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Attenuation of STZ-Induced Damage in Rats via Immune Response and TGF-β/VEGF Pathway Modulation by Quercetin

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ABSTRACT

Diabetes mellitus, a metabolic disorder characterized by hyperglycemia, can lead to severe complications affecting multiple organs, including the heart and liver. Oxidative stress, inflammation, and impaired insulin signaling contribute to the development of diabetic cardiomyopathy and hepatopathy. This study investigated the protective effects of quercetin (Quer) on diabetes-induced cardiac and hepatic dysfunction in rats. Diabetic rats exhibited significant increases in blood glucose levels, impaired insulin sensitivity, and elevated levels of cardiac and hepatic biomarkers, indicating myocardial and hepatic damage. Moreover, diabetic rats showed increased oxidative stress, inflammation, and fibrosis in the heart and liver. Treatment with Quer significantly ameliorated these adverse effects. Quer reduced blood glucose levels, improved insulin sensitivity, and attenuated cardiac and hepatic damage. Quer exerted its protective effects by reducing oxidative stress, inflammation, and fibrosis, as evidenced by decreased levels of oxidative stress markers, inflammatory cytokines, and fibrosis markers. Additionally, Quer upregulated the expression of antioxidant enzymes and activated the Akt/GSK-3β signaling pathway, which plays a crucial role in insulin signaling and cell survival.

These findings suggest that Quer has potential therapeutic benefits in managing diabetes-induced cardio-hepatic complications. Further research is needed to fully elucidate the underlying mechanisms and optimize their clinical application.

Keywords: Diabetes mellitus, Oxidative stress, Insulin resistance, Liver fibrosis, Cardiac dysfunction, Quercetin

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1. INTRODUCTION

Diabetes mellitus, a chronic metabolic disorder characterized by hyperglycemia, poses a significant global health burden (1). This condition arises from impaired insulin production, insulin action, or both, leading to a cascade of metabolic disturbances (2). Hyperglycemia triggers oxidative stress, a state of cellular imbalance characterized by excessive reactive oxygen species (ROS) production (3). These reactive species damage cellular components, including lipids, proteins, and DNA, contributing to the development of various complications (4). One of the most common complications of diabetes is liver disease. Hyperglycemia can lead to hepatic steatosis, inflammation, and fibrosis. These changes can impair liver function and increase the risk of liver failure (5). The disrupted Akt/GSK-3β signaling pathway is a critical factor in the excessive cell death and chronic inflammation observed in diabetes (6). Additionally, activated hepatic stellate

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cells (by inflammatory signals) worsen fibrosis via collagen production (7). This complex interplay between diabetes and liver injury highlights the need for further research.

Another major complication of diabetes is cardiovascular disease. Hyperglycemia induces oxidative stress in the heart, leading to myocardial damage and impaired cardiac function (8). Additionally, diabetes can accelerate atherosclerosis, a condition characterized by the buildup of plaque in the arteries, increasing the risk of heart attacks and strokes (9). Moreover, the balance between transforming growth factor-β (TGF-β) and vascular endothelial growth factor (VEGF) signaling is disrupted in diabetes. Excessive TGF-β signaling can lead to fibrosis and endothelial dysfunction, while excessive VEGF signaling can contribute to vascular dysfunction and inflammation (10). This imbalance can accelerate the development of diabetic cardiovascular complications.

To investigate the effects of diabetes on the heart and liver, researchers often use animal models, such as rats, treated with streptozotocin (STZ) to induce diabetes. STZ is a toxic chemical that destroys pancreatic β-cells, leading to insulin deficiency and hyperglycemia (11). Additionally, a high-fructose diet can exacerbate the development of diabetes-like symptoms, including insulin resistance and hyperlipidemia (12).

In recent years, natural compounds, such as quercetin (Quer), have gained attention for their potential to mitigate the complications of diabetes (13). Quer, a flavonoid found in various fruits and vegetables, possesses potent antioxidant and anti-inflammatory properties (14). It has been shown to protect against oxidative stress, inflammation, and cellular damage, making it a promising therapeutic agent for diabetes.

This study aims to investigate the effects of diabetes on heart and liver function in rats and to evaluate the protective effects of Quer. By understanding the underlying mechanisms of diabetic complications and the therapeutic potential of natural compounds like quercetin, we can develop effective strategies for preventing and treating diabetes-related disorders.

2. MATERIALS AND METHODS

2.1. Chemicals

Streptozotocin (STZ) was acquired from Sigma-Aldrich Chemical Company (St. Louis, MO, USA). All other chemicals used in the study were of analytical status.

2.2. Isolation of quercetin from Trifolium alexandrinum extract

To obtain Quer, the *Trifolium alexandrinum* extract was prepared according to the method described by Newairy et al.(4). The dried extract was then resuspended in distilled water. Subsequently, the residue was diluted and extracted with ethyl acetate. The ethyl acetate fractions were then subjected to silica gel chromatography, yielding eight distinct fractions. Fraction 6 was further purified through capillary electrophoresis and silica gel column chromatography. Finally, Quer was eluted using a mixture of ethyl acetate and methanol, and its structure was confirmed through HPLC analysis.

2.3. Animals and Experimental Design

The study utilized adult male Wistar albino rats weighing approximately 150-170 g. The animals were housed in standard laboratory conditions at the Experimental Animal Center-Medical Research Institute, Alexandria University, Egypt. The rats were kept in conventional cages under a 12-hour light-dark cycle and a temperature of 22 ± 2 °C. They were provided with standard rodent chow and water ad libitum. The experimental protocols employed in this study were approved by the Institutional Animal Care and Use Committee (IACUC) of Alexandria University, Egypt (Approval number: AU04 21 9 23 2 02).

After two weeks of acclimatization, A total of 18 rats were divided into two groups: a control group of 6

rats that received daily oral doses of distilled water for 4 weeks, and a diabetic group of 12 rats. The diabetic group was first given 10% fructose water for two weeks, followed by a single intraperitoneal injection of STZ (40 mg/kg body weight) to induce diabetes, as previously described (15). Three days post-injection, rats with fasting blood glucose levels exceeding 220 mg/dL were considered diabetic. These diabetic rats were randomly assigned to two subgroups of 6 rats each: Diabetic group and Diabetic + Quer group (50 mg/kg/day) (16). Quer was administered once daily for 4 weeks.

2.4. Collection of blood and tissue preparation

After the study, blood samples were collected from the fasting rats via the jugular veins while under anesthesia induced by a ketamine (100 mg/kg) and xylazine (5 mg/kg) mixture. The samples were then allowed to clot at room temperature before being centrifuged at 3000 rpm for 15 minutes. The resulting supernatant sera was aspirated and divided into three Eppendorf tubes. Subsequently, the rats were humanely euthanized using cervical dislocation, and their liver tissues were promptly extracted and rinsed in ice-cold saline. Collected liver tissues were minced, washed, and homogenized by Dounce glass homogenizer in PBS buffer with pH 7.4 (10% M/V). The homogenates were spun down for 10 min at 2,000 rpm. Then the supernatant was collected and kept at-20˚C for further biochemical analysis. Certain portions of the liver tissues were quickly preserved in 10% formalin for histological analysis.

2.5. Biochemical parameters

The serum glucose level was measured using a diagnostic kit that employs a colorimetric method (#GL1320; Biodiagnostic, Egypt) according to the method of Paul Trinder (17). Sandwich ELISA kits (# SE120086; Sigma-Aldrich, USA) were used to measure insulin levels as directed by the manufacturer. In brief, The ELISA assay involves coating wells with an antibody specific to insulin. Serum containing insulin is added to the wells, where it binds to the antibody. An enzyme-linked antibody is then added, which binds to the insulin. Finally, a streptavidin-HRP complex is added, which binds to the enzyme-linked antibody. The amount of color produced by the HRP enzyme is proportional to the amount of insulin present in the sample.

Alanine transaminase (ALT) was estimated using a diagnostic kit (#AL 10 31 (45); Biodiagnostic, Egypt) according to the method of Bergmeyer (18). Pyruvate kinase (pk) was assessed using a diagnostic kit (#ELK1515; ELK biotechnology, USA). Serum glycogen levels were measured using a colorimetric assay Kit (LS-K151-100; LifeSpan Biosciences, USA). Serum albumin and total protein were assessed using Biodiagnostic, Egypt commercial kits (#AB 10 10; # TP 20 20) respectively.

Lactate dehydrogenase (LDH) and Troponin I were assessed using Life Technologies, India kits (#LT860101ETKKBA; # LT910101ETKKBA) respectively. Creatine kinase-MB (CK-MB) was measured using a diagnostic kit (#ER0841; FineTest, China). Total cholesterol and triglyceride levels were measured using Biodiagnostic, Egypt kits (#CH 12 20; # TR 20 30) respectively.

Malondialdehyde (MDA), Glutathione (GSH), and total antioxidant capacity (TAC) were assessed using Biodiagnostic, Egypt kits (#MD 25 29; # GR 25 11; # TA 25 13) respectively. Hydrogen peroxide (H2O2) was measured using kits (#E-BC-K102-M; Elabscience, USA). Advanced Glycation End-products (AGE) were measured using a specific ELISA kit (CSB-E09413r; CUSABIO, USA).

The inflammatory cytokines were estimated by ELISA refer ring to the manufacturer's directions for the corresponding rat immunoassay kits; nuclear factor kappa B (NFκB # E1817Ra; BT LAB, china), interleukin-6 (IL-6 # E0135Ra; BT LAB, china), interleukin-2 (IL-2 # ab100769; Abcam, USA), interleukin-4 (IL-4 # E-EL-R0014; Elabscience,

USA), Interferon γ (INF γ # ER0012; Fine test, USA).

Transforming growth factor beta (TGF-β # ab119558; Abcam, USA), Vascular Endothelial Growth Factor (VEGF# CSB-E04757r; CUSABIO, USA), Caspase 3 (E-EL-R0160; Elabscience, USA), P. Akt (ER0703; FineTest, USA) and Glycogen synthase kinase-3 beta, (GSK-3β # ab123454; Abcam, USA) were estimated using viable kits. These kits employ a sandwich ELISA technique, where specific antibodies capture the target protein, followed by detection with a secondary antibody conjugated to a reporter enzyme. The enzyme catalyzes a colorimetric reaction, and the intensity of the color is proportional to the amount of target protein in the sample.

2.6. Masson's Trichrome Staining

All the liver tissues were fixed in 10% phosphate-buffered formalin. The tissues were processed using an automated tissue processor (Leica, Germany), then embedded in paraffin, sliced into 5 µm sections, and stained with Masson's trichrome at room temperature according to the manufacturer's instruction. All sections were evaluated, and the images were captured under light microscopy (Olympus Corporation, Tokyo, Japan) and the result were interpreted accordingly.

2.7. Statistical Analysis

All values were expressed as mean SE. The statistical significance was evaluated by one-way analysis of variance (ANOVA) using SPSS. The post hoc test Least Significant Difference (LSD) was used to compare different groups. A value of $p<0.05$ was considered to indicate a significant difference between the animal groups.

3. RESULTS

3.1. Changes in serum glucose, insulin, and AGE levels

The results illustrated in Figure (1) reveal that diabetic rats exhibited a substantial ($P < 0.05$) increase in serum glucose levels, coupled with a marked ($P < 0.05$) decrease in serum insulin levels compared to the control group. In contrast, the Diabetic+Quer group displayed a substantial ($P < 0.05$) improvement in these altered parameters when compared to the diabetic group.

3.2. Changes in serum ALT, Pyruvate kinase, Glycogen, Albumin, and total protein

As depicted in Figure (2), the results of liver function markers estimation showed significant ($p < 0.05$) elevation in serum ALT along with a substantial decrease ($p < 0.05$) in glycogen, albumin, total protein, and PK in the diabetic group as compared with the control group. However, the administration of Quer to the diabetic group considerably ameliorated these adverse effects, bringing the liver function parameters closer to the levels observed in the control group.

3.3. Changes in serum LDH, troponin and CK-MB, cholesterol, and triglyceride

Cardiac markers and lipid profile estimation revealed a substantial ($P< 0.05$) escalation in serum LDH, troponin, CK-MB, cholesterol, and triglyceride levels in the diabetic group compared to the control group. Alternatively, the Diabetic+Quer group showed considerable ($P<0.05$) decreases in serum LDH, troponin, CK-MB, cholesterol, and triglyceride levels compared to the diabetic group (Figure 3). These findings suggest that Quer administration ameliorated the adverse effects of diabetes on cardiac function and lipid metabolism.

3.4. Changes in serum MDA, H_2O_2, GSH, TAC, and AGE

The presented data in Figure (4) showed that the diabetic induction caused marked ($P<0.05$) elevation in serum MDA, H_2O_2, and AGE compared to the control group. Concurrently, a significant ($P < 0.05$) decline in serum GSH and TAC levels was observed in the diabetic group. These findings indicate increased oxidative stress in diabetic rats. However, the supplementation of Diabetic+Quer caused

substantial (P<0.05) enhancement in serum oxidative stress markers and AGE compared to the diabetic group. This suggests that Quer possesses antioxidant properties that can mitigate oxidative stress induced by diabetes.

3.5. Changes in hepatic H_2O_2, and TAC

The presented data in Figure (5) showed that the diabetic induction caused marked (P<0.05) elevation in the hepatic H_2O_2 along with marked (P < 0.05) decline in TAC compared to the control group. These findings indicate increased oxidative stress in the liver of diabetic rats. Alternatively, the supplementation of Diabetic+Quer caused substantial (P<0.05) enhancement in hepatic oxidative stress markers compared to the diabetic group, suggesting that Quer possesses antioxidant properties that can mitigate oxidative stress-induced liver damage in diabetes.

3.6. Changes in serum NF-κB, IL-6, IL-2, INF γ, TGF β, and VEGF

As shown in Figure (6), the diabetic induction caused substantial (P<0.05) elevations in serum NF-κB, IL-6, IL-2, INF γ, TGF β, and VEGF levels along with a marked decrease in IL-4 level versus the control group. On the other hand, the Diabetic+Quer group showed a marked (P<0.05) reduction in the levels of serum NF-κB, IL-6, IL-2, INF γ, TGF β, and VEGF along with a marked increase in IL-4 level relative to the diabetic group. These findings suggest that Quercetin administration effectively modulated the inflammatory response in diabetic rats, reducing pro-inflammatory cytokine levels and increasing anti-inflammatory cytokine levels.

3.7. Changes in hepatic NF-κB, IL-4, caspase3, P.AKT, INF γ, TGF β, and GSK3-β

As shown in Figure (7), the diabetic induction caused substantial (P<0.05) elevations in hepatic NF-κB, caspase3, INF γ, TGF β, and GSK3-β levels along with a marked decrease in P.AKT and IL-4 level matched to the control group. On the other hand, the Diabetic+Quer group showed marked (P<0.05) improvement in the levels of hepatic NF-κB, IL-4, caspase3, P.AKT, INF γ, TGF β, and GSK3-β relative to the diabetic group. These findings suggest that Quer administration effectively modulated inflammatory pathways and apoptotic processes in the liver of diabetic rats.

3.8. Masson's trichrome stain

Histological analysis of liver tissue sections, stained with Masson's trichrome, revealed the impact of Quer on liver fibrosis in diabetic rats. The liver tissue of diabetic rats exhibited significant collagen deposition, as indicated by the prominent blue staining (Figure 8). This indicates increased fibrosis in the diabetic liver. However, treatment with Quer resulted in a substantial reduction in fibrosis in the diabetic rats. These findings suggest that Quer possesses hepatoprotective properties, as evidenced by the diminished collagen accumulation observed in the treatment groups.

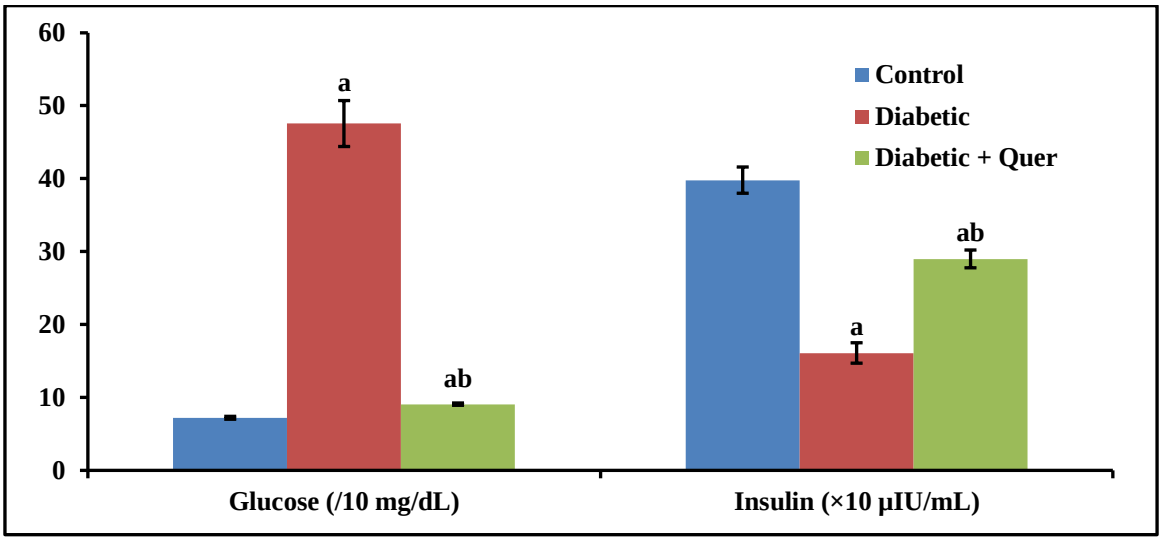

Figure (1): Changes in serum glucose and insulin levels among different experimental groups. ('a' significance with the control group, 'b' significance with a diabetic group).

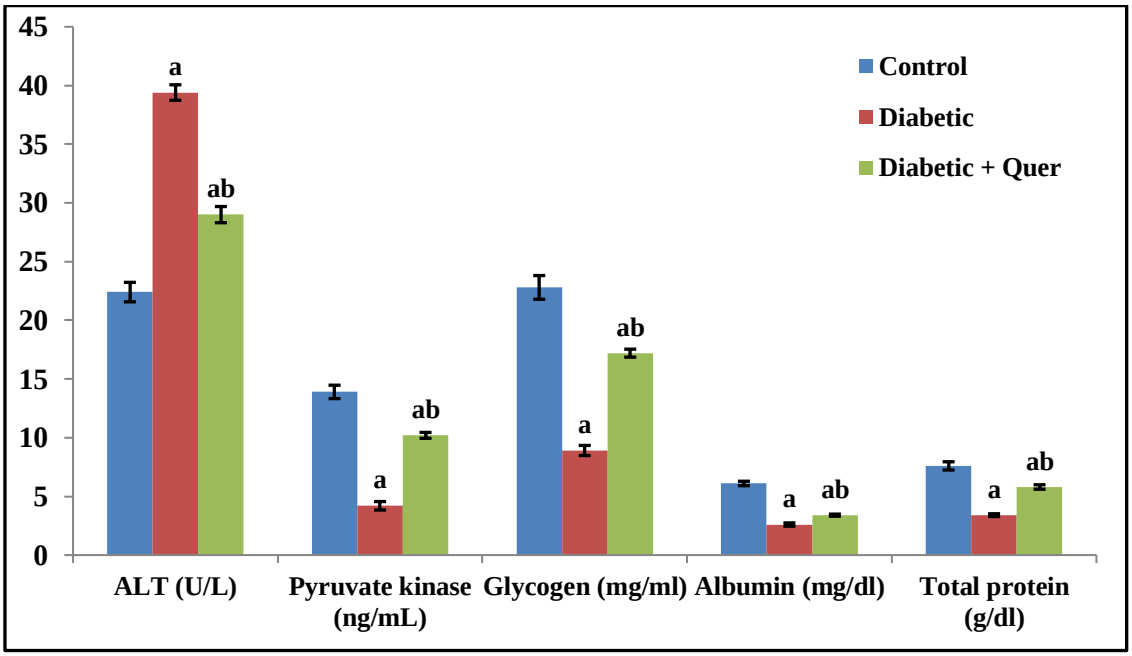

Figure (2): Changes in serum ALT, Pyruvate kinase, Glycogen, Albumin, and total protein among different experimental groups. ('a' significance with the control group, 'b' significance with the diabetic group).

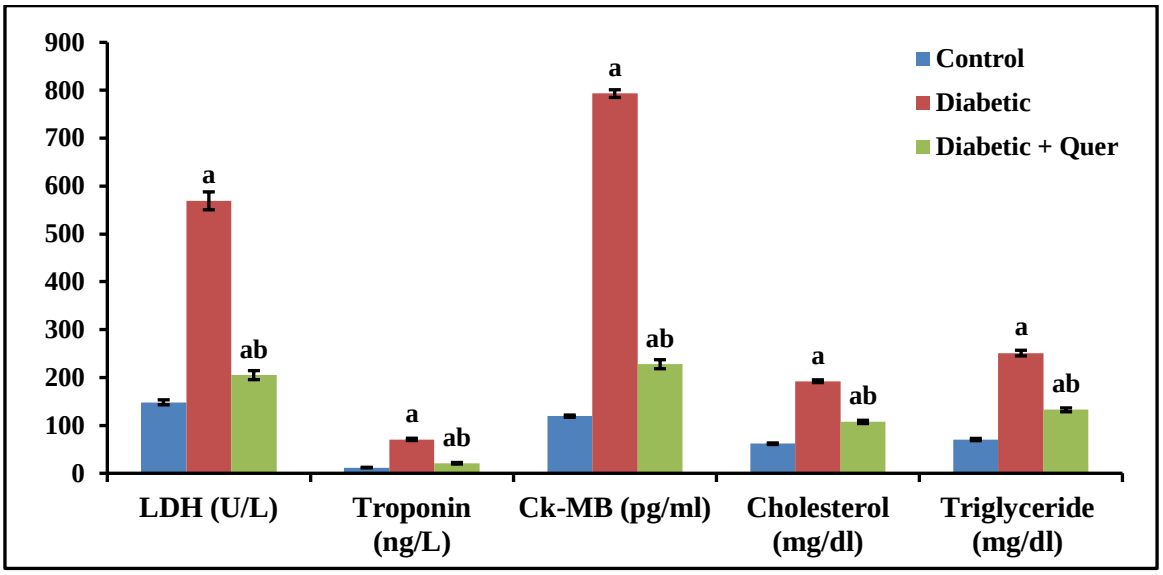

Figure (3): Changes in serum LDH, troponin and CK-MB, cholesterol, and triglyceride among different experimental groups. ('a' significance with the control group, 'b' significance with the diabetic group).

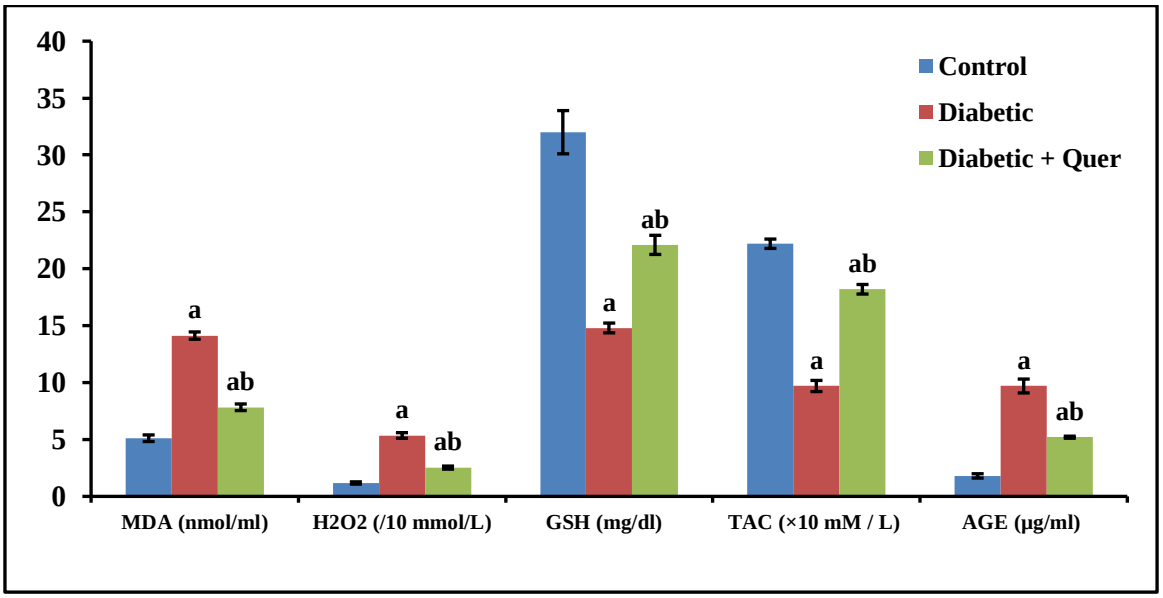

Figure (4): Changes in serum MDA, H_2O_2, GSH, TAC, and AGE among different experimental groups. ('a' significance with the control group, 'b' significance with the diabetic group).

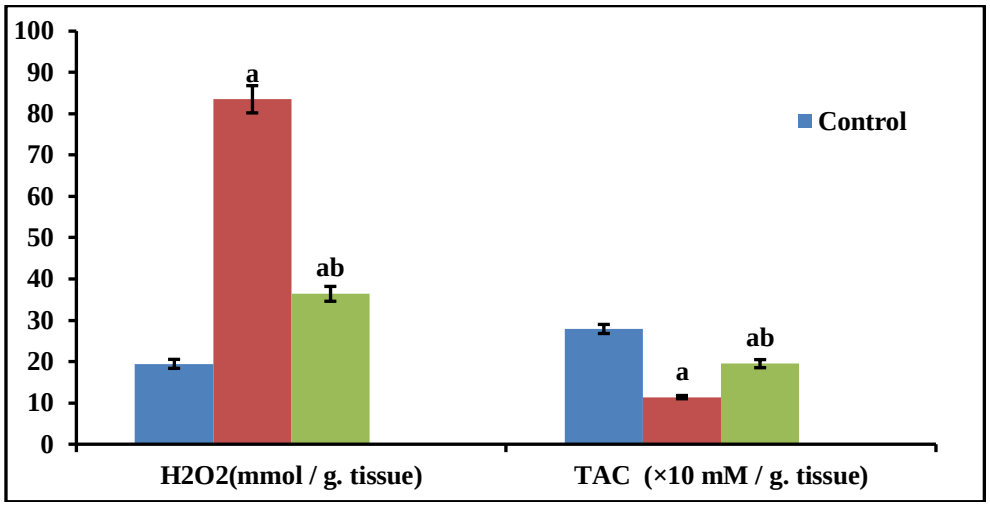

Figure (5): Changes in hepatic H_2O_2 and TAC among different experimental groups. ('a' significance with the control group, 'b' significance with the diabetic group).

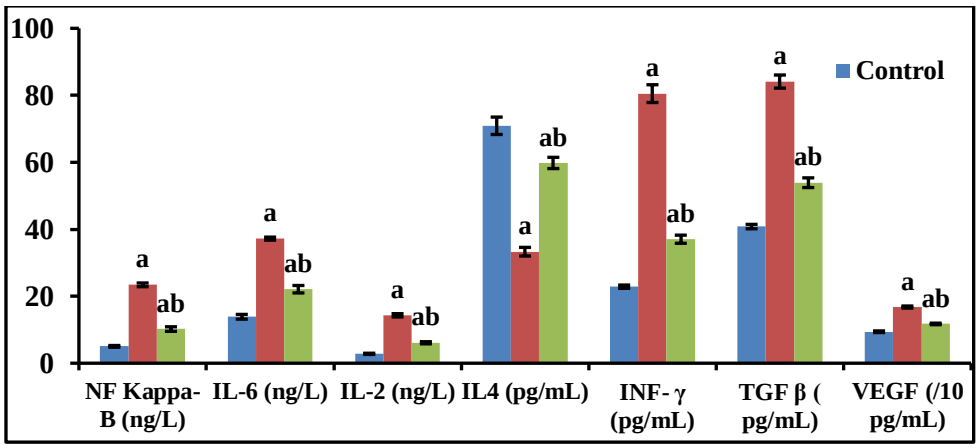

Figure (6): Changes in serum NF-κB, IL-6, IL-2, INF γ, TGF β, and VEGF among different experimental groups. ('a' significance with the control group, 'b' significance with the diabetic group).

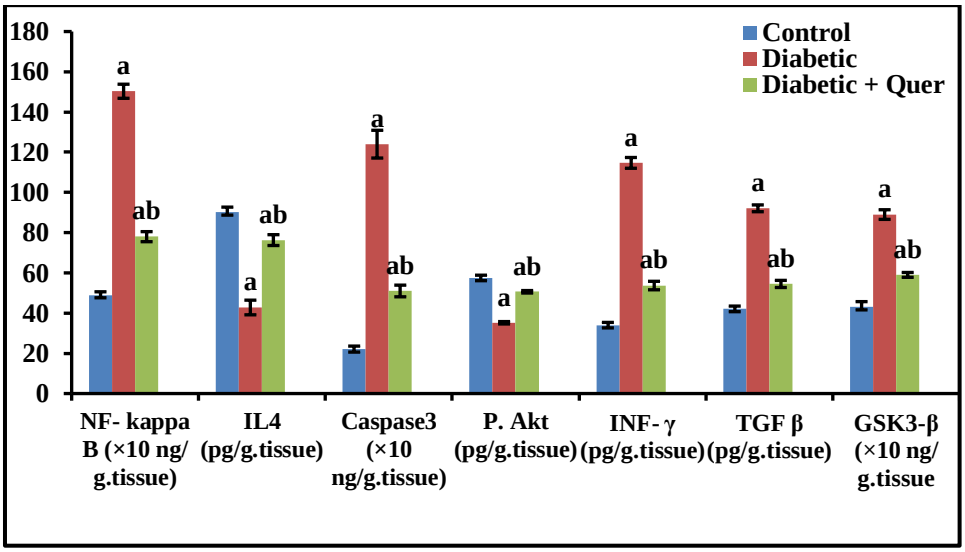

Figure (7): Changes in hepatic NF-κB, IL-4, caspase3, P.AKT, INF γ, TGF β, and GSK3-β among different experimental groups. ('a' significance with the control group, 'b' significance with the diabetic group).

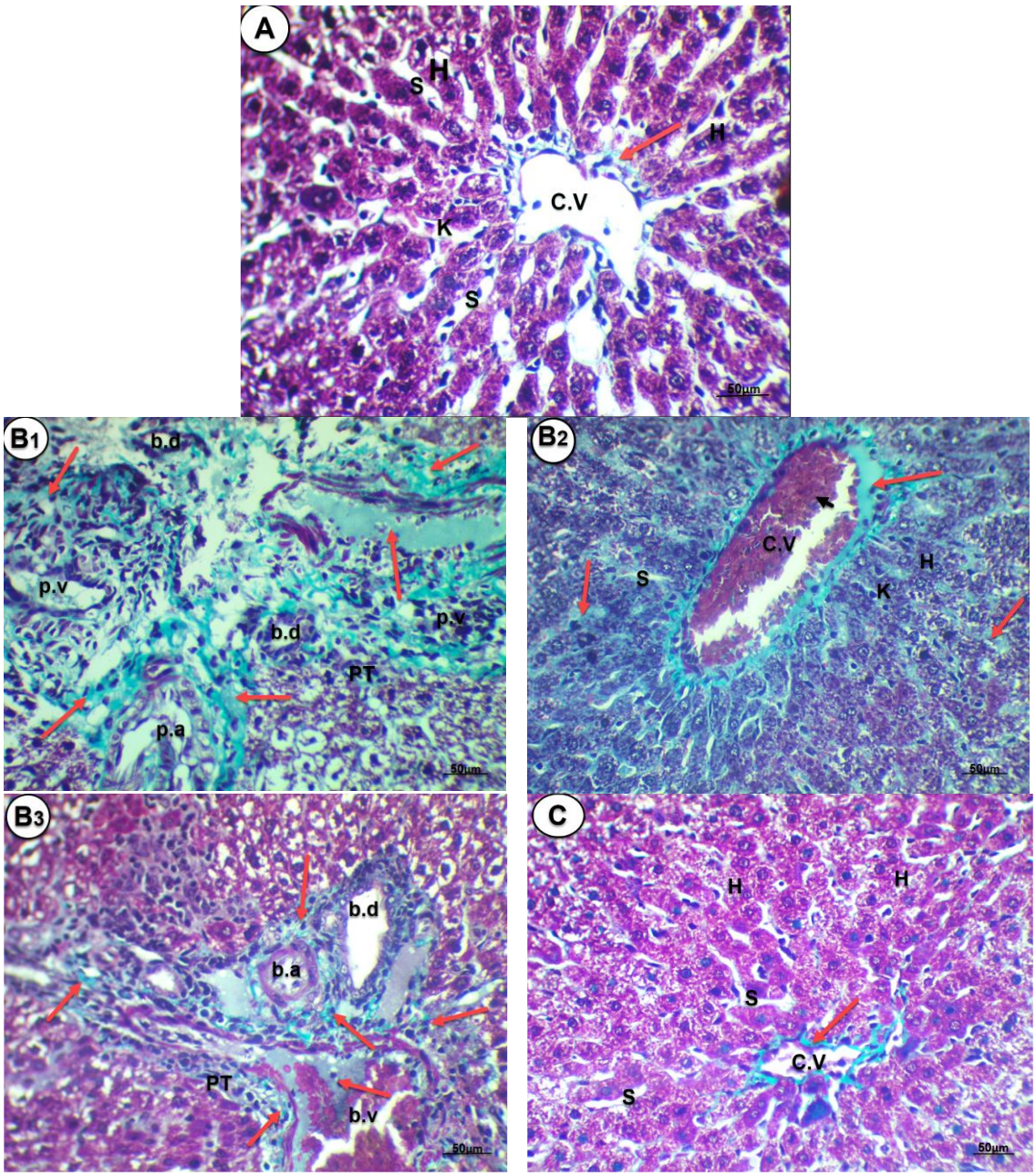

Figure (8): Photomicrographs of Masson trichrome-stained liver sections. A. Section from the control group exhibiting a normal hepatic architecture with minimal collagen fibers deposition (red arrow) around central vein (C.V), hepatocytes (H) and sinusoids (S) and kupffers (k). B1, B2 & B3. Sections from the liver of the STZ-treated group demonstrating increase in collagen fibers deposition (red arrows) which was increased around (c.v), hepatocytes, sinusoids and portal tract (PT) bile duct (b.d), portal artery (p.a) portal vein (p.v) and blood vessel (b.v) . C. Section from the liver of the STZ + Quer elucidating decline in collagen fibers deposition (red arrow) versus to the STZ group (X 400).

4. DISCUSSION

Diabetes is a metabolic disorder characterized by high blood sugar levels due to impaired insulin production or action (2,19). This leads to increased oxidative stress, which damages cells and tissues, including those in the liver and heart (8). STZ is a chemical often used to induce diabetes in animal models. It works by generating excessive ROS, which damages insulin-producing cells in the pancreas (11). A high-fructose diet can also contribute to the development of diabetes-like symptoms, such as insulin resistance and hyperlipidemia (20). Combining STZ injection with a high-fructose diet in the current study can accelerate the development of diabetes in rats, providing a useful model for studying the disease and testing potential treatments.

Diabetes often leads to complications affecting both the heart and liver. These organs share a complex relationship, with the heart releasing signaling molecules called cardiokines that influence liver function and vice versa. This interconnectedness between the heart and liver can exacerbate disease progression in diabetes, highlighting the importance of understanding and addressing these cardio-hepatic interactions for effective disease management (21).

Our results indicated that diabetic rats exhibited abnormal serum glucose and insulin. These findings are consistent with previous research that has shown that STZ primarily targets pancreatic β-cells, leading to their dysfunction and reduced insulin secretion. Moreover, reduced insulin sensitivity impairs glucose uptake by tissues, resulting in hyperglycemia (22). These findings suggest that diabetes is associated with metabolic dysfunction and impaired pancreatic function (23).

Our findings demonstrate that treatment with Quer substantially improved the altered levels of serum glucose and insulin levels in diabetic rats compared to the untreated diabetic group. These findings align with previous studies that reported the beneficial effects of Quer on glycemic control and insulin function in diabetic rats (4). Quer's positive impact on blood glucose levels in diabetes is attributed to its ability to inhibit glucose absorption in the intestine and promote glucose uptake by peripheral tissues (24). Quer promoted pancreatic islet regeneration through hepatic glucokinase activation and increased islet count (25). Quer's antioxidant properties may contribute to its protective effects on pancreatic β-cells and insulin production (26).

The results of the present study demonstrated that diabetic rats exhibited marked elevations in serum ALT levels and substantial reductions in glycogen, albumin, total protein, and PK corresponding to control rats. Given the liver's pivotal role in glucose homeostasis, hyperglycemia can lead to hepatic injury (23). Previous research has demonstrated elevated liver enzymes (ALT), lipid accumulation, lymphocytic infiltration, and fibrosis, in the livers of STZ-treated animals (27). These findings suggest that liver complications in diabetes may be associated with alterations in liver enzymes. In diabetic rats, high levels of ALT may be attributed to liver damage, which allows a large amount of these enzymes to leak out of the liver cells and into the bloodstream (28). Similar to our results, Glycogen content is decreased in STZ-induced diabetic rats. Glycogen levels in tissues like the liver serve as indicators of insulin activity, as insulin regulates glycogen storage by activating glycogen synthase and inhibiting glycogen phosphorylase, decreased insulin results in decreased glycogen content (29). Similar to our results, researchers observed a reduction in serum levels of total protein and albumin resulting from reduced kidney function (30). In a previous study, STZ-induced diabetes was associated with a reduction in PK levels. The decreased PK levels suggest impaired glucose utilization and subsequent alterations in energy balance in these animals (31).

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In contrast, the treatment of diabetic rats with Quer could improve the liver function markers in our diabetic model. These data run in parallel with the results of Ali et al. (32) who reported that Quer has a defensive effect against the hepatotoxicity produced by STZ-induced DM. Maciel et al. (33) reported that Quer increases albumin and total protein in diabetic rats. Consistent with our results, Kandasamy and Ashokkumar (34) concluded that flavonoids restore the reduced albumin level in diabetic nephrotoxic rats. Quer also accelerates the use of glucose in liver cells by activating key glycolysis enzymes, hexokinase, and PK, reducing the activity of glycogen phosphorylase and stimulating glycogen synthesis in the liver (35).

Hyperglycemia in diabetes is believed to trigger the activation of various processes that lead to oxidative stress, endothelial dysfunction, and the development of atherosclerotic changes; it is also an important risk factor for macro- and microvascular complications (35).

Our study demonstrated that diabetic rats exhibited substantial elevations in serum LDH, troponin, and CK-MB levels relative to control rats. These data are similar to several studies (36-38). Cardiac biomarkers are elevated in the serum due to compromised cardiomyocyte cell membrane integrity, leading to the release of these markers into the bloodstream (38,39). The administration of STZ and hyperglycemia associated with diabetes mellitus can increase the production of ROS (36). Excessive ROS generation plays a pivotal role in the development of myocardial damage (38). Once myocardial injury occurs, enzymes such as CK-MB, LDH, and cardiac troponin are released into the circulation and serve as biochemical indicators of myocardial damage (37,40). The activity of CK-MB is the most diagnostic for myocardial infarction because of the marked abundance of this isoenzyme in the myocardium and virtual absence from most other tissues and its consequent sensitivity (36).

However, the administration of Quer counteracted the deleterious effects of diabetes on cardiac markers and lipid profile. This result is consistent with previous reports, showing that treatment with Quer treatment mitigated the elevation of these parameters in the serum, likely by preserving the integrity of the cardiac cell membrane against injuries (41). This suggests that Quer may protect against cardiac cell damage, preventing the leakage of cardiac biomarkers from the cells (42).

Our study demonstrated that diabetic rats exhibited substantial elevations in serum cholesterol and triglyceride levels versus control rats. In concurrence with the present study, Ahmed et al. (43) reported that diabetic rats exhibited notably elevated levels of total cholesterol and triglycerides due to cellular insulin resistance. Moreover, it has been demonstrated that insulin deficiency in diabetes mellitus results in a multitude of disruptions in metabolic and regulatory processes, ultimately leading to the accumulation of lipids, such as triglycerides and total cholesterol, in diabetic patients (44).

Conversely, treatment of the diabetic rats with Quer was able to alleviate the elevation in serum cholesterol and triglyceride levels. These data are in line with a previous study, which has reported a substantial reduction in serum cholesterol and triglyceride levels in diabetic rats treated with Quer (44). The lipid-lowering effects of Quer were attributed to an increase in fecal cholesterol and bile acid excretion, besides the prevention of de novo triglyceride synthesis (45).

The present model of diabetes was associated with an elevation in serum MDA, H_2O_2, and AGE along with a decline in serum GSH and TAC contrasted with the control rats. These results follow earlier findings of diabetic studies that reported a decrease in plasma GSH content, accompanied by an increase in MDA, indicative of elevated oxidative stress in plasma and potentially other tissues of STZ-treated

rats (46). Diabetic individuals often exhibit elevated levels of AGEs, which are oxidative byproducts generated by hyperglycemia and/or dietary intake of AGEs (47). Oxidative stress is a critical factor in the development and progression of diabetic complications. The hyperglycemic environment in diabetes leads to an increased electron flux in the mitochondria, inhibiting complex III and consequently increasing the production of superoxide radicals (48). Chronic ROS generation in diabetic rats can lead to myocardial cell injury by oxidizing lipids and proteins, inactivating antioxidant enzymes, and causing DNA damage. Lipid peroxidation can have severe consequences, disrupting membrane fluidity and permeability, inactivating membrane-bound receptors and enzymes, and ultimately leading to membrane destruction and cell death (38).

Therefore, both the neutralization of ROS and enhancement of the cellular antioxidants can represent a protective approach against hyperglycemia-induced oxidative damage in the tissues. From our results, the treatment of the diabetic rats with Quer caused substantial enhancement in serum oxidative stress markers contrasted with the diabetic group. In this context, the previous study was in accord with the current findings that Quer could potentially contribute to the regulation of elevated oxidative stress, protein glycation, and glucose metabolism in diabetic rats (49).

The liver, a pivotal organ involved in the metabolism of biological molecules and chemicals, has been the subject of previous research regarding its association with diabetes mellitus (48). Diabetes can result in both hepatic dysfunction and metabolic disturbances (50). Our findings indicate that the induction of diabetes led to a substantial increase in the hepatic oxidative stress markers and a decrease in the hepatic antioxidant markers of diabetic rats compared to control rats. Oxidative stress is elevated in diabetes which may serve as a unifying mechanism underlying both insulin resistance and metabolic syndrome within this condition (50).

Treatment with Quer considerably reduced oxidative stress markers in the liver of diabetic rats. Numerous studies have demonstrated Quer's potent antioxidant properties. It acts as a free radical scavenger, neutralizing reactive oxygen species (ROS) that contribute to oxidative stress. This property is particularly beneficial in conditions of chronic hyperglycemia, where oxidative stress is elevated (51, 52).

Oxidative stress can induce inflammation through diverse mechanisms, and inflammation has been implicated as a notable contributor to the pathogenesis of diabetes (53). Our study demonstrated that the induction of diabetes led to substantial impairment in serum levels of NF-κB, IL-6, IL-2, IL-4, INF-γ, TGF-β, and VEGF matched to control rats. In diabetes, the inflammatory process is a consequence of systemic etiological factors, including central obesity and insulin resistance. Subsequently, inflammatory mediators activate various receptors and transcription factors, such as NF-κB, and receptors for AGE, leading to cellular dysfunction, apoptosis, impaired insulin signaling in insulin-sensitive tissues (54). Diabetes-induced alterations in fatty acid metabolism may contribute to the dysregulation of macrophage cytokine release, leading to an upregulation of pro-inflammatory cytokines (55). IL-6 levels have been documented to increase during the acute phase response, a process that can be initiated by diabetes and result in the release of effector molecules with the potential to induce endothelial dysfunction, thereby contributing to atherosclerosis (56). The pro-inflammatory cytokine IL-2 may play an important role in the pathogenesis of DM (55). IL-4 is a significant immunomodulatory cytokine with a crucial role in safeguarding immunity and mitigating inflammatory diseases within the context of diabetes mellitus (54). Our results are similar to Zarfeshani et al. (57) who reported a marked decrease in the IL-4

level among all STZ-treated rats which consequently caused an auto-immune imbalance among diabetics. The hyperglycemia-induced in the rats may have contributed to this reduction in IL-4, potentially dampening the acute inflammatory response (57). This decrease in IL-4 may have shifted the balance of pro-inflammatory and anti-inflammatory cytokines in diabetes mellitus towards a pro-inflammatory state, which could further exacerbate inflammatory complications associated with diabetes. Consistent with the findings of the present study, investigators reported elevated levels of IFN-γ in diabetic rats contrasted with the controls (58). Hyperglycemia-induced oxidative stress in diabetic patients is hypothesized to contribute to elevated levels of pro-inflammatory proteins. Infiltrating macrophages within the affected tissues secrete inflammatory cytokines, leading to both local and systemic inflammation (57). An increase in the secretion of the pro-inflammatory cytokine IFN-γ is often associated with insulin resistance, a well-established risk factor for the development of type 2 diabetes (58). TGF-β, a prominent profibrotic growth factor, is upregulated in individuals with diabetes mellitus due to hyperglycemia. This overexpression is believed to play a pivotal role in the development of diabetic nephropathy (59). Previous research has indicated that VEGF is upregulated in the retinas of animal models with diabetes (60). Additionally, studies have shown increased expression of VEGF mRNA and protein in the kidneys of diabetic rats, as well as upregulated VEGF receptors (61). These findings suggest that VEGF plays a role in various diabetic complications.

Treatment with Quer considerably reduced the levels of serum NF-κB, IL-6, IL-2, IFN-γ, TGF-β, and VEGF, accompanied by a marked elevation in IL-4 levels compared to the diabetic group. In the same line with our study, Albadrani et al. (62) documented that Quer decreased the activation of NF-κB and levels of IL-6 in rats. The protective effect of Quer includes antioxidants and anti-inflammatory effects mediated by the upregulation of endogenous antioxidants and downregulation of NF-κB. Moreover, Quer demonstrated anti-inflammatory properties in chronic prostatitis by reducing the expression of pro-inflammatory cytokines IL-2 and decreasing the activation of the NF-κB signal pathway (63). It was established that Quer mitigated inflammation by attenuating the activation of NF-κB at the upstream inhibitor of kappa B kinase (IKKα/β) level. This attenuation subsequently led to a marked reduction in pro-inflammatory cytokines (IL-6, IL-2, and IFN-γ), while elevating anti-inflammatory cytokine IL-4 (62, 64). Additionally, Quer preconditioning stabilized hypoxia-inducible factor-1 alpha (HIF-1α) and subsequently decreased the expression of its pro-angiogenic target VEGF (64).

Our study demonstrated that the induction of diabetes led to significant elevations in hepatic levels of NF-κB, caspase-3, INF-γ, TGF-β, and GSK-3β along with a marked decrease in P.AKT and IL-4 levels compared to control rats. In this context, the previous study agreed with the current findings that diabetic induction results in the activation of hepatic NF-κB (65). These data indicate that activation of NF-κB is an initial signaling event that leads to cellular dysfunction and damage (66). Hyperglycemia and elevated free fatty acids contribute to oxidative stress, which, in the absence of sufficient antioxidant defense, leads to redox imbalance and the activation of stress-responsive signaling pathways, such as NF-κB, potentially contributing to liver damage and diabetic complications (65). Diabetes is often associated with chronic inflammation, which can lead to a decrease in IL-4, an anti-inflammatory cytokine (67). Additionally, insulin resistance, a hallmark of type 2 diabetes, can also contribute to decreased IL-4 levels. Similar to our result, Khadrawy et al. (68) reported gene expression and protein levels of caspase-3 were markedly elevated in the livers of diabetic rats. Moreover, it was established that the cytokine IFN-γ was elevated in diabetic mice indicating the activation of inflammatory pathways (69,70). TGF-β signaling, a crucial modulator in

diabetes, is activated in diabetic conditions and plays a pivotal role in liver fibrosis (71). Our findings were consistent with these data, showing that diabetes activates the TGF-β pathway resulting in liver damage. P.AKT exerts metabolic effects through the regulation of downstream targets (72). It phosphorylates numerous mediators involved in regulating a variety of biological processes, including apoptosis and hepatic gluconeogenesis. Furthermore, AKT plays a critical role in metabolic regulation and cell survival (6). In diabetes, AKT phosphorylation levels are reduced, leading to impaired insulin signal transduction (73). Consequently, any disruption of the PI3K/Akt signaling pathway can impair insulin signal transduction, leading to the development of insulin resistance and type 2 diabetes mellitus (72). Impaired signal transduction of the insulin/PI3K/Akt signaling pathway is one of the main features of insulin resistance which can lead to an elevation in GSK-3β activity (6)

Treatment with Quer markedly reduced the levels of these inflammatory markers in the liver of diabetic rats. These data are in line with a previous study, which has reported that Quer induced suppression of the release of NF-κB by preventing the degradation of its inhibitor (65). Also, it was established that Quer decreases inflammation markers INF-γ and prevents NFκB activation (74). Another study demonstrated that Quer and naringenin administration significantly increased hepatic IL-4 mRNA expression in rats exposed to diethylnitrosamine/2-acetylaminofluorene, confirming their anti-inflammatory effects (43). Caspase-3 is a key enzyme in apoptosis, initiating a cascade of events leading to cell death by cleaving cellular proteins involved in DNA fragmentation, nuclear breakdown, and cell membrane blebbing (75). A former study discovered that Quer pretreatment significantly inhibited the overexpression of caspase-3 and apoptosis-related proteins resulting from hepatotoxicity (76). Liu et al. (77) reported that the expression level of the TGF-β and its upstream target proteins GSK-3β were also considerably decreased by Quer treatment in rats with glomerulosclerosis. This data suggests that Quer can protect the liver from fibrosis by inhibiting the TGF-β signal pathway.

The present study showed increased collagen fiber deposition around the portal area and in between the hepatocytes in diabetic rats. This indicates that diabetes can induce liver fibrosis due to increased oxidative stress, which damages liver cells and stimulates the production of collagen (78). However, treatment with Quer resulted in a substantial reduction in fibrosis in the diabetic rats. Supporting these findings, QUR prevented CdCl2-induced lipid droplets and collagen from accumulating in the livers of rats by suppressing oxidative stress and oxidation of collagen filaments (79). Additionally, numerous studies have demonstrated the hepatoprotective effects of flavonoids, including Quer (80). These compounds likely exert their protective effects through multiple mechanisms. They may enhance antioxidant defense by upregulating Nrf2/CYP2E1 expression, reduce inflammation by inhibiting MAPK/NF-κB signaling pathways, and mitigate apoptosis by regulating Bcl-2/AKT/caspase expression (80, 81).

5. CONCLUSION

In conclusion, diabetes led to significant metabolic dysregulation, oxidative stress, inflammation, and structural damage in the heart and liver of rats. Treatment with Quer effectively ameliorated these adverse effects by reducing oxidative stress, inflammation, and fibrosis. These findings highlight the potential of Quer as a promising therapeutic agent for mitigating diabetes-induced cardiac and hepatic complications.

Conflict of interest

All authors declared that there were no conflicts of interest.

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