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ANTIHYPERGLYCEMIC EFFECT OF *TRIGONELLA FOENUM-GRAECUM* (FENUGREEK) SEED EXTRACT IN ALLOXAN-INDUCED DIABETIC RATS AND ITS USE IN DIABETES MELLITUS: A BRIEF QUALITATIVE PHYTOCHEMICAL AND ACUTE TOXICITY TEST ON THE EXTRACT.

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Abstract

The effects of ethanol extract of *Trigonella foenum-graecum* (Fenugreek) seeds on the blood glucose levels in alloxan-induced diabetic rats at different doses (2g/kg, 1g/kg, 0.5g/kg and 0.1g/kg) were studied. The hypoglycemic effect of extract was compared with that of the standard antidiabetic drug (glimepiride, 4mg/kg) single dose. The extract showed significant activity against the diabetic state induced by alloxan but the intensity of hypoglycemic effect varied from dose to dose. The most effective dose recognized was 1g/kg but that is still lower than the standard antidiabetic drug. No acute toxicity was observed for ethanol extract of *T. foenum-graecum* seed when it was administered orally at high dose level (3 g/kg body weight), which is higher than effective antihyperglycemic dose, and closely observed for 24 hrs for any mortality and next 10 days for any delayed toxic effects on gross behavioral activities. Phytochemical group tests were also accomplished and presence of alkaloids, steroids and carbohydrates were recognized in the extract.

Key words: alloxan, hypoglycemic effect, toxicity, phytochemical test, alkaloid.

Introduction

Due to the etiopathogenesis of diabetes mellitus, harmful side effects of synthetic drugs, the inability of existing modern therapies to control all the pathological aspects of the diabetic disorder, enormous cost of modern drugs as well as the poor availability of the advanced therapies for many rural populations in developing countries (Tanaka et al., 1992), alternative strategies to current pharmacotherapy of diabetes mellitus are urgently needed. The use of medicinal plants is, therefore, going to be stepped up at primary health care in diabetic mellitus (Udupa, 1987) to make a breakthrough of diabetic treatment. Recent experiences are proving the natural drugs as relatively non-toxic, safe and even free from serious side effects (Momoin, 1987).

Trigonella foenum-graecum (also known as fenugreek, locally as methi), is a well-known traditional medicinal herb in Bangladesh, possesses diverse biological activities and pharmacological functions. *T. foenum-graecum* seeds have been used as traditional medicines not only in diabetes but also in high cholesterol, inflammation and gastrointestinal ailments (Sharma et al., 1990). Preliminary animal (Shani et al., 1974; Amin et al., 1988; Hannan et al., 2003) and human (Sharma et al., 1990; Madar et al., 1988) trials suggested possible hypoglycemic effect and antihyperlipidemic properties of oral fenugreek seed powder. *T. foenum-graecum* seeds have also previously been

shown to have hypoglycemic and hypocholesterolemic effects on type 1 and type 2 diabetes mellitus patients and experimental diabetic animals (Xue et al., 2007). However, the report published so far (Abou El-Soud et al., 2007; Anninda et al., 2000) on the hypoglycemic effect of *T. foenum-graecum* could not establish the optimum dose-level for experimental subjects. In view of the above considerations, the present study has administered ethanol extract of *T. foenum-graecum* at different doses to the alloxan induced diabetic rat and the hypoglycemic effect of respective doses was compared with those of standard antidiabetic drug (glimepiride) to the induced diabetic rat. The study was also undertaken to evaluate some preliminary qualitative phytochemical analysis of crude extract of seeds as well as to examine the level of toxicity of crude extracts.

Materials and methods

Plant Material

T. foenum-graecum seeds were used as a sample for evaluation of antidiabetic properties, toxicological as well as phytochemical tests. Fresh seeds of *T. foenum-graecum* were collected from local market. The sample *T. foenum-graecum* seeds were identified by the Drugs and Toxins Research Division of Bangladesh Council for Scientific and Industrial Research (BCSIR) laboratories, Chittagong.

Extraction

The fresh seeds of *T. foenum-graecum* were air-dried and crushed into powder in a grinding machine. The powder (1.5kg) was extracted in Erlenmeyer flasks with 90% ethanol at room temperature. The maceration was carried out five times each in 48 hrs with occasional shaking and stirring. The whole extract was combined, filtered (Whatman filter paper No.1) and concentrated at 40°C *in vacuo* and finally the extract was freeze-dried to get 150gm of crude extract.

Experimental Animals and their diets

Male Albino rats of Wistar strain (150-250g) obtained from the animal house of BCSIR laboratories, Chittagong were used in the study. The animals were acclimatized to standard laboratory conditions (temperature 24 ± 1°C, relative humidity 55 ± 5%) and a 12 h photoperiod in suspended wire meshed galvanized cages (4-6 rats/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*. All animals were maintained according to the published criteria (Saha et al., 2001).

Diabetes induction and effects on the fasting blood glucose levels

Sixteen Wistar Albino rats were randomly divided into four experimental groups (Gr-A, Gr-B, Gr-C, and Gr-D), where four rats were taken in each group. Diabetes was induced in three of the groups and one was control. Induction of diabetes was performed by intraperitoneal (i.p) injection of alloxan monohydrate (axn, Sigma-Aldrich, Germany, 4 mg/kg body weight) dissolved in 0.9% NaCl saline solution immediate before use to 2.5-3.5 months old rats fasted for 18hrs. After 18 hrs of fasting, normal control (nondiabetic rat), diabetic control (axn induced diabetic rat) and positive control (axn induced diabetic rat treated by antidiabetic drug) of each of the groups will be treated by distilled water (1ml), distilled water (1ml), and standard antidiabetic drug glimepiride (4mg/kg), respectively. The fourth rat of each of the group was treated by *T. foenum-graecum* extract 2g/kg, 1g/kg, 0.5gm/kg, and 0.1g/kg body weight to Gr-A, Gr-B, Gr-C, and Gr-D, respectively.

Blood collection, serum preparation and biochemical analysis

Two hours after drug treatment all the animals were anesthetized with diethyl ether to collect blood from cardiac vessel (heart puncture method) and collected sample was kept undisturbed in room temperature for 20 mins. Serum was separated by centrifugation and the glucose level was measured by oxidase-peroxidase (GOD-POD) method (Trinder, 1969). The absorbance was measured spectrophotometrically at 546 nm based on a red-violet quinoneimine color complex produced by the reaction of 4- aminophenazone and phenol reaction with peroxide. The

estimated amount of glucose was compared with the standard Glucose concentration 100mg/dl or 5.55mol/L.

Toxicity analysis

Acute toxicity of *T. foenum-graecum* extract was carried out on 10 Wister albino rats weighing about 150 ± 10 g of the either sex and 10 Swiss albino mice of both sexes weighting about 25-30gm. Animal of each species were randomly divided into two equal groups, control group (received distilled water) and experimental group (received extracts of *T. foenum-graecum*). Rats and mice were dosed orally with *T. foenum-graecum* seed extract at high dose level (3 g/kg body weight), which is excessively higher than normal effective antidiabetic dose, and closely observed for 24 hrs for any mortality and next 10 days for any delayed toxic effects on gross behavioral activities. Their food consumption and growth rate were also examined once daily up to ten days.

Statistical analysis

Results were expressed as mean blood glucose levels \pm S.E.M. One-way ANOVA followed by Dunnett's test was used to analyze the data. The level of significance (significance level) was set at 0.05.

Phytochemical analysis

Crude ethanol extract of *T. foenum-graecum* seeds was subjected to analyze for checking the occurrence and existence of alkaloid, steroid, flavonoid, carbohydrate, glycoside and glucosides in it.

Alkaloid test. A small amount of each extract was neutralized by adding 1 or 2 drop of dilute H_2SO_4 . The resulting solution was treated with a very small amount of the following reagents in a test tube. The color of precipitates formed in each case was noted: Mayer's reagent- potassiomeric iodide solution, Wagner's reagent- Iodo-potassium iodide, Hager's reagent- 1% picric acid solution, Tannic acid solution-10% tannic acid, and Dragendorff, s iodine solution- bismuth potassium iodide solution

Flavonoid test. To a small amount of the extracts, a few drops of concentrated hydrochloric acid were added and immediate color development was observed keenly.

Steroids test. Existence of steroid was tested through Salkowski test and Liebermann-Burchard test. In the first test, 2 ml of chloroform-dissolved extract was taken in a clean and dried test tube. An equal volume of conc. H_2SO_4 was added to the test tube and color change was observed. For the later one, 2 ml of chloroform-dissolved extract was taken in a clean and dried test tube to which 10 drops of acetic anhydride and then 3 drops of concentrated H_2SO_4 were added. Change of color of the solution was recorded.

Carbohydrate test. Two drops of Molisch's reagents were added to 2-5 ml of chloroform-dissolved extract in a test tube to which 1 ml of concentrated H_2SO_4 was allowed to flow down the side of tube so that the acid formed a layer beneath the aqueous solution without mixing with it. A red ring was formed at the common surface of the two liquids and turned to a dark purple on standing, or shaking. The mixture was shaken and diluted with 5 ml of water to form a dull violet precipitate immediately.

Glycosides test. To a small amount of extract, 1 ml of water and a few drops of aqueous hydroxide solution were added. Development of color was noted.

Glucosides test. A small amount of extract was acidified with diluted H_2SO_4 (2.5ml) and boiled in a water bath for 15 mins. The solution was cooled and neutralized with 20% KOH solution to add 5 ml of Fehling's solution. . A control experiment was carried out using 2.5 ml of water in place of the acid.

Results

The serum blood sugar level in normal control and all experimental groups of induced diabetic rats were analyzed (Table-1). Alloxan is a diabetogenic agent causing the destruction of β -cell and its administration to rats increases the blood sugar level. But the increase of blood sugar and

Table-1: Effect of different doses of *T. foenum-graecum* extract and standard antidiabetic drugs on blood glucose level of alloxan induced diabetic rats.

Treated group	Dose	Blood Glucose Level (mg/dl)	% of increase (+)/decrease (-)
Extract 2 g/kg, antidiabetic drug (glimepiride) 4mg/kg			
Nondiabetic (n=4)	-	61.83 ± 2.15	-
Water control (n=4)	-	96.35 ± 3.6 ^b	55.83 % (+)
Glimepiride control (n=4)	4 mg/kg	62.38 ± 2.8 ^a	35.26 % (-)
Extract (n=4)		63.67 ± 2.8 ^a	33.92 % (-)
Extract 1 g/kg, antidiabetic drug (glimepiride) 4mg/kg			
Nondiabetic (n=4)	-	64.95 ± 1.15	-
Water control (n=4)	-	101.27 ± 3.11 ^b	55.91 % (+)
Glimepiride control (n=4)	4 mg/kg	43.35 ± 1.75 ^a	57.19 % (-)
Extract (n=4)		61.45 ± 1.88 ^a	39.32 % (+)
Extract 500mg/kg, antidiabetic drug (glimepiride) 4mg/kg			
Nondiabetic (n=4)	-	58.65 ± 6.5	-
Water control (n=4)	-	86.23 ± 3.6 ^b	47.02 % (+)
Glimepiride control (n=4)	4 mg/kg	50.38 ± 7.8 ^a	41.17 % (-)
Extract (n=4)	500mg/kg	75.53 ± 5.2 ^a	12.40 % (-)
Extract 100mg/kg, antidiabetic drug (glimepiride) 4mg/kg			
Nondiabetic (n=4)	-	60.23 ± 1.5	-
Water control (n=4)	-	88.50 ± 5.6 ^b	46.93 % (+)
Glimepiride control (n=4)	4 mg/kg	58.65 ± 3.5 ^a	33.73 % (+)
Extract (n=4)	100mg/kg	80.78 ± 2.9 ^a	8.72 % (-)

* Values are mean ± SEM (n = 5, number of rats per group)

Here, ^a P<0.05 compared with diabetic control and ^b P<0.05 compared with normal control

Table-2. Observation of the phytochemical group tests

Name of the group test	Presence (+) / Absence (-)
Alkaloids	+
Flavonoids	-
Steroids	+
Carbohydrate	+
Glycosides	-
Glucosides	-

effect of *T. foenum graecum* seed extract and standard antidiabetic drug varies in different groups. Alloxan increases 55.83 % of the blood sugar level in the treated rat as compared to the normal control. In group A, treatment of induced rat with *T. foenum-graecum* seed extract, significantly (p<0.05) decreased (33.92 %) of the alloxan-induced elevated high blood glucose level as compared with diabetic controls. In the case of standard drug (glimepiride) treatment, the percent of blood sugar decrease was 35.26% as compared to their respective controls.

Administration of alloxan increases the blood sugar level to 57.91 % as compared to normal control in group B. *T. foenum- graecum* seed extract and standard antidiabetic drug (glimepiride) produced a decrease of 39.32 % and 57.19%, respectively. Blood sugar level of alloxan-induced rat increased by 47.02 % in group-C. Ethanol extract of *T. foenum-graecum* seed significantly (p<0.05) decreased blood glucose to 12.40% level in alloxan-induced rats as compared to diabetic controls whereas the glimepiride lowered blood glucose level by 41.17%.

Comparative effect of different dose level of *T. foenum-graecum* on alloxan induced diabetic rats showed that 1gm/kg of extract has highest activity (39.32 %) which has decreased gradually for 2gm/kg (33.92 %), 0.5 gm/kg (12.40 %), and 0.1 gm/kg (8.72 %), respectively.

No visible symptoms of toxicity or mortality in rats and mice administered with very high dose (3g/kg) of *T. foenum-graecum* seeds extract was found.

Result of phytochemical screening tests is summarized in Table 2, which showed the presence of alkaloid, steroid and carbohydrate but no flavonoid, glycoside and glucosides in the crude seed extract.

Discussion

Alloxan is a beta cytotoxin which induces "chemical diabetes" in a wide variety of animal species (Szkudelski, 2001) by damaging the insulin secreting pancreatic-cell resulting from the production of free radicals that undergo dismutation to hydrogen peroxide and cause rapid destruction of the β cells of the pancreas (Raju et al., 2004) causing a diabetic state. Current study focused the effect of different doses of *T. foenum graecum* seed extract and comparison of the effects with those of a single dose standard antidiabetic drug in induced diabetic condition. Existence of phytochemical metabolites in extract was also assessed to presume their role in antihyperglycemic activity. Table-1 showed that different doses of *T. foenum-graecum* extract affected in different intensity the control of the blood glucose level by metabolic alteration of induced-diabetic rat. Among the doses tested, 1gm/kg showed the highest activity (39.32 %) which might be because of its rate of absorption by the experimental size of rat.

The possible mechanism of action of extracts could be correlated with the reminiscent effect of the reference antidiabetic drug glimepiride that promotes insulin secretion by closure of K^+ -ATP channels, membrane depolarization and stimulation of Ca^{2+} influx, an initial key step in insulin secretion (Fuhlendorff et al., 1998). Since alloxan is known to destroy pancreatic cells, the present findings appeared to be in consonance with the earlier suggestion (Jackson and Bressler, 1981) that sulphonylureas (e.g. glimepiride) have extra-pancreatic antihyperglycemic mechanism of action secondary to their insulin secreting effect and the attendant glucose uptake into, and utilization by, the tissues (Jackson and Bressler, 1981). Other probable mechanisms by which the plant extracts lowered blood glucose may be by increasing glycogenesis, inhibiting gluconeogenesis in the liver, or inhibiting the absorption glucose from the intestine.

It is noted that the induction with alloxan of same dose to different groups of rat is also varied. It could be explained by the fact that the metabolic rate could be different with the age, sex and weight of animals. The same artifact may be implied to the administration and effect of extracts and glimepiride. Table 1 showed that the effect of all the doses of *T. foenum graecum* is still lower than that of reference drug glimepiride. Therefore, is the use of *T. foenum graecum* a viable alternative medication for diabetes mellitus?. What encourages us to take *T. foenum graecum* into account as a means of remedy of diabetic mellitus is that the synthetic drugs (glimepiride) possess some side effects, contraindication and adverse reactions which the natural drugs don't have. Rather the *T. foenum-graecum* helps improve diabetic mellitus by the ways detailed henceforth. First, antioxidant circulating activity of *T. foenum-graecum* through significant lipid peroxide level decrease (Devasena and Menon, 2002) exerts beneficial effects (Maxwell, 1995) in oxidative stress increased in patients with diabetes mellitus (Baynes, 1991). Second, our preliminary phytochemical screening of *T. foenum-graecum* suggests that it contains carbohydrate, steroids and alkaloids. These findings validate previous report on the presence of trigonelline, trigocoumarin, and trimecoumarine alkaloids (Al-Habori and Raman, 1998) in the studied plant and they have antihyperglycemic action. Therefore, it is not unreasonable to speculate that some of these compounds present in the plant extracts are probably responsible for hypoglycemic activities. **Third**, the seed fibers of *T. foenum-graecum* reduces the rate of glucose absorption and may also delay gastric emptying, thereby preventing the rise in blood sugar levels following a meal (Gupta et al., 2001). Amino acid, 4-hydroxyisoleucine (Broca et al., 2000) of seed fibre also powerfully stimulates insulin secretion at all levels of cellular organization since the cells are more sensitive to insulin and increase in the number of insulin receptor sites to burn cellular glucose at high fiber diet. Fourth, guar gum of *T. foenum-graecum* prevents the rapid uptake of glucose in the small intestine, aids in blood sugar retention in diabetic patients and may also be effective in the treatment of hypercholesterolemia (Sharma et al., 1996)

Despite the difference in hypoglycemic effect and exact mechanism of action, *T. foenum-graecum* seeds extracts can be considered as an effective alternative in diabetes. Toxicity test result of this plant emphasized to consider it as an alternative to diabetic treatment with no or little side-effect. This study also validated the traditional use of such natural remedies of indigenous plants-origin for the treatment of diabetes mellitus. However, large scale and multicentric clinical trails are still required to establish the usefulness of this indigenous drug. Similarly, activity-directed phytochemical studies are required to isolate pure active compounds from the extracts. Such researches might help in finding new models of chemical compounds, taking *T. foenum-graecum* seeds along with diet as a modulator in the treatment of diabetes mellitus, and finally forming a part of therapy in its management.

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