

Comparison of effects of melatonin, pentoxifylline and dimethyl sulfoxide in experimental liver ischemia-reperfusion injury by three different methods

Zeliha Türkyılmaz¹, Ahmet Hatipoğlu², Mahmut Yüksel³, Nurettin Aydoğdu⁴, Gülara Hüseyinova⁵

¹Department of General Surgery, Edirne 1. Murat State Hospital, Edirne, Turkey

²Department of General Surgery, Trakya University School of Medicine, Edirne, Turkey

³Department of Nuclear Medicine, Altınbaş University Medical Park Bahçelievler Hospital, İstanbul, Turkey

⁴Department of Physiology, Trakya University School of Medicine, Edirne, Turkey

⁵Department of Pathology, Trakya University School of Medicine, Edirne, Turkey

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ABSTRACT

Objectives: Liver transplantation is increasingly being used in the treatment of end-stage liver disease. Ischemia-reperfusion injury is one of the major problems encountered in transplantation. In this study, we aimed to compare the effects of melatonin, pentoxifylline, and dimethyl sulfoxide (DMSO), in hepatic ischemia-reperfusion injury with different methods such as biochemical/ultrastructural changes and hepatobiliary scintigraphy.

Methods: Thirty rabbits were used in the Laboratory of Experimental Animals of Trakya University under appropriate conditions. Sham laparotomy and only ischemia reperfusion group were planned. They were used melatonin, pentoxifylline, and DMSO after I-R in the other three groups. 6 rabbits were randomly selected for each group. Rabbits in all groups were subjected to liver scintigraphy. Following scintigraphy, 2 cm² of liver tissue was removed to examining for liver antioxidant enzyme levels (superoxide dismutase [SOD] and glutathione peroxidase [GPx]) and for liver electron microscopy.

Results: Pentoxifylline and melatonin protected significantly uptake and excretion functions in liver scintigraphy. When the effects of all three substances were examined by electron microscopy, it was found that the three substances protected the liver from the effects of ischemia-reperfusion damage at varying rates. All three agents were found to protect SOD and GPx from falling in various amounts.

Conclusions: Studies to prevent ischemia-reperfusion injury, which may develop as a result of the Pringle maneuver applied to liver transplantations as well as to liver resections or liver injuries, still maintain their popularity. In our study, the effects of agents were identified in three different ways. Ischemia-reperfusion injury-reducing effect of pentoxifylline gave parallel results with three methods.

Keywords: Pentoxifylline, melatonin, dimethyl sulfoxide, ischemia, reperfusion

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Address for correspondence: Zeliha Türkyılmaz, MD., Edirne 1. Murat State Hospital, Department of General Surgery, Edirne, Turkey
E-mail: turkyilmazz@yahoo.com

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Liver transplantation is increasingly being used in the treatment of end-stage liver disease, and as a consequence, any problem of transplantation is becoming popular for researchers. The Pringle maneuver, commonly practiced in liver surgery and first described by Hogarth Pringle in 1908, is the clamping of the portal triad [1]. Hepatic ischemia-reperfusion (I-R) damage is common due to Pringle maneuver applied in liver transplantations and liver resections. Ischemia-reperfusion injury may also occur in sepsis and hemorrhagic shock. Studies have shown that oxygen free radicals (OFRs) are released by ischemia-reperfusion injury. Experimental studies have investigated the activity and mechanisms of many molecules thought to have therapeutic effects in ischemia-reperfusion injury [2-4].

Melatonin is one of the strongest known scavengers of both hydroxyl radicals and other oxygen radicals. Melatonin not only enhances antioxidant enzyme activity but also inhibits some pre-oxidative enzymes. The phosphodiesterase inhibitor, pentoxifylline, is a vasodilator and at the same time has the property of reducing blood viscosity. It is known to increase tissue oxygenation. Pentoxifylline has been shown to protect the liver against normothermic ischemia-reperfusion injury. There are studies indicating that dimethyl sulfoxide (DMSO) is known to be associated with radioprotective and cytoprotective effects, as well as protective effects of ischemia-reperfusion injury [5-7].

In this study, we wanted to determine in different ways whether melatonin, pentoxifylline and DMSO had protective effect on ischemia reperfusion. We aimed to compare the biochemical parameters (superoxide dismutase [SOD] and glutathione peroxidase [GPx]), electron microscopic examination of hepatic tissue and hepatobiliaryscintigraphy for the effects of all three agents.

METHODS

A total of 30 rabbits weighing 1750-2150 g, which were properly cared in the Experimental Animal Laboratory of Trakya University School of Medicine, were used in the study. The rabbits were randomly assigned into 5 groups, with 6 rabbits in each group.

Groups

Group A: Sham laparotomy (control group)

Group B: Only ischemia-reperfusion (I-R) injury

Group C: 10 mg/kg melatonin (Sigma M5250) before I-R

Group D: 50 mg/kg pentoxifylline (PTX) (Sigma P1784) before I-R

Group E: 1 g/kg DMSO (Sigma D8779) before I-R

The rabbits were starved for 12 hours before surgical intervention. Before the operation, the abdominal area was shaved, and the skin was cleaned with povidone-iodine. They were anesthetized with i.m. Ketamin (10mg/kg) and i.m. Rompum (1 ml/kg). Group A underwent sham laparotomy, and then HBS was performed after closure of the abdominal wall. Following scintigraphy, 2 cm² of hepatic tissue was removed to determine hepatic antioxidant enzyme levels and for electron microscopy of the liver from the free edge of the right lobe of the liver. In the rabbits of Groups B, C, D and E, the hepatoduodenal ligament was dissected and then was occluded with microvascular clamps, and the Pringle maneuver was applied for 30 minutes. Group B received no any agent before the operation, Group C received 10 mg/kg intravenous (i.v.) melatonin before the operation, Group D received 50 mg/kg pentoxifylline before the operation, and Group E received 1 g/kg (90%) DMSO before the operation. Microvascular clamps were opened after 30 minutes, and then reperfusion was ensured. Hepatobiliary Scintigraphy (HBS) was performed after intravenous injection of a radiopharmaceutical at 15 minutes of reperfusion. Hepatic tissue was removed to determine hepatic antioxidant enzyme levels and electron microscopy of the liver at all other groups as in the sham laparotomy group.

Hepatobiliary Scintigraphy (HBS)

It was performed by obtaining 45 minutes dynamic images, immediately after 37 MBq Tc99m-Br IDA was administered intravenously via the ear vein of the rabbits at 15 minutes of reperfusion in the Department of Nuclear Medicine, Trakya University School of Medicine. Dynamic images were recorded by the single-head gamma camera equipped with a low energy, high resolution collimator, so that there

would be 12 images in the first 5 minutes and 1 image/2 minutes in the next 40 minutes. A region of interest (ROI) was drawn on the liver parenchyma except for the major biliary ducts and intestines in the right upper quadrant on the obtained dynamic images. Thus, the time-activity curves of the liver parenchyma were obtained. The peak time of liver involvement (Liver PT) and the half-life of the peak activity in the liver (Liver T1/2) were calculated from the obtained curves.

Electron Microscopy

To examine cell organelle damage in the liver, 2 cm² of hepatic tissue which was removed from the free edge of the right lobe of the liver of the rabbits in all groups 60 minutes after reperfusion was used. The obtained tissues were first fixed with 2.5% phosphate buffered osmium tetroxide (OsO₄). They were dehydrated in 30% and 100% alcohols, respectively. The specimens were blocked with Epon 812 after the application of propylene oxide. The blocks which were cut using Raychert ultramicrotome were stained with uranyl acetate. The stained sections were examined using Zeiss-EM-9 and JEM-100B electron microscopes by a pathologist blind to the groups and scintigraphic data in the Department of Pathology, Trakya University School of Medicine.

Biochemical Analysis

Biochemical parameters were studied by a researcher blind to the groups in the Research Laboratories of Department of Chemistry, Faculty of Science and Letters, Trakya University. For the measurement of SOD and GPx values, the liver tissues from each subject were washed with isotonic solution in a standard manner, and then they were dried and stored at -70°C in the eppendorf tubes. Each of the materials removed to be studied were weighed, and the protein values of the tissues were calculated by the Lowry method after homogenization. Using SOD kit (Randox-Ransod SD125-8092H), the baseline and 3 min values of SOD enzyme activity for each tissue were detected by JenWay 6105 UV/visible spectrophotometer. For each tissue, the SOD values were divided by the protein values, and results were expressed as U/mg protein. Using Glutathione peroxidase kit (Randox- Ransel RS504-1315F), it was studied in a similar manner with SOD.

Statistical Analysis

Statistical analysis: The data of our study was obtained using the MiniBit package program (SOO64 minitab release 13) (License No: wcp. 1331.00197) in Trakya University Information Processing Center. All parameters are shown as mean ± standard deviation. *p* values < 0.05 were considered to be statistically significant.

RESULTS

Hepatobiliary Scintigraphy Findings

The HBS data of all groups are given in Table 1. The mean Liver PT value statistically significantly higher in Group B compared to Group A (2.73 ± 0.84 min vs 9.45 ± 4.26 min; *p* = 0.004). The mean Liver PT value statistically significantly higher in Groups C, D, and E compared to Group A (2.73 ± 0.84 min vs 5.25 ± 1.36 min, 4.67 ± 0.30 min, and 5.63 ± 1.06 min, for Groups C, D, and E respectively; *p* < 0.05). The mean Liver PT value was statistically significantly lower in Groups C and D, compared to Group B (9.45 ± 4.26 min vs 5.25 ± 1.36 min, 4.67 ± 0.30 min, for Groups C and D respectively; *p* < 0.05). There was no statistically significant difference in mean Liver PT value between Group E and B (9.45 ± 4.26 min vs 5.63 ± 1.06 min; *p* > 0.05). There was no statistically significant difference in mean Liver PT value between Group C, D, and E (5.25 ± 1.36 min vs 4.67 ± 0.30 min, and 5.63 ± 1.06 min, for Groups D and E respectively; *p* > 0.05). The mean Liver PT value of Group D was closest to that of Group A. There was a statistically significant difference in mean Liver PT value between Groups D and E (4.67 ± 0.30 min vs 5.63 ± 1.06 min; *p* = 0.03).

While the mean Liver T1/2 value was 4.43 ± 1.36

Table 1. Hepatobiliary scintigraphy findings

Groups	Liver PT (min)	Liver T _{1/2} (min)
Group A	2.73 ± 0.84	4.43 ± 1.36
Group B	9.45 ± 4.26	26.98 ± 12.54
Group C	5.25 ± 1.36	14.45 ± 7.15
Group D	4.67 ± 0.30	12.53 ± 3.03
Group E	5.63 ± 1.06	13.28 ± 3.36

Data are shown as mean ± standard deviation. Liver PT = the peak time of liver involvement, Liver T1/2 = the half-life of the peak activity in the liver

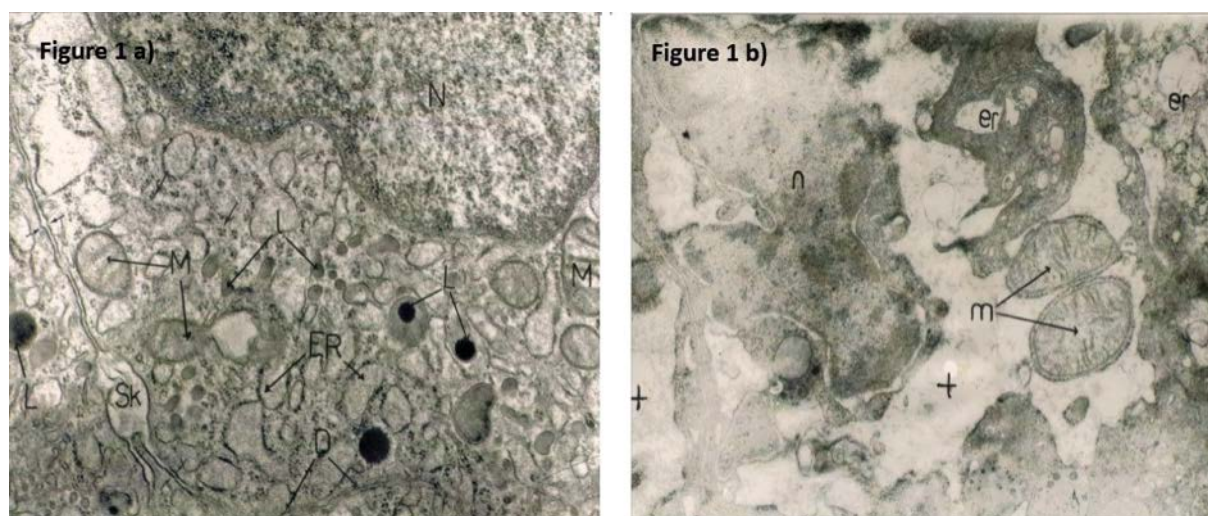


Figure 1. a) Electron microscopy image of Group A. The hepatocytes, sinusoids, space of Disse, bile ducts and other structures were normally observed. b) Electron microscopy image of Group B. Major destructive and degenerative changes in the hepatocytes. The nucleus was pyknotic and the cytoplasm was edematous. glycogen granules decreased, mitochondrial swelling, mitochondrial crystals were destroyed and degenerated. sinusoid and space of Disse extensively expanded, erythrocytes were clustered.

min in Group A, it was 26.98 ± 12.54 min in Group B. The mean Liver T1/2 value statistically significantly higher in Group B compared to Group A ($p = 0.004$). The mean Liver T1/2 value statistically significantly higher in Group C, D and E compared to Group A. (4.43 ± 1.36 min vs 14.45 ± 7.15 min, 12.53 ± 3.03 min, and 13.28 ± 3.36 min; for Groups A, C, D, and E respectively; $p < 0.05$). The mean Liver T1/2 value was statistically significantly lower in Groups C, D and E compared to Group B (26.98 ± 12.54 min, 14.45 ± 7.15 min, 12.53 ± 3.03 min, 13.28 ± 3.36 ; for Groups B, C, D and E respectively; $p < 0.05$).

These data suggest that the uptake and excretion functions were significantly preserved in those treated with melatonin and pentoxifylline in comparison with the I-R group. The uptake times were reduced in those treated with DMSO in comparison with the I-R group, there was no statistically significant difference between the two groups. It was found that the uptake time of those treated with pentoxifylline was statistically significantly lower than that of those treated with DMSO. Despite Liver PT prolong in the DMSO group, Liver T 1/2 time was maintained as in the pentoxifylline and melatonin groups.

Ultrastructural Findings

When Group A was examined by electron microscopy, the hepatocytes, sinusoids, space of

Disse, bile ducts and other structures were normally observed (Figure 1a).

When Group B was examined by electron microscopy, there were the major destructive and degenerative changes in the majority of hepatocytes. It was remarkable that the nucleus was pyknotic and the cytoplasm was edematous. It was seen that the glycogen granules were reduced, the mitochondrial swelling occurred, the mitochondrial crystals were destructed and degenerated, and the endoplasmic reticulum were expanded and disappeared in some places. Moreover, the degeneration and lysis of other organelles were observed. Depending on ischemia-reperfusion injury, it was seen that the sinusoid and space of Disse extensively expanded and the erythrocytes were clustered in it. Macrophage activity was present in the cytoplasm of Kupffer cells and outside the cell. Increased vacuoles and microvesicles were observed. Phagocytosed materials were seen in the cytoplasm (Figure 1b).

When Group C was examined by electron microscopy, it was observed that the majority of organelles in the cytoplasm of hepatocytes were normal. However, it was remarkable that the mitochondria, endoplasmic reticulum and other organelles were destructed in some places. In addition, it was seen that there was an increase in collagen fibrils around hepatocytes. In Group C, it was

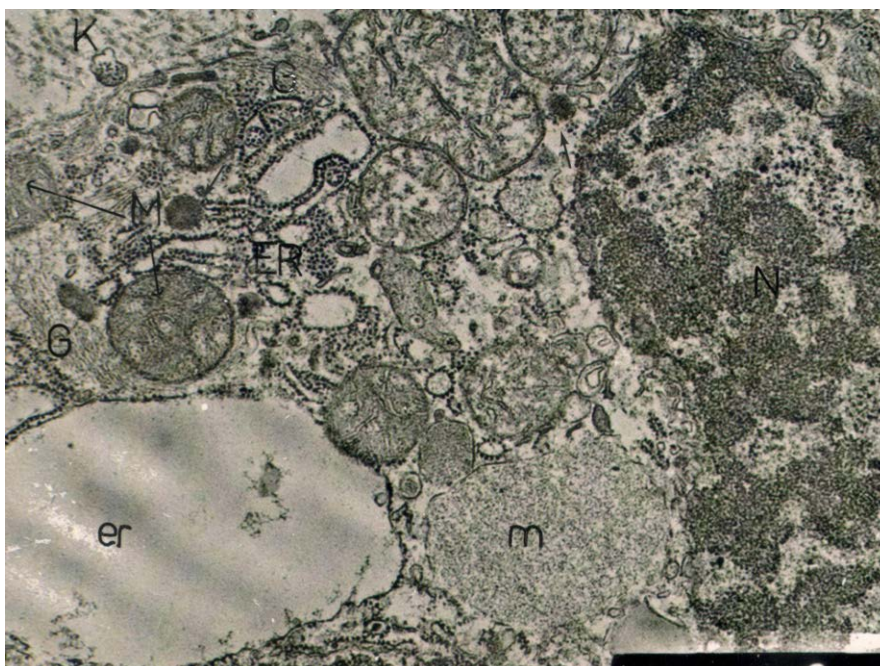


Figure 2. Electron microscopy image of Group C. The majority of organelles in the cytoplasm were normal. However, it was remarkable that the mitochondria, endoplasmic reticulum and other organelles were destructed in some places microscopy.

observed that the erythrocytes were clustered in the lumen of the large vessels, and there were numerous fibroblasts and secreted collagen fibrils around them in the liver parenchyma (Figure 2).

In generally, when Group D was examined by

electron microscopy, normal hepatocytes were observed. The expansions in the space of Disse and bile ducts were remarkable. The outer membranes of hepatocytes and the microvilli of the bile ducts were preserved. There was mild intracellular edema at the

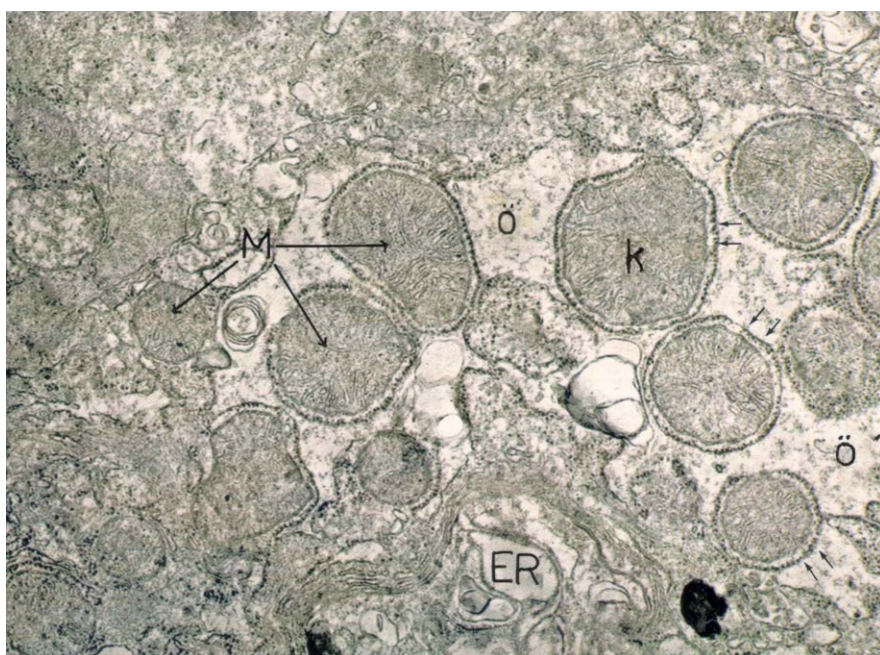


Figure 3. Electron microscopy image of Group D. The outer membranes of hepatocytes and the microvilli of the bile ducts were preserved. The double-layered outer membranes and the crystals of the mitochondria were preserved. Other organelles also were normal.

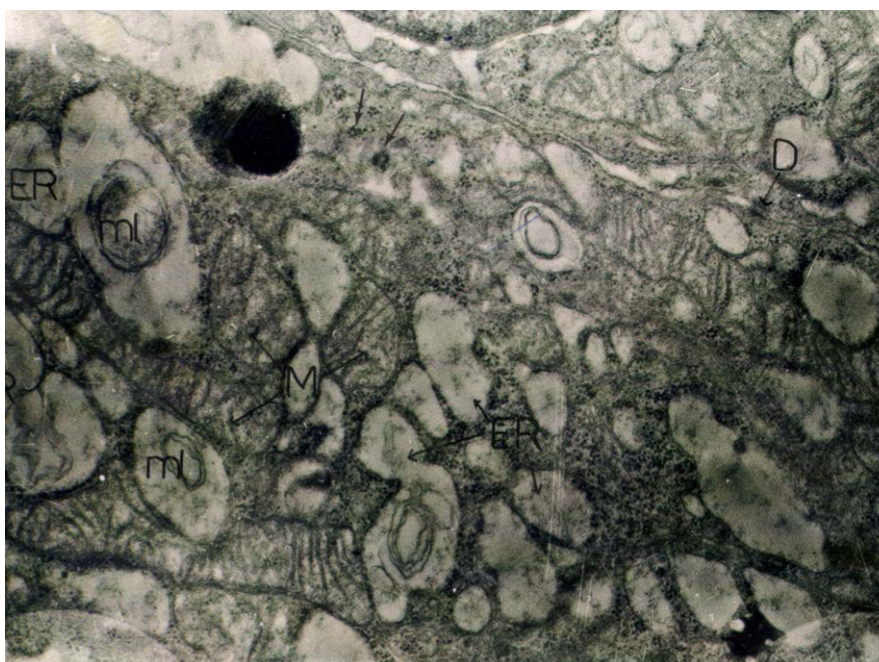


Figure 4. Electron microscopy image of Group E. Mitochondria in hepatocytes swollen, but the outer membranes and the crystals did not deteriorate.

large magnification. The double-layered outer membranes and the crystals of the mitochondria were preserved. Other organelles were normal (Figure 3).

When Group E was examined by electron microscopy, it was observed that the mitochondria in hepatocytes were swollen, but the outer membranes and the crystals did not deteriorate. It was seen that there were endoplasmic reticulum expansion and myelin-like membrane structures in the ER lumen. It was observed that there were abnormal, swollen mitochondria and enlarged ER and other organelles in hepatocyte cytoplasm at the large magnification. Besides, there were irregularities in mitochondrial structures and myelin-like membrane structures in the ER lumen. There was also a proliferation of ribosomes and glycogen granules (Figure 4).

Biochemical Findings

The mean SOD and GPx values of all groups are given in Table 2. The mean SOD value was statistically significantly lower in Group B compared to Group A ($p = 0.004$). The mean SOD value was statistically significantly lower in Group C compared to Group A ($p = 0.004$). However, the mean SOD value was not statistically significantly lower in Groups D and E compared to Group A. It was determined that the mean SOD value was significantly preserved in Groups C, D, and E compared to Group B ($p = 0.004$). And also, there was no statistically significant difference between Groups C, D, and E in terms of mean SOD value.

The mean GPx value was statistically significantly lower in Group B compared to Group A (0.96 ± 0.17 vs 0.40 ± 0.071 , $p = 0.004$). The mean GPx value was statistically significantly lower in Group C compared to Group A (0.96 ± 0.17 vs 0.73 ± 0.005 , $p = 0.06$). However, the mean GPx value was not statistically significantly lower in Groups D and E compared to Group A (0.96 ± 0.17 vs 0.75 ± 0.01 and 0.77 ± 0.087 ; $p = 0.6$). It was determined that the mean GPx value was significantly preserved in Groups C, D, and E compared to Group B ($p = 0.004$). Moreover, there was no statistically significant difference between Groups C, D, and E in terms of mean GPx value.

Table 2. SOD and GPx values of groups

Experimental Groups	SOD (U/mg protein)	GPx (U/mg protein)
Group A	7.44 ± 2.17	0.96 ± 0.17
Group B	3.53 ± 0.47	0.40 ± 0.071
Group C	5.47 ± 0.64	0.73 ± 0.005
Group D	5.70 ± 0.63	0.75 ± 0.01
Group E	5.91 ± 0.84	0.77 ± 0.087

Data are shown as mean \pm standard deviation. SOD = superoxide dismutase, GPx = glutathione peroxidase

DISCUSSION

Studies to prevent I-R injury, which may develop as a result of the Pringle maneuver applied to liver transplantations as well as to liver resections or liver injuries, still maintain their popularity. Studies have been conducted for a long time to preserve the liver under in vitro conditions [3, 5-7]. Liver cells may be resistant to ischemia for 30 to 60 minutes, but irreversible cell damage is usually seen over a longer period of time. After 60 minutes of ischemia, SORs begin to increase at 5 minutes of reperfusion and then peak at 15 minutes of reperfusion [8]. SORs, which occur due to I-R injury, cause damage to both liver parenchyma and other liver cells [2]. The resource of SORs is that the concentration of O₂ suddenly increases in the medium due to reperfusion. The increased amount of SORs interacts with cell membrane lipids, resulting in the formation of arachidonic acid. Inflammatory markers such as prostaglandins (PG) and leukotrienes (LT) are released from arachidonic acid by the activation of lipoxygenase and cyclooxygenase pathways. They contribute to cell damage by disrupting cell membrane permeability. SORs are destroyed by cellular defense mechanisms. When SORs are destroyed, enzymatic pathways such as SOD, GPx, and catalase (Cat) are activated [8-14]. Numerous substances, which are thought to be effective by enzymatic or non-enzymatic pathways, have been experimentally tested in order to reduce ischemic reperfusion injury in the liver and other tissues, but there are not still products that have been actively applied for treatment [3, 5, 6].

Melatonin, a hormone released from the pineal gland, is a strong scavenger for hydroxyl radicals and other oxygen radicals that are toxic in vivo and in vitro. Its passage to the tissues is quite good due to its lipophilic nature, and it is particularly effective on the hydroxyl radical, which is the most toxic radical [15-17]. Some studies have reported that melatonin acts by capturing the superoxide of an indole-derived metabolite and also enhances SOD mRNA expression. Melatonin both stimulates important antioxidant enzymes such as intracellular superoxide dismutases (CuZnSOD and MnSOD) and selenium-containing glutathione peroxidase and catalase (CAT). And also inhibits some pre-oxidative enzymes (such as myeloperoxidase). Because of all of them, many

studies have reported that it reduces lipid peroxidation and protects cells and DNA against oxidative damage [15, 17-19]. Sewerynek *et al.* [20] showed in the mouse model of liver ischemia-reperfusion injury that melatonin reduced leukocyte infiltration and lipid peroxidation products and also increased glutathione reductase (GR) activity. In mice treated with melatonin, less apoptotic (TUNEL positive) cells and DNA fragmentation were observed than only I-R constructed mice. These results show that melatonin reduces the level of apoptotic pathway and oxidative stress and, as a result, improves hepatocytes which are exposed to I-R [21]. Aktöz *et al.* [22] found that SOD and GPx values, which decreased after I-R in rats undergoing renal ischemia reperfusion injury, were significantly preserved in melatonin-treated group. Baykara *et al.* [18] found that there were swollen and vacuolated mitochondria, dilated bile ducts, and several hepatocyte nuclei with heterochromatic and apoptotic appearance in rat liver undergoing I-R injury. They stated that hepatocyte nuclei and mitochondria appeared to be normal in melatonin-treated group. In ultrastructural evaluation of our study, although the majority of organelles in the cytoplasm of hepatocytes were normal in the melatonin-treated group, it was remarkable that the mitochondria, endoplasmic reticulum and other organelles were destructed in some places. In addition, it was seen that there was an increase in collagen fibrils around hepatocytes. Moreover, SOD and GPx values were preserved when compared with the group undergoing only I-R injury. And also, it was observed that the uptake and excretion functions in HBS were significantly preserved in the melatonin-treated group compared to the group undergoing only I-R injury.

Pentoxifylline, a methylxanthine derivative, is a phosphodiesterase inhibitor. Unlike other peripheral vasodilators, it reduces blood viscosity and increases tissue oxygenation. It increases erythrocyte flexibility, as well as reduces platelet aggregation. It increases microvascular blood flow. It has been stated that it selectively directs blood flow to tissues with a disturbed microcirculation in reperfusion [23, 24]. Dinçkan *et al.* [25] revealed that pentoxifylline has protective effect on pneumoperitoneum-induced oxidative stress in rats. There are studies showing that it is effective in I-R injury by decreasing the release of TNF- α , IL-1-, IL-6, SORs and proteases especially

from Kupffer cells [23]. In an experimental study of I-R injury performed using pentoxifylline by El-Ghoneimi *et al.* [7], they reported that it reduced serum levels of TNF- α by providing inhibition of TNF-alpha gene expression. Some studies found that pentoxifylline reduced malondialdehyde and myeloperoxidase activity [26, 27]. Pentoxifylline, a methylxanthine derivative, is a phosphodiesterase inhibitor. Unlike other peripheral vasodilators, it reduces blood viscosity and increases tissue oxygenation. It increases erythrocyte flexibility, as well as reduces platelet aggregation. It increases microvascular blood flow. It has been stated that it selectively directs blood flow to tissues with a disturbed microcirculation in reperfusion [23, 24]. Dinçkan *et al.* [25] revealed that pentoxifylline has protective effect on pneumoperitoneum-induced oxidative stress in rats. There are studies showing that it is effective in I-R injury by decreasing the release of TNF- α , IL1-, IL-6, SORs and proteases especially from Kupffer cells [23]. In an experimental study of I-R injury performed using pentoxifylline by El-Ghoneimi *et al.* [7], they reported that it reduced serum levels of TNF- α by providing inhibition of TNF-alpha gene expression. Some studies found that pentoxifylline reduced malondialdehyde and myeloperoxidase activity [26, 27]. In a clinical trial conducted in 101 noncirrhotic patients by Petrowsky *et al.* [28], they found that PTX which was administered at 12 hours before surgery and at 72 hours after surgery in patients undergoing major liver surgery provided a positive contribution to regeneration in remnant liver tissue. In our study, pentoxifylline has been found to significantly maintain SOD and GPx values in liver I-R injury compared to the group B that undergoing only I-R injury. The electron microscopy findings also confirm these values. Moreover, the HBS findings also showed us the protective feature of pentoxifylline by objective values. HBS has never been studied in identifying changes in liver functions in previous experimental models performed using pentoxifylline. It has been first used in our study. HBS reveals like elektron microcopy an objective finding that shows the efficacy of pentoxifylline.

Some studies have reported that DMSO has protective effect on I-R injury by reducing SORs. DMSO scavenges the hydroxyl radical, which is one

of the most toxic superoxide radicals. In the past decade, there have been studies showing the protective effects of DMSO on liver I-R injury by biochemical parameters as well as histopathological findings [6, 29]. However, the popularity of this agent has decreased due to its nephrotoxic effects [30]. Şahin *et al.* [31] found that DMSO reductions in I-R injury at non-toxic doses and in slow release. In an experimental study conducted by Hatipoğlu *et al.* [6], I-R group and the I-R group after DMSO were compared; hepatic peak time and the half-life of hepatic activity were significantly higher only in the I-R group. In our study, the SOD and GPx values were significantly preserved in the DMSO-treated group compared to the group undergoing only I-R injury. Electron microscope showed that the mitochondria in hepatocytes were swollen, but the outer membranes and the crystals were not deteriorated. There were myelin-like membrane structures in enlarged ER and ER lumens. In HBS, the DMSO group had shorter uptake and excretion times than the I-R group. However, unlike the previous study, the Liver PT was not well protected in the DMSO group. On the other hand, in our study, Liver T1/2 time was preserved in this group.

Today, HBS is sometimes used to assess liver function after transplantation [32]. HBS provides both quantitative and visual appearances when assessing hepatic uptake and excretory functions [6, 33]. HBS as more practical and useful method has been tried to replace serial biopsies to recognize tissue rejection after transplantation. ^{99m}Tc iminodiacetic acid (IDA) analogues, first presented by Ekman *et al.* [34], are now the most commonly used agents in the application of hepatobiliaryscintigraphy. Since HBS shows hepatocyte functions and bile duct dynamics, it is used in bile leaks that occur in liver operations, in partial or complete biliary obstructions, and in post-transplant patients [33-35]. Functional status of the liver affects the uptake of IDA and its derivatives. HBS shows hepatic uptake and excretion [36]. Because of this feature, it is considered to be an important non-invasive alternative in assessing liver I-R injury. Brunot *et al.* [37] found that early biopsy results were consistent with liver uptake functions. However, studies have shown that HBS is a good method for detecting liver complications after transplantation, while it was not found to be safe in distinguishing

tissue rejection from biliary complications [38]. In a study of Tagge *et al.* [39], the effects of ischemia-reperfusion injury on pig liver transplants has been investigated using ^{99m}Tc-diphenyl, and it was found that median transit time of the pharmaceutical agent was prolonged in ischemia. It has also been determined that this condition is associated with duration of ischemia [39]. In a study of Kuni *et al.* [40], liver biopsies were compared by quantitative HBS, and it was shown that the mean damage scores of patients with normal and abnormal uptake were significantly different. In the same study, it was also found that the uptake criteria were insufficient to distinguish pure hepatic damage from cholestasis [40].

In our study, it was found that pentoxifylline and melatonin agents significantly protect uptake and excretion functions in liver scintigraphy, but the uptake functions of the DMSO-treated group were not significantly protected compared to the I-R group. Furthermore, when the scintigraphic parameters were considered, it was found that the protective effect of pentoxifylline was better at a statistically significant level than DMSO. Although there was no statistically significant difference between pentoxifylline and melatonin, uptake and excretion results of pentoxifylline were better. In our study, the effects of all three agents were examined by electron microscopy, it was found that the liver was largely protected from the effects of I-R injury, but the EM findings in the pentoxifylline-treated group were more normal. In particular, the mitochondria and other organelles were observed to be preserved prominently in use of pentoxifylline. Considering only the electron microscopic findings in our study, it can be said that pentoxifylline is more effective in reducing I-R damage than the other two agents. It was determined that these three agents maintained significantly the SOD and GPx values when compared with the I-R group. There was no significant difference between these three agents.

CONCLUSION

When all methods are evaluated in this study, pentoxifylline, melatonin and DMSO have been found to be effective in protecting the hepatic ischemia reperfusion injury. Although there was no difference

between pentoxifylline and DMSO according to biochemical parameters, pentoxifylline was more effective when electron microscopy findings and HBS parameters were taken into consideration. This suggests that it would be safer for the cytoprotective effect of a substance to be assessed in more than one way rather than as a single method. In our study, I-R injury-reducing effect of pentoxifylline, which gives parallel results with three methods, gives more confidence. Reducing I-R injury confirmed by all three ways, provide this agent with confidence and After the experimental works has been replicated, we foresee that it may be used to be in the future in pre-transplant and shock situations.

Authorship declaration

All authors listed meet the authorship criteria according to the latest guidelines of the International Committee of Medical Journal Editors, and all authors are in agreement with the manuscript.

Author contributors

All authors contributed to the design of the study. ZT: performed surgical intervention; wrote the manuscript and contributed design of the study and analysis and interpretation of the data; gives final approval for the submitted version to be published, AH: contributed substantially to the conception and design of the study and analysis and interpretation of the data. MY: contributed design of the study; performed hepatobiliarscintigraphy and interpreted of the hepatobiliarscintigraphy data. NA: studied biochemical parameters and interpreted of the biochemical data. GH: interpreted of the electron microscopy.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

Ethics approval

Medical Faculty Ethics Committee of Trakya University.

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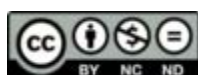
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