

## EFFECT OF AN ANTIDIABETIC EXTRACT OF *TRIGONELLA FOENUM - GRAECUM* ON NORMAL AND ALLOXAN INDUCED DIABETIC MICE

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### ABSTRACT

The present study was carried out to evaluate the antidiabetic effect of *Trigonella foenum - graecum* seed extract (TFSE) and to study the glucose content in blood, liver and pancreas of normal and alloxan induced diabetic mice. Oral administration of 0.5 ml aqueous extract of *T. foenum - graecum* (50 mg/animal) for 7 days. Artificial induction of diabetes resulted in tremendous increase in the glucose content of blood, liver and pancreas. The significance of such increase resulting in hyperglycemia. The administration of TFSE reduced the glucose level of not only blood but also other tissue like liver and pancreas. The significances of this reduction in glucose content and the possible mechanism for such action of TFSE.

**Keywords:** *Trigonella foenum - graecum*, Antidiabetic, Alloxan, Hyperglycemia, Pancreas

### INTRODUCTION

Plants have been considered as sources of medicinal agents for the treatment of many diseases. Before the advent of insulin injections and other pharmaceutical preparation, healers relied heavily upon medicinal plants and herbs to treat diabetes. More than 1200 plants have been described to be experimentally or ethno pharmacologically used in the treatment of diabetes mellitus<sup>1</sup>. Diabetes mellitus is the most important disease involving the endocrine pancreas. Type 2 diabetes is a heterogeneous disease with both genetic and environmental contributory factors, involved multiple defects in insulin action and insulin secretion leads to hyperglycemia and effecting nearly 10% of the population all over the world. In modern medicine, the beneficial effects on glycemic levels are well documented; the preventing activity of these drugs against progressive nature of diabetes and its micro and macro vascular complications was modest and not always effective<sup>2</sup>. Herbal treatments are becoming increasing by popular as the herbal preparations have no or least side effects<sup>3</sup>. Several studies on enzymes involved in hepatic glucose metabolism in rats with alloxan and streptozotocin diabetes have shown well defined changes, which consist primarily of a decrease in the activity of glucokinase, hexokinase<sup>4</sup>.

*Trigonella foenum-graecum* is cultivated throughout India and in certain regions of China. Its seeds are used as condiment in India, a supplement to wheat and maize flour for bread-making in Egypt, and one of the staple foods in Yemen. Its seeds are also be used as herbal medicine in many parts of the world for their carminative, tonic and aphrodisiac effects. Various reports have demonstrated that *Trigonella foenum-graecum* (fenugreek) seeds can lower blood glucose and cholesterol in type 1 and type 2 diabetics and experimental diabetic animals<sup>5, 6, 7, 8</sup>. However, the effects of fenugreek seeds, we intended to investigate the effects of aqueous extract of *Trigonella foenum-graecum* seeds on blood glucose level of normal and alloxan induced diabetic rats.

### MATERIAL AND METHODS

#### Plant material

*Trigonella foenum-graecum* seeds were collected from Arakkonam, Vellore District, Tamil Nadu, India. Collected seeds were shade-dried, cleaned and finely powdered and used for extraction.

#### Extraction of aqueous plant material

Aqueous extract of *T. foenum-graecum* seed powder was prepared by grinding 300mg of dried seeds in 3ml of glass distilled water. 0.5 ml of this solution was administered to each set of ten animals. Freshly prepared extracts alone were administered.

#### Chemicals

Alloxan monohydrate was purchased from SD Fine chemicals (Mumbai, India). All other chemicals used for this study were of analytical grade and obtained from HIMEDIA, SRL and Qualigens (India).

#### Animals

Healthy mature male mice with bodyweight ranging from 25 to 30 grams were selected and maintained in the cages. The mice are fed with commercial pellets supplied by Lipton, India. Food and water were provided ad libitum.

#### Induction of diabetes and study design

The blood glucose levels of normal male mice were determined and allowed to fast overnight. A single intra - peritoneal (i.p.) injection of alloxan monohydrate with a dosage of 120 mg / kg body weight in physiological saline was given<sup>9</sup>. This dosage was prepared because it produced maximum glucose levels. Mice with glucose levels ranging between 200 mg / dl and 350 mg / dl were considered severely diabetic and use for estimations of blood glucose at 1<sup>st</sup>, 3<sup>rd</sup>, 5<sup>th</sup> and 7<sup>th</sup> day after administration of alloxan.

The animals were divided in to four groups of five each.

Group I control mice with normal saline (5ml / animal).

Group II mice with oral administration of TFSE. (50mg / animal).

Group III alloxan induced diabetic mice (120 mg/kg body weight).

Group IV mice treated with TFSE (50mg / animal) after alloxan treatment.

The dosage to be most effective was 50mg / animal (0.5 ml of extract). Animals were segregated after 1<sup>st</sup>, 3<sup>rd</sup>, 5<sup>th</sup> and 7<sup>th</sup> day after administration of seed extract and the samples were collected. The same procedure was followed for alloxan induced diabetic animals. One ml of peripheral blood (PB) was collected from the mice in sterile screw capped glass vials containing EDTA by using sterile disposable syringes. The glucose level of blood, liver and pancreas were estimated by following the method of Sasaki *et al.*, 1972. The values were expressed as mg/gm wet tissues. For blood glucose values were expressed as mg/100ml.

### RESULTS

In the present study, the antidiabetic activity of extract of *T. foenum-graecum* was evaluated in normal and Alloxan induced diabetic rats. The results of the blood glucose content of male mice are given in table 1. The blood glucose level rose significantly to 250.98 ± 0.07

mg / 100 ml in the 1<sup>st</sup> day experimental animals and to 389.03 ± 0.02 mg / 100 ml in the 7<sup>th</sup> day experimental animals, when alloxan was administered to them. These results suggested the induction of diabetes by alloxan. In the 1<sup>st</sup> day experimental animals the blood glucose level dropped to 110.12 ± 0.03 mg / 100 ml and there was a gradual reduction in the 3<sup>rd</sup> day and 5<sup>th</sup> day experimental animals. A near normal value of 96.72 ± 0.05 mg / 100 ml was observed in the 7<sup>th</sup> day experimental animals. It is evident the administration of TFSE has brought down the blood sugar level significantly. However, the administrations of TFSE alone did not show any particular change in blood glucose level of control animals. If the blood sugar level of alloxan induced diabetic mice has been brought down by TFSE then it would be reasonable to expect changes in the liver glucose content as it is one of the sources of blood sugar.

The estimation of liver glucose has been carried out and the results are given in table 2. The liver glucose content varied between 189 ± 0.02 mg / gram wet tissue and 201 ± 0.02 mg / gram wet tissue in control mice. The differences between these results were statistically insignificant. In diabetic animals increased the liver glucose level to 293.55 ± 0.05mg / gram wet tissue in the 1<sup>st</sup> day experimental period from the control value of 191.02 ± 0.02 mg / gram wet tissue and the increase was very gradual from 1<sup>st</sup> day to 7<sup>th</sup>

day experimental periods. When TFSE was given to diabetic animals the value ranged between 190.13 ± 0.03 mg / gram and 201.34 ± 0.04 mg / gram wet tissue in the experimental animals suggesting the impact of TFSE on liver glucose. However, when TFSE was administered to control mice, the liver glucose contents ranged between 176.01 ± 0.04 mg / gram wet tissue to 183.92 ± 0.04 mg / gram wet tissue and the difference was statistically insignificant.

Another tissue which is clearly associated with diabetic is pancreas. Hence, estimation of its glucose content has been carried out and the results are given table 3. The pancreas glucose content of the control animals varied between 79.36 ± 0.03 mg / gram wet tissue and 83.92 ± 0.02 mg / gram wet tissue. When alloxan was given to induce diabetes artificially, the pancreatic glucose content of 1<sup>st</sup> day experimental animals was 130.01 ± 0.05 mg / gram wet tissue which gradually rose up during the 3<sup>rd</sup> and 5<sup>th</sup> day experimental periods before attaining the maximum value of 170.37 ± 0.07 mg / gram wet tissue in the 7<sup>th</sup> day experimental period. However, when TFSE was administered to diabetic animals the pancreatic glucose content in the 1<sup>st</sup> day experimental period was 92.89 ± 0.09 mg / gram wet tissue but in the 7<sup>th</sup> day experimental period it came down to 73.40 ± 0.04 mg / gram wet tissue, the values being nearer to normal ones.

**Table 1: The Effect of *Trigonella foenum-graecum* on blood glucose content in normal and alloxan-induced diabetic rats**

Groups	Blood glucose (mg / 100ml)			
	Experimental periods			
	1 <sup>st</sup> day	3 <sup>rd</sup> day	5 <sup>th</sup> day	7 <sup>th</sup> day
I Control (Received saline water)	96.02±0.02	97.81±0.01	96.13±0.01	95.81 0.01
II <i>Trigonella foenum-graecum</i> only	94.15* ±0.03	93.16* ±0.04	94.81* ±0.03	93.12* 0.04
Change in %	1.95	4.75	1.37	2.81
III Alloxan Administered	250.98**± 0.07	311.22**±0.01	339.11**±0.01	389.03** ±0.02
Change in %	161.38	218.19	252.76	306.04
IV Alloxan + <i>Trigonella foenum-graecum</i>	110.12** ±0.03	107.81** ±0.07	99.02** ±0.01	96.72* ±0.05
Change in %	14.68	10.22	3.01	0.95

\* Not significant    \*\* significant at 0.05 level    ± standard deviation (S.D.)

**Table 2: The Effect of *Trigonella foenum-graecum* on liver glucose content in normal and alloxan-induced diabetic rats**

Groups	Liver glucose (mg / gm of wet tissue)			
	Experimental periods			
	1 <sup>st</sup> day	3 <sup>rd</sup> day	5 <sup>th</sup> day	7 <sup>th</sup> day
I Control (Received saline water)	191.02±0.02	193.60±0.01	201.62±0.03	189.51±0.02
II <i>Trigonella foenum-graecum</i> only	176.01* ±0.04	183.92* ±0.04	179.36* ±0.05	178.70*±0.05
Change in %	7.86	5.00	11.04	5.70
III Alloxan Administered	293.55**± 0.05	294.32**±0.01	314.91**±0.01	324.02** ±0.02
Change in %	53.68	52.02	56.19	70.98
IV Alloxan + <i>Trigonella foenum-graecum</i>	194.01** ±0.05	198.92** ±0.08	201.34** ±0.04	190.13** ±0.03
Change in %	1.57	2.75	0.14	0.33

\* Not significant    \*\* significant at 0.05 level    ± standard deviation (S.D.)

**Table 3: The Effect of *Trigonella foenum-graecum* on pancreatic glucose content in normal and alloxan-induced diabetic rats**

Groups	Pancreatic glucose (mg / gm of wet tissue)			
	Experimental periods			
	1 <sup>st</sup> day	3 <sup>rd</sup> day	5 <sup>th</sup> day	7 <sup>th</sup> day
I Control (Received saline water)	83.92±0.02	79.36±0.03	80.11±0.02	81.32±0.02
II <i>Trigonella foenum-graecum</i> only	81.22* ±0.01	76.54* ±0.06	73.30* ±0.02	70.92*±0.10
Change in %	3.22	3.55	8.50	12.79
III Alloxan Administered	130.01**± 0.05	135.49**±0.34	158.61**±0.04	170.37** ±0.07
Change in %	54.92	70.73	97.99	109.51
IV Alloxan + <i>Trigonella foenum-graecum</i>	92.89** ±0.09	84.02** ±0.05	92.54** ±0.04	73.40** ±0.04
Change in %	10.69	5.87	15.52	9.74

\* Not significant    \*\* significant at 0.05 level    ± standard deviation (S.D.)

## DISCUSSION

In the present study, alloxan has been used to induce diabetes following previous authors. The administration of alloxan resulted in the steady increase in the blood glucose level during seven days of experimental period, indicating hyperglycemia. These observations are similar to those of Dixit *et al.*, 1986, Al - Hader, 1994, and Nagappa *et al.*, 2003 who have used alloxan to induce diabetes in a variety of species. Rerup 1970 and Nakabow *et al.*, 1978 reported that alloxan acted through strecker reaction on the beta cell directly and specifically leading to the destruction of these cells, thus causing cessation of insulin secretion. A similar condition exists in the present study. The increase in the blood glucose noticed in the present case after alloxan administration might be due to the destruction of beta cells of pancreas by alloxan.

The work of Chakravarthy *et al.*, 1982 lend a clue to this problem. They suggested that the damage to beta cells might not be permanent. Regeneration of beta cells of the islets of pancreas in alloxan induced diabetic rats by epicatechin has been cited as the support for above suggestion. The number of functionally intact beta cells in the islet organ is of decisive importance for the development course and outcome of diabetes. The total beta cell mass reflects the balance between the renewal and loss of these cells. Therefore, Gorray *et al.*, 1986 suggested that the regeneration of islets of beta cells following the destruction by alloxan may be the primary cause of the recovery of alloxan injected guinea pigs from the effect of the drug.

The results obtained in the present investigation on the blood glucose level during this period support such a possibility. It is clear from the tables that the administration of TFSE has brought glucose level back to normalcy. It is possible that the compounds present in the extract might have caused regeneration of beta cells effecting normal secretion of insulin.

A point of interest at this juncture will be to understand the sequence of events following increase in blood glucose level. The change in blood glucose level is reflected in the glucose content of the liver tissue. The liver glucose showed a similar increase along with the blood glucose level and hence it is of greater interest to know the reason for the increase in the liver glucose after alloxan treatment. Revathi and Namasivayam 2000 suggested that the liver glucose level increased because of glycogenolysis which was promoted by the hormones like glucagons, catecholamines and growth hormones.

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