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Evaluation of the antidiabetic effect of *Trigonella foenum-graecum* seed powder on alloxaninduced diabetic albino rats

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Abstract: The antidiabetic effect of ethanolic extract of *Trigonella foenum-graecum* seed powder was investigated in alloxan-induced diabetic rats. Oral administration of ethanolic extract of *T. foenum-graecum* seed powder (50mg/100g bodyweight) for 48 days on the blood glucose level, serum cholesterol level, SGOT and SGPT level in normal and alloxan-induced diabetic rats were evaluated. Administrations of the herbal extract decreased blood glucose, serum cholesterol, SGOT and SGPT levels. Thus it is concluded that the ethanolic extract of *T. foenum-graecum* seed powder may be considered as a good candidate for future studies on diabetes mellitus.

Key words: Alloxan, antidiabetic, hypoglycemia, Trigonella foenum-graecum

Introduction

Diabetes mellitus (DM) is in the top 5 of the most significant diseases in the developed world, and is gaining in significance there and elsewhere. Present number of diabetics worldwide is 171 million and this is likely to increase to 340 million or more by the year 2030^{1,2}. Synthetic hypoglycemic agents can produce serious side effects and in addition they are not suitable to use during pregnancy³. Therefore, the search for more effective and safer hypoglycemic agents has continued to be an important area of active research. For a long time, diabetics have been treated with several medicinal plants or their extracts based on the folklore medicine⁴. Furthermore, after the recommendation made by WHO on diabetes mellitus, investigation on hypoglycemic agent from medicinal plants has been more important.

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Trigonella foenum-graecum, commonly known as fenugreek, is extensively used in many preparations of Ayurveda and also against antiulcer $action^5$ and hypocholesterolaemic effects^{6,7,8}. Fenugreek (*Trigonella*) foenum-graecum) is commonly used as a condiment and seasoning in food preparations; is assumed to possess nutritive and restorative properties⁹ and has been used in folk medicine for centuries for a wide range of diseases including diabetes, fever and abdominal colic as a poultice for abscesses, boils, and carbuncles¹⁰. The hypoglycemic property of fenugreek was observed in diabetic patients¹¹. However, no report is available on the effect of fenugreek seed in the alloxan-induced diabetic albino rats. Hence, in the present study of alcoholic extract of T. foenum-graecum seed was administered and antidiabetic effect was evaluated in alloxan-induced diabetic rats.

Material and Methods

Drugs and chemicals - Alloxan was purchased from local scientific company, Trichy and all other chemicals, and reagents used were of analytical grade.

Plant material - *Trigonella foenum-graecum* seed were chosen for the present study.

Preparation of plant extract - Hundred gram of dry powdered of *T. foenum-graecum* was continuously extracted for 48 h with 90% 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.

Experimental rats and induction diabetes -Albino male rats 7 to 8 weeks old and weighing 150 to 200g was used for the present study. The individuals which were obtained from a private animal husbandary, Trichy, brought to the laboratory and were maintained under controlled environment. The rats were randomized into control and experimental groups and housed 4 to 5 cages. Standard pellets obtained from Sai Durga Feeds & Foods, Bangalore were used as a basal diet during the experiment. The control and experimental individuals were provided food and drinking water *ad libitum*.

Diabetes mellitus was induced by single intraperitoneal injection of alloxan (150 mg/kg of body weight) dissolved in 0.1 m. citrate buffer (pH 4.5) to overnight fasted albino rats¹². The diabetes was assessed in alloxan-induced rats by determining the blood glucose level, 48 h after injection of alloxan. The rats with blood glucose level above 250 mg/dl were selected for the experimental studies.

Drug administration - The ethanolic extract of fenugreek seed was suspended in phosphate buffer and administered through pediatric cathedral tube at doses of 500mg/100g body wt. The volume of administered extract was 1 ml for each animal.

Experimental design - In the experiment, 24 rats (18 diabetic rats & 6 normal rats) were used: divided into 4 groups of six each.

Group I: Served as untreated control rats.

Group II: Served as diabetic control rats (150 mg/kg body weight i.p. Alloxan).

Group III: Diabetic rats orally administrated with saline only.

Group IV: Diabetic rats were orally administrated with ethanolic extract of *T. foenum-graecum* seed.

After the experimental period, all individuals were sacrificed for biochemical studies.

Biochemical analysis - Blood glucose was estimated by the methods of Sasaki *et al*¹³, Serum cholesterol was estimated by the methods of Zlatkis *et al*¹⁴, Serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) was estimated by the standard method of practical in biochemistry and clinical pathology.

Statistical analysis-Statistical analyses were carried out by SPSS (Windows based statistical package).

Data were expressed as mean \pm SD. Statistical analysis was performed using one way analysis of variance followed by SNK post hoc test. The criterion for statistical significance was p<0.05.

Results and Discussion

The present study indicated that there was a significant elevation in blood glucose, serum cholesterol, serum glutamate oxaloacetate transaminase (SGOT) level and serum glutamate pyruvate transaminase (SGPT) in alloxan-induced diabetic rats (Tables 1&4). The administration of the herbal extract significantly (p < 0.05) decreased blood glucose level in the diabetic rats when compared to the diabetic control rats (Tables 2&3). The oral dose of the herbal extract significantly (p<0.05) decreased the serum cholesterol level in alloxan-induced diabetic rats compared with the diabetic control rats. The oral dose of the herbal extract significantly (p<0.05) decreased the SGOT level and SGPT in alloxan-induced diabetic rats compared with the diabetic control rats (Tables 5&6). Mean glucose, Serum cholesterol, SGOT and SGPT levels of the group I (control) and group IV (extract treated) were observed to be similar, and significantly differed from that of group II (Diabetic rat) and group III (buffer treated). The result indicated that the effect of fenugreek seed extract tends to bring the values to near those of the non diabetic control rats.

Various reports revealed that fenugreek possess a plethora of benefits under various experimental conditions. The fenugreek seed possess antidiabetic effect¹⁵. The present study indicated that the extract of fenugreek seeds notably decreased blood glucose level in diabetic rats. In agreement with the present results, the hypoglycemic effect of fenugreek seeds has been experimentally proved in induced diabetic rats, dogs, mice and healthy volunteers and type-I and II diabetic patients^{15,16,17}. In recent years, considerable interest has been directed towards the investigation of total cholesterol in diabetes mellitus due to the fact that abnormal lipid levels lead to the development of coronary artery disease in diabetic patients. A marked increase in serum cholesterol level was observed in untreated diabetic rats, whereas the administration of herbal mixture significantly decreased serum cholesterol level in diabetic rats. In continence with the present data, other workers have reported that the administration of fenugreek seed extract lowered the serum cholesterol level in diabetic rats^{18,19} and hypercholesterolemic patients²⁰. Similar to these observations, Noor *et al*²¹ reported the antidiabetic activity of Aloe vera in streptozotocin induced diabetic rats. Further they have also mentioned that there are two possible explanations for this finding. First, A. vera may exert its effect by preventing the death of beta cells and/or second, it may permit recovery of partially destroyed beta cells. Burcelain *et al*²² reported that the hypoglycemic action of the extract of herbal plants in diabetic rats may be possible through the insulinomimictic action or by other mechanism such as stimulation of glucose uptake by peripheral tissue, inhibition of endogenous glucose production of activation of gluconeogenesis in liver and muscles. The antidiabetic activity of *T. foenum-graecum* may be possible through the mechanism as reported by Noor *et al*²¹ and Burcelain *et al*²². This is an interesting finding and suggests that fenugreek seed may have antioxidant or free radical scavenger properties in preventing these changes.

It may be concluded that fenugreek seed extract possess anti diabetic activities and the seeds extract may be used as an anti diabetic agent.

The plant should be considered as an excellent candidate for future studies on diabetes mellitus. In addition, further comprehensive pharmacologic investigations, including experimental chronic studies should be carried out.

Table 1: Changes in blood glucose level and serum cholesterol level in different experimental group rats after treatment with herbal extract.

Parameter	Group-I Mean±SD	Group-II Mean±SD	Group-III Mean±SD	Group-IV Mean±SD
Blood glucose (mg/dl)	81.0±651	333.2±47.12	293.7±17.01	71.8±6.14
Serum cholesterol (mg/dl)	68.6±2.58	175.0±5.56	163.8±6.30	64.2±2.77

Table 2: One way analysis of variance to know the influence of herbal extract on blood glucose and serum cholesterol level in alloxan-induced diabetic rats.

Parameter	Source	SS	DF	MSS	F	P *
Blood glucose level	Between groups	33.102	3	13.034	58.96	P<0.05
	Within groups	3.528	16	0.221		
	Total	36.630	19			
Serum	Between groups	53407	3	17802.333		
Cholesterol level	Within groups	340.300	16	21.269	837.018	P<0.05
	Total	53747.300	19			

Table 3: Multiple comparison test (Student-Newman Keul's Test) to know the influence of herbal extract on blood glucose and serum cholesterol level in alloxan-induced diabetic rats.

Parameter	Groups				
	Mean groups in homogeneous subsets (alpha=0.05)				
Blood glucose	71.6	81.0	293.7	332.2	
level (mg/dl)	Group-1V	Group-I	Group-III	Group-II	
			-		
Serum	64.2	68.6	163.8	175.00	
Cholesterol	Group-1V	Group-I	Group-III	Group-II	
level (mg/dl)					

Similar means are connected by horizontal line.

Table 4: Changes in serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) in different experimental group rats after treatment with herbal extract.

Parameter	Group-I Mean±SD	Group-II Mean±SD	Group-III Mean±SD	Group-IV Mean±SD
SGOT (IU/100ml)	51.0±6.30	136.8±9.41	139.0±7.41	54.0±10.27
SGPT (IU/100ml)	44.2±5.40	52.2±10.11	54.0±3.80	40.4±2.07

Table 5: One way analysis of variance to know the influence of herbal extract on serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) in alloxan-induced diabetic rats.

Parameter	Source	SS	DF	MSS	F	P *
SGOT (IU/100ml)	Between groups	36149.2	3	12049.73		P<0.05
	Within groups	1155.6	16	72.225	166.836	
	Total	37304.8	19			
SGPT (IU/100ml)	Between groups	627.400	3	209.1		
	Within groups	600.800	16	37.6	5.569	P<0.05
	Total	1228.200	19			

* P<0.05

Table 6: Multiple comparison test (Student-Newman Keul's Test) to know the influence of herbal extract on serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) in alloxan-induced diabetic rats.

Parameter	Groups			
	Mean groups in homogeneous subsets (alpha=0.05)			
SGOT	51.8	54.00	136.8	139.00
(IU/100ml)	Group-1	Group-IV	Group-II	Group-III
SGPT	40.4	44.20	52.20	54.00
(IU/100ml)	Group-1V	Group-I	Group-II	Group-III

Similar means are connected by horizontal line.

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