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Effect of *Trigonella foenum-graecum* Against Antidiabetic Activity in Male Albino Wistar Rats

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Abstract: The present study investigated the antidiabetic effect of ethanolic extracts of fenugreek seeds *Trigonella foenum-graecum* (TFG) in streptozotocin (STZ) induced diabetic rats. Diabetes mellitus (DM) was induced in male albino rats of the Wistar strain weighing 200–250 g. The rats were divided into 4 groups. Group 1 served as control. Group 2, 3 and 4 rats were induced with diabetes by administering 60 mg/kg body weight (b wt) of STZ. The antidiabetic activity of TFG was evaluated by administering 500 mg/kg b wt. of extract of TFG in group 3 rats along with 50 mg/kg b wt. of STZ for 0, 7, 14, 21 and 28 days, respectively. Furthermore, group 4 rats received 50 g/kg b wt. of metformin (MET) along with STZ. STZ treatment showed significant changes in the body weight of animals during days 0, 7, 14, 21, and 28. Blood glucose levels on day 0 showed no significant intragroup variation. Administration of 60 mg/kg b wt. of STZ showed a significant increase in fasting blood glucose levels. After 28 days, group 2 rats exhibited significantly higher blood glucose levels as compared to control and TFG treated rats. Moreover, group 4 rats administered with metformin showed a significant decrease in blood glucose levels when compared with the normal control and TFG treated groups at intervals of days 7, 14, 21, and 28. The current study revealed the antidiabetic potential of TFG being effective in hyperglycemia and can protect against other metabolic aberrations caused by DM.

Keywords: *Trigonella foenum-graecum*, Diabetes mellitus, Streptozotocin, Blood glucose, Metformin, Antidiabetic

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Introduction

Diabetes mellitus (DM) is a chronic metabolic disorder in which there is high blood sugar levels over a prolonged period. The term "mellitus" or "from honey" was added by the Briton John Rolle in the late 1700s to separate the condition from

diabetes insipidus, which is also associated with frequent urination. It is a fast-growing global problem with huge social, health, and economic consequences. It was estimated that in 2010, globally, there were 285 million people

(approximately 6.4% of the adult population) suffering from this disease. This number is estimated to increase to 430 million in the absence of better control or a cure. An ageing population and obesity are two main reasons for the increase. Furthermore, it has been shown that almost 50% of the putative diabetics are not diagnosed until 10 years after the onset of the disease; hence, the real prevalence of global diabetes must be astronomically high.

DM is a metabolic disorder characterised by a loss of glucose homeostasis, with disturbances of carbohydrate, fat, and protein metabolism resulting from defects in insulin secretion, insulin action, or both (Imam, 2012). It is also represented by hyperglycemia, lipidemia, and oxidative stress; it predisposes affected individuals to long-term complications affecting the eyes, skin, kidneys, nerves, and blood vessels (Elosta *et al.*, 2012). It is prevalent in all parts of the world and is rapidly increasing worldwide. The estimated number of adults living with DM has scored to more than 371 million, with a prevalence of 8.3% (Ghazanfar *et al.*, 2014).

Globally, DM presents enormous and increasingly important public health issues and its prevalence in all age groups was estimated to be 2.8% (170 million) in 2000. This rate is expected to rise to 4.4% (366 million) in 2030 (Fonseca, 2006). The worldwide survey reveals that among the entire DM population, more than 90% are type II (Ramakrishnamacharya *et al.*, 1996). The overall death rate among people with DM is about twice that of people without diabetes (Harrigan *et al.*, 2001).

Trigonella foenum-graecum (TFG) (also known as fenugreek, locally as methi), a well-known traditional medicinal herb in Bangladesh, possesses diverse biological activities and pharmacological functions. The seeds of TFG have been used as traditional medicines not only for DM but also for high cholesterol, inflammation, and gastrointestinal ailments (Sharma *et al.*, 1990). Preliminary animal and human trials suggested possible hypoglycemic effects and

antihyperlipidemic properties of oral fenugreek seed powder. TFG seeds have also previously been shown to have hypoglycemic and hypocholesterolemic effects on type 1 and type 2 DM patients and experimental diabetic animals (Xue *et al.*, 2007). However, the report published so far (Abou El-soud *et al.*, 2007) on the hypoglycemic effect of TFG could not establish the optimum dose level for experimental subjects. In view of the above considerations, in the present study we administered ethanol extract of TFG at different doses to the alloxan-induced diabetic rat, and the hypoglycemic effects of the respective doses were compared with those of the standard antidiabetic drug Metformin (MET) in the induced diabetic rats.

Materials and Methods

Seeds of *Trigonella foenum-graecum* were purchased from the local market. The seeds were dried and crushed into coarse powder, which was used for extraction with alcohol (95% v/v) using the Soxhlet apparatus. The extracts were evaporated to dryness at a controlled temperature (35–40 °C). The extracts were stored in airtight containers under refrigeration. These dried extracts were dissolved in their respective solvents and used for further analysis.

Experimental Animals:

Male albino rats of the Wistar strain (150–250g) obtained from the animal house of the Rainbow Institute in Kattumannargudi were used in the study. The study protocol was approved by ethical committee of Department of Pharmacology, Periyar College of Pharmaceutical Sciences, Trichy, India. The registration number is CPCSEA/265. The animals were acclimatised to standard laboratory conditions (temperature 24±1°C, relative humidity 55±3%) and a 12 h photoperiod in suspended wire meshed galvanised cages (4–6 rats per cage) for one week before the commencement of the experiment. During the entire period of study, the rats were supplied with a semi-purified basal diet and water *ad libitum*. They were maintained at room temperature under

standard laboratory conditions.

Acute toxicity studies:

Albino rats weighing 200-250 g selected by random sampling technique were used in the study. The dose level of ethanolic extracts of TFG in different concentrations was observed for any symptoms of toxicity for 48 h as per Organisation for Economic Co-operation and Development (OECD) guidelines 423 (Acute toxic class method), and the median lethal dose (LD_{50}) was estimated to be >2000 mg/kg b wt. Based on the results obtained from this study, the doses for further pharmacological studies were fixed at 200 mg/kg and 400 mg/kg, respectively.

Induction of DM:

Sixteen Male Albino Wistar rats were randomly divided into four numerically equal experimental groups 1, 2, 3 and 4. Group 1 served as control. Diabetes was induced in group 2, 3 and 4 on overnight fasted adult rats weighing 200–250 g by a single intraperitoneal (i.p.) injection of 60 mg/kg STZ. STZ induced hyperglycemia has been described as a useful experimental model to study the activity of hypoglycemic agents. After overnight fasting (deprived of food for 16 h, and allowed free access to water), diabetes was induced in rats by i.p injection of STZ dissolved in 0.1 M sodium citrate buffer (pH 4.5) at a dose of 60 mg/kg b wt. After the injection, they have free access to food and water. The animals were allowed to drink a 5% glucose solution overnight to overcome hypoglycemia for 18 h. The development of diabetes was confirmed after 48 h of the STZ injection. The animals with fasting blood glucose levels greater than 200 mg/dl were considered diabetic rats and used for experimentation. After 48 h of fasting, the normal control (nondiabetic rat), diabetic control (STZ-induced diabetic rat), and positive control (STZ induced diabetic rat treated by MET) of each of the groups were treated by distilled water (1 ml) and the standard antidiabetic drug MET (4 mg/kg), respectively. The fourth rat in each group was treated with TFG at 500 mg/kg b.wt. Treatment

was given orally using an intragastric tube once daily for 28 consecutive days. The initial and final body weights were measured. On the 28th day, the animals were fasted for 12 h, anaesthetized using diethyl ether, and sacrificed.

Blood collection and biochemical analysis:

Two hours after drug treatment, all the animals were anaesthetized with diethyl ether to collect blood from the cardiac vessels (heart puncture method), and the collected sample was kept undisturbed at room temperature for 20 min. Serum was separated by centrifugation, and the glucose level was measured by the oxidase-peroxidase (GOD-POD) method (Trinder, 1969). The absorbance was measured spectrophotometrically at 546 nm based on a red-violet quinoneimine colour complex produced by the reaction of 4-aminophenazone and phenol with peroxide. The estimated amount of glucose was compared with the standard glucose concentration of 100 mg/dl.

Statistical analysis:

Data were analysed by the statistics software package (SPSS for Windows v.10). The statistical significance of mean values between different groups was determined by applying one-way ANOVA with post hoc Bonferroni test and P value < 0.05 was considered significant.

Results

Effect of TFG on relative body weight:

In an acute toxicity study, the relative body weight was calculated to determine the effect of an ethanolic extract of TFG on changes in b wt. in STZ induced rats. Some alteration was noticed in daily feed and water intake in rats treated with a single dose of extract as well as in control animals. There is a significant change seen in the body weight of animals after the treatment of diabetes with STZ. The decreased body weight of the animals was significantly regained when compared with the diabetic control animals after treatment for 28 days with the TFG extract, and the body weight of the normal control group was also significantly

Table 1: Effect of TFG on body weight in STZ induced diabetic rats

Groups	Body weight (g)				
	0 day	7 th ay	14 th day	21 st day	28 th day
Group1(Control)	201.5 ± 2.29	206.8± 4.32	212.5 ± 4.21	215.1±2.74	219.2 ± 3.24
Group 2 (Diabetic control)	203.3 ± 3.15 ^{NS}	182.6 ±2.39 ^{NS}	171.5±2.37 ^{NS}	164.8± .22 ^{NS}	145.5 ±5.21 ^{NS}
Group 3(60 mg/kg STZ + 500 mg/kg extract of TFG)	199.7 ± 3.12*	178.2 ± 2.16*	169.8 ± 2.45*	184.1 ± .23*	196.8 ± 2.25*
Group 4 (60 mg/kg STZ + 50 mg/kg MET)	202.5 ± 3.22*	186.7 ± 2.32*	193.5 ± 2.26*	201.4 ± 2.28*	205.1 ± 2.12*

Values are expressed as mean ± S.D. Statistical significance was calculated by comparing Control vs. Diabetic control, Control vs. TGF, TGF vs. MET. *P = 0.001, NS = Non significant

Table 2: Effect of TFG on blood glucose levels in STZ induced diabetic rats

Groups	Blood glucose level (mg/dl)				
	0 day	7 th day	14 th day	21 st day	28 th day
Group1 (Control)	81.2 ± 1.29	86.8 ± 1.31	83.6 ± 1.29	85.7±1.42	84.9 ± 1.25
Group 2 (Diabetic control)	105.4 ± 1.52 ^{NS}	164.9 ± 1.24 ^{NS}	228.1±2.32 ^{NS}	275.5±3.19 ^{NS}	313.8 ±5.12 ^{NS}
Group 3(60 mg/kg STZ + 500 mg/kg extract of TFG)	94.5 ± 1.24*	146.2 ± 1.62*	165.5±2.29*	147.2±1.36*	114.4 ± 1.35*
Group 4 (60 mg/kg STZ + 50 mg/kg MET)	92.7 ± 1.22*	136.5 ± 1.12*	143.7±1.36*	130.5±1.15*	110.5 ± 1.28*

Values are expressed as mean ± S.D. Statistical significance was calculated by comparing Control vs. Diabetic control, Control vs. TGF, TGF vs. MET. *P = 0.001, NS = Non significant

increased compared to the initial body weight. The changes in body weight of the animals during 0, 7, 14, 21, and 28 days are shown in Table 1.

Effect of TFG on blood glucose levels in diabetic rats:

A 28-day chronic study was done in STZ induced diabetic rats with an extract of TFG and the results of blood glucose levels are illustrated in Table 2. Blood glucose levels on day zero showed no significant intragroup variation. Administration of STZ (60 mg/kg, i.p.) showed a significant increase in fasting blood glucose levels. After 28 days, diabetic control rats exhibited significantly higher blood glucose levels (313.8 ± 5.12 mg/dl) as compared to normal control rats (84.9 ± 1.25). A daily treatment of phytocompounds (50 mg/kg, p.o.) for a period of 28 days lowers the blood

glucose levels in diabetic treated rats. Blood glucose levels on 28 days were lower, from 84.9 ± 1.25 to 110.5 ± 1.28. This is less significant in blood glucose levels when compared with the TFG treated groups. Similarly, diabetic rats treated with TFG (500 mg/kg, p.o.) also showed significant activity when compared with the standard drug treated group from the 7th day to the 28th day (94.5 ± 1.24 to 114.4 ± 1.35 mg/dl). Overall, MET 50 mg/kg p.o. showed a significant decrease in blood glucose levels when compared with the normal control and 500 and 50 mg/kg of TFG and phytocompounds treated groups at intervals of days 7, 14, 21, and 28. MET showed its potent antidiabetic activity and reduced the blood glucose levels of diabetic rats significantly (92.7 ± 1.22 to 110.5 ± 1.28 mg/dl).

Discussion

DM is a chronic condition that grows the most, especially in developing countries. The disease is highlighted for the severity of its complications, in addition to being considered a public health problem in terms of population growth and ageing, greater urbanisation, the increasing prevalence of obesity and sedentarism, as well as the increased survival rate of people with DM (Whiting *et al.*, 2011). In the present study, in an acute toxicity study, the relative body weight was calculated to determine the effect of an ethanolic extract of TFG on changes in body weight in STZ induced rats. Some alteration was noticed in daily feed and water intake in rats treated with a single dose of extract as well as in control animals. The decreased body weight of the animals was significantly regained when compared with the diabetic control animals after treatment for 28 days with the extract, and the body weight of the normal control group was also significantly increased compared to the initial body weight. The changes in body weight of the animals during days 0, 7, 14, 21, and 28 were observed. Zhang *et al.* (2016) have also recorded similar observations were noted in many experimental diabetes research studies.

An oral glucose tolerance test was studied in normal rats. The lowering of glucose can be seen well in an assay of glucose tolerance (Versphol, 2002). Fasting blood glucose levels decrease in TFG treated rats. Low doses show reduced activity at 150 min. The lowering of glucose levels may be due to the inhibition of intestinal absorption, or it may act by potentiating the secretion of insulin and increasing the utilisation of glucose levels in muscles (Venkatesh *et al.*, 2010). Blood glucose levels on 28 days were lower, 84.9 ± 1.25 to 110.5 ± 1.28 , which is less significant in reducing blood glucose levels when compared with the TFG treated groups. Similarly, diabetic rats treated with TFG (500 mg/kg, p.o.) also showed significant activity when compared with the standard drug-treated group from the 7th day to the 28th day (94.5 ± 1.24 to 114.4 ± 1.35 mg/dl). This

hypoglycemic activity may be due to the stimulation of surviving β -cells to release more insulin. TFG may act by inhibiting hepatic gluconeogenesis or inhibiting α -glucosidase enzyme in the intestine, which is the enzyme helpful for the breakdown of disaccharides to form glucose (Okwuosa and Unekw, 2011). Overall, MET 50 mg/kg p.o. showed a significant decrease in blood glucose levels when compared with the normal control and 500 and 50 mg/kg of TFG and MET treated groups at intervals of days 7, 14, 21, and 28. MET showed its potent antidiabetic activity and reduced the blood glucose levels of diabetic rats significantly (92.7 ± 1.22 to 110.5 ± 1.28 mg/dl) which may be due to the protective effect in controlling muscle wasting, i.e., reversal of gluconeogenesis, and may also be due to the improvement of glycemic control (Salahuddin and Jalalpure, 2010).

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