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# GLP-1 Receptor Agonist Liraglutide Modulates the Oxidative Stress and SIRT1/PGC1 Alpha Levels on Liver Tissue in Ovariectomized Rats

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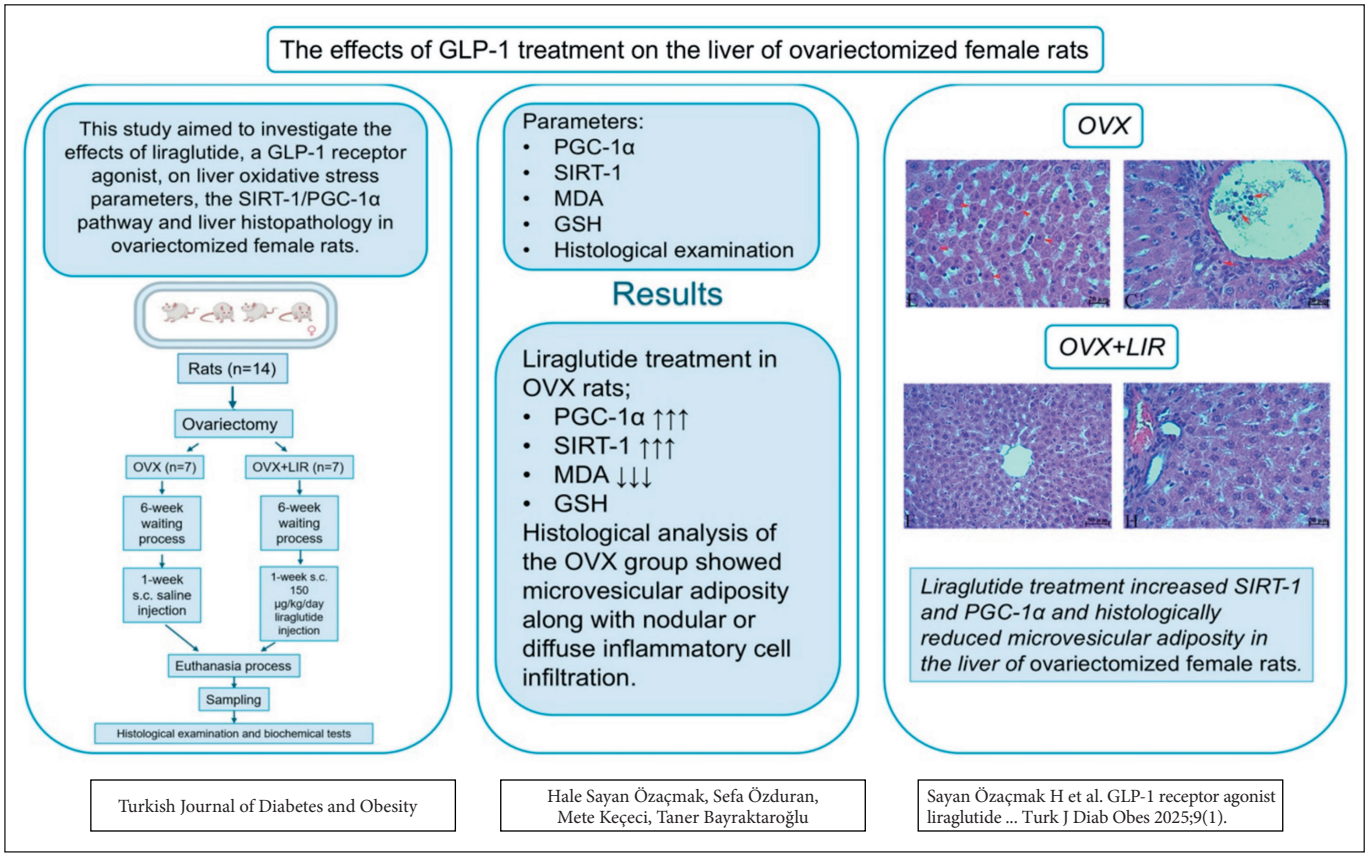
**GRAPHICAL ABSTRACT**

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## ABSTRACT

**Aim:** Non-alcoholic fatty liver disease (NAFLD), characterized by increased hepatic lipid accumulation, inflammation, and oxidative stress, is the most prevalent liver disease worldwide. Menopause is associated with an increased risk of developing NAFLD. The glucagon-like peptide-1 (GLP-1) receptor is present on human hepatocytes, and GLP-1 receptor agonists have a protective effect against liver steatosis and inflammation. Sirtuin 1 (SIRT1) and peroxisome proliferator-activated receptor-α (PPAR-α) are key regulators of hepatic lipid metabolism. This study aimed to determine whether liraglutide exerts a protective effect on the liver of ovariectomized rats through the SIRT1/PGC-1α pathway.

**Material and Methods:** Ovariectomized (OVX) rats were divided into two groups: a control group and a liraglutide-treated group. Liraglutide treatment lasted for five days at a dose of 150 μg/kg/day. Five days after liraglutide treatment, SIRT1 and PGC-1α levels, along with oxidative stress markers, were analyzed in the liver. SIRT1 and PGC-1α levels were measured using the ELISA method, while malondialdehyde (MDA), a marker of lipid peroxidation, and reduced glutathione (GSH) levels were quantified spectrophotometrically. Additionally, liver tissues collected from the animals were stained with hematoxylin and eosin and evaluated histopathologically.

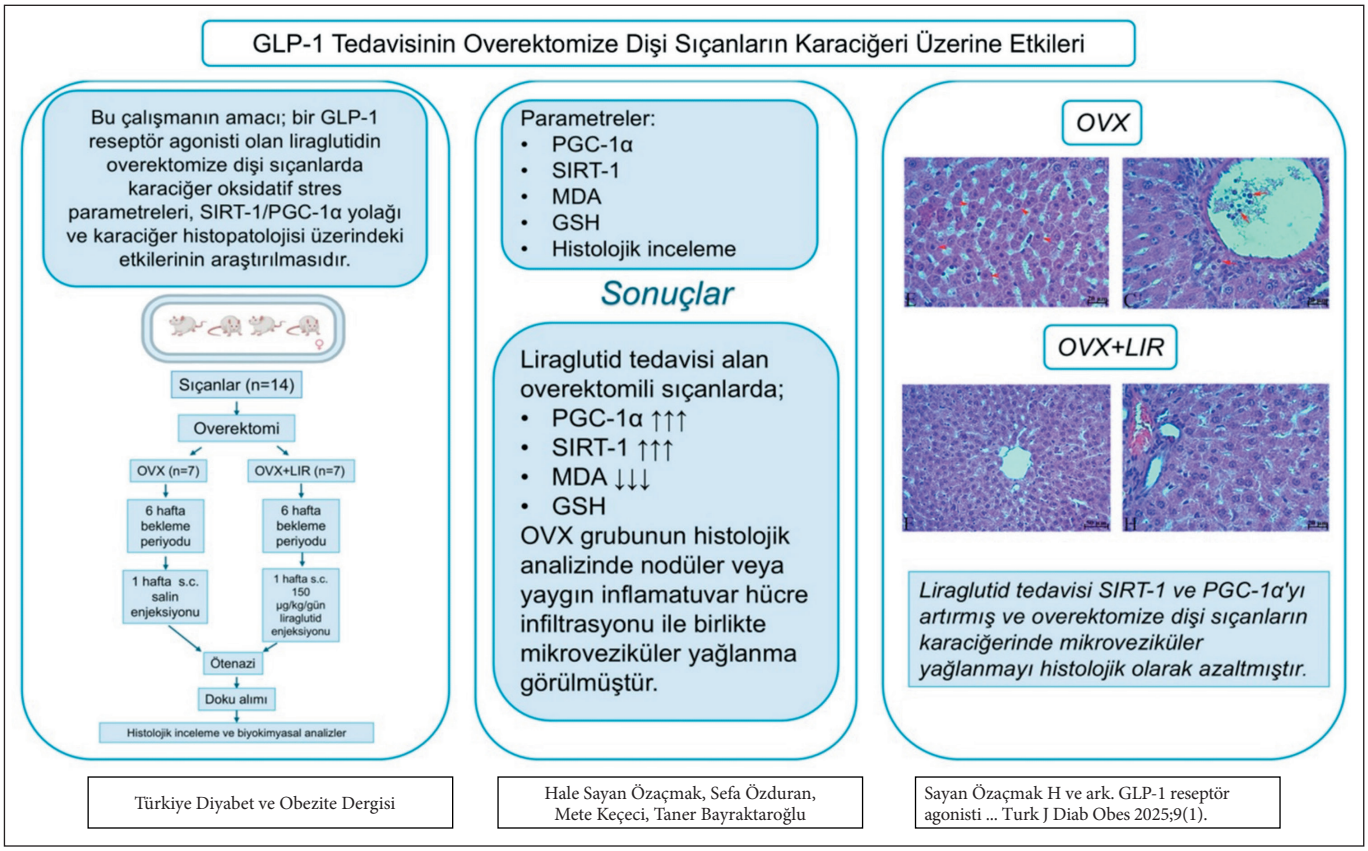
**Results:** In ovariectomized rats, decreased levels of SIRT1 and PGC-1α, along with microvesicular steatosis in liver tissue, apoptotic cellular changes, and heightened oxidative stress were observed (p=0.014). Conversely, in the liraglutide-treated groups, SIRT1 and PGC-1α levels were elevated compared to those in the OVX group, and histological changes showed improvement alongside reduced oxidative stress (p=0.036, p=0.05, respectively).

**Conclusion:** This study suggests that fat metabolism is compromised in ovariectomized rats and that liraglutide treatment may confer protection against NAFLD by upregulating SIRT1 and PGC-1α expression in the liver and reducing oxidative stress.


***Keywords:*** *Liraglutide, Menopause, Liver steatosis, SIRT1, PGC-1α*

# GLP-1 Reseptör Agonisti Liraglutid, Yumurtalıkları Alınmış Sıçanlarda Karaciğer Dokusunda Oksidatif Stresi ve SIRT1/PGC1 Alfa Düzeylerini Düzenler

**GRAFİKSEL ÖZET**



## ÖZ

**Amaç:** Artmış hepatik lipid birikimi, inflamasyon ve oksidatif stres ile karakterize non alkolik yağlı karaciğer hastalığı (NAYKH), dünya çapında en yaygın karaciğer hastalığıdır. Menopoz, NAYKH oluşum riskinin artmasıyla ilişkilidir. Glukagon benzeri peptid-1 (GLP-1) reseptörü insan hepatositlerinde bulunur ve GLP-1 reseptör agonistleri karaciğer yağlanmasına ve inflamasyona karşı koruyucu bir etkiye sahiptir. Sirtuin 1 (SIRT1) ve peroksizom proliferatör aktive reseptör-α (PPAR-α), hepatik lipid metabolizmasının temel düzenleyicileridir. Bu çalışma, liraglutidin SIRT1/PGC-1α yoluyla ovarektomize sıçanlarının karaciğeri üzerinde koruyucu bir etki gösterip göstermediğini belirlemeyi amaçlamıştır.

**Gereç ve Yöntemler:** Ovarektomi (OVX) uygulanan sıçanlar iki gruba ayrıldı: Kontrol grubu ve liraglutid ile tedavi edilen grup. Liraglutid tedavisi 150 µg/kg/gün dozunda beş gün boyunca uygulandı. Beş günlük liraglutid tedavisi sonrasında, karaciğerde SIRT1 ve PGC-1α düzeyleri oksidatif stres belirteçleriyle birlikte analiz edildi. SIRT1 ve PGC-1α düzeyleri ELISA yöntemi kullanılarak ölçülürken, lipid peroksidasyonunun bir belirteci olan malondialdehit (MDA) ve indirgenmiş glutatyon (GSH) düzeyleri spektrofotometrik olarak belirlendi. Ek olarak, hayvanlardan toplanan karaciğer dokuları hemotoksilen eozin ile boyanarak histopatolojik değerlendirildi.

**Bulgular:** Yumurtalıkları alınmış sıçanlarda, karaciğer dokusunda mikroveziküler steatoz, apoptotik hücresel değişiklikler ve artmış oksidatif stresle birlikte SIRT1 ve PGC-1α düzeylerinde azalma gözlemlendi (p=0.014).). Tersine, liraglutid ile tedavi edilen grupta, SIRT1 ve PGC-1α düzeyleri OVX grubuna kıyasla yükseldi ve histolojik değişiklikler oksidatif stresin azalmasıyla birlikte iyileşme gösterdi (sırasıyla, p=0.036 ve p=0.05).

**Sonuç:** Bu çalışma, yumurtalıkları alınmış sıçanlarda yağ metabolizmasının bozulduğunu ve liraglutid tedavisinin karaciğerde SIRT1 ve PGC-1α ekspresyonunu artırarak ve oksidatif stresi azaltarak NAYKH'ye karşı koruma sağlayabileceğini düşündürmektedir.


***Anahtar Sözcükler:*** *Liraglutid, Menopoz, Yağlı karaciğer, SIRT1, PGC-1α*

## INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is a clinical metabolic syndrome defined by the excessive accumulation of fat in liver cells that is not attributed to alcohol consumption. NAFLD is an emerging public health concern, characterized by the abnormal buildup of triglycerides in the liver. NAFLD is predominantly observed in individuals with obesity and metabolic syndrome, both of which are closely linked to insulin resistance. A significant challenge remains the lack of approved pharmacological treatments for NAFLD (1). Notably, NAFLD is more frequently observed in postmenopausal women compared to those who are premenopausal.

The reduction in female hormone levels may contribute to metabolic dysfunction (2). Moreover, menopause is linked to alterations in body composition, particularly an increase in intra-abdominal fat and waist circumference (3). The liver, being a target for sex hormones, expresses estrogen receptors in both sexes. Variations in sex hormone levels and their downstream signaling have been identified as key factors influencing the susceptibility to hepatic diseases. Although the link between reduced estrogen levels and liver fibrosis is well established, the underlying mechanisms driving this effect have not yet been fully understood (4). The ovariectomized (OVX) animal model, which simulates postmenopausal conditions, provides a platform for studying NAFLD-like pathogenesis, including steatosis and liver damage (3). Beyond their role in reproduction, estrogens serve as key regulators of energy homeostasis (5).

The decrease in estrogen levels during menopause leads to increased insulin resistance, dyslipidemia, and an imbalance between fatty acid production and utilization, resulting in steatosis and fat accumulation in the liver, ultimately leading to NAFLD. The primary mechanism behind the development of steatosis and NAFLD is attributed to both enhanced lipid storage and impaired lipid removal (6). On the other hand, enhanced generation of reactive oxygen species (ROS)-induced oxidative stress is implicated in the pathogenesis of NAFL (7).

Glucagon-like peptide-1 (GLP-1), a 30-amino acid gut hormone, is secreted in response to nutrient intake. It enhances insulin release while suppressing glucagon secretion and delaying gastric emptying, ultimately leading to lower postprandial blood glucose levels (8). Liraglutide is a glucagon-like peptide-1 receptor agonist used in the treatment of patients with type 2 diabetes (9). Due to portal circulation, the liver is exposed to higher concentrations of GLP-1 compared to other organs. While the direct effects of this incretin on hepatocytes remain a topic of debate, several effects of its analogs have been well-documented. Most of these effects involve the regulation of glucose and lipid metabolism, resulting in reduced hepatic glucose production and improved hepatic steatosis (7). As a novel class of antidiabetic drugs, incretin mimetics like exenatide (exendin-4) have garnered increasing evidence showing their ability to effectively reduce lipid accumulation in the liver. The presence of the GLP-1 receptor on human hepatocytes and its direct role in reducing hepatic steatosis *in vitro* (10) and *in vivo*

(1) have been reported. A recent study demonstrated that sirtuin 1 (SIRT1) mediates the effect of the GLP-1 receptor agonist exenatide in alleviating liver steatosis (1). However, the precise mechanism of the GLP-1 signaling pathway and its mimetics in improving ovariectomy-induced hepatic steatosis remains unclear.

Nicotinamide adenine dinucleotide (NAD)-dependent protein deacetylase sirtuin 1 (SIRT1) is one of the seven mammalian homologs (SIRT1-SIRT7) of the yeast silent information regulator 2 (SIR2). SIRT1, an NAD+-dependent protein deacetylase, has been shown to play several important roles in cells, including regulating longevity, apoptosis, DNA repair, inflammation, and mitochondrial function (PGC-1α). SIRT1 is a key regulator of hepatic lipid metabolism (11). Peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC-1α) serves as a crucial transcriptional coactivator of energy metabolism, particularly in enhancing lipid catabolism. A previous study indicated that the forced expression of PGC-1α led to increased fatty acid oxidation and reduced lipid accumulation in hepatocytes (12). PGC-1α participates in the activation of nuclear receptors and other transcription factors and plays an important role in mitochondria generation (13). Upon activation of PGC-1α, transcription factors and receptors within the nucleus significantly influence lipid metabolism (14).

The absence of estrogen, as seen in menopause or following ovariectomy, contributes to the onset and progression of obesity-related conditions, including NAFLD. However, estrogen replacement therapy in postmenopausal women has been linked to an increased risk of certain types of cancers (15,16).

Therefore, novel alternative therapies are required to address the metabolic alterations that arise following menopause. This study aimed to investigate the effects of liraglutide on liver changes induced by ovariectomy, with a particular focus on the SIRT1/PGC-1α pathway, liver steatosis, and oxidative stress.

## MATERIAL and METHODS

### Animals

Fourteen *Wistar Albino* female rats (weighing 250-300 g) purchased from Zonguldak Bülent Ecevit University Animal Care Unit were housed in a temperature (22 °C) and lighting (12 h (light) - 12 h (dark) cycle) controlled experimental animal unit. Ethical approval was obtained from the Zonguldak Bülent Ecevit University Experimental Animals Ethics Committee prior to the study (Ethics number: 2024-23-14/11).

### Experimental Design

We examined the effects of liraglutide on hepatic fat accumulation, SIRT1/PGC-1α expression, and oxidative stress in ovariectomized rats. The rats were divided into two groups:

i)OVX rats, and ii) OVX rats treated with liraglutide (150 µg/kg/day, s.c., Saxenda, Novo Nordisk). The liraglutide dose was selected based on previous studies indicating that it does not lower blood sugar while exhibiting cytoprotective activity (17).

After five days of liraglutide treatment, hepatic levels of PGC-1α, SIRT1, and oxidative stress markers were analyzed. Additionally, liver tissue was assessed histologically using hematoxylin and eosin (H&E) staining.

### Ovariectomy procedure

The animals were bilaterally OVX under anesthesia using a mixture of ketamine (90 mg/kg, intraperitoneal (i.p.) injection) and xylazine (10 mg/kg, i.p.) to eliminate endogenous estradiol and progesterone production. Ovariectomies were conducted 6 weeks prior to the study.

### Biochemical Analyses

The oxidant and antioxidant status of the liver of OVX rats was assessed by measuring tissue lipid peroxidation and reduced glutathione (GSH) levels. To evaluate lipid peroxidation, the tissue content of malondialdehyde (MDA), a by-product of lipid peroxidation, was quantified. Briefly, tissue samples were homogenized in ice-cold trichloroacetic acid (TCA) using a motor-driven pestle, with TCA 10 mL of 10% added per gram of tissue. After centrifugation, the supernatant 750 µL of was mixed with an equal volume of thiobarbituric acid 0.67% and heated to 100°C for 15 minutes. The absorbance of the samples was then measured spectrophotometrically at 535 nm (18). For measuring the GSH content in the samples, $Na_2HPO_4$ 2 mL of 0.3 M solution was added to 0.5 mL of the supernatant obtained from the same homogenization procedure described above. A 0.2 mL solution of dithiobisnitrobenzoate was then added to the mixture, and the absorbance at 412 nm was measured immediately after vortexing (19).

### ELISA Assay

The levels of PGC-1α and SIRT1 were measured using an enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's guidelines (ELK Biotechnology, cat# ELK6192 and cat# ELK6194, respectively). Briefly, liver tissues were homogenized in PBS (pH 7.2–7.4) and then centrifuged for 20 minutes at a speed of 2000–3000 rpm. Following the test protocol, standards and samples were added to the wells, followed by the addition of antibody and

streptavidin-HRP to each well. The plate was gently washed and incubated for 60 minutes at 37 °C. After the incubation period, TMB substrate was added, and the plate was incubated again for 10 minutes at 37 °C to allow reactions to occur in the wells. Subsequently, a stop solution was added. Finally, the absorbance was measured at 450 nm using an ELISA plate reader (4300 Chromate Microplate Reader, Awareness Technology, Inc., FL, USA). Tissue concentrations of PGC-1α and SIRT1 were calculated from the standard curves and expressed as ng per gram of tissue.

### Histopathological Analyses

Liver tissues were fixed with 10% formalin fixative, dehydrated using graded alcohols (70%, 90%, 96%, and 100%), cleared with toluene, and embedded in paraffin to obtain paraffin blocks. To determine the general histological characteristics of the liver tissue, 5µm-thick sections were obtained from the paraffin blocks using a Shandon Finesse 325 rotary microtome and stained with hematoxylin-eosin (H&E) and Masson's trichrome staining methods. H&E-stained liver tissues were evaluated under X200 magnification in 10 fields per section and scored based on steatosis, lobular inflammation, and hepatocyte ballooning criteria (20). The following scoring system was applied for evaluation:

**Steatosis**: <5%: 0, 5%-33%: 1, 34%-66%: 2, >66%: 3.

**Lobular inflammation:** No foci: 0, 1 focus per field at X200 magnification: 1, 2-4 foci per field at X200 magnification: 2, >4 foci per field at X200 magnification: 3.

**Hepatocyte ballooning:** No foci: 0, 1 focus at X200 magnification: 1, 2-4 foci at X200 magnification: 2. The amount and distribution of connective tissue in the liver tissue were assessed in Masson's trichrome-stained sections. Stained tissues were visualized using an Axio Lab A1 microscope (Zeiss, Germany).

### Statistical Analyses

Statistical analyses were conducted using the SPSS version 21.0 (IBM Corporation, Somers, NY, USA). Group comparisons were performed using Mann Whitney U tests. Data are presented as median (minimum – maximum) values, with a significance level set at $p < 0.05$.

## RESULTS

### Biochemical Results

As shown in Table 1, liver tissue MDA levels were significantly elevated in OVX rats compared to the liraglutide-treated group (p=0.014). Liraglutide treatment appears to effectively reduce MDA levels elevated by OVX. Additionally, liver tissue SIRT1 and PGC-1α levels were statistically significantly increased in the liraglutide-treated group compared to the OVX group (p=0.05 and p=0.036, respectively). No statistically significant difference was observed in tissue GSH levels between the experimental groups (p=0.055).

### Histopathological Results

Following Hematoxylin & Eosin (H&E) staining of liver tissues obtained from the subjects in the groups, the OVX group exhibited microvesicular steatosis (black arrow), which was focal in some areas and affected all zones, along with nodular or diffuse inflammatory cell infiltration, predominantly in the periportal area (zone 1) (Figure 1A-E). It was noteworthy that inflammatory cells (red arrow) occasionally filled sinusoidal lumens and were also observed in the lumens of portal venous branches in the portal areas. Furthermore, in this group, hepatocyte clusters with pyknotic nuclei, eosinophilic cytoplasm, small size, and characteristics indicating early-stage apoptosis were found, most notably in zone 2, although present in all zones (red arrowhead). Masson's trichrome staining of liver tissues revealed no significant difference in the amount and distribution of connective tissue between the two groups (Figure 2).

Histopathological evaluation of liver tissues from liraglutide-treated rats revealed that the classical liver lobule maintained a histological architecture closely resembling normal structure in all three zones (Figure 1F-H). Hepatocyte ballooning was absent in this group, while only minimal inflammatory cell infiltration was observed.

**Table 1.** Effects of liraglutide on oxidative stress, PGC-1α and SIRT1 level.

| Parameters * | OVX (n=7) | OVX+LIRA (n=7) | p |
|---|---|---|---|
| **PGC-1α** | 292.67 (259.75-383.96) | 464.58 (427.29-673.3) ** | p=0.036 |
| **SIRT-1** | 61.79 (56.36-74.94) | 121.12 (108.39-147.67) ** | p=0.05 |
| **MDA** | 60.16 (52.22-74.24) | 48.13 (39.42-56.32) ** | p=0.014 |
| **GSH** | 23.15 (19.32-27.66) | 18.71 (12.03-24.79) | p=0.055 |

*Data are shown as *median (minimum-maximum) values*. ** Statistical significance compared to OVX group.
**OVX:** Ovariectomized, **OVX+LIRA:** Ovariectomized+Liraglutide, **PGC-1α:** Peroxisome proliferator-activated receptor gamma coactivator-1 α, **SIRT-1:** Sirtuin-1, **MDA:** malondialdehyde, **GSH:** Reduced glutathione.

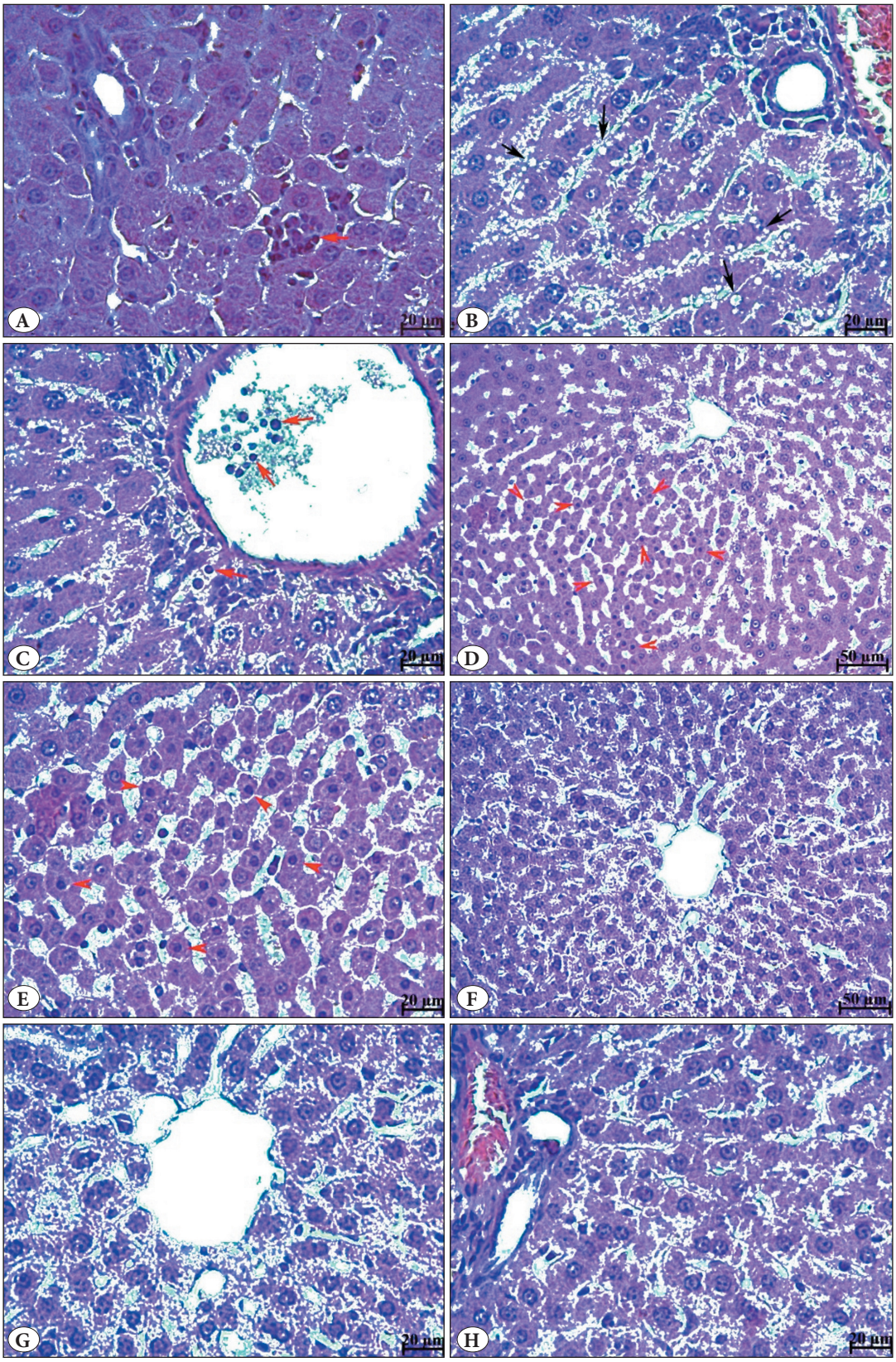



**Figure 1:** H&E staining results of liver tissues of the groups. **A-E)** OVX group; **F-H)** Liraglutide treated group. In the OVX group, macrovesicular steatosis, inflammatory cell infiltration, and early apoptotic changes were observed in hepatocytes. In the liraglutide-treated group, the normal histological structure of hepatocytes was preserved. Microvesicular steatosis (black arrow), inflammatory cell infiltration (red arrow) and early-stage apoptotic hepatocytes (red arrowhead). Scale bar: D, F: 50 µm; A, B, C, E, G, H: 20 µm.

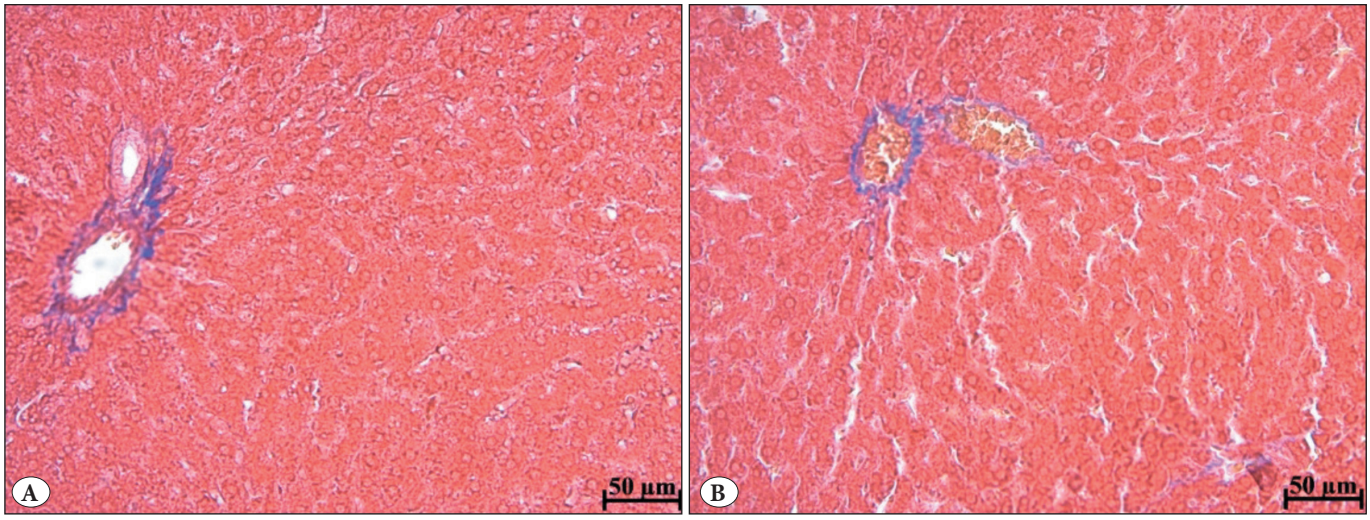



**Figure 2:** Masson trichrome staining results of liver tissues of the subjects. **A)** OVX group, **B)** Liraglutide treated group. No significant difference was observed between the two groups regarding the amount and distribution of connective tissue. Scale bar: 50 µm.

Masson's trichrome staining of liver tissues from the subjects revealed no significant difference between the two groups in terms of the amount and distribution of connective tissue.

## DISCUSSION

In the current study, ovariectomy induced an increase in oxidative stress in liver tissue, accompanied by suppression of the SIRT1/PGC-1α levels. Liraglutide treatment countered these effects by elevating the levels of SIRT1 and PGC-1α and reducing lipid peroxidation. Histologically, liraglutide administration significantly reduced hepatic lipid accumulation and apoptotic changes in hepatocytes. These findings suggest that the suppression of oxidative stress and increasing levels of SIRT1/PGC-1α may represent a key mechanism underlying the protective effects of liraglutide.

Recently, liraglutide has been shown to protect against NAFLD in both human trials and cellular models (21,22). Previous studies have demonstrated that GLP-1 receptor agonists increase the expression of GLP-1 receptors in the liver tissue of rats with liver cirrhosis. Shao et al. reported that exenatide, a GLP-1 receptor agonist, exhibits superior hepatoprotective effects compared to intensive insulin therapy (23). Furthermore, it may serve as a promising additional treatment option for patients with NAFLD who have elevated liver enzymes and Type 2 diabetes mellitus. Throughout these investigations, liraglutide has been demonstrated to have lots of effects, including enhancing cell survival through promoting autophagy inhibition of inflammation, attenuation of endoplasmic reticulum stress and so on (22,24,25); however, the precise molecular mechanisms are still poorly understood.

Accumulating evidence indicates that severe oxidative stress is associated with NAFLD and plays a critical role in its etiology. Reducing excessive oxidative stress has proven effective in ameliorating NAFLD in both cellular experiments and animal models (7). In this study, we demonstrated that ovariectomy induces an increase in oxidative stress in the liver, and that liraglutide treatment effectively reduces this oxidative stress. Our findings are supported by results from Tong et al., which show that liraglutide is effective in decreasing oxidative stress and increasing antioxidant enzymes in high-fat diet-induced fatty liver disease (7). The accumulation of reactive oxygen species and oxidative stress, which disrupt mitochondrial architecture, is implicated in the mitochondrial etiology of age-related metabolic diseases. SIRT1 has been identified as a key factor in maintaining mitochondrial homeostasis by preserving mitochondrial membrane potential, reducing oxidative damage, and promoting mitochondrial biogenesis (26). Additionally, it has been reported that liraglutide can protect mitochondrial architecture by supporting both mitochondrial fusion and fission processes through sirtuins (7).

In our study, liraglutide increased SIRT1 levels in the liver, and this finding is consistent with the findings of many studies in the literature (1,7,27,28). Liraglutide plays a protective role by activating the SIRT1/PGC-1α pathway. For example, liraglutide has been shown to alleviate kidney damage by activating the renal SIRT1/AMPK/PGC-1α signaling pathway in rats rendered obese with a high-fat diet (27). More importantly, studies have shown that the SIRT1/AMPK pathway can be activated by the GLP-1 receptor agonist exenatide, which in turn reduces lipid accumulation

in the liver induced by a high-fat diet (1). In this study, we suggest that the GLP-1 receptor agonist liraglutide may alleviate liver steatosis after ovariectomy by increasing SIRT1 levels in liver tissue. This finding is consistent with the study by Xu et al., which showed that exenatide reduces liver steatosis and inflammation via SIRT1. Additionally, SIRT1 has been reported to be required for the GLP-1 receptor agonist exenatide to reduce hepatic steatosis in mice (1). Another study has shown that hepatocyte-specific deletion of SIRT1 impairs fatty acid metabolism and leads to hepatic steatosis and inflammation (28). The application of liraglutide has been shown to provide protection against myocardial steatosis and oxidative stress, independent of its glucose-lowering effect, through the activation of the AMPK-SIRT1 pathway in a diabetic model (29). After ovariectomy, histological analysis of the rats' livers revealed microvesicular steatosis, inflammatory cell infiltration, and early apoptotic changes. This finding aligns with previous studies indicating that ovariectomy is linked to various liver changes, such as microvesicular formation, apoptotic cell presence, and inflammatory cell infiltration (30).

Specifically, SIRT1 plays a crucial role in energy homeostasis by regulating glucose metabolism in hepatic tissue (31). In the present study, liraglutide treatment may enhance energy metabolism through the activation of PGC-1α/SIRT1 signaling. Hepatic mitochondrial dysfunction is a significant etiological factor in the progression of the first and second stages of NAFLD, characterized by decreased energy production and disrupted redox balance. Research has indicated that oxidative stress-mediated mitochondrial dysfunction. Research has shown that in a mouse model of hepatic steatosis, the expression of the hepatic protein PGC-1α and the activation of mitochondrial biogenesis were diminished (14). Upon activation, SIRT1 regulates PGC-1α-mediated mitochondrial biogenesis and mitosis (32). The overexpression of hepatic PGC-1α may provide additional protective effects as follows: First, PGC-1α acts as a master regulator of lipid metabolism, and its deficiency increases the likelihood of liver steatosis. A positive correlation exists between PGC-1α levels and the ability of cells to fully oxidize fatty acids. Both in vivo and in vitro studies have shown that PGC-1α enhances liver fatty acid oxidation while reducing the storage and release of triacylglycerol. Consequently, liraglutide treatment resulted in a significant decrease in hepatic lipid deposition and histological abnormalities. This intervention may effectively impede the progression of steatosis in the liver (32).

Among the limitations of this study, it is important to note that only ovariectomized experimental animals were utilized, and the effects of liraglutide in subjects with intact gonadal steroids were not assessed. Additionally, another limitation is the lack of measurement of plasma lipid profiles in the experimental animals. A more comprehensive study incorporating these adjustments would be necessary. Furthermore, biochemical assessment of liver enzymes would provide a more precise evaluation of the liver changes and further validate the effects of liraglutide on liver tissue.

Based on these findings, we hypothesized that liraglutide may be effective in ameliorating ovariectomy-induced fatty liver disease via the SIRT1/PGC1α pathway. The GLP-1 receptor agonist liraglutide reduced hepatic steatosis in ovariectomized rats by increasing SIRT1 and PGC-1α levels and reducing lipid peroxidation, suggesting that GLP-1 receptor agonists could serve as potential therapeutic agents for NAFLD. Current findings suggest that the administration of GLP-1 analogs represents a promising treatment for metabolic disorders resulting from estrogen deficiency. The data derived from this study highlight the need for further investigations and phase studies in humans to clarify their clinical correlations.

**Acknowledgment**
None.

**Author Contributions**
**Hale Sayan Özaçmak** and **Sefa Özduran** conceived and designed the experiments; **Hale Sayan Özaçmak** and **Sefa Özduran** performed the animal experiments, collected samples and biochemical analyses; **Mete Keçeci** performed histapathological evaluations, **Hale Sayan Özaçmak, Sefa Özduran** and **Taner Bayraktaroğlu** writing and prepration manuscript; **Hale Sayan Özaçmak** and **Taner Bayraktaroğlu** drafted, edited, and revised the manuscript.

**Conflicts of Interest**
No conflict of interest is reported by authors.

**Financial Support**
None to declare.

**Ethical Approval**
Ethics committee approval numbered 2024-23-14/11 was obtained from Zonguldak Bulent Ecevit University Animal Experiments Local Ethics Committee for the study.

**Review Process**
Extremely and externally peer reviewed.

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