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ORIGINAL ARTICLE

Effects of quercetin on hepatic fibroblast growth factor-21 (FGF-21) and peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) levels in rats fed with high fructose

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Abstract

Background Available studies show that quercetin reduces Metabolic Syndrome (MetS) and its complications, increases insulin sensitivity and improves glucose levels. It has been reported that the increase in hepatic gene expressions of fibroblast growth factor-21 (FGF-21), an important metabolic regulator of insulin sensitivity, glucose and energy homeostasis, and peroxisome proliferator-activated receptor gamma coactivator-1α (PGC-1α), which plays a central role in the regulation of cellular energy metabolism, eliminate the negative effects of fructose in fructose-fed rats. The main purpose of our study is to examine the effects of quercetin on hepatic FGF-21 and PGC-1α expressions and levels, as well as its protective and therapeutic role on MetS components in rats fed with fructose.

Methods and results In our study, 24 Sprague Dawley male rats were divided into 4 groups: control, fructose, quercetin, fructose+quercetin ($n=6$). During the 10-week experiment, quercetin was administered at a daily dose of 15 mg/kg body weight and fructose at a rate of 20%. Blood pressure and weights of all groups were measured and recorded. At the end of week 10, blood and liver tissue samples were taken. Serum insulin, glucose and triglyceride, total, HDL and VLDL cholesterol levels were determined from the samples. Insulin resistance was calculated using the HOMA-IR formula. Hepatic PGC-1α and FGF-21 protein levels and their mRNA expressions were determined. Criteria for metabolic syndrome were successfully established with fructose. It was observed that the administration of quercetin alone and in combination with fructose exerted positive effects and improved MetS criteria. It was determined that the administration of quercetin increased hepatic FGF-21 and PGC-1α protein levels and Messenger RNA (mRNA) expressions of them, which were decreased by fructose application.

Conclusions The results of our study showed that 10-week administration of quercetin at 15 mg/kg exerted beneficial effects on lipid and carbohydrate metabolism in the fructose-mediated MetS model; therefore, quercetin may have great potential in the prevention and treatment of metabolic disorders.

Keywords Fructose · Quercetin · FGF-21 · PGC-1α · Liver

Introduction

Metabolic syndrome (MetS) is characterized by insulin resistance, which is the most predominant, abdominal obesity, glucose intolerance [type 2 Diabetes Mellitus (DM), impaired glucose tolerance (IGT)], hyperglycemia, dyslipidemia (high triglycerides and low HDL), hypertension, and coronary artery disease (CAD). MetS causes high morbidity and mortality due to its cardiac and metabolic complications. It is very important to create experimental animal models that mimic the condition of the disease in humans for evaluating the pathophysiology of MetS. Rats and mice are the most common animal models used in investigating MetS.

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MetS occurs as a result of dietary imbalance in humans. The main cause of MetS is excess fructose intake from sucrose and high-fructose corn syrup. Fructose-based diet causes weight gain, glucose intolerance and insulin resistance, hypertension, metabolic dyslipidemia and is associated with higher cardiometabolic risk indices [1, 2].

Fibroblast growth factors (FGFs) are a large family of polypeptide growth factors found in a variety of organisms and possess many biological activities. FGF-21, a member of this family, is produced predominantly in the liver and has been shown to reduce most of the metabolic diseases associated with obesity in animal models, including hyperglycemia, insulin resistance, dyslipidemia, and hepatosteatosis [3]. FGF-21 has dramatic effects on liver metabolism that includes the induction of fatty acid oxidation, ketogenesis, gluconeogenesis and suppression of lipogenesis [4]. Therefore, FGF-21 is suggested to be a promising potential therapeutic agent for the treatment of T2DM, obesity, and related complications [4].

During the adaptive starvation response, FGF-21 induces hepatic expression of PGC-1α which facilitates fatty acid oxidation, tricarboxylic acid cycle and gluconeogenesis [5]. PGC-1α is a transcription coactivator that interacts with a broad range of transcription factors involved in a wide variety of biological responses including adaptive thermogenesis, mitochondrial biogenesis, glucose/fatty acid metabolism, fiber type switching in skeletal muscle, and heart development [6]. PGC-1α has become a target gene in the design of pharmacological interventions due to its role in major biological processes.

Quercetin (3,3', 4', 5,7-Pentahydroxyflavone), a plant-derived flavonoid found in vegetables and fruits such as onions, broccoli, strawberries, red grapes and tea, is reported to exert anti-inflammatory [7],antioxidant [8], antihypertensive [9], and vasodilator [10] effects as well as antiobesity [11],antihypercholesterolemic [12] and antiatherosclerotic [13] metabolic effects. In the light of the abovementioned data, we think that quercetin may have great potential in the prevention and treatment of metabolic disorders in which obesity, T2DM and insulin resistance play a major role.

Based on the literature, we aimed to investigate the effects of quercetin administration on hepatic FGF-21 and PGC-1α gene expression and levels in rats in the fructose-mediated MetS model, as well as its protective and therapeutic role on MetS components.

Materials and methods

Animals and diet

A total of 24 adult male Sprague Dawley rats, 10 weeks of age, each weighing 220 ± 15 g, were obtained from Gazi University Laboratory Animal Breeding and Experimental Research Center to establish an experimental models of MetS. They maintained in 12-h light/12-h dark cycle at 22 ± 2 °C. Experimental animals were randomly placed into cages, with 6 in each. To all experimental animals for 10 weeks were given standard rat food containing; 88% dry matter,23% protein,7% cellulose,8% raw ash,2% HCl insoluble ash,1.5% calcium, 0.9% phosphorus,0.7% sodium,1% salt,0.3% methionine and 1% lysine. All groups were given tap water as drinking water, no restrictions were placed on the water and feed consumption of animals [14]. Approval for our study was obtained from Gazi University Animal Experiments Local Ethics Committee (G.Ü.ET-19.016).

For the study, quercetin was obtained from Sigma Aldrich (St. Louis, ABD) (≥ 95%) and D-fructose was obtained from Merck (Darmstadt, Germany) (≥ 99%).

Creation of experimental groups

In our study, 4 experimental groups (Control, Fructose, Quercetin, Fructose + Quercetin) with each containing 6 animals were formed and the procedures stated in Table 1 were applied for 10 weeks:

Table 1 Experimental groups and procedures applied to the groups

Experimental groups ($n = 6$)	Procedures
Control	The control group was given a solution of 0.2% Dimethyl Sulfoxide (DMSO) by gavage in proportion to their body weight for 10 weeks from the first day and tap water for drinking
Fructose	Fructose solution prepared by dissolving D-fructose in tap water at a rate of 20% was given to the fructose group for 10 weeks from the first day [14, 15]. A 0.2% (DMSO) solution in proportion to body weight was administered daily by oral gavage
Quercetin	The quercetin group was given quercetin solution in proportion to body weight at a dose of 15 mg/kg by oral gavage for 10 weeks from the first day. Quercetin solution was prepared fresh every day in 0.2% DMSO As a reference in determining the dose of quercetin, studies with significant results in terms of antioxidant, antidiabetic and liver protective effects were used [16]
Fructose + Quercetin	Experimental animals in the fructose + quercetin group were given drinking water with fructose in the same dose and form as the fructose group for 10 weeks from the first day, and quercetin was administered in the same dose and manner as the quercetin group

At the end of week 10, the experimental animals were sacrificed under ketamine (10 mg/kg)-xylazine (90 mg/kg) intraperitoneally anesthesia and blood was collected by cardiac puncture. Then, their livers were removed and frozen in liquid nitrogen and were preserved together with the serums at − 80° C.

Measurement of body weight and systolic blood pressure

Animals' body weight (BW) was measured at the beginning of the experiment, at the end of each week, and before sacrification. Animals' systolic blood pressure was measured from the tail using the Tail-Cuff BIOPAC Systems at the beginning of the experiment, at the end of the first month, and before sacrification. Rats were fasted before weekly body weight measurements and blood tests were performed.

Obesity index

At the end of week 10, the "Lee Index" of each experimental animal was calculated according to the formula BW1/3* [10/Nose-Anus Distance (mm)]. Rats with a reference value equal to or less than 0.3 were considered normal, whereas rats with a value greater than 0.3 were classified as obese [17].

Determination of serum glucose, lipid, insulin and HOMA-IR levels

Glucose and lipid levels including triglyceride, total cholesterol, HDL cholesterol, LDL cholesterol and VLDL cholesterol were determined in the serum of animals using the Beckmann AU5800 biochemistry autoanalyzer. Serum insulin was measured using the sandwich enzyme immunoassay ELISA kit (Millipore, USA). Insulin resistance was calculated according to the mathematical formula of HOMA-IR [insulin (mU / L) x glucose (mmol / L)] / 22.5. [18]

Homogenization of hepatic tissue samples and tissue protein determination

Hepatic tissue samples were homogenized 1/10 in 50 mM ice-cold Tris–HCl (pH 7.4) buffer. Homogenates were centrifuged at 15,000 rpm for 15 min in a refrigerated centrifuge, and total tissue protein in the supernatants was determined according to the Lowry Method [19].

Measurement of hepatic fibroblast growth factor 21 (FGF-21) and peroxisome proliferator-activated receptor gamma coactivator-1α (PGC-1α) protein levels.

In our study, hepatic FGF-21 and PGC-1α protein levels were measured using a quantitative ELISA kit (Cusabio, China).

RNA isolation and mRNA expression analysis with real time RT-QPCR

The mRNA expression levels of the target genes were determined using the Reverse Transcription-Polymerase Chain Reaction (RT-PCR) method. Relative changes in expression were calculated with the $2^{-}\Delta\Delta^{Ct}$ method. GAPDH was used as an endogenous reference and the expression of other genes were normalized based on the level of GAPDH in each sample [20–22].

Primer sequences for reference gene analysis by qPCR:

GAPDH: Forward primer: 5' GATTTGGTCGTATTG GGCGC 3'; (sense)

Reverse primer: 5' AGTGATGGCATGGACTGTGG 3'.(antisense).

Genes whose expression levels are determined by PCR studies:

FGF-21: Forward primer: 5'-CAAATCCTGGGTGTC AAAGC-3'; (sense)

Reverse primer: 5'-GCCTCAGACTGGTACACATTG-3'. (antisense).

PGC-1α: Forward primer: 5'-AGGTCCCCAGGCAGT AGAT-3' (sense)

Reverse primer: 5'-CGTGCTCATTGGCTTCATA-3' (antisense).

Histological method

All liver samples were first fixed in 10% formaldehyde solution for light microscopic examination. Hematoxylin–Eosin staining was applied to the 4–5 micron thick sections obtained from the prepared paraffin blocks for all groups. The sections were evaluated in the Leica DCM 4000 (Germany) computer aided imaging system, the LAS program, and their pictures were taken.

Statistical analysis

IBM Corporation SPSS Statistics 22.0 package program was used for statistical analysis of the data. The Kruskal–Wallis test was used for the comparison of all groups while the Mann–Whitney *U* test was used to compare different parameters in paired groups. A p-value of < 0.05 was considered statistically significant.

Results

Results are given as mean ± standard error in all tables and figures. Body weights, obesity indexes and systolic blood pressure of the experimental groups are shown in Table 2, Serum lipid profiles, Serum glucose, insulin, and HOMA-IR index findings are shown in Table 3, Hepatic

FGF-21 and PGC-1α levels are shown in Table 4, and the folded changes in Real Time QRT-PCR mRNA Expression are shown in Table 5, Histopathology Results are given in Figs. 3, 4, 5, 6, 7 and Degeneration Criteria are shown in Table 6.

When the obesity index and 5th and 10th weeks systolic blood pressure of the fructose group was compared with the all group, significant increase was found to support obesity and hypertension. When the obesity index of the quercetin group was compared with the control group, no statistically significant difference was found, and similarly co-administration of quercetin with fructose significantly improved the lee index and systolic blood pressure (Table 2).

Serum triglyceride levels of the groups

When triglyceride levels of the groups were evaluated, significantly higher values were obtained in fructose ($p=0.006$) and fructose + quercetin ($p=0.037$) groups than in the control group. When we compared the control group with the group receiving quercetin, a non-significant increase in triglyceride levels was observed. In the same way, the quercetin

Table 2 Body weight, Obesity index and Systolic blood pressure results of the groups

Experimental groups ($n=6$)	Body weight (g)		Lee index (mm)	Systolic blood pressure (mmHg)		
	Start	10th Week	10th Week	Start	5th Week	10th Week
Control	200 ± 8	249 ± 8[d]	0.29 ± 0.007	125 ± 5.1	115 ± 7	119 ± 2
Fructose	223 ± 11[a]	288 ± 23[a,d]	0.31 ± 0.007[a]	121 ± 7.8	139 ± 5[a,d]	181 ± 1[a,d,e]
Quercetin	230 ± 7[a]	287 ± 16[a,d]	0.30 ± 0.002[b]	125 ± 2.8	124 ± 2[a,b]	124 ± 4[b]
Fructose + Quercetin	210 ± 12[c]	275 ± 25[d]	0.29 ± 0.021[b,c]	125 ± 2.1	132 ± 6[a,b,c,d]	159 ± 1[a,b,c,d,e]

[a]Compared to the control group $p<0.05$

[b]Compared to the fructose group $p<0.05$

[c]Compared to the quercetin group $p<0.05$

[d]Compared to the initial value $p<0.05$

[e]Compared to the value of the 5th week $p<0.05$

Table 3 Serum Triglyceride, Total, LDL, HDL, VLDL Cholesterol, Glucose, Insulin and HOMA-IR Levels

	Experimental Groups ($n=6$)			
	Control	Fructose	Quercetin	Fructose + Quercetin
Triglyceride(mg / dl)	30 ± 8	51 ± 12[a]	39 ± 4[b]	52 ± 19[a]
Total Cholesterol (mg/dl)	49 ± 7	54 ± 2	52 ± 9	45 ± 5[b]
LDL-Cholesterol (mg/dl)	12 ± 2	10 ± 1	13 ± 2[b]	8 ± 1[a,c]
HDL- Cholesterol (mg/dl)	40 ± 4	42 ± 2	47 ± 1[a,b]	37 ± 2[b,c]
VLDL- Cholesterol (mg/dl)	6 ± 1	10 ± 2[a]	7 ± 1[b]	10 ± 4[a]
Glucose (mg/dl)	152 ± 12	230 ± 16[a]	149 ± 12[b]	207 ± 56[a,b]
Insulin (mU/L)	5.85 ± 1.11	9.16 ± 0.29[a]	6.38 ± 1.40[b]	6.35 ± 1.27[b]
HOMA-IR	2.19 ± 0.38	5.21 ± 0.47[a]	2.37 ± 0.61[b]	3.92 ± 1.22[a]

[a]Compared to the control group $p<0.05$

[b]Compared to the fructose group $p<0.05$

[c]Compared to the quercetin group $p<0.05$

Table 4 Liver tissue FGF-21 and PGC-1α levels

Experimental Groups ($n=6$)	FGF-21 (pg/mg protein)	PGC-1α (ng/mg protein)
Control	2.60 ± 0.70	43.71 ± 3.85
Fructose	2.11 ± 0.52	38.93 ± 8.01
Quercetin	2.62 ± 0.51[b]	59.37 ± 12.04[a,b]
Fructose + Quercetin	2.58 ± 0.20	57.69 ± 6.09[a,b]

[a]Compared to the control group $p<0.05$

[b]Compared to the fructose group $p<0.05$

[c]Compared to the quercetin group $p<0.05$

Table 5 FGF-21 and PGC-1α mRNA levels (multiple change*)

Experimental groups ($n=6$)	FGF-21 mRNA levels			PGC-1α mRNA levels		
Fructose	−1.53[a]			−1.23[a]		
Quercetin	1.60[a]	1.75[b]		1.58[a]	1.77[b]	
Fructose + Quercetin	−0.22[a]	1.31[b]	−1.48[c]	0.82[a]	1.5[b]	−1.23[c]

[a]Multiple changes compared to the control group were expressed as over expression (+) and suppression (–)

[b]Multiple changes compared to the fructose group were expressed as over expression (+) and suppression (–)

[c]Multiple changes compared to the quercetin group were expressed as over expression (+) and suppression (–)

Table 6 Degeneration Criteria

	Control	Quercetin	Fructose	Fructose + Quercetin
V. Centralis dilatation			+++	++
Sinosoid dilatation			+++	++
Congestion			+++	+
Acidophilus (-) hepatocytes			+++	++
Radial disorganization			+++	
Impaired hepatocyte morphology			+++	
Decrease in the number of polynuclear hepatocytes			+++	+
Pycnotic nucleus			+++	+
Vacuolar degeneration			+++	
Lipid droplets in hepatocytes consistent with steatosis			+++	+
Increase in connective tissue density			+++	+
Hepatic artery dilatation			+++	+
Disruption of the bile duct epithelium			+++	
Infiltration			+++	+

group had a significantly lower triglyceride level than the fructose group ($p=0.025$) (Table 3, Online Resource 1).

Serum total cholesterol levels of the groups

When the total cholesterol levels of the groups were evaluated, a non-significant statistical increase was observed in the fructose ($p=0.262$) and quercetin ($p=0.749$) administrated groups and a statistically significant difference was found between the fructose group and the fructose + quercetin group ($p=0.006$) (Table 3, Online Resource 2).

Serum LDL-cholesterol levels of the groups

When the LDL-cholesterol levels of the groups were evaluated, an increase was observed in the quercetin ($p=0.631$) group, and a decrease was observed in the fructose ($p=0.199$) and fructose + quercetin ($p=0.025$) groups compared to the control. A statistically significant difference was found between the fructose group and the quercetin group ($p=0.004$). A statistically significant difference was found between the quercetin and fructose + quercetin group ($p=0.004$) (Table 3, Online Resource 3).

Serum HDL-cholesterol levels of the groups

When the HDL-cholesterol levels of the groups were evaluated, an increase was observed in the fructose ($p=0.423$) and quercetin ($p=0.010$) groups, while a decrease was observed in the fructose + quercetin ($p=0.150$) group compared to the control. A statistical difference was also found between the fructose group and the quercetin group ($p=0.004$) and the fructose + quercetin group ($p=0.016$). A statistically significant difference was also found between the quercetin group and the fructose + quercetin group ($p=0.004$) (Table 3, Online Resource 4).

Serum VLDL-cholesterol levels of the groups

When the VLDL-cholesterol levels of the groups were evaluated, much higher values were obtained in the fructose ($p=0.006$), quercetin ($p=0.078$) and fructose + quercetin

($p=0.045$) groups compared to the control group. A statistically significant difference was found between the fructose group and the quercetin group ($p=0.025$). (Table 3, Online Resource 5).

Serum glucose levels of the groups

When glucose levels were evaluated, it was found that fructose ($p=0.004$) and fructose-accompanied quercetin ($p=0.004$) groups were higher than the control group, while the values of the group that were administered quercetin alone ($p=0.470$) were found to be lower. A statistically significant difference was also found between the fructose group and the quercetin group ($p=0.004$) (Table 3, Online Resource 6).

Serum Insulin and HOMA-IR Levels of the Groups

When insulin and HOMA-IR levels were evaluated, higher values were obtained in the fructose group compared to other groups. The HOMA-IR values of the fructose and fructose + quercetin groups were found to be higher than the control group (Table 3 and Online Resources 7, 8).

Liver tissue FGF-21 levels

When the groups are evaluated; compared to the control group, a decrease in the fructose ($p=0.337$) and fructose + quercetin group, an increase was observed in the quercetin ($p=0.749$) group. When the fructose group and the group taking quercetin were compared, a statistically significant difference was found between them ($p=0.037$). Higher FGF-21 protein levels were detected in the quercetin group (Table 4, Online Resource 9).

Liver tissue PGC-1a levels

When the groups are evaluated; compared to the control group, a decrease was observed in the fructose ($p=0.2$) group, an increase was observed in the quercetin ($p=0.01$) group and the fructose + quercetin ($p=0.04$) group. When the fructose group and the group taking quercetin were compared, a statistically significant difference was found between them ($p=0.037$). The liver tissue PGC-1α protein levels of the fructose + quercetin group were found to be significantly higher than the fructose group ($p=0.004$) (Table 4, Online Resource 10).

Liver tissue mRNA expression levels of FGF-21, which is indicated by literature data to have a protective and therapeutic role on MetS components; compared to control group, decreased 1.53 times in the fructose group, increased 1.6 times in the quercetin group and 1.75 times in the fructose group. Therefore, in line with the literature, it has been shown that fructose suppresses FGF-21 mRNA expression in liver tissue and quercetin stimulates hepatic FGF-21 production in rats fed a fructose diet. It is observed that the mRNA expression level of FGF-21 decreased by 0.22 times in the fructose+quercetin group compared to the control group. When we compare the data we have obtained here with the data in which only the fructose administered group is compared with the control group; it is seen that the deep suppression of mRNA expression caused by fructose improves positively with the application of quercetin. The mRNA expression level of FGF-21 increased 1.31 times in the fructose+quercetin group compared to the fructose group, and the mRNA expression level of FGF-21 was suppressed by fructose and decreased 1.48 times compared to the quercetin group. (Tables 4, 5 and Fig. 1, Online Resources 9, 10).

In our study, it was observed that liver PGC-1α mRNA expression levels were also decreased by 1.23 times in the fructose group compared to the control group. In parallel, it was observed that liver PGC-1α level decreased significantly in the fructose given group compared to the control group. In the quercetin-only group, liver PGC-1α levels increased significantly compared to the control and fructose groups, in addition, PGC-1α mRNA expression level increased 1.58 times compared to the control group and 1.77 times compared to the fructose group. Administration of quercetin alone resulted in a statistically significant increase in the expression of PGC-1α and its amount in the tissue (Tables 4, 5 and Fig. 2, Online Resource 10). The amount of PGC-1α in the tissue was found to be significantly higher in the group to which fructose and quercetin were administered together than in the control and fructose groups. It is observed that the mRNA expression level of PGC-1α increased 0.82 times in the fructose+quercetin group compared to the control group. This increase is actually a more serious increase in expression. It is an indication that the profound decrease

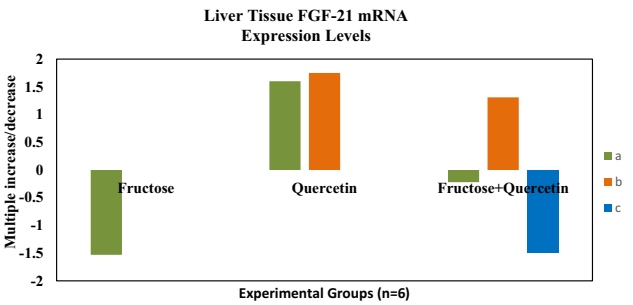

Fig. 1 Liver Tissue FGF-21 mRNA Expression Levels. a: Multiple changes compared to the control group were expressed as over expression (+) and suppression (–). b: Multiple changes compared to the fructose group were expressed as over expression (+) and suppression (–). c: Multiple changes compared to the quercetin group were expressed as over expression (+) and suppression (–)

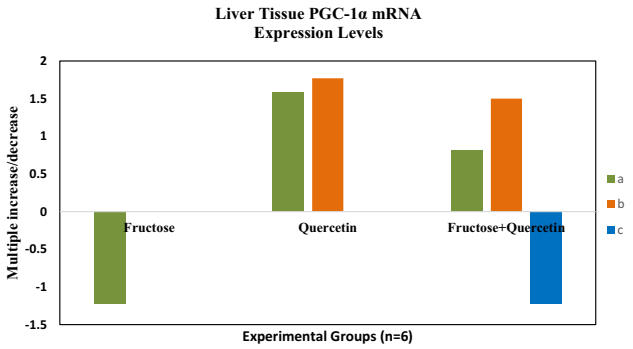

Fig. 2 Liver Tissue PGC-1α mRNA Expression Levels. a: Multiple changes compared to the control group were expressed as over expression (+) and suppression (–). b: Multiple changes compared to the fructose group were expressed as over expression (+) and suppression (–). c: Multiple changes compared to the quercetin group were expressed as over expression (+) and suppression (–)

of fructose in the amount of PGC-1α and mRNA expression in liver tissue is not only recovered by the concomitant quercetin, but also positively increased. While the mRNA expression level of PGC-1α increased 1.5 times in the fructose+quercetin group compared to the fructose group, the mRNA expression level of PGC-1α decreased by 1.23 times compared to the quercetin group due to the suppression of fructose (Tables 4, 5 and Fig. 2, Online Resource 10).

Histopathology results

In the large and small magnification examinations of the liver sections of the Control and Quercetin groups, the vena centralis located in the center of the classical liver lobule structure, hepatocytes showing radial arrangement around the vena centralis and sinusoid structures located between the hepatocytes were observed in their normal appearance. Vena centralis was in normal appearance with peripheral endothelium. The radially arranged hepatocytes were distinguished in their normal structures with their classical polygonal shapes, centrally located and mononuclear or polynuclear nuclei according to their activity status, acidophilic cytoplasm and cytoplasmic glycogen accumulations that vary according to their activity status. Sinusoids located between hepatocytes were also observed in their normal course with surrounding endothelial cells and blood cells in the lumen (Fig. 3).

In the large and small magnification examinations performed in the portal area (portal triad/portal triangle) located in the connective tissue at the junction of the classical liver lobules, belonging to the control and Quercetin groups, the connective tissue density, the hepatic vein with the branch of the vena porta, the branch of the arteria hepatica propria extension of the hepatic artery and bile duct were observed with their normal structures. Endothelial cells surrounding the hepatic vein and artery and single-layered cuboidal epithelial cells surrounding the extension of the bile duct were observed in their normal appearance (Fig. 4).

In small magnification examinations of the fructose group liver tissue, it was determined that the vena centralis was dilated in most of the classical liver lobules, extended by extending into the lobule in a way that disrupts the arrangement of hepatocytes and sinusoids, and the lobule structures were deteriorated especially in these areas. (Fig. 5A). It was observed that the sinusoids located between hepatocytes became dilated in most areas (Fig. 5B), and the presence of congestion was detected in these dilated sinusoids (Fig. 5B). In the large magnification examinations, it was observed that hepatocytes located especially in the vicinity of the vena centralis lost their acidophilia and gained light-colored cytoplasmic staining; it was thought that the metabolic activities of these hepatocytes were impaired. In this group, it was observed that the normal radial arrangement of hepatocytes was disrupted and they were arranged in the form of cell masses in places. It was observed that the normal polygonal morphology of most hepatocytes was disrupted and their cell borders became indistinguishable and size differences were manifested. It was determined that the number of polynuclear hepatocytes was relatively decreased compared to the Control and Quercetin groups, pycnotic nuclei were observed in some hepatocytes, the cytoplasmic glycogen density decreased,vacuolar degeneration occurred, and lipid droplets were found in the cytoplasm in accordance with steatosis. The congestion in the sinusoids was also observed in this magnification (Fig. 5C). It was observed that the connective tissue fiber and cell density increased especially in the areas around the vena centralis, which was described as a progression to liver fibrosis (Fig. 5D).

In the small and large magnification examinations performed in the portal area of this group, dilatation was observed in the hepatic arteries and veins, with the hepatic artery being much more pathological in size. It was determined that the connective tissue density in the portal area increased much more than around the vena centralis (Fig. 6-Fructose Group). It was determined that the single-layered cubic epithelium of the bile duct deteriorated and became stratified (Fig. 6B), and infiltration was generally observed in the portal area (Fig. 6A). In the examinations performed in the portal area of this group, it was determined that the hepatic artery dilatation continued, although it was greatly reduced compared to the fructose group, and infiltration was seen at a low rate. In addition, it was observed that this area returned to its normal course, including the hepatic vein and bile duct epithelium and connective tissue density in the portal area (Fig. 6-Fructose + Quercetin Group).

In the examinations made in the fructose + quercetin group; It was observed that dilatation in the vena centralis and sinusoids, and congestion in some areas continued.

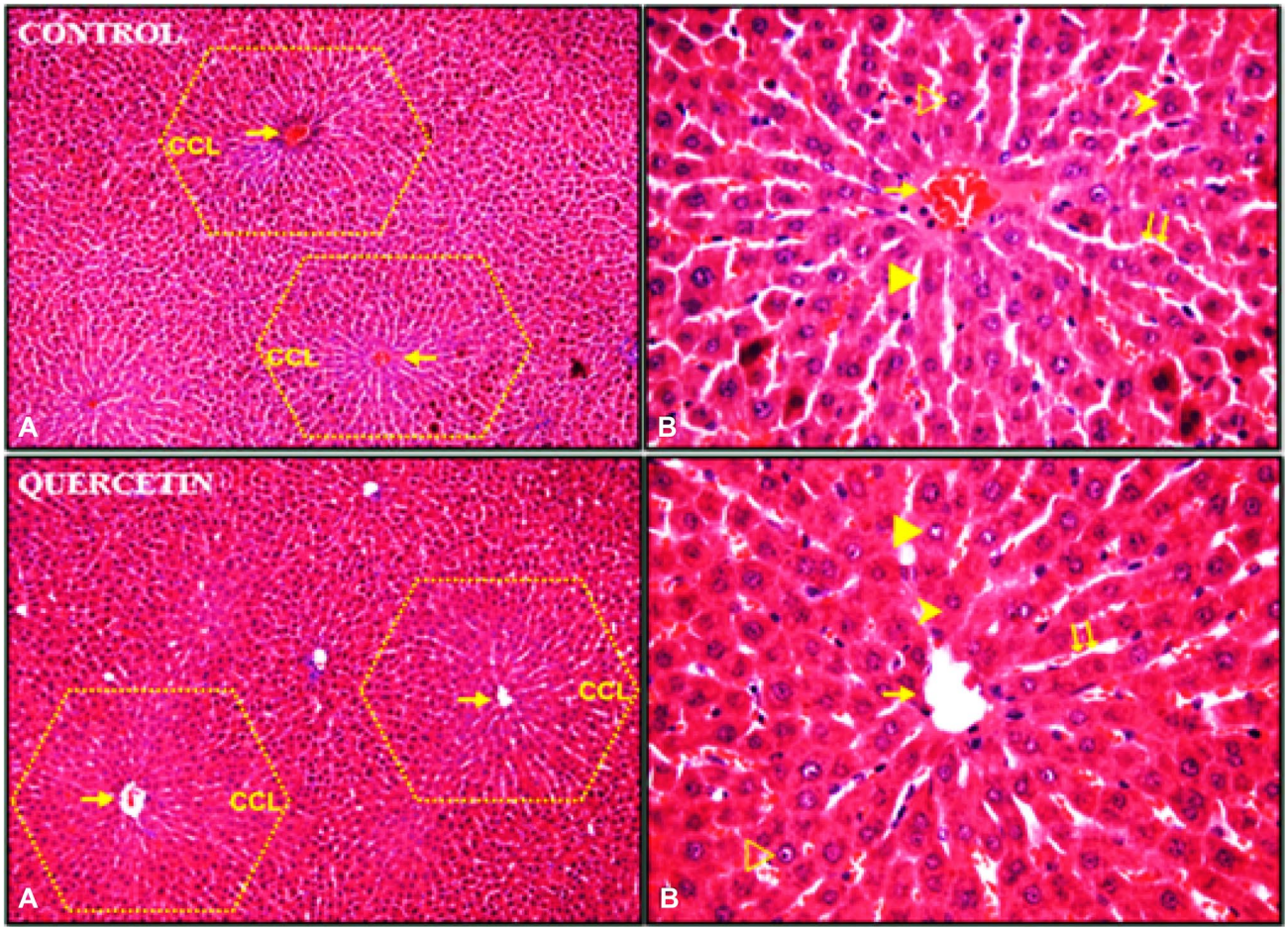

Fig. 3 *CLL in pictures of the control group*: classic liver lobule, → : vena centralis, ▶: hepatocyte, ⇉: sinusoid, ▷: nucleus and ➤: glycogen + acidophilic cytoplasm is seen. (Hematoksilen Eozin × 100 [A], X400 [B]). *CLL in pictures belonging to the quercetin group*: classic liver lobule, → : vena centralis, ▶ : hepatocyte, ⇉ : sinusoid, ▷ : nucleus and ➤ : glycogen + acidophilic cytoplasm is seen. (Hematoksilen Eozin × 100 [A], X400 [B])

On the other hand, it was determined that the morphological deterioration and radial arrangement of hepatocytes returned to normal, vacuolar degeneration and lipid droplets disappeared, pycnotic nuclei continued to be seen in some cells with a small amount of infiltration,but hepatocyte staining properties returned to normal in general. It was observed that the general appearance and degenerative changes in the hepatocyte level returned to normal, except for the dilatation in the vena centralis and sinusoids in the classical liver lobule (Fig. 7).

As a result, it was determined that fructose administration caused structural degenerations compatible with steatosis in the liver and hepatocyte degeneration at a level that would affect the liver function. On these degenerations; it was observed that the applied quercetin had a better regenerative effect, especially in the portal area.

Discussion

The demonstration in animal and human experimental models that MetS can be generated by a high fructose diet is proof that environmental factors should be addressed seriously. In our study, we used Sprague Dawley rats, which are known to be more sensitive to fructose-mediated MetS formation, [23] and constructed MetS by adding 20% fructose to the drinking water. The development of hypertriglyceridemia, hyperglycemia, hypertension, obesity, and insulin resistance was demonstrated in fructose groups.

The importance of quercetin in the prevention and improvement of functional changes in MetS is emphasized in the literature. Research shows that administration of quercetin once a day for five weeks in spontaneously

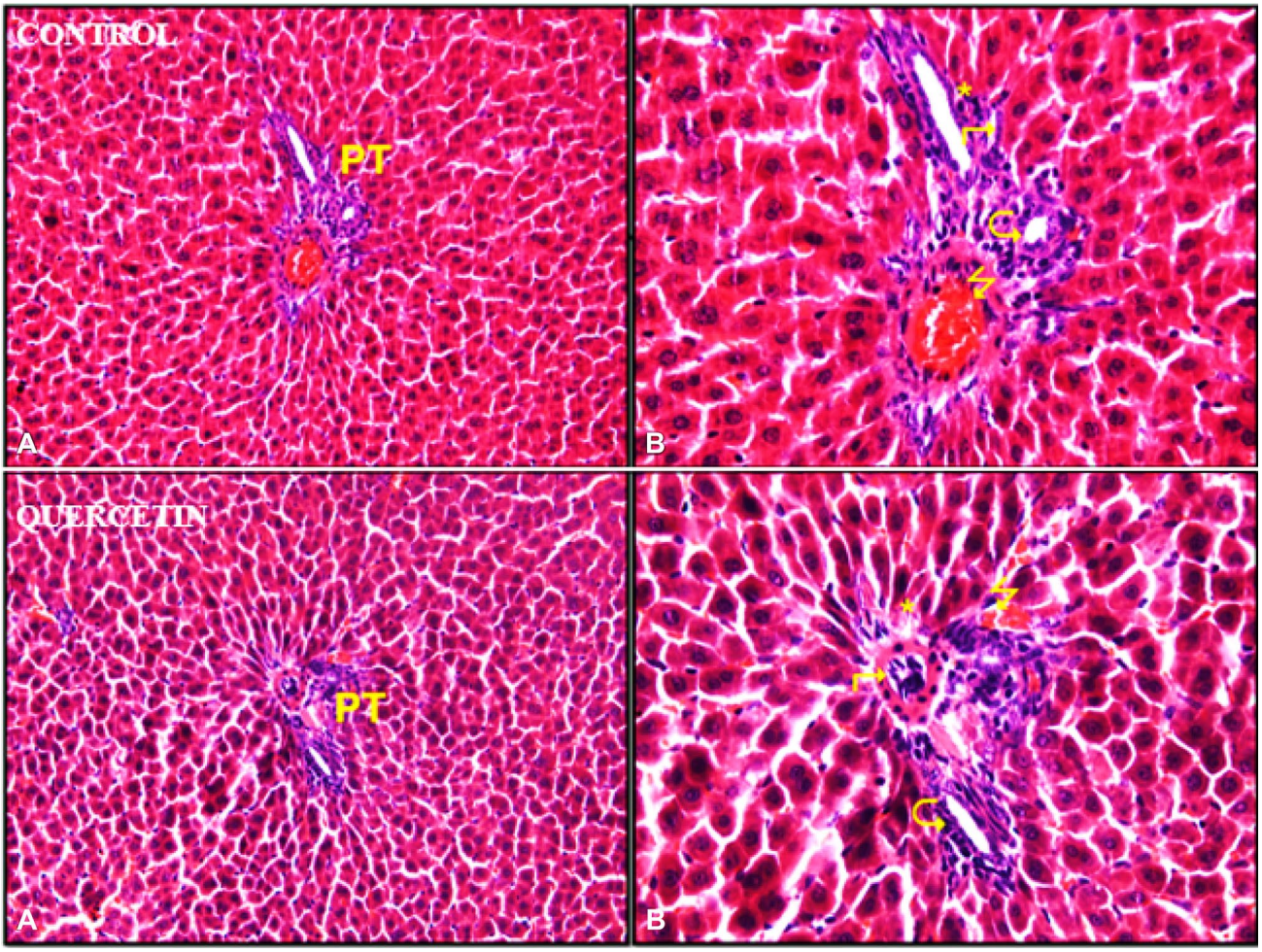

Fig. 4 *PT in the pictures of the control group*: portal alan, *: connective tissue, ↯ : hepatic artery, ↦ : hepatic vein ve ↻ : bile duct is visible. (Hematoxylin Eozin × 200 [A], X400 [B]). *PT in pictures belonging to the quercetin group*: portal area, *: connective tissue, ↯ : hepatic artery, ↦ : hepatic vein ve ↻ : bile duct is visible. (Hematoxylin Eozin × 200 [A], X400 [B])

hypertensive rats significantly decreased systolic blood pressure [24]. A human study reported that 8-week administration of quercetin exhibits anti-hypertensive effects by acting on the endothelium [25]. Mostardo et al. reported that administration of high amounts of fructose to rats caused MetS, while demonstrating that administration of small amounts of quercetin reduced blood pressure and that the anti-hypertensive effect was positively associated with the effect on the endothelial disorder, [26] which was in agreement with the results of our study. Individual comparison of systolic blood pressure in the groups revealed an increase in the fructose group and the fructose + quercetin group at the end of week 5 and 10 compared to the baseline value. A significant decrease was observed in blood pressure in the quercetin group. Decreased blood pressure was observed in the group in which quercetin was administered with fructose (Table 2).

Evaluation of the Lee index data, which is an indicator of obesity in our study, reveals that rats' body weight increased in line with fructose administration, indicating the development of obesity. Administration of quercetin appears to improve fructose-induced obesity. In their study, Kim et al. showed that eating foods containing quercetin reduced body fat mass [27]. Rivera et al. reported that the effect of quercetin on body weight varies depending on the amount administered: while high doses of quercetin may reduce body weight, low doses may be ineffective [26]. There is a difference between the initial weights of the groups in our animals. Rats one week younger than the others were provided to the control group and therefore their height as well as their weight are smaller than the other groups. In addition, since the obesity index is used as a criterion for metabolic syndrome, this difference does not pose a problem in the interpretation of the study. In studies in the literature, it is seen

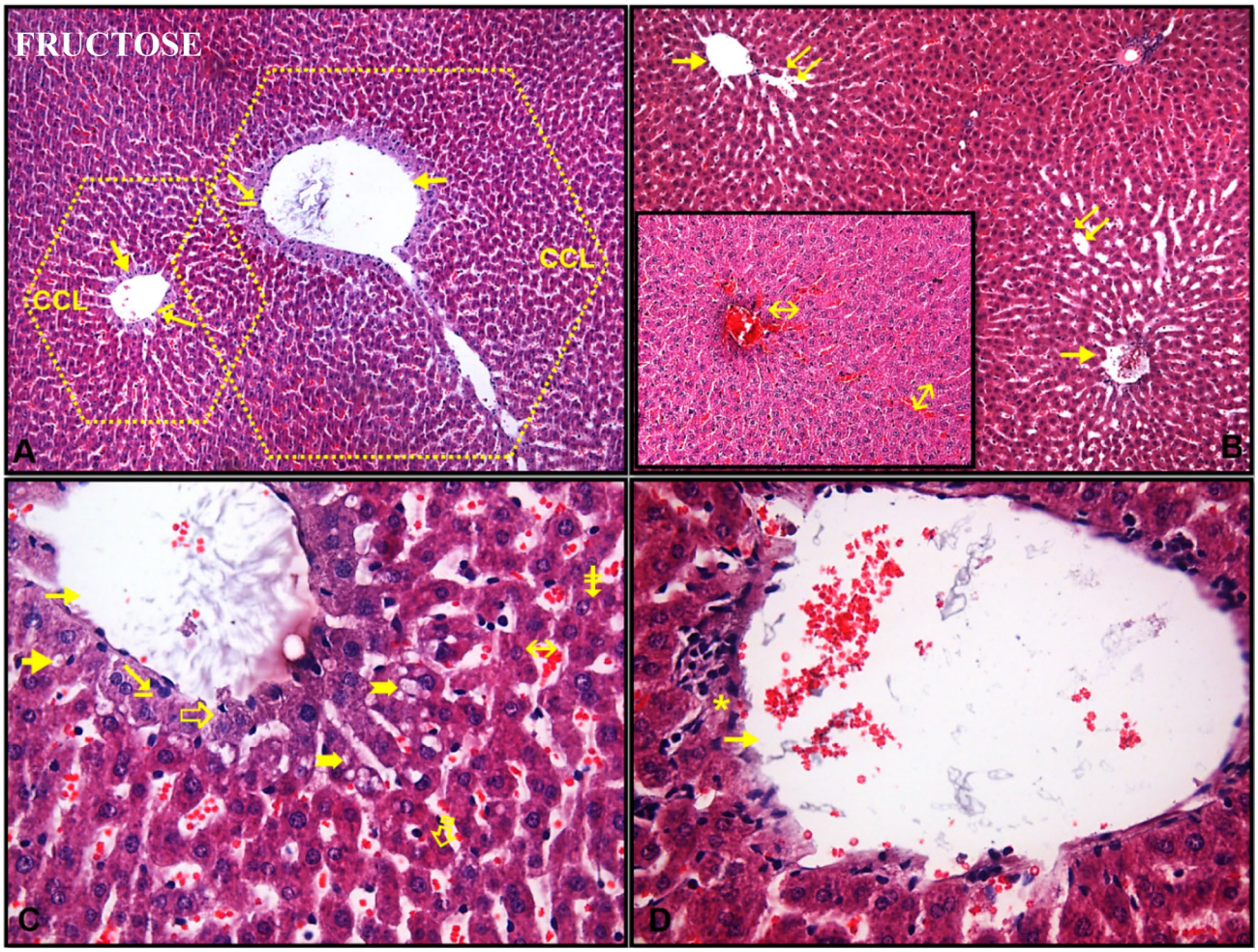

Fig.5 CLL in pictures belonging to the fructose group: classical hepatic lobule (note that their arrangement is disturbed), ➝ : dilated vena centralis, ⇉ : dilated sinüzoid, ↔: congestion (B-Inset), ↘ : hepatocytes that do not show acidophilia, ⇨ : morphologically impaired hepatocytes, ↨ : hepatocyte cell clumps, ⇪ : pycnotic nucleus, ➧ : vacuolar degeneration, ➦ : lipid droplets, *: increased density of connective tissue around the vena centralis and ➧ : infiltration is seen (Hematoxylin Eosinx100 [A], X400 [B])

that 30–500 mg/day quercetin is used. The dose of 15 mg/kg quercetin we applied was considered as the dose that can be taken with a healthy diet, no external quercetin supplements other than food, and no side effects. Studies using higher doses are also included in the discussion section.

In our study, it was determined that fructose resulted in a statistically significant increase in the triglyceride level. Triglyceride was observed to increase in the fructose+quercetin group. Panchal et al. reported that triglyceride levels increased after the administration of quercetin to rats fed with corn syrup. It was argued that the administration of quercetin resulted in lipolysis and inhibited the storage of these fats in tissues [28]. The results of our study are also consistent with this study. In our study, it was observed that when administered alone, quercetin increased HDL and LDL cholesterol, but decreased VLDL cholesterol compared to the fructose group. On the other hand, it was observed that co-administration of fructose and quercetin reduced total cholesterol and LDL cholesterol compared to the fructose group, and had no significant effect on HDL and VLDL cholesterol levels (Table 3). In the light of the literature, the dose of 15 mg/kg quercetin is considered to be the dose that can be taken daily with a healthy diet. With the 15 mg/kg quercetin dose in our study, we found a statistically insignificant increase in triglyceride and total cholesterol in the quercetin-only group. We did not see any improvement in these parameters when quercetin was given together with fructose. During sacrification, we noticed an increase in abdominal adiposity in the guercetin group at this dose. Histologically, there was no visceral fat in the liver. However, in the findings of studies using high-dose quercetin, the increase in abdominal adiposity and lipid profile with the use of quercetin alone was not discussed. In most of the literature, significant improvements in the lipid profile have

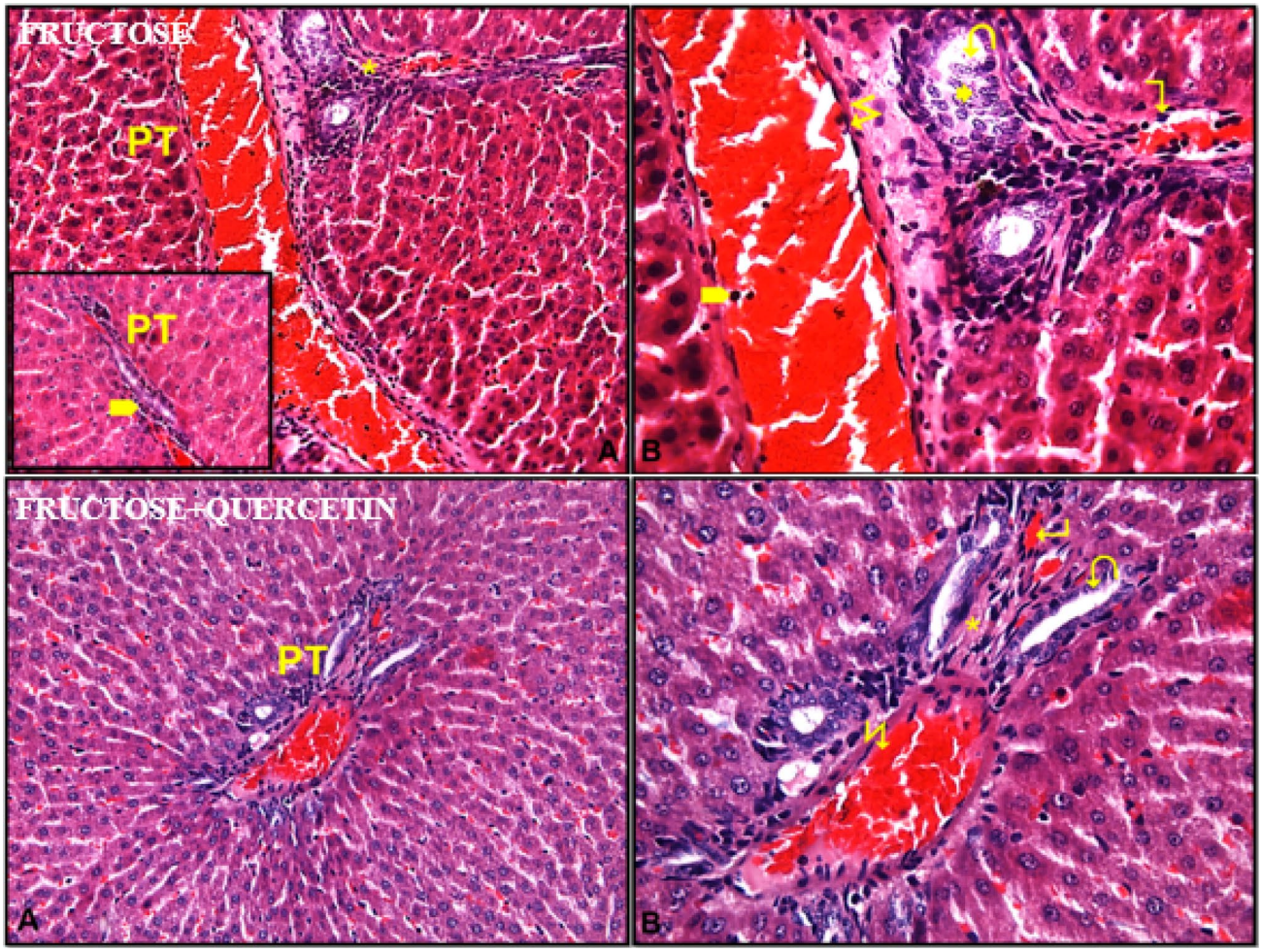

Fig. 6 PT in pictures belonging to the fructose group: portal area, ➡ : infiltration (A-Inset ve B), * : increased density of connective tissue, ↯ : dilated hepatic artery,↦ : hepatic vein, ↻ : bile duct and ➧ : bile duct epithelium that has lost its normal arrangement is seen (Hematoxylin Eosin×200 [A], X400 [B]). *PT in pictures belonging to the Fructose+Quercetin group* : portal area, * : connective tissue, ↯ : dilated hepatic

been reported with the administration of quercetin. This situation, the reason of which we cannot explain, may be due to our very low dose. Since it cannot be supported by similar literature, it is a situation that needs to be clarified.In meta-analyses conducted to explain the reasons for the different metabolic effects of quercetin, it has been stated that its beneficial effects on carbohydrate and lipid metabolism are more pronounced at doses above 500 mg/day, and there are controversial and different results in studies below this dose and as the sample size decreases. In the related meta-analysis, it was determined that there would be no increase in total cholesterol, decrease in triglyceride levels (at 500 mg/day doses), no significant effect on LDL-C and HDL-C [29]. It was observed that fructose caused an increase in serum glucose and insulin levels. Elevated HOMA-IR values were also indicative of the formation of insulin resistance Available studies with fructose-mediated MetS models reported similar changes in these parameters, which was consistent with our study [30–32]. Comparison of the quercetin and fructose groups showed that serum glucose, insulin and HOMA-IR values decreased in the group receiving quercetin similar to the values of the control group Co-administration of quercetin with fructose significantly lowered insulin levels, slightly lowered glucose levels, and have a positive effect on insulin resistance, compared to the fructose group (Table 3). Abo-Youssef argued that quercetin administration reduces glucose entry into enterocytes via glucose transporter-II and its mechanism of action is to increase GLUT-4 activity in muscles. In his study, he administered 50 mg/kg of quercetin to rats, on which he created diabetes, daily for 14 weeks and reported a decrease in insulin and HOMA-IR values [30]. Roslan et al. applied quercetin to diabetic rats at 10, 25 and 50 mg/kg/day for 28 days and found that insulin levels, which were very low at the beginning of the

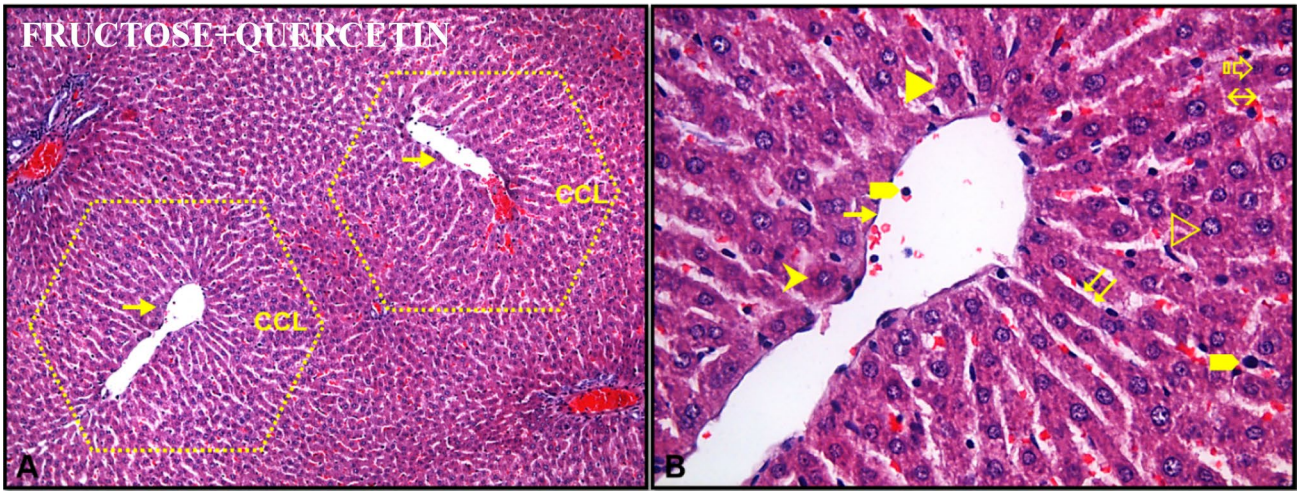

Fig. 7 CLL in pictures belonging to the Fructose + Quercetin group : classic liver lobule, → : vena centralis, ▶ : hepatocyte, ⇉ : sinusoid, ▷ : nukleus, ➤ : glycogen + acidophilic cytoplasm, ↔ : congestion, ⇪ : pycnotic nucleus and ➡ : infiltration is seen (Hematoxylin Eosin × 100 [A], X400 [B])

experiment compared to the control group, increased as a result of the administration of quercetin. In line with these results, they reported that the administration of quercetin exerted a therapeutic effect on rats with diabetes [33]. We think that the reason why our findings differ from the literature is due to the difference in dose and duration of our quercetin administration and the difference in the rat breed.

After the recognition of FGF-21 as a potential novel hepatic hormone in the treatment of T2DM, obesity, liver steatosis and other metabolic disorders, studies have been conducted to investigate the mechanisms underlying its production under physiological and pathophysiological conditions. We think that FGF-21, which was shown to reduce most of the metabolic diseases in animal models associated with obesity, including hyperglycemia, insulin resistance, dyslipidemia and hepatosteatosis [34, 35] may act as a biomarker for MetS, as well as a guide for disease prevention and treatment. Elevated FGF-21 protein levels in rodents has been shown to improve glucose homeostasis, reduce hepatic lipid, increase whole body energy expenditure and reduce body weight [36]. Fasting and ketogenic diets were reported to increase FGF-21 protein levels release in the liver [34, 37]. In our study, it was observed that the level of hepatic FGF-21 protein decreased in the fructose group compared to the control group, and in agreement, it was found that the FGF-21 mRNA expression levels decreased 1.53 times in the fructose group. Although the hepatic FGF-21 protein level did not increase significantly in the quercetin-administered group compared to the control group, the FGF-21 mRNA expression level was found to be 1.6 times higher. Administration of quercetin alone increased FGF-21 expression, but did not increase its amount in tissue. In the group in which fructose and quercetin were administered together, the amount of FGF-21 in the tissue was similar to the control group.FGF-21 mRNA expression was found 0.22 times lower than the control group. This decrease was not actually a decrease in expression, but is an indication that the fructose-induced decrease in hepatic FGF-21 protein level and mRNA expression is recovered and increased by the co-administration of quercetin. It was found that the FGF-21 mRNA expression increased 1.31 times in the fructose+quercetin group compared to the fructose group. At the end, hepatic FGF-21 protein levels were similar to the levels of FGF-21 in the control group as a result of the elevations in expression (Tables 4 and 5).

In agreement with the literature, it was shown that fructose suppresses FGF-21 mRNA expression in liver tissue, and quercetin stimulates hepatic FGF-21 production in rats on fructose diet. Our study demonstrated that fructose-induced suppression of mRNA expression was improved as a result of the administration of quercetin.

PGC-1α, an inducible transcriptional coactivator, is heavily expressed in metabolically active tissues. During starvation, the stimulation of hepatic PGC-lα stimulates the transcription of genes involved in fatty acid oxidation, tricarboxylic acid (TCA) cycle flux, mitochondrial oxidative phosphorylation and gluconeogenesis. PGC-1α is expressed at very low levels in the liver during fed conditions. There is evidence that PGC-1α promoter activity is inhibited by insulin [38]. At the same time, hepatic PGC-1α mRNA expression was shown to increase in animal models of insulin deficiency [39].

In our study, hepatic PGC-1α mRNA expression was also found to decrease 1.23 times in the fructose group. In agreement, it was observed that hepatic PGC-1α protein level decreased in the fructose group compared to the control

group, but it was not statistically significant. In the quercetin-only group, a significant increase was observed in hepatic PGC-1α protein level compared to the control and fructose groups while the PGC-1α mRNA expression was found to be 1.58 times higher than the control group. With the administration of quercetin alone, a statistically significant increase was achieved in the mRNA expression of PGC-1α and its amount in tissue. In the group in which fructose was administered in combination with quercetin, the amount of PGC-1α in tissue was found to be significantly higher than in the control and fructose groups. PGC-1α mRNA expression increased 0.82 times compared to the control group. This elevation is actually more significant in terms of expression. It is an indication that quercetin not only results in recovery but also improvement in the profound fructose-induced reduction in the amount of PGC-1α and mRNA expression in liver tissue. It was observed that PGC-1α mRNA expression of the fructose+quercetin group increased 1.5 times when compared to the fructose group (Tables 4 and 5).

Research shows that stimulation of FGF-21 production involves activation of the nuclear receptor PPARα, which is a key stimulus for hepatic FGF-21 gene transcription. This study demonstrates that fructose-induced FGF-21 resistance, which includes the reduction of FGFR1 and bKlotho expression and the activation of the PPARα//FGF-21// PGC-1α axis, can be overcome by long-term administration of quercetin in fructose-fed rats. [40]

In this study, it was shown that FGF-21 and PGC-1α mRNA expressions, which are important metabolic regulators of glucose and lipid metabolism, exert parallel responses to the administration of fructose, quercetin and quercetin combined with fructose in liver tissue. Our study is one of the first studies on quercetin and PGC-1α. It was demonstrated that the fructose-induced suppression of FGF-21 and PGC-1α in experimental animals was improved by the administration of quercetin. According to the results of our study, we can say that the administration of quercetin increased PGC-1α mRNA expression through FGF-21, which is located in the PPARα pathway, by which it achieved its positive effects on metabolic syndrome components. However, there is a need for further and larger studies where the whole PPARα pathway and its effects on muscle and adipose tissue, two major tissues for insulin resistance, are investigated and revealed to fully prove these effects. Further studies are needed to be conducted in this regard to support the interpretation of data we concluded in our study.

Conclusion

In our study, rat model of MetS was successfully created with the administration of 20% fructose and sufficient criteria were provided. We investigated possible positive effects of quercetin at a dose of 15 mg/kg on hepatic FGF-21 and PGC-1α protein levels and mRNA expression. Administration of quercetin alone or in combination with fructose increased hepatic FGF-21 and PGC-1α protein levels, as well as increasing mRNA expression. The results of our study suggest that the 10-week administration of 15 mg/kg quercetin will be beneficial for lipid and carbohydrate metabolism in the fructose-mediated MetS model, and its effects at this dose may even have greater effects in the long-term use. Future studies with quercetin should focus on revealing the mechanisms of MetS components with different duration and doses, and also elucidating the effects of possible antioxidant drugs on these components. We believe that significant progress can be achieved in the pathogenesis and treatment of MetS by supporting comprehensive projects on the mechanisms of action of quercetin, a promising molecule in the treatment of metabolic syndrome, with transcriptomic, proteomic and metabolomic studies.

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Author contributions All authors contributed equally to this manuscript. All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by KKK, SŞ, BN and CY. The first draft of the manuscript was written by KKK and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Declarations

Competing interests The authors have no relevant financial or non-financial interests to disclose.

Ethical approval Approval for our study was obtained from Gazi University Animal Experiments Local Ethics Committee. Ethics committee number: G.Ü.ET-19.016. The relevant document is shared below. All experiments were performed considering Guide for the Care and Use of Laboratory Animals. This regulation by prepared on the basis of Articles 9 and 17 of the Animal Protection Law No: 5199 dated 24/6/2004 and in parallel with the European Union Directive on the Protection of Animals Used for Scientific Purposes No: 2010/63/EU.

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