Research Article

Extraction and evaluation of *Trigonella foenum-graecum* L. seeds extract for attenuating the progression of nephropathy in diabetic rats

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Abstract

Objective: Many plants are predominantly used to treat diabetes and have been the main source for various synthetic compounds. Literature suggests that not much work has been done using *Trigonella foenum-graecum* plant extracts to check their efficacy in attenuating diabetic nephropathy. So, the objective of present study is to evaluate the effect of the extracts of plant seeds in attenuating the progression of diabetic nephropathy in animal models. **Material and methods:** Diabetic nephropathy was induced in male albino rat by diabetogenic diet and streptozotocin (65 mg/kg b.wt.,i.p.). On the 9th week of the induction of diabetic nephropathy in animals, treatment was given for 40 days to attenuate the condition of diabetic nephropathy in induced rats. Animals were treated with methanolic extract of *Trigonella foenum-graecum* Linn. (METFG) at two dose levels (500 mg/kg and 1 g/kg) and with standard drug Glimipride (2mg/kg) for 40th day in order to analyse its renoprotective effect, which was evaluated by means of metabolic profile, renal function test and electrolyte concentration in blood and urine and renal tissue test with its antioxidant status and histopathological study. **Results and conclusion:** Nephropathy was noted in diabetic induced rats between 8th-9thweeks as assessed by exhibited statistical significant polyuria and increased in serum glucose, creatinine, blood urea nitrogen levels and albuminuria and proteinuria. Nodular glomerularsclerosis, hyaline arteriolosclerosis with chronicglomerulonephritis(GN) was observed by renal morphological study. On 40 days of treatment of METFG; metabolic profile including the renal functions were greatly improved as evidenced by amelioration of creatinine, blood urea nitrogen, total protein concentration, albumin and serum electrolytes and also improvement in antioxidant with decreased in malondialdehyde and increased in catalase, superoxide dismutase and glutathione-s-transferase levels. It ameliorated renal hypertrophy, nodular glomerularsclerosis, hyaline arteriolosclerosis and GN in diabetic nephropathy rats. These studies suggest that *Trigonella foenum-graecum* Linn seed possess a protective effect and attenuates the progression of diabetic nephropathy in rat.

Keywords: *Trigonella foenum-graecum* Linn*,* Diabetic nephropathy, renal function

Introduction

Chronic diabetes is associated with various microvascular complications like nephropathy, neuropathy, cardiomyopathy and retinopathy (Sjoquistet al., 1998). Nephropathy is defined as the loss of functions of kidney associated with nephrotic syndrome, glomerulosclerosis, type IV renal tubular acidosis, persistant albuminuria, declining glomerular filtration rate (GFR). It is associated with risk factors such as high blood glucose, elevated cholesterol levels and proteinuria with the remaining renal function is insufficient to support life (Singh et al*.,* 2006).

Different mechanisms are responsible for induction of nephropathy in diabetes (Blickle et al., 2007). Literature review has shown that lipid deposition in the kidneys play an important role in the pathogenesis of diabetic nephropathy in experimental animals, as well as in diabetic patients (Srividya et al., 2010). From the recent studies indicate that reactive oxygen species (ROS) play a key intermediated role in the pathophysiology of DN (Kumari et al., 2003). Chronic hyperglycemia and progression of DN not only generates more reactive oxygen metabolites but also attenuates an antioxidative mechanism through non-enzymatic

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glycosylation of the antioxidant (Abu et al., 2010).

Trigonella foenum-graecum Linn. (Leguminosae) have a record of safety and efficacy, spanning several centuries. It is used in folklore medicine for the treatment of various diseases in the different states of India and also by alternative medical practitioners for an array of diseases. The plant is used traditionally as a carminative, demulcent, expectorant, laxative and stomachic. The fiber content of fenugreek extract is used for metabolism of glucose in the digestive tract. It is Indian herbal medicine which has been widely used in the diabetes mellitus. The folk of Chotanagpur region in Jharkhand use these plants indigenously mostly for the treatment of diabetes, but so far there is no report regarding their efficacy in attenuating diabetic nephropathy. Hence, the objective of the present study is to evaluate the antihyperglycemic, antihyperlipidemic, antioxidant and renal function protecting effect of *Trigonella foenum-graecum* for attenuating the progression of nephropathy in streptozotocin induced diabetic animals.

Materials and methods

Plant materials

The dried seeds of *T. foenum-graecum* were purchased from the local market of Mesra, Ranchi. The plant material was taxonomically identified and authenticated by K. Karthikeyan, Scientitst 'C', Botanical Survey of India (BSI), Central National Herbarium, Howrah, with ref. no. CNH/103/2011/Tech-II/620. The voucher specimen was deposited in the herbarium section of B.I.T., Mesra, Ranchi, Department of Pharmaceutical Sciences and Technology.

Preparation of extracts

The dried seeds of *T. foenum-graecum* were cleaned and made into coarse powder, passed through a 1-mm sieve and again dried in hot air oven below 50^0C and stored at room temperature in an air tight container. The coarsely powdered material were subjected to successive soxhlet extraction using solvent petroleum ether, chloroform and methanol in the increasing order of their polarity for 48 hours with each solvent (Harborne, 1986).The obtained seed extracts were finally dried at low temperature under reduced pressure in a rotavapor (Buchi Lab ortechnik AG, CH-9230 Flawil 1/Switzerland) to obtain a semi-solid mass and then finally lyophilized by freeze dryer (MPS-55, Korea) (Kokate et al., 2004). The yield of the PEETFG, CETFG and METFG were found to be 1.5, 2.0 and 19.0 % w/w respectively and METFG selected for in vivo animal models for attenuating the diabetic nephropathy in rat.

Preparation of test samples

The dried METFG as well as standard drug Glimipride was suspended in 1% carboxymethyl cellulose (CMC) in distilled water prior to oral admintration to the animals. Glimipride IP was received as a gift sample from Aristo Pharmaceutical Pvt. Ltd., (Rajsen, Madhya Pradesh).

Acute toxicity study

Acute toxicity study was carried out for the METFG following OECD 423 guidelines. METFG was suspended in 1% w/v CMC and was given at a dose of upto to 2000mg/kg P.O. body weight to overnight fasted, healthy mice (n=6). Then the animals were observed for mortality and morbidity for 24 hours. Morbidity like pupil dilatation, tremors, grip strength and convulsions were observed. The animals were observed for 14 days daily and animal body weight was taken (Arulselvan and Subramanian, 2007).

Experimental design and preparation of diabetic nephropathic animals

The inbred animals were housed in standard polypropylene cages and maintained under controlled room temperature (22 ± 2^0C) and relative humidity (55±5%) with 12:12 hour light and dark cycle. All the animals were initiated with commercially available pellet diet (Foster Biotech. India, Ltd. Ambala) and water *ad libitum.* The guidelines were followed for conducting the experiment of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) of the Govt. of India were followed and prior permission and clearance were granted from the Institutional Animal Ethics Committee (BIT/PH/IAEC/32/2011, Dated: 10/12/2011). Diabetic Model with diabetic nephropathy was successfully established after trial and error by diabetogenic diet given for 7 days before the administration of STZ at dose of 65 mg/kg b.wt. i.p. once selected on the basis of middle dose as per the method of Davis *et al*, (2003). The streptozotocin injected i.p. to animals were then given 5% w/v glucose solution for 5-6 hours following the injection to prevent initial drug induced hypoglycaemic mortality. After 72 hours of streptozotocin injection, blood sugar of animals was estimated by Raptis method (Raptis and Viberti, 2001) and animals having fasting blood sugar above 200 mg/dl were considered to be diabetic. After 3^{rd}, 6^{th} and 9^{th} week rats were estimated for the induction of diabetic nephropathy using different renal function test i.e. blood urea nitrogen, creatinine, albuminuria and total protein including blood glucose and body weight (Abu et al., 2010; Muhammad et al., 2007). Nephropathy was noted in diabetic rats between 8-9 week after the administration of STZ (65 mg/kg b.wt, i.p, once) as assessed in terms of above mentioned renal function test and showed a typical increase in blood glucose, kidney dysfunction: proteinuria and glomerular filtration rate which were similar as that of the reported studies (Cooper *et al*, 1988; Davis *et al*, 2003; Pitchal *et al*,

2008). Then animals were included for the treatment. Then biochemical and pharmacological studies were carried out on 0^{th}, 10^{th}, 20^{th}, 30^{th} and 40^{th} day of the experiment.

The animals were divided in the following groups:

Group I- Normal animals received saline and CMC daily, p.o., and served as normal control (NC).

Group II - Diabetic nephropathic animals received saline daily, p.o., and served as a diabetic nephropathic control (DN).

Group III - Diabetic nephropathic animals received 2mg/kg b.wt ,p.o.,of Glimiprideand served as standard group (TS).

Group IV: Diabetic nephropathic animals received METFGat a dose of 500 mg/kg, p.o., and served as test group (T1TFG).

Group V: Diabetic nephropathic animals received METFGat a dose of 1 g/kg, p.o., and served as test group (T2TFG).

The TFG seed extract was suspended and diluted in distilled water using 1% w/v CMC and given orally p.o. everyday starting from 0^{th} to 40^{th} day after the induction of diabetic nephropathic in rat by force-feeding. After randomization, then rats were acclimatized for a period of 7 days before starting of experiment.

Sample collection

Fasting blood sample of animal was collected by retro-orbitally from the eye under light ether anaesthesia using capillary tubes (Micro Hematocrit capillaries, Mucaps). Serum and plasma sample was separated in a cold centrifuge (Remi, C-24 BL) at 2000 rpm for 10 minute. The serum was estimated for glucose, creatinine and urea nitrogen levels. The urine sample of animals was collected by keeping the animal in metabolic cages for 24 hours with proper access to drinking water and food, and then urine estimated for clearance of creatinine and urea.

Biochemical analysis

Serum and urine biochemical analysis

Serum was collected from all the groups and analyzed for metabolic profile including: fasting blood sugar by glucose oxidase-peroxidase method (Ratliff and Hall, 1973; Varley, 1976). The renal function test were determined by other biochemical parameters such as blood urea nitrogen (BUN) based on modified berthelot method, serum creatinine (SCr) based on Jaffe's kinetic method, total protein in urine (TP) by Lowry method, creatinine clearance (CrCl) and urea clearance (UrCl) were determined for measuring glomerular filtration rate (Jewett SL and Rocklin 1993; Nilufer et al, 2006). On the 40^{th} day of treatment, serum electrolytes concentrations (sodium, potassium, chloride, calcium and magnesium) were assayed respectively (Kakkar et al., 1997).

Glycosylated hemoglobin

Upon termination of the treatment studies, animals were anesthetized, blood was obtained from the bifurcation of the aorta for estimation of glycosylated hemoglobin by ion exchange resin method (Yu et al., 2010). The kits used for determination were obtained from Span diagnostics Ltd. Sachin, Surat, India.

Kidney hypertrophy, renal enzymes status and histopathological studies

After 40 days of treatment, then animals were sacrificed by exsanguinations. The animal kidneys were isolated and their fresh weight was determined gravimetrically and the degree of renal hypertrophy (RH) was expressed as the ratio of the weight of the two kidneys to total body weight of animals (Eliza et al., 2010). Then other animal kidney was kept at -20°C and subsequently homogenized in cold potassium phosphate buffer (0.05 M, pH 7.4). Renal cortical homogenates were centrifuged at 5000 rpm for 10-15 minutes at 40°C. The resulting supernatant was used for renal enzyme assays by malondialdehyde (MDA), superoxide dismutase (SOD), catalase and glutathione s-transferase activities (GST)(Matsumoto et al., 2007). The second kidney was fixed in 10% formal saline for renal histopathological examination (Kumari et al., 2016).

Statistical analysis

The significant differences were analyzed using analysis of variance followed by Tukey's multiple comparison test (TMCT). All the values were expressed as Mean ± SEM using graph pad prism software (Trial version 5.01).

Results and discussion

Acute toxicity study

Acute toxicity study revealed no mortality or any toxic reactions with METFG even at the highest dose 2000mg/kg b.wt., p.o.. There were no lethal effects seen in any of an animal of groups. Therefore for the biological evaluation of METFG, two doses namelyT1TFG a low dose ($1/4^{th}$ i.e. 500 mg/kg) and T2TFGa high dose (1/2 i.e. 1g/kg) were selected.

Effect of METFG on metabolic profile in DN animals

Administration of METFG for 40 days caused a significant reduction ($P<0.05$) in fasting blood glucose level in groups IV and V treated with T1TFG and T2TFG respectively (Table 1). Group III (TS) treated with the standard drug glimepiride also showed a significant reduction in FBG level ($P<0.001$) as compared to diabetic nephropathic control group II. In the present study, it has been concluded that METFG showed a decrease in FBG levels in diabetic nephropathic animals as is also supported by the literature of *T. foenum-graecum* having antidiabetic activity (Arulselvan *et al*, 2007; Gupta and Seth et al, 1962).

Table 1. Effect of METFG on fasting blood glucose level in diabetic nephropathic animals

Groups	Fasting blood glucose (mg/dl)				
	0th day	10th day	20th day	30th day	40th day
NC	73.03 ± 5.3	74.10± 4.6	74.51± 4.8	75.12± 5.1	75.08 ± 4.2
DN	401.03± 21.3[a]	403.08± 23.1[a]	404.02± 25.2[a]	404.09± 24.7[a]	406.30 ± 25.1[a]
TS	402.01± 24.2[b]	390.11± 18.2[b]	312.13± 13.2[b]	262.12± 11.3[b]	168.21 ± 10.1[b]
T1TFG	400.10 ± 26.9[c]	390.20 ± 22.7[c]	360.11 ± 16.7[c]	304.02 ± 15.9[c]	239.35 ± 17.0[c]
T2TFG	406.31 ± 27.1[c]	386.70 ± 23.6[c]	345.72 ± 21.0[c]	299.02 ± 19.9[c]	169.23 ± 16.7[c]

Values are given as mean ± SEM, n = 6; [a]P< 0.001, [b]P< 0.01 and [c]P< 0.05

Effect of METFG on renal functions in diabetic nephropathic animals

The result of two doses of METFG on blood urea nitrogen (BUN) and serum creatinine (SCr) level in DN animals has been summarized in Table 2-3. BUN level showed a significant increase (P<0.001) in group II (DN) as compared to group I (NC). Administration of METFG caused a significant decrease in BUN level in groups IV (P<0.05) and V (P<0.01) treated with T1TFG and T2TFG at a dose of 500 mg/kg and 1000 mg/kg body weight respectively. Group III (TS) treated with the standard drug glimepiride also showed a significant decrease in BUN (P<0.01) as compared to group II (DN).

The nephroapthic animals in group II (DN) showed a significant increase (P<0.001) in level of SCras compared to group I (NC). Administration of METFG showed a significant decrease (P<0.05) in SCr level in groups IV and V treated with T1TFG and T2TFG at a dose of 500 mg/kg and 1000 mg/kg body weight respectively after 40 days of treatment. These findings have been justified by reports that BUN and SCr level, which is considered to be the renal function index, was significantly improved in the treatment groups (Gupta et al, 2001; Rajkumar et al, 1997; Ramachandran et al, 2002).

The results of the effects of METFG on total protein (TP) in urine (g/100 ml) in diabetic nephropathic animals have been summarized in (Table 4).Group II (DN) showed a significant increase (P<0.001) in the protein level as compared to group I (NC). Administration of METFG caused a significant decrease in protein level in groups IV (P<0.05) and V (P<0.01) treated with T1TFG and T2TFG at a dose of 500 mg/kg and 1000 mg/kg body weight respectively after 40 days of treatment. Proteinuria is considered to be the determinant factor for diabetic renal disease progression and is recognized as a clinical

Table 2. Effect of BUN level in diabetic nephropathic animals

Groups	Blood urea nitrogen (mg/dl)				
	0th day	10th day	20th day	30th day	40th day
NC	19.01 ± 1.2	19.30 ± 2.5	19.83 ± 1.8	19.13 ± 2.1	20.02 ± 3.6
DN	33.21 ± 2.7[a]	33.23 ± 2.6[a]	40.12 ± 2.7[a]	41.32 ± 3.6[a]	43.25 ± 3.8[a]
TS	34.26 ± 2.4[b]	32.73 ± 2.3[b]	29.72 ± 3.8[b]	26.12 ± 2.9[b]	22.27 ± 3.1[b]
T1TFG	34.02 ± 1.7[c]	33.01 ± 4.9[c]	32.93 ± 1.7[c]	30.91 ± 2.1[c]	29.84 ± 4.7[c]
T2TFG	32.90 ± 2.8[b]	31.82 ± 2.7[b]	29.63 ± 5.6[b]	28.02 ± 1.5[b]	27.90 ± 2.9[b]

Values are given as mean ± SEM, n = 6. [a]P< 0.001, [b]P< 0.01 and [c]P< 0.05

Table 3. Effect of serum creatinine level in diabetic nephropathic animals

Groups	Serum creatinine (mg/dl)				
	0th day	10th day	20th day	30th day	40th day
NC	0.32 ± 0.02	0.32 ± 0.03	0.31 ± 0.04	0.32 ± 0.04	0.32 ± 0.07
DN	0.61 ± 0.05[a]	0.62 ± 0.08[a]	0.64 ± 0.07[a]	0.65 ± 0.09[a]	0.65 ± 0.06[a]
TS	0.62 ± 0.04[b]	0.60 ± 0.07[b]	0.57 ± 0.06[b]	0.51 ± 0.03[b]	0.46 ± 0.05[b]
T1TFG	0.63 ± 0.10[c]	0.62 ± 0.10[c]	0.59 ± 0.06[c]	0.56 ± 0.09[c]	0.49 ± 0.08[c]
T2TFG	0.64 ± 0.09[c]	0.61 ± 0.06[c]	0.58 ± 0.02[c]	0.51 ± 0.02[c]	0.40 ± 0.08[c]

Values are given as mean ± SEM, n = 6. [a]P< 0.001, [b]P< 0.01 and [c]P< 0.05

signature, as well as a risk factor of renal lesions in diabetic nephropathy (Genet *et al*, 1999; Shannon *et al*, 1935). METFG a significant reduction in total protein level in urine was evident. Thus resulted in prevention of the occurrence of renal lesions and its functions in STZ induced diabetic nephropathic animals.

The effect of METFG on glomerular filtration rate (GFR) namely urea clearance (UrCl) and creatinine clearance (CrCl) level in diabetic nephropathic animals have been summarized in Tables 5 & 6. The result showed a significant decrease (P<0.001) in the UrCl and CrCl levels in group II (DN) as compared to group I (NC). Administration of METFG caused a significant increase in UrCl and CrCl levels in groups IV (P<0.05) and V (P<0.05) treated with T1TFG and T2TFG at a dose of 500 mg/kg and 1000 mg/kg body weight respectively after 40 days of treatment. Group III (TS) treated with the standard drug glimepiride also showed a significant increase in UrCl and CrCl (P< 0.01) levels as compared to group II (DN). In the present study, clearance test of urea and creatinine mainly accessed the GFR, which gives the general index of severity of renal damage (Grover *et al*, 2003). On the basis of results, it has been assumed that METFG had an effect on the improvement of GFR index. These findings have been well justified by various reports in the literature stating that GFR, the most prominent renal function index, was significantly improved in the treatment groups with an increase in levels of UrCl and CrCl, which indicated the protective effect of renal function.

Effect of METFG on serum electrolytes concentration in diabetic nephropathic animals

The result (Table 7) showed a significant decrease (P<0.001) in serum electrolytes (sodium, chloride, calcium and magnesium) level and increase (P<0.001) in potassium concentration in group II (DN) as compared to group I (NC). Administration of METFG caused a significant increase of sodium, chloride, calcium and magnesiumin serum and decrease potassium in serum in the groups IV (P<0.05) and V (P<0.01) treated with T1TFG and T2TFG at a dose of 500 mg/kg and 1000 mg/kg body weight respectively after 40 days of treatment. Group III (TS) treated with the standard drug glimepiride also showed a significant increase of sodium, chloride, calcium and magnesium and decrease in potassium in serum (P<0.01) as compared to diabetic nephropathic control animals. One of functions of the kidney is to regulate water, electrolytes and maintain acid-base balance. Measurements of electrolytes are needed for the management of renal disease. In the present study, diabetes was associated with electrolyte imbalance, where a significant decrease in Na, Ca, Cl and Mg and a significant increase in K in serum were observed in diabetic nephropathic animals. This may be attributed with hyperglycemia and

Table 4. Effect of METFG on total protein in urine of diabetic nephropathic animals

Groups	Total protein in urine (g/100 ml)				
	0^{th} day	10^{th} day	20^{th} day	30^{th} day	40^{th} day
NC	0.213 ± 0.01	0.214 ± 0.01	0.212± 0.02	0.213 ± 0.03	0.215 ± 0.04
DN	1.126 ± 0.08[a]	1.184 ± 0.09[a]	1.237 ± 0.05[a]	1.502 ± 0.06[a]	1.726 ± 0.07[a]
TS	1.132 ± 0.05[a]	0.996 ± 0.08[a]	0.628 ± 0.08[a]	0.294 ± 0.07[a]	0.215 ± 0.09[a]
T1TFG	1.012 ± 0.02[c]	0.984 ± 0.04[c]	0.736 ± 0.12[c]	0.598 ± 0.03[c]	0.453 ± 0.08[c]
T2TFG	1.123 ± 0.09[b]	0.998 ± 0.04[b]	0.792 ± 0.03[b]	0.547 ± 0.03[b]	0.242 ± 0.02[b]

Values are given as mean ± SEM, n = 6. [a]P< 0.001, [b]P< 0.01 and [c]P< 0.05

Table 5. Effect of METFG on urea clearance level of diabetic nephropathic animals

Groups	Urea clearance (ml/24 hr)				
	0^{th} day	10^{th} day	20^{th} day	30^{th} day	40^{th} day
NC	0.61 ± 0.03	0.63 ± 0.02	0.70 ± 0.03	0.68 ± 0.03	0.72 ± 0.06
DN	0.30 ± 0.12[a]	0.27 ± 0.13[a]	0.23 ± 0.12[a]	0.22 ± 0.11[a]	0.16 ± 0.15[a]
TS	0.31 ± 0.16[b]	0.35 ± 0.11[b]	0.43 ± 0.15[b]	0.49 ± 0.21[b]	0.52 ± 0.13[b]
T1TFG	0.30 ± 0.06[c]	0.34 ± 0.06[c]	0.41 ± 0.09[c]	0.46 ± 0.10[c]	0.50 ± 0.08[c]
T2TFG	0.32 ± 0.10[c]	0.40 ± 0.12[c]	0.48 ± 0.09[c]	0.52 ± 0.06[c]	0.51 ± 0.05[c]

Values are given as mean ± SEM, n = 6. [a]P< 0.001, [b]P< 0.01 and [c]P< 0.05

produced osmotic diuresis (Oh *et al*, 2007). However in the present study level of potassium in serum was increase which indicated mild acidosis formation in kidneys known as Type IV Renal tubular acidosis because of no failure in kidney system in diabetic nephropathic animals. 40 days of treatment with METFG balanced the concentration of electrolytes to prevent the complication in diabetic nephropathic animals.

Effect of METFG on renal hypertrophy in DN animals

The effect of STZ and the plant extracts on renal hypertrophy is shown in (Table 7). The result shows a significant increase (P<0.05) in the renal hypertrophy in group II (DN) as compared to group I(NC). Administration of METFG caused a significant decrease (P<0.05) in renal hypertrophy in groups IV and V treated with T1TFG and T2TFG at a dose of 500 mg/kg and 1000 mg/kg body weight respectively after 40 days of treatment. Group III (TS) treated with the standard drug glimepiride also showed a significant decrease (P<0.05) in renal hypertrophy as compared to group I(NC). In the present study, 40 days of treatment with METFG showed a decrease in renal hypertrophy which indicated a decrease in weight of the kidneys in nephropathic condition. This finding elaborated the view that METFG had a protective effect in STZ induced DN animals (Steffes et al, 1989).

Renal antioxidant status

The effect of STZ and the plant extracts on renal antioxidant status is shown in (Table 7). In the DN control animals the value of lipid peroxidation increased significantly (P<0.001) prior to the treatment as compared to group I (NC) suggesting formation of free radicals. Administration of METFG caused significant decrease (P<0.05) in lipid peroxidation in groups IV and V treated with T1TFG and T2TFG at a dose of 500 mg/kg and 1000 mg/kg body weight. In the present study, increased level of lipid peroxidation confirmed the presence of oxidative stress in diabetic nephropathic animals. However the level of lipid peroxidation was restored to normal value after treatment with *T. foenum-graecum* seeds which suggested the antioxidant capacity of the extracts (Chatterjee *et al*, 1988).

Table 6. Effect of METFG on creatinine clearance level of diabetic nephropathic animals

Groups	Creatinine clearance (ml/24 hr)				
	0^{th} day	10^{th} day	20^{th} day	30^{th} day	40^{th} day
NC	1.14 ± 0.15	1.17 ± 0.12	1.23 ± 0.11	1.25 ± 0.13	1.18 ± 0.11
DN	1.10 ± 0.03[a]	0.91 ± 0.03[a]	0.71 ± 0.02[a]	0.49 ± 0.07[a]	0.37 ± 0.08[a]
TS	0.78 ± 0.12[b]	0.82 ± 0.09[b]	1.02 ± 0.06[b]	1.23 ± 0.03[b]	1.32 ± 0.07[b]
T1TFG	0.68 ± 0.12[c]	0.71 ± 0.03[c]	0.76 ± 0.20[c]	0.83 ± 0.14[c]	0.93 ± 0.01[c]
T2TFG	0.74 ± 0.09[c]	0.79 ± 0.02[c]	0.83 ± 0.03[c]	0.85 ± 0.04[c]	0.96 ± 0.02[c]

Values are given as mean ± SEM, n = 6. [a]P< 0.001, [b]P< 0.01 and [c]P< 0.05

Table 7. Effect of 40 days treatment of METFG on serum electrolytes concentration, renal hypertrophy and renal antioxidant status in diabetic nephropathic animals

Parameters	NC	DN	TS	T1TFG	T2TFG
Sodium (meq/L)	161.05± 0.27	142.12 ± 0.38[a]	157.16 ± 0.72[b]	147.16 ± 0.42[c]	156.03 ± 0.03[b]
Potassium (meq/L)	5.37 ± 0.03	7.24 ± 0.03[a]	5.38 ± 0.04[b]	5.90 ± 0.06[c]	5.45 ± 0.01[c]
Chloride (meq/L)	95.13 ± 0.16	76.15 ± 0.23[a]	85.21 ± 0.22[b]	86.32 ± 0.24[c]	89.94 ± 0.36[b]
Calcium (meq/L)	9.86 ± 0.07	7.72 ± 0.09[a]	9.03 ± 0.15[b]	10.45 ± 0.09[c]	10.40 ± 0.20[b]
Magnesium (meq/L)	4.17 ± 0.15	1.89 ± 0.12[a]	4.07 ± 0.15[b]	3.70 ± 0.16[c]	3.92 ± 0.16[c]
Two kidney weight	1.10 ± 0.01	1.68 ± 0.01[c]	1.13 ± 0.03[c]	1.42 ± 0.03[c]	1.39 ± 0.03[c]
Renal hypertrophy	0.004 ± 0.01	0.009 ± 0.01[c]	0.004 ± 0.02[c]	0.005 ± 0.01[c]	0.005 ± 0.01[c]
MDA(μM/mg of renal)	1.13 ± 0.04	4.05 ± 0.01[a]	1.67 ± 0.01[b]	2.01 ± 0.03[c]	1.62 ± 0.02[c]
SOD(EU/mg protein)	45.02 ± 0.10	24.21 ± 0.12[a]	48.7 ± 0.03[b]	41.90 ± 0.02[c]	47.32 ± 0.04[c]
Catalase (μM/min/mg)	29.13 ± 0.15	19.38 ± 0.21[a]	28.21 ± 0.20[b]	23.10 ± 0.16[c]	28.41 ± 0.21[c]
GST(nM/mg protein)	0.20 ± 0.01	0.07 ± 0.0[a]	0.19 ± 0.0[b]	0.17 ± 0.0[c]	0.19 ± 0.01[c]

Values are given as mean ± SEM. [a]P< 0.001, [b]P< 0.01 and [c]P< 0.05

The levels of antioxidant enzymes catalase, superoxide-dismutase and glutathione-s-transferase was decreased significantly (P<0.001) in group II (DN) as compared to group I(NC). Administration of METFG caused a significant increase (P<0.05) in the level of antioxidant enzymes in groups IV and V treated with T1TFG and T2TFG at a dose of 500 mg/kg and 1000 mg/kg body weight respectively w.r.t. group II. Group III treated with the standard drug glimepiride also showed significant increase (P<0.05) in level of antioxidant enzymes as compared to diabetic nephropathic control group.

Results of the present study suggested that the DN animals treated with METFG showed a significant improvement in antioxidant enzyme levels and decrease in oxidative stress in diabetic nephropathic animals suggested antioxidant activity of seeds.

Effect of METFG on glycosylated hemoglobin in DN animals

The result of the effect of METFG on glycosylated hemoglobin level in diabetic nephropathic animalsafter 40th day of treatment has been summarized in (Table 8). The results showed a significant increase (P<0.01) in glycosylated hemoglobin level in group II (DN) as compared to group I (NC). Administration of METFG caused a significant decrease (P<0.05) in glycosylated hemoglobin level in groups IV and V treated with T1TFG and T2TFG at a dose of 500 mg/kg and 1000 mg/kg body weight respectively. Group III (TS) treated with the standard drug glimepiride also showed a significant decrease (P<0.05) in glycosylated hemoglobin level as compared to group II (DN). In the present study, 40 days of treatment with METFG showed a decrease in glycosylated hemoglobin levels in diabetic nephropathic animals. On the basis of results, it has been assumed that METFG have an effect on glycemic control. These findings have been well justified by various reports in the literature stating that glycosylated hemoglobin (HbA_1C) is used as a good marker of glycemic control (Nakagawaet al, 2004).

Table 8. Effect of 40 days treatment of METFG on glycosylated hemoglobin (% HbA1C) in diabetic nephropathic animals

Group Parameter	NC	DN	TS	T1TFG	T2TFG
HbA1C (%)	4.69	13.84^b	6.83^c	8.83^c	7.65^c

^{a}P< 0.001,^{b}P< 0.01 and ^{c}P< 0.05

Effect of METFG on the histopathology of kidneys

Figure 1 depicts the histology of the normal kidney showing normal architecture of its cells in group I (NC). Stained renal sections of group II (DN) showed hyaline arteriolosclerosis, nodular glomerulosclerosis, enlargement of glomeruli and subcapsular urinary space with severe renal damage. Study of kidneys of the animals of group III (TS) treated with glimepiride showed the relative decrease in subcapsular urinary space and renal damage in sections stained with H & E. Administration of METFG showed the relative decrease in subcapsular urinary space and renal damage in group IV and normal glomerulus in group V treated with T1TFG and T2TFG at a dose of 500 mg/kg and 1000 mg/kg body weight respectively.

The renal architecture in group III (TS) was comparable to those of the untreated control of group I (NC).These findings have been justified by various reports in the literature on renal histology in diabetic nephropathic animals stating that diabetic nephropathy is associated with nodular glomerulosclerosis, subcapsular urinary space, hyaline arteriolosclerosis, enlargement of glomeruli and severe renal damage with changes in shape and structure of cells, expansion of the mesangial area, extracellular matrix (ECM) accumulation, which gives the general index for the assessment of the damage of the kidneys (Genet *et al*, 1999). However in the present study, the diabetic nephropathic animals treated with METFG showed marked improvement in the histology of the kidneys.

Conclusion

The improvement in metabolic profile (carbohydrate and lipid) exhibited by METFG supports their antidiabetic activity along with antihyperlipidemic activity. Improvement in lipid profile reduces the possibilities of diabetic nephropathy induced cardiovascular diseases as it is

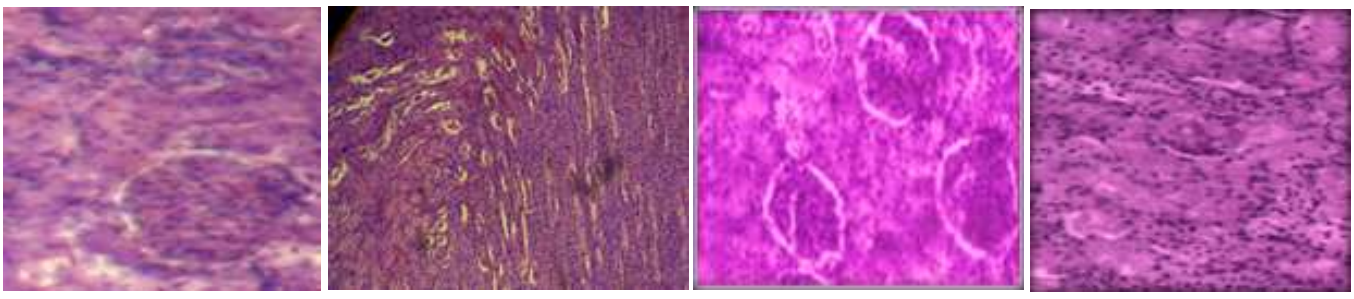

Figure 1. Effect of 40 days treatment of METFG on renal morphology in DN animals

known that diabetes increases the risk for cardiovascular and coronary artery diseases. Antioxidant activity helps in reducing the microvascular complications like diabetic nephropathy, retinopathy, neuropathy and their harmful effect in diabetes. These compounds can also reduce the oxidative stress induced damage of the kidneys and thus improves the renal enzymatic activity. Histopathological studies indicated the nodular glomerulosclerosis, enlargement of glomeruli and subcapsular urinary space and severe renal damage, suggesting development of diabetic nephropathy. These results imply that METFG could be used as an adjuvant therapy with a conventional hypoglycemic regimen to treat diabetic complication.

Conflicts of interest

The authors declare there is no conflict of interests.

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