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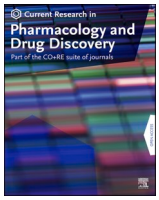

Quercetin prevents rats from type 1 diabetic liver damage by inhibiting TGF-β/apelin gene expression

Gholampour Firouzeh [a,*], Abbasi Susan [a], Karimi Zeinab [b]

[a] Department of Biology, College of Science, Shiraz University, Shiraz, Iran
[b] Shiraz Nephro-Urology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran

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ABSTRACT

Background: Hyperglycemia-induced oxidative stress is a significant contributor to diabetic complications, including hepatopathy. The current survey aimed to evaluate the ameliorative effect of quercetin (Q) on liver functional disorders and tissue damage developed by diabetes mellitus in rats.

Methods: Grouping of 35 male Wistar rats was performed as follows: sham; sham + quercetin (sham + Q: quercetin, 50 mg/kg/day in 1 ml 1% DMSO for 6 weeks, by gavage); diabetic control (Diabetes: streptozotocin (STZ), 65 mg/kg, i.p.); diabetic + quercetin 1 (D + Q1: quercetin, 25 mg/kg/day in 1 ml 1% DMSO for 6 weeks, by gavage after STZ injection); and diabetic + quercetin 2 (D + Q2: quercetin, 50 mg/kg/day in 1 ml 1% DMSO for 6 weeks, by gavage after STZ injection). Body weight, food intake, and water intake were measured. Ultimately, the samples of plasma and urine, as well as tissue samples of the liver and pancreas were gathered for later assays.

Results: STZ injection ended in elevated plasma blood glucose levels, decreased plasma insulin levels, liver dysfunction (increased activity levels of AST, ALT, and ALP, increased plasma levels of total bilirubin, cholesterol, LDL, triglyceride, decreased plasma levels of total protein, albumin and HDL), enhanced levels of malondialdehyde, diminished activities of antioxidant enzymes (superoxide dismutase, and catalase), reduced level of glutathione (GSH) increased gene expression levels of apelin and TGF-β, plus liver histological destruction. All these changes were diminished by quercetin. However, the measure of improvement in the D + Q2 group was higher than that of the D + Q1 group.

Conclusions: Quercetin improved liver function after diabetes mellitus type 1, possibly due to reduced lipid peroxidation, increased antioxidant systems, and inhibiting the apelin/TGF-β signaling pathway.

1. Introduction

Liver disease, as one of diabetic mellitus (DM) complications, can be very considerable (Manna et al., 2010), as the standardized mortality rate of DM patients from end-stage liver disease (i.e., cirrhosis) is more than those for cardiovascular diseases (Harrison, 2006).

The central role of the liver in glucose homeostasis comprises storing glycogen in the fed state and producing glucose via glycogenolysis and gluconeogenesis in the postabsorptive period. Hepatocytes are the main site of glucose metabolism. Approximately 30–60% of all glucose absorbed in the gastrointestinal tract is stored as glycogen or metabolized into amino acids and fatty acids in the liver (Harrison, 2006). Insulin and glucagon as two counter-regulatory hormones regulate energy metabolism (Harrison, 2006). Uncontrolled gluconeogenesis, glycogenolysis, and lipogenesis result from the failure of hepatocytes to respond to insulin brought on by diabetes, which promotes hyperglycemia, dyslipidemia, and systemic insulin resistance (Harrison, 2006; Adiels et al., 2008), leading to steatohepatitis (Takamatsu et al., 2008).

Apelin plays an important role in both physiological and pathophysiological processes, inclusive of inflammation and metabolic balance (Yang et al., 2017). Also, apelin participates in the apoptosis of hepatocytes, synthesis of glycogen, and fibrosis of the liver (Lv et al., 2017). Although apelin expression is low in normal liver (D'Aniello et al., 2009), serum apelin level is enhanced in nonalcoholic fatty liver diseases (NAFLD) and cirrhosis (Lim et al., 2016). Moreover, under hypoxic or proinflammatory conditions, apelin expression is enhanced in hepatic stellate cells (HSCs) which contributes to cirrhosis progression or liver fibrosis (Yokomori et al., 2011; Melgar-Lesmes et al., 2010). Two characteristic features of NAFLD are lipid deposition and fibrosis in the

* Corresponding author. School of Science, Shiraz University, Shiraz, Iran, Department of Biology, School of Science, Adabiat Intersection, Shiraz, Iran.
E-mail address: gholampour@shirazu.ac.ir (G. Firouzeh).

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Abbreviations

CAT	Catalase
DM	Diabetes mellitus
GSH	Reduced glutathione
MDA	Malondialdehyde
Q	Quercetin
SOD	Superoxide dismutase
TBA	Thiobarbituric acid
TGF-β1	Transforming growth factor-β1

liver (Falck-Ytter et al., 2001). Aggravated oxidative stress due to disturbance of lipid metabolism in the liver accompanied by inflammation, insulin resistance, and hyperinsulinemia gives rise to the progress of DM (type 2) and NAFLD which cause fibrosis and possibly cirrhosis (Machado and Cortez-Pinto, 2014). In liver injury, activation of HSCs into fibrogenic cells by the TGF-β/Smad signaling pathway contributes to the process of liver fibrosis (Elpek, 2014).

Apelin has therapeutic potency for diabetes through improving insulin sensitivity and insulin production as well as positive regulation of diabetic kidney hypertrophy (Castan-Laurell et al., 2019; Hu et al., 2016). However, there is some conflict about the direct effects of apelin on the endocrine pancreas such as those initial observations that showed a decrease in insulin output caused by apelin (Guo et al., 2009).

Type 1 Diabetes Mellitus (T1DM) can directly impact liver health, leading to a condition called hepatopathies. This is primarily due to the high blood glucose levels, oxidative stress, and chronic inflammation associated with T1DM, which can cause liver cell damage and fibrosis (Karimi et al., 2024; Heydarpour et al., 2020)[1, 7].

Transforming Growth Factor-beta (TGF-β) is a key player in the development of hepatopathies. Activation of the TGF-β signaling pathway is linked to liver fibrosis, inflammation, and cell proliferation (Heydarpour et al., 2020; Callan et al., 2024) [6, 7]. In T1DM, increased TGF-β expression might contribute to the development of diabetic hepatopathies (Karimi et al., 2024) [3].

Apelin, a potent regulator of energy metabolism and insulin sensitivity, has shown potential in mitigating the effects of T1DM. Research suggests that apelin might be able to counterbalance TGF-β-induced fibrosis in the liver (Wang et al., 2024) [4]. Moreover, apelin has been found to cooperate with TGF-β signaling during the development of diabetic nephropathy (Day et al., 2013) [5], and modulating apelin signaling could be an effective approach for T1DM-induced hepatopathies (Karimi et al., 2024)[3].

However, more research is needed to fully understand the complex interactions between apelin, TGF-β signaling, and T1DM, as well as to develop efficient treatment strategies for T1DM-associated hepatopathies.

T1DM can cause hepatopathies through various mechanisms. One such mechanism is chronic hyperglycemia, which can lead to the accumulation of advanced glycation end products (AGEs). AGEs can damage the liver, resulting in hepatopathies such as cirrhosis, fibrosis, and non-alcoholic fatty liver disease (NAFLD) (Mertens et al., 2021).

Regarding the link between apelin and the TGF signaling pathway, it's been shown that apelin can inhibit the TGF-β1 signaling pathway in various organs, including the liver and kidneys. This inhibition can help protect against acute renal injury and reduce inflammation and fibrosis associated with diabetic nephropathy and hepatopathy (Day et al., 2013; Chen et al., 2015). Moreover, a study suggests that conditioned media derived from human Wharton's jelly mesenchymal stem cells (hWJ-MSCs) can modulate both TGF-β and apelin signaling pathways, potentially improving diabetic nephropathy and hepatopathy in male rats (Karimi et al., 2024). However, the exact mechanisms through which apelin affects TGF signaling in the context of T1DM-induced hepatopathies require further research.

Quercetin, a famous flavonoid derived from fruits and plants (Kim and Park, 2016), possesses anti-inflammatory, antioxidant, and anti-fibrotic activities (Hou et al., 2018). Some animal models have confirmed the helpful effects of quercetin on liver injury and fibrosis (Hernández-Ortega et al., 2012). It has been shown that quercetin inhibits the CYP2E1 enzyme in the course of diabetes development, thereby preventing oxidative damage in the liver (Maksymchuk et al., 2017). Even so, the accurate mechanisms of quercetin against liver injury should be determined. In the current survey, we investigated the ameliorative effects of quercetin against liver functional disorders and tissue damage induced by diabetes mellitus in rats. In addition, the mRNA expression level of apelin and TGF-β was measured to verify further the hypothesis that apelin and TGF-β play a main role in the disease process of DM-induced liver injury and the beneficial therapeutic effect of quercetin should be involved in regulating apelin and TGF-β gene expression.

2. Materials and methods

2.1. Chemicals

Streptozotocin (STZ) and quercetin were obtained from Sigma Chemical Co, USA. All other chemicals were of analytical grade.

2.2. Animals

Male adult Wistar rats (weighing 220–250 g) were obtained from the animal house of Pasteur Institute (Shiraz, Iran). All experimental procedures involving animals were approved by the Health Guide of National Institutes for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978) and the ethical standards of the Animal Ethics Committee of Shiraz University. All animals received a standard laboratory diet and water ad libitum at room temperature (22 ± 2 °C) under 12-h light and dark cycles.

2.3. Streptozotocin-induced diabetes mellitus type 1

Fasted Wistar rats were intraperitoneally injected with 65 mg/kg streptozotocin, dissolved in 0.1 cold citrate buffer (pH = 4.5) (27), while non-diabetic rats were intraperitoneally injected with only citrate buffer. After 72 h of STZ injection, plasma glucose was determined by ACCU-Check glucose meter. The animals with more than 300 mg/dl glucose were considered diabetic animals (28).

2.4. Experimental design

Grouping of 35 male Wistar rats was randomly performed (n = 7) into sham (1 ml 1% DMSO for 6 weeks, by gavage); sham + quercetin (sham + Q: quercetin, 50 mg/kg/day in 1 ml 1% DMSO for 6 weeks, by gavage); diabetic control (Diabetes: STZ, 65 mg/kg, i.p.); diabetic + quercetin 1 (D + Q1: quercetin, 25 mg/kg/day in 1 ml 1% DMSO for 6 weeks, by gavage after STZ injection); and diabetic + quercetin 2 (D + Q2:quercetin, 50 mg/kg/day in 1 ml 1% DMSO for 6 weeks, by gavage after STZ injection. At the termination of the six weeks, the animals were anesthetized using i.p. injection of ketamine (60 mg/kg) and xylazine (5 mg/kg), and *blood sampling was performed by cardiac puncture*. The liver weight was recorded. The liver and pancreas were promptly divided, with some sections fixed in 10% phosphate-buffered formalin and others frozen at −70 C (in liquid nitrogen) for later oxidative stress and molecular assay investigation.

2.4.1. Estimation of blood and urinary glucose

Blood sampling from the lateral tail vein was performed on the first day and at the end of 3rd and 6th weeks (during the same time in the morning) for estimating fasting blood glucose by an ACCU-Check

glucose meter.

On the 42nd day of the experiment, the animal was situated in a metabolic cage individually to collect 24-h urine samples. Urinary glucose value was estimated by an automatic analyzer (Prestige, Biolis 24I, Japan) using commercially accessible kits (Pars Azmoon, Tehran, Iran).

2.4.2. Estimation of body weight

Recording of animaĺs body weight was performed at the beginning of the experimental period and at the termination of the 3rd and 6th weeks (at the same time during the morning).

2.4.3. Liver functional assays

Activity levels of lactate dehydrogenase, alkaline phosphatase, alanine aminotransferase, and aspartate aminotransferase (LDH, ALP, ALT, and AST, respectively), as the levels of total cholesterol, triglyceride, total bilirubin, total protein and albumin in plasma samples were assayed spectrophotometrically using available kits (autoanalyzer; Prestige, Biolis 24I, Japan).

2.4.4. Plasma insulin assay

Plasma insulin was assayed by "Mercodia Rat Insulin ELISA Kit (Mercodia, Uppsala, Sweden)".

2.4.5. Liver homogenate preparation and estimation of lipid peroxidation and antioxidant enzymes

Liver tissue samples were washed thrice in cold normal saline solution (0.9%) and then homogenized using a homogenizer (6.7 mM phosphate buffer, pH 7.4). Afterward, 10% homogenates were centrifuged (10,000 rpm for 10 min at 4^{oC}), and the supernatant was utilized for more assays. For evaluation of lipid peroxidation, malondialdehyde (MDA) level, and assessment of the antioxidant system, enzymatic activities of superoxide dismutase (SOD) and catalase, along with the level of reduced glutathione (GSH) were assessed by calorimetric methods applying Kiazist Kits (Iran) under the manufacturer's protocol.

2.4.6. Real-time polymerase chain reaction (Real-time PCR)

Following the manufacturer's instructions, the CinnaPureTM kit (SinaClon, Iran) was used to isolate total RNA from the stabilized liver tissues. Thermo Scientific nanodrop was used to measure the amount of extracted RNA, and unbroken bands of 18S and 28S rRNA which were run on a 1% agarose gel were used to evaluate the quality of the sample. As directed by the manufacturer, 1 μg of RNA was reverse transcribed to cDNA using the Parstous cDNA synthesis kit (Parstous, Iran).

The sequences of PCR primer pairs (designed using Primer 3 software, such that span an exon-exon junction to avoid amplifying genomic DNA), are shown in Table 1. The mRNA levels of *apelin* and *TGF-β* were assessed using the housekeeping gene *GAPDH*. Amplifying of 100 ng cDNA by Biofact SYBER Green master mix was performed using an ABI 7500 PCR machine. The corresponding expression levels of *apelin* and *TGF-β* to *GAPDH* were computed by the Delta Delta Ct method (Livak and Schmittgen, 2001). Running of all the samples was done thrice. A pretreatment of 95 °C for 10 min was carried out for all PCR reactions. Soon after, 40 cycles of 95 °C pending 15 s, 58 °C for 20 s, and 72 °C for 30 s for *GAPDH*, 40 cycles of 95 °C pending 15 s, 60 °C within 20 s and 72 °C pending 30 s for *TGF-β*, and 95 °C within 15 s, 59 °C within 20 s and 72 °C within 30 s for *apelin* were carried out.

Table 1
Primer pairs used in the current study.

Genes	Primer Sequences
GAPDH	Forward: 5′- AGTGCCAGCCTC GTCTCATA -3′ Reverse: 5′- GAGAAGGCAGCCCTGGTAAC -3′
TGF-β	Forward: 5′- TGACATGAACCGACCCTTCC -3′ Reverse: 5′- TGCCGTACACAGCAGTTCTT -3′
Apelin	Forward: 5′- CTCTGGCTCTCCTTGACTGC -3′ Reverse: 5′- TCGAAGTTCTGGGCTTCACC -3′

2.4.7. Histopathological examination

Fixation in 10% buffered formalin and embedded in paraffin wax was carried out for a portion of liver tissue. Thereafter, staining of 5 μm thick sections was done with hematoxylin and eosin (H&E). The histopathological sections were examined under a light microscope. Quantification of pancreatic histopathological changes was done for necrotic changes in the islets and severe atrophy of the acinar parts. The histopathological changes of the liver were quantified for impairment in the standard arrangement of hepatocyte cords and irregular cell community. Sections were given the following scores based on the adjustments that were made: 0 (no lesions); 1 (less than 20%), 2 (20%–40%), 3 (40%–60%), 4 (60%–80%), 5 (greater than 80%). The overall histopathological score was determined by adding together all of the numerical scores for each group (Gholampour et al., 2022).

2.5. Statistical analysis

All the data were presented as mean ± SEM and were evaluated using one-way ANOVA with Turkey test for multiple comparison tests by the SPSS statistical package (SPSS for Windows v. 26.0, Chicago, IL). A p-value of less than 0.05 was used as the criterion to determine whether an observed difference was statistically significant.

3. Results

3.1. Estimation of mean body weight, plasma glucose and insulin levels, and urine glucose concentration

According to Fig. 1A, the mean body weight of the diabetic group was diminished on the 21st day compared with its value on the 1st day of the experiment period ($P < 0.01$), as well as on the 42nd day compared with its value on the of the experiment period ($P < 0.05$). The mean body weight values of diabetic rats on the 21st and 42nd days were lower than the values in sham ones ($P < 0.001$). However, in quercetin-treated diabetic rats (D + Q1 and D + Q2 groups), body weight values were statistically increased compared with the diabetic control group both on the 21st ($P < 0.01$) and 42nd days ($P < 0.001$).

Fig. 1B shows that diabetes mellitus caused a decrease in plasma insulin level ($P < 0.001$) in the diabetic control group on the 42nd day compared with the sham group. Quercetin administration to D + Q1 and D + Q2 groups caused a significant increase in plasma insulin level ($P < 0.05$ for the D + Q1 group and $P < 0.001$ for the D + Q2 group) compared to diabetic control group. Besides, there was a significant rise ($P < 0.001$) in plasma insulin value of the D + Q1 group compared to the D + Q2 group.

According to Fig. 1C, the mean plasma glucose concentration of the diabetic group was increased on the 21st day compared with its value on the 1st day of the experiment period ($P < 0.001$), as well as on the 42nd day compared with its value on the 21st day of the experiment period ($P < 0.001$). The mean glucose concentration values of diabetic rats on the 21st and 42nd days were higher than those in sham ones ($P < 0.001$). However, in quercetin-treated diabetic rats (D + Q1 and D + Q2 groups), plasma glucose concentration values were statistically decreased compared with the diabetic control group both on the 21st and 42nd days (all $P < 0.001$).

According to Fig. 1D, diabetes mellitus enhanced the value of urine glucose concentration in diabetic control rats compared with sham ($P < 0.01$) and sham + Q ($P < 0.001$) groups. Quercetin administration to D + Q1 and D + Q2 groups decreased urine glucose concentration (both $P < 0.001$) compared with the diabetic control group. Furthermore, the urine glucose level of the D + Q2 group was lower than the D + Q1 group ($P < 0.05$).

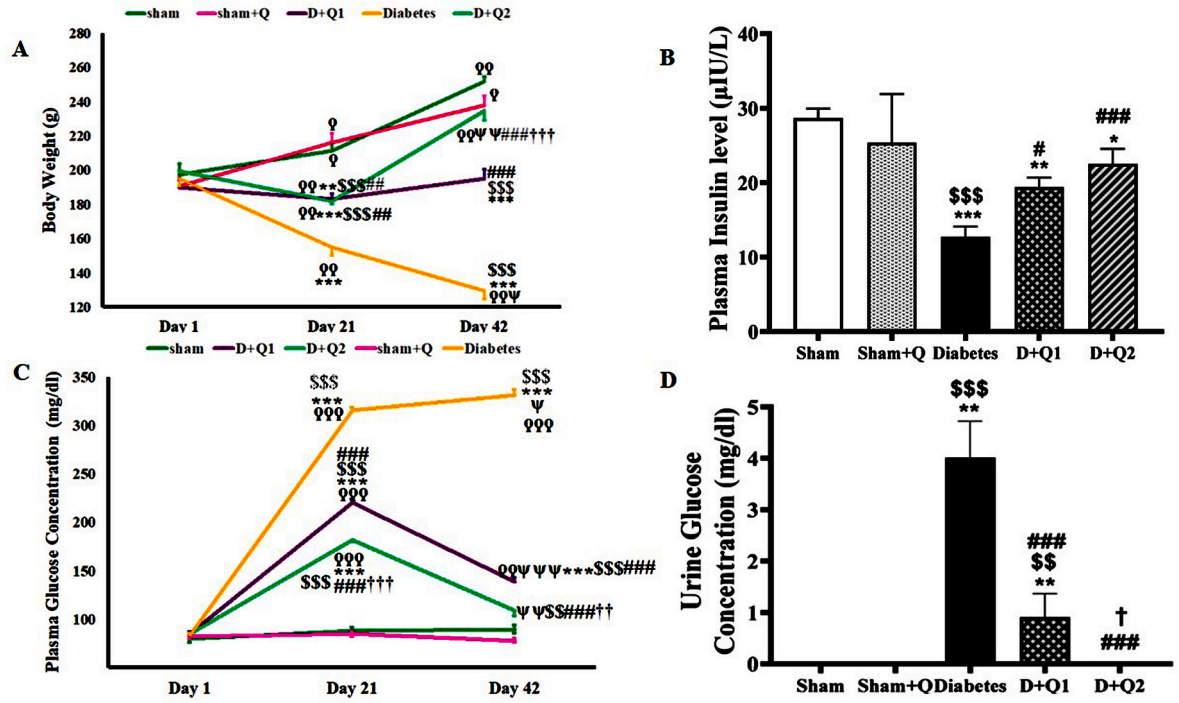

Fig. 1. Effects of quercetin on the values of (A) body weight (ϙP < 0.05, ϙϙP <0.01 vs first day; ψP < 0.05, ψψP <0.01vs 21st day in each group), (B) insulin, (C) plasma glucose concentration (ϙϙP <0.01, ϙϙϙP <0.001 vs first day; ψP < 0.05, ψψP <0.01, ψψψP <0.01 vs 21st day in each group) and (D) urine glucose concentration in each experimental group. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ vs sham group; $$P < 0.01$, $$$ $P < 0.001$ vs sham + Q group; #$P < 0.05$, ##$P < 0.01$, ###$P < 0.001$ vs diabetes group; †$P < 0.05$, †††$P < 0.001$ vs D + Q1 group.

3.2. Biochemical and liver functional parameters

As Table 2 represents, the mean amounts of food and water intake plus urine volume at the end of the 6th week in diabetic rats were higher than sham groups (all $P < 0.001$). However, administration of quercetin to the diabetic groups made significant decreases in water and food intake in D + Q1 ($P < 0.01$, $P < 0.05$ respectively) and D + Q2 ($P < 0.001$) groups along with a diminishment ($P < 0.001$) in urine volume of D + Q1 and D + Q2 animals compared to control diabetic rats.

Table 2 shows that diabetes mellitus caused a significant rise in plasma activity levels of ALT, AST, and ALP in the Diabetes group compared with the sham groups (All P < 0.001). However, quercetin caused significant reductions in plasma activity levels of ALT, AST, and ALP in D + Q1 (P < 0.01 for both ALT and ALP) and D + Q2 (all P < 0.001) groups compared with the control diabetic group.

As shown in Table 2, a significant decrease in plasma levels of protein and albumin along with an enhanced level of total bilirubin was seen in the diabetic control group compared to sham rats (all $P < 0.001$). Giving quercetin to diabetic rats caused enhanced plasma levels of protein in the D + Q2 group ($P < 0.01$) and albumin in both D + Q1 ($P < 0.01$) and D + Q2 ($P < 0.001$) groups, along with the decreased level of total bilirubin in D + Q1 ($P < 0.01$) and D + Q2 ($P < 0.001$) compared with the diabetic control group.

STZ-induced diabetes mellitus caused elevation in the values of LDL (Fig. 2A), total cholesterol (Fig. 2C), and triglyceride (Fig. 2D), along with diminished value of HDL (Fig. 2B) in the diabetic control group compared to normal control group (All $P < 0.001$). Furthermore, quercetin induced reductions in the values of LDL ($P < 0.01$, $P < 0.001$ respectively), total cholesterol, and triglyceride (all $P < 0.001$) and enhancement in the value of HDL (both $P < 0.001$) in D + Q1 and D + Q2 groups.

Table 2
Effect of quercetin on biochemical and liver functional parameters.

Groups	sham	sham + Q	Diabetes	D + Q1	D + Q2
Food intake (g/24h)	52.10 ± 3.41	52.01 ± 3.53	101.56 ± 2.68***$$ $	83.26 ± 2.65***$$ $#	64.84 ± 3.94###†
Water intake (ml/24h)	54.40 ± 1.86	57.69 ± 6.30	405.01 ± 5.38***$$ $	83.50 ± 2.492***$ $##	64.72 ± 4.30###†
Urine volume (ml/24h)	17.16 ± 0.49	16.36 ± 1.04	83.23 ± 2.30***$$ $	44.73 ± 3.62***$$ $###	30.34 ± 1.88**$ $###††
ALT activity (U/L)	25.00 ± 1.73	22.60 ± 2.37	52.00 ± 2.70***$$ $	36.00 ± 3.52$##	27.00 ± 2.47###
AST activity (U/L)	18.60 ± 2.06	21.20 ± 2.08	48.40 ± 2.87***$$ $	46.60 ± 2.73***$$$	25.00 ± 2.84###†††
ALP activity (U/L)	97.36 ± 1.63	80.14 ± 4.78	177.0 ± 9.25***$$ $	143.8 ± 3.99***$$ $##	130.0 ± 5.51**$$ $###
Plasma total protein (mg/dl)	7.00 ± 0.51	7.44 ± 0.74	3.35 ± 0.49**$$ $	5.39 ± 0.67	7.36 ± 0.38###
Plasma albumin (mg/dl)	4.22 ± 0.29	4.80 ± 0.18	1.89 ± 0.24***$$ $	3.22 ± 0.09 $$#	4.51 ± 0.40###†
Plasma total bilirubin (mg/dl)	0.86 ± 0.44	0.80 ± 0.04	1.57 ± 0.10***$$ $	1.10 ± 0.06##$	0.88 ± 0.03###

Sham + Q: sham + quercetin; Diabetes: diabetic control; D + Q1: diabetes + quercetin, 25 mg/kg/day post-treatment; D + Q2: diabetes + quercetin, 50 mg/kg/day post-treatment. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ vs sham group; $ $P < 0.05$, $$ $P < 0.01$, $$$ $P < 0.001$ vs sham + Q group; #$P < 0.05$, ##$P < 0.01$, ###$P < 0.001$ vs diabetes group; †$P < 0.05$, †††$P < 0.001$ vs D + Q1group.

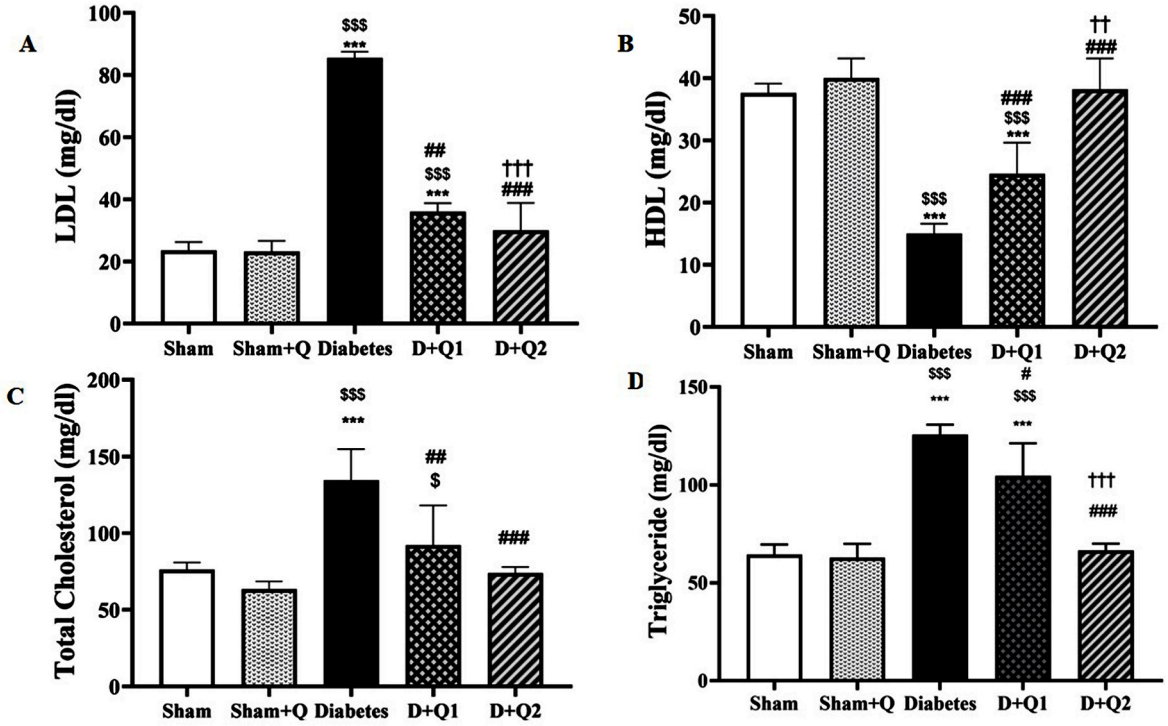

Fig. 2. Effects of quercetin on the values of (A) LDL, (B) HDL, (C) total cholesterol, and (D) triglyceride in each experimental group. ***$P < 0.001$ vs normal control group; \$ $P < 0.05$, \$\$\$ $P < 0.001$ vs normal quercetin group; #$P < 0.05$, ##$P < 0.01$, ###$P < 0.001$ vs diabetic group; †$P < 0.05$, †††$P < 0.001$vs D + Q1 group.

3.3. Estimation of redox markers of liver tissue

Fig. 3 presents the mean values of SOD and CAT activities, as well as GSH and MDA levels of both sham and experimental groups after 6 weeks. In renal tissues of the diabetic group, the activity levels of SOD (Fig. 3A) and CAT (Fig. 3B), as well as the amount of GSH (Fig. 3C) were decreased ($P < 0.001$), even though MDA content (Fig. 3D) was increased ($P < 0.001$) compared with their normal values. However, treating the diabetic group with quercetin lead to significant increase in enzymatic activity levels of SOD ($P < 0.05$ for D + Q2 group) and CAT

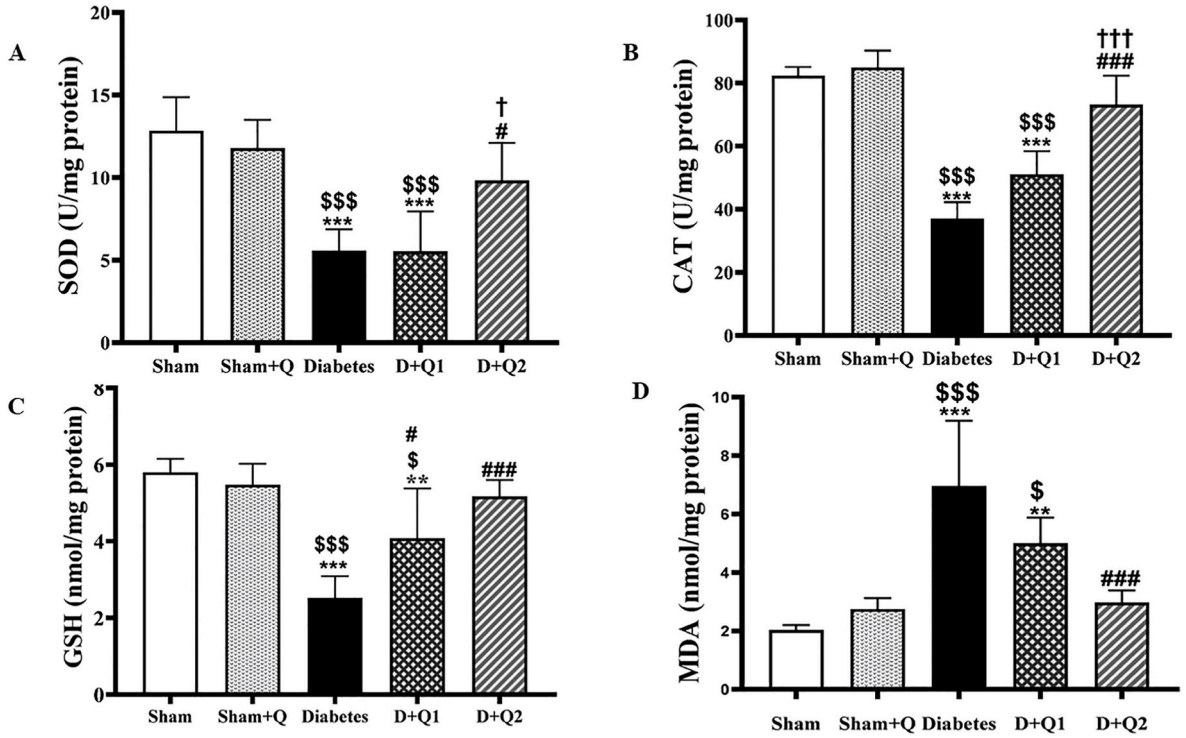

Fig. 3. Effects of quercetin on the enzymatic activity values of (A) SOD, (B) catalase, and the tissue contents of (C) GSH, and (D) MDA in each experimental group. **$P < 0.01$, ***$P < 0.001$ vs normal control group; \$ $P < 0.05$, \$\$\$ $P < 0.001$ vs normal quercetin group; #$P < 0.05$, ###$P < 0.001$ vs diabetic group; †$P < 0.05$, †††$P < 0.001$vs D + Q1 group.

($P < 0.001$ for D + Q2 group), an enhanced level of GSH ($P < 0.05$ for D + Q1 group and $P < 0.001$ for D + Q2 group) and a decreased MDA level ($P < 0.001$ for D + Q2 group) as compared with control diabetic rats. In addition, an increase in SOD ($P < 0.05$) and CAT ($P < 0.001$) activity levels was observed in the D + Q2 group compared with the D + Q1 group.

3.4. Estimation of expression values of mRNA encoding apelin and TGF-β in the liver tissue

Fig. 4 shows that apelin and TGF-β gene expression levels in liver tissues were higher in diabetes mellitus than in normal control liver tissues (both $P < 0.001$). When compared to diabetic rats, the apelin gene expression level (Fig. 4A) was lowered in D + Q2 rats ($P < 0.001$) with quercetin administration. Additionally, compared to diabetic rats, the administration of quercetin resulted in a decrease in the TGF-β gene expression levels (Fig. 4B) in D + Q1 ($P < 0.05$) and D + Q2 ($P < 0.01$) rats.

3.5. Histopathology of pancreas and liver

The control animals' pancreatic tissues showed no signs of injury (Fig. 5A–B). However, there were severe necrotic alterations in the islets and extensive acinar portion atrophy in diabetic rats (Fig. 5C). In diabetic rats, quercetin reduced pancreatic damage (Fig. 5D–E).

In the sham group, the liver had a normal structure (Fig. 6A–B). In the diabetic group, the standard arrangement of hepatocyte cords was impaired and there was an irregular cell community (Fig. 6C). In the D + Q1 and D + Q2 groups, less severe damages were seen compared with the diabetes group (Fig. 6D–E).

4. Discussion

In the current study conducted in rats, DM induced by streptozotocin (STZ, in a single dose) made great liver lesions as affirmed by histopathological damages and biochemical variations such as diminished activity of antioxidant enzymes, enhanced plasma activity of hepatic enzymes (ALT, AST and ALP), declined liver function, as well as enhanced liver oxidant stress and mRNA expression of apelin and TGF-β.

STZ makes nitrative and oxidative stress which have a role in its harmful effects such as hepatotoxicity (Raza and John, 2012). Although the mechanisms of STZ-induced hepatotoxicity are not exactly realized, the connection between STZ toxicity and oxidative stress has been proposed too (Raza and John, 2012). Moreover, apelin and TGF-β participate in hepatic steatosis or fibrosis in NAFLD (Wang et al., 2019; Yang et al., 2014). Thus, increased levels of apelin, TGF-β and free radicals along with decreased levels of endogenous antioxidants may provoke injury to hepatic cells.

Owing to its anti-inflammatory and antioxidant characteristics, quercetin can prevent diabetes and have hepatoprotective effects (Dhanya, 2022). In the current study, STZ-induced DM resulted in elevated MDA levels, decreased CAT and SOD activity levels, and reduced glutathione levels at the termination of the experiment. Other studies have demonstrated that a single dose of STZ (65 mg/kg (Bhardwaj and Modi, 2017) and 60 mg/kg rise (Niazmand et al., 2021), i.p.) increases MDA content while lowering glutathione levels, which is consistent with these findings (Bhardwaj and Modi, 2017). Decreased enzyme activity could result from non-enzymatic glycation of the enzymes or structural damage from excessive ROS generation in hyperglycemia (Giacco and Brownlee, 2010). According to our findings, administering quercetin at doses of 25 and 50 mg/kg intraperitoneally for 42 days caused a decrease in MDA levels (in this sense, quercetin at the larger dose of 50 mg/kg worked better than the lower dose of 25 mg/kg), an increase in GSH content, and higher levels of CAT and SOD activity (more so in the D + Q2 group than in the D + Q1 group) when compared to the diabetic group. Quercetin is believed to decrease malondialdehyde (MDA) levels and increase Glutathione (GSH) content due to its antioxidant properties. It accomplishes this by acting as a free radical scavenger, reducing oxidative stress, and increasing endogenous antioxidant levels (Xu et al., 2019; Qi et al., 2022; Zhang et al., 2016). By doing so, it can help protect cells from damage caused by reactive oxygen species (ROS) (Ashari et al., 2022). Additionally, some evidence suggests that quercetin may also increase the activity of antioxidant enzymes like copper/zinc superoxide dismutase (CuZn-SOD) and glutathione peroxidase (Dong et al., 2014). These findings are supported by various research studies (Cruz-Gregorio and Aranda-Rivera, 2023; Yang et al., 2020; Çelik et al., 2017).

Additionally, quercetin improved the histological alterations in the rats' livers that were treated with STZ in the current study. The documents indicate that STZ-induced hepatic damage is directly caused by ROS (Ghosh et al., 2015). Furthermore, ROS scavengers offer potent defense against acute hepatic damage (Takemoto et al., 2014). Quercetin likely improved histological alterations in the diabetic rats' livers through its primary antioxidant properties and its potential role in reducing oxidative stress and damage, as well as protecting against lipid and protein damage (Shamsi et al., 2019).

According to our findings, oxidative stress caused by a single dose of 60 mg/kg, i.p. of STZ raised ALT, AST, ALP, and MDA levels (Abolfathi et al., 2012). In the current study, the plasma level of albumin decreased, consistent with previous research (Abolfathi et al., 2012), whereas the plasma total bilirubin level increased in the diabetes group. Current research suggests that quercetin treatment improves raised levels of ALT, AST, and ALP activity in the livers of STZ-induced diabetic rats through several mechanisms involving antioxidative (Ahmed et al.,

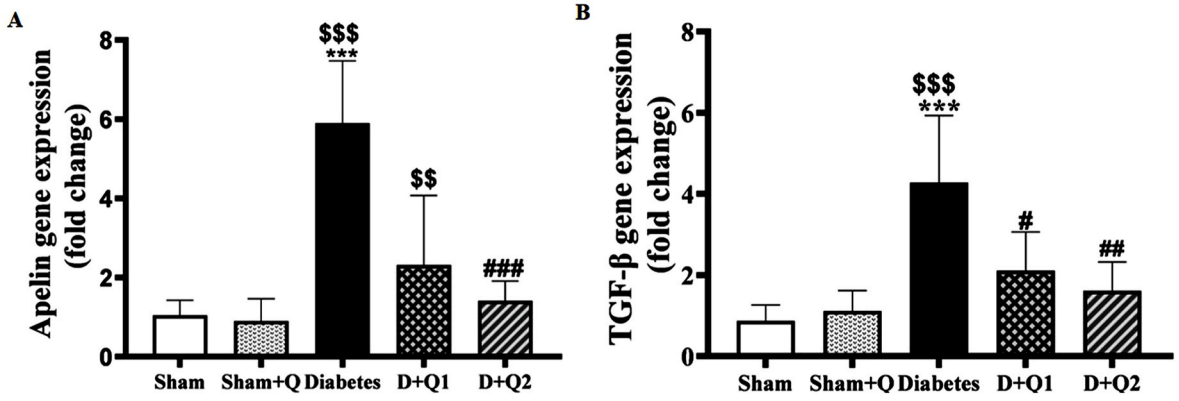

Fig. 4. Effects of quercetin on the gene expression levels of (A) apelin, and (B) TGF-β in each experimental group. ***$P < 0.001$ vs normal control group; $$ $P < 0.01$, $$$ $P < 0.001$ vs normal quercetin group; #$P < 0.05$, ##P < 0.01, ###$P < 0.001$ vs diabetic group.

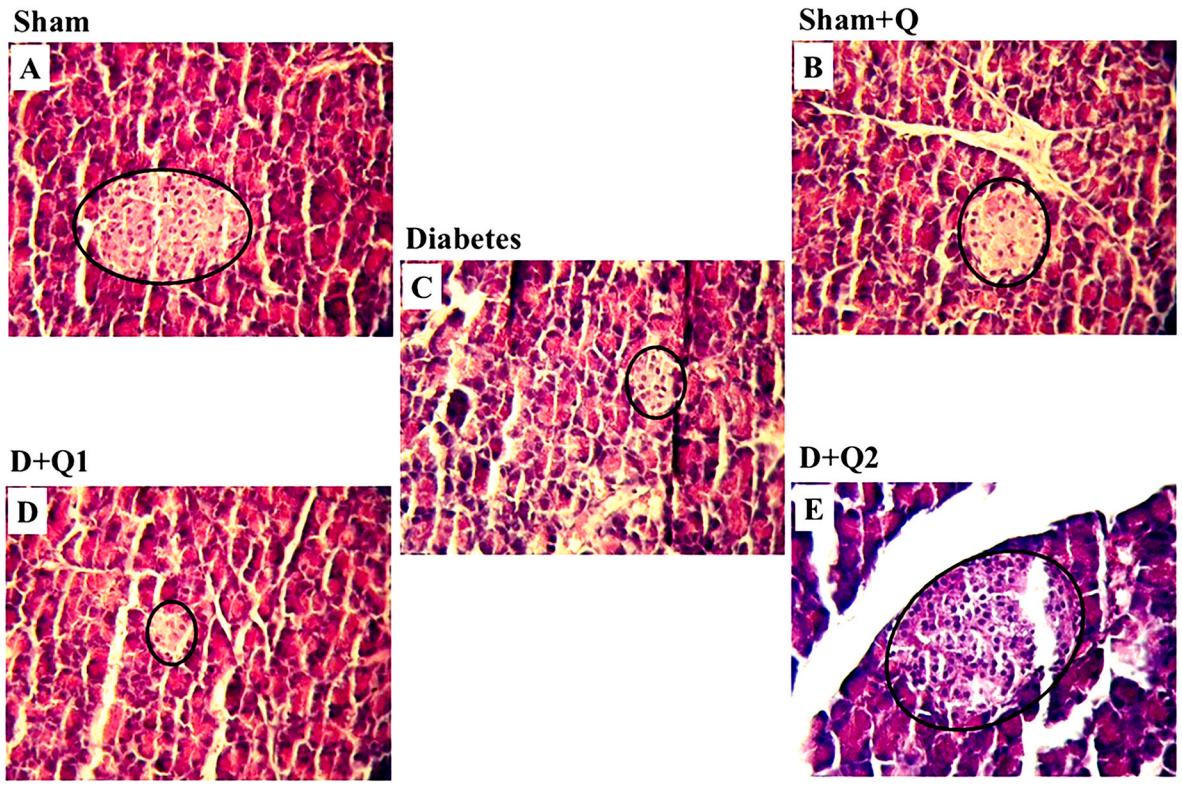

Fig. 5. Quercetin improved DM-induced histological changes of pancreatic sections demonstrated in the sham (A), sham + Q (B), diabetes (C), D + Q1 (D), and D + Q2 (E) groups. Histological changes of pancreatic sections (stained with H&E) were examined at the end of the 43rd day inclusive of severe necrotic changes in the islets (black circle) and severe atrophy of the acinar parts (red arrow). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

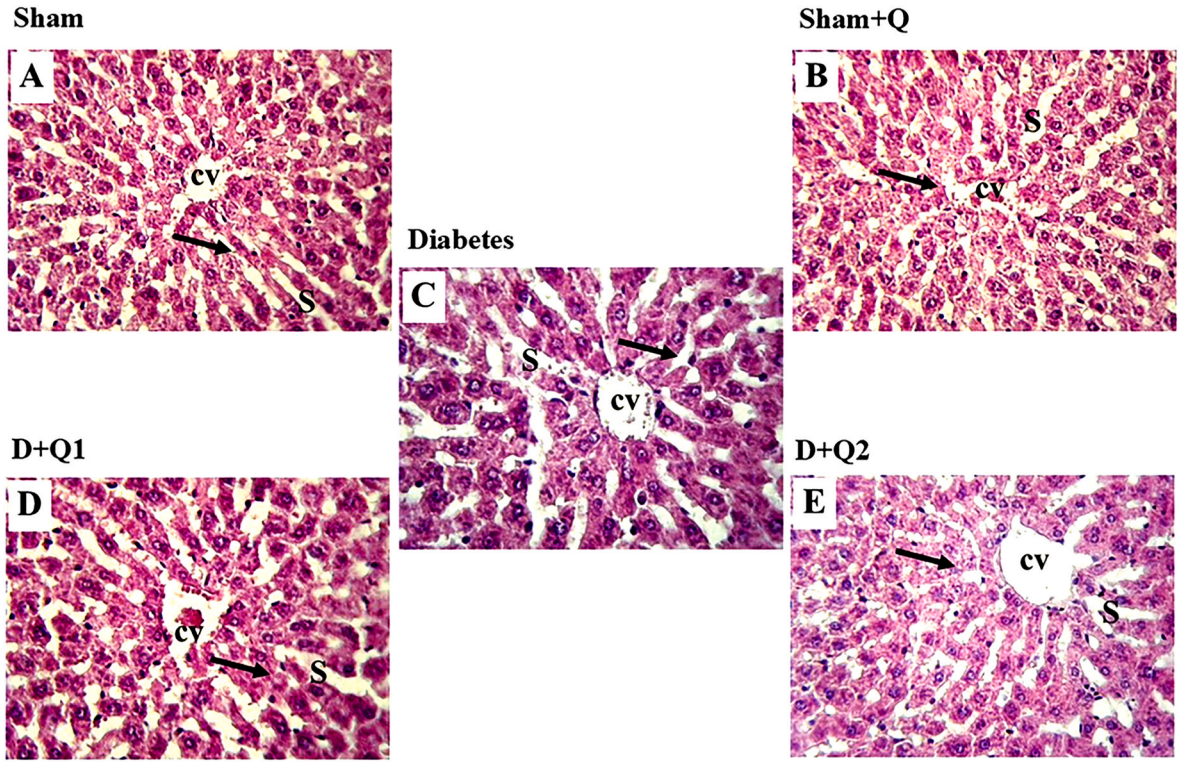

Fig. 6. Quercetin improved DM-induced histological changes of liver sections demonstrated in the sham (A), sham + Q (B), diabetes (C), D + Q1 (D), and D + Q2 (E) groups. Histological changes of liver sections (stained with H&E) were examined at the end of the 43rd day inclusive of derangement of hepatocyte cords and an irregular cell community. CV: central vein; black arrow shows the standard arrangement of hepatocyte cords; S: sinusoid space.

2022; Wang et al., 2013), anti-inflammatory (Ahmed et al., 2022; Ying et al., 2020), and hepatoprotective effects (Ahmed et al., 2022). In this regard, quercetin significantly decreased levels of oxidative stress markers (Ying et al., 2020), TNF-α (Ying et al., 2020), and IL-1β (Ying et al., 2020) in STZ-induced diabetic rats and reduced liver histology damage (Liu et al., 2010). Additional studies suggest that quercetin aids in the reduction of blood glucose levels (Wang et al., 2013), which lessens the oxidative stress created by high glucose, ultimately protecting the liver. Furthermore, investigations have demonstrated that quercetin may reduce serum total bilirubin, ALP, ALT, and AST while ameliorating liver lesions in the doxorubicin-induced liver injury model (Ahmed et al., 2022).

The current study's findings, which are consistent with those of another investigation, show that STZ injection significantly decreased the body weight of the rats. (Hakim et al., 1997). The loss of muscle (proteolysis) and fat (lipolysis), tissues that rely on insulin to use glucose for fuel, may be the cause of the drop in body weight on day 42 after STZ injection (Dimitriadis et al., 2011). Quercetin administration prevented diabetic rats from body weight reduction induced by STZ-induced DM by improving glucose tolerance (Ansari et al., 2022; Türedi et al., 2024; Rahmani et al., 2023; Chellian et al., 2022), preserving pancreatic β-cell function (Yi et al., 2021), reducing insulin resistance (Mahmoud et al., 2013), exhibiting antioxidant activity (Dias et al., 2005), and anti-inflammatory effects (Dias et al., 2005). Also, according to our results, all of the rats had significant hyperglycemia 72 h after receiving the STZ injection (Fig. 1B). STZ, which is most frequently used to induce diabetes in rats, promotes pancreatic ***β***-cell death through DNA alkylation, which lowers insulin synthesis and release (Eleazu et al., 2013). In this study, STZ-induced diabetes reduced the insulin secretion. However, quercetin prevented from reduction in plasma insulin levels, which was more apparent in the D + Q2 group compared to the D + Q1 group. According to our findings, quercetin has been shown in other recent research to have a hypoglycemic effect on diabetes, and in type 1 diabetic rats, it could considerably lower blood glucose levels (Ha-Neul et al., 2010). A way by which quercetin lowers blood glucose levels is the increase in glucose uptake by peripheral tissues (Alam et al., 2014). In a different study, quercetin treatment helped lower fasting blood glucose levels by increasing pancreatic beta cell counts and promoting insulin production in rats with induced diabetes using alloxan (Jeong et al., 2012). In the current research, measuring blood glucose and weight was done at 3 and 6 weeks in diabetic rats to monitor the progression and treatment effects of diabetes. The rationale behind this data was that bi-weekly measurements help evaluate the impact of diabetes and interventions on the rats' blood glucose levels and overall body weight (Pournaghi et al., 2012). By the 3rd week, most rats would have developed severe diabetes with blood glucose concentrations typically >250–600 mg/dl (Furman, 2021).

Several studies have indicated that TGF-β may be crucial for the onset of insulin resistance in type 1 and type 2 diabetes mellitus, as well as for the problems associated with the disease (Hou et al., 2018; Beaudoin et al., 2014). Besides, the apelin/APJ system is changed in the liver under the condition of obesity and diabetes (Butruille et al., 2013). Patients with NAFLD had higher apelin plasma levels associated with MDA and ROS generation. (Ercin et al., 2010). In our study, rats with STZ-induced diabetes exhibited high gene expression levels of TGF-β1 and apelin, which were diminished by quercetin. This significant reduction in TGF-β1 and apelin, accompanied by less liver injury, was more prominent in the D + Q2 group than the D + Q1 group. These findings are consistent with previous studies in hepatic fibrosis (Wu et al., 2017) and cholestatic liver injury (Lin et al., 2014). Quercetin inhibits the phosphorylation of Smad3, a key transcription factor in the TGF-β signaling pathway, thereby reducing the expression of genes involved in liver fibrosis (Shakerian et al., 2022; Liu et al., 2019). Thus, the preventive effect of quercetin in diabetic hepatopathy may be associated with the inhibition of the TGF-β and apelin signaling pathways, as quercetin decreased the DM-induced renal expression of TGF-β and apelin (Hossein et al., 2024). Quercetin may inhibit the TGF-B/apelin pathway by reducing the expression of genes associated with fibrosis and inflammation, thereby protecting the liver from further damage.

In conclusion, the results of this study demonstrated that quercetin reduced histological alterations, oxidative stress, and apelin and TGF-β mRNA expression to lessen liver dysfunction in type 1 diabetic rats. This protective action against diabetic hepatic injury is associated with antioxidant system enhancement and apelin and TGF-β mRNA expression suppression.

CRediT authorship contribution statement

Gholampour Firouzeh: Investigation, designing the experiments, analyzing the data and writing the manuscript. **Abbasi Susan:** Investigation, designing the experiments, the experimental work of the study. **Karimi Zeinab:** assisted with the designing of primers, molecular study, and histopathological evaluation.

Declaration of competing interest

None.

Data availability

Data will be made available on request.

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References

Abolfathi, A.A., Mohajeri, D., Rezaie, A., Nazeri, M., 2012. Protective effects of green ea Extract against hepatic tissue injury in streptozotocin-induced diabetic rats. Evid Based Complement Alternat Med 2012, 740671.
Adiels, M., Taskinen, M.R., Borén, J., 2008. Fatty liver, insulin resistance, and dyslipidemia. Curr Diab Rep 8 (1), 60–64.
Ahmed, O.M., Elkomy, M.H., Fahim, H.I., et al., 2022. Rutin and quercetin counter doxorubicin-induced Liver toxicity in Wistar rats via their modulatory effects on inflammation, oxidative stress, apoptosis, and Nrf2. Oxid. Med. Cell. Longev. 2022, 2710607.
Alam, M.M., Meerza, D., Naseem, I., 2014. Protective effect of quercetin on hyperglycemia, oxidative stress and DNA damage in alloxan induced type 2 diabetic mice. Life Sci. 109 (1), 8–14.
Ansari, P., Choudhury, S.T., Seidel, V., et al., 2022. Therapeutic potential of quercetin in the management of type-2 diabetes mellitus. Life 12 (8).
Ashari, S., Karami, M., Shokrzadeh, M., et al., 2022. Quercetin ameliorates Di (2-ethylhexyl) phthalate-induced nephrotoxicity by inhibiting NF-κB signaling pathway. Toxicol. Res. 11 (2), 272–285.
Beaudoin, M.S., Snook, L.A., Arkell, A.M., et al., 2014. Novel effects of rosiglitazone on SMAD2 and SMAD3 signaling in white adipose tissue of diabetic rats. Obesity 22 (7), 1632–1642.
Bhardwaj, A., Modi, K.P., 2017. Antidiabetic and antihyperlipidaemic activity of Nelumbo nucifera gaertn ethanol seed extract in streptozotocin induced diabetic rats. Int. J. Pharm. Pharm. Sci. 9 (10).
Butruille, L., Drougard, A., Knauf, C., et al., 2013. The apelinergic system: sexual dimorphism and tissue-specific modulations by obesity and insulin resistance in female mice. Peptides 46, 94–101.
Callan, A., Jha, S., Valdez, L., Baldado, L., Tsin, A., 2024. TGF-β signaling pathways in the development of diabetic retinopathy. Int. J. Mol. Sci. 25 (5).
Castan-Laurell, I., Masri, B., Valet, P., 2019. The apelin/APJ system as a therapeutic target in metabolic diseases. Expert Opin. Ther. Targets 23 (3), 215–225.
Çelik, N., Vurmaz, A., Kahraman, A., 2017. Protective effect of quercetin on homocysteine-induced oxidative stress. Nutrition 33, 291–296.
Chellian, J., Mak, K., Chellappan, D.K., Krishnappa, P., Pichika, M.R., 2022. Quercetin and metformin synergistically reverse endothelial dysfunction in the isolated aorta of streptozotocin-nicotinamide- induced diabetic rats. Sci. Rep. 12 (1), 21393.
Chen, H., Wan, D., Wang, L., et al., 2015. Apelin protects against acute renal injury by inhibiting TGF-β1. Biochim. Biophys. Acta 1852 (7), 1278–1287.
Cruz-Gregorio, A., Aranda-Rivera, A., 2023. Quercetin and ferroptosis. Life 13 (8), 1730.
D'Aniello, C., Lonardo, E., Iaconis, S., et al., 2009. G protein-coupled receptor APJ and its ligand apelin act downstream of Cripto to specify embryonic stem cells toward the

cardiac lineage through extracellular signal-regulated kinase/p70S6 kinase signaling pathway. Circ. Res. 105 (3), 231–238.
Day, R.T., Cavaglieri, R.C., Feliers, D., 2013. Apelin retards the progression of diabetic nephropathy. Am J Physiol Renal Physiol 304 (6), F788–F800.
Dhanya, R., 2022. Quercetin for managing type 2 diabetes and its complications, an insight into multitarget therapy. Biomed. Pharmacother. 146, 112560.
Dias, A.S., Porawski, M., Alonso, M., Marroni, N., Collado, P.S., González-Gallego, J., 2005. Quercetin decreases oxidative stress, NF-κB activation, and iNOS overexpression in liver of streptozotocin-induced diabetic rats. J. Nutr. 135 (10), 2299–2304.
Dimitriadis, G., Mitrou, P., Lambadiari, V., Maratou, E., Raptis, S.A., 2011. Insulin effects in muscle and adipose tissue. Diabetes Res. Clin. Pract. 93 (Suppl. 1), S52–S59.
Dong, Ys, Wang, Jl, Feng, Dy, et al., 2014. Protective effect of quercetin against qxidative stress and brain edema in an experimental rat model of subarachnoid hemorrhage. Int. J. Med. Sci. 11 (3), 282–290.
Eleazu, C.O., Eleazu, K.C., Chukwuma, S., Essien, U.N., 2013. Review of the mechanism of cell death resulting from streptozotocin challenge in experimental animals, its practical use and potential risk to humans. J. Diabetes Metab. Disord. 12 (1), 60.
Elpek, G.Ö., 2014. Cellular and molecular mechanisms in the pathogenesis of liver fibrosis: an update. World J. Gastroenterol. 20 (23), 7260–7276.
Ercin, C.N., Dogru, T., Tapan, S., et al., 2010. Plasma apelin levels in subjects with nonalcoholic fatty liver disease. Metab., Clin. Exp. 59 (7), 977–981.
Falck-Ytter, Y., Younossi, Z.M., Marchesini, G., McCullough, A.J., 2001. Clinical features and natural history of nonalcoholic steatosis syndromes. Semin. Liver Dis. 21 (1), 17–26.
Furman, B.L., 2021. Streptozotocin-induced diabetic models in mice and rats. Curr Protoc 1 (4), e78.
Gholampour, F., Masoudi, R., Khaledi, M., et al., 2022. Berberis integerrima hydro-alcoholic root extract and its constituent berberine protect against cisplatin-induced nephro- and hepato-toxicity. Am. J. Med. Sci. 364 (1), 76–87.
Ghosh, S., Bhattacharyya, S., Rashid, K., Sil, P., 2015. 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. Toxicol Rep 2, 365–376.
Giacco, F., Brownlee, M., 2010. Oxidative stress and diabetic complications. Circ. Res. 107 (9), 1058–1070.
Guo, L., Li, Q., Wang, W., et al., 2009. Apelin inhibits insulin secretion in pancreatic beta-cells by activation of PI3-kinase-phosphodiesterase 3B. Endocr. Res. 34 (4), 142–154.
Ha-Neul, C., Jung-In, K., Ji-Hye, K., Heejeong, J., Young-Hee, K., 2010. Hypoglycemic effect of quercetin in animal models of diabetes. Faseb. J. 24.
Hakim, Z.S., Patel, B.K., Goyal, R.K., 1997. Effects of chronic ramipril treatment in streptozotocin-induced diabetic rats. Indian J. Physiol. Pharmacol. 41 (4), 353–360.
Harrison, S.A., 2006. Liver disease in patients with diabetes mellitus. J. Clin. Gastroenterol. 40 (1), 68–76.
Hernández-Ortega, L.D., Alcántar-Díaz, B.E., Ruiz-Corro, L.A., et al., 2012. Quercetin improves hepatic fibrosis reducing hepatic stellate cells and regulating pro-fibrogenic/anti-fibrogenic molecules balance. J. Gastroenterol. Hepatol. 27 (12), 1865–1872.
Heydarpour, F., Sajadimajd, S., Mirzarazi, E., et al., 2020. Involvement of TGF-β and autophagy pathways in pathogenesis of diabetes: a comprehensive review on biological and pharmacological insights. Front. Pharmacol. 11, 498758.
Hossein, A., Firouzeh, G., Zeinab, K., Gholamreza, D., 2024. Quercetin prevents kidney against diabetes mellitus (type 1) in rats by inhibiting TGF-β/apelin gene expression. Mol. Biol. Rep. 51 (1), 677.
Hou, B., Zhao, Y., Qiang, G., et al., 2018. Puerarin mitigates diabetich hepatic steatosis and fibrosis by snhibiting TGF-β Signaling pathway activation in Type 2 diabetic rats. Oxid. Med. Cell. Longev. 2018, 4545321.
Hu, H., He, L., Li, L., Chen, L., 2016. Apelin/APJ system as a therapeutic target in diabetes and its complications. Mol Genet Metab 119 (1–2), 20–27.
Jeong, S.M., Kang, M.J., Choi, H.N., Kim, J.H., Kim, J.I., 2012. Quercetin ameliorates hyperglycemia and dyslipidemia and improves antioxidant status in type 2 diabetic db/db mice. Nutr Res Pract 6 (3), 201–207.
Karimi, Z., Daryabor, G., Masjedi, F., 2024. Effects of conditioned media derived from human Wharton's jelly mesenchymal stem cells on diabetic nephropathy and hepatopathy via modulating TGF-beta and apelin signaling pathways in male rats. BMC Endocr. Disord. 24 (1), 6.
Kim, Y.J., Park, W., 2016. Anti-inflammatory effect of quercetin on RAW 264.7 mouse macrophages induced with polyinosinic-polycytidylic acid. Molecules 21 (4), 450.
Lim, Y.L., Choi, E., Jang, Y.O., et al., 2016. Clinical implications of the serum apelin level on portal hypertension and prognosis of liver cirrhosis. Gut Liver 10 (1), 109–116.
Lin, S.Y., Wang, Y.Y., Chen, W.Y., Chuang, Y.H., Pan, P., Chen, C.J., 2014. Beneficial effect of quercetin on cholestatic liver injury. J. Nutr. Biochem. 25 (11), 1183–1195.
Liu, C.M., Zheng, Y.L., Lu, J., et al., 2010. Quercetin protects rat liver against lead-induced oxidative stress and apoptosis. Environ. Toxicol. Pharmacol. 29 (2), 158–166.
Liu, Y., Dai, E., Yang, J., 2019. Quercetin suppresses glomerulosclerosis and TGF-β signaling in a rat model. Mol. Med. Rep. 19 (6), 4589–4596.
Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 25 (4), 402–408.
Lv, X., Kong, J., Chen, W.D., Wang, Y.D., 2017. The role of the apelin/APJ system in the regulation of liver disease. Front. Pharmacol. 8, 221.
Machado, M.V., Cortez-Pinto, H., 2014. Non-alcoholic fatty liver disease: what the clinician needs to know. World J. Gastroenterol. 20 (36), 12956–12980.
Mahmoud, M.F., Hassan, N.A., El Bassossy, H.M., Fahmy, A., 2013. Quercetin protects against diabetes-induced exaggerated vasoconstriction in rats: effect on low grade inflammation. PLoS One 8 (5), e63784.
Maksymchuk, O., Shysh, A., Rosohatska, I., Chashchyn, M., 2017. Quercetin prevents type 1 diabetic liver damage through inhibition of CYP2E1. Pharmacol. Rep. 69 (6), 1386–1392.
Manna, P., Das, J., Ghosh, J., Sil, P.C., 2010. Contribution of type 1 diabetes to rat liver dysfunction and cellular damage via activation of NOS, PARP, IkappaBalpha/NF-kappaB, MAPKs, and mitochondria-dependent pathways: prophylactic role of arjunolic acid. Free Radic. Biol. Med. 48 (11), 1465–1484.
Melgar-Lesmes, P., Casals, G., Pauta, M., et al., 2010. Apelin mediates the induction of profibrogenic genes in human hepatic stellate cells. Endocrinology 151 (11), 5306–5314.
Mertens, J., De Block, C., Spinhoven, M., Driessen, A., Francque, S.M., Kwanten, W.J., 2021. Hepatopathy associated with type 1 diabetes: distinguishing non-alcoholic fatty liver disease from glycogenic hepatopathy. Fron. Pharmacol. 12.
Niazmand, S., Mirzaei, M., Hosseinian, S., et al., 2021. The effect of Cinnamomum cassia extract on oxidative stress in the liver and kidney of STZ-induced diabetic rats. J. Complement. Integr. Med. 19 (2), 311–321.
Pournaghi, P., Sadrkhanlou, R.A., Hasanzadeh, S., Foroughi, A., 2012. An investigation on body weights, blood glucose levels and pituitary-gonadal axis hormones in diabetic and metformin-treated diabetic female rats. Vet. Res. Forum 3 (2), 79–84.
Qi, W., Qi, W., Xiong, D., Long, M., 2022. Quercetin: its antioxidant mechanism, antibacterial properties and potential application in prevention and control of toxipathy. Molecules 27 (19).
Rahmani, A.H., Alsahli, M.A., Khan, A.A., Almatroodi, S.A., 2023. Quercetin, a plant flavonol attenuates diabetic complications, renal tissue damage, renal oxidative stress and inflammation in streptozotocin-induced diabetic rats. Metabolites 13 (1).
Raza, H., John, A., 2012. Streptozotocin-induced cytotoxicity, oxidative stress and mitochondrial dysfunction in human hepatoma HepG2 cells. Int. J. Mol. Sci. 13 (5), 5751–5767.
Shakerian, E., Akbari, R., Mohammadtaghvaei, N., Mohammadi Gahrooie, M., Afarin, R., 2022. Quercetin reduces hepatic fibrogenesis by inhibiting TGF-β/Smad3 signaling pathway in LX-2 cell line. Jundishapur J. Nat. Pharm. Prod. 17 (1), e113484.
Shamsi, M., Ranjbar, R., Khaksary Mahabady, M., Tabandeh, M.R., Khazaeel, K., Najafzadeh, H., 2019. Protective effect of quercetin on morphological and histometrical changes of placenta in streptozotocin-induced diabetic rat. Zahedan Journal of Research in Medical Sciences 22 (1).
Takamatsu, S., Noguchi, N., Kudoh, A., et al., 2008. Influence of risk factors for metabolic syndrome and non-alcoholic fatty liver disease on the progression and prognosis of hepatocellular carcinoma. Hepato-Gastroenterology 55 (82–83), 609–614.
Takemoto, K., Hatano, E., Iwaisako, K., et al., 2014. Necrostatin-1 protects against reactive oxygen species (ROS)-induced hepatotoxicity in acetaminophen-induced acute liver failure. FEBS Open Bio 4, 777–787.
Türedi, S., Çelik, H., Dağlı, Ş.N., Taşkın, S., Şeker, U., Deniz, M., 2024. An examination of the effects of propolis and quercetin in a rat model of streptozotocin-induced diabetic peripheral neuropathy. Curr. Issues Mol. Biol. 46 (3), 1955–1974.
Wang, W., Wang, C., Ding, X.Q., et al., 2013. Quercetin and allopurinol reduce liver thioredoxin-interacting protein to alleviate inflammation and lipid accumulation in diabetic rats. Br. J. Pharmacol. 169 (6), 1352–1371.
Wang, Y., Song, J., Bian, H., et al., 2019. Apelin promotes hepatic fibrosis through ERK signaling in LX-2 cells. Mol. Cell. Biochem. 460 (1–2), 205–215.
Wang, Q., Liu, X., Zhai, A., Xu, H., Ma, S., Liu, Y., 2024. Expression of apelin-13 and its negative correlation with TGF-β1 in patients with diabetic kidney disease. Exp. Ther. Med. 27 (3), 110.
Wu, L., Zhang, Q., Mo, W., et al., 2017. Quercetin prevents hepatic fibrosis by inhibiting hepatic stellate cell activation and reducing autophagy via the TGF-β1/Smads and PI3K/Akt pathways. Sci. Rep. 7 (1).
Xu, D., Hu, M.J., Wang, Y.Q., Cui, Y.L., 2019. Antioxidant activities of quercetin and its complexes for medicinal application. Molecules 24 (6).
Yang, L., Roh, Y.S., Song, J., et al., 2014. Transforming growth factor beta signaling in hepatocytes participates in steatohepatitis through regulation of cell death and lipid metabolism in mice. Hepatology 59 (2), 483–495.
Yang, P., Read, C., Kuc, R.E., et al., 2017. Elabela/Toddler is an endogenous agonist of the apelin APJ receptor in the adult cardiovascular system, and exogenous administration of the peptide compensates for the downregulation of its expression in pulmonary arterial hypertension. Circulation 135 (12), 1160–1173.
Yang, D., Wang, T., Long, M., Li, P., 2020. Quercetin: its main pharmacological activity and potential application in clinical medicine. Oxid. Med. Cell. Longev. 2020, 8825387.
Yi, H., Peng, H., Wu, X., et al., 2021. The therapeutic effects and mechanisms of quercetin on metabolic diseases: pharmacological data and clinical evidence. Oxid. Med. Cell. Longev. 2021, 6678662.
Ying, L., Chaudhry, M.T., Xiao, F., et al., 2020. The effects and mechanism of quercetin dietary supplementation in streptozotocin-induced hyperglycemic arbor acre broilers. Oxid. Med. Cell. Longev. 2020, 9585047.
Yokomori, H., Oda, M., Yoshimura, K., Machida, S., Kaneko, F., Hibi, T., 2011. Overexpression of apelin receptor (APJ/AGTRL1) on hepatic stellate cells and sinusoidal angiogenesis in human cirrhotic liver. J. Gastroenterol. Hepatol. 46 (2), 222–231.
Zhang, Y., Mei, H., Shan, W., et al., 2016. Lentinan protects pancreatic β cells from STZ-induced damage. J. Cell Mol. Med. 20 (10), 1803–1812.