

RESEARCH ARTICLE

Received on: 02/05/2016

Published on: 25/07/2016

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Conflict of Interest: None Declared !

Evaluation of the antidiabetic activity of hydro-alcoholic extract of *Trigonella foenum-graecum* Linn. in alloxan induced diabetic rats

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ABSTRACT

Background: *Trigonella foenum graecum* Linn. (Commonly known as Methi or Fenugreek) seed extract belonging to family Leguminosae have been documented to elicit hypoglycemic activity. Herbal preparations of *T. foenum graecum* have been considered as safe, effective and economical for various ailments in Indian traditional system of medicine. Its seed extract is used for the treatment of diabetes. The present study was undertaken to evaluate the hypoglycemic potential and effect on various biochemical parameters of hydro-alcoholic extract (70% v/v) of seeds of *T. foenum graecum* (TGE) in alloxan induced diabetic rats.

Materials and method: Albino Wister male rats of weighing between 150 to 200 gms of were used for the study. Diabetes was induced by injecting alloxan (120 mg/kg, i.p.). Rats were divided in different groups for the study. Group I served as normal control, Group II served as diabetic control, Group III served as standard control and treated by Tolbutamide 100 mg/kg p.o. Group IV served as diabetic rats treated with hydro-alcoholic extract of *T. foenum graecum* seeds at a dosage of 1000 mg/kg body weight. All the treatments were given for 21 days. At the end of study, overnight fasted rats were sacrificed and blood was collected to determine fasting blood glucose and biochemical findings.

Result: Diabetic rats treated with TGE at a dose of 1000 mg/kg significantly ($P < 0.01$) reduced fasting blood glucose and normalize the lipid profile, renal profile and hepatic profile. Improvement in the histopathology of pancreas & liver of TGE treated rats confirmed its protective role in alloxan induced diabetes.

Conclusion: It can be concluded that hydro-alcoholic extract (70% v/v) of seeds of *T. foenum graecum* possess antidiabetic activity and may be beneficial in improving complications associated with diabetes mellitus.

Key Words: Antidiabetic activity, *Trigonella foenum graecum*, TGE, Seed extract, Alloxan-induced diabetes.

Introduction

Diabetes mellitus is a group of syndromes characterized by hyperglycemia, altered metabolism of lipids, carbohydrates and proteins, and an increased risk of complications from vascular disease¹. More than 400 species of plants have been reported to display hypoglycemic effects, but only a few of them have been investigated.² Non-insulin dependent diabetes mellitus (NIDDM) accounts for more than 85% of the prevalence of diabetes worldwide. NIDDM is associated with morbidity &

mortality, resulting from its microvascular, macrovascular and neuropathic complications.³ The derangement in lipid metabolism in diabetes mellitus are often important determinants of the course and status of the disease. The abnormalities of the lipid metabolism generally leads to elevation in the levels of serum lipids and lipoproteins that in turn play an important role in occurrence of premature and severe atherosclerosis, which affects patients and diabetes.⁴

Fenugreek seeds (*Trigonella foenum graecum*) have long been described in the Greek and Latin pharmacopoeias for the treatment of diabetes. More recently, several studies have demonstrated hypoglycemic properties of fenugreek seeds in both animal and human studies, thus, lending support to its traditional use.^{5,6}

Fenugreek seeds contain alkaloids (mainly trigonelline) and protein high in lysine and L-tryptophan. Its steroidal saponins (diosgenin, yamogenin, tigogenin and neotigogenin) and mucilaginous fibers are thought to account for its beneficial effects. Seeds contain Flavonoids, including vitexin, vitexin-7-glucoside, orientin arabinoside, homo-orientin, isovitexin, vicenin-1, vicenin-2, quercetin, luteolin and vitexin cinnamate.⁷

MATERIAL AND METHODS

Plant material

The seeds of *T. foenum graecum* were purchased from local market Hapur (U.P.). The specimen was given for authentication in Raw Material and Laboratory of National Institute of Science Communication and Information Resources (NISCAIR), New Delhi (voucher no. NISCAIR/Consult/RHMD/-2010-11/1620/218).

The seeds of *T. foenum graecum* were washed and dried in an electric hot air oven at a temperature of 40°C.

Preparation of extract

Dried seeds were coarsely powdered and defatted with petroleum ether by soxhlet apparatus. Defatted drug then extracted with 70% hydroalcoholic solution in soxhlet apparatus. The extract was concentrated under reduced pressure.

Standardization of plant drug and extract

The morphological and microscopical studies, ash value, extractive value in different solvents, qualitative heavy metal analysis of the extract and test for microbial contamination were carried out for the purity of drug. The Phytochemical screening of the extract was carried out for the presence of Alkaloids, Proteins & Amino acids, Carbohydrates, Flavonoids, Phenolic group, Glycosides, Saponins, Tannins, Steroids, Triterpenoids. Determination of microbial count included total viable aerobic count, total yeast and mould, *E. coli*, *S. typhi*, *P. aeruginosa* and *S. aureus* count have been determined.⁸⁻¹⁵

Animals

Albino wistar male rats of weighing between 150 to 200 gms were procured from Indian Veterinary Research Institute Bareilly U.P.(IVRI).The animals were housed under standard conditions of temperature (25 ± 2°C) and relative humidity (30-70%) with a 12:12 light-dark cycle and acclimatized under ambient conditions in the animal house facility of the department of pharmacology, Siddhartha Institute of pharmacy, Dehradun (CPCSEA Approval no. 1435/PO/a/11/CPCSEA). The animals were fed with standard diet (Amrut Rat Feed, India) and water *ad libitum*. The Institutional Animal Ethics Committee approved all the experimental protocols with approval no. SIP/IAEC/12/Polyherbal.

Acute Toxicity studies

The acute oral toxicity study was carried out as per the guidelines set by Organization for Economic Co-operation and Development (OECD), draft guidelines 420, received from committee for the purpose of control and supervision of experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India. A total of six female albino rats were used for the study. The extract was administered at a single dose of 2000 mg/kg by gavage. All animals were observed individually after dosing during first 30 minutes, periodically during the first 24 hours and daily for 14 days.¹⁶

Induction of Diabetes:

The albino male rats 150-200 gm, were allowed to fast overnight prior to experimentation and rendered diabetic by injection of a single dose of Alloxan 120 mg/kg body weight (Manufactured by Loba Chemie Company) administered as a 0.9% w/v in saline solution by I.P. route. It produces diabetes by selective necrosis of β - cells of islets of langerhans of pancreas. Since alloxan could evoke fatal hypoglycemia as a result of massive insulin release, rats received 20% of glucose solution for first 6 hours then simple tap water was given. The rats were then kept for next 24 hours with free access of 5 % glucose solution to prevent hypoglycemia.¹⁷ After 48 hrs of injection of Alloxan, Blood glucose level was measured for the evidence of diabetes by using commercially available kit "ACCU-CHEK ACTIVE" Glucometer from Roche Diagnostics GmbH, Germany. The rats which showed blood glucose level

more than 200 mg/dl were considered as diabetic. The animals with sugar level more than 200mg/dl were selected. Animals were maintained for 72 hrs in diabetic condition for well establishment of diabetes.

Experimental design

Hydroalcoholic extract of the drug was suspended in 2% acacia solution and the dose of 1000 mg/kg extract was given by oral route using a catheter¹⁸. Tolbutamide 100mg/kg was used as a standard drug.¹⁹

Animals were divided into four groups of six each.

Group-1: Healthy normal animals received only water served as Normal control (NC).

Group-2: Untreated alloxan induced diabetic animals served as a Diabetic control (DC) also received water.

Group-3: The Reference Standard group (STD) was treated with Tolbutamide at a dose of 100 mg/kg b.wt., p.o.

Group-4: Diabetic animals treated with 70 % v/v hydro alcoholic extract of seeds of *T. foenum graecum* (TGE) at a dose of 1000 mg/kg b.w, p.o/day.

Blood samples were collected by retro-orbital plexus puncture method and blood glucose levels were estimated using an electronic glucometer (ACCU-CHEK ACTIVE™ Glucometer from Roche Diagnostics GmbH, Germany). Blood samples were drawn at weekly intervals till the end of study (i.e. 3 weeks). Blood glucose estimation was done on day 0, 7, 14 and 21 of the study. On day 21, blood was collected by cardiac puncture under mild ether anesthesia from overnight fasted rats and fasting blood sugar²⁰ was estimated. Serum was separated and estimation of serum cholesterol,²¹ serum triglycerides,²² serum HDL,²³ serum LDL,²⁴ serum creatinine,²⁵ serum urea,²⁶ serum alkaline phosphatase (ALP),²⁷ bilirubin, serum glutamate oxalate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT)²⁸ carried out.

Statistical analysis

All values of blood sugar and biochemical estimations were expressed as mean ± Standard error means (S.E.M.) and analysed for ANOVA and post test TUKEY-One Way ANOVA. Differences between groups were considered at $P < 0.01$ levels.

Histopathology of isolated liver and pancreas:

Small pieces of liver and pancreas tissues were collected in 10% formalin for proper fixation. Tissues were fixed in Bouin's fixative (without acetic acid) for

histopathological studies. Sections of tissues (6 microns in thickness) were stained with haematoxylin and eosin (H & E) for histological examination.²⁹ The photomicrographs of histological studies are presented in fig. 2 and 3.

RESULTS

Plant material, Standardization and preparation of extract

The plant material was identified by Dr. H.B. Singh in Raw Material and Laboratory of National Institute of Science Communication and Information Resources (NISCAIR), New Delhi. A certificate of identification was issued by NISCAIR. (NISCAIR/Consult/RHMD/-2010-11/1620/218).

The colour of the *T. foenum graecum* seed powder was yellowish brown, odour was characteristic and taste was bitter. Moisture content was found to be 4.23% w/w. Total ash, acid insoluble & water soluble ashes were determined as 3.56%, 0.44% and 1.92% w/w respectively. Alcohol soluble and water soluble extractive values were determined as 5.62% and 4.23% w/w respectively. The percentage yield of hydroalcoholic extracts of seeds of *T. foenum graecum* was found to be 15.035 %.

Results of phytochemical screening showed that carbohydrates, flavonoids, phenolic group, alkaloids, Saponins, Tannins, Steroids and Triterpinoids were present in the extract. Glycosides and protein & amino acids were found to be absent in the extract.

The results of heavy metal analysis showed that hydroalcoholic extract of *T. foenum graecum* seeds passed the limit test for heavy metals.

The total aerobic count was found to be 285 CFU/g, yeast and mould, *E. coli*, *S. typhi*, *P. aeruginosa* and *S. aureus* were found to be absent in the extract.

Acute Toxicity studies

TGE treated rats showed no discernible behavioral changes given by oral route. No mortality was observed when it was administered orally at a high dose (2g/kg bw) which was higher than effective Antihyperglycemic dose during observation period.

Antidiabetic activity

Table 1- Effect of 3-week treatment with standard drug and TGE on blood glucose level on alloxan induced diabetic rats

S.N.	GROUP	0-DAY	7-DAY	14-DAY	21-DAY
1.	NC	79.33 ± 2.692	79.17 ± 1.740	79.33 ± 1.333	77.67 ± 0.988
2.	DC	318.2 ± 4.175	355.7 ± 6.097	367.8 ± 9.372	370.2 ± 9.928
3.	STD	358.3 ± 4.780*#	244.2 ± 21.60*#	199.0 ± 12.70*#	138.0 ± 13.43*#
4.	D+TGE	349.3 ± 7.544*#	313.5 ± 6.922*	281.7 ± 6.323*#	243.2 ± 9.502*#

* P<0.01 (Tukey test) significant when treated with Normal control

P<0.01 (Tukey test) significant when treated with Diabetic control

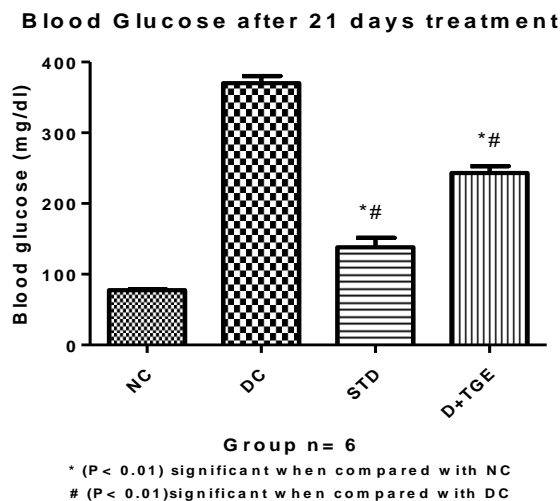


Fig.1: Graphical representation of effect of TGE on blood glucose.

Table 2- Effect of TGE on serum profile in alloxan induced diabetic albino rats after 21 days treatment

S.N.	Serum profile	Normal control	Diabetic control	Standard control	TGE treated
1.	Cholesterol	73.45±6.376	145.1±10.58	88.75±4.804#	120.8±10.15*
2.	Triglycerides	40.75±3.262	92.33±4.645	51.98±3.975#	82.50±7.331*
3.	HDL	26.72±0.8157	14.40±0.9980	21.98±0.9769#	16.50±0.9397*
4.	LDL	29.50±3.896	94.80±6.225	41.91±3.373#	63.0±11.71*#
5.	Urea	72.13±4.858	127.3±14.31	69.40±4.925#	82.67±8.408#
6.	Creatinine	0.525±0.02598	1.070±0.1060	0.6575±0.2287#	0.7025±0.02780#
7.	Albumin	3.854±0.03202	1.775±0.1377	3.193±0.04230#	2.650±0.2021*
8.	SGPT	56.75±5.963	149.8±4.090	68±2.972#	88±4.340#
9.	SGOT	52.15±4.614	139±14.08	82.13±6.514#	97.75±3.945*#
10.	ALP	79.50±6.193	145.8±5.528	94.38±2.348#	103.8±5.234*#
11.	Bilirubin	0.2125±0.01315	0.4475±0.04820	0.2450±0.02363#	0.3475±0.3119*

* P<0.01 (Tukey test) significant when treated with Normal control

P<0.01 (Tukey test) significant when treated with Diabetic control

Histopathology

Photomicrographs (Fig.2) shows normal acini and normal cellular population in the islets of langerhans in pancreas of normal control (A) and lesions in diabetic rats (B) which maintained significantly after treatment by standard drug (C) and TGE (D) up to normal.

Photomicrographs (Fig.3) shows normal hepatocytes (A) and lesions in diabetic rats (B) which maintained significantly after treatment by standard drug (C) and TGE (D).

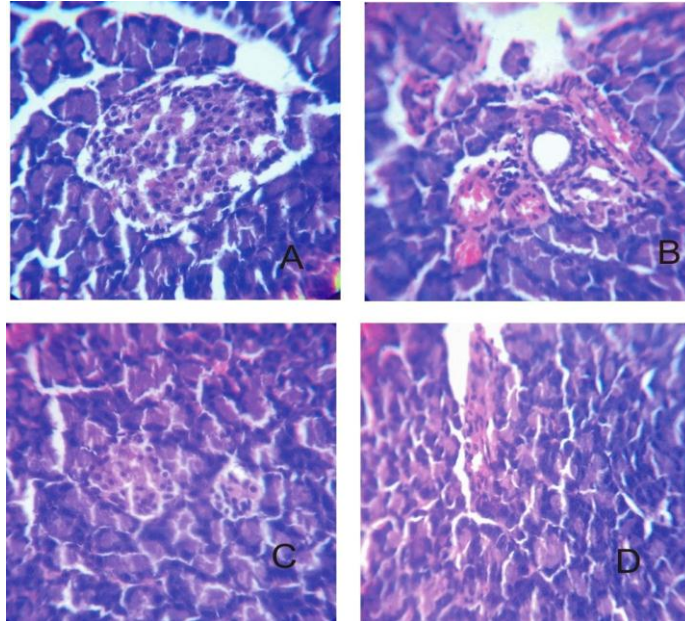


Fig. 2: Photomicrograph of rat pancreas stained by haematoxylin and eosin of normal control (A) diabetic control (B) standard (tolbutamide) treated (C) TGE treated (D)

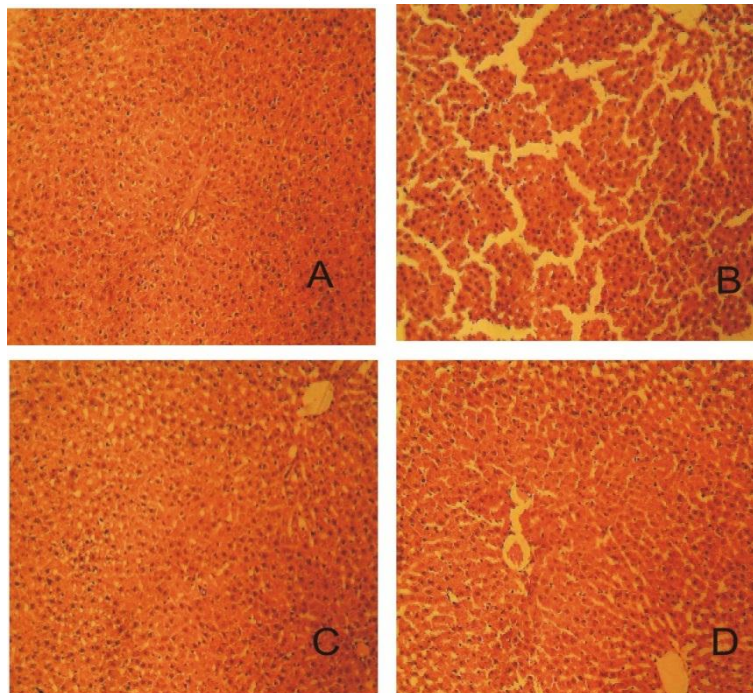


Fig. 3: Photomicrograph of rat liver stained by haematoxylin and eosin of normal control (A) diabetic control (B) standard (tolbutamide) treated (C) TGE treated (D)

Discussion

Medicinal plants are the potential source of bioactive agents and gaining acceptability worldwide. The ethnobotanical prospect can help for the development of drugs to treat human diseases like diabetes. Safe, effective and inexpensive indigenous remedies are gaining popularity equally among the people of both the urban and rural areas, especially in developing countries like India.³⁰

Alloxan induced diabetes has been commonly utilized as an animal model to study antidiabetic agents in experimental animals. Alloxan exerts its diabetogenic actions when administered intravenously, intraperitoneally or subcutaneously. The action of alloxan in the pancreas is preceded by its rapid uptake by the insulin-secreting beta cells.³¹ The cytotoxic action of alloxan is mediated by reactive oxygen species which leads to rapid destruction of beta cells, thereby reducing levels of insulin and increasing the blood glucose.^{32, 33} However there is possibility of survival of a few beta cells and this has been proved by several workers who observed antihyperglycemic activity with oral hypoglycemic agents in alloxan induced diabetes mellitus.^{34, 35} Fenugreek has primarily been described as an antihyperglycemic herb in humans as well as in laboratory animals.^{36, 37} Its cholesterol-reducing effect is also well established.³⁸

The present study indicates the effects of hydroalcoholic extract of *T. foenum graecum* seeds on alloxan induced diabetic rats. The plant material and extract was standardized before starting the experiment (results are given). Administration of alloxan led to elevation of blood glucose levels, which was maintained over a period of 3 weeks. Three weeks of daily treatment of TGE (1000 mg/kg bw per day) significantly reduce blood sugar levels. The anti-hyperglycemic effect of the TGE extract on the blood sugar levels of diabetic rat is shown in Table 1 and Fig. 1.

Serum cholesterol, serum triglycerides, serum, serum LDL, serum creatinine, serum urea, serum alkaline phosphatase (ALP), bilirubin, serum glutamate oxalate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT) were significantly decreased by Tolbutamide and TGE. HDL and albumin levels were significantly increased by Tolbutamide and TGE. Effects on serum profile are shown in Table 2.

Histopathological findings of pancreas of the diabetic rats showed necrosis, atrophy and fibrotic changes, but the pancreas of diabetic rats treated with TGE and Tolbutamide showed significant improvement. Histopathological findings of liver of the diabetic rats showed complete destruction of hepatocytes. Histopathological changes are restored near to normal in the group treated by the TGE extract.

Conclusion

Thus our study proves the beneficial effects of 70% v/v hydroalcoholic extract of *T. foenum graecum* seeds in the management of diabetes and its associated complications. Our findings support the long term use of the extract at the dose of 1000 mg/kg bw per day for better control of blood glucose and restorations of diabetes associated changes. After completing the preclinical studies the herbal products need to be tried on human diabetic patients to ascertain its efficacy and safety.

Acknowledgement: The author wish to thank to CEO, NKBR College of Pharmacy & Research Centre, Meerut and the Director, Siddhartha Institute of Pharmacy, Dehradun for providing facilities for the study.

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Cite this article as:

Alimuddin Saifi, Rajani Chauhan and Jaya Dwivedi. Evaluation of the antidiabetic activity of hydro-alcoholic extract of *Trigonella foenum-graecum* Linn. in alloxan induced diabetic rats. Asian Journal of Pharmacology and Toxicology, 04(15), 2016, 26-32.
