ORIGINAL ARTICLE

Effect of ischemia-reperfusion injury on elafin levels in rat liver

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ABSTRACT

BACKGROUND: The aim of this study was to quantify serum levels of elafin, a serine protease inhibitor, and to assess its effects on histopathological and biochemical parameters in hepatic ischemia-reperfusion injury.

METHODS: Forty female Wistar albino rats were divided into five groups: Group 1 served as the control group. Liver ischemia was induced for 30 minutes in the other four groups. An additional 1-hour, 2-hour, and 3-hour reperfusion was induced in Groups 3, 4, and 5, respectively. At the end of the experiment, intracardiac blood samples were obtained for biochemical examination, and tissue samples from the liver were taken for histopathological examination. Levels of elafin, ischemia-modified albumin (IMA), total antioxidant status (TAS), and total oxidant status (TOS) were also examined.

RESULTS: Serum elafin levels decreased beginning from Group 2, with the lowest level reached in Group 5 ($p<0.01$). The IMA level was the lowest in the control group and the highest in Group 5 ($p<0.01$). TOS, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) levels were lowest in the control group and highest in Group 5 ($p<0.01$). Group 5 had the highest IMA/albumin ratio, although no significant differences were found between these four groups. The lowest TAS level was found in the control group, but a stable and significant increase was not detected in the other groups. No significant differences were found between the groups in terms of alkaline phosphatase (ALP) and albumin levels. A negative correlation was observed between serum elafin levels and AST, ALT, and TOS levels ($p<0.01$). The number of Grade 1 histopathological results was found to be higher in the groups with reperfusion (Groups 3, 4, 5). In histopathological subgroup analysis, while the elafin level was lower in Grade 1 group, AST, ALT, and TOS levels were higher ($p<0.01$). Additionally, the IMA/albumin ratio was found to be higher in the Grade 1 group ($p=0.02$).

CONCLUSION: In hepatic ischemia-reperfusion injury, elafin levels decreased as the reperfusion time increased. As the reperfusion time increased, both hepatocyte damage and oxidant capacity increased, with a negative correlation observed between these findings and elafin levels. Therefore, elafin may play a protective role in hepatic ischemia-reperfusion injury and could assist clinicians in assessing liver injury.

Keywords: Elafin; ischemia reperfusion; liver; liver injury.

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INTRODUCTION

One of the scenarios in which ischemia-reperfusion injury is commonly observed in humans is during liver surgery. Liver ischemia-reperfusion injury is most frequently caused by shock, trauma, the Pringle maneuver during liver surgery, and liver transplantation. Ischemia-reperfusion injury occurs if the tissue cannot tolerate the free oxygen radicals (FORs) generated during reperfusion following ischemia.[1,2] Numerous mechanisms related to the pathophysiology of ischemia-reperfusion injury have been described. These include increased consumption of Adenosine Triphosphate (ATP), elevated intracellular Na+ and Ca+2 levels, reduced oxidative phosphorylation, decreased intracellular pH, and activation of proteases and phosphatases that lead to phospholipids degradation. Consequently, excessive amounts of FORs are formed, causing oxidative stress.[3-6]

One of the identified serine protease inhibitors against these FORs is elafin. Elafin was first described in bronchial mucus in 1981, and consequently in skin and lung in 1990.[7-9] Three primary targets of elafin include neutrophil elastase, proteinase 3, and pancreatic elastase, which play crucial roles in inflammatory damage mechanisms. Elafin, a polypeptide, inhibits these proteases by blocking the reactive serine amino acid at their center.[10] It is synthesized in various tissues including the colon, lung, skin, salivary gland, vaginal mucosa, placenta, and fetal membranes.[11,13]

In addition to its diverse effects, elafin has been recognized as a substrate for the cross-linking activity of tissue transglutaminase (TG-2), which plays important roles in wound healing, cell migration, apoptosis, and tissue homeostasis.[14] Since elafin appears to interact with TG-2 and moderately inhibits its activity, reduced elafin levels may lead to mucosal damage.[15]

The aim of this study was to investigate elafin levels and their impact on liver ischemia-reperfusion injury in rat liver, under conditions of fixed ischemia time (30 minutes) and prolonged reperfusion. To our knowledge, this is the first study to report serum elafin levels in rat liver ischemia-reperfusion injury.

MATERIALS AND METHODS

Approval for this study was granted by the Animal Research Ethics Committee of Akdeniz University, according to the protocol dated January 18, 2021, decision no. 12 and protocol number 1243/2021.01.01. All procedures were conducted at the Akdeniz University Experimental Medicine and Animal Laboratory between July and October 2021, in compliance with National Principles on the Experimental Use of Laboratory Animals.

Surgery and Experimental Protocol

In this study, 40 female Wistar albino rats, each weighing between 250-300 grams, were used. The rats were provided standard laboratory food and tap water and were kept under constant environmental conditions (temperature: 23°C, humidity: 55.5%) both preoperatively and postoperatively.

The rats were randomized into five groups:
Group 1 (Sham, Control): Only laparotomy was performed. The animals were sacrificed after blood and tissue samples were obtained.

Group 2 (30 min ischemia, followed by 0 min Reperfusion): Following laparotomy, the common bile duct, portal vein, and hepatic artery (liver pedicle) were clamped to induce ischemia for 30 minutes. After this, the clamps were opened but no reperfusion was induced (0 minutes). Tissue and blood samples were then obtained, and the rats were subsequently sacrificed.

Group 3 (30 min Ischemia, followed by 1 h Reperfusion): Following laparotomy, the common bile duct, portal vein, and hepatic artery (liver pedicle) were clamped to induce ischemia for 30 minutes. Afterward, the clamps were removed to initiate reperfusion for 1 hour. Tissue and blood samples were collected before the rats were sacrificed.

Group 4 (30 min Ischemia, followed by 2 h Reperfusion): After laparotomy and clamping the bile duct, portal vein, and hepatic artery (liver pedicle), ischemia was induced for 30 minutes. Following 2 hours of reperfusion post clamp removal, tissue and blood samples were collected and the rats were then sacrificed.

Group 5 (30 min Ischemia, followed by 3 h Reperfusion): Following laparotomy, the common bile duct, portal vein, and hepatic artery (liver pedicle) were clamped, after which ischemia was induced for 30 minutes. Tissue and blood samples were obtained after 3 hours of reperfusion after the clamp was opened, and the rats were then sacrificed.

Biochemical Analysis

Routine parameters were measured using the following kits: Rat serum albumin (Beckman Coulter Diagnostics, Brea, CA, USA; catalog no. OSR6602), alkaline phosphatase (ALP) (Beckman Coulter Diagnostics, Brea, CA, USA; catalog no. OSR6504), aspartate aminotransferase (AST) (Beckman Coulter Diagnostics, Brea, CA, USA; catalog no. OSR6509), and alanine aminotransferase (ALT) (Beckman Coulter Diagnostics, Brea, CA, USA; catalog no. OSR6607).

Rat serum elafin levels were quantified using a commercially available Enzyme-Linked Immunosorbent Assay (ELISA) kit (Bioassay Technology Laboratory, Shanghai, China; catalog no. E2890Ra), following the manufacturer's instructions. The analysis range was 7-1500 ng/mL, with a sensitivity of 8.01 ng/mL. Precision was defined as intra-assay Coefficient of Variation (CV) < 8% and inter-assay CV < 10%. Assay results were expressed as ng/mL.

Serum Total Antioxidant Status (TAS) levels were analyzed using an automated colorimetric measurement method developed by Erel.[16] This method involves the reduction of the dark blue-green colored 2,2'-azino-bis (3-ethylbenzthiazoline-

6-sulfonic acid) (ABTS) radicals to a colorless reduced ABTS form by antioxidants in the sample. The change in absorbance at 660 nm is related to the total antioxidant level in the sample. This method determines the anti-oxidative effect of the sample against potent free radical reactions initiated by the hydroxyl radical produced. The results are expressed as micromolar Trolox equivalent per liter (µmol Trolox equiv./L).

Serum Total Oxidative Status (TOS) levels were analyzed using an automated colorimetric measurement method developed by Erel.[17] In this method, oxidants in the sample oxidize the ferrous ion-chelated complex into a ferric ion, which forms a colored complex with a chromogenic in an acidic medium. The color intensity, measurable by spectrophotometry, is related to the total amount of oxidant molecules present in the sample. The results were expressed in micromolar hydrogen peroxide equivalent per liter (µmol H_2O_2 equiv./L).

Reduced Cobalt-Albumin-Binding Capacity (Ischemia-Modified Albumin level (IMA) level) was measured using the rapid and colorimetric method developed by Bar-Or et al.[18] Initially, approximately 200 µl of rat serum was transferred into glass tubes. To this, 50 µl of 0.1% $CoCl_2$ * $6H_2O$ (lot S38901-248, Sigma Aldrich, St Louis, MO, USA) was added. After gentle shaking, the mixture was incubated for 10 minutes to ensure sufficient cobalt-albumin binding. Then, 50 µl of 1.5 mg/ml dithiothreitol (DTT) (lot D5545-1G, Sigma-Aldrich) was added as a coloring agent. After 2 minutes, 1 ml of 0.9% NaCl was added to stop the binding between cobalt and albumin. A blank for each specimen was prepared at the DTT addition step, using 50 µl of distilled water instead of 50 µl of 1.5 mg/ml DTT to obtain a blank without DTT. The absorbance was measured at 470 nm using a spectrophotometer (UV1201, Shimadzu, Kyoto, Japan). Color formation in specimens with DTT was compared with that in the blank tubes, and the results were expressed as absorbance units. To maintain the IMA rate, the formula IMA value/individual serum albumin concentration was used, to mitigate the impact of albumin concentration differences between groups.

Histopathological Evaluation

Liver samples were preserved in a 10% formaldehyde solution. Paraffin blocks of approximately 4-5 microns were prepared (Leica RM 2125, Leica Microsystems Nussloch GmbH, Germany) and stained with hematoxylin and eosin. The preparations were evaluated blindly by a pathologist using a light microscope.

Hepatic bleeding, necrosis, leukocyte infiltration, vacuolization in hepatocytes, and connections between hepatocytes were assessed similarly to methods described in the literature and were graded pathologically as follows: GRADE 0: Slight or no damage; GRADE 1: Mild damage, including focal nuclear pyknosis and cytoplasmic vacuolization; GRADE 2: Moderate to severe injury, characterized by diffuse nuclear pyknosis, cytoplasmic hypereosinophilia, and loss of intercellular bridges; GRADE 3: Severe necrosis, separation of hepatic cords, bleeding, and neutrophil infiltration.[19]

Statistical Analysis

The statistical analyses of the study were conducted using the Statistical Package for the Social Sciences version 25.0 software for Windows (IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp., USA).

The normality of the variables was tested using the Kolmogorov-Smirnov and Shapiro-Wilk tests. Descriptive statistics for the variables were presented as mean ± standard deviation, median (25th and 75th percentiles), and frequencies (n, %), depending on the type of variable and the fulfillment of assumptions. For the univariate analyses of the variables in the study, the Fisher-Freeman-Halton exact test, Kruskal-Wallis test, and Mann-Whitney U test were used, depending on the variable type and the fulfillment of assumptions. Pairwise comparisons of groups with significant differences, as identified by the Kruskal-Wallis test, were conducted using the Mann-Whitney U test and evaluated by applying the Bonferroni correction (0.05 divided by the number of groups). The relationships between elafin and other parameters were investigated using Spearman Correlation analysis.

RESULTS

Group comparisons and descriptive statistics of the data obtained are presented in Table 1. Pairwise comparisons of means, which showed statistically significant differences between the groups, were performed using the Mann-Whitney U test. In the results of these pairwise comparisons, means that did not differ significantly are indicated with the same letter.

The difference in serum elafin levels between the groups was statistically significant ($p<0.01$). The lowest mean elafin level was observed in Group 5, while the highest was in Group 2. The difference in serum elafin values between Groups 1 and 3 was not statistically significant ($p>0.05$). However, the differences between the other groups were statistically significant ($p<0.01$) (Fig. 1).

The difference in the Ischemia-Modified Albumin to Albumin (IMA/ALB) ratio between the groups was statistically significant ($p<0.01$). The lowest IMA/ALB ratio was observed in Group 1. No statistically significant differences were found

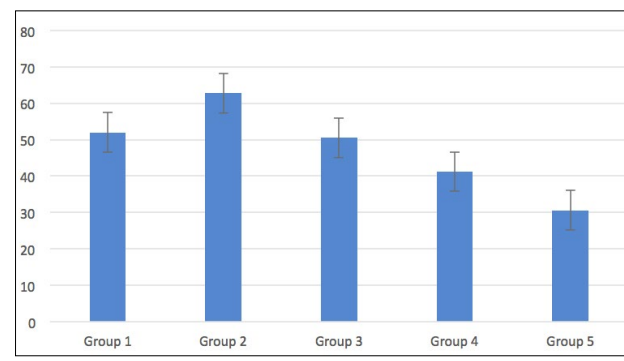

Figure 1. Elafin levels in groups.

Table 1. Group comparisons of variables and descriptive statistics

	Group 1 Mean±SD Median (25th-75th)	Group 2 Mean±SD Median (25th-75th)	Group 3 Mean±SD Median (25th-75th)	Group 4 Mean±SD Median (25th-75th)	Group 5 Mean±SD Median (25th-75th)
Elafin (ng/mL)	52.00±5.39[c]	62.75±10.49[d]	50.50±10.19[c]	41.25±7.20[b]	30.62±4.53[a]
	52.0 (47.0–56.2)	66.0 (52.0–71.0)	49.5 (60.5–41.5)	42.0 (35.5–47.7)	32.5 (27.2–34.0)
IMA/ALB	2.97±1.75[a]	5.45±3.14[b]	5.40±4.81[b]	5.76±2.09[b]	9.56±3.63[b]
	3.0 (1.2–4.2)	4.8 (3.1–7.2)	3.6 (2.2–7.7)	5.9 (3.6–7.7)	9.2 (6.3–13.5)
Albumin (g/dL)	3.27±0.13[a]	3.73±0.21[b]	3.30±0.26[ab]	3.16±0.14[a]	3.37±0.33[b]
	3.3 (3.1–3.4)	3.8 (3.5–3.9)	3.3 (3.1–3.5)	3.1 (3.0–3.2)	3.4 (3.2–3.6)
IMA (absorbance unit)	0.09±0.06[a]	0.20±0.12[b]	0.16±0.13[b]	0.18±0.06[b]	0.32±0.14[c]
	0.10 (0.03–0.13)	0.17 (0.11–0.26)	0.12 (0.07–0.24)	0.19 (0.11–0.24)	0.28 (0.20–0.46)
TOS	10.17±2.12[a]	20.21±4.90[b]	23.67±2.92[b]	29.53±3.46[c]	43.37±10.79[d]
(µmol H2O2 equiv./L)	10.2 (7.9–12.3)	20.5 (16.2–24.2)	22.8 (21.8–25.2)	29.9 (26.3–30.5)	44.6 (32.2–52.3)
TAS	0.62±0.04[a]	1.32±0.12[c]	0.76±0.05[b]	1.10±0.31[c]	1.02±0.21[c]
(µmol Trolox equiv./L)	0.60 (0.60–0.67)	1.30 (1.22–1.37)	0.80 (0.70–0.80)	0.95 (0.82–1.40)	1.0 (0.85–1.07)
AST					
(U/L)	68.37±14.37[a]	207.12±82.88[b]	527.87±317.97[c]	1389.37±386.83[d]	2173.25±864.58[e]
	72.0 (57.0–78.0)	190.0 (132.0–292.25)	471.0 (306.5–544.5)	1440.5 (977.0–1722.2)	1854.0 (1662.0–3063.0)
ALT	45.25±18.50[a]	167.75±56.18[b]	332.87±175.92[c]	847.87±239.79[d]	2240.62±510.97[e]
(U/L)	40.0 (36.0–44.25)	151.0 (128.5–232.25)	265.5 (257.5–320.75)	842.0 (661.25–1023.25)	2020.0 (1884.5–2627.0)
ALP	117.12±57.24	112.37±33.77	72.87±36.97	86.50±26.69	107.75±39.23
(U/L)	106.5 (73.25–156.0)	109.0 (84.75–119.25)	63.5 (39.75–113.0)	82.0 (74.0–85.0)	112.5 (81.5–136.25)
Histopathological Grade 0	8 (100%)	8 (100%)	6 (75.0%)	2 (25.0%)	2 (25.0%)
Histopathological Grade 1	0 (0.0%)	0 (0.0%)	2 (25.0%)	6 (75.0%)	6 (75.0%)

*Kruskal-Wallis tests, pairwise comparisons, and Mann-Whitney U test were used. There is no significant difference between the averages indicated by the same letter on the same line (p>0.05). SD: Standard Deviation.

between Groups 2, 3, and 4 (p>0.05). The IMA/ALB ratio in Group 5 was significantly higher than in all other groups (p<0.01). The difference in albumin levels between the groups was statistically significant (p<0.01). The highest mean albumin value was observed in Group 2. Albumin values in Groups 2, 3, and 5 were similar (p>0.05). The difference in albumin values between Groups 1, 3, and 4 was not statistically significant (p>0.05). However, the albumin values of Groups 2 and 5 were significantly different from those of Groups 1 and 4 (p<0.01). The lowest mean albumin value was found in Group 4. The difference in the IMA value between the groups was statistically significant (p<0.01). The lowest mean IMA value was found in Group 1, and the highest IMA value was observed in Group 5.

The difference in mean TOS value between the groups was statistically significant (p<0.01). The average TOS value was lowest in Group 1 and highest in Group 5. The difference in mean TOS value between Groups 2 and 3 was not statistically significant (p>0.05) (Fig. 2). The difference in mean TAS value between the groups was statistically significant (p<0.01). The mean TAS value was lowest in Group 1 and highest in Group 2. The difference in mean TAS value between Groups 2, 4, and 5 was not statistically significant (p>0.05) (Fig. 3).

The difference in mean AST value between the groups was statistically significant (p<0.01). The mean AST value was

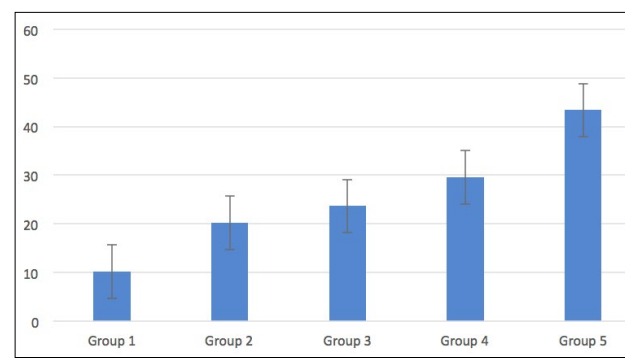

Figure 2. TOS levels in groups.

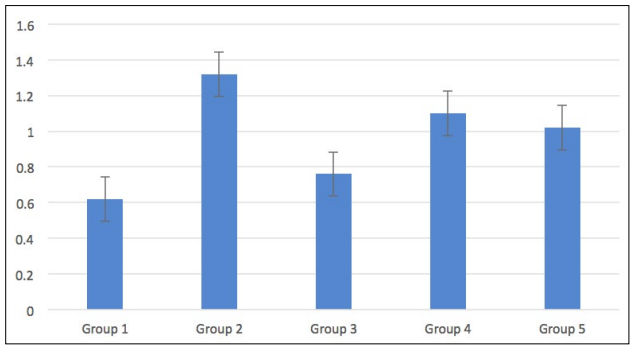

Figure 3. TAS levels in groups.

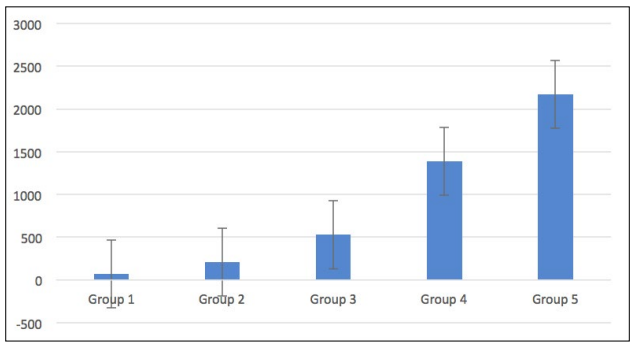

Figure 4. AST levels in groups.

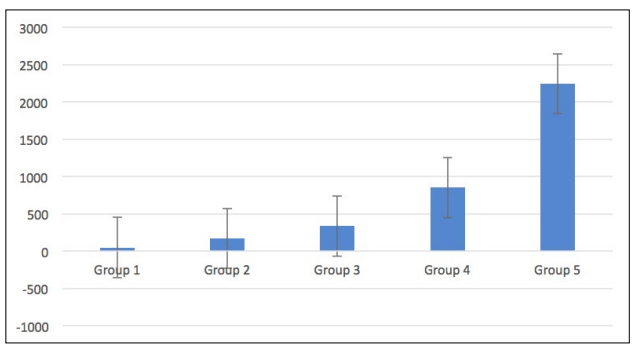

Figure 5. ALT levels in groups.

lowest in Group 1. The mean AST value of Group 5 was considerably higher than that of the other groups ($p<0.01$) (Fig. 4). Similarly, the difference in mean ALT value between the groups was statistically significant ($p<0.01$). The mean ALT value was lowest in Group 1. The mean ALT value of Group 5 was considerably higher than that of the other groups ($p<0.01$) (Fig. 5). The difference in mean ALP value between the groups was not statistically significant ($p>0.05$).

The numbers and percentages of the histopathological damage grades of the liver tissues of the rats in the groups (n, %) are presented in Table 1. According to these results, there was a significant relationship between the groups and the grades ($p<0.01$). In Groups 1 and 2, 100% of the rats were classified in Grade 0. In Group 3, 75% of the rats were classified in Grade 0 and 25% in Grade 1. In Groups 4 and 5, 75% of the rats were classified in Grade 1. The number of rats classified in Grade 1 gradually increased from Group 3 to Group 5. In addition, microscopic images of histopathological Grade 0 and Grade 1 liver damage are presented in Figures 6 and 7.

Table 2. The relationship between elafin levels and serum parameters

	Elafin	p
Albumin	0.115 N.S.	0.48
IMA/ALB	-0.193 N.S.	0.23
IMA	-0.129 N.S.	0.43
TOS	-0.600**	<0.001
TAS	-0.137 N.S.	0.4
AST	-0.708**	<0.001
ALT	-0.730**	<0.001
ALP	0.013 N.S.	0.94

Spearman Correlation Analysis. **p<0.01; N.S.: Non-Significant (no difference).

Relationships between elafin levels and serum parameters are presented in Table 2. The relationship between elafin and AST was negative and significant (Rho=-0.708, $p<0.001$). A significant negative correlation was found between elafin and ALT (Rho=-0.730, $p<0.001$). There was a negative and significant relationship between elafin and TOS (Rho=-0.600, $p<0.001$). No significant relationship was found between elafin and albumin (Rho=0.115, $p=0.48$). The relationship between elafin and IMA/ALB was not statistically significant (Rho=-0.193, $p=0.23$). The relationship between elafin and IMA was not significant (Rho=-0.129, $p=0.43$). The relationship between elafin and TAS was not statistically significant (Rho=-0.137, $p=0.4$). The relationship between elafin and ALP was not statistically significant (Rho=0.013, $p=0.94$).

The test results regarding the differences in serum parameters between the grades are provided in Table 3. According to these results, the difference in elafin values between Grade 0 and Grade 1 rats was statistically significant ($p<0.001$), with lower values observed in Grade 1 rats. The difference in the IMA/ALB value between the grades was statistically significant ($p=0.02$), with higher values in Grade 1 compared to Grade 0. The difference in albumin value between the grades was not

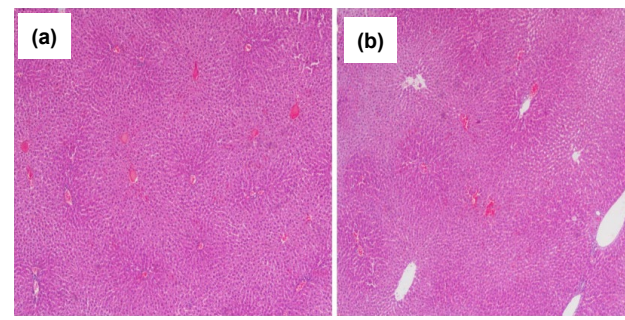

Figure 6. Histopathological Grade 0 liver damage (a and b: Hematoxylin Eosin (HE) x40).

Table 3. Comparison between grades

	Grade 0 Mean±SD Median (25th-75th)	Grade 1 Mean±SD Median (25th-75th)	p*
Elafin (ng/mL)	52.96±11.02	37.14±11.03	
	51.50 (46.75–61.25)	35.0 (28.75–43.25)	<0.001
IMA/ALB	4.90±3.46	7.54±3.84	
	3.95 (2.65–6.25)	7.60 (3.95–9.25)	0.02
Albumin (g/dL)	3.43±0.29	3.25±0.26	
	3.40 (3.20–3.62)	3.25 (3.10–3.40)	0.08
IMA (absorbance unit)	0.16±0.11	0.24±0.13	
	0.13 (0.10–0.21)	0.23 (0.12–0.28)	0.05
TOS (µmol H2O2 equiv./L)	19.56±8.22	36.22±11.62	
	21.0 (12.35–24.80)	30.0 (29.10–48.50)	<0.001
TAS(µmol Trolox equiv./L)	0.93±0.32	1.02±0.26	
	0.80 (0.67–1.30)	1.0 (0.80–1.17)	0.15
AST (U/L)	467.15±645.27	1627.28±864.59	
	214.0 (78.0–503.25)	1501.0 (1111.25–1797.25)	<0.001
ALT (U/L)	379.76±577.08	1371.50±924.19	
	176.5 (44.25–304.25)	1004.5 (665.75–2001.0)	<0.001
ALP (U/L)	100.80±48.13	96.57±27.08	
	99.0 (64.50–132.75)	85.0 (78.25–114.75)	0.94

significant (p=0.07). The difference in IMA value between the grades was not significant (p=0.05). The difference in TOS values between Grade 0 and Grade 1 rats was statistically significant (p<0.001), with higher values in Grade 1 rats. The difference in TAS value between the grades was not significant (p=0.15). The difference in AST values between Grade 0 and Grade 1 rats was statistically significant (p<0.001), with higher values in Grade 1 rats. Similarly, the difference in ALT values between Grade 0 and Grade 1 rats was statistically significant (p<0.001), with higher ALT values found in Grade 1 rats. The difference in ALP value between the grades was not significant (p=0.94).

DISCUSSION

Human tissues can tolerate ischemia-reperfusion injury up to a physiological threshold, but damage occurs once this

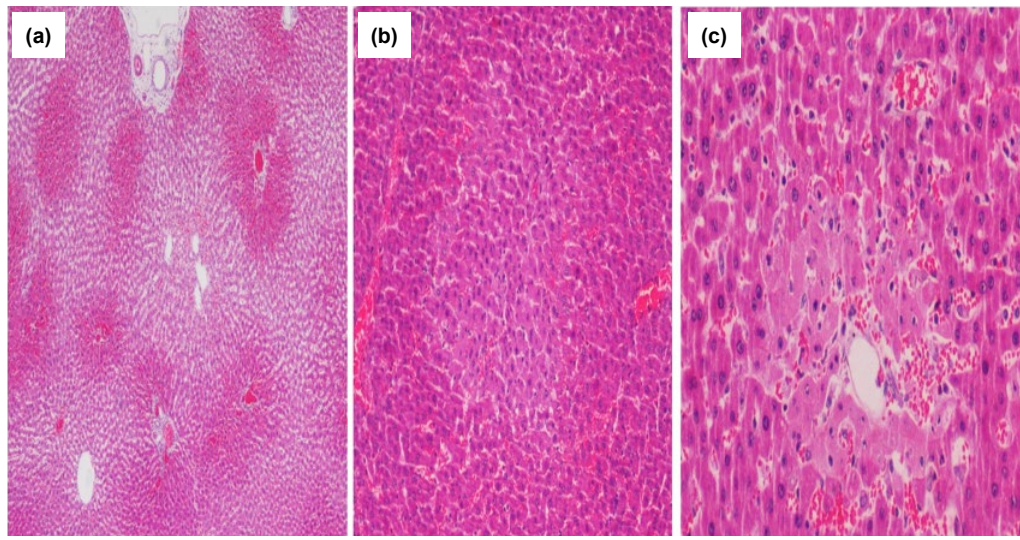

Figure 7. Histopathological Grade 1 liver damage (a: HE x40, b: HE x100, c: HE x200).

threshold is exceeded. A variety of markers and strategies have been developed to detect and prevent ischemia-reperfusion injury. Among these, serine protease inhibitors have been the subject of many studies. Elafin, a serine protease inhibitor, has been observed to undergo changes in tissue and serum levels in various diseases and conditions.

It has been shown that serum elafin levels increase in skin diseases such as systemic sclerosis, pemphigus vulgaris, and psoriasis.[20-23] Wang et al. reported that a decrease in elafin level is effective in predicting the prognosis in acute respiratory distress syndrome (ARDS), a respiratory system disease.[24] Elafin has been shown to have a very important diagnostic and prognostic role in graft-versus-host disease (GVHD), a major complication of allogeneic bone marrow transplantation that affects many systems.[25,26] Elafin has also been identified as a potential molecular biomarker in various malignancies. Its overexpression in certain types of breast, ovarian, and hepatocellular cancer (HCC) has been associated with a worse prognosis.[27-29]

In contrast to these studies, elafin has shown anti-cancer effects in some studies. Caruso et al. demonstrated that elafin expression in breast cancer led to retinoblastoma-dependent growth arrest, which inhibits cell proliferation by inducing apoptosis.[30] Another study revealed that elafin triggered cell death by apoptosis in human melanoma cells, though this effect was not observed in normal melanocyte cells.[31]

Furthermore, elafin has been shown to be effective in acute or chronic diseases affecting the gastrointestinal system. Motta et al. demonstrated that elafin inhibited intestinal inflammation in different mouse models of colitis.[32] As a result of clinical studies conducted following animal experiments, it was determined that elafin expression in the intestinal mucosa and serum elafin levels were decreased in patients with Crohn's disease, ulcerative colitis, and celiac disease.[33-35] To understand this phenomenon, it is important to comprehend the relationship between tissue transglutaminase (TG-2) and elafin, both of which play roles in tissue homeostasis and wound healing. Elafin has been identified as a substrate that plays an important role in the cross-linking activity of TG-2.[14] The association of TG-2 with gluten peptides is an important discovery in the pathogenesis of Celiac disease.[36] Tridegin inhibits plasma factor XIIIa and also has a lower potency in inhibiting transglutaminase in an enzyme concentration-independent manner.[33] It has been shown that elafin interacts with TG-2, and a decrease in elafin levels correlates with mucosal damage.[15]

The effects of elafin have been investigated in many aforementioned fields in the literature. However, there are very few studies on the effect of elafin on ischemia-reperfusion injury. These studies generally investigate the therapeutic, rather than diagnostic, effects of elafin. In one such study, Alam and colleagues investigated whether elafin could prevent myocardial ischemia-reperfusion injury in coronary artery bypass graft surgery.[37] However, they did not find strong evidence that elafin reduces myocardial damage and inflammation.

Numerous studies have investigated the effects of serine protease inhibitors other than elafin on ischemia/reperfusion (I/R) injury in different tissues.

It has been reported that one of these inhibitors, Lex032, may be useful in treating pancreatic I/R tissue injury, as it significantly improves microcirculation in the pancreas during ischemia-reperfusion injury.[38] In another study, it was reported that inhibition of mannan-binding lectin (MBL)-associated serine protease-1 (MASP-1) improved cardiac function and alleviated damage to myocardial tissue (such as infarct size, enzymes, histology, and fibrosis) in myocardial ischemia/reperfusion injury.[39] Neuroserpin, another serine protease inhibitor, has been reported to reduce brain damage by decreasing post-ischemic inflammation in brain tissue.[40]

ONO-5046, a neutrophil elastase inhibitor, was shown to improve lung functions in a study examining its effects on I/R injury in lung tissue.[41] In an animal study, sivelestat, another neutrophil elastase inhibitor, was reported to alleviate the effects of intestinal I/R injury caused by supravisceral aortic clamping and increase survival rates.[42]

Regarding liver I/R injury, studies have shown that sivelestat sodium hydrochloride and L-658,758, both neutrophil elastase inhibitors, reduce I/R-induced liver injury.[43] Sivelestat has been found effective in repairing hepatocellular I/R injury.[44,45] Gabexate mesylate was shown to be effective in preventing I/R injury in the human liver in another study.[46] In a study investigating the effect of nafamostat mesylate, another serine protease inhibitor, on liver I/R injury, it was found effective in preserving sinusoidal microcirculation in liver grafts.[47] A study measuring secretory leukocyte protease inhibitor (SLPI) during liver I/R injury in mice reported that SLPI messenger Ribonucleic Acid (mRNA) expression decreased in ischemia but gradually increased during reperfusion. It was also shown that exogenously administered SLPI protects against hepatic ischemia.[48] In our study, it was determined that elafin, a serine protease inhibitor, increased during ischemia and gradually decreased during reperfusion. This contrasts with secretory leukocyte protease inhibitor (SLPI), another serine protease inhibitor, whose levels have been measured in liver I/R injury in the literature.

A decreased elafin level was negatively correlated with ALT and AST, which are indicators of liver parenchymal damage. Additionally, the decrease in elafin was negatively correlated with TOS, indicating oxidant capacity. This shows that as elafin levels decrease, both the total oxidant capacity of the liver and hepatocyte damage increase.

When evaluating the histopathological features of the liver tissues examined for liver ischemia-reperfusion, it was observed that the damage increased as the reperfusion time increased. Compared with subjects having mild liver damage

(Grade 1) or no damage (Grade 0), those with mild liver damage had lower elafin values. This indicates that the effectiveness of elafin in demonstrating ischemia-reperfusion injury is also supported by histopathological examination results. However, as expected, other markers of liver damage, including ALT, AST, and TOS, were higher in Grade 1 subjects with mild liver injury than those in Grade 0.

The structure of albumin changes when exposed to ischemia, acidosis, and free oxygen radicals, forming what is known as "ischemia-modified albumin" (IMA). IMA may be elevated in conditions such as infections, stroke, liver damage, trauma, and acute coronary syndromes.[49,50] Normally, the IMA/albumin ratio is approximately 1-2%, but this ratio increases to 6-8% in cases of ischemia.[50] In our study, both the IMA value and the IMA/albumin ratio increased as reperfusion time increased, which is consistent with the literature. The IMA/albumin ratio was also found to be high in rats with Grade 1 histopathological liver damage.

No study in the literature was found to have measured elafin levels in the liver at different reperfusion times during constant ischemia. This study is the first to do so. Undoubtedly, the most important result of this study is the decrease in elafin levels in liver ischemia-reperfusion injury. Particularly in this study, where reperfusion time gradually increased from Group 2 to Group 5, elafin levels decreased in parallel with this gradual increase, suggesting that elafin may be effective in determining ischemia-reperfusion duration. With more studies on this subject in the literature, the diagnostic value of serine protease inhibitors in I/R situations will be better understood.

Our study has some limitations. First, as the subjects are female, menstrual cycle changes have the potential to affect wound healing. Second, as our study is an animal experiment, the results may not directly apply to humans.

CONCLUSION

In conclusion, elafin levels decreased as the reperfusion time increased in liver ischemia-reperfusion injury induced in rats in our study. Furthermore, as the reperfusion time increased, both hepatocyte damage and oxidant capacity increased, and these findings showed a negative correlation with elafin levels. Therefore, elafin may play a protective role in hepatic ischemia-reperfusion injury and assist clinicians in assessing liver injury.

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Ethics Committee Approval: This study was approved by the Akdeniz University Hospital Ethics Committee (Date: 18.01.2021, Decision No: 12).

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REFERENCES

1. Lu TF, Yang TH, Zhong CP, Shen C, Lin WW, Gu GX, et al. Dual effect of hepatic macrophages on liver ischemia and reperfusion injury during liver transplantation. Immune Netw 2018;18:e3. [CrossRef]
2. Nastos C, Kalimeris K, Papoutsidakis N, Tasoulis MK, Lykoudis PM, Theodoraki K, et al. Global consequences of liver ischemia/reperfusion injury. Oxid Med Cell Longev 2014;2014:906965. [CrossRef]
3. Aragno M, Cutrin JC, Mastrocola R, Perrelli MG, Restivo F, Poli G, et al. Oxidative stress and kidney dysfunction due to ischemia/reperfusion in rat: attenuation by dehydroepiandrosterone. Kidney Int 2003;64:836–43. [CrossRef]
4. Jaeschke H, Woolbright BL. Current strategies to minimize hepatic ischemia-reperfusion injury by targeting reactive oxygen species. Transplant Rev 2012;26:103–14. [CrossRef]
5. Siriussawakul A, Zaky A, Lang JD. Role of nitric oxide in hepatic ischemia-reperfusion injury. World J Gastroenterol 2010;16:6079–85. [CrossRef]
6. Kumar V, Abbas AK, Aster JC. Robbins basic pathology. Elsevier Health Sciences; 2017. [CrossRef]
7. Hochstrasser K, Albrecht GJ, Schonberger OL, Rasche B, Lempart K. An elastase-specific inhibitor from human bronchial mucus. Isolation and characterization. Biol Chem Hoppe Seyler 1981;362:1369–75. [CrossRef]
8. Schalkwijk J, Chang A, Janssen P, De Jongh GJ, Mier PD. Skin-derived antileucoproteases (SKALPs): characterization of two new elastase inhibitors from psoriatic epidermis. Br J Dermatol 1990;122:631–41. [CrossRef]
9. Wiedow O, Schroder JM, Gregory H, Young JA, Christophers E. Elafin: an elastasespecific inhibitor of human skin. Purification, characterization, and complete amino acid sequence. J Biol Chem 1990;265:14791–5.
10. Shaw L, Wiedow O. Therapeutic potential of human elafin. Biochem Soc Trans 2011;39:1450–4. [CrossRef]
11. Guyot N, Zani ML, Berger P, Dallet-Choisy S, Moreau T. Proteolytic susceptibility of the serine protease inhibitor trappin-2 (pre-elafin): evidence for tryptase-mediated generation of elafin. Biol Chem 2005;386:391–9.
12. Williams SE, Brown TI, Roghanian A, Sallenave JM. SLPI and elafin: one glove, many fingers. Clin Sci (Lond) 2006;110:21–35. [CrossRef]
13. Marischen L, Wesch D, Schroder JM, Wiedow O, Kabelitz D. Human gammadelta T cells produce the protease inhibitor and antimicrobial peptide elafin. Scand J Immunol 2009;70:547–52. [CrossRef]
14. Baranger K, Zani ML, Labas V, Dallet-Choisy S, Moreau T. Secretory leukocyte protease inhibitor (SLPI) is, like its homologue trappin-2 (pre-elafin), a transglutaminase substrate. PLoS One 2011;6:e20976. [CrossRef]
15. Galipeau HJ, Wiepjes M, Motta JP, Schulz JD, Jury J, Natividad JM, et al. Novel role of the serine protease inhibitor elafin in gluten-related disorders. Am J Gastroenterol 2014;109:748–56. [CrossRef]
16. Erel O. A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. Clin Biochem 2004;37:277–85. [CrossRef]
17. Erel O. A new automated colorimetric method for measuring total oxidant status. Clin Biochem 2005;38:1103–11. [CrossRef]
18. Bar-Or D, Lau E, Winkler JV. A novel assay for cobalt-albumin binding and its potential as a marker for myocardial ischemia-a preliminary report. J Emerg Med 2000;19:311–5. [CrossRef]
19. Sözen S, Kisakürek M, Yildiz F, Gönültaş M, Dinçel AS. The effects of glutamine on hepatic ischemia reperfusion injury in rats. Hippokratia

2011;15:161–5.
20. Elgharib I, Khashaba SA, Elsaid HH, Sharaf MM. Serum elafin as a potential inflammatory marker in psoriasis. Int J Dermatol 2019;58:205–09. [CrossRef]
21. Alkemade HA, De Jongh GJ, Arnold WP, van de Kerkhof PC, Schalkwijk J. Levels of skin-derived antileukoproteinase (SKALP)/elafin in serum correlate with disease activity during treatment of severe psoriasis with cyclosporin A. J Invest Dermatol 1995;104:189–93. [CrossRef]
22. Olewicz-Gawlik A, Trzybulska D, Graniczna K, Kuznar-Kaminska B, Katulska K, Batura-Gabryel H, et al. Serum alarm antiproteases in systemic sclerosis patients. Hum Immunol 2017;78:559–64. [CrossRef]
23. Aya BY, Rofaida SR, Alaa AES, Amira KA, Fatma AES, Ahmed GS. Trappin-2/Elafin and Clusterin serum levels in pemphigus vulgaris and correlation with the severity score: a case–control study. Egypt J Dermatol Venerol 2022;42:53. [CrossRef]
24. Wang T, Zhu Z, Liu Z, Yi L, Yang Z, Bian W, et al. Plasma neutrophil elastase and elafin as prognostic biomarker for acute respiratory distress syndrome: a multicenter survival and longitudinal prospective observation study. Shock 2017;48:168–74. [CrossRef]
25. Paczesny S, Braun TM, Levine JE, Hogan J, Crawford J, Coffing B, et al. Elafin is a biomarker of graft-versus-host disease of the skin. Sci Transl Med 2010;2:13ra2. [CrossRef]
26. Solán L, Carbonell D, Muñiz P, Dorado N, Landete E, Chicano-Lavilla M, et al. Elafin as a predictive biomarker of acute skin graft-versus-host disease after haploidentical stem cell transplantation using post-transplant high-dose cyclophosphamide. Front Immunol 2021;12:1. [CrossRef]
27. Bouchard D, Morisset D, Bourbonnais Y, Tremblay GM. Proteins with whey-acidic-protein motifs and cancer. Lancet Oncol 2006;7:167–74.
28. Labidi-Galy SI, Clauss A, Ng V, Duraisamy S, Elias KM, Piao HY, et al. Elafin drives poor outcome in high-grade serous ovarian cancers and basal-like breast tumors. Oncogene 2015;34:373–83. [CrossRef]
29. Clauss A, Ng V, Liu J, Piao H, Russo M, Vena N, et al. Overexpression of elafin in ovarian carcinoma is driven by genomic gains and activation of the nuclear factor κB pathway and is associated with poor overall survival. Neoplasia 2010;12:161-IN15. [CrossRef]
30. Caruso JA, Hunt KK, Keyomarsi K. The neutrophil elastase inhibitor elafin triggers rb-mediated growth arrest and caspase-dependent apoptosis in breast cancer. Cancer Res 2010;70:7125–36. [CrossRef]
31. Yu KS, Lee Y, Kim CM, Park EC, Choi J, Lim DS, et al. The protease inhibitor, elafin, induces p53-dependent apoptosis in human melanoma cells. Int J Cancer 2010;127:1308–20. [CrossRef]
32. Motta JP, Magne L, Descamps D, Rolland C, Squarzoni-Dale C, Rousset P, et al. Modifying the protease, antiprotease pattern by elafin overexpression protects mice from colitis. Gastroenterology 2011;140:1272–82.
33. Finney S, Seale L, Sawyer RT, Wallis RB. Tridegin, a new peptidic inhibitor of factor XIIIa, from the blood-sucking leech Haementeria ghilianii. Biochem J 1997;324:797–805. [CrossRef]
34. Motta JP, Bermúdez-Humarán LG, Deraison C, Martin L, Rolland C, Rousset P, et al. Food-grade bacteria expressing elafin protect against inflammation and restore colon homeostasis. Sci Transl Med 2012;4:158ra144. [CrossRef]
35. Zhang W, Teng G, Wu T, Tian Y, Wang H. Expression and clinical significance of elafin in inflammatory bowel disease. Inflamm Bowel Dis 2017;23:2134–42. [CrossRef]
36. Molberg Ø, Mcadam SN, Körner R, Quarsten H, Kristiansen C, Madsen L, et al. Tissue transglutaminase selectively modifies gliadin peptides that are recognized by gut-derived T cells in celiac disease. Nat Med 1998;4:713–7. [CrossRef]
37. Alam SR, Lewis SC, Zamvar V, Pessotto R, Dweck MR, Krishan A, et al. Perioperative elafin for ischaemia-reperfusion injury during coronary artery bypass graft surgery: a randomised-controlled trial. Heart 2015;101:1–7. [CrossRef]
38. Von Dobschuetz E, Hoffmann T, Messmer K. Inhibition of neutrophil proteinases by recombinant serpin Lex032 reduces capillary no-reflow in ischemia/reperfusion-induced acute pancreatitis. J Pharmacol Exp Ther 1999;290:782–8.
39. La Bonte LR, Pavlov VI, Tan YS, Takahashi K, Takahashi M, Banda NK, et al. Mannose-binding lectin-associated serine protease-1 is a significant contributor to coagulation in a murine model of occlusive thrombosis. J Immunol 2012;188:885–91. [CrossRef]
40. Gelderblom M, Neumann M, Ludewig P, Bernreuther C, Krasemann S, Arunachalam P, et al. Deficiency in serine protease inhibitor neuroserpin exacerbates ischemic brain injury by increased postischemic inflammation. PLoS One 2013;8:e63118. [CrossRef]
41. Ishikawa N, Oda M, Kawaguchi M, Tsunezuka Y, Watanabe G. The effects of a specific neutrophil elastase inhibitor (ONO-5046) in pulmonary ischemia–reperfusion injury. Transpl Int 2003;16:341–6. [CrossRef]
42. Fujimura N, Obara H, Suda K, Takeuchi H, Miyasho T, Kawasako K, et al. Neutrophil elastase inhibitor improves survival rate after ischemia reperfusion injury caused by supravisceral aortic clamping in rats. J Surg Res 2013;180:e31–6. [CrossRef]
43. Okajima K, Harada N, Uchiba M, Mori M. Neutrophil elastase contributes to the development of ischemia-reperfusion-induced liver injury by decreasing endothelial production of prostacyclin in rats. Am J Physiol Gastrointest Liver Physiol 2004;287:G1116–23. [CrossRef]
44. Uchida Y, Freitas MCS, Zhao D, Busuttil RW, Kupiec-Weglinski JW. The protective function of neutrophil elastase inhibitor in liver ischemia and reperfusion injury. Transplantation 2010;89:1050. [CrossRef]
45. Nakano Y, Kondo T, Matsuo R, Murata S, Fukunaga K, Ohkohchi N. Prevention of leukocyte activation by the neutrophil elastase inhibitor, sivelestat, in the hepatic microcirculation after ischemia-reperfusion. J Surg Res 2009;155:311–7. [CrossRef]
46. Kim YI, Hwang YJ, Song KE, Yun YK, Lee JW, Chun BY. Hepatocyte protection by a protease inhibitor against ischemia/reperfusion injury of human liver. J Am Coll Surg 2002;195:41–50. [CrossRef]
47. Miyagi S, Ohkohchi N, Oikawa K, Satoh M, Tsukamoto S, Satomi S. Effects of anti-inflammatory cytokine agent (FR167653) and serine protease inhibitor on warm ischemia-reperfusion injury of the liver graft. Transplantation 2004;77:1487–93. [CrossRef]
48. Lentsch AB, Yoshidome H, Warner RL, Ward PA, Edwards MJ. Secretory leukocyte protease inhibitor in mice regulates local and remote organ inflammatory injury induced by hepatic ischemia/reperfusion. Gastroenterology 1999;117:953–61. [CrossRef]
49. Yin M, Liu X, Chen X, Li C, Qin W, Han H, et al. Ischemia-modified albumin is a predictor of short-term mortality in patients with severe sepsis. J Crit Care 2017;37:7–12. [CrossRef]
50. Gaze DC. Ischemia modified albumin: a novel biomarker for the detection of cardiac ischemia. Drug Metab Pharmacokinet 2009;24:333–41.

ORİJİNAL ÇALIŞMA - *ÖZ*

Sıçan karaciğerinde iskemi reperfüzyon hasarının elafin düzeyine etkisi

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AMAÇ: Bu çalışmanın amacı, bir serin proteaz inhibitörü olan elafinin hepatik iskemi-reperfüzyon hasarında serum düzeylerini ve histopatolojik ve biyokimyasal parametreler üzerindeki etkilerini ölçmektir.

GEREÇ VE YÖNTEM: Kırk adet dişi Wistar albino sıçanı 5 gruba ayrıldı: Grup 1 kontrol grubuydu. Diğer 4 grupta 30 dakika süreyle karaciğer iskemisi oluşturuldu. Grup 3, 4 ve 5'e sırasıyla 1 saatlik, 2 saatlik ve 3 saatlik ek olarak reperfüzyon uygulandı. Deney sonunda biyokimyasal inceleme için intrakardiyak kan örnekleri, histopatolojik inceleme için ise karaciğerden doku örnekleri alındı. Elafin, iskemi modifiye albümin (IMA), toplam antioksidan durumu (TAS) ve toplam oksidan durumu (TOS) düzeyleri de incelendi.

BULGULAR: Serum elafin düzeyi grup 2'den itibaren azalarak en düşük düzeye grup 5'te ulaştı ($p<0.01$). İMA düzeyi kontrol grubunda en düşük, grup 5'te ise en yüksekti ($p<0.01$). TOS, aspartat aminotransferaz (AST) ve alanin aminotransferaz (ALT) düzeyleri kontrol grubunda en düşük, grup 5'te ise en yüksekti ($p<0.01$). Grup 5 en yüksek IMA/albümin oranına sahipti, ancak bu dört grup arasında anlamlı bir fark yoktu. En düşük TAS düzeyi kontrol grubunda bulundu ancak diğer gruplarda stabil ve anlamlı bir artış saptanmadı. Alkalen fosfataz (ALP) ve albümin düzeyleri açısından gruplar arasında anlamlı bir artış saptanmadı. Serum elafin düzeyi ile AST, ALT ve TOS düzeyleri arasında negatif korelasyon mevcuttu ($p<0.01$). Reperfüzyon yapılan gruplarda (grup 3, 4, 5) grade 1 histopatolojik sonuç sayısı daha fazla bulundu. Histopatolojik alt grup analizinde; grade 1 grupta elafin düzeyi daha düşük iken, AST, ALT ve TOS düzeyleri yüksek bulundu ($p<0.01$). Ayrıca grade 1 grupta İMA/Albümin oranının daha yüksek olduğu görüldü ($p=0.02$).

SONUÇ: Hepatik iskemi-reperfüzyon hasarında reperfüzyon süresi arttıkça elafin düzeyinin azaldığı görüldü. Reperfüzyon süresi arttıkça hem hepatosit hasarı hem de oksidan kapasitenin arttığı, bu bulgular ile elafin düzeyi arasında negatif korelasyon olduğu tespit edildi. Dolayısıyla elafinin hepatik iskemi-reperfüzyon hasarında koruyucu bir rolü olabilir ve klinisyenlere karaciğer hasarını göstermede yardımcı olabilir.

Anahtar sözcükler: Elafin; iskemi reperfüzyon; karaciğer; karaciğer hasarı.

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