EFFECTS OF BORIC ACID IN AN EXPERIMENTAL RAT MODEL OF HEPATIC ISCHEMIA-REPERFUSION INJURY

MURAT BASBUG[1], MURAT YILDAR[1], İSMAIL YAMAN[1], ÖMER FARUK ÖZKAN[2], HASAN AKSIT[3], FARUK CAVDAR[2], FATMA BAHAR SUNAY[5], MUSA OZGUR OZYIGIT[6], HAYRULLAH DERICI[1]

[1]Department of Surgery, Balikesir University, Faculty of Medicine, Balikesir - [2]Department of Surgery, Onsekizmart University, Faculty of Medicine, Canakkale - [3]Department of biochemistry, Balikesir University Faculty of Veterinary Medicine, Balikesir - [4]Department of Surgery, Yalova State Hospital, Yalova - [5]Department of Histology and Embryology , Balikesir University, Faculty of Medicine, Balikesir - [6]Department of Patology, Uludag University Faculty of Veterinary Medicine Bursa - [6]Department of Patology, Uludag University Faculty of Veterinary Medicine, Turkey

ABSTRACT

Introduction*: Hepatic ischemia-reperfusion injury can cause serious damages and affect distant organs. Boric acid is a antioxidant agent in ischemia/reperfusion injury. The aim of this study was to investigate the effects of boric acid in a rat model of hepatic ischemia-reperfusion injury.*

Material and methods*: 30 rats were divided into three groups: sham, ischemia reperfusion and ischemia-reperfusion+boric acid. The sham group underwent only the surgical stress procedure. In the ischemia-reperfusion group, liver ischemia was induced by clamping the hepatic pedicle for 45 minute, followed by reperfusion for 1 hour. In the ischemia-reperfusion +boric acid group, the therapeutic agent boric acid was administered intraperitoneally, 10 minute before clamping the hepatic pedicle. Serum levels of malondialdehyde, superoxide dismutase, glutathione, total antioxidant capacity, tumor necrosis factor-α, interleukin-6, aspartate aminotransferase, alanine aminotransferase, Gamma-glutamyl transferase were determined. Liver tissues were taken for histopathological examination, DNA fragmentation, and TUNEL staining to determine the apoptotic index.*

Results*: Boric acid moderately reduced serum levels of malondialdehyde, tumor necrosis factor-α, interleukin-6, aspartate aminotransferase, gamma-glutamyl transferase in the ischemia-reperfusion injury group. Superoxide dismutase and alanine aminotransferase levels were decreased significantly in the boric acid-administered group ($P < 0.05$). The histopathological injury scores and the rate of apoptosis were significantly higher in the ischemia-reperfusion group; these injuries were reduced by boric acid administration.*

Conclusion*: Our results demonstrate that boric acid decreases lipid peroxidation and enhances the antioxidant defense mechanism. This study showed that boric acid might protect against ischemia-reperfusion injury in this rat model.*

Key words*: Hepatic Ischemia Reperfusion, Boric Acid.*

Received *November 30, 2014;* ***Accepted*** *May 02, 2015*

Introduction

Hepatic ischemia/reperfusion (IR) injury is a common and serious problem, especially in elective and emergency surgery of the liver, such as liver transplantations, trauma, or resection[(1)]. Hepatic pedicle occlusion, the Pringle maneuvre, minimİzes blood loss for haemostasis, but also induces IR injury. Cellular death and liver dysfunction in the aftermath of hepatic IR injury affect morbidity and mortality after surgery.

The pathogenesis of hepatic IR injury is multifactorial[(2)]. During the IR injury process, various cellular events occur, such as Kupffer cell activation, neutrophil infiltration, increases in reactive oxygen species (ROS), cytokine release, and separation of sinusoidal endothelial cells[(3)]. Overproduction of ROS leads to a reduction in antioxidant levels and the emergence of an oxidant/anti-oxidant imbalance. Greater in vivo production of ROS during oxidative stress, also causes damage to nucleic acids, cellular proteins, and

lipids[4]. Such damage can cause cell death, liver dysfunction, and organ loss[5]. To date, many anti-oxidant and anti-inflammatory agents have been assessed for prevention of IR injuries[6].

Boric acid occurs in nature as a mineral. It has uses in many clinical situations, including cancer treatment (boron capture therapy). Epidemiological studies also suggest that boron may reduce the risk of prostate cancer[7].

Recently, it has been shown that boric acid has protective and anti-oxidant effects[8-11]. It is unknown whether boric acid can prevent or ameliorate liver IR-induced injury. Thus, in the present experimental study, we investigated whether boric acid had protective effects against liver IR injury.

Materials and methods

Protocol

This study was conducted in the Experimental Research Application and Research Center of Canakkale Onsekiz Mart University, Faculty of Medicine, Turkey, after the approval of the local Ethics Committee. All experimental manipulations were conducted in accordance with the US National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Drug

Boric acid (H3BO3), purchased from Sigma-Aldrich (Sigma-Aldrich Chemical Co., St. Louis, MO, USA), was used as the test compound. All other chemicals and reagents were of analytical reagent grade and were from commercial sources.

Animals and experimental protocol

In total, 30 female Wistar albino rats (11 12 weeks of age, weighing 200 224 g) were acclimatized for 1 week before the experiments. The animals were kept in individual cages, housed at constant room temperature (24±2°C) with 12/12-h light/dark cycles, and given standard rat chow. Only water was provided in the 12 h preceding the experiments. The rats were divided randomly into three groups as: sham, ischemia/reperfusion, ischemia /reperfusion + boric acid.

Surgical protocol

Rats were anesthetized with 90 mg/kg ketamine hydrochloride (Ketalar, ParkeDavis, Eczacibasi, Istanbul, Turkey) and 10 mg/kg xylazine (Rompun, Bayer AG, Leverkusen, Germany), given intramuscularly before the surgical procedures. All animals breathed spontaneously throughout the procedures. The mid-abdominal area was shaved and prepared with povidone-iodine. In Group 1 (n = 10), only a laparotomy was performed and was closed after 1 h, 45 min. In Group 2 (n = 10), a laparotomy was performed and the hepatic pedicle was occluded for 45 min, using a vascular clamp. The clamp was then removed and reperfusion was established subsequently for 1 h. In Group 3 (n = 10), boric acid was administered, 200 mg/kg intraperitoneally (ip), 10 min before induction of the IR procedure in the liver. The other groups were administered saline. All rats were sacrificed after the reperfusion period. Serum and tissue samples for biochemical analyses were stored at 80°C. Tissue samples for histopathological examinations were stored in 10% formaldehyde solution until examination.

Biochemical evaluation

Blood samples were centrifuged (3000 rpm, 10 min) to obtain serum, which was stored at 20°C until analysis. Serum samples were subjected to determination of alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), urea, creatinine, tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6) malondialdehyde (MDA), superoxide dismutase (SOD), glutathione (GSH), and total antioxidant capacity (TAC) levels.

Enzyme-linked immunosorbent assay (ELISA) results were measured using a microplate reader at 450 nm. Serum ALT (Archem, A2221, Istanbul, Turkey), AST (Archem, A2212), and GGT (Archem, A2171) activities were measured using commercially available kits and a biochemistry auto analyzer (D280; Sinnowa, Nanjing, China). Serum creatinine (Archem, A2162) and serum urea (Archem, A2331) levels were measured similarly using commercially available kits on the same auto analyzer. Double sandwich enzyme-linked immunosorbent assay kits (eBioscience) were used to measure the serum concentrations of TNF-α and IL-6.

SOD measurements were performed according to the method of Sun et al.[12]. The results are shown as % inhibition/mg. SOD estimation was based on the generation of superoxide radicals, produced by xanthine with xanthine oxidase, which reacts with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride to form a red formazan dye. SOD activity was then measured by the degree of inhibition of this reaction. GSH measurements were performed by the method of Fairbanks and Klee[13]. GSH levels in

the supernatant were estimated using 5,5'-bis-dithionitrobenzoic acid reagent. The results were determined against an aqueous standard solution of GSH (Sigma Chemical Co.), and are expressed as mg/mg tissue protein.

Lipid peroxidation was assayed in terms of MDA levels reacting with thiobarbituric acid, according to the method of Yoshioka et al.[14]. Serum TAC was determined using an automated measurement method with a commercially available kit (Rel Assay Diagnostics, Turkey).

Histopathological examination

Liver tissue samples were taken from all groups and fixed in 10% neutral buffered formalin. After routine dehydration and clearing procedures they were embedded in paraffin. The 5 μm hick sections were stained with hematoxylin and eosin (H&E). Sections were examined and photos were taken by a histologist who was unaware of the groups by using a Olympus BX51 light microscope, the Olympus DP72 camera and DP2 BSW software.Pathologist blindly analyzed sections. Histological appearance of tissues in different groups was compared among themselves. Hepatic injury was evaluated for severity of hepatic injury using an ordinal scale as follows

Grade 0 = minimal or no evidence of injury; grade 1 = mild injury with cytoplasmic vacuolation and focal nuclear pyknosis; grade 2 = moderate to severe injury with enlarged nuclear pyknosis, cytoplasmic hypereosinophilia and loss of intercellular borders; grade 3 = severe necrosis with disintegration of hepatic cords, hemorrhage and neutrophil infiltration[15].

Terminal Deoxynucleotidyl Transferase dUTP Nick End Labelling Staining For Detection Of Apoptotic Cells and Apoptotic Index

Apoptosis was detected by the TdT-mediated dUTP-biotin nick end labelling (TUNEL) method using ApopTag® Peroxidase In Situ Apoptosis Detection Kit (ApopTaq Peroxidase In Situ Apoptosis Detection Kit, S7101-KIT, Millipore). The tissue sections were deparafinized and fixed. Thereafter, the sections were incubated with 3% H2O2 for 5 minutes at room temperature followed by washing with PBS (2 x 5 minutes). Sections were incubated with equilibration buffer for at least 10 minutes at room temperature and then with working strength TdT enzyme at 37°C for 60 minutes, at which point the sections were incubated with working strength stop/wash buffer for 10 minutes after being agitated for 15 seconds at room temperature, followed by washing with PBS (2 x 1 minute). Sections were then incubated with anti-digoxigenin conjugate for 30 minutes at room temperature followed by washing with PBS (4 x 2 minutes). Sections were stained with 3,3'-Diaminobenzidine (DAB) solution, dehydrated, and mounted. The apoptotic index (AI) was obtained as the ratio of TUNEL-positive cells relative to the total number of counted cells. Apoptotic index was counted on 10 randomly selected ×40 high-power fields containing representative sections. The apoptotic index, that is, the percentage of apoptotic cells displaying a specific lineage antigen (LAg) within a population of cells that remain unfragmented and retain the expression of the LAg was defined as follows: apoptotic cells/total cells X 100.

DNA fragmentation

The measurement of cytoplasm histone-associated DNA fragments was performed with the Cell Detection ELISA plus (Manheim, Germany). Briefly, tissues were homogenised in cold lysis buffer for 3 minutes and centrifuged at 20 000 g for 10 minutes at 4ºC. Cytoplasm lysates (supernatants) from tissues were transferred to a streptavidin coated plate supplied by the manufacturer. A mixture of antihistone-biotin and anti-DNA-POD (anti-DNA antibody conjugated with peroxidase) were added to tissue lysates and incubated 2 hours at room temperature. The complex was then simultaneously conjugated with the peroxidase substrate (ABTS) to form an immune complex on the plate, whose the absorbance was read at 405 nm using an ELISA-reader (Thermo Multiskan FC, USA).

Statistical analysis

The data were expressed as the mean standard deviation. The Statistical Package for the Social Sciences for Windows (SPSS 21 Inc., Chicago, IL, USA) was

used for statistical analysis. The one-way analysis of variance was used to test the differences between the groups. The tukey's honestly significant difference (HSD) test was used for multiple comparisons. In all analyses, a P value <0.05 was considered statistically significant.

Results

All animals survived the experimental procedures.

Histological changes

Tissues from the sham group showed the normal structure of liver tissue. The IR group showed central vein and sinusoidal congestion, hepatocellular hypertrophy, and dissociation of hepatocyte cords. Administration of boric acid decreased the severity of all these changes (Figure 1 C1,2). Liver IR injury scores were significantly correlated with serum TAC and MDA levels. DNA Fragmentations and apoptosis levels were also positively correlated with liver histopathological scores. This score was significantly lower in the boric acid-administered group ($p < 0.05$). Histological scores are shown in Figure 2.

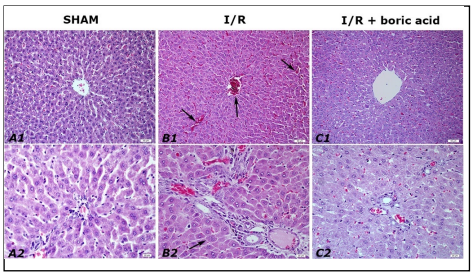

Fig 1: Representative liver photos from sham group (A1 and A2), ischemia and reperfusion group (B1 and B2) and ischemia reperfusion + boric acid group (C1 and C2). Pictures at the upper line (A1, B1 and C1) show central veins of the related groups and pictures at the lower line (A2, B2, C2) show portal areas. Normal liver histology is seen in sham group (A1 and A2). In ischemia and reperfusion group, central vein and sinusoidal congestions (arrows at B1), hepatocellular hypertrophy, and dissociation of the hepatocyte cords (arrow at B2) are seen. Boric acid administration improved all these pathological changes (C1 and C2).

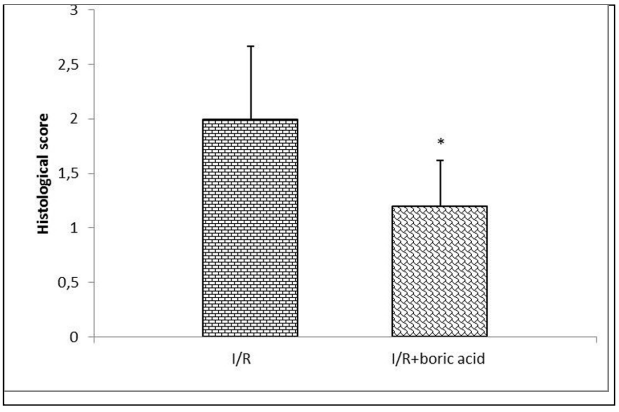

Fig 2: Mean histologic scores in all experimental groups. Data are expressed as the mean ± standard deviation.

** P < 0.001 compared with the I/R group.*

I/R,ischemia/reperfusion group; I/R+boric acid, ischemia/reperfusion+boric acid group

Biochemical changes

ALT, AST, GGT, urea and creatinine levels increased after reperfusion in the IR group versus the sham group. Levels of these markers were decreased in the IR + boric acid group versus the IR group (Table 1). These biochemical values were not significantly different, with the exception of the ALT value in the IR + boric acid group versus the IR group ($P < 0.05$).

Groups	ALT	AST	GGT	UREA	CR
Sham	54±10.75	152.1±26.13	3.3±0.82	43.5±9.15	0.36±0.11
I/R	58±6.91	201.2±5.53	7.8±1.98	70.5±4.14	0.72±0.11
I/R+BA	48±6.94**	192.8±13.53	6.5±1.43	66.1±6.55	0.62±0.14

Table 1: The mean AST, ALT, GGT, Urea and creatinine levels and statistical results in all groups. Results are expressed as the mean _ standard deviation.

***P < 0.05 compared with the sham group.*

AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma-glutamyl transferase; CR, creatinin

Mean serum GSH and SOD levels were lower in the IR group than in the sham group. Serum SOD levels differed significantly among the groups ($P < 0.05$). Decreases in these markers in I/R were augmented by intraperitoneally(i.p.) administration boric acid (Table 2).

Groups	TNF-α	SOD	MDA	TAC	IL-6	Glutation
Sham	28.66±5.59	59.50±2.14	26.22±3.34	2.72±0.49	7.91±1.88	22.70±2.90
I/R	38.68±7.91	51.83±2.06	31.77±5.08	2.64±0.45	13.01±2.71	17.80±2.39
I/R+BA	32.57±4.39	56.26±2.98*	28.06±4.16	2.47±0.26	12.38±3.25	19.50±1.35

Table 2: The mean levels of serum TNF-α, SOD, MDA, TAC, IL-6,Glutation levels and statical results in all experimental groups.Results are expressed as the mean _ standard deviation.

** P < 0.05 compared with the I/R group*

TNF-α,tumor necrosis factor-α, IL-6, interleukin-6; malondialdehyde (MDA), SOD, superoxide dismutase; .MDA, malondialdehyde; TAC, total antioxidant capacity

The MDA level, as a marker of lipid peroxidation, was increased after reperfusion in the IR group and the IR + boric group compared with the sham group. Boric acid prevented the increase in MDA levels in the IR + boric acid group versus the IR group.

Plasma IL-6 and TNF-α levels were increased in the IR groups. Boric acid caused a moderate decrease in IL-6 and TNF α levels (Table 2).

Levels of total antioxidant capacity

The mean TAC levels in serum were lower in the IR group than in the sham group. The IR induced decrease in these markers was moderately increased in the IR + boric acid group. However, the difference was not statistically significant ($P > 0.05$).

Apoptotic DNA fragmentation changes

DNA fragmentation, a hallmark of apoptotic death, showed a ~fourfold increase in the IR group compared with the sham group. Boric acid moderately reduced DNA fragmentation in the treatment group. The apoptotic index and DNA fragmentation results are shown in Table 3.

	SHAM	I/R	I/R+boric acid
DNA Fragmentions	0.1150±0.83	0.4787±0.19	0.3832±0.91
Apopitotic index	11%	47%	38%
HS	0	2±0.67	1.2±0.42***

Table 3: DNA fragmentations and histopathologic evaluation of liver for each group Data are given as mean±SD. HS: Histological score. S: Sham.
I/R: Ischemia/Reperfusion. I/R+boric acid: Ischemia/Reperfusion + Boric Acid
**** $P < 0.05$ compared with the I/R group*

Effects of boric acid on TUNEL staining

Livers from the IR group animals exhibited extensive nuclear changes indicating apoptotic cell death, as shown by the TUNEL-positive cells. Treatment with boric acid reduced the number of TUNEL-positive cells (Figure 3).

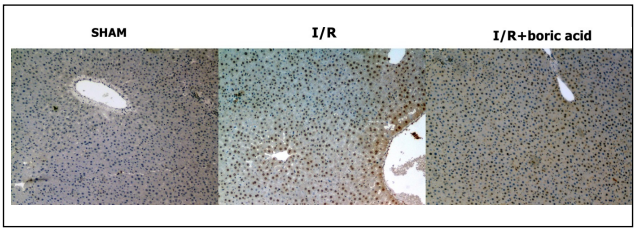

Fig 3: TUNEL analysis for apoptotic cells shown in (Sham), (I/R), and (I/R+boric acid). Nuclei of TUNEL-positive cells are stained brown in contrast to blue-stained TUNEL negative cells.

Discussion

This is the first reported study to reveal a protective effect of boric acid against hepatic IR injury. Boric acid may affect various steps of the pathophysiological pathways that lead to hepatic IR injury. The most probable mechanism is inactivation of ROS by acting as a ROS scavenger, because boric acid reduced MDA levels in this study. SOD, glutathione, and ROS levels were maintained. Boric acid also suppressed inflammation by reducing the release of pro inflammatory cytokines, such as TNF-α and IL-6, and protected the cells from the pathway(s) leading to apoptosis.

Several mechanisms are involved in hepatic IR injury. A decrease in blood flow to the liver leads to a reduction in oxidative phosphorylation, increased ATP consumption, and compromise of intracellular calcium level[16]. ROS levels increase with reperfusion. Changes, such as pro-inflammatory cytokine release, cell membrane damage, and greater compromise of calcium hemostasis occur as ROS levels increase. Injury at the cellular level occurs when these changes are accompanied by an increase in DNA fragmentation, Kupffer cell activation, neutrophil and macrophage infiltration, vasoconstriction, and chromosomal damage. Cellular damage increases as a result of a decrease in the levels of antioxidant agents and cytoprotective substances, such as prostaglandins[17,18].

An increase in ROS, one of the major mediators of IR injury, leads to lipid peroxidation in both the cell and organelle membranes. MDA is an important marker of oxidative stress occurring through lipid peroxidation and is used to assay cell damage. MDA levels in this study were lower in the group given boric acid than in the IR group. As shown by Turköz et al., boric acid may reduce lipid peroxidation, and, therefore, MDA concentrations, by scavenging ROS[19].

ROS in the intracellular space are inactivated by antioxidants. SOD, in particular, an antioxidant enzyme, plays an important role in antioxidant defence mechanisms by reducing the effects of ROS[20]. Ince et al. showed a rise in the level of antioxidant enzymes following administration of boric acid in livers with induced hepatotoxicity[21]. In our study, TAS, and particularly SOD levels, that decreased with IR increased significantly with the administration of boric acid.

Together with the activation of macrophages in tissues, reperfusion causes an increase in pro-inflammatory cytokine levels. Increases in the levels of IL-6 and TNF-α, pro-inflammatory cytokines significantly involved in reperfusion injury, lead to injury in both reperfused and distant organs[22]. TNF-α and other cytokines activate many proteases, such as caspases-3 and 8, during the reperfusion

period. The subsequent cascade of events leads ultimately to DNA destruction[23,24]. In this study, TNF-α and IL-6 levels that increased with IR decreased with boric acid administration. Thus boron may also have anti inflammatory effects.

In a study of experimentally induced hepatic IR injury, Crockett et al. reported changes, such as sinusoidal congestion in the liver, cytoplasmic vacuolization, and neutrophil infiltration[25]. Similarly, the histopathological changes in livers exposed to IR in this study were compatible with hepatic injury. The histopathological findings in hepatic tissue improved significantly with the administration of boric acid (Figure 1 C1,2). The increase in ALT levels following IR injury in liver function tests decreased significantly following boric acid administration.

The first lesions occurring in oxidative injury in DNA are branch fragmentation. Increasing free oxygen radicals enter into reactions with thymine in nuclear and mitochondrial DNA, causing single-strand breaks. Necrotic cell death then occurs as cells lose energy. Apoptotic cells that are too infrequent to be detected in DNA investigations can be observed using TUNEL staining(26). In our study, boric acid reduced the degree of DNA fragmentation occurring following IR, in part by preventing oxidative damage. This finding is supported by the decrease in the apoptotic cell index, as determined by TUNEL staining.

In conclusion, i.p. administration of boric acid before induction of IR protects against hepatocyte injury leading to apoptosis by reducing oxidative stress and lipid peroxidation in the liver. Boric acid can be used as an antioxidant agent against hepatic IR. This is the first report of a protective effect of boric acid against hepatic IR injury. More rigorously designed studies are required to further investigate the role of boric acid in preventing IR injury.

References

1) Gao Z, Li Y-H. *Antioxidant Stress and Anti-Inflammation of PPARα on Warm Hepatic Ischemia-Reperfusion Injury*. PPAR Research 2012; 2012: *738785,8* pages
2) Nicoud IB, Knox CD, Jones CM, 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(3): *623-630*.
3) Bahde R, Spiegel H-U. *Hepatic ischaemia-reperfusion injury from bench to bedside*. Br J Surg 2010; 97(10): *1461-1475*.
4) Aksit H, Bildik A: *Determination of DNA Damage in Experimental Liver Intoxication and Role of N-Acetyl Cysteine*. Cell Biochem Biophys 2014. doi:10.1007/s12013-014-0031-4.
5) Baykara B, Tekmen I, Pekcetin C, et al. *The protective effects of carnosine and melatonin in ischemia-reperfusion injury in the rat liver*. Acta Histochem 2009; 111(1): *42-51*.
6) Sahin T, Begeç Z, Toprak Hİ, et al. *The effects of dexmedetomidine on liver ischemia-reperfusion injury in rats*. J Surg Res 2013; 183(1): *385-90*.
7) Gallardo-Williams MT, Maronpot RR, Wine RN, Brunssen SH, Chapin RE. *Inhibition of the enzymatic activity of prostate-specific antigen by boric acid and 3-nitrophenyl boronic acid*. Prostate 2003; 54(1): *44-49*.
8) Ince S, Kucukkurt I, Cigerci IH, Fatih Fidan a, Eryavuz A. *The effects of dietary boric acid and borax supplementation on lipid peroxidation, antioxidant activity, and DNA damage in rats*. J Trace Elem Med Biol. 2010; 24(3): *161-164*.
9) Turkez H, Geyikoglu F, Tatar A, Keles MS, Kaplan I. *The effects of some boron compounds against heavy metal toxicity in human blood*. Exp Toxicol Pathol. 2012; 64(1-2): *93-101*.
10) Türkez H, Geyikoğlu F, Tatar A, Keleş S, Ozkan A. *Effects of some boron compounds on peripheral human blood*. Z Naturforsch C 2007; 62(11-12): *889-896*.
11) Colak S, Geyikoglu F, Keles ON, Türkez H, Topal A, et al. *The neuroprotective role of boric acid on aluminum chloride-induced neurotoxicity*. Toxicol Ind Health. 2011; 27(8): *700-710*.
12) Sun Y, Oberley LW, Li Y: *A simple method for clinical assay of superoxide dismutase*. Clin Chem 1988; 34(3): *497-500*.
13) Fairbanks SW, Klee GG: *Biochemical aspects of hematology*. In: Burtis CA, Ashwood ER, editors. Tietz textbook of clinical chemistry 3rd ed. Philadelphia: WB Saunders; 1999: *1690*.
14) Yoshioka T, Kawada K, Shimada T, Mori M. *Lipid peroxidation in maternal and cord blood and protective mechanism against activated-oxygen toxicity in the blood*. Am J Obstet Gynecol. 1979; 135(3): *372-376*.
15) Önder A, Kapan M, Gümüfi M, Yüksel H, Böyük A, et al. *The protective effects of curcumin on intestine and remote organs against mesenteric ischemia / reperfusion injury*. Turk J Gastroenterol 2012; 23(2): *141-147*.
16) Montalvo-Jave EE, Escalante-Tattersfield T, Ortega-Salgado J a, Piña E, Geller D: *Factors in the pathophysiology of the liver ischemia-reperfusion injury*. J Surg Res 2008;147(1): *153-159*.
17) Kaplowitz N: *Mechanisms of liver cell injury*. J Hepatol 2000; 32(1 Suppl): *39-47*.
18) Jaeschke H: *Molecular mechanisms of hepatic ischemia-reperfusion injury and preconditioning*. Am J Physiol Gastrointest Liver Physiol 2003; 284(1): *15-26*.
19) Türkez H, Geyİkoğlu F, Çolak S: *The protective eff ect of boric acid on aluminum-induced hepatotoxicity and genotoxicity in rats*. Turk J Biol 2011; 35: *293-301*.
20) Abu-Amara M, Yang SY, Tapuria N, Fuller B, et al. *Liver ischemia/reperfusion injury: processes in inflammatory networks-a review*. Liver Transpl 2010;16(9): *1016-1032*.

21) Ince S, Keles H, Erdogan M, Hazman O, Kucukkurt I. *Protective effect of boric acid against carbon tetrachloride - induced hepatotoxicity in mice*. Drug and Chemical Toxicology 2012; 35: *285-292*.
22) 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(2): *683-689*.
23) Selzner N, Rudiger H, Graf R, Clavien P-A. *Protective strategies against ischemic injury of the liver*. Gastroenterology 2003; 125(3): *917-936*.
24) Arumugam T V, Shiels IA, Woodruff TM, Granger DN, Taylor SM. *The role of the complement system in ischemia-reperfusion injury*. Shock 2004; 21(5): *401-409*.
25) Crockett ET, Galligan JJ, Uhal BD, Harkema J, Roth R, Pandya K. *Protection of early phase hepatic ischemia-reperfusion injury by cholinergic agonists*. BMC Clin Pathol. 2006; 6: *3*.
26) Fak TIP, Cerrah G, Tez U, Yavuzer N. *Karaciğerde iskemi reperfüzyon ile indüklenmiş rejenerasyon modelinde kompleman inhibitörünün rolü*. 2008.

Acknowledgement
This study was supported by The Scientific and Technological Research Council of Turkey (Project no: 113S445)

Correspoding author
MURAT BASBUG, Assos Prof,
Department of Surgery, Balikesir University
Faculty of Medicine
10145, Balikesir
(Turkey)