Original Article

Investigation of the Effects of Curcumin on GLP1-R in Liver Tissue of Diabetic Rats

Uğran, R[1*], Koral Taşçı, S[2]

1. *Department of Veterinary Medicine, Vocational School of Technical Sciences. Batman University, Batman, Turkey*
2. *Department of Histology and Embryology, Faculty of Veterinary Medicine, Kafkas University, Kars, Turkey*

How to cite this article: Uğran R, Koral Taşçı S. Investigation of the Effects of Curcumin on GLP1-R in Liver Tissue of Diabetic Rats. Archives of Razi Institute Journal. 2024;79(4):815-826. DOI: 10.32592/ARI.2024.79.4.815

ABSTRACT

The study was designed to investigate the effect of curcumin, known for its antidiabetic properties, on the immunohistochemical localization and gene expression of glucagon-like peptide-1 receptor (GLP-1R) in the liver tissues of experimental diabetic rats using reverse transcription polymerase chain reaction (RT-PCR). For this, 24 Sprague–Dawley rats were divided into four groups—control, sham, diabetic, and diabetic + curcumin groups. The control group received no treatment, and 50 mg/kg streptozotocin was administered to the rats in the diabetic and diabetic + curcumin groups received 50 mg/kg streptozotocin. Once diabetes had been established, 100 mg/kg of curcumin was administered intraperitoneally to rats in the diabetic + curcumin group for a period of 21 days. Thesham group was administeredintraperitoneal ethanol and isotonic sodium chloride solution. At the ends of the experiment,tissues were subjected to histological and immunohistochemical examination to ascertain the localization of GLP-1R. Additionally, RT-PCR was employed to determine the levels of GLP-1R gene expression.The histological examinations revealed that the tissue samples from the control and sham groups exhibited a normal histological structure. In contrasr, the diabetic group displayed a range of degenerative changes, including enlargement of the sinusoidal wall enlargement and vacuolization of the hepatocytes. Furthermore, these degenerative findings were mitigated in the diabetic + curcumin group. In the immunohistochemical examinations, the majority of hepatocytes surrounding the vena centralis, as well as some endothelial, and some Kupffer cells,exhibited positively for GLP-1R. The diabetic group exhibited reduced immunoreactivity, while the diabetic + curcumin group demonstrated elevated immunoreactivity compared to the diabetes group. With regard to the molecular analysis, the mean expression level was observed to be higher in the diabetes + curcumin group. However, no significant difference in GLP-1R gene expression was identified between the groups. In conclusion, the administration of curcumin was observed to enhance GLP-1R expression in the liver of the rats with diabetes. Given that GLP-1R is a targets for diabetes treatment, curcumin can be used as a viable therapeutic agent for treating diabetes and alleviating its complications.

Keywords: Diabetes, Immunohistochemistry, GLP-1R, RT-PCR, Curcumin

Article Info:
Received: 26 October 2023
Accepted: 4 February 2024
Published: 31 August 2024

Corresponding Author's E-Mail:
resit.ugran@batman.edu.tr

1. Introduction

Curcumin is the most important among the major bioactive components derived from the roots of the turmeric (*Curcuma longa*) plant, a perennial herbaceous plant belonging to the ginger family (Zingiberaceae). In traditional medicine, curcumin is employed in the treatment of various diseases, including indigestion, urinary system infections, liver diseases, and rheumatoid arthritis. Moreover, it has been identified as a potential therapeutic option for diabetes due to its anti-inflammatory and antioxidative properties (1). The hypoglycemic effect of curcumin was initially documented in 1972, and it has subsequently been recognized as a potential therapeutic agent for treating various complications of patients with diabetes (2). It has been reported that curcumin can prevent the development of diabetes, reduce in vivo insulin resistance, and improve cellular functions (3). The therapeutic potential of curcumin in treating various diabetic complications, including nephropathy, retinopathy, and diabetes-related neurochemical changes in the brainstem, has been documented in the literature (4). It has been demonstrated that curcumin increases postprandial insulin levels and exerts beneficial effects on insulin secretion (5). Additionally, curcumin may sever to mitigate the likelihood of developing type 2 diabetes in individulas with prediabetes (2). The proglucagon gene is responsible for the production of the peptide hormone known as glucagon-like peptide-1 (GLP-1), with thirty amino acids. GLP-1 is primarily secreted by intestinal endocrine L cells in the distal ileum and colon (6, 7). It exerts its effects through certain receptors, targeting pancreatic islet cells (6). Additionally, it prompts pancreatic β-cells to secrete greater amounts of insulin, stimulates the proliferation of these cells by activating genes associated with cell division, and reduces the cell's apoptosis by inhibiting the production of caspase-3 (8). Consequently, GLP-1 exerts an influence on the release of pancreatic hormones and directly affects organs that are sensitive to glucose, such as the liver, thereby assisting in the regulation of blood sugar levels. Furthermore, because the portal vein represents the site at which GLP-1 achieves its maximum intravenous dosage, the liver is regarded as a potential target for the protein. The GLP-1 receptor (GLP-1R) is activated by GLP-1, which elicits a series of physiological effects such as glucose-dependent secretion of insulin and its biosynthesis, the improvement of insulin sensitivity in peripheral tissues,the maintenance of β-cell mass, and weight loss. These effects make GLP-1 an effective treatment for type 2 diabetes (9). It is established that GLP-1R is a target molecule for the treatment of type 2 diabetes. Consequently, further information regarding the interaction mechanisms of this receptor is required (10). GLP-1R has been identified in many cells and organs such as the pancreatic α and β islet cells, the liver, spleen, and adipocytes as well as in the central and peripheral nervous system tissues, the heart, kidney, lungs, and gastrointestinal tract of rats (11). The expression level of GLP-1R varies between various tissues and cell types (12). In the liver, GLP-1R plays an active role in lipid metabolism and controls ectopic lipid accumulation in the hepatocytes (20). The objective of this study was to examine the impact of curcumin, a compound with antidiabetic properties, on GLP-1R in the liver tissues of rats with diabetes.

2. Materials and Methods

2.1. Experimental Implementation

The study was conducted in accordance with the ethical standards set forth by the Kafkas University Animal Experiments Local Ethics Committee, which approved accepted the study protocol (IRB number: 081, Date:03/09/2017). A total of 24 male Sprague–Dawley rats were utilized in the present study. The rats were divided into four groups: diabetic + curcumin, diabetic, sham, and control groups. First, the rats in the diabetic + curcumin group (n = 6) were administered 50 mg/kg streptozotocin (STZ) dissolved in 0.1 M sodium citrate buffer (pH 4.5) as a single dose intraperitoneally (IP) to establish diabetes. Subsequently, 100 mg/kg/day curcumin, dissolved in ethanol and diluted with isotonic sodium chloride solution, was administered IP (intraperitoneally) for a period of 21 days. The rats in the diabetic group (n = 6) were administered 50 mg/kg STZ dissolved in 0.1 M sodium citrate buffer (pH 4.5) as a single dose IP. The rats in the sham group (n = 6) were administered the same amount of ethanol and isotonic sodium chloride mixture that was administered to the diabetic + curcumin group, in proportion to their body weight, via the same route, following the administration of a single dose of sodium citrate solution, according to their body weight. The control group (n = 6) didn't receive any administrations.

2.2. Blood Glucose Levels

The blood glucose levels of the rats in all the groups were measured using a glucometer device (Vital Plus) and a single drop of bloodcollected from the tail veins on days 0, 3, 7, 14, and 21, following a 6 hours of fast. In the diabetic and diabetic + curcumin groups, the rats with blood glucose levels > 200 mg/dL at 72 h after STZ administration were considered to have diabetes, and the study was initiated (13).

2.3. Tissue Sampling

At the end of the 21-day experimental period, the rats from all the groups were euthanized via the cervical dislocation method under ether anesthesia, and liver tissues were collected. The collected liver tissue samples were divided into two portions. Tissues intended for molecular analysis were homogenized using the TRI Reagent (Sigma-Aldrich) and a homogenizer (Wiggen Hauser),after which they were stored at 4°C. The tissues intended for histological examinations were fixed in 10% formaldehyde solution.

2.4. Histological Examination

First, the tissue-tracking protocol was followed after fixation. Then, the tissues were embedded in paraffin. Tissue sections (5µm thick) were cut from the paraffin blocks. Crossman's trichrome staining technique (trichrome

staining) was used to stain the tissue sections for performing histological examination. Furthermore, the prepared slides were examined under a light microscope (Olympus BX51) and photographed.

2.5. Immunohistochemical Examination

The localization of GLP-1R in the liver(hepatic) tissues was investigated through the use of ABC (avidin, biotin, and peroxidase complex) approach. Subsequently, the tissue sections were subjected to a deparaffinization and rehydration process. To prevent endogenous peroxidase activity, 3% hydrogen peroxide was applied to the tissues. To expose antigens, heat application in sodium citrate buffer (pH: 6.0) solution was performed using a microwave oven. The ABC technique was performed using an antipolyvalent horseradish peroxidase kit (Thermo Scientific, TP-125-HL) in accordance with the manufacturer's instructions. To prevent non-specific binding, the were incubated with Ultra V Block solution (10%) for 10 minutes. At the end of the specified period, the tissues were rinsed with phosphate-buffered saline and incubated with an anti-GLP-1R antibody (Abcam: 188602, 1:250 dilution) at room temperature for 1 hour. Subsequently, a biotinylated secondary antibody (goat anti-rabbit antibody) produced in the same species as the primary antibody was produced was added to the tissues and left for 30 minutes. Subsequently ,the tissues were rewashed and streptavidin–horseradish peroxidase was added to them and left for 15 minutes. After performing these procedures, diaminobenzidine-hydrogen peroxidase was introduced to facilitate visualization of the reaction. Subsequently, the sections were counterstained with Mayer's hematoxylin. Furthermore, a negative control was employed to ascertain the specificity of the GLP-1R immunoreactivity. The tissue preparations from all the groups were examined under a light microscope (Olympus BX51) and evaluated according to the intensity of the reaction. The semiquantitative method entailed grading th reaction intensity in the scale of 0 to 3 with 0 indicating no reaction, 1 denoting a weak reaction, 2 signifying a moderate reaction, and 3 representing a very intense reaction. For the purpose of statistical analysis, 10 randomly selected areas of each preparation and 60 areas from each group were graded and evaluated in terms of reaction intensity.

2.6. Gene Expression Evaluated by RT-PCR

For RNA isolation (14), chloroform was added to the samples that had been kept in TRI Reagent. The samples were then centrifuged at 12,000 rpm and 4°C for 15 minutes. Subsequently, the upper RNA layer was removed. Subsequently, 0.5 ml of isopropanol was added to the tubes containing RNA, and centrifugation was performed at 13,000 rpm and 4°C for 8 minutes. Afterwards, 1 ml of 75% ethanol was added to the formed RNA pellet, facilitating the washing of RNA molecules. The resulting pellet was then dissolved by adding 80 µl of double-distilled water to the isolated RNA samples. The concentration of RNA per microliter was determined using a spectrophotometer per µl at a wavelength of 260 nm. Therefore, the quantity of the solution containing 3 µg of RNA was then determined. The extraction of messenger RNA (mRNA) was conducted using oligo-dT primers. Subsequently, the solution containing 3 µg of RNA for each sample was diluted to a volume of 12 µl with nuclease-free water, after which 1 µl of oligo-dT primers were added. Following incubation, of the samples in a PCR apparatus (GenePro, TC-E-96G) at 70°C for 5 minutes and at 4°C for at minimum of 2 minutes, mRNA extraction was performed. To obtain cDNA, the Fermentas Revert Aid First Strand cDNA Synthesis kit (K1622) was employed. Four milliliters of 5X reaction buffer, one milliliter of RNase inhibitor, two milliliters of a 10 mM dNTP mix (including dATP, dCTP, dGTP, and dTTP), and one milliliter of Revert Aid M-MuLV reverse transcriptase enzyme were added to the mRNA samples that had been previously acquired. The 20-µl mixture was subjected to the following program in a PCR device (GenePro, TC-E-96G): 42°C for 60 minutes and 70°C for 5 minutes. The cDNA molecules were stored at −20°C. To the 3 µl of cDNA, 5 µl of 10× buffer, 4 µl of $MgCl_2$, 4 µl of dNTP, 5 µl of the GLP-1R/β-actin forward primer, 5 µl of the GLP-1R/ β-actin reverse primer (Table 1), 0.5 µl of the Taq DNA polymerase, and double-distilled water were added to make up a total volume of 50 µl. The prepared samples were subjected to the following program in a PCR device: 94°C for 5 min, 94°C for 1 minutes, 52°C for 1 minute, 72°C for 1 minute(40×), 72°C for 10 minutes, and 4°C for 5 minutes. The obtained PCR products were stored at −20°C. The obtained products were subjected to electrophoresis on a 1.5% agarose gel in an electrophoresis device (CBS Scientific, WSGE-014) at 100 V for 60 minutes. Subsequently, the bands were visualized and photographed in the UV-imaging device (DNR, MiniLum). The intensities and pixel areas of the bands in the images were converted into numerical data using the Plot Lanes analysis tool included in the ImageJ 1.52a software (7). In this study, the expression of GLP-1R in the liver tissues of the rats from all groups was determined using the RT-PCR method, with β-actin gene expression used as the control. The results of the molecular analysis of the groups were subjected to densitometry analysis, and the obtained numerical data were evaluated.

2.7. Statistical Analyses

Following the completion of a one-way analysis of variance on the acquired data, the SPSS 16.0 software was employed to conduct the Tukey and Duncan tests for multiple comparisons. The mean values of the groups were deemed be exhibit statistically significant differences when the p-values were <0.05.

Table 1. Primers utilized for RT-PCR

GLP-1R - forward	5′-ACGCACTTTCTTTCTCTGCC-3′
GLP-1R - reverse	5′-CAAACAGGTTCAGGTGGATG-3′
β-Actin - forward	5′-CTAAGGCCAACCGTGAAAAG-3′
β-Actin - reverse	5′-TACATGGCTGGGGTGTTGA-3′

3. Results

3.1. Blood Glucose Levels

The blood glucose levels obtained for the rats of all the groups on the 0th (the day of beginning the experiment), 3rd, 7th, 14th, and 21st (the day ofsigni concluding the experiment) days were examined. The statistical evaluations revealed no significant difference between the control and sham groups and between the diabetic and diabetic + curcumin groups .However, a significant difference was identified between these groups(Table 2).

3.2. Histological Findings

The histological examination revealed that the liver tissues of the control group rats were observed to be externally surrounded by Glisson's capsule. The Remark's cords, in which hepatocytes aggregate radially around the vena centralis and the hepatic sinusoids between them, were identified. Endothelial and Kupffer cells were observed on the walls of the sinusoids. In the portal area between the lobules, the hepatic artery, portal vein, and bile duct, which collectively constitute the liver triad, as well as the lymphatic vessel, which typically accompanies this triad, were seen (Figure 1A). Upon examination of the tissue samples from the diabetic group, it was observed that there were localized enlargements in the hepatic sinusoids. Additionally, in certain parts of the liver, lipid vacuoles were present within the cytoplasm of the hepatocytes in the vicinity of vena centralis (Figure 1B). In the histological examinations of the diabetic + curcumin group, occasional enlargements of the hepatic sinusoids were observed, though these enlargements were less prominent than those observed in the diabetic group. Furthermore, the number of lipid vacuole-containing hepatocytes observed in the diabetic group was reduced, and they did not elicit the same level ofattention as those observed in the diabetic group (Figure 1C). Upon histological examination, it was observed that the general structure of the liver tissue samples from the sham group was similar to that of those from the control group (Figure 1D).

3.3. Immunohistochemical Findings

With regard to the immunohistochemical examinations of the liver tissue samples from all the groups, hepatocytes exhibited GLP-1R immunoreactivity, which typically elicited a cytoplasmic response. In some hepatocytes, immunoreactivity was observed in both the cytoplasm and the nucleus. Immunoreactivity was typically observed in the individual hepatocytes in the vicinity of the vena centralis. The degree of immunoreactivity observed in the hepatocytes of the control group rats exhibited a range, withthe highest degree of immunoreactivity also observed in this group. In addition, mild reactions were observed in the endothelial cells on the sinusoidal walls (Figure 2A). The presence of GLP-1R positivity was r observed in both hepatocytes and, on occasion, the endothelial cells within the diabetic group. The results demonstrated that the level of immunoreactivity observed in the hepatocytes was less than that seen in the control group. However, the immunoreactivity observed in the sinusoidal walls of the diabetic group rats was more pronounced than that of the control group rats (Figure 2B). The intensity of GLP-1R immunoreactivity in the hepatocytes was greater in the diabetic + curcumin group than in the diabetic group. Additionally, immunoreactivity was observed in some endothelial cells (Figure 2C). The intensity of GLP-1R immunoreactivity observed in the sham group was found to be comparable to that observed in the control group. Nevertheless, as with the control group, immunoreactivity was also observed in some endothelial and Kupffer cells (Figure 2D).

Table 2. Comparison of the blood glucose levels (mg/dL) between the groups (P < 0.05).

GROUP	N	Mean ± SD	P	F
Control	30	78,83±25,11[a]	0,000	46,258
Sham	30	87,63±18,67[a]		
Diabetic	30	259,16±127,2[b]		
Diabetic + Curcumin	30	254,16±94,2[b]		

[a,b]At the same line, different superscript letters mean significant differences (P<0.05)

The negative controls used for determining the GLP-1R immunoreactivity specificity exhibited no observable reaction (Figure 1E). Furthermore, a comparison of the groups in terms of GLP-1R immunoreactivity revealed a similarity was observed between the control, sham, and diabetic + curcumin groups. However, a statistically significant decrease was observed in the diabetic group compared to the other groups (Table 3).

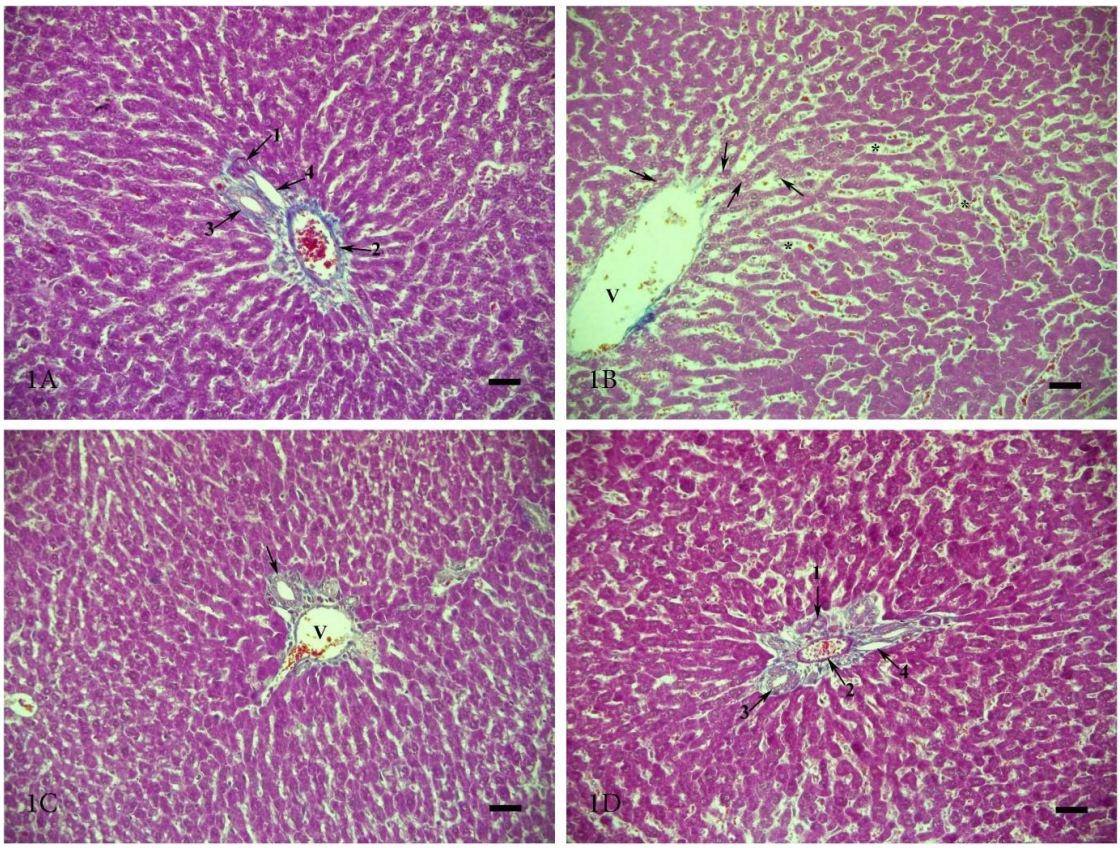

Figure 1. A) depicts the histological appearance of control group liver tissue. The tissue was subjected to triple staining. The arrows indicate: 1- Hepatic Artery, 2 - Interlobular Vein, 3 - Bile Duct, 4 - Lymph Vessel. The scale bar represents a length of 100 micrometers **B)** Histological appearance of liver tissue from the diabetic group. The tissue was subjected to triple staining. The arrows indicate the presence of lipid vacuoles in hepatocytes . *: The hepatic Sinusoids are visible. The scale bar represents a length of 100 micrometers. **C)** Histological Appearance of Liver Tissue from the group receiving diabetes+curcumin therapy. V: vena centralis. The arrow indicates the location of thebile duct. The samples were subjected to triple staining. The scale bar is 100 micrometers. **D)** Histological Appearance from the Sham Group. The tissue was subjected to triple staining. The arrows indicate:: 1- Hepatic Artery, 2- Portal Vein, 3- Bile Duct, 4 - Lymph Vessel. The scale bar is 100 micrometers.

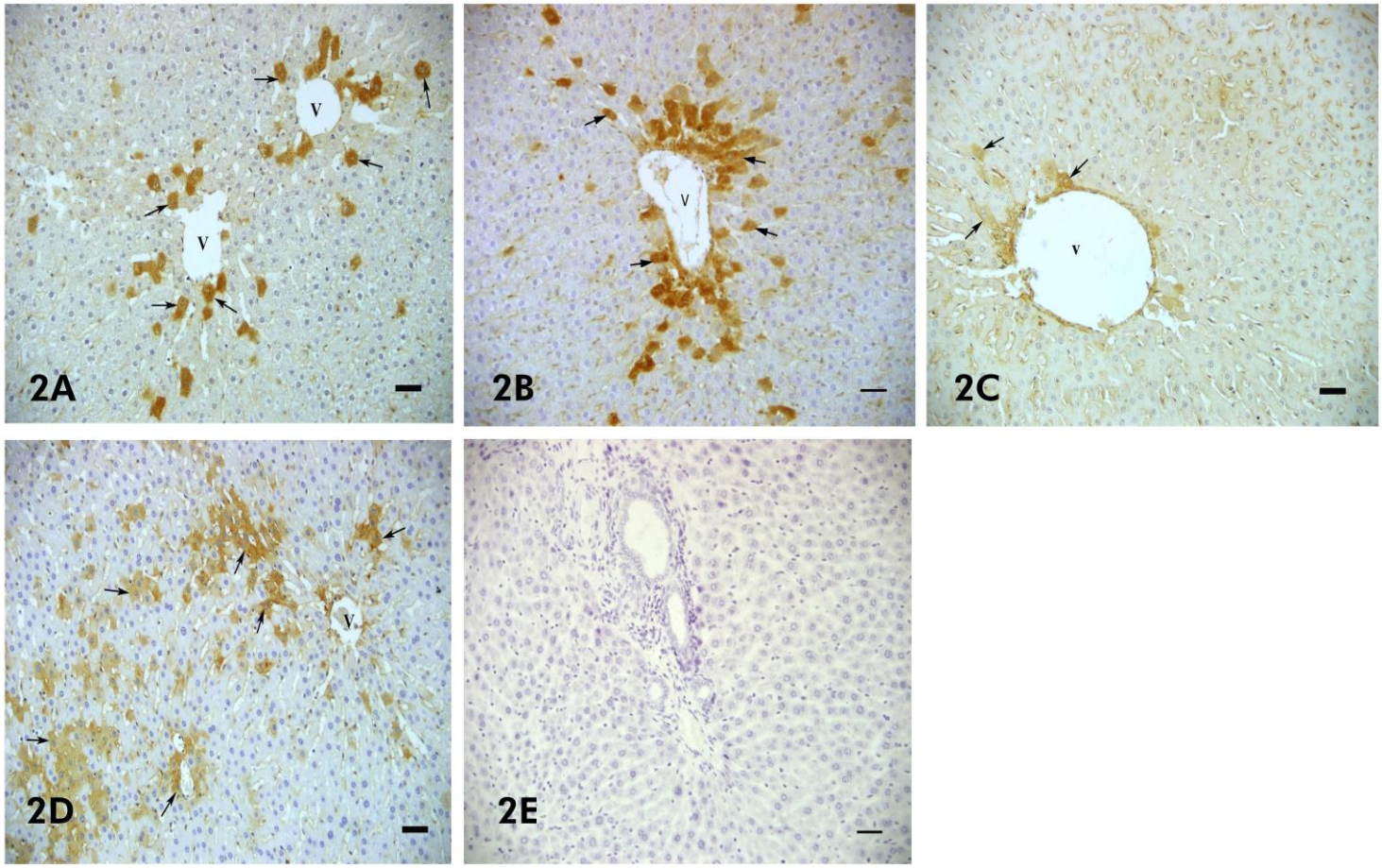

Figure 2. A) depicts a general view of the Control Group Liver Tissue withGLP-1R immunoreactivity. V: Central vein. The arrows indicate : hepatocytes exhibiting with GLP-1R immunoreactivity. The scale bar represents a length of 100 micrometers. **B)** General View of Diabetic Group Liver Tissue GLP-1R Immunoreactivity. V: Central vein. The arrows indicate: Immunopositive Hepatocytes. The scale bar represents a length of 100 micrometers. **C)** General View of Diabetic+Curcumin Group Liver Tissue GLP-1R Immunoreactivity. V: Central vein. The arrows indicate: Immunopositive Hepatocytes. The scale bar represents a length of 100 micrometers. **D)** General View of Sham Group Liver Tissue GLP-1R Immunoreactivity. V: Central vein. The scale bar represents a length of 100 micrometers. **E)** Control Group Liver Tissue. Negative Control. The scale bar represents a length of 100 micrometers.

Table 3. Intergroup comparison of liver GLP-1R immunoreactivity

GROUP	N	Mean±SD	P
Control	60	1,9167± ,69603 [a]	0,000
Sham	60	1,8500± ,73242 [a]	
Diabetic	60	1,3333± ,57244 [b]	
Diabetic+Curcumin	60	1,7667± ,74174 [a]	

P: p-value, P < 0.05, Mean: GLP-1R immunoreactivity in the liver samples, SD: standard deviation, superscripts indicate significant differences between the groups. [a,b]At the same line, different superscript letters mean significant differences (P<0.05)

3.4. Molecular Analysis

The results of the assessments indicate thatthe average expression of GLP-1R was low, despite the absence of a significant difference in GLP-1R expression between the diabetic group and the control, sham, and diabetic + curcumin groups (Figures 3,and4). The data demonstrated that curcumin elevated the mean expression level of GLP-1R in the diabetic rats, despite the absence of a statistically significant difference between the groups (Table 4). Table 4 presents a comparative analysis of the expression levels of GLP-1R in the liver tissues of the control, sham, diabetic, and diabetic + curcumin groups.

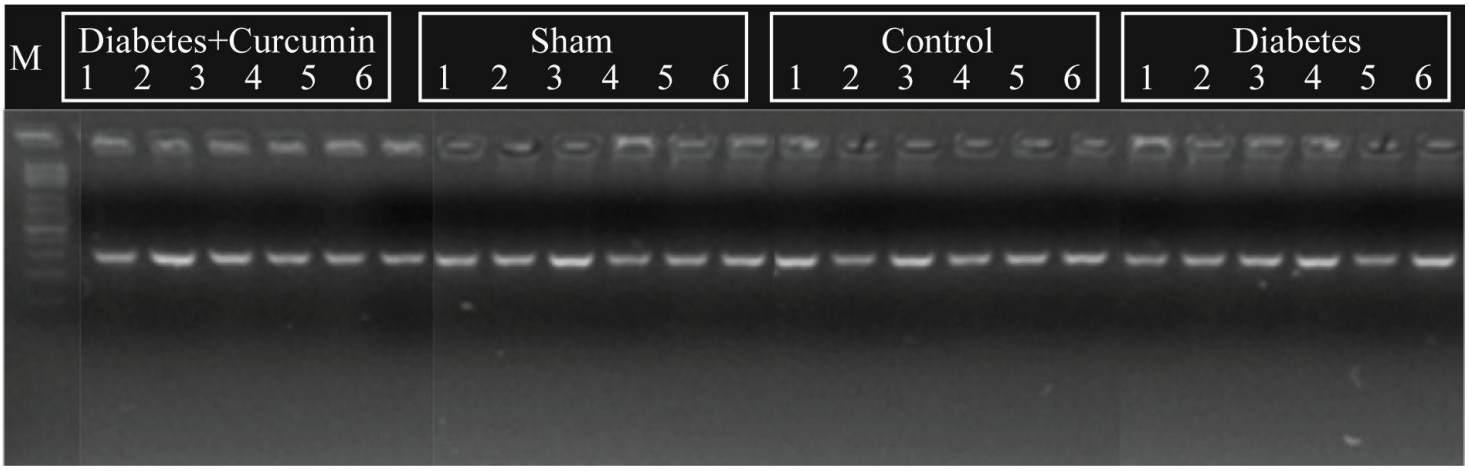

Figure 3. Image of PCR results of the β-actin (control) gene.

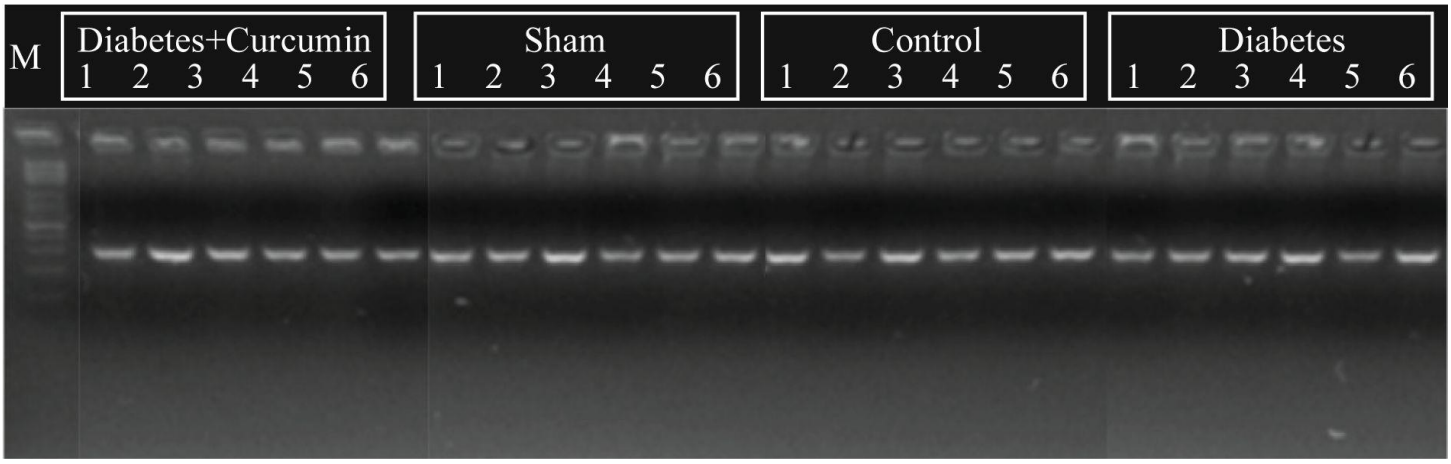

Figure 4. Image of PCR results of the GLP-1R gene.

Table 4: Comparison of GLP-1R Gene Expression Level in Liver Between Groups.

GROUP	N	GLP-1R Gene Expression Level (AU**) Mean±SD	P*
Control	6	154,09±18,76	0,157
Sham	6	144,80±8,37	
Diabetic	6	132,49±14,12	
Diabetic+Curcumin	6	140,82±14,53	

*One-Way ANOVA, **Arbitrary Units SD: Standard Deviation

4. Discussion

Herbal methods have been employed for many years in the treatment of diabetes, which is becoming increasingly prevalent in response to shifting environmental factors, living conditions, and dietary trends. However, there is currently no definitive treatment for this disease. The objective of our study was to examine the impact of curcumin, derived from the roots of the turmeric plant, on GLP-1R. Curcumin is renowned for its antidiabetic properties and has been utilized extensively in the treatment of numerous disorders, including cancer and diabetes, in recent times.

4.1. Administration of Curcumin

Following the discovery by Srinivasan (2) that curcumin affects glycaemia in a patient, many studies have been conducted with the objective of determining the effect of curcumin in controlling blood glucose levels. In studies examining the effects of curcumin, various methods of curcumin administration were employed with different doses and durations (15). In accordance with the methodology employed in analogous study, in line with similar studies (16), rats in which diabetes was induced through the administration of STZ were IP administered 100 mg/kg of curcumin on a daily basis for a period of 21 days.

4.2. Curcumin and Blood Glucose

The effect of curcumin on blood glucose levels has been the subject of numerous studies. These studies have indicated that curcumin can regulate blood glucose levels in individuals with diabetes (17). Conversely, some studies have indicated that curcumin has no significant effect on blood glucose levels in cases of STZ-induced diabetes (18). Our investigation yielded results consistent with the those of Kim et al. (18) and Palma et al. (19), indicating that curcumin did not significantly alter the blood glucose levels in the rats with experimental diabetes.

4.3. Histological Evaluation

The cytotoxic effect of STZ is mediated by free radicals, which affect the pancreas, liver, and kidneys. The liver plays a pivotal role in insulin-mediated physiological processes, including the maintenance of blood glucose, and lipid homeostasis (20). It has been documented that STZ administration results in damage to liver tissue along the cell cords a surrounding the vena centralis (1). In experimental diabetes, generally, the radial arrangement of the cells around the vena centralis is typically deteriorated, the vessel walls widen and thicken, degenerates widely, lipid accumulates and vacuolize , hydropic changes occur, the organelles degenerate and reduces in number, fibrosis develops , and sinusoids dilute (21). These observations are made in liver tissue. Additionally, fibrosis and inflammatory cell infiltration were observed in the portal regions. The histological alterations observed in rats with STZ-induced diabetes are including inflammatory cell infiltration surrounding the portal triad. Additionally, the hepatocytes of in addition, these rats' hepatocytes stored less glycogen than those of the control group (22). In diabetic animals, hepatocytes with densely stained nuclei and vacuoles were observed, and local fibrous areas were noted between these cells; In severely affected hepatocytes, it has been reported that the nuclei were degenerated and appeared very small, and the chromatin was clumped in the nuclei and appeared dark (23). In a distinct investigation, the levels of collagen and connective tissue surrounding the arteries in the periportal regions were demonstrated to have significantly increased. In our study, the liver tissue from the control group rats exhibited a normal histological structure. In contrast,the liver tissue from the diabetic group rats displayed vacuolization in the hepatocytes, particularly in those around the vena centralis, as well as dilatation in the sinusoids. These observations align with those reported by others. (21, 23). In the diabetic + curcumin group, occasional dilatation in the sinusoids was observed, though it was less pronounced than that seen in the diabetic groups.Additionally, and the vacuolization in the hepatocytes was also less severe. It is considered that this might be related to the reduction of hepatic fat accumulation through the activation of GLP-1R (24) and the hypolipidemic effect of curcumin (15).

4.4. GLP-1R Immunoreactivity Evaluation

GLP-1R was identified in the colon and intestinal walls, stomach muscle, and submucosal layers, but it was not discovered in the hepatocytes, according to Richards et al. (25) who also reported that it was detected in certain fibers in the portal vein walls close to the liver hilum. In their study investigating the localization of GLP-1R in human and monkey tissues, Pyke ,et al reported that GLP-1R was not found in the monkey liver, but was present in the pancreas, stomach, duodenum, jejunum, colon, ileum, and heart of monkeys. Additionally, Ronveaux et al. (27) also reported that GLP-1R immunoreactivity was undetectable in the liver in their

study on the ability of GLP-1 to reduce food intake in rats. Coşkun et al. (28) observed that GLP-1R was expressed in the liver during immunohistochemical analyses, and that the diabetes group exhibited a significantly lower number of GLP-1R-immunopositive cells than the control group. A review of literature revealed very little information regarding the immunoreactivity of GLP-1R in the liver of individuals with diabetes. In contrast with the findings of Ronveaux et al. (27), our study revealed the presence of GLP-1R immunoreactivity in the liver. Our findings are supported by the fact that the liver is one of the target tissues of GLP-1 and is also a glucose-sensitive organ, as well as the fact that a high concentration of GLP-1 is found in the portal vein. In contrast with unlike the findings of Richards et al. (25), the immunoreactivity in the liver was predominantly observed in the hepatocytes. The present study revealed that the localization of GLP-1R localization in the liver was immunoreactive in a similar manner in the control, sham, diabetic, and diabetic + curcumin groups. With regard to immunohistochemical examinations, GLP-1R immunoreactivity was found to be similar in the hepatocytes for all the groups, and it generally showed a cytoplasmic reaction. Additionally, the vena centralis exhibited individual hepatocyte immunoreactivity, with the diabetic group exhibiting a more pronounced response in endothelial cells than the other groups. In addition, our findings align with those of Coşkun et al. (28) indicating that the GLP-1R immunoreactivity observed in the hepatocytes of the diabetic group rats was diminished in comparison to that of the control and sham group rats. It was found that the GLP-1R immunoreactivity observed in the hepatocytes of the diabetic + curcumin group rats was higher than that observed in the those of the diabetic group rats.

4.5. Evaluation of the Molecular analysis

There is a certain degree of controversy surrounding the GLP-1R expression in hepatocytes, as evidenced by certain research findings. According to Vahl et al. (29) RT-PCR analysis of RNA extracts derived from tissues obtained from the portal vein and liver demonstrated that the GLP-1R gene was not expressed in these tissues. In a study on diabetic dogs in which RT-PCR was employed, it was reported that GLP-1R was expressed in the pancreas, fat, and muscle cells but not in the liver (30).Additionally, Thomas and Habener (31) also reported the inability to detect GLP-1R mRNA in mouse hepatocytes in their study employing the PCR technique.Similarly, Bullock et al. (32) reported that they could not identify GLP-1R in the rat liver through molecular analysis examining the tissue distribution of GLP-1R mRNA. In contrast, Dunphy et al. (12) examined the tissue distribution of glucagon receptor and GLP-1R gene expression in rats and reported that they identified GLP-1R in the liver. Similarly, Svegliati-Baroni et al. (24) reported that they identified GLP-1R in human and rat liver hepatocytes using both Western blot analysis and RT-PCR in their study. Gupta et al. (33) were able to detect both the GLP-1R mRNA and its protein in human hepatocytes. Lee et al. (34) determined GLP-1R expression in the liver as a result of molecular analyses and reported a high-fat diet treatment decreased GLP-1R expression in mouse hepatocytes. Conversely, exendin-4 therapy elevated GLP-1R expression it in a dose-dependent manner in human hepatocytes. The impact of diabetes on GLP-1R expression in different tissues has been the subject of investigation in several studies. A study (25) has reported a decrease in GLP-1R mRNA expression in the pancreas of neonatal rats with STZ-induced diabetes compared to the control group. In a study by Broide et al. (35), the examination of the expression of GLP-1R was done in the gastric glands of patients with type 2 diabetes. The results indicated a significant decrease in mean GLP-1R mRNA levels in diabetic patients compared to those in the non-diabetic control group. As reported by Coşkun et al. (22), GLP-1R mRNA expression in rat liver was found to be significantly lower in the diabetes group as compared to the control group. In our investigation, GLP-1R gene expression was observed in the liver, which differs from the findings of Bullock et al. (32), Sandhu et al. (30), and Thomas and Habener (31). However, it is consistent with the results reported by Dunphy et al. (12), Svegliati-Baroni et al. (24), Gupta et al. (33), and Lee et al. (34). Additionally, the GLP-1R gene expression in the diabetic group decreased, though not statistically significantly, in line with the observations made by Coşkun et al. (28), while the GLP-1R gene expression in the diabetic + curcumin group was comparable to that observed in the control group. The low average expression level observed in the diabetic group and the higher average expression level noted in the curcumin+diabetic group in our molecular analysis indicates that curcumin exerts an influence onGLP-1R gene expression in diabetes, despite the absence of a statistically significant difference in GLP-1R gene

expression between the groups. A multitude of queries pertaining to abundance and tissue specificity can be addressed with minimal cost and effort through the utilization of gel-based RT-PCR (37). It has been determined that curcumin is beneficial for diabetes at both the tissue and cellular levels in the liver, as well as for GLP-1R, which has recently been identified as a potential target for diabetes treatment. This finding led to the conclusion that curcumin, which is readily available and has few side effects, can be used as a therapeutic agent to treat diabetes and mitigate its complications. This conclusion is supported by the historical use of curcumin for centuries in traditional medicine to treat a wide range of illnesses.

Acknowledgment
We would like to acknowledge the financial support of Kafkas University, BAP (Project No: 2018-TS-06), which made this research possible. The findings presented in this work were taken from a student thesis from the Medical Science Institute at Kafkas University (Reşit Uğran's doctoral thesis, 2020).

Authors' Contribution
SKT and RU designed the study. The data were analyzed and a draft manuscript was prepared.: RU. The paper was subjected to a critical revision.: RU and SKT. The research was supervised by: RU and S KT. The final draft of the work has been reviewed and approved by all authors.

Ethics
The authors of this study certify that every ethical guideline was adhered to during the preparation of the final manuscript.

Conflict of Interest
We declare that we have no conflicts of interest as authors of the present study.

Data Availability
The data that support the findings of this study are available on request from the corresponding author.

References

1. Ghosh S, Bhattacharyya S, Rashid K, Parames CS. Curcumin protects rat liver from streptozotocin-induced diabetic pathophysiology by counteracting reactive oxygen species and inhibiting the activation of p53 and MAPKs mediated stress response pathways. Toxicology Rep. 2015;2:365-376.
2. Srinivasan M. Effect of curcumin on blood sugar as seen in a diabetic subject. Indian J Med Sci. 1972;26(4):269-270.
3. Maheshwari RK, Singh AK, Gaddipati J, Srimal RC. Multiple biological activities of curcumin: a short review. Life Sci. 2006;78(18):2081-2087.
4. Sharma S, Kulkarni SK, Chopra K. Curcumin, the active principle of turmeric (Curcuma longa), ameliorates diabetic nephropathy in rats. Clin Exp Pharmacol Physiol. 2006;33 (10): 940-945.
5. Wickenberg J, Ingemansson SL, Sandra J. Effects of Curcuma longa (turmeric) on postprandial plasma glucose and insulin in healthy subjects. Nutrition J. 2010;9: 43.
6. Gautier JF, Choukem SP, Girard J. Physiology of incretins (GIP and GLP-1) and abnormalities in type 2 diabetes. Diabetes Metab. 2008;34 Supplement 2:S65-S72.
7. Taşçı SK, Bingöl SA. GLP-1 localisation and proglucagon gene expression in healthy and diabetic mouse ileum. J Vet Res. 2018;62(2):237-242.
8. Holst JJ. Glucagon and glucagon-like peptides 1 and 2. Cellular Peptide Hormone Synthesis and Secretory Pathways. Results Probl Cell Differ. 2009:221-234.
9. Baggio LL, Drucker DJ. Biology of incretins: GLP-1 and GIP, Gastroentrology. 2007;132:2131-2157.
10. Donnelly D. The structure and function of the glucagon-like peptide-1 receptor and its ligands. Br J Pharmacol. 2012;16 (1):27-41.
11. Orskov C, Poulsen SS. Glucagonlike peptide-I-(7-36)-amidereceptors only in islets of Langerhans. Autoradiographic survey of extracerebral tissues in rats. Diabetes. 1991;40(10):1292-1296.
12. Dunphy JL, Taylor RG, Fuller PJ. Tissue distribution of rat glucagon receptor and GLP-1 receptor gene expression. Mol Cell Endocrinology. 1998;141(1-2):179-186.
13. Kanitkar M, Bhonde R. Existence of islet regenerating factors within the pancreas. The Review of Diabetic Studies. 2004;1(4):185-192.
14. Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem. 1987;162(1):156-159.

15. Babu PS, Srinivasan K. Hypolipidemic action of curcumin, the active principle of turmeric (Curcuma longa) in streptozotocin induced diabetic rats. Mol Cell Biochem. 1997;66(1-2):169-175.
16. Kaur G, Meena C. Amelioration of obesity, glucose intolerance, and oxidative stress in high-fat diet and low-dose streptozotocin-induced diabetic rats by combination consisting of "curcumin with piperine and quercetin." international scholarly research. Network Pharmacol. 2012;1-7.
17. Seo KI, Choi MS, Jung UJ, Kim HJ, Yeo J, Jeon SM, et al. Effect of curcumin supplementation on blood glucose, plasma insulin, and glucose homeostasis related enzyme activities in diabetic db/db mice. Mol Nutr Food Res. 2008;52(9):995-1004.
18. Kim T, Davis J, Zhang AJ, He X, Suresh TM. Curcumin activates AMPK and suppresses gluconeogenic gene expression in hepatoma cells. Biochemical and Biophysical Research Communications. 2009;388(2):377-382.
19. Palma HE, Wolkmer P, Gallio M, Corrêa MMB, Schmatze R et al. Oxidative stress parameters in blood, liver, and kidney of diabetic rats treated with curcumin and/or insulin. Mol Cell Biochem. 2014;386(1-2):199-210.
20. Wójcik-Cichy K, Koślińska-Berkan E, Piekarska A. The influence of NAFLD on the risk of atherosclerosis and cardiovascular diseases. Clin Exp Hepatol. 2018;4(1):1-6.
21. Alshathly MR. Efficacy of ginger (Zingiber officinale) in ameliorating streptozotocin-induced diabetic liver injury in rats: histological and biochemical studies. J Microsc Ultrastruct. 2019;7(2):91-101.
22. Taslidere E, Gul M, Elbe H, Cetin A, Vardi N, Ozyalın F et al. The effects of caffeic acid phenethyl ester on streptozotocin-induced diabetic liver injury. Bratislavske Lekarske Listy. 2016;117(5):276-282.
23. Khidr BM, El-Sokkary GH, Saleh SMM. Study on morphological changes induced by aspartame on liver of normal and diabetic male albino rats. Journal of Histology. 2017;4(1):1
24. Svegliati-Baroni G, Saccomanno S, Rychlicki C, Agostinelli L, De Minicis S, Candelaresi C et al. Glucagon-like peptide-1 receptor activation stimulates hepatic lipid oxidation and restores hepatic signalling alteration induced by a high-fat diet in nonalcoholic steatohepatitis. Liver International. 2011;31(9):1285-1297.
25. Richards P, Parker HE, Adriaenssens AE, Hodgson JM, Cork SC, Trapp S et al. Identification and characterization of GLP-1 receptor-expressing cells using a new transgenic mouse model. Diabetes. 2014;63(4):1224-1233.
26. Pyke C, Heller RS, Kirk RK, Ørskov C, Reedtz-Runge S, Kaastrup P et al. GLP-1 receptor localization in monkey and human tissue: novel distribution revealed with extensively validated monoclonal antibody. Endocrinology. 2014;155(4):1280-1290.
27. Ronveaux CC, de Lartigue G, Raybould HE. Ability of GLP-1 to decrease food intake is dependent on nutritional status. Physiology and Behavior. 2014;135:222-229.
28. Coşkun ZM, Beydogan AB, Bolkent S. Changes in the expression levels of CB1 and GLP-1R mRNAs and microRNAs 33a and 122 in the liver of type 2 diabetic rats treated with ghrelin. Journal of Biochemical and Molecular Toxicology. 2019;33(10):e22388.
29. Vahl TP, Tauchi M, Durler TS, Elfers EE, Fernandes TM, Bitner RD et al. Glucagon-like Peptide-1 (GLP-1) receptors expressed on nerve terminals in the portal vein mediate the effects of endogenous GLP-1 on glucose tolerance in rats. Endocrinology. 2007;148 (10):4965-4973.
30. Sandhu H, Wiesenthal SR, MacDonald PE, McCall RH, Tchipashvili V, Rashid S et al. Glucagon-like peptide 1 increases insulin sensitivity in depancreatized dogs. Diabetes. 1999;48(5):1045-1053.
31. Tomas E, Habener JF. Insulin-like actions of glucagon-like peptide-1: a dual receptor hypothesis. Trends Endocrinol Metab. 2010;21(2):59-67.
32. Bullock BP, Heller RS, Habener JF. Tissue distribution of messenger ribonucleic acid encoding the rat glucagon-like peptide-1 receptor. Endocrinology. 1996;137(7):2.9682978.
33. Gupta NA, Mells J, Dunham RM, Grakoui A, Handy J, Saxena NK et al. Glucagon-like peptide-1 receptor is present on human hepatocytes and has a direct role in decreasing hepatic steatosis in vitro by modulating elements of the insulin signaling pathway. Hepatology 2010;51(5):1584-1592.
34. Lee J, Hong SW, Chae SW, Kim DH, Choi JH, Bae JC et al. Exendin-4 improves steatohepatitis by increasing Sirt1 expression in high-fat diet-induced obese C57BL/6J mice. PLoS ONE. 2012;7(2):e31394.

35. Thyssen S, Arany E, Hill DJ. Ontogeny of regeneration of β-cells in the neonatal rat after treatment with streptozotocin. Endocrinology 2006;147(5):2346-2356.
36. Glare EM, Divjak M, Bailey MJ, Walters EH. Beta-actin and GAPDH housekeeping gene expression in asthmatic airways is variable and not suitable for normalising mRNA levels. Thorax. 2002;57(9):765-770.
37. Mitchell K, Iadarola MJ. RT-PCR analysis of pain genes: use of gel-based RT-PCR for studying induced and tissue-enriched gene expression. Methods Mol Biol. 2010;617:279-295.