Kafkas Univ Vet Fak Derg
17 (2): 183-190, 2011

RESEARCH ARTICLE

Effects of Gemfibrozil and L-carnitine on PON1 Activity, Oxidative/Anti-Oxidative Parameters and Hepatosteatosis in Rabbits Feed With Fat-Rich Diet [1]

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[1] *This investigation was supported by a grant from TUBITAK (Research Project No: TOVAG 106 O 160)*
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Makale Kodu (Article Code): KVFD-2010-2636

Summary

The scope of this study was to determine the effect of gemfibrozil, l-carnitine and their combination on paraoxonase/arylesterase activity, oxidative/anti-oxidative parameters and hepatosteatosis in rabbits on fat-rich diet. The study involved 35 New Zealand albino white rabbits. Animals were equally divided into 5 groups (n=7); group I: control (standard diet), group II: rabbits received only fat-rich diet, group III: rabbits received fat-rich diet plus gemfibrozil (100 mg/kg/day, oral), group IV: rabbits received fat-rich diet plus L-carnitine groups (200 mg/kg/day, oral), and group V: rabbits received fat-rich diet plus the combination of gemfibrozil (100 mg/kg/day, oral) and L-carnitine (200 mg/kg/day, oral) for 9 weeks. The results revealed marked increases in ALT and AST activities in group III ($P<0.05$) throughout the experiment and a gradual increase in activities of paraoxonase/arylesterase in group III, IV and V when compared to group I and II ($P<0.05$) and a decrease in OSI and TOS levels in group IV and V when compared to group I and II ($P<0.05$) over the experimental period. A significant negative correlation between serum OSI and paraoxonase and arylesterase activities in group IV ($r= -0.36$, $P<0.012$ and $r= -0.41$, $P<0.009$, respectively) and V ($r= -0.31$, $P<0.022$ and $r= -0.37$, $P<0.014$, respectively). A similar correlation was determined for liver samples. Changes in paraoxonase and arylesterase activity at 0, 3, 6 and 9 weeks were positivelly correlated with the changes in high density lipoprotein-cholesterol (HDL-C) in group III ($r=0.63$, $P<0.001$ and $r=0.57$, $P<0.003$; respectively). The present study disclosed hepatosteatosis and hyperlipidemi due to fat-rich diet in rabbits, and treatment of gemfibrozil is required in order to increase PON1 activity.

Keywords: *Paraoxonase, Arylesterase, TAS, TOS, OSI, Hepatosteatosis, Gemfibrozil, L-carnitine*

Yüksek Yağlı Diyetle Beslenen Tavşanlarda Gemfibrozil ve L-carnitine Uygulamalarının PON1 Aktivitesi, Oksidatif/Anti-Oksidatif Parametreleri ve Hepatosteatozis Üzerine Etkileri

Özet

Bu çalışmanın amacı; yüksek yağlı diyetle beslenen tavşanlarda gemfibrozil ve L-carnitine ile her iki ilacın kombinasyonlarının paraoksonaz/arilesteraz aktivitesi, oksidatif/antioksidatif parametreleri ve hepatosteatozis üzerine etkilerinin araştırılmasıdır. Çalışmada 35 adet Yeni Zelanda tavşanı kullanıldı. Tavşanlar 5 eşit gruba (n=7) ayrıldı. Grup I: standart diyet verilen kontrol grup, grup II: yalnızca yağlı diyet verilen grup, grup III: yağlı diyet +gemfibrozil (100 mg/kg/day) verilen grup, IV: yağlı diyet + L-carnitine (200 mg/kg/day) verilen grup ve grup V: yağlı diyet ile gemfibrozil + L-carnitine kombinasyonun verildiği grup olarak dizayn edildi. Tavşanlara bütün uygulamalar oral yolla günlük olarak 9 hafta süresince yapıldı. Elde edilen bulgulara göre III. grupta ALT ve AST enzim aktivitelerinde deneme boyunca önemli düzeyde ($P<0.05$) artışların olduğu belirlenirken, paraoxonase/arylesterase aktivitesinin ise grup III, IV ve V'te kademeli olarak artış gösterdiği ve grup I ve II'ye göre $P<0.05$ düzeyinde istatistiksel olarak yüksek olduğunu belirlendi. Grup IV ve V'te Oksidatif Stres İndeks (OSI) ve Total Antioksidan Durum (TOS) düzeylerinin bütün çalışma boyunca grup I ve II'ye göre istatistiksel olarak anlamlı derecede ($P<0.05$) düşük olduğu ortaya konuldu. İlaçların kombine uygulandığı grup V'te OSI düzeyleri ile paraoksonaz/arilesteraz aktivitesi arasında sırasıyla $r=-0.36$, $P<0.012$, $r=-0.41$, $P<0.009$; ve grup IV'te sırasıyla $r= -0.31$, $P<0.022$ and $r= -0.37$, $P<0.014$ düzeylerinde negative korelasyonlar tespit edildi. Karaciğer örneklerinden de benzer korelasyonların olduğu belirlendi. Bu sonuçlara ilave olarak grup 3'teki tavşanlarda 0, 3, 6 ve 9 haftalarda yüksek dansiteli lipoprotein-kolesterol (HDL-C) düzeylerinin paraksonase ve arylesterase aktivitesi arasında sırasıyla $r=0.63$, $P<0.001$ ve $r=0.57$, $P<0.003$ düzeylerinde pozitif korelasyonlara sahip olduğu belirlendi. Yüksek yağlı diyetle besleme ile tavşanlarda hepatosteatosis ve hyperlipidemi için model oluşturulduğu bu çalışmada gemfibrozil uygulamalarının PON1 aktivitesinde artışlara yol açtığı ortaya konuldu.

Anahtar sözcükler: *Paraoksonaz, Arilesteraz, TAS, TOS, OSİ, Hepatosteatozis, Gemfibrozil, L-carnitine*

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INTRODUCTION

Increased lipid intake generally results in significant increases in lipid oxidation and deposition in animals. Many researchs have suggested that hyperlipidemi, oxidative stress and its consequent oxidation of low density lipid (LDL) has a key role in the development and progression of the atherosclerotic lesions [1,2]. Oxidized low density lipids (Ox-LDL) are taken up by macrophages in the arterial wall via scavenger receptors, leading to foam cell formation and atherosclerotic plaque [3,4]. However, available surrounding antioxidants (enzymes or compounds), such as PON1 or vitamin E have been shown to prevent oxidative stress to protect LDL from oxidation and to reduce the development of atherosclerotic lesions [5].

Studies have shown that serum paraoxonase of rabbit is similar to high activity human alloenzyme and has a positively charged amino acid in position 192, lysine. Acid-equence of highly purified human and rabbit serum paraoxonase have been found identical at the proportion 85% [6]. PON1 activity is a calcium-dependent esterase that hydrolyzes a broad spectrum of substrates including organophosphates, aryl esters, and lactones [5]. PON1 a high density lipid (HDL) bound ester hydrolase has been demonstrated to have a key role in the protection against oxidative stress, foam cell formation by macrophage, and development of atherosclerosis [7]. Studies have also shown that anti-hyperlipidemic therapy may play an important role in reducing the risk of atherosclerosis and associated coronary heart disease (CHD) [8]. The Helsinki Heart Study has demonstrated that reducing total and LDL cholesterol is effective in the primary prevention of CHD [9]. Gemfibrozil is a fibric acid derivative that is widely used for anti-hyperlipidemic treatment. The clinical benefit of gemfibrozil has been attributed to its ability to reduce plasma total cholesterol (TC), LDL-cholesterol (LDL-C) and trigliseride (TG) and to increase high density lipoprotein-cholesterol (HDL-C) concentrations [9,10].

L-carnitine is a vital compound routinely used for the management of obesity and hyperlipidemi [11]. L carnitine is naturally synthesized from lysine and methionine amino-acids in tissues and also externally taken from diet [12]. The primary function of L-carnitine is to transport long-chain fatty acids across iner mitochondrial membran into the mitochondria for β-oxidation [13] and secondary functions include; i) buffering and removing potentially toxic acyl groups from cells, ii) equilibrating ratios of free CoA and acetyl-CoA between mitochondria and cytoplasm and iii) participating in biological processes such as gluconeogenesis, stimulation of fatty acid synthesis and ketone bodies, branched-chain amino acid, triglyceride and cholesterol metabolism [14]. Over the last years, L-carnitine has been shown to posses an antioxidant promoting action and to protect cells from reactive oxygen species (ROS). Thus, L-carnitine acts as a free radical scavenger and protects cells from reactive oxygen species [15-18].

No studies, to date, exist regarding to the effect of gemfibrozil, L-carnitine and their combination on PON1 activity, oxidative/antioxidative parameters and hepatosteatosis in rabbits fed with fat-rich diet. This study therefore evaluated the efficacy of gemfibrozil, L-carnitine and their combination in reducing the development of atherosclerosis in hyperlipidemic subjects.

MATERIAL and METHODS

Animals, Diet and Experimental Protocol

Gemfibrozil and L-carnitine were purchased from sigma chemical Co. (St. Louis, MO, USA). This study involved 35 healthy New Zealand albinos white rabbits, aged 6-9 months, weighing around 2-2.5 kg and were obtained from the Experimental Animal Care Center, Faculty of Veterinary Medicine, Kafkas University (Kars, Turkey).

Rabbits were kept under standard conditions (20±1°C, 12-h light/12-h dark cycles) and were randomly divided into 5 equal groups. Animals in group I, II, III, IV and V received normal diet (standart pellet diet, 4.5% fat), fat-rich diet (15% fat), fat-rich diet (15% fat) plus 100 mg/kg/day gemfibrozil, fat-rich diet (15% fat) plus 200 mg/kg/day L-carnitine and fat-rich diet (15% fat) plus combination of 100 mg/kg/day gemfibrozil and 200 mg/kg/day, respectively.

All rabbits were fed commercical rabbit feed (Bayramoglu Yem Inc., Erzurum Turkey) and water *ad libitum*. The experiment comprised a period of 9 weeks. At the beginning and every three weeks (0, 3, 6 and 9) after a feed deprivation of 10 h with free access to water, the rabbits were weighed and blood was taken from auricular vein into 5 ml plain tubes and stored at 4°C. Serum was harvested by centrifugation at 3.500 rpm for 10 min, and stored at -80°C until analysis for analysis of paraoxonase, arylesterase, total antioxidant status (TAS), total oxidant status (TOS), and oxidative stress index (OSI).

At the end of the experiment, rabbits were deprived of food and sacrificed by decapitation under anestheise with pentobarbital (60 mg/kg, IP). Livers were dissected out for histological examination (fixing 10% formol solution) and biochemical analysis (homogenasing in 0.9% NaCl). Then 10% of homogenate in 0.9% NaCl was centrifuged at 3500 rpm for 10 min and supernatant was harvested and stored at -80°C until analysis of TAS, TOS and OSI.

Measurement of the Total Antioxidant Status

Serum TAS level was determined spectrofotometrically on an autoanalyzer (Aeroset®, Abbott®, Illinois, USA) using commercial kits (Rel assay diagnostic kits, Gaziantep, Turkey) as described by Erel [19]. The assay had excellent precision values lower than 3% and results were expressed as mmol Trolox Eq/L.

Measurement of Total Oxidant Status

Serum TOS level of was measured spectrofotometrically on an autoanalyzer (Aeroset®, Abbott®, Illinois, USA) using commercial kits (Rel assay diagnostic kits, Gaziantep, Turkey) as described by Erel [19]. The assay was calibrated with hydrogen peroxide and the results were expressed in terms of micromolar hydrogen peroxide equivalent per liter (μmol H_2O_2 Eq/L).

Oxidative Stress Index

Percentage ratio of TOS to TAC level was accepted as OSI. For calculation, the resulting unit of TAC was converted to mmol/L, and the OSI value was calculated according to the following formula; OSI (Arbitrary Unit) = TOS (μmol H_2O_2 Eq/L) / TAC mmol Trolox Eq/L) [20].

Measurement of Paraoxonase and Arylesterase Activity

Paraoxonase activity was measured in the absence (basal activity) and presence of NaCl (salt-stimulated activity), using paraoxon substrate. The rate of paraoxon hydrolysis (diethyl p-nitrophenylphosphate) was measured by monitoring the increase of absorbance at 412 nm. Paraoxonase activity was expressed as U/L serum [21,22]. The ratio of the salt-stimulated paraoxonase activity to the arylesterase activity was used to assign individuals to one of the three possible phenotypes [21,22].

Other Parameters

The concentrations of HDL-C, LDL-C, TC, TG, AST and ALT were measured using commercially available assay kits (Abbott®, Illinois, USA) on an autoanalyzer (Aeroset®, Abbott®, Illinois, USA).

Histopathological Examination

Livers were dissected out and fixed in 10% formol saline. Histopathological specimens were made by embedding in parafin and were stained with routine hematoxylin and eosin (H&E). Tissue sections were then examined microscopically by a pathologist blind to clinical data and experimental procedure.

Statistical Analysis

All data were expressed as mean±standard deviation (X±SD). The comparisons of groups were performed using repeated measure of ANOVA. Differences of between liver groups were evaluated by ANOVA. Assosciations between parameters were determined by pearson's correlation analysis. P-values <0.05 was accepted as significant. The X^2 test was used to compare distributions of phenotypes in all rabbits. Data were analyzed using the SPSS® for Windows computing program (Version 11.0).

RESULTS

Biochemical Findings

A significant increase in the concentrations of TC (P<0.001), TG (P<0.001), LDL-C (P<0.01), HDL-C (P<0.01) were observed in group given fat-rich diet (group II) when compared to control group (group I). A statistically significant increase in HDL-C levels (P<0.014) and significant decreases in TC (P<0.001), TG (P<0.001) and LDL-C levels (P<0.015) were determined in fat-rich diet + gemfibrozil group (group 3) compared to fat-rich group (group II) *(Table 1)*. In addition, significant increases were determined in ALT and AST activity in the fat-rich diet + gemfibrozil group (group III) compared to control group (P<0.05) throughout the experiment *(Table 1)*.

Evaluation of Paraoxonase and Arylesterase Activity

Serum paraoxonase and arylesterase activity significantly increased throughout the study in group III, IV and V while decreased in the group given fat-rich diet (P<0.05) *(Table 2)*. In addition, the changes in paraoxonase and arylesterase activity at 0, 3, 6 and 9 weeks were positivelly correlated with the changes in HDL-C in gemfibrozil group (r=0.63, P<0.001 and r=0.57, P<0.003; respectivelly). Serum paraoxonase/arylesterase activity significantly increased through the experimental period when compared to control (P<0.05) and fat-rich diet group (P<0.05) in group IV and V *(Table 2)*.

A significant negative correlation between OSI and paraoxonase and arylesterase activities in group IV (r= -0.36, P<0.012 and r= -0.41, P<0.009, respectively) and V (r= -0.31, P<0.022 and r= -0.37, P<0.014, respectively). Serum PON1 phenotype distribution did not significantly differ between the groups (P>0.05).

In addition, high PON1 activity was found in liver *(Table 3)* but tissue PON1 activity did not correlate with serum activity. However, liver paraoxonase and arylesterase activities were significantly higher in group IV and V than that of control (P<0.05) and group II (P<0.05) *(Table 3)*.

A significantly negative correlation between paraoxonase and arylesterase activity and OSI parameters in group IV and V (r= -0.32, P<0.041 and r= -0.27, P<0.036; respectivelly) was noted.

Assessment of TAS, TOS and OSI

A marked increase in serum TOS levels in group II was determined when compared to control group (P<0.05) *(Table 2)*. OSI levels determined both in serum and liver tissue decreased gradually in group IV (P<0.05) and V (P<0.05) compared control and group II over the experimental period *(Table 2, 3)*.

Table 1. *Biochemical parameters levels in all groups (n=7, X±SD)*
Tablo 1. *Bütün gruplarda biyokimyasal parametre düzeyleri (n=7, X±SD)*

Parameters	Weeks	Groups				
		I	II	III	IV	V
AST (U/L)	0	22.8±0.4	31.3±1	32.6±1.6	32.4±0.8	32.0±1
	3	26.4±1.1	32.8±1.4	39.2±0.9	31.6±1.1	32.6±1.3
	6	25.7±1.7	33.8±1.4	43.1±1.2	32.6±0.9	32.3±0.9
	9	25.4±2.1	33.7±1.1	45.1±1.8	32.5±1.8	31.9±0.8
	P values			P<0.05 *		
ALT (U/L)	0	17.8±1.8	27.2±0.7	26.7±0.4	25.8±1.2	26.4±0.6
	3	19.8±2.4	30.2±1.6	33.8±1.7	26.8±1.4	28.1±1.8
	6	19.0±1.2	31.7±2.0	35.3±1.6	28.1±0.9	29.1 ±1.7
	9	23.9±2.2	32.3±2.2	37.7±2.0	27.0±1.0	26.9 ±1.3
	P values			P<0.05 *		
HDL-C (mg/dL)	0	23.7±1.1	20.5±2.4	20.4±0.8	21.8±1.3	19.5±2.1
	3	24.2±0.8	24.8±1.1	26.5±1.6	25.2±1.7	26.1±1.0
	6	24.0±0.7	26.4±2.2	29.7±1.7	29.4±0.8	29.7±1.0
	9	24.2±0.8	24.8±2.2	33.7±1.6	30.2±0.9	31.1±1.5
	P values		P<0.01*	P<0.014 **		
TC (mg/dL)	0	51.4±2.6	51.1±2.3	50.1±2.0	49.7±1.4	49.1±2.1
	3	53.4±1.7	70.1± 2.8	52.7±2.3	53.0±1.9	51.7±2.4
	6	53.6±2.3	75.1±6.2	58.6±3.4	57.1±1.7	55.7±1.5
	9	54.6±2.3	82.8±8.3	57.7±1.5	54.8±1.4	55.1±2.2
	P values		P<0.001*	P<0.001 **		
TG (mg/dL)	0	120.7±3.6	125.1±6.7	122.3±5.1	123.3±4.8	123.0±5.6
	3	117.9±1.9	165.1±9.3	126.6±5.4	121.1±4.9	123.1±2.1
	6	117.7±2.9	187.0±12.4	135.4±2.8	126.1±2.9	128.0±4.8
	9	118.8±3.9	211.1±11.0	135.4±3.8	129.0±4.5	128.9±3.0
	P values		P<0.001*	P<0.001 **		
LDL-C (mg/dL)	0	3.5±0.3	3.8±0.8	4.7±0.5	4.8 ±0.4	4.8±0.7
	3	3.6±0.3	6.1±1.6	4.1±0.9	2.6±0.4	2.2±0.2
	6	3.7±0.8	7.7±1.0	4.3±0.6	4.0±0.6	3.5±0.3
	9	4.0±0.6	10.5±2.2	3.6±0.6	3.1±0.4	3.3±0.6
	P values		P<0.01*	P<0.015 **		

** Data compared to control; ** Data compared to fat-rich diet rabbit*
I: *Control group or standart diet group,* ***II:*** *Fat-rich diet group,* ***III:*** *Fat-rich diet + Gemfibrozil group,* ***IV:*** *Fat-rich diet + L-Carnitine group,* ***V:*** *Fat-rich diet + Gemfibrozil + L-Carnitine combination group,* ***AST:*** *Aspartate amino transferase,* ***ALT:*** *Alanin amino tranferase,* ***HDL-C:*** *High-density lipoprotein cholesterol,* ***TC:*** *Total cholesterol,* ***TG:*** *Triglyseride,* ***LDL-C:*** *Low-density lipoprotein-cholesterol*

Assessment of Histopathological Examination

Macrovesicular steatosis was particularly observed in group II and hepatosteatosis was more marked in periacini *(Fig. 1)*.

There were no histopathological changes apart from moderate steatosis in the liver of group IV. In addition, marked degenerative changes were observed in group III and these changes included both hydropic and vacuolar degeneration. Histopathological changes determined in group V were closer to the control group *(Fig. 1)*.

DISCUSSION

The link among cardiovascular disease, lipids and PON1 activity have been appreciated for a long time, and the underlying cause of increased tendency towards cardiovascular diseases in animals with hyperlipidemia is probably related to the enhanced atherogenic potential of lipoproteins. Possible mechanisms contributing to this increased atherogenicity include the increase of LDL and its enhanced susceptibility to oxidative modification. Oxidized LDL has gained a prominent role in the pathogenesis of

***Table 2**. PON1 and oxidative stress parameters levels in all groups (n=7, X±SD)*
***Tablo 2.** Bütün gruplarda PON1 ve oksidatif stres parametre düzeyleri (n=7, X±SD)*

Parameters	Weeks	Groups				
		I	II	III	IV	V
Paraoxonase (U/L)	0	619±91	685±184	617±130	672±93	639±142
	3	631±115	660±219	676±43	737±112	774±83
	6	677±134	651±93	776±59	767±111	804±119
	9	677±48	579±87	798±105	822±127	830±70
	P values			P<0.045 **	P<0.024 * P<0.01 **	P<0.05 * P<0.032 **
Salt stimulated Paraoxonase (U/L)	0	711±111	794±281	725±193	684±159	708±176
	3	656±174	779±323	713±126	857±224	877±237
	6	739±213	700±136	862±209	906±324	930±291
	9	686±179	602±133	890±287	1032±249	982±214
	P values			P<0.040 **	P<0.019 * P<0.01 **	P<0.043 * P<0.029 **
Arylesterase (kU/L)	0	385±20	401±18	383±21	396±11	390±23
	3	400±11	395±10	390±15	397±16	400±21
	6	403±22	392±8	402±16	412±13	404±19
	9	404±6	370±16	406±18	423±8	417±20
	P values			P<0.038 **	P<0.041 * P<0.019 **	P<0.40 * P<0.048 **
TAS (mmol Trolox Eq/L.)	0	0.77±0.06	0.94±0.04	0.87±0.04	0.88±0.05	0.86±0.05
	3	0.87±0.08	0.88±0.08	0.86±0.05	0.88±0.04	0.82±0.04
	6	0.87±0.07	0.90±0.04	0.84±0.04	0.90±0.04	0.90±0.05
	9	0.89±0.06	0.85±0.06	0.89±0.03	0.94±0.04	0.92±0.05
	P values					
TOS (μmol H_2O_2 Eq/L)	0	6.13±1.31	4.5±0.73	4.6±1.22	5.2±1.24	6.4±1.72
	3	6.9±2.71	5.3±1.04	5.2±0.71	4.8±1.07	4.8±1.11
	6	6.5±1.82	5.3±1.13	5.8±1.23	4.1±1.09	4.6±1.13
	9	5.7±0.51	6.3±1.06	6.2±0.52	3.4±0.51	3.4±1.01
	P values		P< 0. 017*			
OSI (Arbitrary Unit)	0	0.73±0.18	0.48±0.05	0.53±0.14	0.59±0.13	0.75±0.16
	3	0.61±0.11	0.60±0.10	0.58±0.06	0.55±0.12	0.57±0.11
	6	0.66±0.14	0.58±0.13	0.70±0.14	0.45±0.11	0.51±0.12
	9	0.64±0.11	0.62±0.11	0.70±0.07	0.35±0.10	0.38±0.13
	P values				P<0.029 * P<0.047 **	P<0.040 * P<0.030 **

** Data compared to control; ** Data compared to fat-rich diet rabbit*
***I:** Control group or standart diet group, **II:** Fat-rich diet groups, **III:** Fat-rich diet + Gemfibrozil group, **IV:** Fat-rich diet + L-Carnitine group, **V:** Fat-rich diet + Gemfibrozil + L-Carnitine combination group, TAS: Total antioxidant status, TOS: Total oxidant status, OSI: Oxidative stress index*

atherosclerosis [23,24]. PON1 deficiency is related to increased susceptibility to LDL oxidation and development of atherosclerosis. Therefore, PON1 activity clinically is a very important parameter which may be modified by diet, drugs [25] or antioxidants [26].

As the structure of rabbit and human paraoxonase enzyme is greatly similar [6] a hyperlipidemi model was developed in rabbits by feeding fat-rich diet. To the best of our knowledge, this was the first study where baseline effects of gemfibrozil and L-carnitine, alone and in combination on paraoxonase activity, oxidative/antioxidative parameters and hepatosteatosis were determined in animals with hyperlipidemia.

Clinical studies suggested that gemfibrozil have a significant protective effect against cardiovascular disease due to both lipid-lowering effect and stimulating PON1 activity [9,10,25]. Gemfibrozil act as an indirect anti-atherogenic due to increased HDL-C and modulation of plasma

Table 3. *Oxidative and antioxidative parameters levels in liver tissue of rabbits (n=7, X±SD)*
Tablo 3. *Tavşanlarda karaciğer dokusu oksidatif ve antioksidatif parametre düzeyleri (n=7, X±SD)*

Parameters		Groups				
		I	II	III	IV	V
Paraoxonase (U/g)		3.6±2.2	2.7± 1.3	3.9±1.7	5.4±1.3	3.3±1.2
	P values				P<0.01 **	
Salt Stimulated Paraoxonase (U/g)		10.6±4.2	7.3±3.1	11.3±3.3	15.4±4.7	11.5±4.9
	P values				P<0.01 **	
Arylesterase (kU/g)		57±12	43±10	63±11	125±33	71±14
	P values				P<0.001 * P<0.001 **	P<0.039 **
TAS (mmol Trolox Eq/g)		3.2±0.49	3.0±0.47	2.5±0.27	4.2±0.27	3.0±0.21
	P values			P<0.07 * P<0.05 **	P<0.03 * P<0.001 **	
TOS (μmol H_2O_2 Eq/g)		30±6.3	47±4.3	50±7.3	34±5.8	39±3.9
	P values		P<0.050*	P<0.032 * P<0.03 **		P<0.01 * P<0.046 **
OSI (Arbitrary unit)		11.8±2.7	15.8±2.2	20.6±3.3	9.0±1.6	13.6±3.1
	P values		P<0.050 *	P<0.006 * P<0.001 **	P<0.001 * P<0.006 **	P<0.003 *

** Data compared to control; ** Data compared to fat-rich diet rabbit*
I: *Control group or standart diet group,* ***II:*** *Fat-rich diet groups,* ***III:*** *Fat-rich diet + Gemfibrozil group,* ***IV:*** *Fat-rich diet + L-Carnitine group,* ***V:*** *Fat-rich diet + Gemfibrozil + L-Carnitine combination group,* ***TAS:*** *Total antioxidant status,* ***TOS:*** *Total oxidant status,* ***OSI:*** *Oxidative stress index*

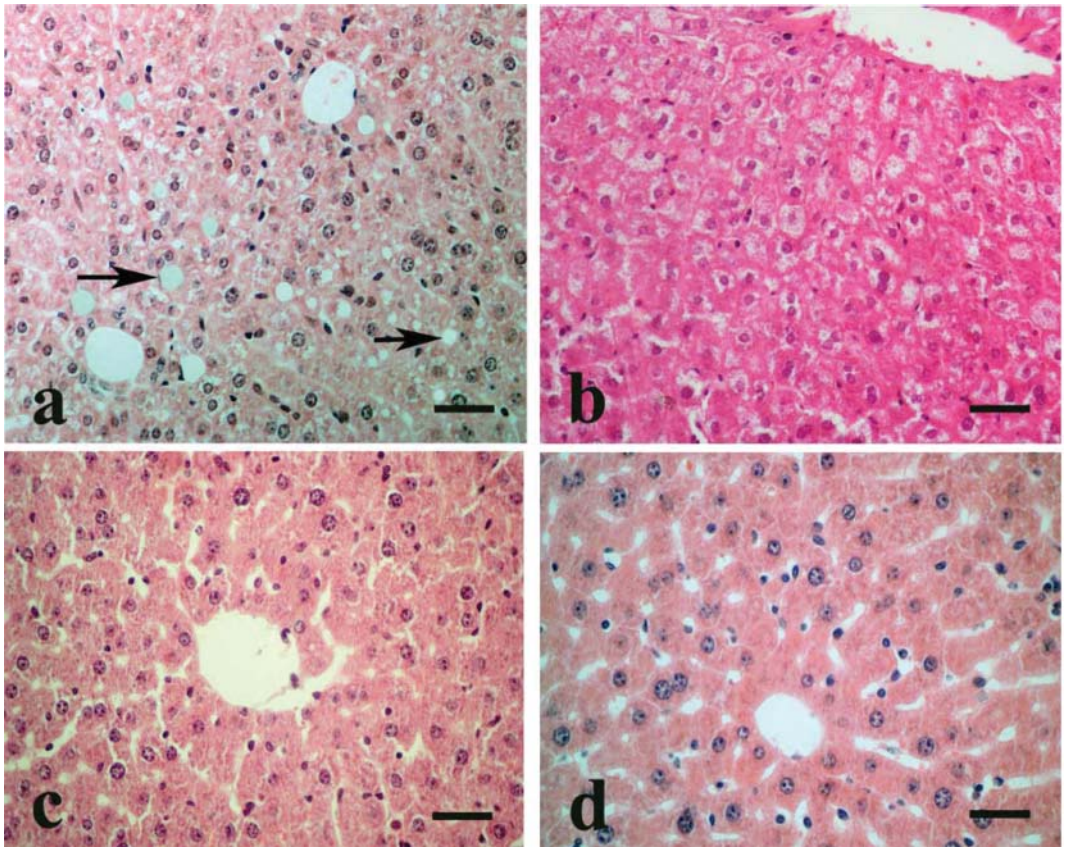

Fig 1. In rabbits applied fat-rich diet showing histopathological of liver (H&Ex64). In rabbits applied fat-rich diet showing definitive limited oil vacuolar in hepatocytes (a), in rabbits applied fat-rich diet + gemfibrozil showing degenerative changes (b), in rabbits applied fat-rich diet + gemfibrozil+ l-carnitine (c) and standart diet (d) showing oil vacuolar in hepatocytes (Bar= 30 μm)

Şekil 1. Yüksek yağlı diyetle beslenen tavşanlarda karaciğerin histopatolojik görünümü (H&Ex64). Yüksek yağlı diyetle beslenen tavşanlarda hepatositlerde yağ vakuolleri (a), yüksek yağlı diyete ilave olarak verilen gemfibrozile bağlı gelişen dejeneratif değişiklikler (b), yüksek yağlı diyete ilave olarak verilen gemfibrozil + L-carnitine ilavesinin etkisi (c) ve standart diyet (d)hepatositlerde yağ vakuolleri (Bar= 30 μm)

lipids [25,27]. Data obtained here showed a significantly positive correlation between HDL-C and paraoxonase and arylesterase activities during gemfibrozil therapy. On the other hand, changes in paraoxonase/arylesterase activity were negatively correlated with the changes in triglycerides. Lipid-lowering therapy with gemfibrozil in the present study not only increased PON1 activity but also reduced atherogenic lipids (TC, TG and LDL). Thus, increasing PON1 activity by gemfibrozil might play an important role in decreasing LDL-C oxidation and its related CHD.

Many studies have shown that there is a decrease in the concentration of L-carnitine in blood and tissues in hyperlipidemic conditions and carnitine derivates have been shown to protect liver in atherosclerotic rats [28]. In atherosclerotic condition, there is a depletion of myocardial carnitine, resulting in a decrease in the transport of fatty acid into mitochondria. L-carnitine reduces the lipid hydroperoxides levels, inhibits the microsomal peroxidation, and has a role in chelating free Fe^{+2} ions so, by this way, reduces free radical generation [29].

In this study, decreased serum OSI and increased TOS levels in L-carnitine group might add credence to L carnitine's capacity as a free radical scavenger in the blood [15]. Many studies have demostreated that purified human and rabbit PON1 contained a single free sulfhydryl group in the native state and cysteine-284 as the active site of the PON1 for its antioxidant effect, and a close association between PON1's free sulfhydryl groups and its antioxidant capacity has already been reported. PON1 activity has been shown to be inactivated under oxidative stres, and its activity is preserved by dietary antioxidant [30,31]. In this study, a correlation between OSI and serum paraoxonase/arylesterase activity in rabbits given L-carnitine was determined. Elevated PON1 activity has been associated with decreased OSI levels, and thus, decreased atherosclerosis and CHD.

Fat-rich diet can stimulate ROS production and consequently lipid peroxidation in cell membrane resulting in tissue damage including liver [32]. In the present study, particularly liver TOS levels were significantly higher in rabbits given fat-rich diet compared to control but no significant difference in liver TOS levels between L-carnitine, combination and control groups was noted.

In this study, an increase was observed in serum AST and ALT activities though within reference range [33] over the experimental period in gemfibrozil treated rabbits while the activites did not differ among other groups. Lipid-lowering drugs are already known to cause alteretions in liver as was the case in gemfibrozil group in this study. L-carnitine was proven to act as a protective agent against hepatosteatosis caused by high fat diet and against liver damage caused by gemfibrozil treatment in this study as ALT and AST activities and TOS and OSI levels decreased in L- carnitine given rabbits. These effects of L-carnitine may explain the mechanism of protection against of ROS. Thus, carnitine treatment exerts both free radical scavenger and thiol sparing activity, and maintains normal cell function by decreasing OSI. In conclusion, L- carnitine may act as an anti-atherosclerotic agent trough minimizing oxidative stress and thus resulting in minimizing ox-LDL and inhibition oxidation of thiol groups of paraoxanase that in turn leads an increase in PON1 activity. All these events may prevent development of therosclerosis. L-carnitine may also play role in hepatoprotection in rabbits with hyperlipidemia through possibly several mechanisms, I) by activating PON1 enzyme, ii) by decreasing OSI and iii) by decreasing lipidemia.

In light of the results of this study, it may be concluded that gemfibrozil and L-carnitine combination reduce hepatic degeneration and increase PON1 activity. Therefore, side effects gemfibrozil may be avoided by administration of L-carnitine.

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