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# Immunohistochemical and Electron Microscopic Changes in the Liver of Rats Fed High-Fructose and High-Fat Diet

## Yüksek Fruktoz ve Yüksek Yağlı Diyetle Beslenen Sıçanların Karaciğerinde İmmünohistokimyasal ve Elektron Mikroskobik Değişiklikler

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## ABSTRACT

**Objective:** This study aimed to investigate the effect of high-fructose and high-fat diets on advanced glycation end products (AGE) and their receptors (RAGE) in rat liver immunohistochemically and ultrastructurally.

**Materials and Methods:** Rats were divided into three groups as the control group, high-fructose group, and high-fat group. The high-fructose group was given 60% fructose diet, and the high-fat group was fed 45% fatty diet. After 12 weeks, rats were sacrificed, and liver tissues were taken for light and electron microscopic examinations. Expressions of AGE, RAGE, nuclear factor kappa B (NF-κB), and 8–hydroxyguanosine (8-OhdG) were detected immunohistochemically. Liver function enzymes, namely, aspartate transaminase, alanine transaminase, alkaline phosphatase, and gamma-glutamyl transferase, were measured from serum samples.

**Results:** Prominent degenerative changes were detected in the liver sections of both diet groups. Immunostaining of AGE, RAGE, NF-κB, and 8-OHdG was more evident than that of the control group. Activities of the liver function enzymes of these groups were higher than those of the control group. Ultrastructurally, accumulations of glycogen and lipid droplets in hepatocytes were observed in the diet groups.

**Conclusion:** High-fructose and high-fat diets can cause ultrastructural changes in the liver, and the damage can be related with increased AGE.

**Keywords:** Advanced glycation end products, electron microscopy, liver, receptor for advanced glycation end products

## ÖZ

**Amaç:** Bu çalışma, sıçan karaciğerinde ileri glikasyon son ürünleri (AGE) ve reseptörleri (RAGE) üzerine yüksek fruktozlu ve yüksek yağlı diyetlerin etkisini immünohistokimyasal ve ultrastrüktürel olarak araştırmayı amaçlamıştır.

**Gereç ve Yöntem:** Sıçanlar kontrol grubu, yüksek fruktoz grubu ve yüksek yağlı grup olarak üç gruba ayrıldı. Yüksek fruktozlu gruba%60 fruktozlu diyet, yüksek yağlı gruba ise %45 yağlı diyet uygulandı. 12 hafta sonra, sıçanlar sakrifiye edildi ve ışık ve elektron mikroskobik incelemeler için karaciğer dokuları alındı. AGE, RAGE, nükleer faktör kappa B (NF-κB) ve 8-hidroksiguanozin (8-OhdG) ekspresyon düzeyleri immünohistokimyasal olarak tespit edildi. Serum örneklerinden aspartat transaminaz, alanin transaminaz, alkalin fosfataz ve gama-glutamil transferaz gibi karaciğer fonksiyon enzimleri ölçüldü.

**Bulgular:** Her iki diyet grubunun karaciğer dokularında belirgin dejeneratif değişiklikler saptandı. AGE, RAGE, NF-κB ve 8-OHdG'nin immün boyanması, kontrol grubuna göre daha belirgindi. Bu grupların karaciğer fonksiyon enzim aktiviteleri, kontrol grubuna göre daha yüksekti. Ultrastrüktürel olarak diyet gruplarında hepatositlerde glikojen ve lipid damlacıklarının birikimleri gözlendi.

**Sonuç:** Yüksek fruktozlu ve yüksek yağlı diyetler karaciğerde ultrastrüktürel değişikliklere neden olabilir ve artmış hasar AGE ile ilişkili olabilir.

**Anahtar Kelimeler:** İleri glikasyon son ürünleri, elektron mikroskobu, karaciğer, ileri glikasyon son ürünleri için reseptör

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## INTRODUCTION

As a result of the high caloric intake in Western diets, increased intra-abdominal fat content may contribute to liver fat accumulation (1). Abnormal fat deposition in the liver is associated with liver diseases such as non-alcoholic fatty liver disease (NAFLD) (2). NAFLD is a common chronic liver disease and is characterized by increased fat deposition in the liver in individuals without excessive alcohol consumption. NAFLD includes many liver disorders, such as steatosis, steatohepatitis, hepatocellular carcinoma, lobular inflammation, and fibrosis (3, 4). The liver is also an organ involved in advanced glycation end product (AGE) metabolism and elimination. This function is performed through receptors in liver sinusoids and Kupffer cells. The liver also contributes to the production of inflammatory molecules caused by oxidative stress with AGEs (5). AGEs are produced both exogenously in foods and endogenously in the body. High amounts of exogenous AGE is found in highly processed food. Dietary AGE restriction significantly reduces inflammation and oxidative stress in patients with type 2 diabetes (6). AGEs and their receptors (RAGE) are compounds that play an essential role in liver disease pathogenesis (7). AGEs interact with cell surface receptors RAGE. This interaction stimulates the transcription factor nuclear factor-kappa B (NF-κB), which increases the secretion of inflammatory cytokines and oxidative stress markers such as 8-hydroxy-2-deoxyguanosine (8-OHdG). The increase in the severity of inflammation contributes to cellular dysfunction and tissue destruction. Furthermore, AGEs destroy the intracellular detoxification mechanism (8, 9). The level of alanine aminotransferase (ALT) is elevated in liver diseases, and its test is most useful in detecting damage due to hepatitis, liver-toxic drugs, and other substances. Compared with aspartate aminotransferase (AST), ALT is more specific for the liver and is less affected by diseases affecting other body parts (10, 11). Gamma-glutamyl transferase (GGT) is an enzyme found in many organs such as the spleen and pancreas. However, the primary source of GGT is the liver, and it is very sensitive to liver function. Alkaline phosphatase (ALP) is an enzyme in protein structure found in various body tissues. Levels of this enzyme increase in pathologies such as liver damage and obstruction of the biliary tract (12, 13).

This study aimed to investigate the ultrastructural changes in diet-induced liver damage and the relationship between the injury and AGE and their receptors and to determine the liver enzyme levels and molecular pathways involved in this pathophysiology to evaluate the effect of fructose and fat diet on the liver.

## MATERIAL and METHODS

### Chemicals

For this study, 60% fructose feed and 45% fat feed were purchased from Altromin International (Germany). Other chemicals were taken from Sigma-Aldrich International (St. Louis, MO, USA), Biorbyt Explore Bioreagents (CA, USA), and Santa Cruz Biotechnology (CA, USA).

### Animal categorization and treatment

Male Sprague-Dawley rats (150–250 g) were obtained from the Atatürk University Experimental Research and Application Center. The rats were housed on sawdust bedding in plastic cages. The animals were housed under laboratory conditions at room temperature of 22 ± 2°C, humidity of 55%, and 12-h light and 12-h dark environment. During the experiment, the animals were allowed ad libitum food and water consumption.

This study was approved by Atatürk University Animal Experiments Local Ethics Committee (13/179). According to the NIH guidelines for the use of laboratory animals, all applications were carried out by protecting animal rights. Twenty-four rats were equally divided into three groups. G1 was given with standard rodent diet for 12 weeks (control group). G2 was fed only 60% fructose diet for 12 weeks (high-fructose diet group) (14, 15). G3 was fed only 45% fatty diet for 12 weeks (high-fat diet group) (16, 17). After the experiment, the rats were sacrificed with administration of a combination of 100 mg/kg of ketamine and 15 mg/kg of xylazine, and liver tissues and blood samples were taken.

### Histopathological examinations and assessments

In preparation for light microscopic examination, the liver tissues were fixed for 72 h in 10% formaldehyde solution. Later, the tissues were dehydrated in increasing graded series of ethyl alcohol and embedded in paraffin. Liver tissues embedded in paraffin blocks were cut at a thickness of 5 μm. The sections were stained with hematoxylin-eosin (H&E) and Gomori trichrome stains for evaluating of morphological structure and detecting collagen fibers, respectively. Histological examination of liver tissues was performed under the Nikon Eclipse E600 microscope (Nikon, Tokyo, Japan) and photographed.

### Immunohistochemical assessments

For immunohistochemistry tests, sections were collected on poly-L lysine-coated slides and then deparaffinized. AGE immunostaining was performed using a rabbit polyclonal antibody (1:500 dilution) (Biorbyt Explore Bioreagents). The expression of RAGE was determined by the rabbit polyclonal antibody (1:200 dilution) (Biorbyt Explore Bioreagents). Immunohistochemical staining procedures of 8-OHdG and NF-κB were performed using mouse monoclonal antibodies (1:500 dilution) (Santa

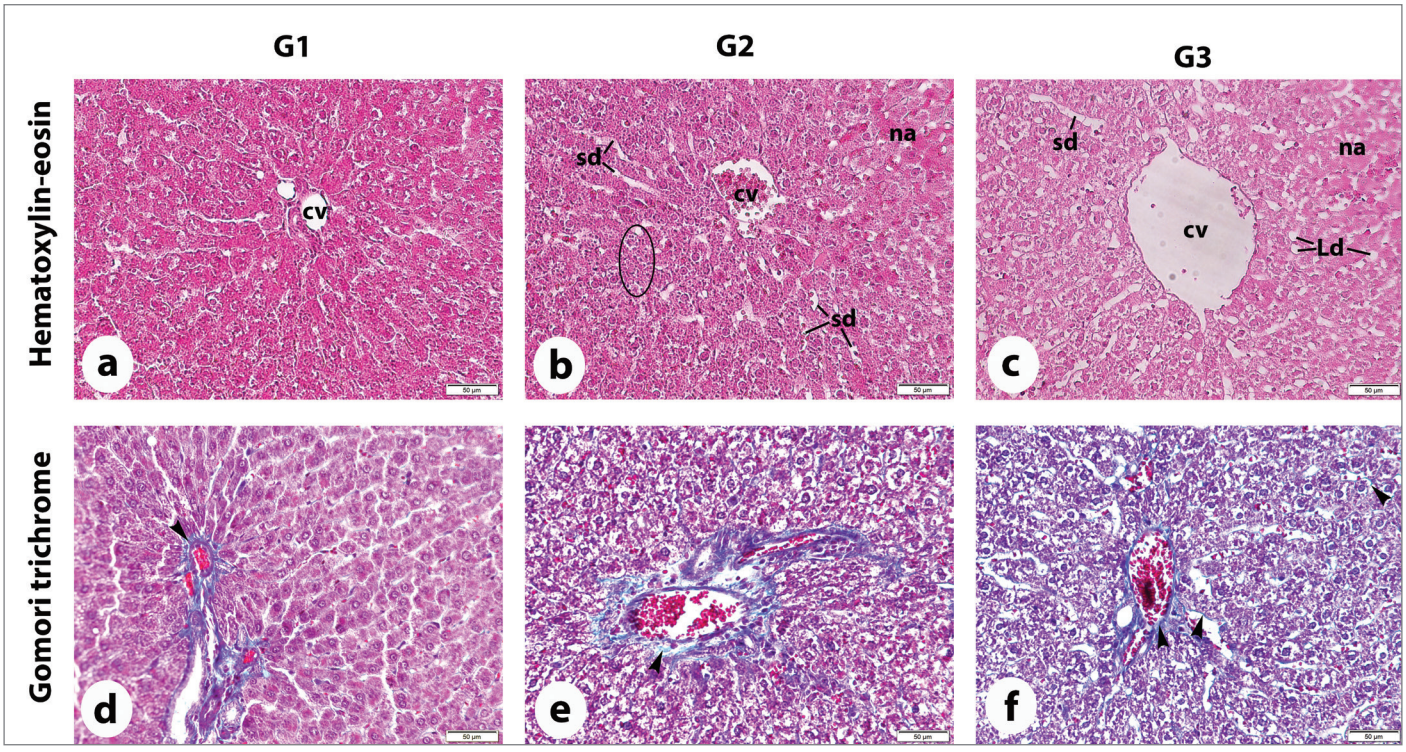


**Figure 1. a-f.** Light micrographs of Hematoxylin-eosin and Gomori trichrome stained liver sections (a, d); G1(Control), (b, e); G2 (High Fructose Diet), (c, f); G3 (High-Fat Diet)
cv; central vein, sd; sinusoidal dilatation, circle; inflammatory cells, na; necrotic area, Ld; lipid droplets, arrow head; collagen fibrils. Bars; 50 µm.

Cruz Biotechnology). The sections were loaded onto an automatic immunohistochemistry staining machine (Ventana Benchmark GX). The slides were then incubated with the diluted antibody, followed by the application of an ultraView Universal DAB Detection Kit (Ventana Medical Systems, Inc., Cat. No. 5269806). DAB was used as the chromogen, and hematoxylin (Ventana Medical Systems, Inc., Cat No. 5266726) was used as the counter-stain. After the slides were dehydrated, they were covered by coverslips. Photographs were taken by two investigators using a light microscope (Nikon Eclipse E600, Tokyo, Japan). The immunohistochemical evaluation was made by two researchers, using a semi-quantitative immunoreactivity scoring method. Five areas were evaluated and scored in each liver slide by taking the mean staining intensity into account (18). The immunostaining score (19) was obtained as described below: very little or no immune reactivity,–(0%); mild immune positivity, + (0%–30%); moderate, ++ (30%–60%); severe, +++ (60%–100%).

### Estimation of serum biochemical parameters

Blood samples were centrifuged at 4000 rpm for 10 min. For the blood biochemistry analysis of ALT, AST, ALP, GGT, serum samples were stored in the refrigerator at -80°C until analyzed. The enzyme activities were measured by the spectrophotometric method in Beckman Coulter AU 5800 (CA, USA) clinical chemistry analyzer.

### Preparation for electron microscopic examination

The liver tissues were cut approximately 1 mm in thickness and fixed quickly in 2.5% phosphate-buffered glutaraldehyde (pH 7.4). The specimens were then post-fixed in 1% osmium tetroxide for 1 h and washed in 0.1 M phosphate buffer. Tissue samples were then dehydrated in graded ethanol series and embedded in Araldite-Epon (20). The liver sections (0.5 µm thick) were cut using an ultramicrotome (LKB NOVA, Bromma, Sweden). The ultrathin sections were cut (60 nm) and then stained with uranyl acetate and lead citrate.

### Statistical analysis

Data were evaluated statistically by using GraphPad Prism 6.01 (GraphPad Software, Inc.). The significance between groups was analyzed according to Tukey's post-hoc tests from the one-way analysis of variance multiple comparison test. A value of $p<0.05$ was considered statistically significant.

## RESULTS

### H&E and Gomori trichrome staining results

H&E-stained liver sections of G1 were examined histologically, showing hepatocyte cords that extend radially around the central vein. Sinusoidal spaces were normal in size (Figure 1a). In the liver sections of G2, prominent degeneration in cells was noted, cell boundaries could not be distinguished, and nuclei were not seen. In addition to

**Table 1.** Immunohistochemical staining scores of liver tissue

| Groups | AGE | RAGE | NF-kB | 8-OhDG |
|---|---|---|---|---|
| G1 | - | - | + | - |
| G2 | +++ | +++ | +++ | ++ |
| G3 | +++ | +++ | +++ | +++ |

According to immunohistochemical findings: – none (-), mild (+), moderate (++) and severe (+++).
G1(Control), G2 (High Fructose Diet), G3 (High-Fat Diet).

these necrotic sites, inflammatory cells were seen commonly (Figure 1b). In the liver sections of G3, widespread intracytoplasmic swelling, vacuolization in hepatocytes, and pyknosis in the nuclei were observed. Besides, the presence of abundant lipid droplets in the liver was remarkable (Figure 1c). Histological evaluation of G2 samples revealed irregular hepatocyte plaques. Sinusoidal dilatation and lipid droplets were occasionally present, although not as much as in G3.

In Gomori trichrome staining, connective tissue areas consisting of small amounts of collagen fibers were observed both in the central vein and around the portal area of G1 (Figure 1d). In G2 and G3, collagen deposition in the portal area was observed in Gomori trichrome-stained sections, and collagen deposition in sinusoidal areas was remarkable (Figure 1e and 1f).

### Immunohistochemistry results

Representative images of AGE expression are depicted in Figure 2a-2c. The immunostaining levels of AGE protein were prominent in liver sections of both G2 and G3 compared with those in G1 (Figure 2a-2c, and Table 1). RAGE expression was weaker in G1 (Figure 2d, and Table 1). However, G2 and G3 had a strong RAGE expression (Figure 2e, 2f and Table 1). NF-κB expression is shown in Figure 2g–2i. The immune reactivity level of NF-κB protein was significant in liver sections in G2 and G3 (Figure 2g–2i and Table 1). Thus, NF-κB expression increased in liver cells of rats fed high-fructose and high-fat diets. When the diet groups were compared with each other, immunostaining of all proteins in G3, especially 8-OHdG, was higher than those in G2 (Figure 2j-2l and Table 1). Thus, compared with the high-fructose diet, the high-fat diet markedly stimulated 8-OHdG formation in rat liver cells.

### Biochemical results

High-fructose and high-fat diets can increase the serum activities of liver function enzymes, namely, AST, ALT, ALP, and GGT, in rats (Figure 3a-3d). Serum AST activities increased significantly in both G2 and G3 compared with G1 ($p<0.0001$) (Figure 3a). Serum ALT activities in G2 and G3 were statistically higher ($p<0.0001$) than that in G1. Serum ALT l activity in G3 increased statistically ($p<0.05$) compared with that in G2 group (Figure 3b). Serum ALP activities in G2 and G3 were increased compared with that in G1 ($p<0.01$ and $p<0.001$, respectively) (Figure 3c). The increase in serum GGT activities in G2 and G3 was not statistically significant ($p>0.05$) (Figure 3d).

### Electron microscopic results

Examination results of ultrathin liver sections of G1 showed that hepatocytes had normal structure and moderate glycogen. Their cytoplasm had round or oval mitochondria (Figure 4a), while those of G2 and G3 showed ultrastructural changes in the hepatocytes (Figure 4b, 4c). There was abundant glycogen accumulation in hepatocytes of G2 (Figure 4b). In G3, enlargement of mitochondria and high amounts of lipid droplets in the hepatocytes were noticeable. Matrix and cristae losses in the mitochondria were observed (Figure 4c). While high amounts of glycogen granules accumulated around the nucleus of hepatocytes of G2 (Figure 4b), the cytoplasm of hepatocytes in G3 contained few glycogen granules (Figure 4c).

## DISCUSSION

Diet has an essential role in the development of liver diseases. Fructose can cause obesity, type 2 diabetes mellitus, cardiovascular diseases, liver and kidney diseases, and cancer depending on the dose and duration of intake (21). A high-fat diet can induce obesity and metabolic diseases (22). NAFLD is a clinical and pathological condition that progresses in end-stage liver disease, and it is related with increased consumption of saturated fatty acids, cholesterol, and fructose-rich products (23). Nowadays, an intake of high-fructose and high-fat foods has been considered an important health threat in the emergence of chronic diseases. Thus, this study aimed to investigate the possible effects of high-fructose and high-fat diet on rat liver.

Studies have shown that inflammation, hepatic steatosis, hepatocyte necrosis, and fibrosis structure disrupt the cellular structure through micro- and macrovascular mechanisms in liver damage (24). In this study, H&E staining showed deterioration of hepatocyte cords and dilatation of sinusoids. Necrotic areas and inflammatory cells were observed in some places. In G3, the presence of abundant lipid droplets in the liver was remarkable. Both high-fructose and high-fat diets caused degenerative changes in the liver. In many liver diseases, formation of fibrous tissue leads to the final stage called cirrhosis (25). In this study, we used Gomori trichrome staining method to show collagen accumulation in the livers of rats. Gomori trichrome staining showed a higher level of

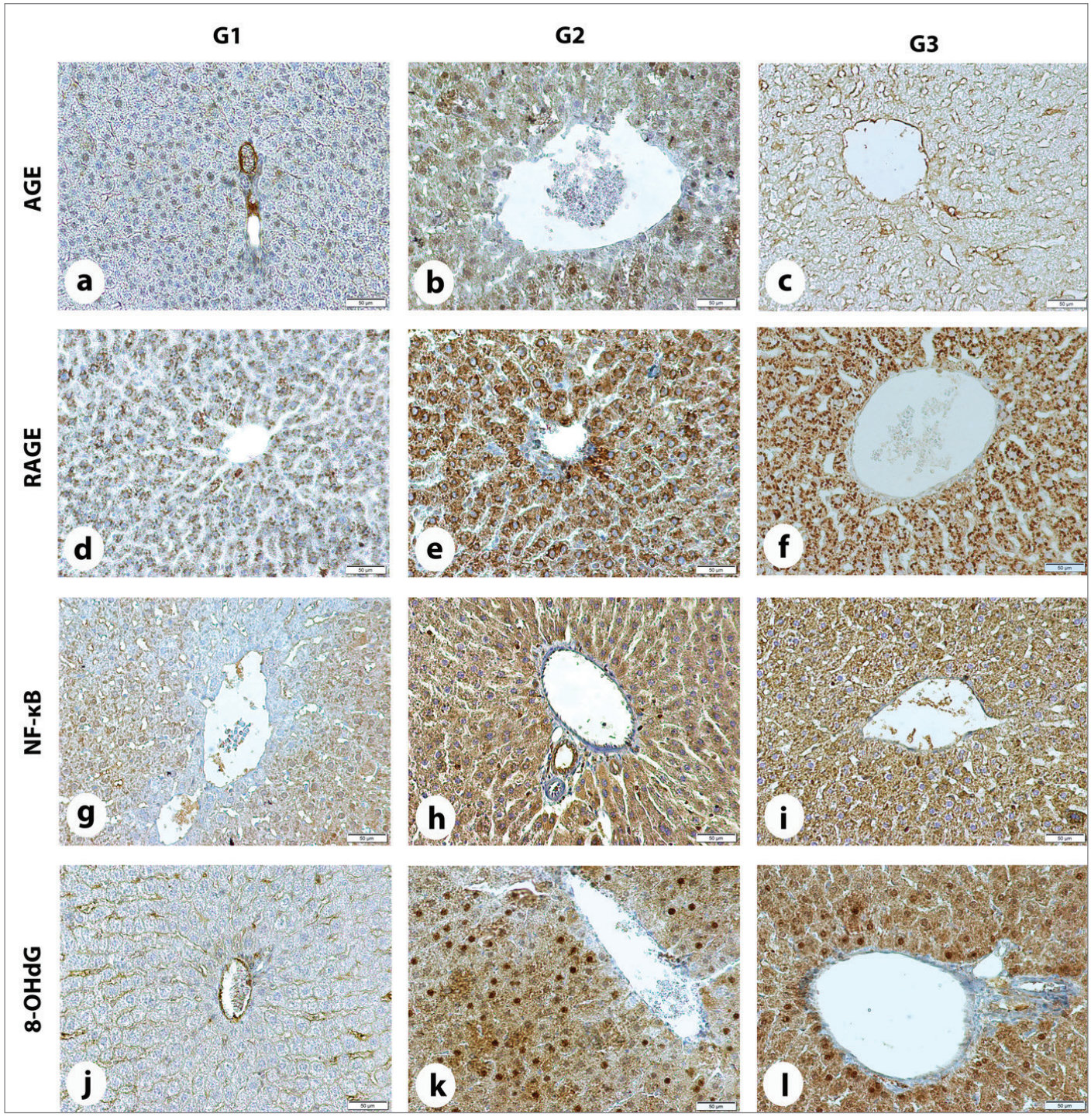


**Figure 2. a-l.** Light micrographs of immunohistochemical staining for AGE (a-c), RAGE (d-f), NF-κB (g-i) and 8-OHdG (j-l) in liver sections. G1(Control) (a, d, g, j), G2 (High Fructose Diet) (b, e, h, k), G3 (High-Fat Diet) (c, f, i, l).
Bars; 50 µm.

collagen fiber distribution around the sinusoids and portal areas in G2 and G3 than in G1. Especially, fatty diet could cause fibrosis in the liver.

Processed foods containing fructose and fatty acids are the primary sources of endogenously developed AGEs. Increased intake of processed foods with high fructose and fat contents increases AGE formation and accumulation (26, 27). The interaction of increased AGEs levels with the cellular receptor RAGE activates the cellular signaling cascade, resulting in oxidative stress and chronic inflammation. High AGE levels also increase liver damage through the activation of hepatic stellate cells involved in fat storage and fibrosis (28). In our study, compared with

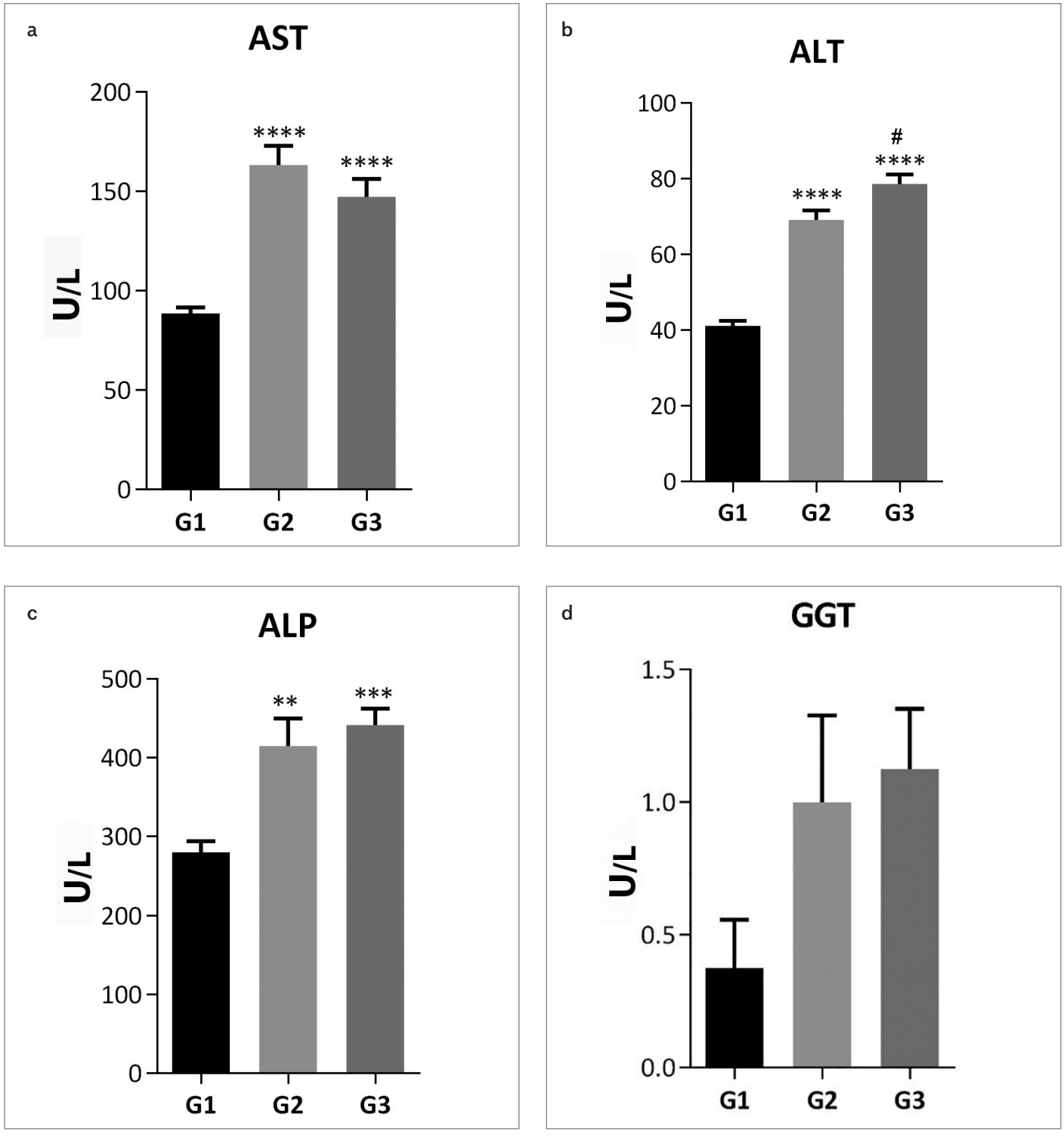


**Figure 3. a-d.** Liver function enzymes, AST (a), ALT (b), ALP (c) and GGT (d) activities in the serum. Data are given as mean ± SEM (Standard Error of Mean) (n=8).* indicates differences between control and other groups. (p<0.05:*, p<0,01: ** , p<0,001 : *** ve p<0,0001 :* ***). # refers to the differences between G2 and G3 groups (p <0.05: #). G1; Control Group; G2; High Fructose Diet Group; G3; High-Fat Diet Group. AST; Aspartate aminotransferase, ALT; Alanine aminotransferase, ALP; Alkaline phosphatase and GGT; Gamma-glutamyl transferase.

the low expression in G1, AGE and RAGE immunostaining significantly noticeable in G2 and G3. NF-κB is an important protein that is expressed by regulating many genes for differentiation, immune response, proliferation, cell adhesion, apoptosis, and angiogenesis. NF-κB stimulates the release of proinflammatory cytokines in the liver as well as in liver cell damage (29). 8-OHdG is a primary biomarker of oxidative DNA damage, and oxidative damage

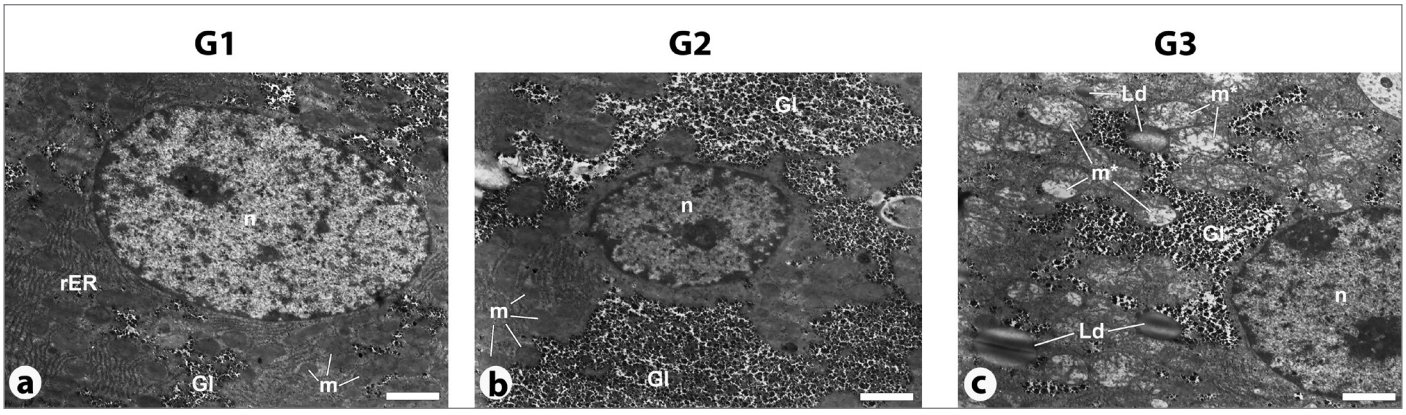


**Figure 4. a-c.** Electron photomicrographs of rat livers. (a) G1(Control), (b) G2 (High Fructose Diet), (c) G3 (High-Fat Diet) Gl; glycogen, m; mitochondria, n; nucleus, rER; rough endoplasmic reticulum, Ld; lipid droplet, m*; degenerated mitochondria. Stain; Lead citrate and uranyl acetate. Bars; 2 µm.

in DNA is determined mostly by this method. Reactive oxygen species is the most common mutagen in base DNA damage products (30). In this study, 8-OHdG and NF-κB proteins were immunohistochemically positive in G2 and G3 liver sections. However, 8-OHdG protein immunoreactivity was the highest in G3. The increase in 8-OHdG expression, which is an indicator of damage in DNA structure, signifies tissue damage. In this case, both high-fructose and high-fat diets caused damage to rat liver tissues and increased AGE and RAGE expressions were determined to be due to this damage.

Parenchymal cells (hepatocytes) are the main structural component of the liver. They regulate carbohydrate, protein, lipid metabolism, and synthesize proteins and enzymes such as albumin and clotting factors. Besides, bile regulation and secretion and detoxification of drugs and chemicals occurred in the hepatocytes. ALT, AST, ALP, GGT, and liver-related enzymes are used to determine liver function. ALT and AST are indicators of hepatocellular damage, while GGT and ALP are indicators of cholestasis (31, 32). Our results showed that high-fructose and high-fat diet increased activities of liver enzymes, such as AST, ALT, and ALP, in rat serum samples. These results supported that both fructose and fatty diets caused liver damage. The GGT value, which is one of the liver function enzymes, increased in rats fed high-fructose and high-fat diet, but this increase was not statistically significant.

Accumulation of glycogen particles in hepatocytes was observed in liver hepatocytes of patients with NAFLD by electron microscopic examination (33). In the control group, most hepatocytes had unimpaired organelles, whereas in the high-fat diet group, hepatocytes exhibited dense accumulation of lipid droplets. Impaired mitochondria were often found near lipid droplets in hepatocytes in the high-fat diet group (34), and degenerative mitochondria have matrix and cristae loss (35), and such losses were observed in the mitochondria of G3. In addition, numerous lipid droplets are noted in the cytoplasm. In G3, the cytoplasm of hepatocytes contained few glycogen granules, while in G2, many glycogen granules were deposited around the nucleus. In our electron microscopic examination, the observation of glycogen accumulation in the hepatocyte cytoplasm is an essential finding in liver damage caused by a high-fat diet. Intake of a high-fat diet may cause fatty liver, through the formation of insulin resistance (36). Insulin resistance, which eventually increases in the blood, stimulates insulin secretion, which results in hyperglycemia (37). Hyperglycemia is defined as a blood glucose level >110 mg/dl after 8–12 h of fasting (38). The liver ensures that the blood glucose concentration is maintained at approximately 90 mg/dl. When the body blood glucose level rises, some of this glucose is converted into glycogen and stored in the liver (39).

While no significant cholestasis was observed, steatohepatitis was evident in both diets. The increase in AGE and RAGE levels in these diets caused hepatocyte damage by increasing inflammatory and oxidative factors. High-fructose diet leads to storage of glycogen in addition to fat. While no significant cholestasis was observed, steatohepatitis was evident in both diets.

Although this study is limited by the non-evaluation of insulin resistance, many studies have shown that insulin resistance develops. Therefore, in this study, investigating how AGE and RAGE levels change, rather than insulin resistance, is a priority.

**Ethics Committee Approval:** Ethics committee approval was received for this study from the ethics committee of Atatürk University Animal Experiments Local Ethics Committee (13/179).

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## REFERENCES

1. Kaur J. A comprehensive review on metabolic syndrome. Cardiol Res Pract 2014; 2014: 943162. [Crossref]
2. Tarantino G, Finelli C. What about non-alcoholic fatty liver disease as a new criterion to define metabolic syndrome? World J Gastroenterol 2013; 19: 3375. [Crossref]
3. Farrell GC, Teoh NC, McCuskey RS. Hepatic microcirculation in fatty liver disease. Anat Rec (Hoboken) 2008; 291: 684-92. [Crossref]
4. Schindhelm RK, Diamant M, Heine RJ. Nonalcoholic fatty liver disease and cardiovascular disease risk. Curr Diab Rep 2007; 7: 181-7. [Crossref]
5. Guimarães EL, Empsen C, Geerts A, van Grunsven LA. Advanced glycation end products induce production of reactive oxygen species via the activation of NADPH oxidase in murine hepatic stellate cells. J Hepatol 2010; 52: 389-97. [Crossref]
6. Wang H, Li H, Jiang X, Shi W, Shen Z, Li M. Hepcidin is directly regulated by insulin and plays an important role in iron overload in streptozotocin-induced diabetic rats. Diabetes 2014; 63: 1506-18. [Crossref]
7. Chang JS, Lin SM, Chao JC, Chen YC, Wang CM, Chou NH, et al. Serum ferritin contributes to racial or geographic disparities in metabolic syndrome in Taiwan. Public Health Nutr 2014; 17: 1498-506. [Crossref]
8. Dixit VA, Bharatam PV. SAR and computer-aided drug design approaches in the discovery of peroxisome proliferator-activated receptor γ activators: a perspective. Journal of Computational Medicine 2013; 2013. [Crossref]
9. Matsui T, Higashimoto Y, Nishino Y, Nakamura N, Fukami K, Yamagishi SI. RAGE-Aptamer Blocks the Development and Progression of Experimental Diabetic Nephropathy. Diabetes 2017; 66: 1683-95. [Crossref]
10. Chou D. Henry's Clinical Diagnosis and Management by Laboratory Methods. JAMA 2007; 297: 1827-33. [Crossref]
11. Calvaruso V, Craxì A. Implication of normal liver enzymes in liver disease. J Viral Hepat 2009; 16: 529-36. [Crossref]
12. Anderson D. Harrison's Principles of Internal Medicine. AAN Enterprises; 2005.
13. Pagana KD, Pagana TJ. Mosby's diagnostic and laboratory test reference. 8th ed. St Louis: Mosby Elsevier; 2007.
14. Kho MC, Lee YJ, Park JH, Cha JD, Choi KM, Kang DG, et al. Combination with Red ginseng and Polygoni Multiflori ameliorates highfructose diet induced metabolic syndrome. BMC Complement Altern Med 2016; 16: 1-11. [Crossref]
15. Ding X-Q, Wu W-Y, Jiao R-Q, Gu T-T, Xu Q, Pan Y, et al. Curcumin and allopurinol ameliorate fructose-induced hepatic inflammation in rats via miR-200a-mediated TXNIP/NLRP3 inflammasome inhibition. Pharmacol Res 2018; 137: 64-75. [Crossref]
16. Maciejewska D, Skonieczna-Zydecka K, Lukomska A, Gutowska I, Dec K, Kupnicka P, et al. The short chain fatty acids and lipopolysaccharides status in Sprague-Dawley rats fed with high-fat and high-cholesterol diet. J Physiol Pharmacol 2018; 69: 205-10. [Crossref]
17. Xie L, Zhang K, Rasmussen D, Wang J, Wu D, Roemmich JN, et al. Effects of prenatal low protein and postnatal high fat diets on visceral adipose tissue macrophage phenotypes and IL-6 expression in Sprague Dawley rat offspring. PLoS One 2017; 12: e0169581. [Crossref]
18. Fedchenko N, Reifenrath J. Different approaches for interpretation and reporting of immunohistochemistry analysis results in the bone tissue-a review. Diagn Pathol 2014; 9: 221. [Crossref]
19. Akaras N, Abuc OO, Koc K, Bal T, Geyikoglu F, Atilay H, et al. (1→ 3)-β-d-glucan enhances the toxicity induced by Bortezomib in rat testis. J Food Biochem 2020; 44: e13155. [Crossref]
20. Bancroft JD, Gamble M. Theory and practice of histological techniques: Elsevier health sciences; 2008.
21. Jin R, Welsh J, Le N-A, Holzberg J, Sharma P, Martin D, et al. Dietary fructose reduction improves markers of cardiovascular disease risk in Hispanic-American adolescents with NAFLD. Nutrients 2014; 6: 3187-201. [Crossref]
22. Song GY, Gao Y, Di YW, Pan LL, Zhou Y, Ye JM. High-fat feeding reduces endothelium-dependent vasodilation in rats: differential mechanisms for saturated and unsaturated fatty acids? Clin Exp Pharmacol Physiol 2006; 33: 708-13. [Crossref]
23. Peng Q, Zhang Q, Xiao W, Shao M, Fan Q, Zhang H, et al. Protective effects of Sapindus mukorossi Gaertn against fatty liver disease induced by high fat diet in rats. Biochem Biophys Res Commun 2014; 450: 685-91. [Crossref]
24. Antonucci L, Porcu C, Iannucci G, Balsano C, Barbaro B. Non-alcoholic fatty liver disease and nutritional implications: special focus on copper. Nutrients 2017; 9: 1137. [Crossref]
25. Stains CUS. Special stains in interpretation of liver biopsies. Connection 2010: 92.
26. Goldin A, Beckman JA, Schmidt AM, Creager MA. Advanced glycation end products: sparking the development of diabetic vascular injury. Circulation 2006; 114: 597-605. [Crossref]

27. Karachalias N, Babaei-Jadidi R, Ahmed N, Thornalley P. Accumulation of fructosyl-lysine and advanced glycation end products in the kidney, retina and peripheral nerve of streptozotocin-induced diabetic rats. Biochem Soc Trans 2003; 31: 1423-5. [Crossref]
28. Sharma M, Mitnala S, Vishnubhotla RK, Mukherjee R, Reddy DN, Rao PN. The riddle of nonalcoholic fatty liver disease: progression from nonalcoholic fatty liver to nonalcoholic steatohepatitis. J Clin Exp Hepatol 2015; 5: 147-58. [Crossref]
29. Gavrilova O, Haluzik M, Matsusue K, Cutson JJ, Johnson L, Dietz KR, et al. Liver peroxisome proliferator-activated receptor γ contributes to hepatic steatosis, triglyceride clearance, and regulation of body fat mass. J Biol Chem 2003; 278: 34268-76. [Crossref]
30. De Martinis BS, Bianchi MdLP. Methodology for urinary 8-hydroxy-2′-deoxyguanosine analysis by HPLC with electrochemical detection. Pharmacol Res 2002; 46: 129-31. [Crossref]
31. Ruhl CE, Everhart JE. Upper limits of normal for alanine aminotransferase activity in the United States population. Hepatology 2012; 55: 447-54. [Crossref]
32. Ceriotti F, Henny J, Queraltó J, Ziyu S, Özarda Y, Chen B, et al. Common reference intervals for aspartate aminotransferase (AST), alanine aminotransferase (ALT) and γ-glutamyl transferase (GGT) in serum: results from an IFCC multicenter study. Clin Chem Lab Med 2010; 48: 1593-601. [Crossref]
33. Ahishali E, Demir K, Ahishali B, Akyuz F, Pinarbasi B, Poturoglu S, et al. Electron microscopic findings in non-alcoholic fatty liver disease: Is there a difference between hepatosteatosis and steatohepatitis? J Gastroenterol Hepatol 2010; 25: 619-26. [Crossref]
34. Kurosaka Y, Shiroya Y, Yamauchi H, Kitamura H, Minato K. Characterization of fat metabolism in the fatty liver caused by a high-fat, low-carbohydrate diet: A study under equal energy conditions. Biochem Biophys Res Commun 2017; 487: 41-6. [Crossref]
35. Deng XQ, Chen LL, Li NX. The expression of SIRT1 in nonalcoholic fatty liver disease induced by high-fat diet in rats. Liver Int 2007; 27: 708-15. [Crossref]
36. Jump DB. Fatty acid regulation of hepatic lipid metabolism. Curr Opin Clin Nutr Metab Care 2011; 14: 115-20. [Crossref]
37. Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, et al. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute scientific statement. Circulation 2005; 112: 2735-52. [Crossref]
38. Altınışık M. Karbonhidrat metabolizması bozukluklarına biyokimyasal yaklaşım. 2010; 11: 51-9.
39. Koeppen BM, Stanton BA. Berne & Levy Physiology, Updated Edition E-Book: Elsevier Health Sciences; 2009.