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# Hypoglycemic and nephroprotective activity of of *Trigonella foenum* seeds

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## *ABSTRACT*

*The aim of the present study was to evaluate the hypoglycemic and nephroprotective activity of Trigonella foenum-graecum in alloxan induced diabetic rats. The antidiabetic effect of Trigonella foenum-graecum was studied against Alloxan (140mg/k g b.w., i.p.) induced diabetes in wistar rats for doses 250 mg/kg b.w. and 500 mg/kg b.w. (p.o.) for four weeks .effect was compared with oral dose of 10mg/kg, b.w. glibenclamide. Diabetes caused by Alloxan treatment increases the level of glucose and biochemical parameter in blood sample but treatment with Trigonella foenum-graecum significant decrease the elevated glucose and blood biochemical parameter. Hence, the results obtained in the present study indicate that Trigonella foenum-graecum has the potential to treat diabetes mellitus and prevent diabetes mellitus associated renal damage.*

***Key words:*** *Trigonella foenum-graecum, Glibenclamide, Alloxan, Renal damage, Hypoglycemic and Nephroprotective.*

## INTRODUCTION

Diabetes mellitus is a common chronic disease affecting millions of people worldwide. Standard treatment is failing to achieve required correction of blood glucose in many patients. Therefore, there is a need for investigating potential hypoglycemic drugs or herbs to improve glycemic control in diabetic patients (Abdullah et al.,2010). The present number of diabetics worldwide is over 150 million and this is likely to increase to 300 million or more by the year 2025 (Shaw et al., 2010). Non-insulin-dependent diabetes mellitus or adult-onset diabetes is a metabolic disorder that is characterized by high blood glucose in the context of insulin resistance and relative insulin deficiency (Kumar et al., 2005).

Trigonella foenum-graecum [fenugreek] belong to Famil Fabaceae. Trigonella foenum-graecum is one such plant that has been extensively used as a source of anti-diabetic compounds, from its seeds, leaves and extracts.in different model systems(v et al., 2010). Preliminary human trials and animal experiments suggest possible hypoglycemic and anti-hyperlipedemic properties of fenugreek seed powder taken orally (Baquer et al., 2011). Broca et al. reported that 4-hydroxyisoleucine (4-OH-Ile), an amino acid extracted and purified from fenugreek seeds, displays an in vitro insulin tropic activity, which is of great interest, and that its stimulating effect is related to the immolation of glucose concentration in the medium as shown in isolated pancreatic beta cells . 4-Hydroxyisoleucine is only found in plants, and owing to its particular insulin tropic action (Broca et al., 2000), it might be considered as a novel secretagogue with potential interest for the treatment of type II diabetes, a disease characterized by defective insulin secretion associated with various degrees of insulin resistance (Baquer et al., 2009). Basch et al. had reviewed the literature on the safety and adverse effects of T. f oenum-gra ecum (Basch et al., 2003)although fenugreek has traditionally been considered safe and well tolerated; some side effects have been associated with its use (Patil et al., 1997). Other reported side effects include transient diarrhea and flatulence (Abdel et al., 2000). Toxicological evaluation of diabetic patients taking fenugreek seed powder at a dose of 25 gm per day for 24 weeks showed no clinical hepatic or renal toxicity and no hematological abnormalities (Sharma et al., 1996b).

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## MATERIALS and METHODS

**Collection and Authentication of Plant Material:** The seeds of *Trigonella foenum-graecum* were collected in the month of May from the surrounding fields of Acharya N.G. Ranga Agricultural University, Rajendra Nagar and Authenticated by Dr. A. Manohar Rao.

**Preparation of plant extract:** 100gram of *T.foenum-graecum* was powdered, dried and continuously extracted for 48hrs with ethanol in a Soxhlet apparatus. The collected extract was stored at 0-4°C until used. The plant extract was pooled and evaporated to dry at 60°C.

**Preliminary Phytochemical Screening:**

Preliminary phytochemical investigation was carried out on ethanolic extract of *Trigonella foenum-graecum* for detection of various phytochemicals by following standard methods (Kokate 2007 and Khandelwal 2004).

**Experimental Animals: Alloxan induced diabetic model** (Ahmed et al., 2005)

Rats were made diabetic by a single intraperitoneal injection of alloxan monohydrate (150 mg/kg). Alloxan was first weighed individually for each animal according to the body weight and then solubilized with 0.2 ml saline (154mM NaCl) just prior to injection. Two days after alloxan injection, rats with plasma glucose levels of >140 mg/dl were included in the study. Treatment with plant extracts was started 48 h after alloxan injection.

**The various groups used in experiment:**

Total of 30 rats were divided in to 5 groups (n=6) as follows

Group I -Served as normal control and did not receive any treatment.
Group II - Served as diabetic control and received alloxan monohydrate and vehicle
Group III - Alloxan + Glibenclamide (10 mg/kg p.o.) and served as standard.
Group IV - Alloxan + TF (250 mg/kg, p.o.)
Group V - Alloxan + TF (500mg/kg, p.o.)

Group-I nondiabetic animals: received only 1% gum acacia (1 ml/kg/day, p.o.) for four weeks, and served as control. Group-II to V were rendered diabetic by single intraperitonial dose of alloxan monohyderate 150 mg/kg, in citrate buffer (pH 4.5). Group II received 1 % gum acacia (1 ml/kg/day, p.o.) for four weeks and served as diabetic control. Group-III received glibenclamide (10 mg/kg/day, p.o.) for four weeks. Group-IV and V received two different doses of TF (250 and 500 mg/kg/day, p.o.) for four weeks respectively.

**Care of Diabetic Animals:** Since diabetic animals drink large amount of fluid and produce large volume of urine, the bedding is changed frequently, usually every day and in some circumstances, more than once per day. Diabetic rats should have sufficient food and water.

**Collection blood and serum samples:** The blood was drawn from the retro orbital plexus of the rats (fasted for 14 h) under light ether anesthesia on different occasion, i.e., 0, 10th, 20th and 30th day. The blood samples were allowed to clot for 30mins at room temperature and then they were centrifuged at 3000 rpm for 10mins. The resulting upper serum layer was collected in properly labeled, clean and dry micro-centrifuge tubes. The serum samples were stored at -400 C and analyzed either immediately or within two weeks.The parameters studied were as follows:

a. Serum total cholesterol.
b. Serum and creatinine.
c. Serum urea.
d. Serum and Urine total protein.

- Body weight of an animal.
- Histopathological studies.

**Statistical Analysis:**

Results were expressed as mean ± SEM, (n=6). Statistical analyses were performed with one way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test by using Graph Pad Instat Software. P value less than 0.05 was considered to be statistically significant. *P<0.05, **<0.01 and ***<0.001, when compared with control and toxicant group as applicable.

## RESULTS

Results of the preliminary phytochemical investigation on *Trigonella foenum* are shown in **Table 1**.

**Table No. 1: Preliminary phytochemical screening**

| S.No. | Name of Group Test | Inference |
|---|---|---|
| 1. | Alkaloids | + |
| 2. | Carbohydrates | ++ |
| 3. | Flavonoids | + |
| 4. | Saponins | +++ |
| 5. | Steroids | + |
| 6. | Tannins | - |
| 7. | Glycosides | + |
| 8. | Amino acids | + |

– Absent; + Indicates Present; ++ Clearly present; +++ Better response

***a) Body weight:***

The Diabetic control showed significant decrease in the body weight during the treatment period. The diabetic animals treated with TF (250mg/kg) showed slight reduction in body weight but not much when compared to control. The group that received TF 500mg/kg had shown significant results (**Table 2 & Fig. 1**).

**Table No. 2: Effect of TF on body weight in alloxan induced diabetic rats**

| Groups | Body weight of the animal (gms) | | | |
|---|---|---|---|---|
| | Initial | 10th day | 20th day | 30th day |
| Normal | 156±2.16 | 160±4.01 | 170±3.33 | 175±2.67 |
| Alloxan+vehicle | 184±1.22 | 172±2.30 | 164±4.60 | 149±3.80 |
| Alloxan+Glibenclamide 10mg/kg | 162±3.60 | 160±6.20 | 163±2.4** | 166±1.80*** |
| Alloxan+ TF 250mg/kg | 171±2.5 | 169±3.60 | 160±2.80 | 156±4.20 |
| Alloxan+ TF 500mg/kg | 166±2.5 | 155±6.82** | 160±8.60* | 164±2.20*** |

Values are Mean ±S.E.M; n=6; ns= non-significant; *P <0.05; **P < 0.01 and ***P<0.001 vs. Diabetic Control

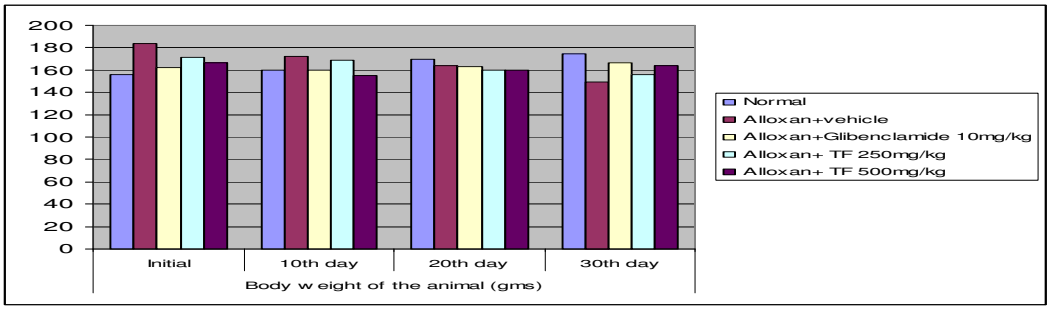



**Fig. 1: Effect of TF on body weight in alloxan induced diabetic rats**

***b) Biochemical parameters:***

Diabetic animals treated with TG showed significant decrease in serum creatinine (p<0.05), serum cholesterol (p<0.01) and urea, and significant increase in serum albumin and total protein (p<0.01) when compared with diabetic control (**Table 3 & Fig. 2-5**).

**Table No. 3: Effect of TF on biochemical parameters in alloxan induced diabetic rats**

| Groups | Serum protein(mg/dl) | Serum urea(mg/dl) | Serum creatinine | Serum cholesterol |
|---|---|---|---|---|
| Normal | 6.6±0.2 | 36.5±0.4 | 0.71±0.02 | 72.50±2.2 |
| Alloxan+vehicle | 4.2±0.4 | 81.8±2.2 | 1.6±0.01 | 130.34±1.8 |
| Alloxan+Glibenclamide 10mg/kg | 6.5±0.4** | 40.6±1.4** | 0.74.0.06*** | 74.26±0.6*** |
| Alloxan+ TF 250mg/kg | 4.6±2.7 | 65.4±2.6 | 1.21±0.04 | 102.08±0.8 |

| | | | | |
|---|---|---|---|---|
| **Alloxan+ TF 500mg/kg** | 5.4±0.4** | 46.2±0.4** | 0.80±0.02*** | 75.26±2.6*** |

Values are Mean ± S.E.M; n=6; * P<0.05; **P < 0.01 and ***P < 0.001 vs. Diabetic Control

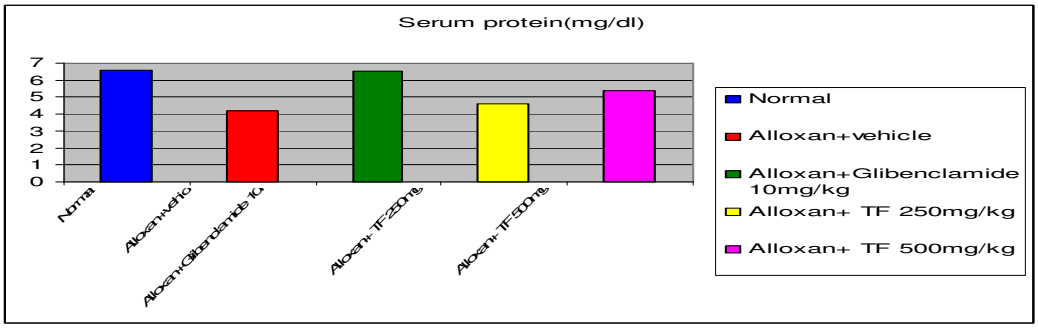



**Fig. 2**

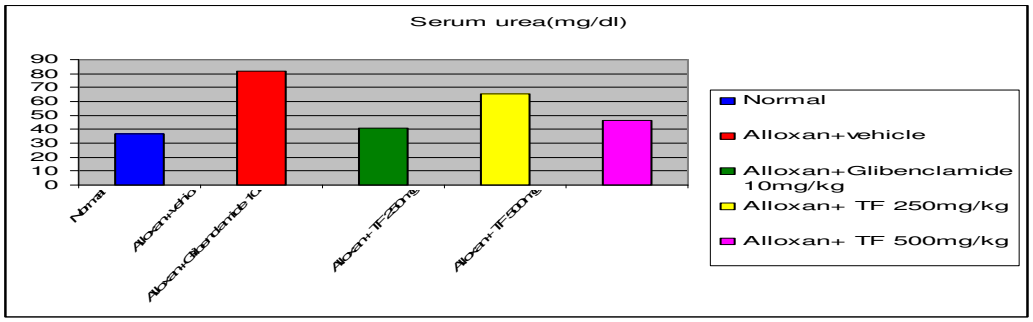



**Fig. 3**

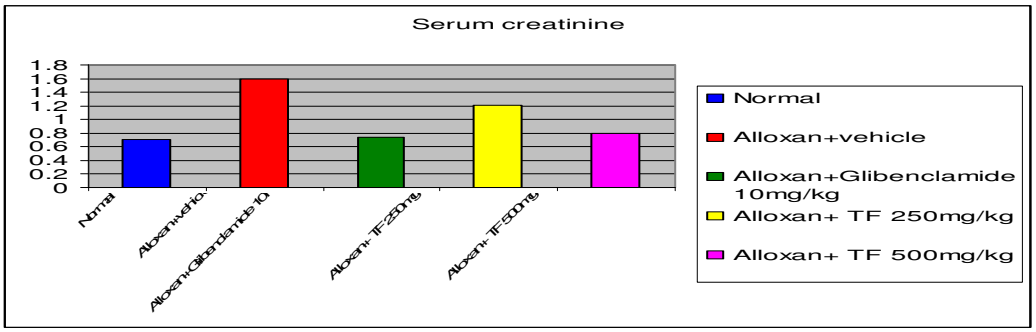



**Fig. 4**

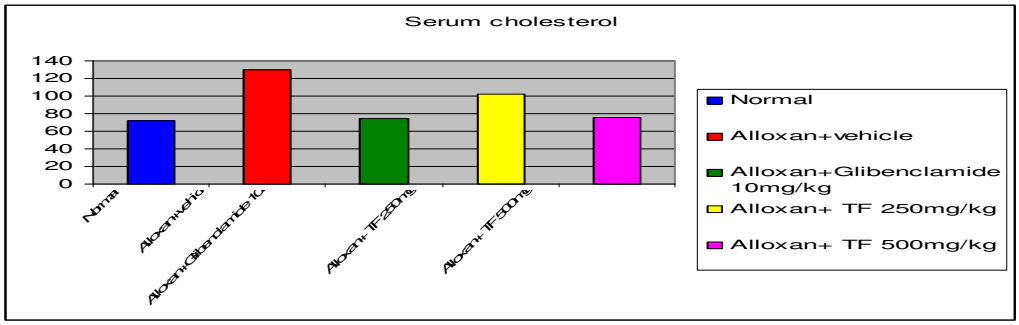
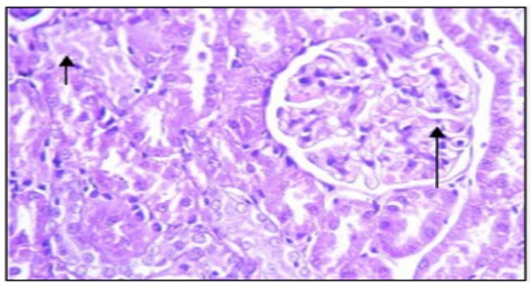


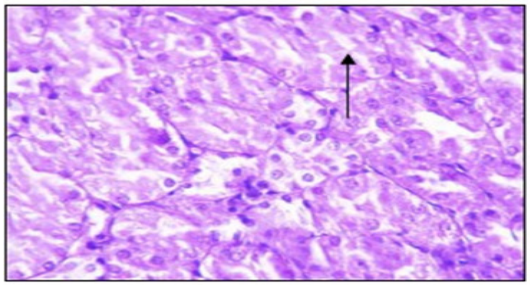

**Fig. 5**

**Effect of TF on histopathology of kidney:**

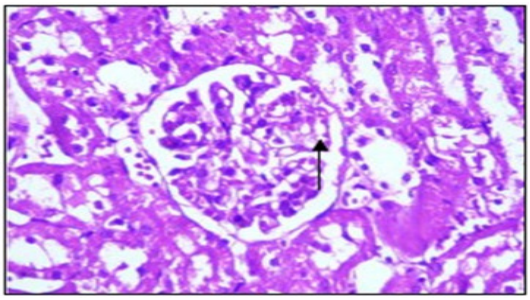
In present study, histopathology of control group showed normal structure of glomerulus, while diabetic control group showed significant mark of glomerulosclerosis and hyalinization which occurs because of severe diabetic condition (diabetic nephropathy). Diabetic group treated with TF 500mg/kg, p.o. showed absence of the sclerotic lesions produced by diabetic condition. While Glibenclamide treated diabetic groups showed partial prevention of the hyalinization but failed to recover the glomerulosclerosis to the normal condition. Diabetic group treated with TF 250mg/kg, p.o. did not show any protection against necrosis of kidney produced by diabetic condition (**Fig. 6**).



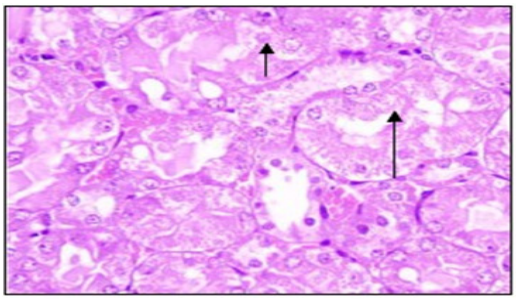
a) Control kidney (Group I) showing normal structure of glomerulus



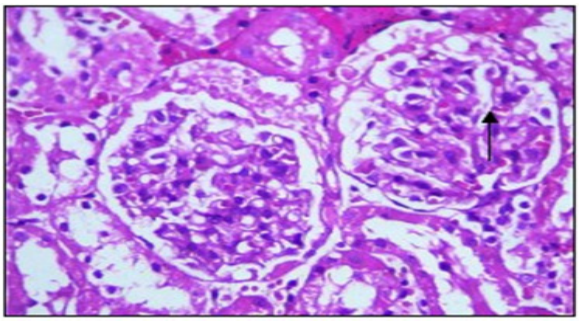
b) Diabetic group kidney (Group II) showing significant mark of nephritis



C) Group III (shows mild nephritis)



d) Group III (shows significant nephritis)



E)Group V(shows very mild nephritis)

**Fig. 6: Effect of TF on Histopathological studies of kidney in alloxan-induced diabetic rats**

## DISCUSSION

Diabetes mellitus ranks highly among the top ten disorders which cause mortality throughout the world. Diabetes mellitus being chronic disorder, treatment without side effect for long term control is important. Present antidiabetic agent possess side effect as risk of hypoglycemia, anemia, choestatic jaundice (Schimmer et al., 2001) There has been growing public interest in herbal medication for treatment of diabetes.

In the present study the periodic estimation of plasma glucose revealed that TG produced significant antihyperglycemic activity which began from 22nd day of treatment and it progressed throughout the study. The antidiabetic effect of the TG could possibly be due to presence of glycosides, terpenoids, tannins and saponins. Substances like glycosides, alkaloids, terpenoids, tannins and saponins are frequently implicated as having antidiabetic effects (Matsuda et al., 2002).

Various reports suggest that there is reduction in the body weight in diabetic rats. Loss of body weight could be due to, dehydration and catabolism of fats and protein seen during diabetes mellitus (Hofteizer et al., 1973). It is reported that the recovery in body weight is far less in the poorly controlled diabetic rats as compared to well-controlled diabetic rats. In the present study diabetic control group rats showed significant loss of body weight. All animals treated with TF showed significant prevention of the loss in body weight throughout the study. This prevention of loss in body weight by TF may be due to increasing glucose uptake in peripheral tissues or inhibiting catabolism of fat and protein or by glycemic control.

Diabetes produces qualitative and quantitative changes in the composition of the basement membrane and this altered material undergoes accelerated glycosylation and further rearrangement to form advanced glycation end-products (AGEs), which stimulate protein synthesis, further decrease degradability of the basement membrane, increase its permeability and cause endothelial dysfunction. Hyperglycemia increases the expression of transforming growth factor beta (TGFβ) in the glomeruli and of matrix protein specifically stimulated by cytokine. TGFβ may contribute to both the cellular hypertrophy and enhanced collagen synthesis is observed in diabetic nephropathy (Vishwanathan et al., 2004).

During diabetes, there is increased protein catabolism with inflow of amino acids to liver, which feed gluconeogenesis and accelerate ureagenesis, resulting in hypoproteinemia and hypoalbuminemia (Bhavpriya et al., 2000). Diabetic hyperglycemia induces elevation of the levels of serum creatinine, urine total protein and urine albumin which are considered as significant markers of renal dysfunction (Bretzel et al., 1997).

In the present study, diabetic animals treated TF showed reduction in proteinurea and albuminurea and also showed improvement in the serum total protein and albumin level. Treatment with TF also prevented the rise in serum creatinine levels. These results indicate that TF attenuates the progression of renal damage in alloxan induced diabetic rats. The use of typical antioxidants alone or in combination may retard or even prevent the normal progression of diabetic complications (Sabu et al., 2002).

In case of uncontrolled diabetes there is accumulation of lipids in kidney. Excessive production and accumulation of lipids can have devastating effect on renal structure and function (Yotsumoto

et al., 1997). Changes in the fractions of the lipid in renal cortex and medulla readily show its abnormality in diabetes (Rajlingam et al., 1993). In the present study it was found that concentrations of total lipids were significantly increased in cortical and medullary region of the kidney; concentration of total cholesterol was significantly increased in cortical region. TF treated diabetic rats showed significant reduction in the total cholesterol and triglyceride level in the kidney homogenate. The glycemic control exerted by TF may have affected the dislipidemia and the subsequent accumulation of the lipids in the kidney.

The histopathological study of diabetic control group showed significant mark of glomerulosclerosis and hyalinization which was probably due to severe diabetic condition (diabetic nephropathy); and the diabetic groups treated with TF showed absence of the sclerotic lesions produced by diabetic condition indicating the protective effect of TF on the kidneys of the diabetic animals.

Hence, the results obtained in the present study indicate that Trigonella foenum-graecum has the potential to treat diabetes mellitus and prevent diabetes mellitus associated renal damage.

## CONCLUSION

In the present study the ethanolic extract of *Trigonella foenum-graecum* seeds shown better Anti-diabetic and Nephroprotective activities in experimental rat models, it may be due to the presence of flavonoids and other poly phenolic compounds. Hence, the research justifies that the seed extract can be effectively used in treatment of diabetes as well as nephrotoxicity. Further studies are needed to isolate and characterize the active component(s) responsible for the anti-diabetic and nephroprotective properties of the test extract and findings should be confirmed by performing clinical studies.

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