2-APB has been shown to effectively inhibit Ca²⁺ passage from the extracellular space into the cell and restrict the calcium accumulation in the cytoplasm and mitochondria.^[10]

Recent evidence gained from experimental models targeting isolated organ systems has demonstrated

that 2-APB can reduce IR injury and it is suggested that this effect is related to its antioxidant and anti-inflammatory properties.^[11-13] However, the impact of 2-APB on aortic clamping-induced organ inflammation and injury has not been evaluated in detail, yet. In the current study, we aimed to investigate the impact of 2-APB administration on organ damage through the evaluation of histology, oxidative stress parameters, cytokine responses, and NF- κ B activations in lung and kidney tissues in experimental supra-celiac aortic occlusion.

MATERIALS AND METHODS

Animals

The experimental design and procedure were reviewed and approved by Adnan Menderes University, Experimental Animals Local Ethics Committee (No: 64583101/2019/078). The procedures regarding the animal care and experimental surgery were carried out in accordance with the National Health Institute (NIH) Guide to the Care of Laboratory Animals.

A total of 28 adult female Wistar albino rats (250 to 350 g) were used in the study. The animals were simply randomized to four groups including seven animals in each group. The rats were kept in cages including four animals per cage. The room humidity (45 to 50%) and temperature (22°C \pm 2°C) were controlled where the animals were kept prior to the experiment. Animals had a regular diet for rats and unrestricted access to tap water; however, 12 h prior to experimental surgery, food and water access were ceased.

Experimental IR injury

The rats were anesthetized intraperitoneal (i.p.) injection with a mixture of ketamine (90 mg/kg) and xylazine (10 mg/kg) previous to each experiment. The surgical field was shaved, and the povidone-iodine solution was used for sanitizing. The abdominal aorta was, then, exposed proximal to the level of renal arteries via median laparotomy and heparin was administered. Subsequently, the aorta was occluded with a non-traumatic clamp (Vascu-Statt® II; Scanlan, MN, USA) at the supra-celiac level for 45 min. At the end of the occlusion, the non-traumatic clamp was removed and reperfusion was allowed for 60 min as described previously.^[14]

2-Aminoethoxydiphenyl borate

Dimethyl sulfoxide (DMSO), an aprotic solvent, can dissolve poor soluble molecules. It has a low toxicity risk at concentrations less than 10% and,

thus, is widely used in experimental studies.^[15,16] 2-APB (D9754; Sigma Aldrich, MO, USA) was at first mixed with DMSO (D5879; Sigma Aldrich, MO, USA) to dissolve. This initial solution was diluted with physiological saline to obtain a concentration of 5% DMSO in the final solution.^[13,17] 2-APB was given 10 min before experimental IR at a dose of 2 mg/kg (i.p.) as described in previous studies.^[9,11]

Study groups

Control (C) (n=7): Median laparotomy was performed and saline (i.p.) was applied without aortic clamping.

IR group (n=7): Saline was given (i.p.) 10 min before aortic clamping, and ischemia-reperfusion was induced.

DMSO group (n=7): 5% DMSO (i.p.) diluted with saline was administered 10 min before aortic clamping, and IR was induced.

2-APB group (n=7): 2-APB (i.p.) was given 10 min before aortic clamping, and IR was induced.

Lung and Kidney Tissue Sampling

After reperfusion, the animals were sacrificed under deep anesthesia by aortic puncture exsanguination. The right lungs were harvested for histopathological examination and the left lungs were harvested for biochemical studies.

Similarly, the right kidneys for biochemical analysis were harvested, and the left kidneys for histopathological evaluation were harvested.

Biochemical analysis

Tissue samples were stored in Eppendorf tubes, rapidly refrigerated, and preserved at -80°C for biochemical analysis. On the day of study, frozen renal and lung tissue samples were thawed and, then, weighed and homogenized at 50 mM pH 7.4 phosphate buffer (PBS) (PRO 250 Scientology Inc., CT, USA). Homogenized samples were centrifuged at 20,000 g for 15 min, and supernatants were collected for biochemical analyses.

Tissue levels of glutathione (GSH) were assessed based on the method of Beutler et al.^[18] The results were presented in micromoles per gram of protein ($\mu\text{M/g}$ protein). Total antioxidant status (TAS) and total oxidant status (TOS) levels were tested with purchased kits (Rel Assay Diagnostics Kit; Mega Tip, Gaziantep, Turkey) using the method described by Erel.^[19] The results were obtained in terms of Trolox equivalents per gram of protein

(μmol Trolox Eq/g protein) for TAS and micromole hydrogen peroxide equivalents per gram of protein (μmol H_2O_2 Eq/g protein) for TOS.^[19]

Tumor necrosis factor alpha (TNF- α) and interleukin-1 beta (IL-1 β) concentrations in tissue samples were identified by enzyme-linked immunosorbent assay (ELISA). Rat-specific ELISA kits (E-EL-R0019, E-EL-R0012, E-EL-R0016, Elabscience Biotechnology Co. Wuhan, PRC) were used. The ELISA microplate reader (DAR 800, Diagnostic Automation, CA, USA) was used for the measurements and the results were expressed in picograms per milligrams of protein (pg/mg protein).

Histopathological evaluation

Evaluation of histological damage in lung and kidney tissue samples

Samples of lung and kidney were removed and placed in buffered formalin solution (10%) for analysis. Tissue samples were stained with hematoxylin-eosin (H-E) for regular microscopic examination (Olympus BX53, Olympus Co., Tokyo, Japan). A pathologist who was blinded to the experimental groups carried out all examinations. The Image Analysis Software version 2 (Olympus DP-22 Microscope digital camera software program, Tokyo, Japan) was used for assessment.

The sections of lung tissue were assessed using high magnification ($\times 200$); intra-alveolar hemorrhage, intra-alveolar edema, and interstitial-perivascular infiltrations of neutrophils were examined. The severity of parenchymal damage in the lungs was determined by scoring the tissue samples on the basis of a four-point semi-quantitative scale as follows: (0) absent, (1) focal-mild, (2) focal-moderate, and (3) diffuse-severe changes.^[20]

The kidney tissue sections were evaluated in terms of focal glomerular necrosis, Bowman's capsule dilatation, tubular epithelial degeneration, necrosis of the tubular epithelium, tubular dilatation, and interstitial inflammatory infiltration. The changes in histopathology were scored on a semi-quantitative scale according to a method previously described: (0) none, (1) focal-mild changes, (2) multifocal-intermediate changes, and (3) widespread-severe changes.^[21]

Immunohistochemical staining for NF- κ B

An automatic stainer system (Autostainer Link 48 DAKO, Glostrup, Denmark) was used to perform all immunohistochemical staining. Sections from paraffin-embedded blocks were placed on positively charged slides (Superfrost Plus Slides, Thermo

Fisher Scientific, CA, USA) treated with xylene and dehydrated. Antigen removal was, then, performed in a thermostatic bath (PT Link) (Dako Envision Flex Target retrieval solution High pH 50×). The sections were incubated for 60 min with NF-κB (sc-7386; Santa Cruz Biotechnology, TX, USA, 1/500 dilution) at room temperature. Streptavidin-biotin enhanced immunoperoxidase technique (Sensi Tek HRP, ABF 125) was used in an automated system to detect immune reactions.

Evaluation of the immunohistochemical staining

Examination of NF-κB immunohistochemical staining was performed under a light microscope (Olympus BX53, Olympus Co., Tokyo, Japan) at x400 magnification. Brown color staining in the cytoplasm and/or nuclei was accepted as a positive indicator for NF-κB expression. The following scoring system was used to grade tubular staining intensity for NF-expression: (Grade 0) no expression; (Grade 1) less than 25% positive; (Grade 2) 25-50% positive; (Grade 3) 51-75% positive; and (Grade 4) 76-100% positive.^[22]

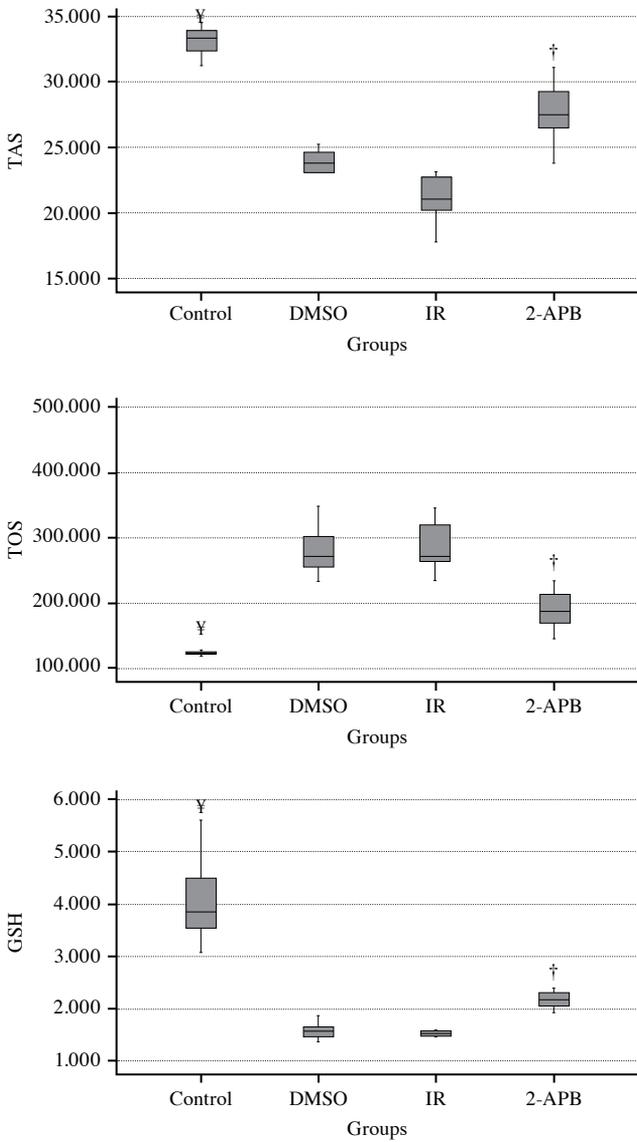


Figure 1. Lung tissue levels of TAS, TOS and GSH. TAS: Total tissue antioxidant status; TOS: Total oxidant status; GSH: Glutathione; DMSO: Dimethyl sulfoxide, IR; Ischemia-reperfusion; 2-APB: 2-Aminoethoxydiphenyl borate; ¥ p<0.008 compared to DMSO, IR and 2-APB groups; † p<0.008 compared to DMSO and IR groups.

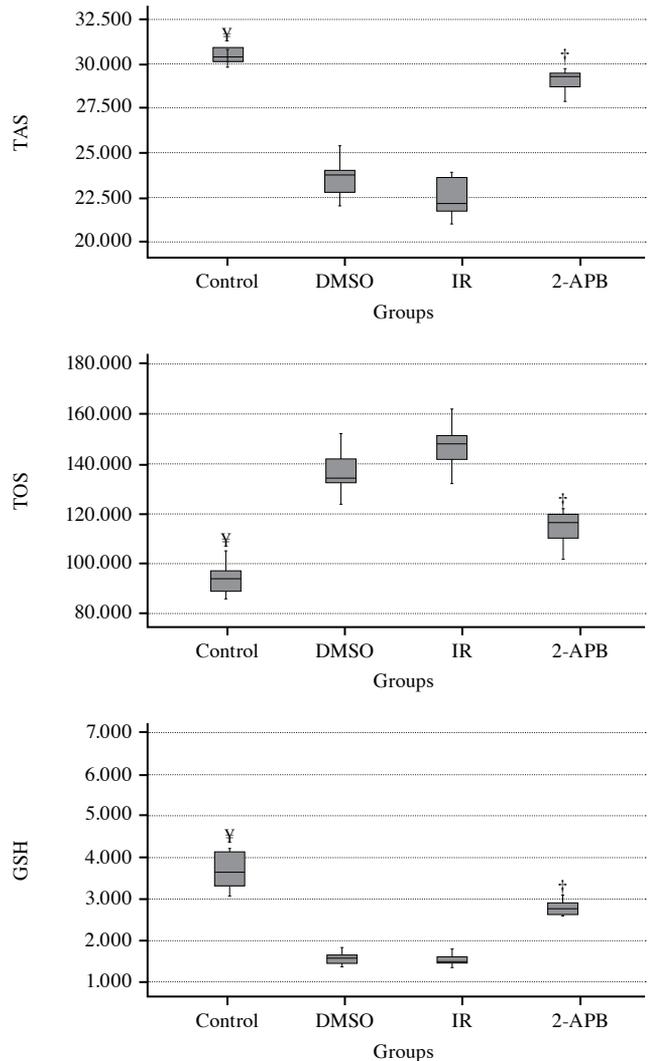


Figure 2. Kidney tissue levels of TAS, TOS and GSH. TAS: Total tissue antioxidant status; TOS: Total oxidant status; GSH: Glutathione; DMSO: Dimethyl sulfoxide, IR; Ischemia-reperfusion; 2-APB: 2-Aminoethoxydiphenyl borate; ¥ p<0.008 compared to DMSO, IR and 2-APB groups; † p<0.008 compared to DMSO and IR groups.

In lung tissue samples, NF-expression was evaluated semi-quantitatively according to the percentage of positive cells per five microscopic areas in the parenchyma. The following scoring was taken into account in determining the intensity of immunohistochemical staining in tissue sections: (0) less than 5%; (1) 6-25%; (2) 26-50%; (3) 51-75%; and (4) more than 75%.^[23]

Statistical analysis

Statistical analysis was performed using the IBM SPSS version 25.0 software (IBM Corp., Armonk, NY, USA). The distribution of normality was tested using the Shapiro-Wilk test. Descriptive data were expressed in median (min-max) values. Variables between groups were compared using the Kruskal-Wallis variance analysis. A 0.05 type-1 error level was adjusted for statistical significance. The Mann-Whitney U test was performed for paired comparison of the groups and using the Bonferroni correction. A *p* value of <0.008 was considered statistically significant.

RESULTS

The results of lung and kidney tissue biochemical assays

Tissue TAS, TOS, and GSH levels

Tissue levels of TAS, TOS, and GSH for lung and kidney tissues are depicted in Figures 1 and 2. In the control group tissue, TAS and GSH levels in both lung and kidney tissues were significantly higher compared to the remaining groups (*p*<0.008). The TOS levels in the lungs and kidneys were found to be significantly lower in the control group than the other study groups (*p*<0.008). The levels of TAS and GSH in the lung and kidney tissues were significantly increased in the 2-APB group, compared to the IR and DMSO groups (*p*<0.008). Furthermore, the levels of TOS in the lungs and kidneys were significantly reduced in the 2-APB group, compared to the IR and DMSO groups (*p*<0.008).

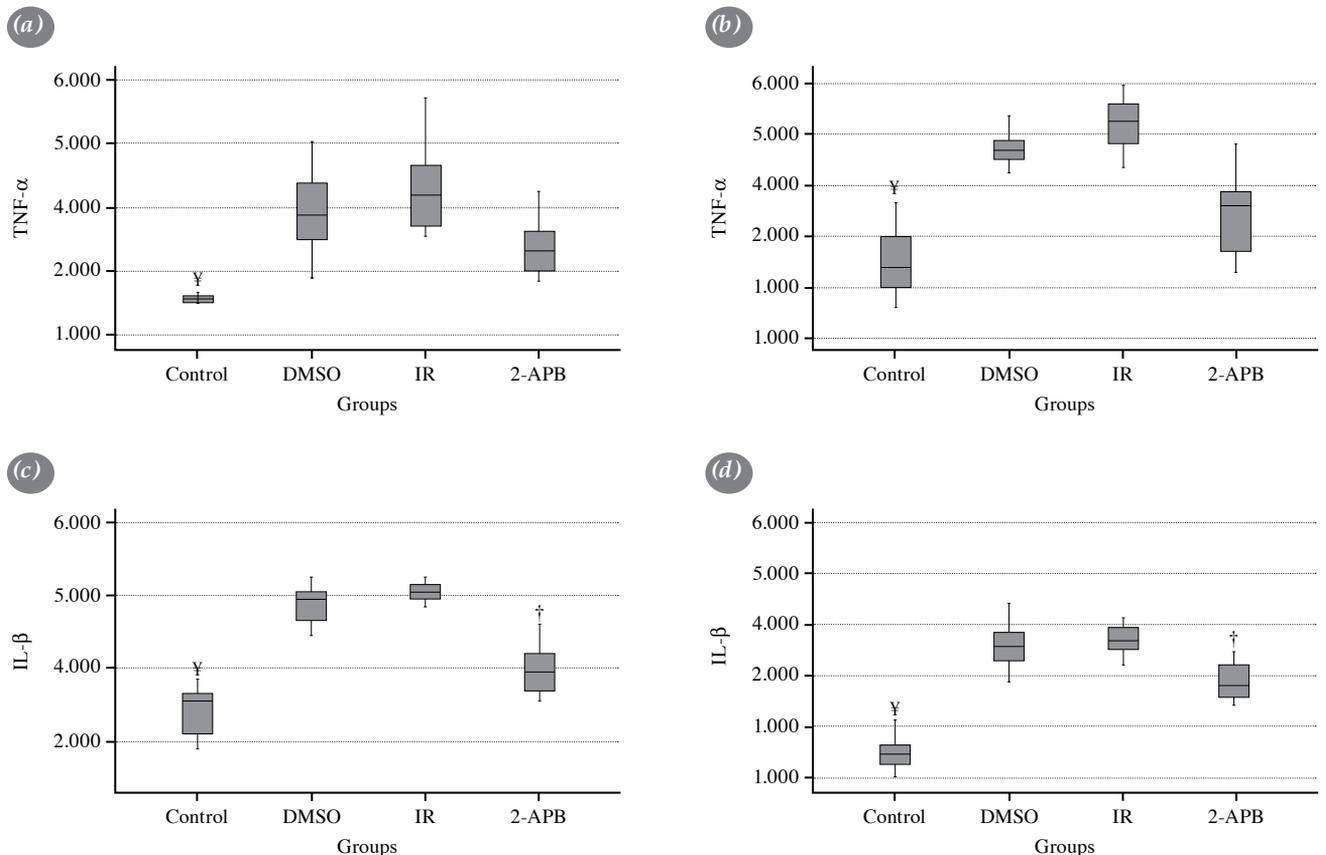


Figure 3. Lung and kidney tissue cytokine levels. **(a)** Lung TNF- α levels. **(b)** Kidney TNF- α levels. **(c)** Lung IL-1 β levels. **(d)** Kidney IL-1 β levels.

TNF- α : Tumor necrosis factor alpha; IL-1 β : Interleukin-1 beta; DMSO: Dimethyl sulfoxide; IR: Ischemia-reperfusion; 2-APB: 2-Aminoethoxydiphenyl borate; ¥ *p*<0.008 compared to DMSO, IR and 2-APB groups; † *p*<0.008 compared to DMSO and IR groups.

Table 1. Histopathological damage scores of lung tissue

Groups	Intraalveolar hemorrhage		Intraalveolar edema		Neutrophil infiltration	
	Median	Min-Max	Median	Min-Max	Median	Min-Max
Control	0*	0	0*	0-1	0*	0
DMSO	3	2-3	2	2-3	2	2-3
IR	3	2-3	3	2-3	3	2-3
2-APB	2	1-3	2	1-3	2	1-3

* Compared to DMSO, IR and 2APB groups; DMSO: Dimethyl sulfoxide, IR; Ischemia-reperfusion; 2APB: 2-Aminoethoxydiphenyl borate; Data were analyzed using the Kruskal-Wallis test. When significant at the 5% level, post-hoc comparisons were performed by Mann-Whitney U ($p < 0.008$).

Table 2. Histopathological damage scores of kidney tissue

Groups	Focal glomerular necrosis		Dilatation of Bowman's capsule		Degeneration of tubular epithelium		Necrosis in tubular epithelium		Tubular dilatation		Interstitial inflammatory infiltration	
	Median	Min-Max	Median	Min-Max	Median	Min-Max	Median	Min-Max	Median	Min-Max	Median	Min-Max
Control	0*	0	0*	0-1	0*	0	0*	0	0*	0-1	0*	0
DMSO	2	1-3	3	2-3	2	1-3	2	1-3	3	2-3	2	1-3
IR	1	1-3	2	2-3	1	1-3	1	1-3	2	2-3	1	1-3
2-APB	2†	1-2	2	1-2	1†	1-2	1†	1-2	2	1-2	1†	1-2

* Compared to DMSO and IR groups; † Compared to the control group; DMSO: Dimethyl sulfoxide, IR; Ischemia-reperfusion; 2APB: 2-Aminoethoxydiphenyl borate; Data were analyzed using the Kruskal-Wallis test. When significant at the 5% level, post-hoc comparisons were performed by Mann-Whitney U ($p < 0.008$).

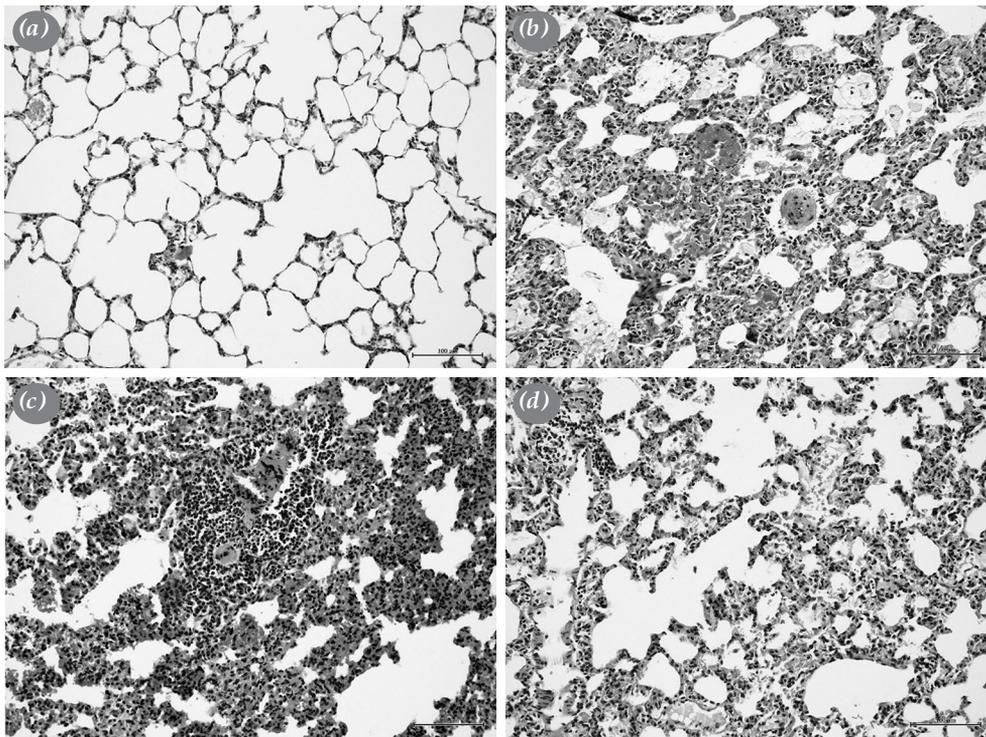


Figure 4. Representative histological samples of lung tissue. Photomicrographs of tissue samples ($\times 200$ magnification, scale bar 100 μm , H-E staining): (a) Control group, (b) DMSO group, (c) IR group, (d) 2-APB group.

DMSO: Dimethyl sulfoxide; IR: Ischemia-reperfusion; 2-APB: 2-Aminoethoxydiphenyl borate.

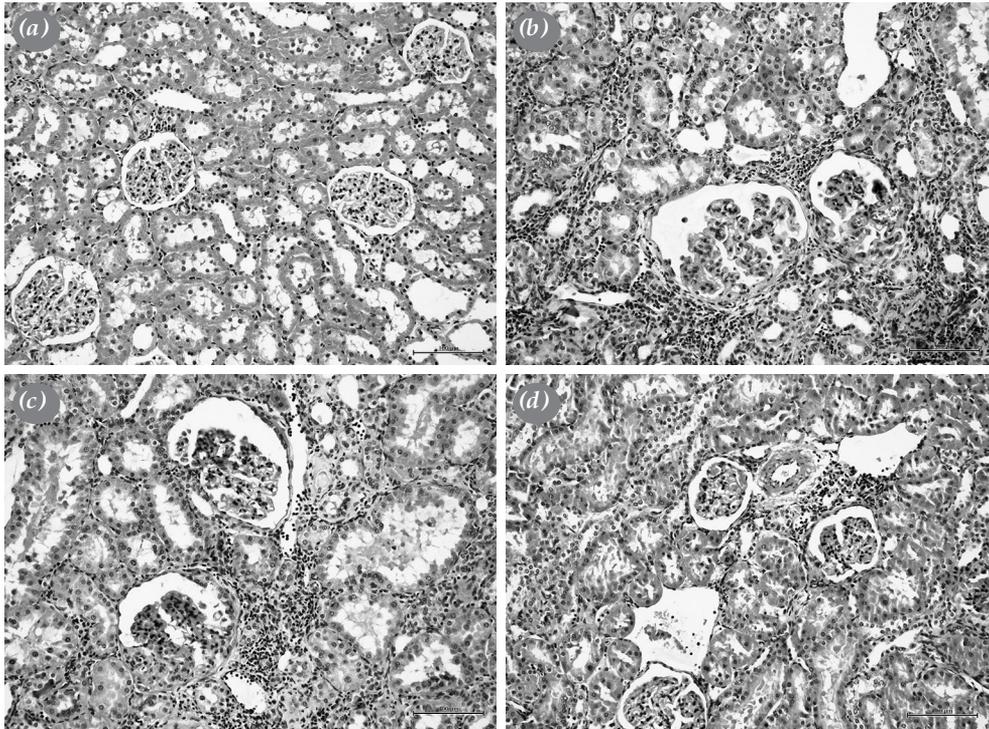


Figure 5. Representative histological samples of kidney tissue. Photomicrographs of tissue samples ($\times 200$ magnification, scale bar 100 μm , H-E staining): (a) Control group, (b) DMSO group, (c) IR group, (d) 2-APB group.

DMSO: Dimethyl sulfoxide; IR: Ischemia-reperfusion; 2-APB: 2-Aminoethoxydiphenyl borate.

Tissue TNF- α and IL-1 β levels

The TNF- α tissue levels in the lung (Figure 3a) and kidney (Figure 3b) were significantly reduced in the control group compared to the remaining study groups ($p < 0.008$). No other significant differences were observed in the TNF- α levels among the groups. In the control group, IL-1 β tissue levels in the lungs (Figure 3c) and kidneys (Figure 3d) were also significantly higher than the other study groups ($p < 0.008$). However, tissue IL-1 β levels were significantly lower in the 2-APB group, compared to the IR and DMSO study groups ($p < 0.008$).

The results of lung and kidney tissue histopathological evaluation

Histological damage scores

The results of histological damage scores of lung tissue are shown in Table 1. The scores regarding the histological damage in the lungs were significantly lower in the control group than the other study groups ($p < 0.008$). Table 2 presents the results of histological damage scores of kidney tissue samples in study groups. Histopathological damage scores of kidney tissue in the control group were also lower, compared to the IR and DMSO groups ($p < 0.008$). There were

Table 3. NF- κ B immunostaining scores of experimental groups

	Control		DMSO		IR		2-APB	
	Median	Min-Max	Median	Min-Max	Median	Min-Max	Median	Min-Max
Kidney	0*	0-0	3	2-4	3	2-4	2	2-3
Lung	0*	0-0	3	2-4	3	2-4	2	1-3

DMSO: Dimethyl sulfoxide; IR: Ischemia-reperfusion; 2-APB: 2-Aminoethoxydiphenyl borate; * Compared to DMSO, IR and 2APB groups; Data were analyzed using the Kruskal-Wallis test. When significant at the 5% level, post-hoc comparisons were performed by Mann-Whitney U test ($p < 0.008$).

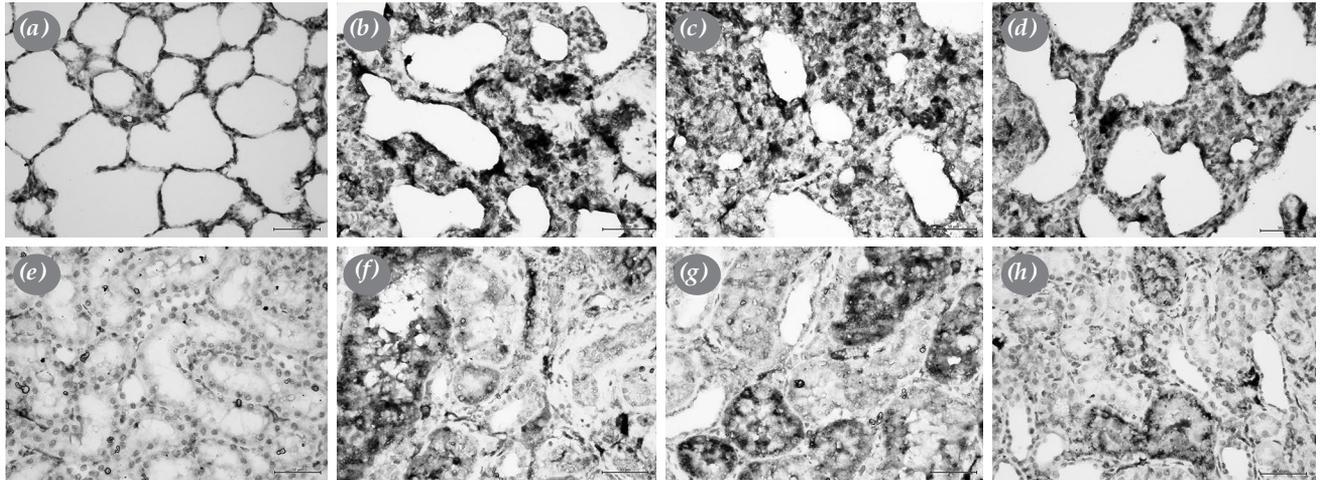


Figure 6. Representative images of NF- κ B immunohistochemical staining in groups ($\times 400$ magnification, scale bar 50 μ m). Lung tissue samples: (a) Control group, (b) DMSO group, (c) IR group, (d) 2-APB group. Kidney tissue samples: (e) Control group, (f) DMSO group, (g) IR group, (h) 2-APB group.

no significant differences in the scores for tubular dilatation and Bowman's capsule dilatation among the control and 2-APB groups. On the other hand, the other histological scores of kidney tissue damage were significantly increased in the 2-APB group, compared to the control group ($p < 0.008$). The representative histological sections of each group included in the study for lung and kidney tissue samples are demonstrated in Figures 4 and 5.

NF- κ B immunostaining intensity scores

The scores of the intensity of immunostaining for NF- κ B in both the lung and kidney tissues were significantly increased in the IR, DMSO, and 2-APB groups compared to the control group ($p < 0.008$) (Table 3). The representative sections from study groups showing the NF- κ B immunostaining are demonstrated in Figure 6 (a-d) lung and (e-h) kidney tissue samples.

DISCUSSION

In the current experimental study, we formed distant and target organ damage via supra-celiac aortic clamping-induced IR. Following ischemia and reperfusion, we observed a significant amount of oxidation and utilization of antioxidant capacity in lung and kidney tissues detected via an increment of TOS and a decrement of TAS and GSH levels. As a consequence of these changes, we found increased levels of tissue cytokines (TNF- α and IL-1 β) which, in turn, were accompanied by the increased NF- κ B immunoreactivity in tissue samples. Histological examination of lung and kidney samples revealed

that severe tissue damage occurred as a response to ischemia and reperfusion.

Administration of 2-APB prior to the induction of IR period prevented the reduction of TAS and GSH tissue levels and the increment of TOS. These findings confirm the antioxidant effects of 2-APB in ischemia. The 2-APB treatment resulted in suppression of tissue IL-1 β levels, but did not cause a significant decrement of tissue TNF- α levels and NF- κ B immunoreactivity. Furthermore, it did not cause a considerable attenuation of tissue damage on histological evaluation of lung and kidney samples. These findings suggest that 2-APB limits aortic clamping-induced inflammatory responses to some extent; however, this effect is not sufficient enough to prevent tissue inflammation and injury in our model.

Various experimental studies have shown that 2-APB has beneficial effects on IR damage. In an animal model of renal artery clamping, 2-APB given before ischemia reduced the levels of circulating oxidative and inflammatory markers and attenuated renal cellular damage.^[11] In another study, 2-APB administration in acute mesenteric ischemia decreased inflammation and protected from gut injury in rats.^[12] Similarly, another study showed that 2-APB reduced oxidative damage and prevented apoptosis in an experimental IR model induced by ovarian torsion.^[13]

In our animal model of aortic IR, we observed that 2-APB significantly attenuated oxidative stress in kidney and lung tissues. There are two postulated mechanisms for the antioxidant effect of

2-APB in IR injury: 1. Limitation of the intracellular Ca^{2+} loads, 2. Direct inhibition of extracellular ROS production. Increased cytosolic calcium causes an increment in mitochondrial calcium via transportation into the organelle through mitochondrial calcium uniporter channels. Consequently, the increment of mitochondrial calcium can lead to the formation of mPT and activate further mitochondrial ROS production.^[4] Therefore, it is suggested that the antioxidant effect of 2-APB may be related to the prevention of cytosolic and mitochondrial calcium overload.^[10] On the other hand, it has been also suggested that the antioxidant effect of 2-APB in IR injury may be associated with the extracellular ROS scavenging.^[24] Although the mechanism of action is not clear, 2-APB seems to have a beneficial antioxidant effect, as evidenced by inhibition of the decrease in tissue GSH and TAC levels in the reperfusion period.

Temporary clamping of the aorta or its major visceral branches at the infradiaphragmatic segment can cause damage to organs that are directly exposed to ischemia including the bowel and kidney, and to the distant target organs, particularly the lungs. Aortic occlusion induces a systemic inflammatory process primarily mediated by TNF- α and IL-1 β , and these cytokines can consequently activate NF- κ B, which is a key regulator molecule in intracellular signal transduction.^[25] As expected, we observed prominent oxidative stress associated with the significant increase of tissue cytokine levels and the activation of NF- κ B as a response to aortic clamping ischemia.

Our results are conflicting with the results of the previous experimental studies reporting a reduction in the levels of circulating cytokines and attenuation of the target organ histopathological damage in the experimental renal artery and superior mesenteric artery clamping models by using the same 2-APB dosage.^[10] Several factors may be related to the relative inefficiency of 2-APB in the modulation of the tissue inflammation and the failure of this agent in reducing the histological damage. The magnitude of the inflammatory responses and the severity of the organ injury provoked by supra-celiac aortic clamping in our model is probably more amplified than the ones generated by clamping of the isolated arterial pedicles of the organs. Therefore, this experimental design may have caused a profound degree of cellular damage and tissue inflammation which are far beyond the capacity of the 2-APB to overcome the reperfusion-induced deterioration. Since we did not determine the circulating mediators of inflammation and the effect of the higher doses of 2-APB in this particular experimental setting, it is not possible to suggest that

the agent is not effective in reducing proximal aortic clamping-induced inflammation. Another explanation for interpretation of our results may be that the antioxidant effect of 2-APB is predominantly formed via scavenging of extracellular ROS. In such a scenario, the antioxidant capacity of 2-APB may be insufficient in the reduction of intracellular ROS production and blockage of NF- κ B activation.

Nonetheless, there are several limitations to this study. First, we did not use methods to measure cellular calcium deposition and intracellular ROS production. These data would have yielded valuable information regarding the mechanism of action of the agent. Second, we did not determine the post-ischemic organ functions using physiological evaluations such as glomerular filtration rate in the kidney and alveolar oxygen diffusion capacity in the lungs. Therefore, it is not possible to speculate on the effect of 2-APB on IR-induced organ dysfunction.

In conclusion, our study results showed that 2-aminoethoxydiphenyl borate treatment given before supra-celiac aortic clamping in rats reduced oxidative stress in kidney and lung tissues which can be attributed to the antioxidant potential of the agent. The 2-aminoethoxydiphenyl borate treatment caused a decrease of tissue interleukin-1beta; however, it did not result in a significant reduction of tissue tumor necrosis factor-alpha and nuclear factor kappa beta activation and histological damage in the lung and kidney. We believe that further studies are needed to clarify the antioxidant effect of 2-aminoethoxydiphenyl borate and to determine its impact on inflammatory responses in ischemic conditions.

Declaration of conflicting interests

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REFERENCES

1. İnce İ, Akar İ, Arıcı A. Renoprotective effect of edaravone in acute limb ischemia/reperfusion injury. *Turk Gogus Kalp Dama* 2020;28:274-81.
2. Mittal M, Siddiqui MR, Tran K, Reddy SP, Malik AB. Reactive oxygen species in inflammation and tissue injury. *Antioxid Redox Signal* 2014;20:1126-67.
3. Wu MY, Yiang GT, Liao WT, Tsai AP, Cheng YL, Cheng PW, et al. Current mechanistic concepts in ischemia and reperfusion injury. *Cell Physiol Biochem* 2018;46:1650-67.
4. Honda HM, Korge P, Weiss JN. Mitochondria and ischemia/reperfusion injury. *Ann N Y Acad Sci* 2005;1047:248-58.

5. Koc ER, Gökce EC, Sönmez MA, Namuslu M, Gökce A, Bodur AS. Borax partially prevents neurologic disability and oxidative stress in experimental spinal cord ischemia/reperfusion injury. *J Stroke Cerebrovasc Dis* 2015;24:83-90.
6. Naghii MR, Mofid M, Asgari AR, Hedayati M, Daneshpour MS. Comparative effects of daily and weekly boron supplementation on plasma steroid hormones and proinflammatory cytokines. *J Trace Elem Med Biol* 2011;25:54-8.
7. Donoiu I, Militaru C, Obleagă O, Hunter JM, Neamtu J, Biță A, et al. Effects of boron-containing compounds on cardiovascular disease risk factors - A review. *J Trace Elem Med Biol* 2018;50:47-56.
8. Maruyama T, Kanaji T, Nakade S, Kanno T, Mikoshiba K. 2APB, 2-aminoethoxydiphenyl borate, a membrane-penetrable modulator of Ins(1,4,5)P₃-induced Ca²⁺ release. *J Biochem* 1997;122:498-505.
9. Karademir M, Gonul Y, Simsek N, Eser O. The neuroprotective effects of 2-APB in rats with experimentally-induced severe acute pancreatitis. *Bratisl Lek Listy* 2018;119:752-6.
10. Nicoud IB, Knox CD, Jones CM, Anderson CD, Pierce JM, Belous AE, et al. 2-APB protects against liver ischemia-reperfusion injury by reducing cellular and mitochondrial calcium uptake. *Am J Physiol Gastrointest Liver Physiol* 2007;293:G623-30.
11. Yildar M, Aksit H, Korkut O, Ozyigit MO, Sunay B, Seyrek K. Protective effect of 2-aminoethyl diphenylborinate on acute ischemia-reperfusion injury in the rat kidney. *J Surg Res* 2014;187:683-9.
12. Basbug M, Yildar M, Yaman İ, Cavdar F, Özkan ÖF, Aksit H, et al. Effect of different doses of 2-aminoethoxydiphenyl borate on intestinal ischemia-reperfusion injury. *Eur Surg* 2017;49:32-7.
13. Taskin MI, Hismiogullari AA, Yay A, Adali E, Gungor AC, Korkmaz GO, et al. Effect of 2-aminoethoxydiphenyl borate on ischemia-reperfusion injury in a rat ovary model. *Eur J Obstet Gynecol Reprod Biol* 2014;178:74-9.
14. Koçarslan A, Koçarslan S, Aydın MS, Gunay Ş, Karahan MA, Taşkın A, et al. Intraperitoneal administration of silymarin protects end organs from multivisceral ischemia/reperfusion injury in a rat model. *Braz J Cardiovasc Surg* 2016;31:434-9.
15. Galvao J, Davis B, Tilley M, Normando E, Duchon MR, Cordeiro MF. Unexpected low-dose toxicity of the universal solvent DMSO. *FASEB J* 2014;28:1317-30.
16. Syed M, Skonberg C, Hansen SH. Effect of some organic solvents on oxidative phosphorylation in rat liver mitochondria: Choice of organic solvents. *Toxicol In Vitro* 2013;27:2135-41.
17. Du K, Williams CD, McGill MR, Xie Y, Farhood A, Vinken M, et al. The gap junction inhibitor 2-aminoethoxy-diphenylborate protects against acetaminophen hepatotoxicity by inhibiting cytochrome P450 enzymes and c-jun N-terminal kinase activation. *Toxicol Appl Pharmacol* 2013;273:484-91.
18. Beutler E, Duron O, Kelly BM. Improved method for the determination of blood glutathione. *J Lab Clin Med* 1963;61:882-8.
19. Erel O. A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. *Clin Biochem* 2004;37:277-85.
20. Aydın MS, Caliskan A, Kocarlan A, Kocarlan S, Yildiz A, Günay S, et al. Intraperitoneal curcumin decreased lung, renal and heart injury in abdominal aorta ischemia/reperfusion model in rat. *Int J Surg* 2014;12:601-5.
21. Kurtoglu T, Durmaz S, Akgullu C, Gungor H, Eryilmaz U, Meteoglu I, et al. Ozone preconditioning attenuates contrast-induced nephropathy in rats. *J Surg Res* 2015;195:604-11.
22. Li Z, Nickkholgh A, Yi X, Bruns H, Gross ML, Hoffmann K, et al. Melatonin protects kidney grafts from ischemia/reperfusion injury through inhibition of NF-κB and apoptosis after experimental kidney transplantation. *J Pineal Res* 2009;46:365-72.
23. Song M, Xia B, Li J. Effects of topical treatment of sodium butyrate and 5-aminosalicylic acid on expression of trefoil factor 3, interleukin 1beta, and nuclear factor kappaB in trinitrobenzene sulphonic acid induced colitis in rats. *Postgrad Med J* 2006;82:130-5.
24. Morihara H, Obana M, Tanaka S, Kawakatsu I, Tsuchiyama D, Mori S, et al. 2-aminoethoxydiphenyl borate provides an anti-oxidative effect and mediates cardioprotection during ischemia reperfusion in mice. *PLoS One* 2017;12:e0189948.
25. Suo T, Chen GZ, Huang Y, Zhao KC, Wang T, Hu K. miRNA-1246 suppresses acute lung injury-induced inflammation and apoptosis via the NF-κB and Wnt/β-catenin signal pathways. *Biomed Pharmacother* 2018;108:783-91.