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Investigation of the Physiological and Histopathological Effects of Omega Acids (3, 6, 9) and Stearic Acid on Rats in Ischemia Reperfusion

Tugba KARAHAN GÜR[1]✉, Okan ARIHAN[2], Gokhan OTO[3], Serkan YILDIRIM[4], Ahmet Ufuk KOMUROGLU[1], Fatih KARAHAN[5]

[1] Vocational School of Health Services, Van Yuzuncu Yil University, Van, Turkey
[2] Department of Physiology, Hacettepe University Faculty of Medicine, Ankara, Turkey
[3] Department of Pharmacology, Faculty of Medicine, Van Yuzuncu Yil University, Van, Turkey
[4] Department of Pathology, Faculty of Veterinary Medicine, Atatürk University, Erzurum, Turkey
[5] Department of Chemistry, Faculty of Science, Van Yuzuncu Yil University, Van, Turkey

✉: tugbakarahan@yyu.edu.tr [1]0000-0001-7220-0210

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ABSTRACT

Ischemia causes reversible or irreversible cell or tissue damage due to insufficient blood flow to the organ or tissue. In this study, our aim is to investigate the protective effect of omega 3, 6 9 and stearic acid application before ischemia reperfusion injury in the leg muscles. For this purpose, 70 female albino rats were divided into 10 groups. The study continued at the same dose for 14 days. In addition, these fatty acids were given to other groups without ischemia-reperfusion. After the application different faty acid, blood biochemical parameters of different fatty acids, oxidative stress parameters and histopathology of tissues (liver, kidney, muscle) were examined in rats. As a result, it was observed that omega 9 fatty acid has better protective properties compared to other omega fatty acids and stearic acid in terms of histopathological properties and oxidative stress index. Additionally, other fatty acids and stearic acid provided some degree of protection against the deleterious effects of ischemia-reperfusion.

Keywords: Ischemia-reperfusion, total antioxidant status, total oxidant status, omega fatty acids, stearic acid, oxidative stress

Omega Asitleri (3, 6, 9) ve Stearik Asidin Sıçanlarda İskemi Reperfüzyon Hasarında Fizyolojik ve Histopatolojik Etkilerinin Araştırılması

ÖZ

İskemi, organ veya dokuya yetersiz kan akışı nedeniyle geri dönüşümlü veya geri dönüşümsüz hücre veya doku hasarına neden olur. Bu çalışmada 10 gruba ayrılan 70 adet dişi Wistar-albino sıçanın bacak kaslarına (kuadriseps) iskemi-reperfüzyon hasarı öncesi uygulanan omega 3, 6, 9 ve stearik asit yağ asitlerinin etkinliği araştırıldı. Çalışma 14 gün boyunca aynı dozda devam etti. Ayrıca bu yağ asitleri iskemi-reperfüzyon yapılmadan diğer gruplara da verildi. Uygulama sonrasında sıçanlarda farklı yağ asitlerinin kan biyokimyasal parametreleri, oksidatif stres parametreleri ve histopatoloji incelendi. Sonuç olarak omega 9 yağ asidinin histopatolojik özellikler ve oksidatif stres indeksi açısından diğer omega yağ asitleri ve stearik asit ile karşılaştırıldığında daha iyi koruyucu özelliklere sahip olduğu gözlendi. Ayrıca diğer yağ asitleri ve stearik asit, iskemi-reperfüzyonun zararlı etkilerine karşı bir dereceye kadar koruma sağlamıştır.

Anahtar Kelimeler: İskemi-reperfüzyon, toplam antioksidan durumu, toplam oksidan durumu, omega yağ asitleri, stearik asit, oksidatif stres.

INTRUDUCTION

Ischemia is defined as the hampered blood flow to an organ due to various reasons. This type of blood flow decrease and ischemia-reperfusion (IR) damage in tissues may occur in clinical conditions such as vascular and transplantation surgery, tourniquet application, tissue transfers and surgery of the amputated limb [1,2]. Depending on the ischemia, the tissue remains in hypoxia, resulting in tissue damage. Ischemia causes a decrease in the energy level in the cell and accumulation of toxic metabolites in the tissue, initiating a series of biochemical reactions that can lead to cell dysfunction and subsequent cell death [3].

Reperfusion is the reviving of tissue blood supply. In the case of blood flow in an ischemic tissue (reperfusion), free oxygen radicals released by the polymorphonuclear leukocytes, which come and settle in the tissue, have more enhancing effects on tissue destruction. The severity of the damage varies depending on the duration of ischemia, temperature of the tissue, and tissue-specific factors [4].

During ischemia, toxic oxygen radicals are produced in ischemic tissue. Free oxygen radicals and superoxide radicals after reperfusion cause endothelial damage and increased vascular permeability. In addition, activated adhesion molecules and cytokines initiate a systemic inflammatory response [5]. In order to prevent potential damages of free oxygen radicals, antioxidant substances counter with many cell protective enzymes and molecules in the body. In a healthy body, cellular antioxidant enzymes, antioxidant substances and free radicals have a balanced relationship between oxidants [6,7].

Free oxygen radicals can disrupt the structural elements of tissue in an organism and cause harmful effects. Oxidative stress is closely related to complications such as myocardial damage, pulmonary edema, kidney and liver failure, and increased mortality [8]. There are many antioxidant defense mechanisms in the cell to remove free radicals. Free radicals are not stationary and they often attenuate their radical feature in a short time period. In addition, many enzymatic and nonenzymatic systems cause inactivation of free radicals. With the catalytic effect of superoxide dismutases (SOD) found in most cells, scavenging of the radicals is significantly accelerated. Enzymes such as glutathione peroxidase are protective against free radicals. Catalase in peroxysomes enzymatically breaks down hydrogen peroxide. In addition, sulfhydryls such as cysteine, glutathione, ceruloplasmin, and vitamins A, C and E are endogenous and exogenous antioxidants that prevent or inactivate free radicals. [9].

Omega fatty acids are important fatty acids that play an important role in human nutrition. Since omega 3 (O3) and omega 6 (O6) fatty acids cannot be produced in the organism, they must be taken with diets. Omega 3 has anti-inflammatory properties and is a fatty acid used to explain why Eskimos are better for cardiovascular health than populations fed other diets [10]. Omega 6 fatty acid metabolites have pro-inflammatory properties [11]. Omega 9 (O9) fatty acid can be produced from these two fatty acids in the human body, and its external intake is especially recommended for cardiovascular and intestinal health [12]. Stearic acid (SA) is a saturated fatty acid found in butter, and saturated fatty acids are associated with cardiovascular health problems, such as atherosclerosis. It has been demonstrated that O3 fatty acids can be incorporated into breast cancer and lung cancer treatment [13,14]. Consuming foods containing O3 fatty acids reduces the risk of colon cancer [15]. In recent studies, O3 fatty acids have been shown to have beneficial effects on cancer, cardiovascular diseases, immune system, cirrhosis and neural system [16-21]. It has also been reported that O3 essential fatty acids have antioxidant properties that reduce oxidative stress and prevent the production of reactive oxygen species [22-26].

Ischemia/reperfusion is a common condition in humans. Oxy, sedative stress, which occurs during the reperfusion, can damage tissues and organs, while at the same time, hadar can occur in organs and tissues that are far from the area where ischemia occurs. With this study, the protective effect of different fatty acids will be determined to determine whether there is distant organ damage caused by ischemia and to prevent possible damage to these organs.

In our study, different fatty acids, namely O3, O6, O9 and SA was tested to compare their effect on rats in blood biochemical and oxidative stress parameters solely or in case of ischemia-reperfusion (IR). At the same time, histopathological examinations were performed on samples taken from certain tissues (quadriceps muscle, liver and kidney) of rats with or without ischemia reperfusion injury and it was aimed to examine and compare protective effects of afformentioned fatty acids.

MATERIAL AND METHODS

Animal and Ethics Statement

In our study, 70 adult, female Wistar-Albino rats were used. Rats obtained from Van Yuzuncu Yil University Experimental Medicine Application and Research Unit weighed approximately 200-250 gr were used. Rats were housed in cages made of polycarbonate material that can be autoclaved at 121 oC. The dimensions of the cage body are 425x266x175 mm, the floor area is 800 cm^2 and 7 animals were kept in each cage. The animals were randomly distributed in cages and standard rat pellet food and water were given ad libitum. Omega acids and SA was purchased from a commercial firm and their purity was chosen as above 95%. During the application period of the experiment, the rats were housed in laboratory conditions with 20 ± 2°C temperature, 50% relative humidity, 12 hours of light/12 hours of dark photo period from 7 am to 7 pm. The test protocol was approved by the Van Yuzuncu Yil University Animal Research Local Ethics Committee and the experimental studies were carried out by adhering to the animal ethics committee directive (Ethics Committee number: 2018/03).

Surgical Procedure

In the study, a total of ten different groups were formed including 7 rats in each group.

1. Control group. No application was made. 2. IR group. 3. O3 group. 300 mg / kg O3 daily. 4. O6 group. 300 mg / kg O6 daily. 5. O9 group. 300 mg / kg O9 daily. 6. SA group. 300 mg / kg SA daily. 7. IR+O3 group. 300 mg / kg O3 daily and ischemia reperfusion at 14^{th} day. 8. IR+O6 group. 300 mg / kg O6 daily and ischemia reperfusion at 14^{th} day. 9. IR+O9 group. 300 mg / kg O9 daily and ischemia reperfusion at 14^{th} day. 10. IR+ SA group. 300 mg / kg SA daily and ischemia reperfusion at 14^{th} day. All fatty acids were given with oral gauge for 14 days and dosage of 300 mg / kg was chosen as the standard comparison dose for all fatty acids [27]. For ischemia reperfusion, 2 hours of

ischemia and 2 hours of reperfusion were performed [28].

Biochemical Analysis

At the end of this process, animals were sacrificed under anesthesia. Blood glucose, LDL, Cholesterol, Triglyceride, ALT and AST measurements were performed as biochemical parameters from blood serum. From the obtained blood samples, Total Antioxidant Status (TAS) (Rel Assay Diagnostics Kit, Catalog No: RL0017) , Total Oxidant Status (TOS) (Rel Assay Diagnostics Kit, Catalog No: RL0024) were measured spectrophotometrically with a commercial kits as the oxidative stress parameters, and the oxidative stress index (OSI) has been determined by using TOS and TAS data. OSI value was calculate according tı the following Formule: OSI (arbitrary unit) = TOS/TAS.

Histopathological Analysis

Tissue samples taken at the end of the experimental procedure were fixed in 10% formalin solution for 48 hours. As a result of routine tissue follow-up procedures, it was embedded in paraffin blocks. 4 mm thick sections were taken from each block. Preparations prepared for histopathological examination were stained with hematoxylin-eosin (HE) and examined by light microscopy (Olympus BX 51, Germany). Sections were evaluated according to histopathological features as none (-), mild (+), moderate (++), severe (+++) and very severe (++++) tissue damage.

Statistical Analysis

SPSS statistical analysis program was used for statistical evaluations (SPSS 20.0 (IBM Corp., NY, USA). As the method of statistics, different tests were applied to different findings. Kruskal-Wallis and post hoc tests were used for non-parametric continuous variables. Histopathological results were evaluated with convenient categorical tests. Statistical significance was set as $p<0.05$.

RESULTS

Serum Biochemical Results

The ALT values of the control group, O3, O6, O9 and SA fatty acid groups are close to each other and do not differ statistically. On the other hand, the highest values were observed in ischemia groups and there is a significant difference between ischemia, IR+O9 and IR+O3 groups with the control group. Applied fatty acids did not reverse ischemia induced ALT increase.
In terms of AST, no significant difference was observed between the control group and the fatty acid groups administered alone. Similar to ALT values, the highest values in AST values were observed in the groups that had ischemia. AST values in all other ischemia groups except O9 ischemia are significantly higher than the control group and O3, O6, O9 and SA groups. Data related with serum biochemical results are presented in table 1.

Table 1. Serum biochemical values of the groups The results are given as mean ±SD.IR= ischemia reperfusion, O3= Omega 3, O6= Omega 6, O9= Omega 9, SA= Stearic acid, GLU= Fasting blood sugar (mg/dL), ALT= (U/L), AST= (U/L), HDL= High Density Lipoprotein (mg/dL), CHOL= Cholesterol (mg/dL), TRIG= Triglycerides, LDL= Low Density Lipoprotein (mg/dL). Differe t letters in the column show statistical significance. P<0.05.

	GLU (mg/dl)	ALT (U/L)	AST (U/L)	CHOL (mg/dl)	TRIG (mg/dl)	LDL (mg/dl)
Control	**159.71**	**34.86^{c}**	88.00^{c}	56.14^{ab}	131.07^{a}	36.50^{a}
O3	177.29	51.29^{abc}	106.00^{c}	43.29^{b}	73.20^{ab}	22.45^{abc}
O6	183.00	39.00^{bc}	115.00^{c}	56.71^{ab}	55.75^{b}	16.48^{bc}
O9	201.00	48.57^{abc}	120.43^{c}	53.86^{ab}	85.05^{ab}	25.29^{abc}
SA	171.86	43.71^{bc}	120.57^{c}	47.43^{ab}	102.60^{ab}	28.76^{ab}
IR	162.86	58.71^{ab}	294.43^{ab}	50.00^{ab}	77.41^{ab}	18.76^{bc}
IR+O3	165.20	70.00^{a}	342.00^{a}	43.60^{b}	80.78^{ab}	25.77^{abc}
IR+O6	185.60	52.00^{abc}	284.20^{ab}	65.40^{a}	44.94^{b}	10.80^{c}
IR+O9	234.50	61.25^{ab}	190.00^{bc}	63.00^{a}	94.77^{ab}	29.50^{ab}
IR+SA	169.83	56.67^{abc}	238.33^{ab}	59.67^{ab}	65.68^{b}	15.35^{bc}

Total Antioxidant Status (TAS), Total Oxidant Status (TOS) And Oxidative Stress İndex (OSI) Results

Among the groups, the lowest TAS value was observed in the IR+O6 group and the highest in the O9 group. IR+O6 TAS level was significantly lower than SA, O9 and IR+O9 groups. TAS level of O9 group was found significantly higher than all other groups. TOS levels in IR+O9 and IR+SA groups were significantly lower than O3, IR+O3 and IR+O6 groups. In terms of OSI values, the highest values were observed in O6 and IR+O6 groups. On the other hand, the lowest values were observed in O9 and IR+O9 groups. OSI values in O9 and IR+O9 groups were significantly lower than the O3 and O6 as well as IR+O3 and IR+O6 groups. The results are given in table 2.

Histopathological Results

Muscle tissue histopathological results

In control, Omega fatty acid groups and SA normal histological structure was observed (Figure 1 A, C, D,

E, F). In IR group, very severe edema and congestion in the interlobuler area, hyaline degeneration and zenker necrosis in the muscle fibers were observed (Figure 1 B). In IR+O3 group, severe edema in the interlobuler area, severe hyaline degeneration and zenker necrosis in the muscle fibers were determined (Figure 1 G).

Table 2. TAS, TOS and OSI values of the gruops

	TAS	TOS	OSI
Control	1.13±0.30 $^{a. b}$	11.78±3.23 $^{a. b}$	10.42 $^{a.b}$
O3	1.34±0.45 $^{a. b}$	19.59±5.43 ^{b}	14.62 ^{b}
O6	1.14±0.28 $^{a. b}$	18.45±10.30 $^{a. b}$	16.33 ^{b}
O9	2.19±0.46 ^{c}	14.11±6.33 $^{a. b}$	6.47 ^{a}
SA	1.50±0.30 ^{b}	15.34±5.68 a. b	10.29 $^{a.b}$
IR	1.24±0.15 $^{a. b}$	14.98±2.84 $^{a. b}$	12.17 $^{a.b}$
IR+O3	1.20±0.49 $^{a. b}$	19.59±6.17 ^{b}	16.32 ^{b}
IR+O6	0.92±0.15 ^{a}	20.14±12.90 ^{b}	21.88 ^{b}
IR+O9	1.45±0.63 ^{b}	9.39±0.37 ^{a}	6.47 ^{a}
IR+SA	1.22±0.16 $^{a. b}$	10.34±5.27 ^{a}	9.40 $^{a.b}$

The results are given as mean ±SD.
IR= Ischemia reperfusion, O3= Omega 3, O6= Omega 6, O9= Omega 9, SA= Stearic acid, TAS= Total Antioxidant Status, TOS= Total Oxidant Status, OSI= Oxidative Stress Index. Note: Different letters in the same column represent statistical significance.

In IR+O6 group, moderate edema, congestion, mild hyaline degeneration and zenker necrosis were observed in the interlobuler area (Figure 1H). In IR+O9 group, mild edema and congestion were observed in the interlobuler area (Figure 1 I). In IR+SA group, severe edema in the interlobuler area, medium intensity hyaline degeneration and zenker necrosis in the muscle fibers were determined (Figure 1 J). Histopatological findings of the quadriceps muscle are summarized in table 3.

Liver tissue histopathological results

Control, omega groups and SA group liver tissue had normal histological appearance (Figure 2 A, C, D, E, F). In IR group, congestion in the veins and sinusoids, dilatation in the sinusoids, and moderate degeneration and necrosis in hepatocytes were detected (Figure 2 B). In IR+O3 group, severe dilatation in sinusoids, congestion in vessels and sinusoids, and moderate degeneration and necrosis in hepatocytes were observed (Figure 2 G). In IR+O6 group, moderate dilatation in sinusoids, congestion in vessels and sinusoids, and mild degeneration in hepatocytes were detected (Figure 2 H). In IR+O9 group, mild dilatation and congestion were determined in sinusoids (Figure 2 I). In IR+SA group, severe dilatation and congestion in sinusoids, congestion in the vessels, and moderate degeneration and necrosis in hepatocytes were observed (Figure 2 J). Liver histopathological findings are summarized in table 4.

Table 3. Scoring of histopathological findings observed in quadriceps muscle tissues

	Edema at interlobuler parts	Congestion in the veins	Hyaline degeneration	Zenker necrosis
Control	-	-	-	-
O3	-	-	-	-
O6	-	-	-	-
O9	-	-	-	-
SA	-	-	-	-
IR	++++	++++	++++	++++
IR+O3	+++	+++	++++	+++
IR+O6	++	++	++	++
IR+O9	+	+	+	-
IR+SA	+++	+++	+++	++

Table 4. Scoring of histopathological findings in liver tissues

	Edema at interlobuler area	Hyperemia in the veins	Hyaline degeneration	Zenker necrosis
Control	-	-	-	-
O3	-	-	-	-
O6	-	-	-	-
O9	-	-	-	-
SA	-	-	-	-
IR	+++	+++	++	++
IR+O3	++	+++	+	-
IR+O6	+	++	-	-
IR+O9	++	++	+	-
IR+SA	++	+++	+	-

Kidney tissue histopathological results

Control group, omega groups and SA group kidney tissue was observed in normal histological appearance (Figure 3A, C, D, E, F). In IR group, moderate congestion was observed in the interstitial and

glomerular vessels (Figure 3B). In IR+O3 group, moderate congestion was determined in the interstitial and glomerular vessels (Figure 3G). In IR+O6 group, mild congestion was observed in the interstitial and

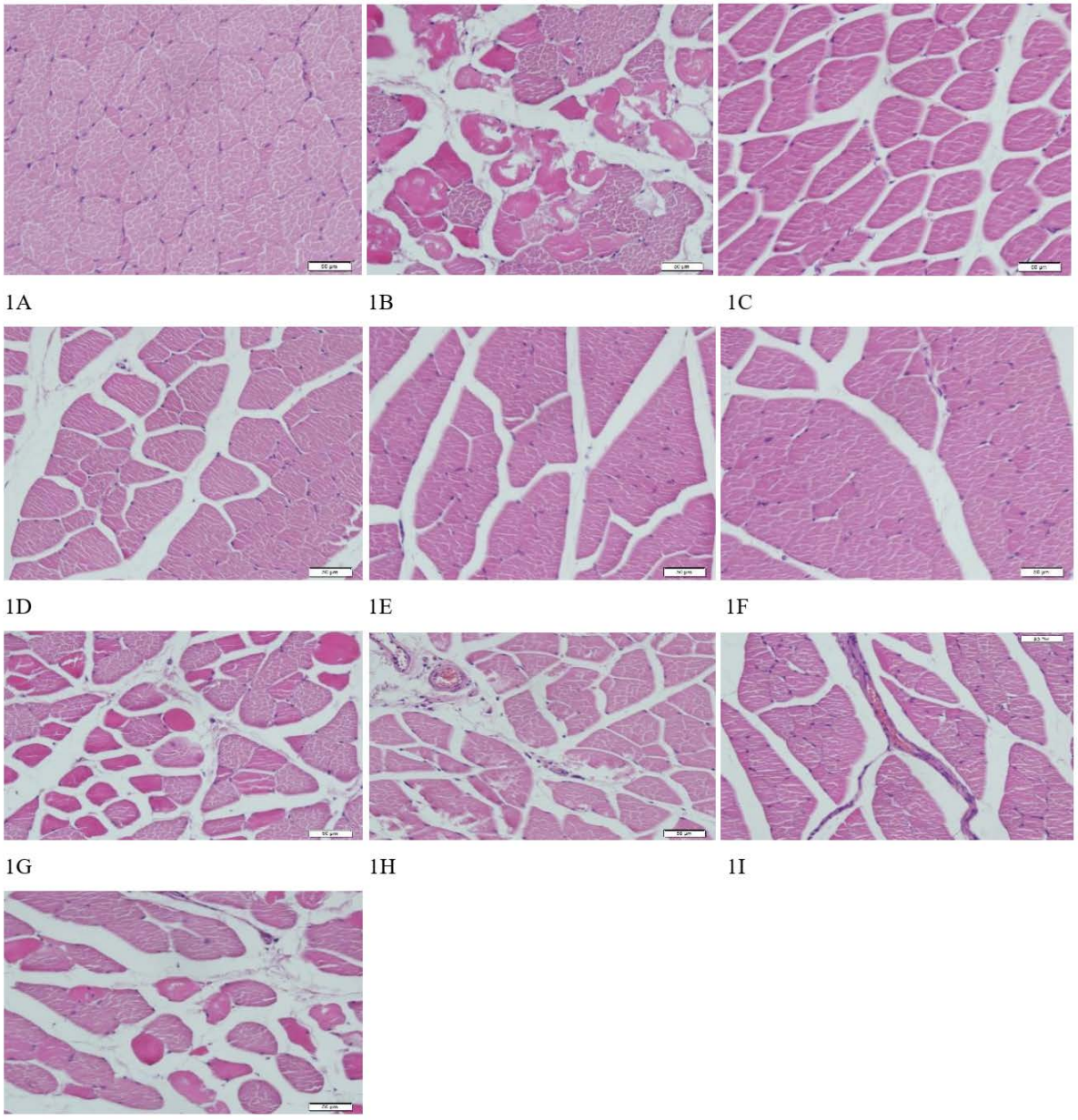

Figure 1A: Control group, quadriceps muscle tissues, normal histological appereance, H&E, Bar: 50 µm.
Figure 1B: Ischemia-reperfusion group, Very severe edema in interlobuler area, hyaline degeneration and Zenker necrosis in the muscle fibers H&E, Bar: 50 µm.
Figure 1C: Omega 3 group, quadriceps muscle tissue, normal histological apperance, H&E, Bar: 50 µm.
Figure 1D: Omega 6 group, quadriceps muscle tissue, normal histological apperance, H&E, Bar: 50 µm.
Figure 1E: Omega 9 group, quadriceps muscle tissue, normal histological apperance, H&E, Bar: 50 µm.
Figure 1F: Stearic acid group, quadriceps muscle tissue, normal histological apperance, H&E, Bar: 50 µm.
Figure 1G: Ischemia-reperfusion + omega 3 group, quadriceps muscle tissue, severe histological appearance, severe edema in the interlobuler area, severe hyaline degeneration and zenker necrosis in the muscle fibers, H&E, Bar: 50 µm.
Figure 1H: Ischemia-reperfusion + omega 6 group, quadriceps muscle tissue, normal histological appearance, moderate edema in interlobuler area, mild level hyaline degeneration in muscle fibers, H&E, Bar: 50 µm.
Figure 1I: Ischemia-reperfusion + omega 9 group, quadriceps muscle tissue, normal histological appearance, mild edema and hyperemia in interlobuler area, H&E, Bar: 50 µm.
Figure 1J: Ischemia-reperfusion + stearic acid group, quadriceps muscle tissue, normal histological appearance, severe edema in the interlobuler area, medium intensity hyaline degeneration and Zenker necrosis in the muscle fibers, H&E, Bar: 50 µm.

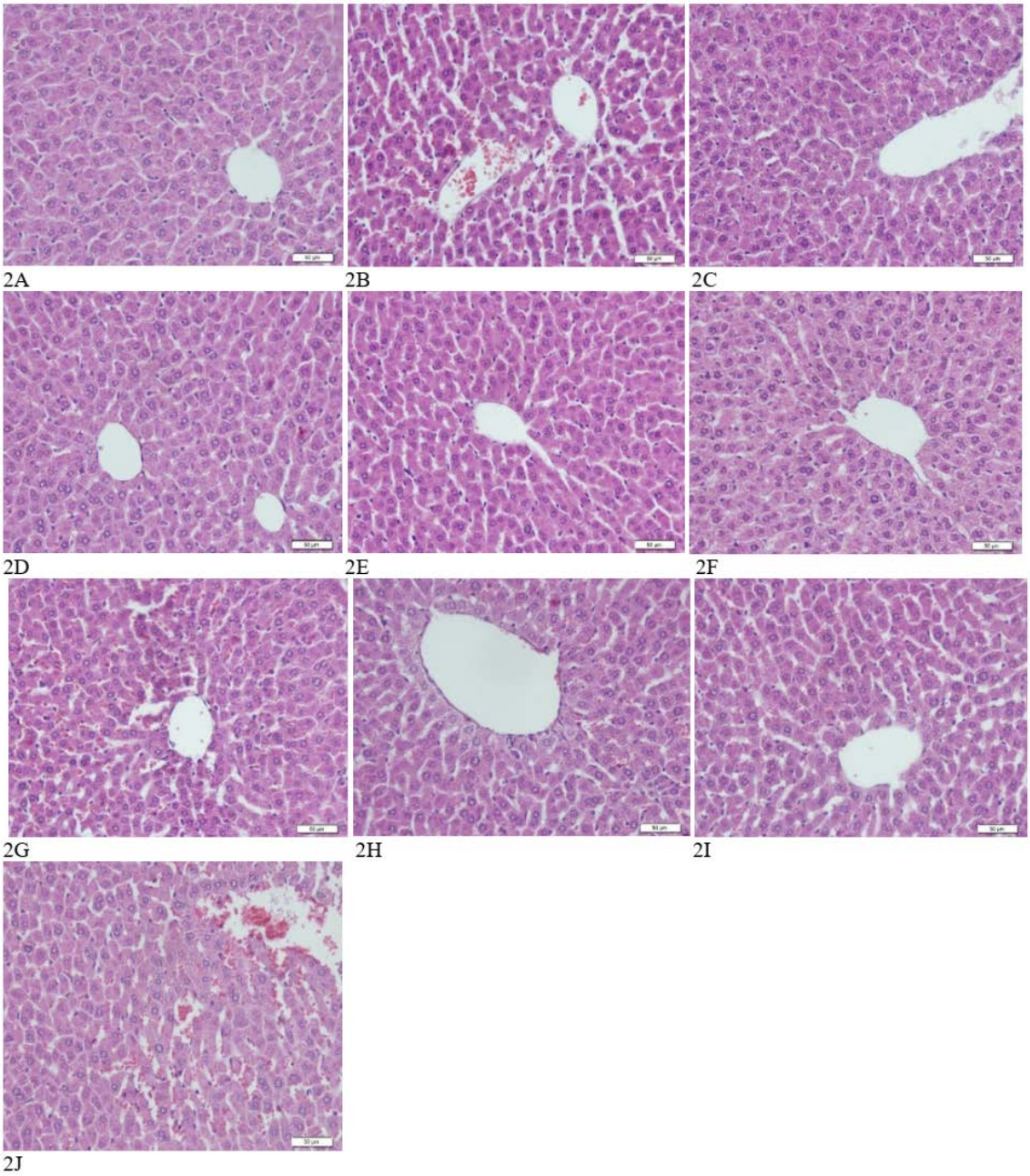

Figure 2A: Control group, liver tissue in normal histological apperance, H&E, Bar: 50 µm.
Figure 2B: Ischemia-reperfusion group, liver tissue, Very severe edema in interlobuler area, hyaline degeneration and zenker necrosis H&E, Bar: 50 µm.
Figure 2C: Omega 3 group, liver tissue, normal histological apperance, H&E, Bar: 50 µm
Figure 2D: Omega 6 group, liver tissue, normal histological apperance, H&E, Bar: 50 µm.
Figure 2E: Omega 9 group, liver tissue, normal histological apperance, H&E, Bar: 50 µm.
Figure 2F: Stearic acid group, liver tissue, normal histological apperance, H&E, Bar: 50 µm.
Figure 2G: Ischemia-reperfusion + omega 3 group, liver tissue, severe dilatation in sinusoids, congestion in vessels and sinusoids, moderate degeneration and necrosis in hepatocytes, H&E, Bar: 50 µm.
Figure 2H: Ischemia-reperfusion + omega 6 group, liver tissue, normal histological appearance, moderate dilatation in sinusoid, congestion, mild degeneration in hepatocytes, H&E, Bar: 50 µm.
Figure 2I: Ischemia-reperfusion + omega 9 group, liver tissue, normal histological appearance, mild dilatation and congestion in sinusoids, H&E, Bar: 50 µm.
Figure 2J: Ischemia-reperfusion + stearic acid group, liver tissue, severe dilatation and congestion in sinusoid, congestion in vessels, moderate degeneration and necrosis in hepatocytes, H&E, Bar: 50 µm.

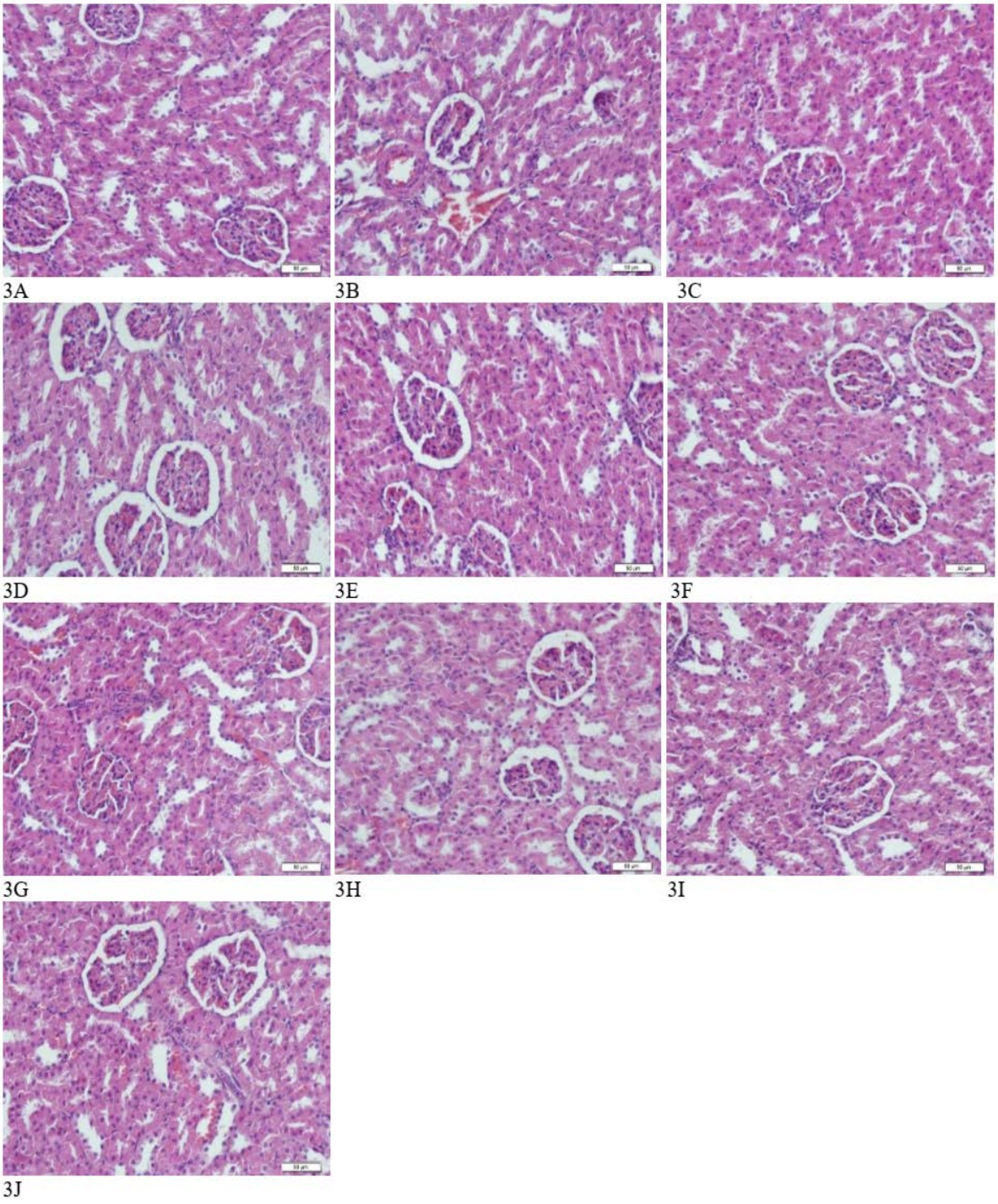

Figure 3A: Control group, kidney tissue, normal histological apperance, H&E, Bar: 50 µm.
Figure 3B: Ischemia-reperfusion group, kidney tissue, moderate congestion in vessels, H&E, Bar: 50 µm.
Figure 3C: Omega 3 group, kidney tissue, normal histological apperance, H&E, Bar: 50 µm.
Figure 3D: Omega 6 group, kidney tissue, normal histological apperance, H&E, Bar: 50 µm.
Figure 3E: Omega 9 group, kidney tissue, normal histological apperance, H&E, Bar: 50 µm.
Figure 3F: Stearic acid group, kidney tissue, normal histological apperance, H&E, Bar: 50 µm.

Figure 3G: Ischemia-reperfusion + omega 3 group, kidney tissue, moderate congestion in interstitial vessels, H&E, Bar: 50 µm.
Figure 3H: Ischemia-reperfusion + omega 6 group, kidney tissue, mild congestion in interstitial vessels, H&E, Bar: 50 µm.
Figure 3I: Ischemia-reperfusion + omega 9 group, kidney tissue, normal histological apperance, H&E, Bar: 50 µm.Figure 3J: Ischemia-reperfusion + stearic acid group, kidney tissue, moderate congestion in interstitial and glomerular veins, H&E, Bar: 50 µm.

glomerular vessels (Figure 3H). In IR+O9 group, normal histological apperance was observed (Figure 3I). In IR+SA group, moderate congestion was detected in the glomerular and interstitial vessels (Figure 3J). Histopathological findings are summarized in Table 5.

Table 5. Scoring of histopathological findings in kidney tissues

	Congestion in glomerular vessels	Congestion in interstitial vessels
Control	-	-
O3	-	-
O6	-	-
O9	-	-
SA	-	-
IR	++	++
IR+O3	++	++
IR+O6	+	+
IR+O9	-	-
IR+SA	++	++

DISCUSSION

The current study results show that the ischemia/reperfusion model we had applied causes significant physiological and histopathological changes in rats and the findings reveal that the desired ischemia/reperfusion model can be achieved. The main purpose of the study is to test and compare protective effects of different fatty acids applied during the 14 days before ischemia/reperfusion injury in investigated histological, physiological and biochemical parameters.

Histopathological results of the study the ischemia reperfusion procedure caused marked damage to the muscle, liver and kidney tissues. In muscle and liver tissue such fatty acids show an attenuation of damage caused by IR which is visible when histopathological data is compared with IR. Excessive saturated fatty acids and also excessive unsaturated fatty acids are mentioned for their adverse effects on cultured hepatocytes [29]. In addition high fat content in the diet may result with a deposition in the muscle cells and causing malfunctions due to overabundance of this fat may overwhelm the oxidizing capacity of myocytes [30]. Our results showed no damage occurred to liver tissue due to our lone Omega acids and SA administration. On the contrary, our administered fatty acids exerted protection in the liver and muscle tissues. Although fatty acids are blamed for cardiovascular harmful effects for a long time stearic acid (0,02 mg/kg) was found to improve functional outcomes after cardiac arrest in rats [31]. Stearic acid is a saturated fatty acid however, it is protective against inflammation [32]. These properties are unlike the other fatty acids such as palmitic and myristic acids [33]. In our study SA and all other fatty acids protected liver tissues against Zenker necrosis. In a study by Skrzep-Poloczek et al. [34] they administered 8 weeks of high fat diet to rats and then animals were underwent for different gastrointestinal surgery methods. They found that a shift in diet regardless of its content causes soleus muscle oxidative stress in this surgery procedure. However our administered faty acids for 14 days did not cause an additional stress to tissue. Protective properties in O9 fatty acid (especially in muscle and kidney tissue) were prominent. In muscle, liver and kidney tissue, O6 also showed protective properties. SA showed no better protective property compared to other fatty acids. In further studies, dose-dependent studies on O9 fatty acid have the potential to give more detailed results. 12 weeks of supplementation of elderly people with a combination including O-3 caused a better protection against sarcopenia compared with other administrations avoiding O-3. This states a positive role for protection of muscle cells for O-3 [35]. In a study by Ali, Rifaai, [36], 400 mg/kg fish oil (not pure O-3 but used as a source of O-3) for 4 weeks (p.o.) improved liver damage caused by acute cold restrain stress which is administered at the end of 4 week oil administration. In a study the researchers showed that 1 mL per day (containing ~25% O-3 fatty acids with a mix of EPA, DHA and octadecatetraenoic acids) O-3 supplementation to male wistar rats caused a beneficial effect against heart ischemia/reperfusion injury [37]. Our data reveals that O3 provides an attenuation of histopathological parameters in muscle such as edema in interlobular parts, congestion in the veins and Zenker necrosis compared to lone IR group. Although this protection is not as prominent as the one in O9, still this finding is in parallel with the literature records. In addition O3 also shows a protective activity in the liver however it was not as much as the one exerted by O6 administration.

In this study we have chosen ALT and AST values to compare liver function in this experimental protocol. These two important parameters showing the functions and health of the liver are important parameters that increase in conditions such as cirrhosis, acute liver injury, necrosis and side effects of drugs such as acetaminophen (paracetamol). ALT and AST values were close to the control group of all applied fatty acids and did not differ statistically. This shows us that the 14 days of administration of fatty acids does not have a negative effect on these two parameters of the liver. On the other hand, the highest values were observed in ischemia groups and there is a significant difference between ischemia, IR+O9 and IR+O3 groups and the control group. Applied fatty acids were unable to alleviate the increase of ALT and AST caused by ischemia. A decrease in ALT and AST values compared to control was given in literature [38]. However their administration period is higher (6

weeks) and dosage was higher (400 mg/kg) compared to our experimental protocol. Stearic acid is known for its anti-inflammatory property. 10 days of administration of wistar rats with pellets containing 180g/kg stearic acid food pellet causes a significant reduction in ALT and AST in 3rd day compared to therapy model without stearic acid [39]. In our experiments no such decrease was observed in ALT and AST due to SA administration but this difference may be related with administration schemes. We had administered oral gauge in mg/kg doses whereas they administered via pellet containing SA. In a study by Majeed, Al-Shawi [40], 14 days of oral O-3 administration to rats caused an insignificant increase in ALT and AST values which is in parallel with our findings. Another study which found similar results with ours is a study by [36] 400 mg/kg fish oil (not pure O-3 but used as a source of O-3) for 4 weeks (p.o.) caused an insignificant increase in ALT and AST values compared to control [36]. In a study by Li et al. [41], pellets containing SA and OA were prepared separately and given to mice for 21 days and showed no significant change for body weight, lean mass and fat mass however SA was found to cause increased food intake without altering body weight significantly compared to OA. Serum fatty acid concentration was also not changed significantly between those two groups. O-9 is a monounsaturated fatty acid and shown to exert protective activity against insulin resistance and causing anti-inflammatory activity. O-9 can bind to peroxisome proliferator activated receptor which acts as a sensor for lipids [42].

Another biochemical parameter evaluated was glucose. Glucose was observed at high values as rats were not fasted during the time blood was drawn. O6 and O6 ischemia groups, SA and SA ischemia groups, and O9 and O9 ischemia groups showed close values to each other. This shows that the fatty acids applied do not show any significant difference when compared to ischemia condition. O-3 PUFAs are shown to contribute brain glucose regulation [43]. El-Fayoumi et al. [44] administered 500mg/kg of eicosapentaenoic acid i.p. for 16 weeks to mice. Such administration increased fasting blood glucose, serum insulin level significantly compared to control but caused no significant alteration in triglyceride level. Variations in the result of different studies concerning O3 fish oil may rise from the composition of fish oil in EPA and DHA content in addition their higher dosage and longer administration period may be the result of this significant glucose increase. In a study by Sahadewa et al. [45], 300 mg/kg of daily oral O-3 administration for 28 days caused a decrease in fasting blood glucose decreased in O-3 group.

One of the important component of the study was the evaluation of how the applied fatty acids, ischemia/reperfusion and the combination of both will affect the oxidative stress parameters. It is observed that ischemia increases oxidative stress (based on OSI value). It has been determined that O9 fatty acid prevents oxidative stress in case of ischemia. In this sense, it is possible to suggest that O9 fatty acid has a protective potential agains an ischemic damage. This situation can be evaluated in studies including dose – response comparisons. Although O3 fatty acids are known to have anti-inflammatory and O6 acids have proinflammatory properties Simopoulos, [46], they may have shown such an effect at these doses, although they were initially expected to respond differently in terms of oxidative stress. It has been known for a long time that people with a marine based diet face less cardiovascular events compared to diets filled with saturated animal fats [10]. There are increasing studies concerning protective activity of O3 in experimental ischaemic conditions in rats [47]. In a study, rats were given two different doses of O3 (100 and 300 mg/kg orally). Their O3 capsules contain an EPA/DHA ratio of3/2. They found that O-3 posttreatment reversed diclofenac induced total antioxidant capacity decrease [48]. In a study showed that 400 mg/kg fish oil (not pure O3 but used as a source of O3) for 4 weeks (p.o.) caused an insignificant increase in total antioxidant capacity compared to control [36]. In a study 300 mg/kg of daily oral O3 administration for 28 days did not cause a significant change in MDA levels compared to control [45].

Another possible reason for our results is the mode of administration of fatty acids to rats. In some of the studies fatty acids are given in a mixture of those acids [49]. Therefore in such studies there is no single fatty acid administration was made which causes and O3, O6, O9 rich fatty acid mixture administration. However in our study we have administered single fatty acid in every other group.

Furhter possible explanation for our observed findings is the gender of animals. In a study by Wendy et al. [50], in Langendorff perfusion experiments dietary O6 fatty acid replacement was found to impair cardiac functional recovery after ischemia in female rats. However this situation was not observed in male rats. They have concluded that O6 dietary lipid intake in females may be an adverse condition for cardioprotection. Since most of the studies concerning protective activity of Omega acids against certain experimental conditions are performed on male animals there is still a need to clarify such actitivies also on female animals.

As with experimental septic shock models, the protective effects of antioxidant and anti-inflammatory molecules have been shown in many experimental models. Fish consumption is recommended for the purpose of a protective effect against heart diseases in adults. It has been demonstrated that consumption of O3 fatty acids in pill form alone is not as effective as consumption as fish. It has been shown with a comprehensive meta-analysis that consuming more fish reduces the risk of dementia, but fish oil consumption does not decrease it [51]. The proposed mechanisms to make fish oil intake more effective through fish consumption are not fully clear. However, due to the

synergistic effects of other compounds in fish meat, absorption in the gut and its interaction with the gut microbiota, it has been shown to be effective in its doses. These situations reveal that more research is needed on synergistic effect and dose-response issues. Co-supplementation of O-3 acids and iron may cause more damage to type 2 diabetes mellitus patients therefore protective effect of O-3 acids may change according to experimental conditions [52].

The mentioned "O-3, 6, 9 and stearic fatty acids" are the types of fatty acids consumed in our diet. However, there are different opinions about how much of these fatty acids should be consumed in our diet. High-dose uses as well as low doses are recommended according to results of different studies. Ratio of O-6 to O-3 in the diet of prehistoric populations was estimated almost equal whereas, modern populations have much higher O-6: O-3 ratio varying between 10:1 to 30:1 which arises some questions about their impact on health [53]. Our work has limitations. It is a single dose of the fatty acids used and investigating their effect in only two tissues. Considering that ischemia reperfusion damage is common in human, the protective effect of fatty acid should be determined by investigating the effects of ischemia/reperfusion damage in other tissues as well. The fatty acids that we used in this study also evolved into various lipid mediators in the body. In other studies, the effects of lipid mediators (such as resolvins, lipoxins and meracins) that these fatty acids have formed can be investigated.

CONCLUSION

The findings of the current study reveal that daily consumption of 300 mg / kg of O9 oil can be protective against oxidative stress and tissue damage in ischemia reperfusion injury in rats. In the light of these findings, new researches may be suggested in the IR model considering the dose-response curve of O9 fatty acid. Thus, IR injuries, which are a life-threatening health problem, research on the possibility of using O9 fatty acids for the purpose of preventing and treating damage may yield valuable results.

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