Research Article

Trigonella foenum-graecum Extract's Effectiveness of Improvements in Renal Functions and Histological Profiles in Male Rats with Streptozotocin-Induced Diabetes

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Abstract | Among the most common metabolic endocrine disorders is diabetes mellitus. Nephropathy may be one of the causes of continuous uncontrolled non-insulin-dependent diabetic mellitus (NIDDM). It has been shown that streptozotocin (SZ)-induced oxidative stress damages pancreatic beta cells and results in hyperglycemia in rats, which leads to diabetic nephropathy. The goal of this investigation was to see if *Trigonella foenum-graecum (TFG)* flavonoid extract could protect the kidneys from the effects of (SZ). The goal of the current study was to determine if the ethanolic extract of (TFG) exhibited antioxidant activity against nephropathy resulting from the effects of diabetes due to (SZ). Animals in groups 2, 3, 4, and 5 were employed as experimental groups, whereas rats in group 1 received orally gavages of tap water and served as negative controls. Group 2 developed diabetes as a result of a single intramuscular injection of (SZ) at a dose of 50 mg/kg body weight, In contrast, rats in groups 3 and 4 were given oral gavages of TFG herbal extracts (0.15 g/kg BW) in addition to being kept on an identical experimental protocol to that of the rats in groups 1 and 2, respectively. While group 5's diabetic rats that received orally gavages 3.5 mg/kg of glucophage XR treatment served as positive controls. Treatment with TFG of groups 3 and 4 recorded significantly ($p \geq 0.05$) enhanced of renal functions during blood urea nitrogen (BUN), creatine (CT), and urea (UR) as well as noted hyperactivity of antioxidant enzyme, SOD, and GSHt, while CAT and GPx have remained within the boundaries of the negative and positive control group 1 and 5 respectively. The above results were reflected in the acute histopathological changes of the kidneys in the diabetic animals of group 2, while showed an improvement in the appearance of renal histology in diabetic rats of groups 3 and 4 treated with TFG. The group of rats treated with Glucophage XR had a relatively significant effect on kidney tissue compared to group 3. Fenugreek extract (TFG) extract can ameliorate the effects of the oxidative damages of SZ-induced diabetes.

Keywords | Rats, Histopathology, Herbal medicine, Biochemical, *T. foenum-graecum,* Antioxidant streptozotocin diabetes, Nephropathy

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INTRODUCTION

Trigonelline is found in many foods, including melon, peas, onions, soybeans, and corn, as well as botanical medications, like coffee (Nick *et al.*, 2023). TFG is a component of dietary supplements, which has antihyperlipidemic and hypocholesterolemia properties (Arvindkumar *et al.*, 2012). The World Health Organization (WHO) defines diabetes mellitus long-term disorder brought about by either the body's inadequate

utilization of insulin or an inability to create insulin (Samar *et al*., 2023). Diabetes mellitus, when uncontrolled, causes several microvascular and macrovascular issues (Todd, 2008). Diabetic nephropathy is one of the microvascular disorders that diabetics can have (Alsharidah, 2022). In experimental rat models, streptozotocin (STZ) has been widely utilized to produce diabetes mellitus due to its specific cytotoxicity on pancreatic islet-cells. STZ causes renal damage in diabetic animals as models, persistent hyperglycemia harms renal anatomy and function (Wenting et. al., 2023). The early changes in diabetic nephropathy are characterized by an increase in renal hypertrophy, and renal physiological disorder, followed by increased urine albumin elimination, glomerular sclerosis, tubular fibrosis, and increasing renal insufficiency (Tadahisa *et al*., 2007). Hyperglycemia-mediated oxidative stress may make the tissue more vulnerable to damage caused by oxidative stress and accelerate progression of disease in the renal glomerulus (Patricia *et al*., 2023). Generally, diabetes is usually associated with elevated levels of lipid peroxidation, and according to Klein *et al*. (2015), lipid peroxidation and the induced activity of free radicals in cellular structures are associated with aging, atherosclerosis and late consequences of diabetes (Surapon, 2015). Impaired radical remover function has been linked to decreased efficiency of enzymes (catalase, superoxide dismutase, and glutathione peroxidase) and non-enzymatic free radical scavengers (glutathione) (Ighodaro and Akinloye, 2018). Consequently, the study aimed to make a comprehensive evaluation of functions and antioxidative mechanisms in the kidney. Additionally, we also studied whether alterations in oxidative stress exacerbate kidney damage, leading to the advancement of diabetic nephropathy in this paradigm, as well as the protective effect of TFG extract against early diabetic nephropathy in diabetic animals caused by SZ.

MATERIALS AND METHODS

Plant components and extraction preparation

From the local market were provided fresh and healthful TFG leaves, based on taxonomy standards the sample was identified. Distilled water was used to properly wash the leaves and air-dried in the shade to remove surface water. Initially, 600 ml of ethanol was used to extract 100 grams of powdered material over 24 hours at 24 ± 3˚C, The initial extract was filtered via sterilized filter paper and stored in a sterile conical flask. The same solvent was utilized for the second extraction at 23±2 ˚C for an additional twenty-four hours, and it was filtered. For the solvents to evaporate, the extracts were combined and placed within the bearer of the sample of a rotating flash evaporator. Subsequent usage, the evaporated extract was kept in an airtight bottle at 4˚C. (Okigbo *et al*., 2005).

Experimental design

After taking into consideration the requirements of the ACUC Center for Animal Care, the Scientific Research Ethics Committee of Basrah University's Faculty of Veterinary Medicine gave its approval to the experimental protocols. Male Sprague-Dawley rats, weighing between 225±25 grams were acquired from the experimental laboratory animal house at Basrah university's veterinary medicine college. Every animal experiment was carried out in accordance with globally accepted standards for animal care. The animals were split up into five groups: Seven animals each for the diabetic groups, whereas the negative control group had five rats. Group 1 (negative control): for five weeks, rats were received tap water orally and provided a single intramuscular injection of buffer solution. Group 2 (diabetic control): For five weeks, and according to Venkateswaran and Pari (2003), to cause diabetes, 50 mg/kg body weight of streptozotocin (STZ) was injected intramuscularly along with administered tap water. Group 3: according to Ramya *et al*. (2012), TFG (0.15 g/kg) was received orally administered for 5 weeks, and it followed the identical experimental protocol as the rats in group 1. Group 4: TFG (0.15 g/kg) was administered for 5 weeks on the same experimental regimen as that of rats in group 2. Group 5: according to John *et al*. (2016) diabetic rats receiving glucophage XR drug treatment served as positive controls. The concentrations used in the current study for TGF extract and SZ material did not record any deaths during the duration of the experiment. Rats treated with SZ showed lower activity and a decrease in feed initiation rates compared to rats treated with TFG extract, which showed normal activity similar to the group of control sample rats. After the conclusion of the experimental period, all the rats were fasted overnight and then slaughtered. Using a 10 mL syringe, blood was drawn from the heart and placed in EDTA tubes for plasma collection. Following a 15-minute centrifugation at 3,000 rpm, the serum and blood plasma were separated and then placed into bottles with the appropriate labels. The kidney tissues were removed, washed by the solution of normal saline, blotted on filter paper, and kept at (–80 °C), until analysis was done.

Kidney functions assay

Using the specific kit, the enzymatic kinetic approach was used to measure the amounts of BUN, creatinine, and urea in blood serum. The results were expressed as mg/dL for each of these three parameters (Eda *et al*., 2016).

Enzymatic assessment

Homogenates of hepatic (10% w/v) were prepared in a phosphate buffer solution and then centrifuged at 10,000 g (4 °C) for ten minutes. The resultant supernatant was stored at -80 °C for enzymatic evaluations. The kidney's antioxidant enzymes' effectiveness and activity was assessed

according to the procedure described below: Superoxide dismutase (SOD) activity has been identified, according to (Elizabeth *et al*., 2017), adapted for a micro plate reader operating at (490 nm) and represented as the quantity of protein (µg) required to inhibit 6-hydroxydopamine autoxidation by 50%. The samples total glutathione (GSHt) levels were determined using the Asensi *et al*. (1999) method. By monitoring the breakdown of H_2O_2, the activity of the enzyme catalase (CAT) was measured spectrophotometrically at (240nm) and represented as µmole H_2O_2/min/u g protein (Hilaire *et al*., 2012). While, Ellerby and Bredesden (2000) method was used to measure the glutathione peroxidase (GPx) activity using spectrophotometry at (340 nm) in wavelength, the activity was represented as nmol NADPH/min/ug protein.

Histopathological study

The kidneys of all animals sacrificed at the end of the experiment were dissected and preserved with a 10% neutral buffered formalin solution, following a 72-hour fixation period, the specimens were rinsed under running water for two hours. Subsequently, they underwent dehydration by varying the alcohol concentration from 70% to 100% for two hours at a time. Xylene was used for clearing the specimens, and paraffin wax was applied. Finally, the samples were cut using a microtome at a thickness of 5 um for every tissue. Hematoxylin and eosin stains (H and E) were used for staining (Daniel, 2022). Histopatrhological changes were assessed using a basic grading system and categorized into (-) none, (+) mild changes, (++) strong change, and (+++) very strong changes (Kenneth *et al*., 2018).

Abbreviations

Streptozotocin (SZ), *Trigonella foenum-graecum* (TFG), blood urea nitrogen (UBN), Creatine (CT), Urea (UR), superoxide dismutase (SOD), total glutathion (GSHt), catalase (CAT), glutathione peroxidase (GPx), and nicotinamide adenine dinucleotide phosphate (NADPH).

Analytical statistics

The mean and standard deviation (SD) for all of the parameters were calculated using basic programming techniques in SPSS. If the P-value was higher than 0.05, it was deemed insignificant (Abatan and Olayemi, 2014).

RESULTS AND DISCUSSION

The effect of TFG on BUN, CR, and UR concentrations, displayed in Table 1. SZ-induced diabetic nephropathy resulted in a significant high ($p \leq 0.05$) of BUN, CR, and UR values as compared to data of the 1 and 3 groups. While, administration of TFG of group 4 appeared a significantly restored the normal values of renal function indicators (BUN, CR, and UR). Group 5 shows a significant increase ($p \leq 0.0.5$) in the values of renal function indicators above compared to the 1 and 3 group data.

Antioxidant enzymes

As revealed in Table 2, SZ- induced a significant decrease ($P \leq 0.05$) in the SOD, GSHt, CAT, and GPx enzymes level of kidney tissue in group 2, in comparison to the other groups. Oral doses of TFG extract for 5 weeks created a marked increase in the activities of the enzymes in both 3 and 4 groups compared to other groups, In general, the level of activity of SOD, CAT, and GSHt increased more (15.35 %, 16.21, and 21.80% $P \leq 0.05$), respectively, in diabetic rats treated with 0.15 mg/kg b.w. of TFG extract, than animals treated with glucovans. While, when group 5 animals were compared to those in group 2 as well, there was a rise in their enzyme activity.

Table 1: Illustrates the impact of TFG extract on the BUN, CR, and UR values in negative control group and SZ-induced diabetic groups.

Group	Exposure modality	BUN(mg/dl)	CR (mg/dl)	UR (mg/dl)
1	Control	32.5 ±3.21a	0.67 ±0.024a	21.5 ±2.32a
2	Diabetes (STZ)	86.4±7.55d	4.47 ±0.98d	51.3 ±5.87c
3	TFG	30.4 ±2.87a	0.64 ±0.016a	23.1 ±1.87a
4	TFG + STZ	44.7 ±3.19b	1.98 ±0.15b	28.9 ±2.47b
5	Glucophage XR + (STZ)	59.6 ±7.22c	2.77 ±0.99c	33.4 ±2.91b

The data are displayed as the mean±SD. Different letters among groups are mean significant variations at the likelihood level $P \leq 0.05$.

Table 2: Illustrates the impact of TFG leaf extracts on antioxidant enzymatic and total glutathione activities in the renal tissue of the experimental groups.

Treatment groups	Control	STZ	TFG	TFG + STZ	GV + (STZ)
SOD (units/µ protein)	0.279 ±0.086	0.154 ±0.033	0.314 ±0.063	0.241 ±0.054	0.205 ±0.031
CAT (µmolH2O2/min/µg protein)	0.77 ±0.23	0.71 ±0.26	0.83 ±0.21	0.74 ±0.18	0.62 ±0.15
GPx (nmol NADPH/ min/µg protein)	0.003 ±0.001	0.003 ±0.001	0.0038 ± 0.001	0.003 ±0.001	0.003 ±0.001
GSHt (µmol/g tissue)	4.11 ±0.97	2.62 ±0.21	3.99 ±0.72	3.76 ±0.66	2.97 ±0.77

The values are represented as the mean±SD. Different letters among groups indicate significant differences at the likelihood level $P \leq 0.05$

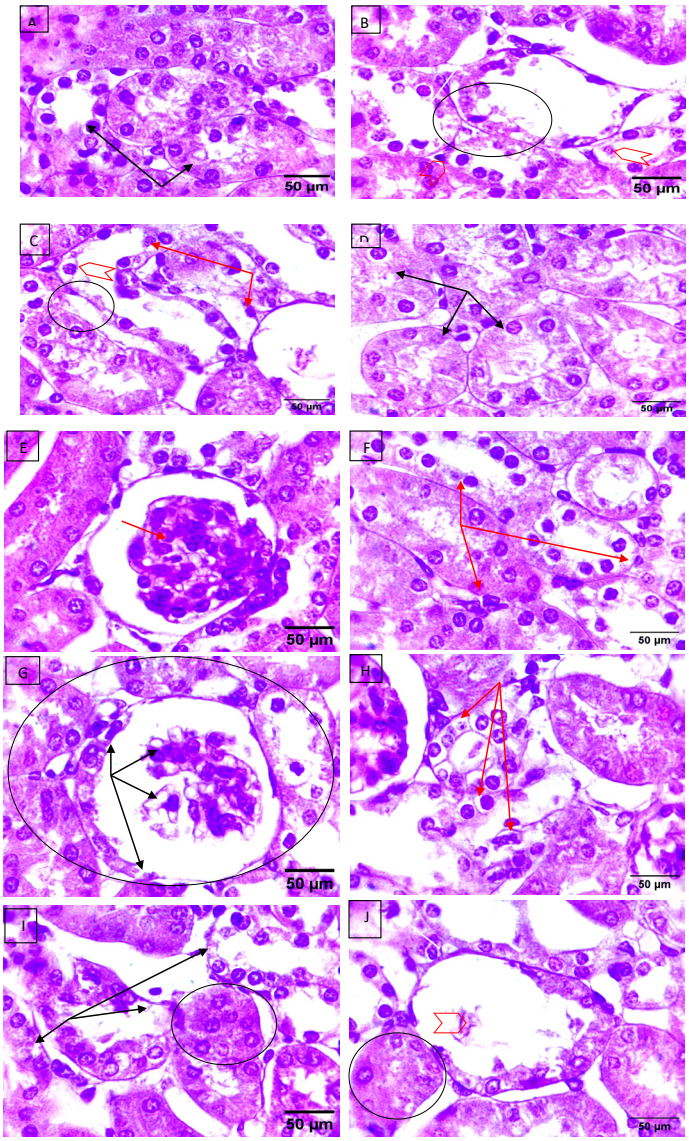

Figure 1: Light micrographs of the rat's kidney sections, (stained with H and E. magnification power 1000X). Group I- A and Group 3- D and E show normal proximal and distal convoluted tubule structures (black arrows) and glomerulus structures (red arrows). Groups 2 B and C, show severe renal injury in the proximal and distal tubules, represented by lumen dilation (black arrows), vacuolization of tubule lining cells (red arrows head), degeneration of some tubules cells (red arrows), and necrosis (circle). Groups 4- F and G show much improvement in the kidney glomerulus and renal tubule structure (circle), except some histological damage was still seen in the proximal tubules (red arrow) and renal corpuscle (black arrows). Group 5- H, I, and J, shows the renal injury was shown loss of glomerulus (arrowhead), renal tubular necrosis (black arrows), cytoplasmic vacuolation (red arrows), and hypertrophy of proximal convoluted lining cells and close of tubules lumen (circle).

Histological analysis

As indicated in Figure 1 and histological signs in Table 3 the rat kidney of the negative control group 1 is shown in (Figure 1A), the typical structure of the interstitial and renal convoluted tubules. Diabetic group 2, cytoplasmic degeneration and necrosis of renal proximal and distal convoluted tubules lining cells, and absence of glomerulus (Figure 1B, C). In animals treated with TFG 0.15 mg/kg b.w. of the extract in groups 3 and 4, the restoration of the normal shape of the renal proximal and distal tubules lining cells and normal renal corpuscle size. The slight cytoplasmic degeneration of renal proximal tubules lining cells was noted with positive group 5.

Table 3: Illustrates the distribution of damage characteristics in the kidneys of the experimental groups.

Histological Signs	Experimental groups				
	1	2	3	4	5
Loss of glomerulus	-	++	-	+	+
Degeneration of PCT and DCT	-	+++	+	+	+
necrosis of PCT and DCT	-	+++	-	+	+
hemorrhage	-	+	-	-	+
Congestion and hyperemia	-	+++	+	+	-
inflammatory cells	-	++	-	-	-
PCT and DCT lumen dilatation	-	+++	+	+	++

(-) none or light pathological changes; (+) moderate pathologic changes; (++) severe pathologic changes; and (+++) extremely severe pathologic changes in the kidney tissue.

The streptozotocin N-[methylnitrocarbamoyl]-D-glucosamine (SZ) was used in the our investigation, which functions as a pancreatic cell's source of nitric oxide and a strong methylating agent for DNA, which as a result, it damages beta cells, causing diabetes. In our study, streptozotocin-induced hyperglycemia in rats is an excellent model of human physiology and histology (Olawale *et al.*, 2021). *In vivo*, SZ produces oxygen radicals that injure the kidney, liver, pancreatic, and hematopoietic systems through oxidative stress effect (Cheng *et al.*, 2018). This ultimately triggers the onset of diabetes and its associated consequences, particularly diabetic nephropathy (Kaur *et al.*, 2017). The damage and weakness of kidney functions associated with diabetes, which were confirmed by the results of our study, were consistent with the interpretation that says: A persistent hyperglycemic condition increases the stress on the glomerulus and complicates the glomerular filtration mechanism. The bioaccumulation of glucose in the glomerulus raises the osmotic pressure, impairs renal cell functions, and leads to glomerular cell necrosis. When renal cells are injured, several chemical factors are carried out into efferent arterioles and released, effectively destroying renal tubule epithelial cells (Vijayaraj *et al.*, 2019). Generally, the most popular method for detecting kidney-related illnesses is to analyze renal specimens, biochemical and histological studies, and important kidney function markers (Severin *et al.*, 2019), this is consistent with the aim of our current study. In the

current investigation, diabetic-positive group rats (group 2) had significantly lower values of SOD, CAT, GPx, and GSHt in rat kidney homogenate than normal group rats, reflecting an imbalance in the antioxidant defense system, these results agreed with the study he conducted by Mahmoodnia *et al*. (2017), during which them confirmed that the high quantities of reactive oxygen species (ROS) are generated in response to hyperglycemia as a result of the disruption of mitochondrial oxidative phosphorylation and the activation of many enzymatic and non-enzymatic pathways. Generally, there are four types of antioxidant defense systems against oxidative stress: physical defenses, antioxidant, preventative, and repair mechanisms (Peter *et al*., 2019). The main objective of our current study was to determine the effectiveness of *T. foenum-graecum* extract's in controlling the diabetes nephropathy system and improving its functions. Furthermore, Kaur *et al*. (2017) showed that the compounds of flavonoids reduced the severity of glycation accumulation in SZ-induced diabetes in rats, as evidenced by a decrease in BUN, UR and CR levels in the diabetic rats that received the proper dosage of TFG. as well as oral dosage of the ethanol TFG extract was caused for improved SOD, catalase, GPx, and GSHt enzymes level in TFG, and TFG-STZ group rats' kidney homogenates compared to the glucophage XR -SZ group and control group. Generally, in diabetic rats, flavonoid compounds were found to dramatically increase total renal GSHt content as well as the mRNA and total antioxidant enzymes CAT and SOD (Sarah *et al*., 2023), also TFG leaves contain phenolic substances that may act as antioxidants (Neffe-Skocinska *et al*., 2023). The flavonoid compounds in TFG leaf extract, act the generation of nitric oxide by inhibiting the transcription of the iNOS gene in several tissues, as well as directly scavenging free radicals (Bayan *et al*., 2018). Also, the flavonoids may protect against oxidative stress through a variety of mechanisms, including the active scavenging of free harmful radicals, preventing of the activity and interplay with several enzymatic systems (Annia, 2015). One noteworthy finding from our investigation was that TFG demonstrated independent antioxidant activity by positively affecting SOD, GPx, CAT, and GSHt in the TFG group as well. The histology analysis in this study revealed improved renal glomerulus and tubules in treatment groups of TFG extract in comparison to all groups. The extract dosage led to a reduction in damage areas and injury indicators in all group. Our results showed degeneration and necrosis of the diabetic rats' kidneys were observed similarly to the observation of Raval *et al*. (2018), similar outcomes in diabetic animals injected with SZ were documented also in (Xiao-Xuan *et al*., 2018). Also, the groups treated with fenugreek leaf extract showed a noticeable improvement in the histological aspect of the kidney, and this was confirmed Rina *et al*. (2021) the rats suffering from nephropathy as a result of treatment with alloxan and underwent treatment with doses of flavonoid extract, appeared a improved their kidney tissue.

CONCLUSIONS AND RECOMMENDATIONS

In summary, our findings indicate that *T. foenum graecum* leaf extract has strong antidiabetic properties. It was observed to be beneficial in lowering BUN, CR, and UR levels and thereby restoring function of renal, which was compromised in SZ-induced diabetic rats. Furthermore, as evidenced by the typical BUN, CR, and UR values in the extract-fed non-diabetic animals, no adverse effects were observed. TFG extract has been demonstrated that the extract promote renal histological recovery and regeneration. Alongside the renal function state, the TFG extract demonstrated significant antioxidative capacity and improved the antioxidant status of diabetic animals. According to the results of our investigation, TFG leaves may be used to treat diabetes. However, further research is needed to identify the precise composition of the active components and how they function.

Conducting more in-depth studies on the possibility of using fenugreek as a natural herbal treatment to help control diabetes of all types.

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NOVELTY STATEMENT

Our study supported the possibility of using T. foenum-graecum extract and the antioxidants, it contains to preserve kidney functions as much as possible for people with diabetic nephropathy without any side effects of the extact used.

AUTHOR'S CONTRIBUTION

EAH: Writing and analyzing the physiological results of the research as well as analyzing and interpreting biochemical results. MFM: Design and analysis of experiments, as well as analysis of data statistically and interpret histopathological results.

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Conflict of interest

The researchers declare that there are no conflicts of interest regarding this manuscript. The style of writing and subject matter of the article are the sole responsibility of the authors.

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