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Histopathological Changes In Diabetic Nephropathy Treated With Fenugreek In Streptozotocin Induced Rat Models

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ABSTARCT:
Background: DN is the primary cause of kidney diseases in patients requiring renal replacement therapy, with approximately 40% of these patients also experiencing diabetes. Research proves that fenugreek seed extractions possess antioxidant, hypocholesterolemic, and antidiabetic properties. This study was conducted to observe effect of fenugreek on Streptozotocin induced diabetic nephropathy and also to compare histopathological studies **Methods:** This is an experimental study conducted among 12 rats (4 control rats, 4 diabetic rats, 4 diabetic rats treated with fenugreek) in an animal house belonging to pharmacology department of a tertiary care medical college situated in South India. Blood glucose control was assessed using serum blood glucose and HbA1c concentrations. The renal function was studied using dimethyl formamide (DMF), 24 h urinary albumin excretion (albuminuria), Blood Urine Nitrogen, Serum Creatinine and Kidney Index **Results:** There was significant difference in blood glucose levels, HbA1c concentration and renal function parameters among diabetic rats treated with fenugreek between both control group and diabetic control groups. PAS positive staining and glomerular was significantly low among DN Fenugreek group microscopy signifying the prevention of ECM accumulation when compared to both control and DN control group. Conclusion: Our study proves both protective effect as well as the significant histopathological changes of fenugreek on diabetic nephropathy.

Introduction:

Diabetes mellitus is a prevalent medical condition that has become a significant public health

challenge of the twenty-first century, despite its potential for catastrophic consequences.[1] Both developed and developing countries worldwide are experiencing a rapid rise in the number of patients diagnosed with type 2 diabetes. Macrovascular conditions, such as peripheral arterial disease, stroke, and coronary heart disease, and microvascular conditions, including diabetic nephropathy , retinopathy, and peripheral neuropathy, are complications that have historically been linked to diabetes mellitus.[2] Diabetes is a life-threatening condition due to its significant increase in the risk of cardiovascular complications, including hypertension, myocardial infarction, coronary artery disease, and dyslipidemia Diabetic nephropathy (DN) is one of the most prevalent complications among diabetic patients, as the cardiovascular injury primarily affects the eye and kidney.[3] Diabetic nephropathy (DN) is also referred to as diabetic kidney disease (DKD). It is the chronic impairment of kidney function that occurs in individuals with diabetes mellitus.[4]

DN is the primary cause of kidney diseases in patients requiring renal replacement therapy, with approximately 40% of these patients also experiencing diabetes. implying that diabetes mellitus is one of the most prevalent causes of nephropathy. The risk of mortality is elevated by DN.[5] Microalbuminuria (UAE 20>g/min and ≤199 μg/min) and macroalbuminuria (UAE ≥200 μg/min) are the two stages into which the DN can be divided.2 The glomeruli of the kidney are damaged by the reactive oxygen species produced in the diabetic patient, resulting in albuminuria. The presence of proteinuria exceeding 0.5 g/24 h has been used to characterize diabetic nephropathy. The glomerular filtration barrier (GFB), a structure in the glomeruli, is progressively damaged as diabetic nephropathy advances. The onset of DKD in the diabetic patient is indicated by the excretion of urinary albumin. The progression of DKD is from normal albuminuria to micro and macro albuminuria. In the initial stages, where microalbuminuria is present, the DKD is clinically detectable and can even be reversed.[6]

DN is histologically defined by the accumulation of extracellular matrix (ECM) in the interstium and glomerulus. The renal dysfunction and morphological changes of the glomerulus have been regarded as critical due to the ECM accumulation and subsequent fibrosis in diabetic kidneys, despite the fact that the mechanisms underlying DNdevelopment have not been fully elucidated. [7] In diabetic patients and animal models, renal dysfunction and ECM accumulation are consistently associated with severe oxidative stress in the diabetic kidney. Additionally, the pathological alterations in the glomeruli and albuminuria in diabetic rats are reduced by treatments with antioxidants, including vitamin E, taurine, or lipoic acid. [8] It has been discovered that the oxidative stress induced by hyperglycemia exacerbated the ECM accumulation of diabetic kidneys by upregulating TGF-β1. This is due to the fact that the increased TGF-β1 could stimulate the production of ECM and reduce its degradation.[9,10] There is an additional mediator, CTGF, who transmits signals received from TGF-β1 to promote renal fibrosis and ECM synthesis during the development of DN. Consequently, it may be advantageous to pursue an agent that can mitigate renal oxidative stress and obstruct the TGF-β1/CTGF signaling pathway in the context of DN therapy.[11]

Fenugreek (Trigonella foenum-graecum) is a condiment, a supplement to wheat and maize flour for breadmaking, and a component of the daily diet of the general population in numerous countries. [12] At present, a variety of studies conducted on both animals and humans have demonstrated that fenugreek seed extractions possess antioxidant, hypocholesterolemic, and antidiabetic properties. Consequently, fenugreek is an optimal choice for DN therapy. The pharmacological characteristics of fenugreek have been demonstrated in diabetes mellitus research through the detection of its functions on peripheral glucose utilization, insulin secretagogue actions, and the effect on the gumfiber in the intestines.[13-15] In the current investigation, our objective is to investigate the potential protective effects of fenugreek on the kidney in diabetic rats that have been induced with streptozotocin (STZ) and also

to compare the histopathological changes

Methods:

This is an experimental study conducted in animal research centre associated with department of pharmacology belonging to Dhanalakshmi Srinivasan medical college and hospital situated in South India. The study duration was months between November 2022 and January 2023. The experiment was conducted after getting the approval from Institutional Animal Ethics committee (IAEC) of the study setting. It was ensured that this study was conducted as per CPCSEA guidelines.[16]

Drug. Fenugreek seed is a commonly used condiment and easily available in India. Fenugreek seeds were purchased from National institute of Siddha, Tambaram, Chennai. These seeds were ground to powder. This grounded powder weighing 200g was put into 2 liters distilled water and boiled for 30 minutes to obtain the decoction. Then this decoction was cooled for 1 hour at room temperature and filtered twice through a coarse sieve to get the filtrate.

Animals. Wistar albino (8 weeks old, male sex, each weighing 180-200 gm) Rats were obtained from Mass Biotech, Chengalpattu, Tamilnadu and were housed in pathogen free facility in Animal house, belonging to the study setting. The animal house had free access to standard diet and water throughout the whole study period.

Induction of diabetes and fenugreek treatment: The rats were acclimatized for 7 days. Intraperitoneal injection of 60 mg/kg streptozotocin (Sisco Research Laboratories Pvt. Ltd, Chennai) in citrate solution (0.1 M citric acid and 0.2 M sodium phosphate, pH 4.5 was administered to all rats after 12 hours fasting. Following this (after 3 days), blood glucose levels were detected using One-Touch strip. Rats with blood glucose concentrations above 250mg/dl are considered as diabetic. A week later, 4 diabetic rats were randomly administered fenugreek (9 g seed powder/kg), daily for 12 weeks (DNF group) and 4 diabetic rats were treated with vehicle control for 12 weeks (DN group). 4 Normal rats as control (Control group), were administered citrate buffer alone and treated with vehicle control for 12 weeks.

Measurement of Blood Glucose and Renal Function: Blood glucose & HBA1c will be measured using one touch test strips & chromatography method respectively**. For** Renal function tests**,** dimethyl formamide (DMF) and 24 h urinary albumin excretion (albuminuria) will be determined by kit Blood Urine Nitrogen & Serum Creatinine will be measured using autoanalyzer. Kidney Index calculated as per the formula – 1000 × kidney weight / body weight

Histopathological examination: Microscopic changes in kidney were studied using light microscope as follows. One portion of kidney tissue is fixed in 10% buffered formalin and embedded in paraffin. The kidney tissue sections were stained with periodic acid-Schiff reagent (PAS) and examined under a light microscope[4] (Olympus). The degrees of mesangial matrix expansion in different groups of rats were determined as PAS-positive staining in the mesangial region excluding cellular elements. The following parameters were also checked in histopathological studies Glomerular capillary wall thickening, Kimmelstiel Wison nodule, Glomerulosclerosis, Tubular atrophy, Interstitial fibrosis, Interstitial inflammation, Arteriolar hyalinosis and Arteriosclerosis

Measurement of Blood Glucose and Renal Function. The rats were sacrificed at 12 weeks after STZ injection and their kidney tissues were dissected and weighed. The kidney index (KI) was calculated as 1000 × kidney weight/body weight. Blood glucose, hemoglobin A1c (HbA1c), dimethyl formamide (DMF), and 24 h urinary albumin excretion (albuminuria) were determined. All kits were used in accordance with the manufacturer's instructions. Blood urine nitrogen (BUN) and serum creatinine (Scr) concentrations were measured.

Results:

A total of 12 rats were included in the study (4 control rats, 4 diabetic rats, 4 diabetic rats treated with fenugreek). Table 1 shows that fenugreek Reduced Blood Glucose Levels and Improved Renal Functions of Diabetic Rats. In the present study, diabetic rat model with blood glucose level ≥13.9 mmol/L was successfully established by intra peritoneal injection of STZ. The mean ± SD of blood glucose concentration in the Diabetic nephropathy group (DN) was 18.04+3.77 mmol/L which is nearly thrice than that of in the Control group (6.16+1.41). By contrast, the elevated blood glucose level of diabetic rats treated with fenugreek (DNF group) was evidently decreased following fenugreek treatment ($P < 0.05$), which demonstrated that fenugreek could reduce blood glucose levels of the diabetic rats. Similar differences were observed in HbA1c levels as shown in table 1.

To examine the renal injury and dysfunction induced by STZ injection, and whether fenugreek could improve renal function of diabetic rats or not, indicators of renal function were detected, including albuminuria, HbA1c, DMF, BUN, Scr, and KI. As a result, each of the indicators was significantly elevated in the DN group compared with the Con group ($P < 0.05$, resp.). as shown in table 2. These data further indicated that the STZ-induced DN rat model had been successfully established, and the diabetic rats had suffered from renal dysfunction. While, when fenugreek treatment was performed in the DNF group, each of the elevated parameters decreased strikingly to a normal range compared with the DN group. Therefore, these findings demonstrated that the STZ-induced diabetic rats were subjected to renal injury and dysfunction and that fenugreek exerts a protective effect on kidneys of the diabetic rats

Fenugreek mitigated Glomerular Morphological Alterations of Diabetic Rats. Three typical alterations were detected through light microscope in the glomeruli of diabetic rats in DN group, including segmental thickening of glomerular basement membranes, widely fused foot processes podocytes, and excessively deposited mesangial matrix, while these ultra structural abnormalities were evidently prevented by fenugreek in DNF group-Figure 1(a) Therefore, the findings demonstrated that fenugreek could attenuate morphological alterations by inhibiting the ECM accumulation in glomeruli of diabetic rats. Also, PAS staining positive cells were significantly low among DNF group on comparison with DN control group as shown in table 3..In our further investigations, glomerular volume was measured under electron microscopy and quantified to be as the measure index of glomerular hypertrophy. As a result, glomerular volume was significantly enlarged in DN group versus Con group and distinctly reduced by fenugreek treatment in DF group versus DN group as shown in fig-1(b). This suggested that fenugreek could evidently mitigate glomerular hypertrophy of diabetic rats

Table 1- Blood glucose parameters among the study subjects

Parameters	Control group (n=4)	STZ induced Diabetic nephropathy(n=4)	Fenugreek treated diabetic group (n=4)
Blood glucose (mg/dl)	110.88±25.38	324.72±67.86*	134.1±46.44#
HbA1c(%)	6.35±1.46	12.54±4.23*	8.18±1.34#

*p value <0.05 for DN versus Control, # p value < 0.05 for DNF versus DN

Table 2- Renal function parameters of the study subjects

Parameters	Control group (n=4)	STZ induced Diabetic nephropathy(n=4)	Fenugreek treated diabetic group (n=4)
Albuminuria (mg/day)	7.22±3.19	17.86±4.7*	10.01±2.89#
BUN(mmol/L)	118.8±39.78	318.06±44.1*	169.92±31.68#
DMF(mmol/L)	31.86±4.14	51.84±9.54*	36.54±2.52#
Scr(umol/L)	449.64±49.68	916.02±89.82*	447.66±48.06#
KI(g/g)	2.67±0.54	5.98±0.38*	4.59±0.36#

*p value <0.05 for DN versus Control, # p value < 0.05 for DNF versus DN

Fig 1- PAS staining and glomerular volume of kidney samples among study subjects

Control (n=4)	Diabetic rats (n=4)	Diabetic rats treated with fenugreek(n=4)
Fig 1-a) PAS staining under a light microscope (×400).		

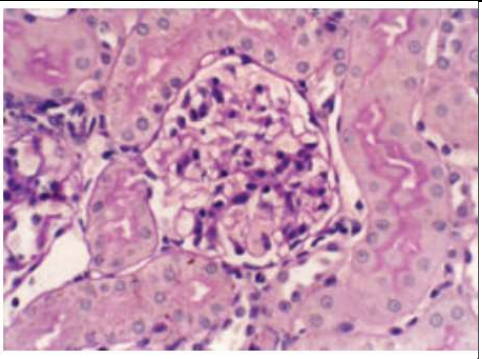
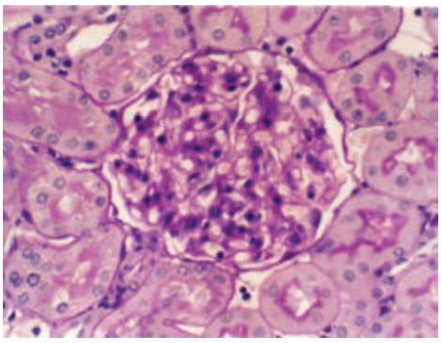
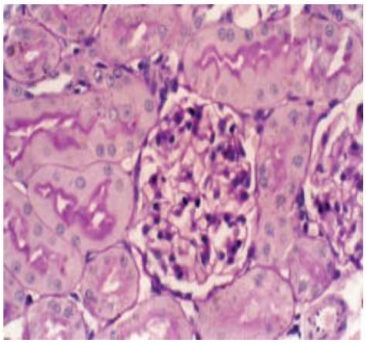

		

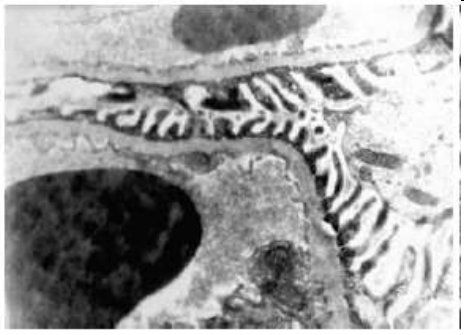
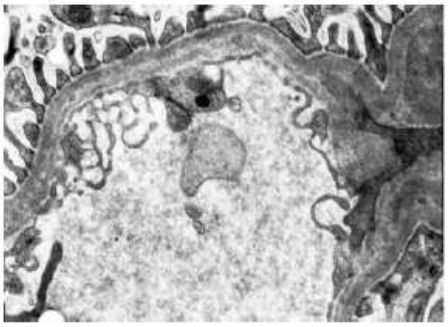
Fig 1.b) Glomerular volume under electron microscopy		
		

TABLE 3- PAS STAINING POSITIVE CELLS AMONG THE STUDY SUBJECTS

Parameters	Control group (n=4)	STZ induced Diabetic nephropathy(n=4)	Fenugreek treated diabetic group (n=4)	P value
PAS positive cells	7.5 ±2.3	30±6.2	15±3.1	<0.01

Discussion:

This study was conducted to compare the protective effect of fenugreek on diabetic nephropathy and to compare the histopathological changes of fenugreek in the diabetic nephropathy with control group. DN is a significant cause of end-stage renal failure worldwide, as it is one of the major complications of diabetes mellitus. Typical morphological changes in the DN kidney include glomerular basement membrane (GBM) thickening, mesangial expansion, broadening and effacement of podocyte foot processes, glomerular hypertrophy, glomerulosclerosis, and tubule-interstitial fibrosis. The glomeruli and interstitium of diabetic kidneys are primarily responsible for these alterations in the ECM accumulation. [17-19] In the present study, oxidative stress induced by hyperglycemia was regarded as the promoter of DN development, as indicated by the results of previous research. The TGF-β1/CTGF pathway was used to deliver signals induced by oxidative stress, which subsequently activated ECM accumulation and the subsequent morphological alterations. Ultimately, these changes led to the development of DN and renal dysfunction in diabetic rats. Fenugreek was chosen as a potential drug against DN due to its nontoxicity and antioxidant properties. The present study was conducted to investigate the protective effect of fenugreek against DN development and to provide evidence for the hypothesis that fenugreek may reduce DN risk by alleviating renal oxidative stress and inhibiting the TGF-β1/CTGF signaling pathway. The hypothesis was preliminarily confirmed.

The STZ-induced diabetic rat model has been extensively employed in the study of DN. The rat model of diabetes was successfully established in the current study in accordance with the previous description. In general, renal dysfunction and elevated urinary protein levels are the functional characteristics of DN. The DN group demonstrated a substantial increase in albuminuria, HbA1c, DMF, BUN, Scr, and KI in comparison to the Con group in the current study (Table 1). This demonstrated that the diabetic rats induced by STZ had experienced renal dysfunction. Renal dysfunction is typically the result of structural/cellular changes in the DN kidney, such as the accumulation of ECM, the thickening of the GBM, the deposition of mesangial cell matrix, and the effacement of podocyte foot processes.[10,20] The DN group exhibited the morphological changes previously mentioned in the present study. Most significantly, they were evidently attenuated by fenugreek treatment (F), which was accompanied by an improvement in renal function. Consequently, it is inferred that renal dysfunction is linked to renal morphological changes, and fenugreek may mitigate the risk of renal dysfunction by preventing injuries to the structural/cellular basis. The above mentioned findings are comparable to similar experiments done by Qiu D, Gupta AK et al and Kalaf AY et al in which also proved the effect of fenugreek on blood glucose , renal function , ECM accumulation as well as glomerular volume proving it to be protective and could be widely used for the treatment of diabetic nephropathy.

Conclusion:

The protective effect of fenugreek against the development and progression of DN is confirmed by the results of the present study. In addition, this research also confirms the positive histo-patholgical changes observed in the diabetic rats treated with fenugreek. However further exploration is needed in terms of exploring other positive as well as the negative effects which could be helpful in the future clinical trials.

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