DOI: https://doi.org/10.31351/vol34iss4pp13-22

Liquiritin Attenuates Diabetic Nephropathy in Type 2 Diabetic Male Rats Irrespective of Antihyperglycemic Effect

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Received 21/3/2024, Accepted 10/6/2024, Published 20/12/2025

Abstract

Diabetic nephropathy (DN) represents one of the common and serious complications of diabetes mellitus, and it is a leading cause of renal failure. Liquiritin is one of the main flavonoids found in Glycyrrhiza glabra and has been shown to have several pharmacological effects, such as antioxidant, anti-inflammatory, antitumor, cardiovascular protection, hepatoprotection, and attenuation of rheumatoid arthritis in experimental animals. The present study investigated the protective effect of liquiritin against diabetic nephropathy in male rats fed a high fat diet and with streptozotocin (STZ) induced diabetes mellitus.

In the present study, type 2 diabetes was induced in the animals by feeding them a high-fat diet for four weeks. Afterward, the animals were fasted overnight and injected with a single intraperitoneal dose of STZ (30 mg/kg). Liquiritin was administered daily at a dose of 40 mg/kg for 15 weeks. At the end of the experimental period, blood glucose level, glycated hemoglobin A1 (HbA1c), lipid profile markers, serum renal function parameters, renal index were assessed, levels of inflammatory and oxidative markers namely interleukin-1beta (IL-1β), interleukin -6 (IL-6), tumor necrosis factor-alpha (TNF- α), and transforming growth factor-beta (TGF-β1), glutathione (GSH), and malondialdehyde (MDA) were determined in the renal homogenate. The histopathological examination was performed using hematoxylin and eosin staining, and the histopathological changes in kidney tissues were semi-quantitatively calculated according to a renal pathology scoring system.

The diabetic control group showed a significant increase in glycemic control parameters and renal function parameters, with significant histopathological changes compared to the normal control group. The results revealed that liquiritin significantly improved histopathological changes but did not affect the glycemic control parameters, with a reduction in the inflammatory markers and oxidative stress in the renal homogenate.

Liquiritin has an ameliorating effect against DN, and it can significantly decrease most of the histopathological changes, as well as oxidative and inflammatory markers.

Keywords: Diabetes Mellitus, Diabetic Nephropathy, Flavonoids, Liquiritin, Oxidative Stress.

Introduction

Diabetic nephropathy (DN) is one of the serious and life-threatening microvascular complications of diabetes that is characterized by permanent albuminuria (>300 mg/d) and a gradual decline in the glomerular filtration rate (GFR) [(1)]. It represents a leading cause of morbidity and mortality in both Type 1 and Type 2 diabetes patients [(2–5)]. The pathogenesis of DN is a complicated and progressive process that involves multiple overlapping biological pathways that initiate and maintain the pathogenesis of DN. These pathways include hemodynamic, metabolic, growth, and proinflammatory or profibrotic pathways. These pathways act simultaneously and in an overlapping manner to increase oxidative stress, inflammatory factors, and fibrotic factors, which induce lesions in the various compartments of the kidney [(6)].

The most important cytokines involved in DN are IL-1β, IL-6, TNF- α, and TGF-β1. They have been involved in increasing the intraglomerular pressure, promoting the hypertrophy of the kidney, increasing the glomerular basement membrane thickening, increasing the hypertrophy of the podocytes, glomerular hemodynamic alterations, increasing oxidative stress, and increasing vascular endothelial permeability [(7-10)]. Oxidative stress represents one of the essential mechanisms involved in the development of diabetes and its complications, including DN (11). In Diabetes mellitus, oxidative stress is developed by hemodynamic factors, and by hyperglycemia-induced activation of alternative glucose metabolic pathways such as hexosamine, protein kinase C, and polyol pathways.

These activated pathways enhanced the expression of inflammatory cytokines (IL-1, 6, 18) that led to oxidative stress. Reactive oxygen species (ROS), such as malondialdehyde (MDA), which are increased by hyperglycemia, produce significant functional and structural changes in glomerular and renal tubular cells and can induce oxidative stress-related renal damage. Various enzymatic antioxidants, such as catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx); and nonenzymatic antioxidants, such as glutathione (GSH), normally protect renal cells from pathological damage caused by ROS, the activity of these antioxidant enzymes is reduced by activation of polyol pathway that decreases the concentration of intracellular NADPH [(12)]. The protective approaches against DN involve the use of antihyperglycemic, antihypertensive, and renoprotective drugs, however, the control of hyperglycemia results only in modest reduction of the onset or progression of DN in the patients with long-term diabetes, and although the regulation of blood pressure, and the use of renin-angiotensin system inhibitors alone or in combination with sodium-glucose transport inhibitors or a mineralocorticoid receptor antagonist can decrease the incidence and the progression of DN, considerable residual risks of DN remain and unfortunately, a large number of patients remain uncontrolled and progress to end stage renal disease (ESRD), in addition, these drugs have some limitations due to their renal and heart side effects [(13–15)]. Therefore, searching for more effective and safe therapies to inhibit the occurrence or slow the progression of DN is a vital mission to reduce the morbidity and mortality associated with diabetes mellitus.

Natural products constitute a major source in the area of drug discovery that have shown primary activity in animal studies against many diseases and conditions, including diabetes and nephroprotection against DN [(16-18)]. Flavonoids have numerous pharmacological effects, including treatment of chronic kidney disease [(19)]. Liquiritin is one of the main flavonoids found in Glycyrrhiza glabra (Licorice). Licorice has numerous pharmacological properties against different disease conditions, as provided by many clinical studies. Various studies concluded that licorice treats patients with asthma, gastric ulcers, and glucose intolerance and improves insulin sensitivity [(20, 21)]. Liquiritin exhibits several useful pharmacological effects, such as antioxidant, anti-inflammatory, anti-tumor, cardiovascular protection, antitussive, hepatoprotection, and skin protective effects, and attenuation of rheumatoid arthritis in experimental animals via reduction of inflammation and angiogenesis [(22, 23)]. To the best of our knowledge, the protective effect of liquiritin hasn't been studied against diabetic nephropathy, and the present study aims to investigate the protective effect of liquiritin against diabetic nephropathy in high fat diet and streptozotocin induced diabetes in rats.

Materials and Methods

Chemicals and reagents

Liquiritin (>98%) was purchased from (Hefei TNJ Chemical Industry Co., Ltd., Hefei, China), STZ, metformin, and protease inhibitor were purchased from (MedChemExpress, USA. The enzyme-linked immunosorbent assay kit (ELISA) for insulin, (IL-1β), (IL-6), (TNF-α), (TGF- β), (GSH), and (MDA) were supplied from (elk technology, China). All other reagents and chemicals were of analytical grade.

Animals

The animals used in the present study were procured from the College of Veterinary Medicine, University of Basrah. Apparently, healthy adult male Wistar rats weighing 150-220 gm were housed in plastic cages at the animal house of the College of Medicine-University of Basrah with the supply of the diet and water ad libitum, a photoperiod of 12 h/12 h Light/dark cycle, and a temperature of 21 ± 4 ◦C. Rats were acclimatized for one week before the commencing of the study. The best effort was made to reduce the animals' distress and pain throughout all experimental procedures (No. of ethical approval statement for using animals in the present study: UNCOMIRB20240518).

Induction and assessment of diabetes

In the present study, the type 2 diabetes model was induced by using the method described by Liu et al with minor modifications: the method included feeding the animals with the high fat diet for four weeks, after which the animals were fasted overnight and injected with a single intraperitoneal dose of STZ 30 mg/kg that was dissolved in a freshly prepared sodium citrate buffer (0.1M, pH 4.5) [(24)]. Because STZ is heat and light sensitive, and unstable even in acidic solution, both STZ and citrate buffer solutions were kept as cold as possible by the use of ice bags containing heat insulated case, STZ was covered with aluminum foil during the work to protect it from the light induced degradation, and dissolved in cold citrate buffer until immediately before the injection. A 10% sucrose solution was used to replace the drinking water after STZ injection for 48 h. To avoid fatal hypoglycemia that may occur as a result of massive β-cell necrosis and sudden release of an enormous quantity of insulin [(25)]. Random blood glucose level was measured in blood samples taken through tail vein puncture by the use of Accu-Check blood glucose meter (Roche Diagnostics/Germany) after seven days of STZ injection and the animals with glucose levels more than 250 mg/dl were included in the study, while those with less than 250 were reinjected with STZ [(25)].

Experimental design

48 male Wister rats were divided into four groups with 12 animals for each group as follows:

1. Normal control group (Con): rats were fed with normal control diet (The caloric density is 2.960 Kcal/g., carbohydrate 70%, 20% protein, 10% fat) for the 19 weeks of study duration, and injected intraperitoneally with citrate buffer at the end of the fourth week, and then given 10 ml/kg normal saline by oral administration daily for 15 weeks.
2. Diabetic control group (DM): diabetes was induced by feeding the rats with high fat diet (4.000 kcal/g., carbohydrates 35 %, protein 20 %, fat 45%) for four weeks and then injected intraperitoneally with 30 mg/kg of STZ and continued on HFD for 15 weeks.
3. Diabetic-Metformin group (Met): diabetes was induced by feeding the rats with high fat diet for four weeks, injected intraperitoneally with 30 mg/kg of STZ, and then treated orally with 100mg/kg of metformin for 15 weeks, and continued on HFD for 15 weeks.
4. Diabetic + liquiritin (Liq): diabetes was induced by feeding the rats with high fat diet for four weeks and injected intraperitoneally with 30 mg/kg of STZ, and then treated orally with 40 mg/kg of liquiritin daily and continued on HFD for 15 weeks. Liquiritin was administered daily by dissolving each 40 mg in 10 ml of normal saline and given orally at a dose of 10 ml/kg, the selection of liquiritin dose was based on previous studies [26]. The body weight of the animals was measured at the beginning and the end of the study on the day of sacrifice, while the kidney index was calculated by using the following formula:

$$\textbf{Kidney index}\ (mg/g) = \frac{\textbf{Kidney weight}\ (mg)}{\textbf{body weight}\ (g)}$$

Measurement of Serum biochemical parameters and blood HbA1c

At the end of the study period, the animals were fasted overnight, sacrificed by using ketamine and xylazine for anesthizination, dissected, and the heart was punctured for blood sample collection by using of 10 ml syringe, and 2 ml of the blood sample was transferred into EDTA-containing tubes for the measurement of HbA1c, by the use of ion exchange HPLC D-100s HbA1c analyzer from Bio-Rad Laboratories. the other part was transferred into gel tube and allowed to stand for 30 min. at 37 ◦C to coagulate and centrifuged at 4000 for 15 min. and the serum was collected to measure serum glucose concentration, serum lipid profile that includes triglycerides (TG), total cholesterol (TC), high density lipoprotein (HDL), low density lipoprotein (LDL), serum albumin; renal function indicators including serum creatinine, and urea.by the use of automated analyzer (ARCHITECT c4000 Chemistry Analyzer, Abbot Diagnostics, USA). The enzyme-linked immunosorbent assay (ELISA) kit was used for the detection of serum insulin level.

ELISA assay of the renal inflammatory and oxidative stress markers

The measurement of IL-1β, IL-6, TNF-α, TGF-β1, GSH, and MDA in the renal tissue was performed by ELISA. After the blood collection both kidneys were harvested and washed with cold phosphate buffer saline and the right kidney was placed in a plain tube containing cold phosphate buffer saline and stored in deep freeze while the left kidney was divided into two parts, one part was placed in a plain tube containing cold phosphate buffer saline and stored in deep freeze to be used later for the preparation of tissue homogenates and other part was used for histopathological examination. For the preparation of tissue homogenate, tissue samples were weighed and homogenized in a PBS solution with a pH of 7.4 containing 1% protease inhibitor cocktail and 1% triton X-100 (1:10 weight of tissues/ volume of lysis buffer) using a high intensity liquid processor. Then the homogenates were centrifuged at 4 C for 5 minutes at a speed of 10000 rpm. Subsequently, the supernatants were collected and subdivided into different aliquots to avoid repeated freeze-thaw cycles. The aliquots were then kept at the deep freeze and were used later for the ELISA assay [27]. The steps briefly include adding 50 µL of standard (gradually diluted according to the instructions) or 50 µL of sample to each well, and after incubation and washing, 100 µL of biotinylated antibody solution was added to each well, and the plate was incubated and washed. Then 50 µL of Streptavidin-HRP (Horseradish peroxidase) was added to each well, and the plate was incubated and washed. Finally, 50 µL of stop solution was added and optic density was recorded at 450 nm by the use of a microplate reader (Thermoscientific/China).

Histopathological examination of the renal tissue

The parts of the left kidneys were fixed in 10% formaldehyde. The fixed tissues were dehydrated by using a tissue processor in which the tissue was passed into 70%, 80%, 95%, and finally 100% ethanol for 2 hours each. After that, the tissue was cleared by immersing it in xylol for 2 hours. Then the tissues were impregnated in melting paraffin (55-60°C) for two hours in two steps to saturate the tissue, replenish the cleaning agent, and remove bubbles. Molten paraffin was then poured into a metal template to allow the samples to take place. Paraffin blocks were removed from the metal once they solidified. Then, a rotary microtome was used for sectioning, and sections were cut at a 5 micrometer thickness. Finally, the sections were stained with Hematoxylin and Eosin. Examination of the hematoxylin and eosins stained sections were examined with the assistance of a histopathologist utilizing a light microscope (Olympus/Japan) and

digital camera (Canon/Japan), at a final magnification of 100 and 400x. The histopathological changes in kidney tissues were semi-quantitatively calculated according to a renal pathology scoring system as previously described [28]. The pathological changes that were examined include interstitial inflammation, glomerular congestion, glomerular atrophy, interstitial vascular congestion, hyaline cast formation, acute tubular necrosis, epithelial vacuolar degeneration, and epithelial fatty change. The scoring system was performed by calculating the averages of 10 fields per section for each animal in the group, and the mean of the group was calculated to be compared with the means of the other groups. The scoring system consists of a total of five scores, which are defined according to the severity of the pathological damage of the renal tissues (score 0: no change, 1: 1-25%, 2: 26-50%, 3: 51-75%, 4: >75%).

Statistical analysis

Both SPSS (Statistical Package for Social Sciences) version 23 and Microsoft Office Excel 2013 were used to summarize, analyze, and display the information, the experimental data were expressed as the mean ± standard deviation (SD). The one-way Analysis of Variance (ANOVA) was used to examine the difference in the means of numeric variables among more than two groups, followed by the Tukey post hoc test to compare the means of any two groups and a *P* value less than 0.05 and 0.001 was considered statistically significant.

Results

Effect of liquiritin on glycemic control, lipid profile, renal function parameters, and body Weight

The DM group showed a significant increase in blood glucose and HbA1c levels compared to the Con group. The DM group showed significantly lower body weight compared to the Con group. There was no significant difference in the glycemic parameters and body weight among DM, Met, and Liq groups ($P > 0.05$) as shown in Table 1. DM group showed a significant increase in the serum level of TG, TC, HDL, and LDL compared with the Con group. There was no significant difference in the lipid parameters among DM, Met, and Liq groups ($P > 0.05$) except for LDL of the Met group, which was significantly higher than that of DM. The DM groups showed a significant increase in serum creatinine, urea, and renal index in comparison with the Con group ($P < 0.05$). There was no significant difference in the serum level of creatinine, albumin, and renal index among DM, Met, and Liq., while the urea levels of Met and Liq groups were significantly lower compared with the DM group.

Table 1. Effect of liquiritin on serum glycemic control, lipid profile, and renal function parameters

Parameter	Con	DM	Met	Liq
Body weight	329.23 ± 20.30	232.40 ± 60.21 [a]	288.76 ± 31.54	264.00 ± 55.31
FBS (mg/dL)	130.54 ± 24.90	593.60 ± 39.56 [a]	661.34 ± 62.50	563.83 ± 89.46
HbA1c %	6.80 ± 0.61	10.26 ± 0.8[a]	9.9 ± 1.20	8.82 ± 0.91
Insulin (mU/L)	2.27 ± 0.63	4.00 ± 0.90	6.0 ± 1.08	6.68 ± 1.49
TG (mg/dL)	39.43 ± 8.87	181.76 ± 20.76 [a]	161.6 ± 24.5	138.00 ± 31.81
TC (mg/dL)	40.54 ± 1.86	63.76 ±16.32 [a]	60.30 ± 9.90	58.50 ± 10.97
HDL (mg/dL)	10.54 ± 0.89	21.60 ± 2.70 [a]	18 ± 2.48	24.33 ± 4.27
LDL (mg/dl)	5.40 ± 0.55	9.40 ± 4.72 [a]	17.67 ± 4.67 [b]	9.00 ± 2.18
creatinine (mg/dL)	0.27 ± 0.04	0.45 ± 0.09 [a]	0.47 ± 0.02	0.41 ± 0.03
Urea (mg/dL)	21.90 ± 3.29	81.60 ± 12.48 [a]	47.05 ± 3.59 [b]	43.00 ±14.32 [b]
Albumin (g/L)	19.40 ± 1.34	25.60 ± 2.23 [a]	24.36 ± 1.46	24.67 ± .94
Renal index	2.61 ± 0.35	4.65 ± 0.31[a]	4.58 ± 0.47	4.32 ± 0.72

Con: normal control group, DM: diabetic control group, Met: diabetic + metformin group, Liq: diabetic + liquiritin group, FBS: fasting blood sugar; HbA1c: glycated hemoglobin A1, TC: Total cholesterol, TG: triglyceride, HDL: High density lipoprotein LDL: Low density lipoprotein.Values are mean ± SD., n=12, (a): Significantly different ($P \leq 0.05$) compared with the Con group, (b): Significantly different ($P<0.05$) compared with the DM group.

Histopathological Findings

The kidney sections in all animals of the Con group show normal glomerular architecture, normal interstitial vascular thickening and normal renal tubules in the cortex. In the medulla, renal collecting tubules appear within normal limits, where the tubular epithelium was simple cuboidal, with a well-defined lumen, and a normal thickness of the tubular basement membrane. All changes that were recorded in DM and other treatment groups were scored 0 in this group, as shown in Figure 1(A-D) and Table 2. The kidney sections in the DM group showed marked glomerular congestion, with an average change of 81±2 (score 4), as well as glomerular atrophy, with an average of 13±2.16 (score 1). Renal tubules showed fatty degeneration of renal tubular epithelium of the distal renal tubules (average 28±0.14, score 2), while proximal renal tubular epithelium showed focal areas of acute tubular necrosis (average 40±1.3, score 2). Renal interstitium showed vascular congestion; (average 93±1, score 4), and spots of intensive inflammation; (average 67±3.2, score 3) as shown in Figure 1 (E-H) and Table 2. In the metformin-treated group, there is marked glomerular congestion (average 56±0.18, score 3), proximal tubules showed hyaline cast (average 36±1.6, score 2), and some sites appear with marked sloughing of the tubular epithelium (score 3). Interstitium showed severe vascular congestion (average 59±4, score 3) and mild inflammation (average 19±3, score1). No features of glomerular atrophy, vacuolar and fatty degeneration of the renal tubular epithelial cells, as shown in Figure 1 (I-L), and Table 2. as shown in Figure 1 (M-P), and Table 2, Liq did not affect glomerular atrophy and hyaline cast formation, while showing significantly lower histopathological changes compared with DM, and Met groups. Statistical analysis showed that the scored changes of the Met and Liq groups were significantly lower than those of DM at ($p \leq 0.05$); however, the Liq group showed superior improvement in comparison with the Met group in most of the scored changes.

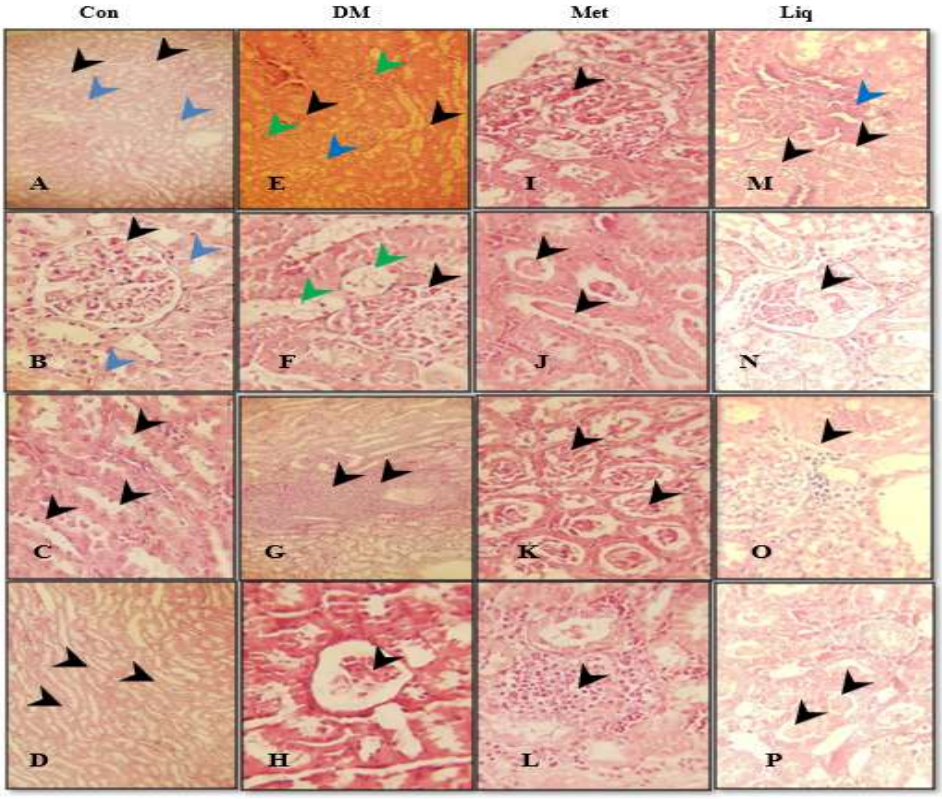

Figure 1. Histopathological findings in the study groups. Kidney of Con group shows A and B: normal glomerular tuft (black arrow), normal renal tubular epithelium (blue arrow) in the renal cortex, C: normal renal tubular epithelium (black arrow) in the renal medulla. Kidney of DM group shows E: necrotic renal tubular epithelium (black arrow), hyaline cast in the tubular lumen (blue arrow) and glomerular congestion (green arrow) in the renal cortex, F: glomerular congestion (black arrow) and fatty degeneration of renal tubular epithelium (green arrow) in the renal cortex, G and H: intensive interstitial inflammation (black arrow) in the renal cortex. The kidney of the Met group shows I: glomerular congestion in the cortex, J: accumulation of hyaline cast in the tubular lumen, K: marked sloughing of the renal tubular epithelium, L: mild interstitial inflammation in the renal medulla. The Kidney of the Liq group shows M: marked glomerular congestion (blue arrow), destruction of renal tubular epithelium (black arrow), N: glomerular atrophy (black arrow), O: mild peri-glomerular inflammation (black arrow), P: tubular hyaline cast (black arrow), destruction of renal tubular epithelium (blue arrow). H&E, A, D, E 40X, all others 40X.

Table 2. Effect of liquiritin on histopathological changes compared with DM and Met groups

pathological changes	DM % (Score)	Met % (Score)	Liq % (Score)
Inflammation	67±3 (3)	19±3 [b] (1)	47±3 [b] (2)
Glomerular congestion	81±2 (4)	56±1 [b] (3)	41±2 [b] (2)
Glomerular atrophy	13±2 (1)	0 (0)	11±0.12 (1)
Interstitial vascular congestion	93±1 (4)	59±4 [b] (3)	0 (0)
Hyaline cast formation	0 (0)	36±1 [b] (2)	23±3.44 (1)
Tubular necrosis	40±1 (2)	52±1 [b] (3)	22±1.53 [b] (2)
Epithelial vacuolar degeneration	0 (0)	0 (0)	0 (0)
Epithelial fatty change	28±0 (2)	0 (0)	0 (0)

DM: diabetic control group, Met: diabetic + metformin group, Liq: diabetic + liquiritin group. Values are mean ± SD., n=12, (b): Significantly different (P<0.05) compared with the DM group.

Effect of liquiritin on renal level of IL-1β, IL-6, TNF-α, TGF-β1

As shown in Figure 2, the DM group showed a significant increase in the renal level of IL-1β, IL-6, TNF-α, and TGF-β1 in comparison with the Con group, after 15 weeks of treatment and except for TGF-β1, liquiritin significantly lowered these inflammatory factors, which is in contrast to the effect of metformin that did not affect their renal level.

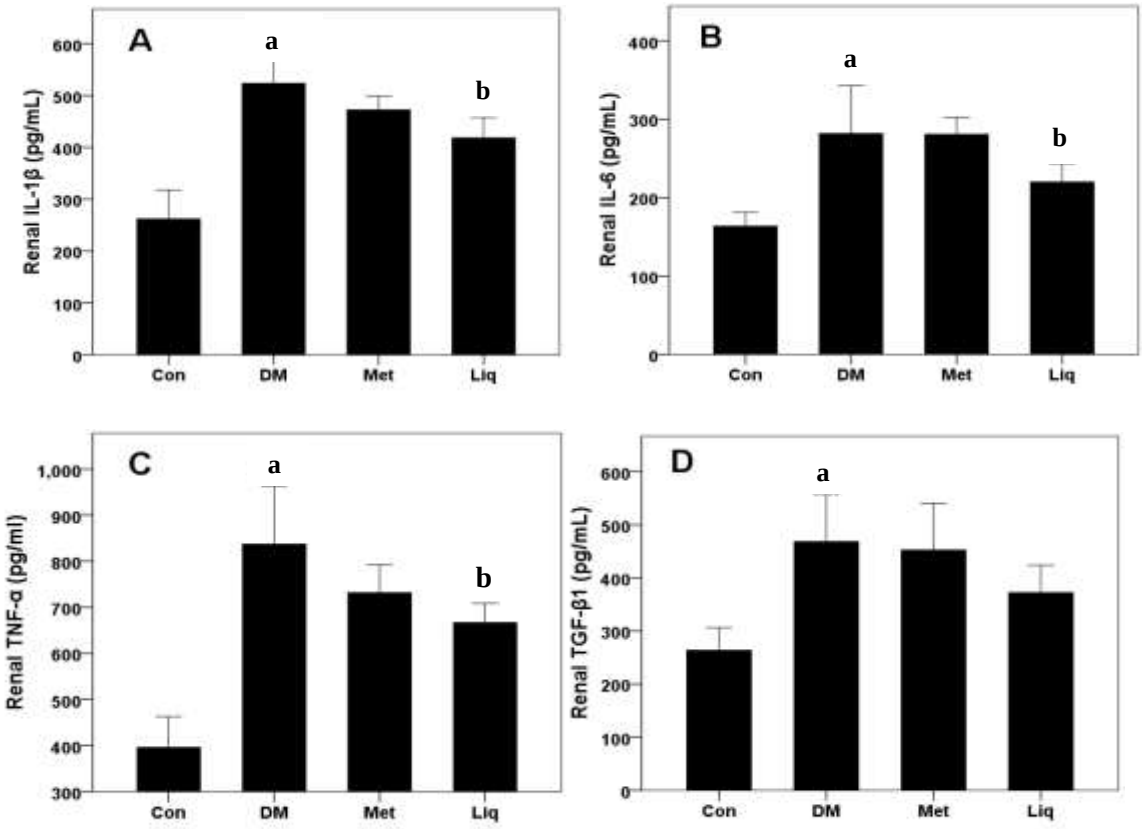

Figure 2. The effect of liquiritin on renal IL-1β, 1L-6, TNF- α, TGF-β1. , (a): Significantly different (P ≤ 0.05) compared with the Con group, (b): Significantly different (P<0.05) compared with the DM group.

Effect of liquiritin on renal oxidative stress

In comparison with the Con group, the DM group showed significantly higher renal level of MDA, and lower level of GSH, and treatment with liquiritin for 15 weeks significantly improved these oxidative markers. Metformin did not affect these oxidative markers, as shown in Figure 3.

Discussion

Diabetes mellitus is a common metabolic disorder that is associated with numerous serious and life-threatening complications. DN is one of the major diabetes microvascular complications and a leading cause of ESRD. The pathogenesis of DN is a complicated and progressive process that involves multiple overlapping biological pathways, and there are continuously newly discovered pathophysiological contributors and molecular mechanisms involved. The control of hyperglycemia results only in a modest reduction of the onset or progression of DN in patients with long-term diabetes [29]. In the present study, we use a low dose of STZ and a high-fat diet to induce type 2 diabetes in rats, which is a stable and efficient model that is commonly used in the published literature. Compared to the normal rats, the diabetic rats develop significantly higher blood glucose levels and typical signs of diabetes, i.e., polyuria, polydipsia, and polyphagia. 15 weeks post diabetes induction, the diabetic control rats develop obvious functional and histopathological changes represented by a significant increase in the serum creatinine, urea level, glomerular hypertrophy, and vacuolar degeneration, which indicate the success of the DN model. Except for glomerular atrophy and hyaline cast formation, liquiritin significantly decreased all other histopathological changes. Interestingly, liquiritin did not lower the blood glucose level; nevertheless, it improved the pathological changes in diabetic rats, suggesting that the renoprotective effect of liquiritin is independent of blood glucose control, and this comes in agreement with several studies that have found that the natural polyphenols could have a renoprotective action against experimental DN irrespective of antihyperglycemic effect (30). In diabetes, the long-term hyperglycemia is the principal trigger of DN, where the inflammation and oxidative stress are the major mechanisms that play an important role in the occurrence and development of DN [31]. Oxidative stress can stimulate the production of inflammatory cytokines, which in turn lead to the production of more free radicals to exacerbate oxidative stress and eventually form a vicious cycle [32, 33]. Many studies illustrated that diabetic nephropathy is related to the excessive production of reactive oxygen species, epithelial cell interstitial transformation, and is closely associated with renal oxygen species under hyperglycemia [34].

MDA is an important marker of lipid peroxidation that results from the attack of accumulated ROS of the unsaturated fatty acids, so it is an important index that reflects the degree of lipid peroxidation and oxidative stress. GSH and SOD act as important antioxidants that could counteract the harmful effects of oxidative damage from free radicals in the body. A considerable number of studies have reported that a decrease in GSH and SOD and an increase in MDA were observed in rats with DN induced by STZ [35, 36]. The present results were in agreement with these findings, and administration of liquiritin significantly improves the levels of GSH and MDA. The proinflammatory cytokines have an important role in the DN pathogenesis [7]. The most important cytokines in DN are IL-1β, IL-6, TNF-α, and TGF-β1. These cytokines have been involved in increasing the intraglomerular pressure by increasing the prostaglandins, inducing neutrophil infiltration, promoting the hypertrophy of the kidney, increasing the glomerular basement membrane thickening, increasing the induction and differentiation of the inflammotory cells, increased toxicity to various kidney cells through apoptosis activation, glomerular hemodynamics alteration, increased oxidative stress, and increased vascular endothelial permeability [8]. The TGF-β1 has been recognized to play an important role in the pathogenesis of DN as it is reported to be included in podocyte injury, basement membrane thickening, mesangial cell proliferation, renal cell fibrosis, and glomerulosclerosis [9, 10]. In the present study, although liquiritin resulted in significant reduction of these inflammatory cytokines but they still higher than that of normal control group which may explain its modest protective activity against pathological alterations and taking into consideration that it did not affect TGF-β1 level, a finding that come in agreement with other studies which had been demonstrated that these inflammatory cytokines had been significantly increased in the experimental DN and the improvement in the function and structure of the kidneys was associated with reduced cytokines expression [37-38]. Another issue that cannot be explained in the present study, and it may be the cause behind this modest nephroprotective activity, is the pharmacokinetic characteristic of liquiritin, as it is a glycoside flavonoid a class of flavonoids that has uncertainty about their enteric absorption and bioavailability, however they are used orally in many experimental studies with significant activity against induced disease models, while used in others via parenteral route [22, 23]. It is concluded from the present study that liquiritin can ameliorate DN.

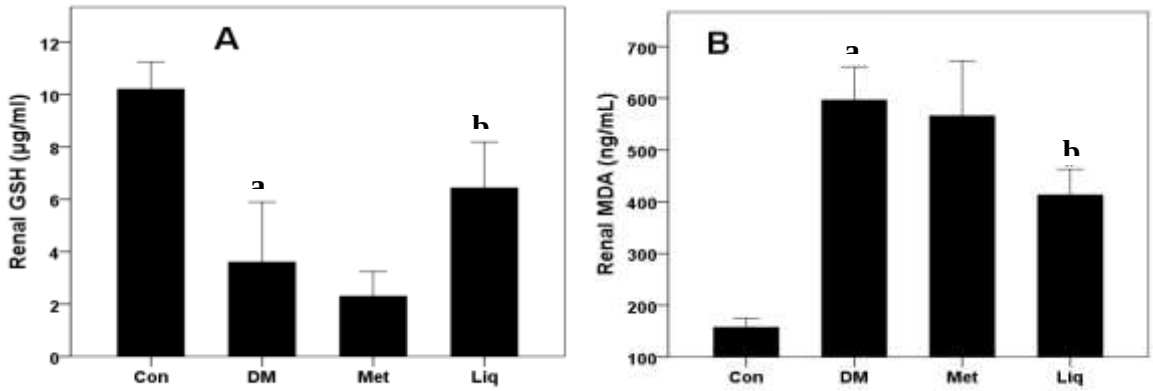

Figure 3. The effect of liquiritin on renal GSH, and MDA (a): Significantly different (P ≤ 0.05) compared with Con group, (b): Significantly different (P<0.05) compared with DM group.

Conclusion

It is concluded from the present study that 15 weeks is a reasonable duration to develop an experiment.

DN with significant functional and histopathological findings. Liquiritin has an ameliorating effect against DN, and it can significantly decrease most of the histopathological changes, oxidative, and inflammatory markers.

Acknowledgment

The author is thankful to Dr. Alfred Sulaka Karomy, College of Agriculture, University of Basrah, for his efforts in the design and formulation of the diet types used in the study.

Conflicts of Interest

The authors declare no conflict of interest.

Funding

No funding source.

Ethics Statements

All experiments were carried out according to the study protocol, which was reviewed by the Institutional Review Board of the College of Medicine/Al-Nahrain University after permission from the scientific committee of the Department of Pharmacology at the College of Medicine/Al-Nahrain University. The best effort was made to reduce the animals' distress and pain throughout all experiment procedures.

Author Contribution

The authors confirm contribution to the paper as follows: study conception and design: Raad Saad Luty, Adeeb Ahmed Al-Zubaidy, and Arif Sami Malik; data collection: Adeeb Ahmed Al-Zubaidy; analysis and interpretation of results: Adeeb Ahmed Al-Zubaidy and Arif Sami Malik; draft manuscript preparation: Raad Saad Luty, Adeeb Ahmed Al-Zubaidy. All authors reviewed the results and approved the final version of the manuscript.

References

1. Tang SCW, Chan GCW, Lai KN. Recent advances in managing and understanding diabetic nephropathy. F1000Research. 2016; 5: 1-9.
2. Warren AM, Knudsen ST, Cooper ME. Diabetic nephropathy: an insight into molecular mechanisms and emerging therapies. Expert Opinion on Therapeutic Targets. 2019; 23(7):579–91.
3. Doshi S, Society AF-CJ of the A. Diagnosis and management of type 2 diabetic kidney disease. Am Soc Nephrol. 2017; 12(8): 1366–1373.
4. Johansen KL, Chertow GM, Gilbertson DT, et al. US Renal Data System 2021 Annual Data Report: Epidemiology of Kidney Disease in the United States. American Journal of Kidney Diseases. 2022;79(4):A8–12.
5. Stoumpos S, Jardine AG, Mark PB. Cardiovascular morbidity and mortality after kidney transplantation. Transplant International. 2015; 28(1):10–21.
6. Herman-Edelstein M, Doi SQ. Pathophysiology of diabetic nephropathy. Proteinuria: Basic Mechanisms, Pathophysiology and Clinical Relevance. 2016; 4:1–65.
7. Navarro-González JF, Mora-Fernández C, De Fuentes MM, García-Pérez J. Inflammatory molecules and pathways in the pathogenesis of diabetic nephropathy. Nature Reviews Nephrology 2011 7:6. 2011;7(6):327–40.
8. Alicic RZ, Johnson EJ, Tuttle KR. Inflammatory Mechanisms as New Biomarkers and Therapeutic Targets for Diabetic Kidney Disease. Advances in Chronic Kidney Disease. 2018; 25(2):181–91.
9. Bhattacharjee N, Barma S, Konwar N, Dewanjee S, Manna P. Mechanistic insight of diabetic nephropathy and its pharmacotherapeutic targets: An update. European Journal of Pharmacology. 2016;791: 8–24.

10. Loeffler I, Wolf G. Mechanisms of interstitial fibrosis in diabetic nephropathy. Diabetic Nephropathy: Pathophysiology and Clinical Aspects. 2018; 227–51.

11. Tuttle KR, Agarwal R, Alpers CE, et al. Molecular mechanisms and therapeutic targets for diabetic kidney disease. Kidney International. 2022;102(2): 248–60.

12. Chen HW, Yang MY, Hung TW, Chang YC, Wang CJ. Nelumbo nucifera leaves extract attenuate the pathological progression of diabetic nephropathy in high-fat diet-fed and streptozotocin-induced diabetic rats. Journal of Food and Drug Analysis. 2019;27(3):736–48.

13. Hu Q, Chen Y, Deng X, et al. Diabetic nephropathy: Focusing on pathological signals, clinical treatment, and dietary regulation. Biomedicine & Pharmacotherapy. 2023;159:114252.

14. Barrera-Chimal J, Jaisser F. Pathophysiologic mechanisms in diabetic kidney disease: A focus on current and future therapeutic targets. Diabetes, Obesity and Metabolism. 2020; 22(S1):16–31.

15. Naaman SC, Bakris GL. Slowing Diabetic Kidney Disease Progression: Where Do We Stand Today? Compendia. 2021; 2021(1): 28–32.

16. Swayeh, Noor Hassoon; Kadhim HM. Effects of iraqi abelmoschus esculentus methanol extract on high fat diet plus streptozotocin induced type 2 diabetes mellitus in rats. Biochemical & Cellular Archives. 2022; 22(1): 1281.

17. researchgatenetAR Abu-Raghif, BJ Qasim, AH Abady, HB Sahib. Effects of aqueous thyme extract against cisplatin induced nephrotoxicity in rabbits. International Journal of Pharmaceutical Sciences Review and Research. 2015; 30(1):190–4.

18. Zangeneh MM, Mohammadi G, Salmani S, Razeghi Tehrani P, Rashidi K, Zangeneh* A. A Comparative Evaluation of Nephroprotective Property of Urtica dioica L. Aqueous Extract and Glibenclamide in Diabetic Mice. Research Journal of Pharmacognosy. 2020; 7(1):31–40.

19. Lin -L ;, Hammes J-H;, Zhang H-P;, et al. Flavonoids in Treatment of Chronic Kidney Disease. Molecules. 2022; 27(7):2365.

20. Wahab S, Annadurai S, Abullais SS, et al. Glycyrrhiza glabra (Licorice): A Comprehensive Review on Its Phytochemistry, Biological Activities, Clinical Evidence and Toxicology. 2021;10(12):2751.

21. Noreen S, Mubarik F, Farooq F, Khan M, Khan AU, Pane YS. Medicinal uses of licorice (Glycyrrhiza glabra L.): A comprehensive review. Open Access Macedonian Journal of Medical Sciences. 2021; 9:668–75.

22. Zhai KF, Duan H, Cui CY, et al. Liquiritin from Glycyrrhiza uralensis Attenuating Rheumatoid Arthritis via Reducing Inflammation, Suppressing Angiogenesis, and Inhibiting MAPK Signaling Pathway. Journal of Agricultural and Food Chemistry. 2019; 67(10):2856–64.

23. Qin J, Chen J, Peng F, et al. Pharmacological activities and pharmacokinetics of liquiritin: A review. Journal of Ethnopharmacology. 2022; 293:115257.

24. Liu L, Tang D, Zhao H, Xin X, Aisa HA. Hypoglycemic effect of the polyphenols rich extract from Rose rugosa Thunb on high fat diet and STZ induced diabetic rats. Journal of Ethnopharmacology. 2017; 200:174–81.

25. Furman BL. Streptozotocin-Induced Diabetic Models in Mice and Rats. 2021; 1: e78.

26. Jia SL, Wu XL, Li XX, et al.Neuroprotective effects of liquiritin on cognitive deficits induced by soluble amyloid-β1–42 oligomers injected into the hippocampus. 2016; 18(12):1186-1199.

27. Aal-Aaboda M, Raghif AR, Almudhafer RH, Hadi NR. Lipopolysaccharide from Rhodobacter spheroids modulate toll-like receptors expression and tissue damage in an animal model of bilateral renal ischemic reperfusion injury. Journal of Medicine and Life. 2022; 15(5):685.

28. Kuttancheri T, Das SK, Shetty MS, Satish S, Bathrenathh B. Renal resistive index as a marker of histopathological damage in diabetic and non-diabetic chronic kidney disease. Egyptian Journal of Radiology and Nuclear Medicine. 2023; 54:159.

29. Wong MG, Group for the A-OC, Perkovic V, et al. Long-term Benefits of Intensive Glucose Control for Preventing End-Stage Kidney Disease: advance-on. Diabetes Care. 2016; 39(5):694–700.

30. Wang X, Gao L, Lin H, et al. Mangiferin prevents diabetic nephropathy progression and protects podocyte function via autophagy in diabetic rat glomeruli. European Journal of Pharmacology. 2018; 824:170–8.

31. Olatunji OJ, Chen H, Zhou Y. Lycium chinense leaves extract ameliorates diabetic nephropathy by suppressing hyperglycemia mediated renal oxidative stress and inflammation. Biomedicine & Pharmacotherapy. 2018; 102:1145–51.

32. Han Y, Xu X, Tang C, et al. Reactive oxygen species promote tubular injury in diabetic nephropathy: The role of the mitochondrial ros-txnip-nlrp3 biological axis. Redox Biology. 2018; 16:32–46.

33. Shahzad K, Bock F, Dong W, et al. Nlrp3-inflammasome activation in non-myeloid-derived cells aggravates diabetic nephropathy. Kidney International. 2015; 87(1):74–84.

34. Lee MJ, Rao YK, Chen K, Lee YC, Chung YS, Tzeng YM. Andrographolide and 14-deoxy-11,12-didehydroandrographolide from Andrographis paniculata attenuate high glucose-induced fibrosis and apoptosis in murine renal mesangeal cell lines. Journal of Ethnopharmacology. 2010; 132(2):497–505.
35. Wang T, Zheng L, Liu X, Zhao M. Sea cucumber (Holothuria nobilis) hydrolysates improve diabetic nephropathy via suppression of oxidative stress in high-fat diet/streptozotocin-induced diabetic rats. Journal of Functional Foods. 2023; 106:105589.
36. Zhang C, Li Q, Lai S, et al. Attenuation of diabetic nephropathy by Sanziguben Granule inhibiting EMT through Nrf2-mediated anti-oxidative effects in streptozotocin (STZ)-induced diabetic rats. Journal of Ethnopharmacology. 2017; 205:207–16.
37. Song W, Wei L, Du Y, Wang Y, Jiang S. Protective effect of ginsenoside metabolite compound K against diabetic nephropathy by inhibiting NLRP3 inflammasome activation and NF-κB/p38 signaling pathway in high-fat diet/streptozotocin-induced diabetic mice. International Immunopharmacology. 2018; 63:227–38.Wang DD, Zhang GY, Chen X, Wei T, Liu CX, Chen C. et al. Sitagliptin ameliorates diabetic nephropathy by blocking TGF-β1/Smad signaling pathway. 2018; 41:2784–92.
38. Gao F, He X, Liang S, et al. Quercetin ameliorates podocyte injury via inhibition of oxidative stress and the TGF-β1/Smad pathway in DN rats. RSC advances. 2018; 8(62):35413–21.

اللكيورتين يخفف من الاعتلال الكلوي السكري في ذكور الفئران المصابة بداء السكري من النوع الثاني بغض النظر عن التأثير المضاد لارتفاع الكلوكوز في الدم

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الخلاصة

يمثل اعتلال الكلية السكري أحد المضاعفات الشائعة والخطيرة لمرض السكري وهو سبب رئيسي للفشل الكلوي. اللكيورتين هو أحد مركبات الفلافونويد الرئيسية الموجودة في نبات العرقسوس والذي ثبت أن له العديد من التأثيرات الدوائية، مثل ضاد الأكسدة، وضاد الالتهاب، ومضادات الأورام، وحماية القلب والأوعية الدموية، وحماية الكبد، وتخفيف التهاب المفاصل الروماتويدي في حيوانات التجارب. بحثت الدراسة الحالية في التأثير الوقائي للكيوريتين ضد اعتلال الكلية السكري في النظام الغذائي عالي الدهون وداء السكر المستحدث بالستربتوزوتسين في هذه الدراسة، تم تحفيز نموذج مرض السكري من النوع ٢ عن طريق تغذية الحيوانات بنظام غذائي عالي الدهون لمدة أربعة أسابيع، بعد ذلك تم تصويم الحيوانات طوال الليل وحقنها بجرعة واحدة من الستربتوزوتسين داخل الصفاق قدرها 30 ملغم / كغم. تم إعطاء اللكيورتين يوميًا بجرعة ٤٠ مجم / كجم لمدة ١٥ أسبوعًا. في نهاية الفترة التجريبية، تم تقييم مستوى السكر في الدم، والهيموجلوبين السكري A1 (HbA1c)، وعلامات مستوى الدهون، ومعلمات وظائف الكلى في الدم، ومؤشر الكلى، ومستويات علامات الالتهابات والأكسدة وهي إنترلوكين -١ بيتا (IL-1β)، والإنترلوكين. تم تحديد -٦ (IL-6)، وعامل نخر الورم ألفا (TNF- α)، وعامل النمو المحول بيتا (TGF-β1)، والجلوتاثيون (GSH)، والمالونديالدهيد (MDA) في المتجانس الكلوي، وتم تحديد تم إجراء الفحص النسيجي المرضي باستخدام صبغة الهيماتوكسيلين والأيوسين، حيث تم حساب التغيرات النسيجية المرضية في أنسجة الكلى بشكل شبه كمي وفق نظام تسجيل أمراض الكلى.

أظهرت مجموعة السيطرة على مرض السكري زيادة كبيرة في معايير التحكم في نسبة السكر في الدم، ومعلمات وظائف الكلى، وأظهرت تغيرات نسيجية مرضية كبيرة مقارنة مع مجموعة السيطرة الطبيعية. كشفت النتائج أن اللكيورتين يحسن بشكل كبير التغيرات النسيجية المرضية بينما لا يؤثر على معايير التحكم في نسبة السكر في الدم مع انخفاض في علامات الالتهاب والإجهاد التأكسدي في جناسة الكلى. اللكيورتين له تأثير مخفف ضد اعتلال الكلية السكري ويمكن أن يقلل بشكل كبير من معظم التغيرات النسيجية المرضية والعلامات المؤكسدة والالتهابية.

الكلمات المفتاحية: : داء السكري، اعتلال الكلية السكري، الفلافونويدات، اللكيورتين ، الإجهاد التأكسدي.