EXPERIMENTAL BIOMEDICAL RESEARCH

http://www.experimentalbiomedicalresearch.com

Research article

Effects of magnesium sulphate on liver ischemia/reperfusion injury in a rat model

Kerem Akkoca[1], Hamit Yoldas[1], Mustafa Sit[2], Ibrahim Karagoz[1], Isa Yildiz[1], Abdullah Demirhan[1], Murat Bilgi[1], Ozgur Mehmet Yis[3], Aysel Kukner[4], Ayhan Cetinkaya[5], Oguz Catal[2], Bahri Ozer[2]

Department of Anesthesia and Reanimation[1], General Surgery[2], Biochemistry[3], Histology and Embryology[4] and Physiology[5], Bolu Abant Izzet Baysal University, Faculty of Medicine, Bolu, Turkey
[4]Department of Histology and Embryology, Near East University, Faculty of Medicine, Nicosia, TRNC

ABSTRACT

Aim: To investigate the protective efficacy of magnesium sulphate in a model of rat liver ischemia-reperfusion (I/R) injury.

Method: 32 adult female Wistar-Albino rats (250 to 350 g) were used in this experimental study. Rats were divided into 4 groups according to liver ischemia and magnesium sulfate application methods. Group 1 (C); control, group 2 (M); magnesium sulphate, group 3 (I/R); liver I/R, group 4 (I/R+M); I/R + magnesium sulphate treated. The blood samples were centrifuged for the study of aspartate aminotransferase (AST), alanine aminotransferase, prothrombin time (PT), international normalized ratio (INR) troponin I, total antioxidant status (TAS), total oxidant status (TOS) assays. The livers of the animals were removed at the end of the study and samples were taken for histopathological examination.

Results: AST and INR values were significantly decreased in I/R+M group compared to I/R group. There was no significant difference in ALT values of the groups. Although not statistically significant, the TAS values were increased in I/R + M group compared to I/R group rats. In addition, the value of TOS was found to be lower in I/R + M group rats. In the histopathological examination, the mean values of apoptosis and necrosis were lower in the IR+M group compared to the I/R group.

Conclusion: The main finding of the present study suggested that magnesium sulphate pretreatment moderately decreased the liver damage through its anti-inflammatory and anti-oxidant effects in a rat model of liver I/R.

Keywords: Liver ischemia/reperfusion injury, magnesium sulfate, oxidative stress, rat.

Corresponding Author: Dr. Mustafa Sit
Department of General Surgery, Bolu Abant Izzet Baysal University, Faculty of Medicine, Bolu, Turkey
E mail: drmustafasit@yahoo.com.tr

ORCID ID: https://orcid.org/0000-0002-7475-7298

Introduction

Ischemia is a pathological condition in which the oxygen and metabolic products needed by the tissues cannot be supplied and the metabolites produced cannot be removed by the blood flow due to the inadequate perfusion of the organ or tissues. Reperfusion is the recovery of blood circulation in ischemic tissue. While the resumption of blood flow in an ischemic tissue may supply the tissue's oxygen and other metabolic requirements, it may also cause more damage to the tissues than ischemia. This sequence of events is called ischemia and reperfusion (I/R) damage. There are many conditions that cause I/R damage, such as, trauma, organ transplantation, myocardial infarction, stroke, hypovolemic shock and sepsis, which all are usually common clinical manifestations [1]. Metabolic changes that occur in cells during ischemia include cell death, depletion of cellular energy stores and accumulation of toxic metabolites. Moreover, oxidative phosphorylation decreases in oxygen-depleted cells. The synthesis of high-energy phosphates such as adenosine triphosphate (ATP) and phosphocreatine is reduced while anaerobic metabolites accumulate in the cell. The intracellular free Ca^{2+}, K^+ and Na^+ increase due to the non-functioning ATP-dependent Na^+/K^+ and Ca^{2+} pumps in the cell membrane. Activation of phospholipase, cell membrane damage, mitochondrial dysfunction and prolongation of the ischemic process irreversibly lead to lysosomal enzyme activation and ultimately cell death by apoptosis [2].

Reperfusion takes place right after ischemia by restoration of blood supply to the tissues. In this period, the damage increases in the tissue and the cell by the high O_2 concentration. Lipid peroxidation, membrane damage, deoxyribonucleic acid damage, protein denaturation and mitochondrial damage occur due to elevated levels of reactive oxygen species (ROS) and reactive nitrogen species (RNS). During reperfusion free oxygen radicals cause endothelial damage, increased micro-vascular permeability and tissue edema. Activated adhesion molecules and cytokines may induce systemic inflammatory response. By ongoing inflammatory reaction, leukocyte-associated cell damage is followed by deterioration in microcirculation. The process that begins with tissue ischemia produces an inflammatory response after reperfusion and it advances to cell damage even after restoration of blood-flow [3].

Liver is an essential organ in maintaining proper nutrition status in healthy individuals through the regulation of protein, carbohydrate and fat metabolism. Liver damage caused by ischemia and reperfusion represents a process continuity that causes hepatocellular damage. Ischemia-reperfusion injury in the liver is usually seen during hemorrhagic shock, advanced sepsis, liver transplantation, surgical intervention for a major trauma and hepatic resection. The most common procedure to reduce bleeding during hepatic resection is clamping the liver pedicle to intercept the blood flow to the liver, which is known as Pringle maneuver, however, it may also cause potentially dangerous hepatic parenchymal ischemia [4]. Understanding the mechanisms of this damage and investigating the measures and drugs that may reduce the damage are of great importance in order to increase the success of liver surgery and to reduce the damage of chronic liver diseases [4].

Magnesium is the 4th most common cation in the body, which mainly place in intracellular space. Magnesium has effects on enzymatic reactions, cell channels, receptors and

intracellular signaling molecules in various cells, including mononuclear blood cells. It also acts as an ion channel regulator. It contributes to maintaining the cellular ion balance. It provides smooth muscle relaxation and vasodilatation by reducing intracellular calcium concentration. Magnesium is also required for endocrine functions and protein synthesis [5]. Magnesium sulfate ($MgSO_4$), as a powerful antioxidant, has significant anti-inflammatory effects. It inhibits up-regulation of endotoxin-dependent inflammatory molecule. Magnesium is an L-type calcium channel blocker and may be effective in preventing I/R damage by reducing calcium overload associated with tissue damage in this manner [6-9].

The literature is lacking knowledge about the protective efficacy of magnesium sulfate in the rat liver I/R injury model. Therefore, we investigated whether pre-treatment with magnesium sulfate attenuates liver injury induced by I/R in an in vivo rat model.

Methods

Experimental procedure was started after obtaining approval from Ethics Committee of Animal Research of Faculty of Medicine, Bolu Abant Izzet Baysal University (BAIBU) (Decision no: 2017/14). The procedures were conducted according to routine animal care guidelines, and all experimental procedures complied with the Guide for the Care and Use of Laboratory Animals (1996). 32 adult female Wistar-Albino rats (250 to 350 g) were used in BAIBU Experimental Animals Multidisciplinary Laboratory. Rats were kept in a 12-h light/12-h dark environment to maintain their adaptation to the beginning of the study and fed with standard food and water. They were only allowed to drink water for 12 h prior to surgery.

The weight of all rats was determined by the sensitive weighing and the drugs to be used were prepared according to their weight. Anesthesia was provided with intraperitoneal 90 mg/kg body weight of ketamine (Ketalar®, Pfizer Pharma GMBH, and Germany) and 10 mg/kg xylazine hydrochloride (Alfazine®, 2%, Alfasan International, 3440 AB, Woerden, Holland). 15 minutes after the drug injection, hair on the anterior abdominal wall were shaved.

Experimental design

Rats were divided into 4 groups: Group 1 (C, n=8); control group. After the laparotomy, hepatic pedicle was seen and kept under anesthesia without any intervention during 140 minutes (surgery time of groups 3 and 4). Group 2 (M, n=8); magnesium sulphate group. 200 mg/kg of magnesium sulfate was administered intraperitoneally after appropriate anesthesia. Laparotomy was performed after 20 minutes. Hepatic pedicle was seen and kept under anesthesia without any intervention during 140 minutes (surgery time of groups 3 and 4). Group 3 (I/R, n=8); ischemia-reperfusion. After waiting 20 minutes from initiation of anesthesia, laparotomy was performed. Liver ischemia provided for 60 minutes and followed by reperfusion during another 60 minutes. Group 4 (I/R + M, n=8); ischemia-reperfusion + magnesium sulphate group. After anesthesia with 200 mg/kg of intraperitoneal magnesium sulfate, laparotomy performed in 20th minute of anesthesia. Liver ischemia provided for 60 minutes and followed by reperfusion during another 60 minutes.

Rat model of hepatic I/R injury

Rats group received magnesium were dispensed 200 mg/kg of magnesium sulfate

intraperitoneally 20 minutes before laparotomy. Laparotomy was performed with an incision of 3-4 cm from the midline. After the organs in the abdomen became visible, the portal vein and the hepatic artery were explored in the groups other than the control group and the magnesium group, and the blood supply to the liver was discontinued by the atraumatic vascular clamp. With the aid of atraumatic vascular clamp, ischemia was sustained for 60 minutes. Sufficient occlusion was confirmed by the absence of pulsation in the hepatic artery and by fading of the tissue. The clamps were removed and the ischemia was terminated after 60 minutes. Therefore, after the interval of another 60 minutes reperfusion was achieved. The intra-cardiac blood sample was taken from the right atrium of the rat. Blood samples were centrifuged for the study of aspartate aminotransferase (AST), alanine aminotransferase, prothrombin time (PT), international normalized ratio (INR) troponin I, total antioxidant status (TAS), total oxidant status (TOS) assays. The liver was removed and samples were taken for histopathological examination. Samples stained with hematoxylin and eosin dye and the liver injury score was evaluated.

Measurement of biochemical parameters
The blood sample was centrifuged for 10 minutes at 3000 rpm after coagulation of the blood and serum samples obtained. These samples were stored at -20 °C until laboratory assays done. Serum AST, ALT, troponin and INR levels were measured in the Architect C8000 device (Please write company and country name) in the Biochemistry Laboratory of BAIBU Hospital and the measured values were expressed as U/L for ALT and AST, and as pg/ml for troponin. INR assays were performed in the same laboratory by KOAG

SYMEX device.

Total oxidant status (TOS) analysis
Total Oxidant Status analysis was performed by Total Oxidant Status kit (Rel Assay Diagnostics, Gaziantep, Turkey) and in an Olympus AU 400 biochemistry auto analyzer. Unit of TOS was µmol H_2O_2 equivalent/L.

Total antioxidant status (TAS) analysis
Total antioxidant status analysis was done by Total antioxidant commercial kit (Rel Assay, Gaziantep, Turkey) in an Olympus AU 400 biochemistry auto analyzer. Unit of TAS was Trolox equivalent/L.

Calculation of oxidative stress index (OSI)
Total oxidative stress values of the samples were calculated according to the total antioxidant status values in terms of percentage and OSI values were calculated with following formula:
OSI = 100 x TOS (µmol/L H_2O_2) / TAS (µMol/L Trolox).

Histopathological evaluation
Hepatic tissue was fixed in a 10% formaldehyde solution for 24 h for evaluation in light microscope. To be followed by tissue trimming, the tissue was washed for overnight in order to remove the fixative. For dehydration, samples were kept in a series of an ethanol/water (v/v) in order 70%, 80% 96% and 100% each for one hour. After one hour of exposure to two different xylenes for the purpose of transparency, two to three hours of immersion with paraffin was achieved 2 times. The tissues were then embedded in paraffin blocks. Paraffin sections of 4 µm (Feather S35) were obtained by means of the rotary microtome (RM 2255, Leica).
The fixed tissue sections were left in oven at

60°C for 45 minutes for deparaffinization process. Then they were subjected to xylene three times, initially 20 minutes in oven and the 10 minutes each for the remaining 2 processes. They were passed through the alcohol series from absolute to 96% to 70% of alcohol for rehydration process. The sections were washed with distilled water for 5 min. Then rehydrated tissues were stained with hematoxylin (01562E, Surgipath, Bretton, Peter Borough, Cambridgeshire). The excess of dye was removed from the samples by placing under water stream for 10 minutes and then, 2 minutes staining with Eosin (01602E, Surgipath, Bretton, Peter Borough, Cambridgeshire) was applied to the samples. After dyeing, Entellan coating used for sections which were passed through 70%, 80%, 96% and 2 series of absolute alcohol series, and those kept in three changes of xylene each for 20 minutes.

Liver tissue samples stained with HE were examined at least 10 sites with 40x lenses under light microscopy. Changes in cell histology were evaluated by light microscope. Using the tissues scoring table, scoring 0-3 was performed in terms of cytoplasmic vacuolization, sinusoidal dilatation and cell necrosis [10] (Table 1).

Statistical Analyses
Statistical Package of Social Sciences 24 (SPSS 24.0, Chicago, IL, USA) software was used for statistical analyses. Normal distribution of the data was analyzed by means of Shapiro-Wilks test. Since ALT and OSI have normal distribution, One Way ANOVA test was applied in comparison of these variables between study groups. Kruskal-Wallis analysis of variance was performed in the analysis of other data. The Mann-Whitney U test was used in dual comparison of the groups and all data

were expressed as mean ± standard deviation (mean ± SD). A p value of <0.05 was considered statistically significant.

Table 1. Scoring of liver histopathological injury.

Histopathological indication of liver damage	Score	Interpretation of score
Cytoplasmic vacuolization	0	none
	1	rarely
	2	in some lobules
	3	many lobules
	4	widespread
Sinuzoidal dilatation	0	none
	1	rarely
	2	frequent perivenul
	3	frequent perivenul-midzone
	4	frequent panlobule
Cell necrosis	0	none
	1	1-2 apoptosis cell
	2	>3 apoptosis cell
	3	1-2 focal necrosis area
	4	>3 focal necrosis area

Results
The biochemical and oxidative values of the different groups are given in Table 2. Mean serum ALT, AST, troponin I, INR values was significantly different in I/R+M and I/R group compared to C and M groups.

When the mean serum AST levels of I/R + M and I / R groups were compared, a statistically significant difference was found *(p=0.035)*. In additionally, the mean serum INR levels were significantly different in IR+M group compared to I/R group *(p=0.009)*. Although there was an increase in TAS values and decrease in TOS values with magnesium sulphate treatment, there was no statistically significant difference between these two experimental groups.

**Table 2.** The biochemical and oxidative values in the different groups.

Groups	ALT (IU)	AST (IU)	Troponin (pg / ml)	INR	TAS	TOS	OSI
C	58.1 ±13.3	158.2±25.2	412±488	0.81±0.77	1.53±0.47	3.42 ±1.34	2.28±0.87
M	49.2 ±12.7	182.2±42.7	483±429	0.86±0.76	1.76±0.46	5.13±5.43	2.60±1.88
I/R	717±344*	1280±666*	23629±21716*	1.71 ±0.55*	2.40±1.09*	28.9±16.1 *	9.38±3.9*
IR+M	561±307*	713±309* ▲	2898±2946*	0.94±0.17¶	2.96±0.92*	20.4±13.5 *	8.28±3.6*

C: Control group, M: magnesium sulphate group, I/R: Liver ischemia-reperfusion group, I/R+M: Liver ischemia-reperfusion+ magnesium sulphate treated group, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, INR: International normalized ratio, TAS: Total antioxidant status, TOS: Total oxidant status, OSI: Oxidative stress index. ▲I/R vs IR+M *p= 0.035,* ¶I/R vs IR+M *p= 0.009.*

The liver histopathological injury scores of rats were shown in figure 1. In the control group, almost normal histopathological appearance was obtained. Minimal sinusoidal dilatation was observed in M group. Histopathological examinations revealed vacuolization, sinusoidal dilatation, apoptosis and necrosis in all experimental groups. These pathological findings were more pronounced in the I/R group than in the IR + M group (Figure 2. A-F). However, there was no statistical significance in these parameters.

Figure 1. The liver histopathological injury score of different groups.
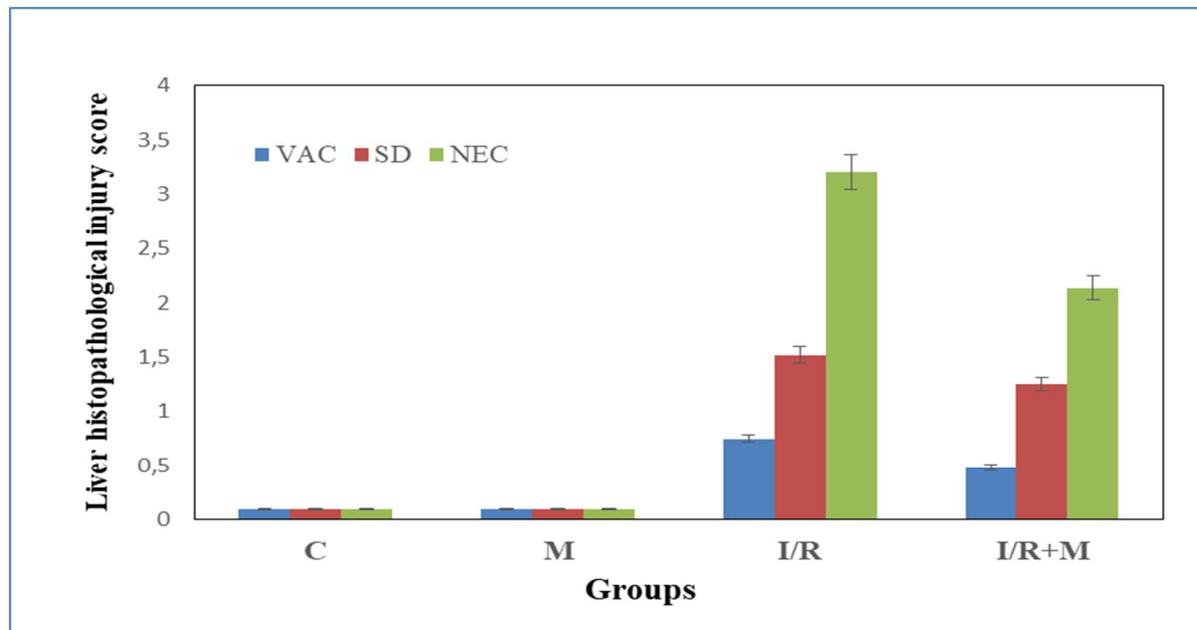
C: Control group, M: magnesium sulphate group, I/R: Liver ischemia-reperfusion group, I/R+M: Liver ischemia-reperfusion+ magnesium sulphate treated group, VAC: Cytoplasmic vacuolization, SD: Sinusoidal dilatation, NEC: Necrosis and apoptosis.
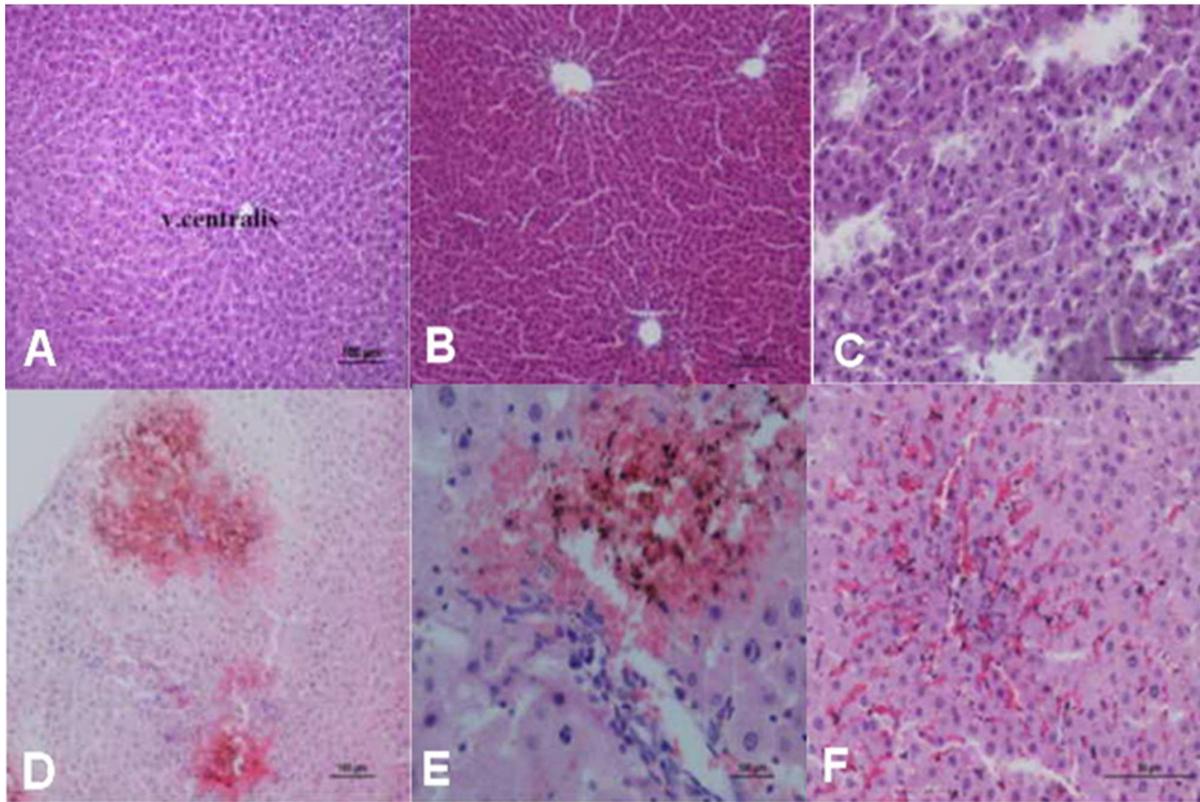
Figure 2. Microscopic observation of liver damage; A; Normal liver parenchyma (10X, H&E) (Group C), B; Sinosoidal dilatation (10X, H&E) (Group M), C; Apoptosis (10X, H&E) (I/R group), D; Sinosoidal dilation (20X, H&E) (I/R group) E; Necrosis (20X, H&E) (I/R group), F; Sinozoid dilatation (20X, H&E) (I/R-M group).

Discussion

One of the most important complications of liver surgery is intraoperative bleeding. Various vascular occlusion techniques are used to solve this problem. A retrospective study of the surgical techniques used in bleeding control of liver transplantation by Nakajima Y et al. [11] and the techniques of Bilt et al. [12] in vascular clamping techniques in liver surgery shows the frequencies of vascular occlusion techniques. While hepatic vascular clamp technique is based on total occlusion of blood flowing into and out of the liver. Pringle maneuver is applied in portal triangle to prevent blood flow into the liver and this technique is more recommended.

Gurusamy and colleagues compared vascular occlusion methods for elective liver resection and reported that hepatic vascular clamp technique caused more destructive changes in hemodynamic indicators than portal triad clamp technique [13]. In our experimental study, we used portal triad clamping technique to create ischemia in the rat liver.

In a study demonstrating the importance of laboratory tests in the diagnosis and monitoring of liver damage, the sensitivity of transaminases (AST, ALT) in demonstrating hepatocyte injury was emphasized [14]. In their study, Seeto et al. [15] found that aminotransferase levels increased in ischemic and toxic liver damage. Changes in hemostatic parameters are common after major hepatic

resection. Clotting balance deteriorates due to the breakdown of the synthesis of regulatory proteins and inflammatory mediators following ischemia reperfusion [16]. In our study, serum ALT, AST and INR which are the most reliable parameters showing cell damage and destruction in liver were evaluated. AST and INR values were significantly decreased in I/R+M group compared to IR group. There was no significant difference in ALT values of the groups.

Serum troponin I was found to be elevated in 74% of patients with acute hepatic failure [17]. In our study, there was no significant difference between the I/R+M and I/R groups in terms of Troponin I values.

Kandis et al. [18] found a decrease in TAS levels and an increase in TOS levels in liver I/R injury. Tüfek et al. [19] found higher TOS values and lower TAS values in the IR group in their studies comparing liver, lung and kidney tissues. In our study, TAS and TOS values were measured and OSI was calculated to determine the level of oxidative stress. In our study, when compared to I/R group of I/R+M group, TAS value was found to be low compared to the studies. The value of TOS was found to be lower in accordance with the results in other studies. OSI values were lower compared to the I/R group of I/R+M group. This shows that oxidative stress is proportionally less in the I/R+M group.

Magnesium sulfate, which has a strong anti-inflammatory effect, is also a powerful antioxidant. Magnesium sulfate inhibits endotoxin-dependent inflammatory molecule up-regulation. It was suggested that magnesium, an L-type calcium channel blocker, may be effective in preventing IR damage by reducing calcium overload related to tissue damage [6-9]. There are studies showing that magnesium sulfate is used at different doses in different organ irradiation therapies [6-8,20]. In our study, magnesium sulfate was administered 200 mg/kg intraperitoneally 20 minutes before ischemia.

Various histopathological damage scoring systems have been defined for detection of ischemia-reperfusion injury and measurement of damage level. These scoring systems examine tissue changes in various stages of cell damage and score them according to the level of histological findings. Selzner et al. [21] showed that the ATP deficiency caused by the oxygenation of the cell induce Na-K pump failure in the cell membranes and dilatation in the SECs with intracellular Na accumulation, the progression of the damage by the complex mechanisms, the disappearance of the current in the sinuses, and ultimately the cell went into apoptosis and necrosis. For this reason, many researchers have investigated the pathophysiological changes and therapeutic efficacy of hepatic I/R injury in this mechanism [10,21].

The most commonly used parameters in this study are vacuolization, sinusoidal dilatation and apoptosis/necrosis. In our study, we used vascularization, sinusoidal dilatation and apoptosis/necrosis parameters to determine the I/R damage and to investigate the effects of the drugs we use on the histopathological damage. In this experimental study, when I/R+M group of I/R group were compared with I/R group in rats with liver ischemia reperfusion, there was no significant difference in terms of histopathological level vacuolization, sinusoidal dilatation apoptosis and necrosis. In the histopathological examination, the mean values of apoptosis and necrosis were lower in the I/R+M group compared to the I/R group.

Conclusion
In this experimental study, the effects of

magnesium sulfate given before ischemia on liver I/R injury were evaluated with ALT, AST, INR, Troponin I, TAS, TOS and histopathological examination. Histopathological examination of rats; It was observed that magnesium sulfate decreased necrosis and apoptosis in I/R+M group compared to I/R group. In the evaluation of liver damage, the results of AST and INR biochemistry were statistically lower in the I/R+M group compared to the I/R group. We believe that early administration of magnesium sulfate, which is not routinely involved in the clinical treatment of ischemia-reperfusion, may decrease liver damage. This result should be supported by more extensive experimental and clinical studies.

References

[1]Ugras D, Mustafa U, Oguzhan O, Mehmet Y, Seckin A, Orhan GO, et al. Protective effects of escin on hepatic ischemia/reperfusion injury in rats Acta Medica Mediterr. 2016: 32(4):973-79.

[2]Weigand K, Brost S, Steinebrunner N, Büchler M, Schemmer P, Müller M. Ischemia/Reperfusion injury in liver surgery and transplantation: pathophysiology. HPB Surg. 2012;2012:176723.

[3] Erturk E. Ischemia-reperfusion injury and volatile anesthetics. Biomed Res Int. 2014;2014:526301.

[4] Girgin S, Gedik E, Taçyildiz IH. [Evaluation of surgical methods in patients with blunt liver trauma]. Ulus Travma Acil Cerrahi Derg. 2006;12(1):35-42.

[5] de Araujo M, Andrade L, Coimbra TM, Rodrigues AC Jr, Seguro AC. Magnesium supplementation combined with N-acetylcysteine protects against postischemic acute renal failure. J Am Soc Nephrol. 2005;16(11):3339-49.

[6] Akan M, Ozbilgin S, Boztas N, Celik A, Ozkardesler S, Ergur B, et al. Effect of magnesium sulfate on renal ischemia-reperfusion injury in streptozotocin-induced diabetic rats. Eur Rev Med Pharmacol Sci. 2016;20(8):1642-55.

[7] Kaptanoglu AF, Arca T, Kilinc K. Magnesium sulfate protects fetal skin from intrauterine ischemia reperfusion injury. Arch Dermatol Res. 2012;304(7):529-32.

[8] Kao M-C, Jan W-C, Tsai P-S, Wang T-Y, Huang C-J. Magnesium sulfate mitigates lung injury induced by bilateral lower limb ischemia-reperfusion in rats. J Surg Res. 2011;171(1):e97-e106.

[9]Lin C, Tsai P, Hung Y, Huang C. L-type calcium channels are involved in mediating the anti-inflammatory effects of magnesium sulphate. Br J Anaesth. 2009;104(1):44-51.

[10]Abu-Amara M, Gurusamy K, Hori S, Glantzounis G, Fuller B, Davidson BR. Systematic review of randomized controlled trials of pharmacological interventions to reduce ischaemia-reperfusion injury in elective liver resection with vascular occlusion. HPB (Oxford). 2010;12(1):4-14.

[11]Nakajima Y, Shimamura T, Kamiyama T, Matsushita M, Sato N, Todo S. Control of intraoperative bleeding during liver resection: analysis of a questionnaire sent to 231 Japanese hospitals. Surg Today. 2002;32(1):48-52.

[12] van der Bilt JD, Livestro DP, Borren A, van Hillegersberg R, Rinkes IB. European survey on the application of vascular clamping in liver surgery. Dig Surg. 2007;24(6):423-35.

[13] Gurusamy KS, Kumar Y, Pamecha V, Sharma D, Davidson BR. Ischaemic pre-conditioning for elective liver resections performed under vascular occlusion. Cochrane Database Syst Rev. 2009;(1):CD007629.

[14] Dufour DR, Lott JA, Nolte FS, Gretch DR, Koff RS, Seeff LB. Diagnosis and monitoring of hepatic injury. I. Performance characteristics of laboratory tests. Clin Chem. 2000;46(12):2027-49.

[15] Seeto RK, Fenn B, Rockey DC. Ischemic hepatitis: clinical presentation and pathogenesis. Am J Med. 2000;109(2):109-13.

[16] Shimada M, Matsumata T, Kamakura T, Suehiro T, Itasaka H, Sugimachi K. Changes in regulating blood coagulation in hepatic resection with special references to soluble thrombomodulin and protein C. J Am Coll Surg. 1994;178(1):65-68.

[17] Parekh NK, Hynan LS, De Lemos J, Lee WM, Group ALFS. Elevated troponin I levels in acute liver failure: is myocardial injury an integral part of acute liver failure? Hepatology. 2007;45(6):1489-95.

[18] Kandis H, Karapolat S, Yildirim U, Saritas A, Gezer S, Memisogullari R. Effects of Urtica dioica on hepatic ischemia-reperfusion injury in rats. Clinics. 2010;65(12):1357-61.

[19] Tüfek A, Tokgöz O, Aliosmanoglu İ, Alabalik U, Evliyaoglu O, Çiftçi T, et al. The protective effects of dexmedetomidine on the liver and remote organs against hepatic ischemia reperfusion injury in rats. Int J Surg. 2013;11(1):96-100.

[20] Hwang J, Kim J, Park S, Cho S, Park S, Han S. Magnesium sulfate does not protect spinal cord against ischemic injury. J Invest Surg. 2011;24(6):250-6.

[21] Selzner M, Selzner N, Jochum W, Graf R, Clavien PA. Increased ischemic injury in old mouse liver: an ATP-dependent mechanism. Liver Transpl. 2007;13(3):382-90.