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BIOCHEMISTRY

Prevention of Tissue Injury with *Olea europaea* L. Leaf Extract after Partial Liver Ischemia/Reperfusion

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Abstract—The present study, the protective effect of *Olea europaea* L. leaf extract containing high oleuropein on oxidative damage in ischemic and non-ischemic parts of rat liver was investigated. The Spraque-dawley male rats were separated into five groups. The physiological water was given to Group I and Group II. Group III, IV, and V animals received orally 150, 200 and 400 mg kg^{-1}/day olive leaf extract for 15 days, respectively. Then, except for Group I rats, partial (2/3) liver Ischemia (45 min)/Reperfusion (60 min) (I/R) was applied in the other groups. In addition to histopathological and cytological examination, serum Alanine transaminase (ALT) and Aspartate transaminase (AST) levels in liver tissues as well as activities of the Superoxide dismutase (SOD), Catalase (CAT) and Glutathione peroxidase (GPx) enzymes were investigated. As a result of the statistical evaluations of all parameters, the oxidative damage in ischemic and non-ischemic parts of the liver was same in all experimental groups. 200 mg kg^{-1}/day *Olea europaea* L. leaf extract was found to have more protective effect against I/R induced oxidative damage when compared to other doses.

Keywords: Liver, Ischemia/Reperfusion, *Olea europaea* L., Antioxidant enzymes

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INTRODUCTION

Stopping the blood supply of the liver in whole or in part to control bleeding during surgical procedures in the clinical field results in I/R damage and increased Reactive Oxygen Species (ROS) are held responsible as the cause of tissue damage after I/R (Seo et al., 2002). The most common ROSs are known as superoxide anions, hydroxyl radicals, hydrogen peroxide, and peroxynitrite (Abdel-Ghany et al., 2018). Such factors as neutrophil activation, hyperoxia, drugs with redox potential, radiation, and I/R increase the effect of ROS on protein, nucleic acid, and lipid damage (Hanawa et al., 2009). The main causes of damage by I/R are free radicals containing and derived from oxygen, uptake of calcium into cell, reduction in ATP as a result of mitochondrial dysfunction, and the activation of lysosomal enzymes (Araya et al., 2003). Polymorphonuclear leukocytes (PMNLs) are one of the important sources of ROS that cause I/R related oxidative damage (Delibas et al., 2018) and transmigration occurs in synosoid (Marubayashi et al., 2000). Certain mediators released by PMNL into the tissue during ischemia trigger ROS formation during reperfusion. In the meantime, I/R damage occurs by various proteases and lipases gaining activity thanks to increased calcium in cell (Kurian et al., 2016).

Distortion of the sensitive balance between ROS and antioxidants in the body in favor of ROS creates oxidative stress (Öztürk et al., 2002). The inhibition of ROSs that are potentially dangerous in the body to, or their elimination can be achieved by a defense mechanism called "antioxidant system" that the body creates itself (Shah et al., 1999).

Also formed during normal cellular metabolism, ROS is also inhibited by antioxidant-containing endogenous enzymes such as superoxide dismutase, catalase, glutathione peroxidase, phospholipid hydroperoxide glutathione peroxidase, glutathione S transferase, glutathione reductase, and cytochrome -C oxidase. Non-enzymatic small antioxidant molecules such as uric acid, ascorbic acid, α-tocopherol, β-carotene, glutathione, and protein SH groups also support this response of the body in the form of oxidant concentration (Shah et al., 1999). Flavonoids, anthocyanins, and phenolic compounds that can exogenously show antioxidant properties are taken into the body by nutrition (Zhang et al., 2002).

The compounds found in olive leaves show a wide range of biological activities including antimicrobial, antiviral, and anti-inflammatory agents, lipid stabilizers, and blood pressure regulators that strengthen the body's immune system (Rigacci and Stefania, 2016). It is reported that these biological effects of olive leaves containing approximately one hundred different chemicals are caused by phenolic compounds and their derivatives in its content, and that the most sig-

nificant of these five main groups of phenolic compounds is oleuropeocides (oleuropein and verbascoside) (Benavente-Garcia et al., 2000). Oleuropein, which belongs to a group of polyphenols called secoiridoids (Supplement 1) (Klimova et al., 2019), is more in olive leaves than other parts of olive (between 0.005–0.12% in olive oil, 0.87% in alperujo, and 1–14% in olive leaves. oleuropein) and has the highest antioxidant and scavenging power (Japon-Lujan et al., 2006). On the other hand, it was reported that tea prepared from olive leaves showed antioxidant effect against DPPH and hydrogen peroxide radicals (Buyukbalci and El, 2008).

In our study, partial I/R induced oxidative stress damage was generated in rat liver in order to in vivo test whether there is a difference in oxidative damage between ischemic and non-ischemic parts of an organ and also the usability of the *Olea europaea* L. leaf extract with high oleuropein content in antioxidant therapy. In addition to histopathological examinations, serum ALT and AST levels and semi-quantitative activities of SOD, CAT, GPx enzymes in liver tissues were established and evaluated statistically in order to determine damage and possible protective effect.

MATERIALS AND METHODS

Animals and Experimental Design

Male *Spraque Dawley* albino rats, weighing between 220 ± 20 g, were used in the experiment. All experimental animals were obtained from the medical and surgical experimental animals application and research center and all experiments were carried out at the same center. They were housed in polycarbonate cages in an airconditioned room (12 Light/12 Dark, 22 ± 2°C, 50 ± 5% humidity). They were fed with laboratory pellet chows and water was given ad libitum.

The rats were randomly divided into five groups, each consisting of 7 animals:

Group I (Sham group): Physiologic water (0.9% NaCl) was given by oral gavage for 15 days at 2 mL/day without performing any surgical procedure on this group of experimental animals. After median laparotomy was performed, dissections were performed without I/R operation.

Group II: In this group of experimental animals, 2/3 partial liver I/R (45/60 min) was performed in addition to Group I. At the end of the reperfusion period, a dissection was performed.

Group III, IV and V animals, *Olea europaea* L. leaf extract for 15 days, respectively 150, 200 and 400 mg kg^{-1}/day orally by gavage. The daily freshly prepared olive leaf extract was dissolved in physiological water and applied to the experimental animals. Then, 2/3 partial liver I/R (45/60 min) was performed after the abdomen was opened with median laparotomy. At the end of the reperfusion period, a dissection was performed.

In our study, olive (*Olea europaea* L.) leaf ethanol extract was used with high oleuropein content (Supplement 2). The herbal extract was produced by Kale Naturel Herbal Products, Food Cosmetics and Agricultural Products, Foreign Trade and Industry Limited Company.

Anesthesia and Surgical Procedures

All surgical procedures were performed in sterile environment and using sterile surgical instruments. Animals in the experimental groups received intramuscular 10 mg kg^{-1} xylazine and 70 mg kg^{-1} ketamine anesthesia. In all groups except Group I, the blood vessels and bile ducts entering the middle and left lobes of the liver were blocked using atraumatic bulldog microvascular clamps and the fluid flow was stopped for 45 minute. At the end of the ischemia period, clamps were removed and reperfusion was performed for 60 minutes.

Peripheral blood samples from each animal in all groups were centrifuged for 10 min at 3000 rpm (MSE Mistral 2000) to obtain serum samples and stored in a −80°C freezer for biochemical analysis.

The I/R-administered 2/3 liver (ischemic) and 1/3 liver parts without I/R (non-ischemic) were dissected and rapidly washed in cold physiological water. Some of the liver tissue samples were stored in a −80°C freezer for use in the determination of SOD, CAT and GPx enzyme activity by native polyacrylamide gel electrophoresis (Native-PAGE). The other parts of the liver tissue samples were chemically fixed with 10% neutral formaldehyde for use in histopathological examinations.

Biochemical Analysis

The ALT and AST enzyme values in blood serum were performed using HITACHI-917 auto analyzer and Human brand (Human Gesellschaft für Biochemica und Diagnostica mbH, Wiesbaden Germany) was made using commercial kits.

Determination of Isozyme Activity

Prior to electrophoretical analysis, each liver sample (100 mg/mL buffer) was homogenized in 50 mM phospate buffer (pH 7.4). The homogenate was then centrifuged at 10000 rpm for 25 min and the supernatant obtained was used for electrophoretical analysis. All liver parameters were expressed as activity per mg protein. The protein concentration in each fraction was determined by the method of Lowry using crystalline bovine serum albumine as a standard (Mæhre et al., 2018).

Native-PAGE was performed on samples of liver, essentially as described by Laemmli (1970). The enzymes were run on the basis of equal amounts of protein (70 μg) in a 10% gel for SOD and GPx and 8% gel for CAT. Electrophoretic separation was performed at 4°C with a constant power supply of 50 V for stacking gel and 100 V for separating gel. Staining for the activity of each enzyme was performed separately as follows:

CAT activity was detected using the method of Woodbury et al. (1971). Here, the gel was soaked in 5 mM H_2O_2 solution for 10 min and then washed with water and stained with a reaction mixture containing 1% potassium ferricyanide (w/v) and 1% ferric chloride. The enzyme appeared as a yellow band superimposed on a dark green background. The reaction as terminated by adding water and the gel was photographed at once.

SOD activity was identified using the method of Beauchamp and Fridovich (1971). The gel was soaked in 50 mM Tris-HCl buffer (pH 8.0) containing 10 mg nitroblue tetrazolium (NBT), 1 mg ethylene diamine tetraacetic acid (EDTA) and 2 mg riboflavin (50 mL final volume), and kept in the dark for 30 min. The gel was then placed on an illuminated light box to locate the area of SOD activity, which appeared as a clear zone on a bluish-violet background.

GPx isozymes were separated using the method of Lin et al. (2002). The gel was soaked in 50 mL of 50 mM Tris-HCl buffer (pH 8.0) containing 200 mg reduced glutathione and 8 μL of 30% H_2O_2 for 20 min. The gel was then transferred to 50 mL of 50 mM Tris-HCl buffer (pH 8.0) containing 25 mg NBT and 25 mg phenazine methosulphate (PMS). The appearance of white bands in the gel was taken to indicate the presence of GPx isozymes.

Band areas, which were created by each isozyme activity, were measured by using the Kodak Gel Logic 1500 Imaging System and Kodak Molecular Imaging Software.

Histopathological Analysis

5 μm thick sections were taken from the ischemic and non-ischemic liver samples with microtome (Leica RM 2025) and stained with standard Hematoxylin and Eosin (H & E). Randomly selected 20 different cross-sectional areas in each of the liver tissue sections were investigated by light microscopy (Olympus CX31) by using the Spot Advanced Software (V.4.0.6; Diagnostic Instruments, Sterling Heights, USA). Sections were digitally photographed using a Olympus Camedia C-5060 compact dijital camera.

Statistical Analysis

"SPSS 24.0 for windows" version package program was used to evaluate the data obtained as a result of our studies. The One Way Analysis of Variance (ANOVA) and TUKEY tests were used for the analysis and comparison of data within and between groups. The Mann–Whitney *U* test was used to compare the scores of histopathological changes for the groups. The results were expressed as the mean ± Standard deviation (SD) of seven animals per group. Differences were considered significant at $P < 0.05$.

RESULTS

In our study, ALT and AST levels in the serum samples of the experimental animals of the groups; in liver tissue samples, SOD, CAT and GPx enzyme activity measurements as well as histopathological analyzes were performed.

Biochemical Evaluation

Serum ALT and AST levels were found as higher in all partial I/R groups compared to Group I (Sham group). While serum ALT and AST levels were the highest in Group II, they were observed as closer in Group IV that received 200 mg kg^{-1}/day *Olea europaea* L. leaf extract to Group I. ALT and AST levels of Group V were established as decreased compared to Group III (Fig. 1).

As a result of our study, in terms of serum ALT levels obtained from animals that received 2/3 partial liver I/R (45/60 min), it was found that Group II and III were only statistically different from Group I ($P < 0.05$), and Group IV and V were found as different from both Group I and Group II ($P < 0.05$). The ALT level of Group IV was found as the closest to Group I. When evaluated statistically the serum AST levels, Group II was observed as different from Group I ($P < 0.05$). AST levels established from Group III, IV, and V animals were found as different from Group I and II ($P < 0.05$). When compared the AST levels between groups, it was found that Group IV was the closest group to Group I.

Isozyme Activity in Liver

The activities of SOD, CAT, GPx antioxidant enzymes detected in 2/3 partial I/R and in non-I/R liver tissue samples using the native-PAGE method were compared between the groups by measuring the band area images formed on polyacrylamide gel (Table 1).

SOD isozyme was shown on two bands of polyacrylamide gel in all groups (Fig. 2). The mean SOD activity that was lower in Group I increased in ischemic liver samples of Groups II, III, IV, and V. While SOD activity had the highest band area in Group II, it was the lowest in Group IV that received 200 mg kg^{-1}/day olive leaf extract. SOD activity of Group V was found as lower than SOD activity of Group III. Similar findings were found in samples taken from non-ischemic liver sections.

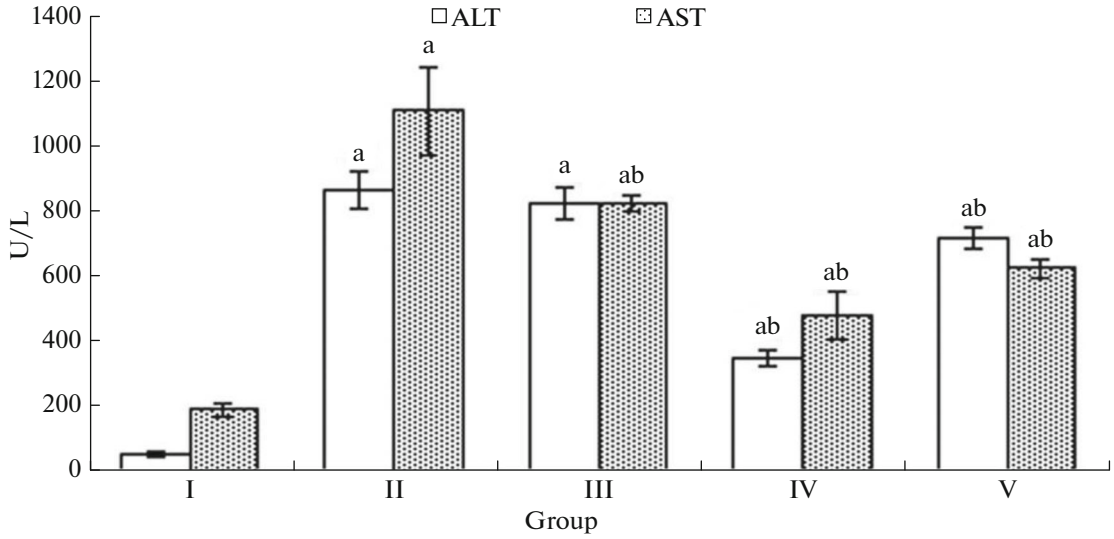


Fig. 1. ALT and AST levels in blood serum samples of animals belonging to experimental groups (mean ± SD) ($n = 7$). ($P < 0.05$ (a) There is a significant difference from Group I; (b) from Group II).

CAT isozyme activity was observed as a single band on polyacrylamide gel in all groups in native-PAGE analyses (Fig. 3). CAT activity that was at baseline in Group I was established as increased in the 2/3 partial I/R and non-I/R liver samples of Groups II, III, IV and V separately. CAT activity in ischemic and non-ischemic liver samples was found as the highest in Group II, and as the lowest in Group IV. In ischemic liver samples, CAT activity of Group V was established as lower than that of Group III.

GPx isozyme was observed as five bands on polyacrylamide gel in all groups (Fig. 4). Mean GPx activity that was at a low level in Group I was increased in liver samples of Group II, III, IV and V that had 2/3 partial I/R, whereas GPx activity had the highest mean band area in Group II, and it was the lowest in Group IV. The mean GPx activity of Group V was also lower than that of Group III. Similar findings were found in samples taken from non-ischemic liver sections.

Table 1. Band areas of CAT, SOD, GPx enzyme activities determined in liver tissue of groups (mm^2)

Group	Liver tissue example	Activity band areas average, mm^2		
		SOD	CAT	GPx
I	□	122.60	119.31	99.06
II	Non-I/R	172.97	185.78	120.18
	2/3 I/R	156.63	160.76	180.00
III	Non-I/R	161.03	177.26	118.35
	2/3 I/R	144.71	157.95	175.04
IV	Non-I/R	151.01	145.98	103.02
	2/3 I/R	132.02	117.71	140.41
V	Non-I/R	151.66	161.00	111.19
	2/3 I/R	139.36	123.58	167.94

Histopathological Evaluation

Ischemic and non-ischemic in liver tissue sections of the animals in all experimental groups were evaluated for sinusoidal dilatation, cytoplasmic vacuolization and PMNL infiltration findings. Histological changes were scored (1; no evidence of injury, 2; mild, 3; moderate, 4; severe) in order to perform a statistical comparison between the groups (Fig. 5).

When examined the ischemic and non-ischemic liver sections, it was observed that sinusoidal dilatation score was higher in Group II, and that the Group II was statistically different from Group I ($P < 0.05$). Compared to Group II, sinusoidal expansion was less common in the groups that received *Olea europaea* L. leaf extract. Sinusoidal dilatation score was lower in Group IV compared to Group III and V, and lower in Group V compared Group III. The number of sinusoidal dilatations in Group III liver sections was statistically different from Group I ($P < 0.05$). Partial I/R liver sections of Group IV and V were found as statistically different from Group II in terms of sinusoidal dilatation score ($P < 0.05$). Sinusoidal dilatation score detected in tissue sections of non-I/R liver sections was similar to I/R treated liver sections. In terms of this parameter, non-I/R liver tissue sections of Group II, III, and V were statistically different than Group I ($P < 0.05$). On the other hand, Group IV was statistically different from Group II ($P < 0.05$), and similar to Group I.

When examined ischemic liver parts, intense cytoplasmic vacuolization in Group II was found as

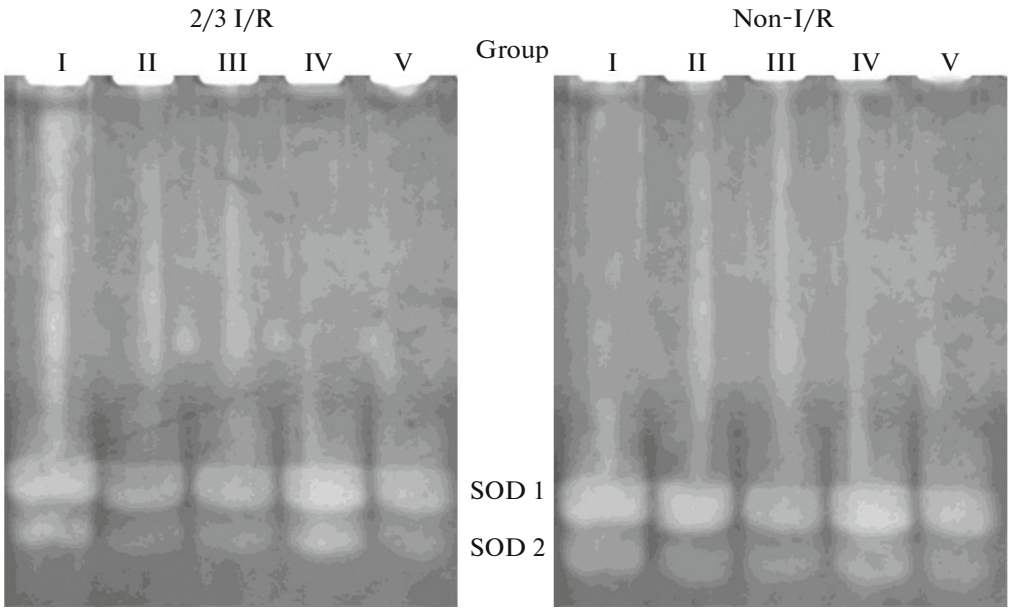


Fig. 2. Electrophoresis bands of SOD isozyme in ischemic and non-ischemic liver tissue samples.

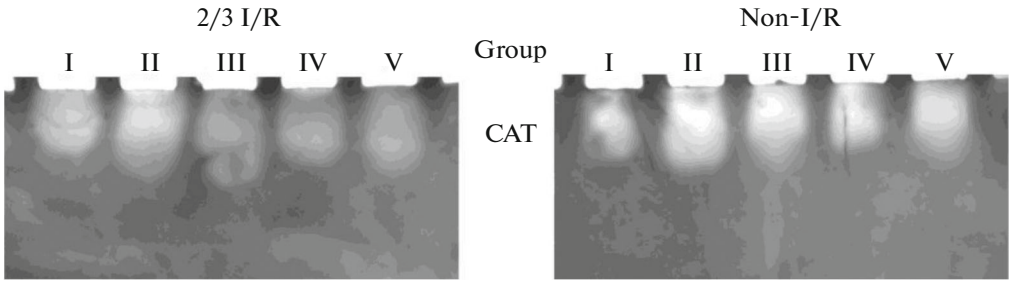


Fig. 3. Electrophoresis bands of CAT enzyme in ischemic and non-ischemic liver tissue samples.

decreased in groups that received *Olea europaea* leaf extracts. Group IV had lower levels than Group III and V; however, the same concentration of cytoplasmic vacuolization was observed in Group III and Group V. In terms of cytoplasmic vacuolization scores, it was observed that Group II was statistically different only from Group I, and Group III, IV and V were different from both Group I and Group II ($P < 0.05$). The cytoplasmic vacuolization density of non-I/R sections of the liver was higher in Group III compared to Group II and Group V. Cytoplasmic vacuolization was established as having lower intensity in Group IV. Cytoplasmic vacuolization in non-I/R liver sections of Group IV animals showed statistical difference from Group I and II ($P < 0.05$), and Group II, III and V manifested a difference ($P < 0.05$) from Group I.

In both of the 2/3 partial I/R and non-I/R sections, PMNL infiltration that was found as dense in Group II was established as lower in groups that received *Olea europaea* leaf extracts. PMNL infiltration score in ischemic liver sections was lower in Group IV than in Group III and V, and it was observed as lower in Group V than Group III. Statistically, it was observed that only Group II was different ($P < 0.05$) in the ischemic liver sections compared to Group I ($P < 0.05$), and that the other groups were not different. In non-ischemic liver sections, it was found that PMNL infiltration finding decreased dose-dependently in Group III, IV and V, respectively, compared to Group II. PMNL infiltration scores of Group II and III were statistically different than Group I, and those of Group V than Group II ($P < 0.05$).

No pathological findings were found at light microscope level in liver sections of Group I animals that was the Sham group of our study (Fig. 6a). In Group II animals, while a difference was not observed in classic lobule structure of ischemic and non-ischemic liver sections, a quite dense sinusoidal dilatation, cytoplasmic vacuolization, and PMNL filtration was found (Figs. 6b–6c). These histopathological findings

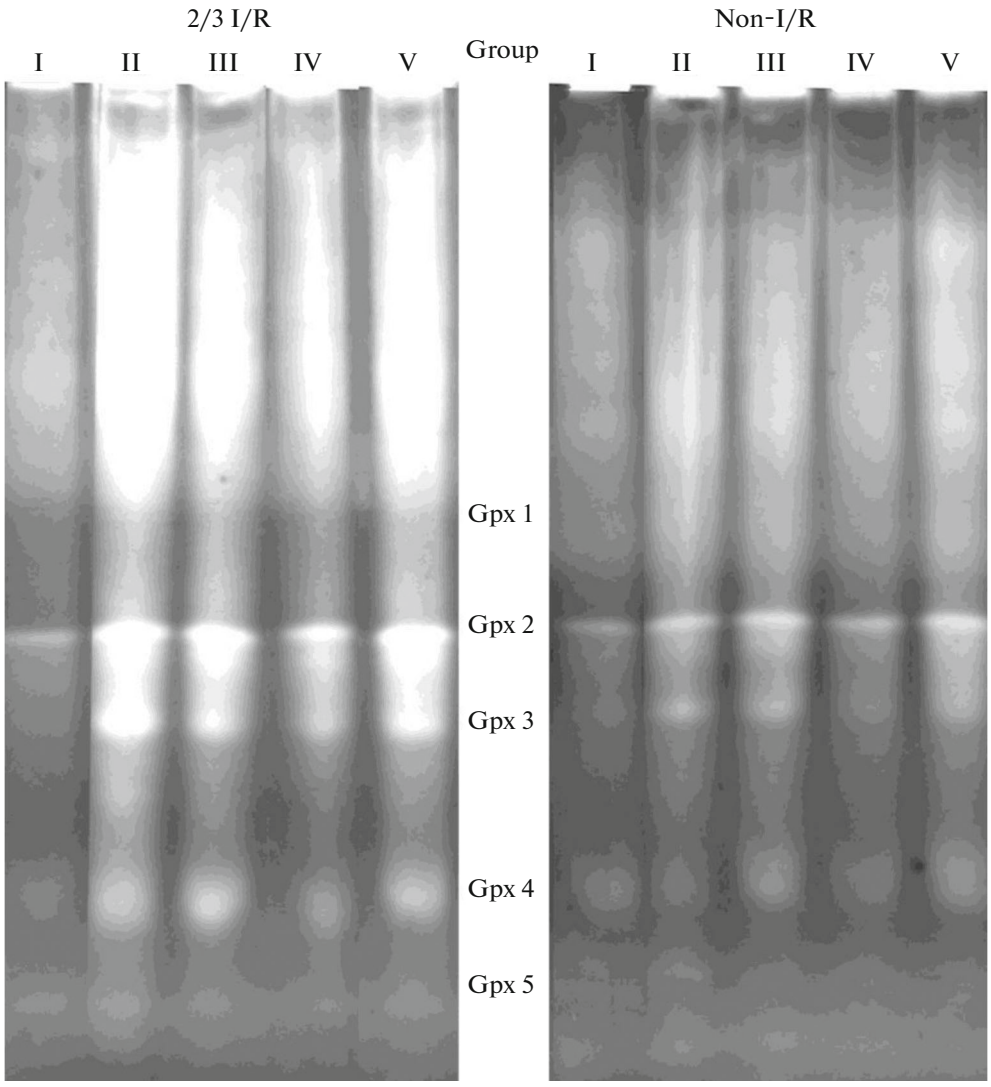


Fig. 4. Electrophoresis bands of GPx isozyme in ischemic and non-ischemic liver tissue samples.

were similar in ischemic and non-ischemic liver sections.

It was established that classic lobule structures were preserved in liver sections of animals that received 150 mg kg^{-1}/ day olive leaf extract and 2/3 partial I/R and non-I/R in Group III, but that they had higher amount of sinusoidal dilatation, cytoplasmic vacuolization, and PMNL filtration (Figs. 6d–6e).

It was found in liver sections of animals that received 200 mg kg^{-1}/day olive leaf extract and 2/3 partial I/R and non-I/R in Group IV that they had decreased sinusoidal enlargement, cytoplasmic vacuolization, and PMNL filtration compared to other groups. It was found that classic liver lobule structure was preserved in Group IV (Figs. 6f–6g). These histopathological findings were found as approximately similar in the ischemic and non-ischemic liver tissue samples.

In Group V with 400 mg kg^{-1}/day olive leaf extract, ischemic and non-ischemic liver tissue samples were observed to retain the classical lobule structure, and that sinusoidal dilatation, cytoplasmic vacuolization, and PMNL filtration findings were found as lower (Fig. 6h).

DISCUSSION

In liver surgery, total or partial ischemia should be performed in order to control the bleeding that may occur for various reasons. The toxic products formed during ischemia, especially the ROS are spread throughout the organism during reperfusion. ROS is first to cause damage to tissue with ischemia and then to other tissues and organs. Researchers reported that I/R effects can be demonstrated by looking at levels of enzymes and molecules such as GSH, MDA, CAT, SOD, and NO, and of apoptotic gene expressions, as

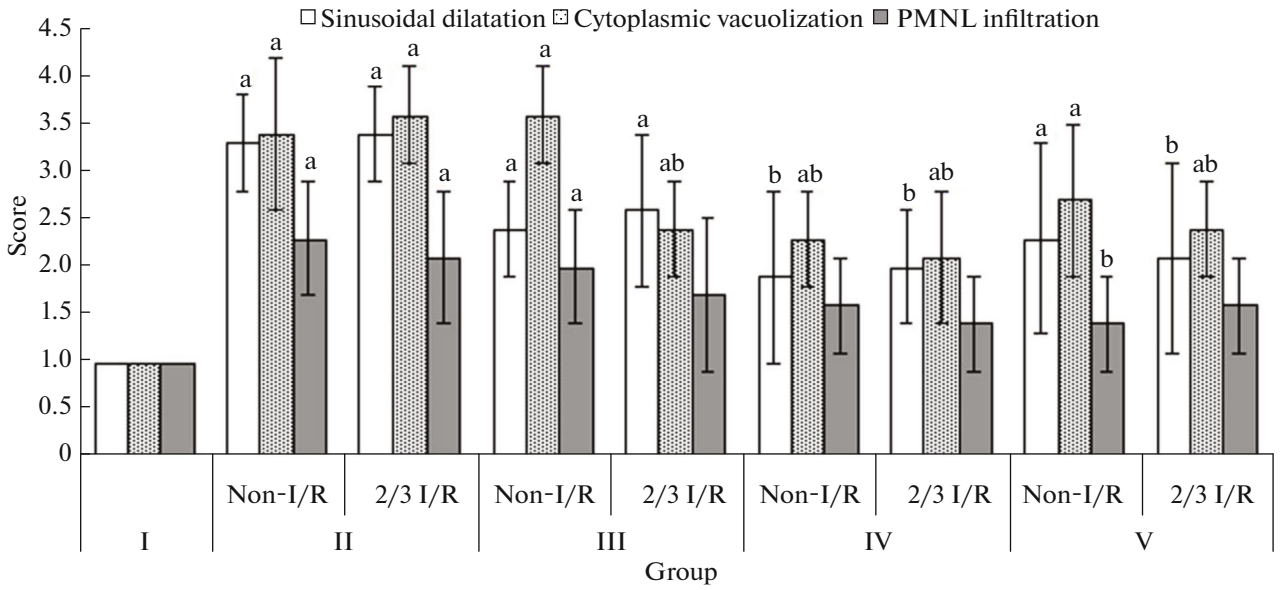


Fig. 5. Histopathological evaluations of liver tissue sections of animals in all experimental groups (mean ± SD) ($n = 7$). (Score: 1; No evidence of injury, 2; mild, 3; moderate, 4; severe) ($P < 0.05$ (a) There is a significant difference from Group I; (b) from Group II).

well as histological tissue sections (Sener et al., 2003). Ahmad et al. (2020) while examining the effects of a polyherbal extract mixture on rats with hepatorenal toxicity with CCl_4, similar to our study, they determined the levels of ALT and AST as well as endogenous antioxidant enzymes and made histopathological evaluations.

The importance of increased plasma levels of enzymes such as AST, and ALT in the liver is emphasized in the evaluation of the effects of both partial hepatectomy and hepatic I/R injury on hepatocyte function (Fouad et al., 2007). As a result of oxidative damage caused by liver I/R, it is reported that serum ALT and AST levels increase with time compared to the control group (Sener et al., 2003; Fouad et al., 2007). Ozmen et al. (2012) reported that serum ALT and AST levels increased as a result of liver I/R in rats. According to the results of our study, serum ALT and AST levels in Group II that received I/R were statistically higher than those of Sham (Group I) group, and that these values were observed to decrease in the groups that received *Olea europaea* L. leaf extract.

Vidičević et al., (2019) reported that *Olea europaea* L. leaf extract also decreased CAT activity in addition to serum ALT and AST levels in their study that they formed damage on rat liver by CCl_4. The electrophoresis results of our study showed that SOD, CAT, GPx enzyme activities determined in Group II were higher than Group I, and that enzyme activities decreased groups that orally received *Olea europaea* L. leaf extract. In their study on the antioxidant effects of phenolic compounds found in olive leaves, Lee and Lee (2010) revealed that olive leaf extract also has a radical scavenging effect like SOD. Similarly, in our study, oleuropein-high olive leaf extract administered to experimental animals before surgical procedures can be considered to cause SOD activity to decrease with radical scavenging effect. Also, Jin et al. (2018) reported that oleuropein increased SOD inhibition in oxidative damage caused by myocardial I/R in rats.

On the other hand, SOD and CAT enzyme activities found in ischemic liver sections of each experimental group in our study were observed as higher in non-ischemic sections, and that the GPx activity was lower in non-ischemic sections. When evaluated separately the ischemic and non-ischemic liver sections among the experimental groups, it may be noted that 200 mg kg^{-1}/day *Olea europaea* L. leaf extract administered to Group IV animals had more positive effect in terms of endogenous antioxidant enzyme activity. The enzyme activity results of our study showed that ischemic damage occurring in only one section of the same organ affects the other sections of the organ at the same rate.

Fadillioglu et al. (2008) reported that I/R-related oxidative damage stress occurring in one organ may also be observed in another organ established oxidative damage histopathologically as a result of renal I/R. In the same manner, Uyanoglu et al. (2011) reported renal I/R-induced oxidative damage affects SOD, CAT and GPx enzyme activities in the liver, in addition to it causing hepatocyte dysfunction, histological sinusoidal congestion, and vacuolization in hepatocytes. In their study of total liver I/R, Araya et al. (2003) reported the occurrence of histological sinusoidal congestion and vacuolization in hepatocytes.

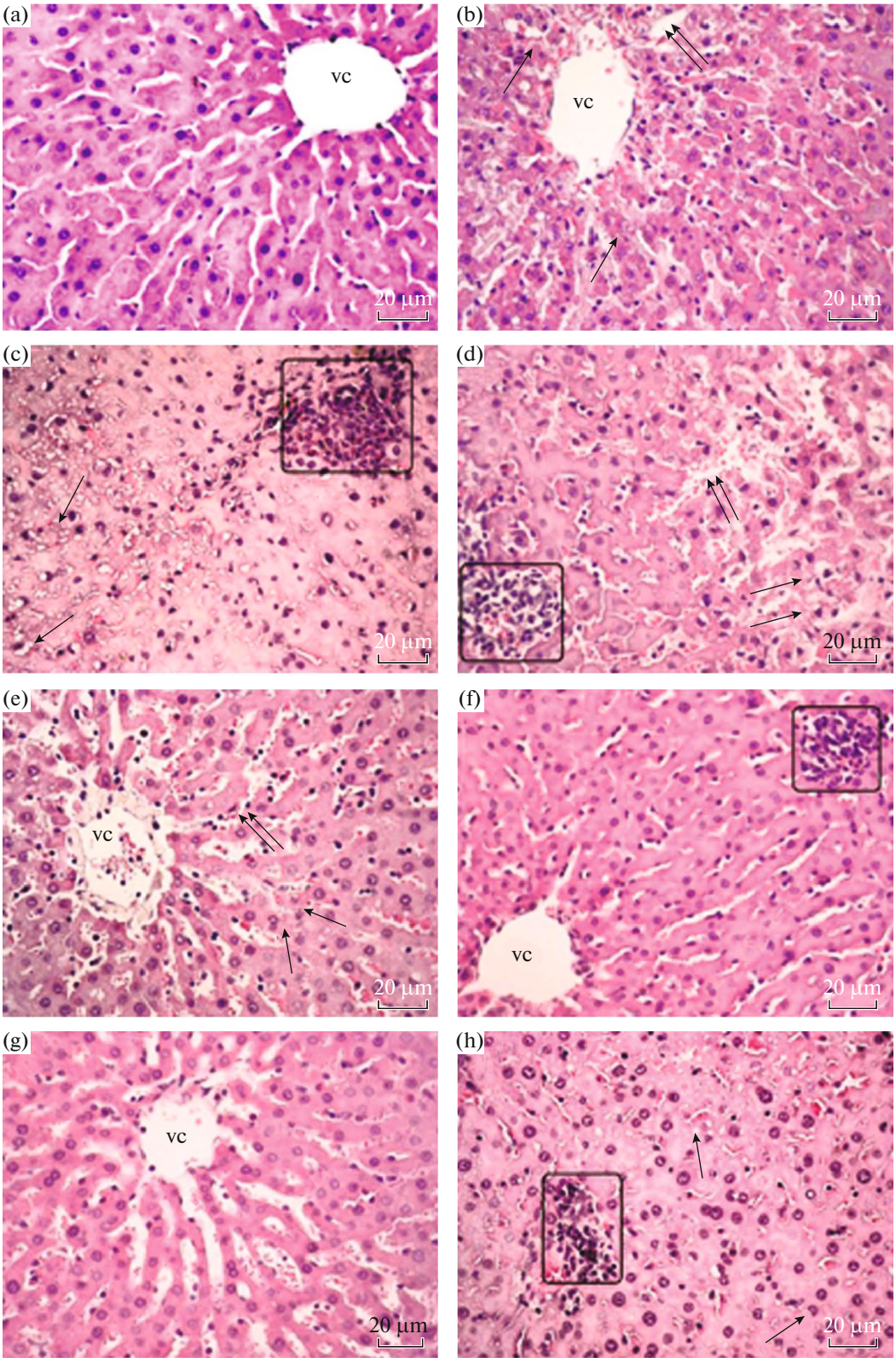


Fig. 6. Liver sections of all experimental animals (H & E). Group I (a); Group II (b and c); Group III (d and e); Group IV (f and g); Group V (h). Cytoplasmic vacuolization (↗); sinusoidal dilatation (↗↗); PMNL infiltration (□).

It is stated that the critical time for recovery of liver cell damage is 90 min ischemia and subsequent reperfusion (Marubayashi et al., 2000). It was observed that oxidative stress damage detected in parenchymal cells in the periportal area after 30 or 60 min ischemia and 30 min reperfusion of the liver was spread to the middle sections after 60 min reperfusion and cell death was further increased (Kato et al., 2001). In our study that we formed a recoverable damage to the 2/3 of the liver by administering I/R (45/60 min), cytoplasmic vacuolization, sinusoidal dilatation, and PMNL infiltration findings were observed in the ischemic and non-ischemic liver sections of Group II animals. The cytoplasmic vacuolization and sinusoidal dilatation concentrations in the ischemic and non-ischemic liver sections were found as statistically decreased in Group III and V compared to Group II and as significantly lower in Group IV. In our study, while especially 200 mg kg^{-1}/day *Olea europaea* L. leaf extract was protective against the formation of sinusoidal dilatation and cytoplasmic vacuolization, the effect of 400 mg kg^{-1}/day dose had a smaller effect. In their study with renal I/R, Senturk and Yildiz (2018) reported that 200 mg kg^{-1}/day *Olea europaea* L. leaf extract was less protective compared to 100 mg kg^{-1}/day dose. These researchers reported that CAT, SOD and GPx activities in renal tissue of the treatment groups were decreased and that 200 mg kg^{-1}/day dose prevented vacuolization and tubular cell deformations. It was noted that the regional tissue destruction commenced with the release of protease and ROS along with endothelial I/R damage, and in addition, migratory neutrophils adversely affected the delicate balance in microcirculation (Fondevila et al., 2003). Vidičević et al. (2019) reported that *Olea europaea* L. leaf extract reduced/prevented such pathological findings as cytoplasmic vacuolization and PMNL infiltration in rat liver that they created damage by CCl_4. Lapi et al. (2015) noted that oleuropein reduced microvascular leakage, leukocyte adhesion, and ROS formation. In our study, it was observed that the density of PMNL infiltration detected in the ischemic liver sections was higher in Group II, but it decreased further in Group III and IV in a dose-dependent manner without any statistical difference. 400 mg kg^{-1}/day dose of olive leaf extract used in our study did not comply with this dose-dependent order, but PMNL infiltration density detected in the non-ischemic liver sections decreased further in a dose-dependent manner in Group III, IV, and V, respectively, compared to Group II. Parallel to our study, Cerig et al. (2016) reported in their study in which they administered 50, 100, and 200 mg kg^{-1}/day commercial oleuropein to rats that they formed oxidative stress with cisplatin that sinusoidal dilatation, cytoplasmic vacuolization, and PMNL infiltration findings decreased in a dose-dependent manner.

The results of this study showed that I/R-induced oxidative damage in a part or lobe of the liver can affect the entire organ. In our study, the effects of *Olea europaea* L. leaf ethanol extract with high oleuropein content administered 200 mg kg^{-1}/day orally through gavage for 15 days were evaluated. As a result, it provided a protective effect against I/R (45/60 min) induced oxidative stress damage by reducing both SOD, CAT and GPx enzyme activities and serum ALT and AST levels. In addition, liver lobule and cell structure are protected from the effects of oxidative damage.

FUNDING

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COMPLIANCE WITH ETHICAL STANDARDS

The author declares no conflict of interest. This article does not contain any studies involving animals or human participants performed by the author.

The experimental protocols were approved by the Institutional Ethical Committee for Animal Care and Use at Eskisehir Osmangazi University, Eskisehir, Turkey (protocol number: 2013/336).

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