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EFFECTS OF AQUEOUS EXTRACT OF *TRIGONELLA FOENM – GRAECUM* SEEDS ON PROTEIN CONTENT OF NORMAL AND ALLOXAN-INDUCED DIABETIC MICE

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ABSTRACT : The aim of the present study was to evaluate the antidiabetic potential of aqueous extract of *Trigonella foenm – graecum* seeds in normal and alloxan induced diabetic mice (*Mus musculus*) by administering oral dose (50mg / animal) of the extract for seven days. The administration of *T. foenm – graecum* seeds extract reduced the protein content of blood and also other tissue like liver and pancreas. The significant that *T. foenm – graecum* seeds extract has got tremendous effect on biochemical parameter protein content of blood, liver and pancreas towards near normal range.

Keywords: *Trigonella foenm – graecum*, antidiabetic, alloxan, liver, pancreas

INTRODUCTION

Diabetes mellitus (DM) is a group of syndrome characterized by hyperglycemia and altered metabolism of carbohydrates, lipids and proteins. Chronic hyperglycemia during diabetes causes glycation of body proteins that in turn leads to secondary complications affecting eyes, kidneys, nerves and arteries (Sharma 1993).Increasing evidences from both experimental and clinical studies suggest that oxidative stress plays a major role in the pathogenesis of DM. Free radicals are formed disproportionately in diabetes by glucose oxidation, nonenzymatic glycation of proteins and the subsequent oxidative degradation of glycated proteins (Maritim *et al* 2003). Without enough insulin, the cells of the body cannot absorb sufficient glucose from the blood; hence blood glucose levels increase, which is termed as hyperglycemia. If the glucose level in the blood remains high over a long period of time, this can result in long-term damage to organs, such as the kidneys, liver, eyes, nerves, heart and blood vessels (Mahendra and Bisht 2011). Diabetes mellitus is a metabolic disorder initially characterized by a loss of glucose homeostasis with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both (Barcelo and RajaPathak 2001). *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 (Kumar *et al*., 2005, Puri *et al* ., 2002). In the indigenous system of medicine, Ayurveda, a mention has been made on a number of plants for controlling diabetes but only a few have been scientifically evaluated and the active principles are isolated (Ivorra *et al*., 1989).

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 protein content 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 (Pandey and Khan 2002). 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 protein content at 1st, 3rd, 5th and 7th 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 1st, 3rd, 5th and 7th day after administration of seed extract and the samples were collected. The same procedure was followed for alloxan induced diabetic animals. The protein content level of blood, liver and pancreas were estimated by following the method of Lowry *et al.,* 1951. The values are expressed as µg/ 100mg of tissue and µg/100ml of blood.

RESULTS

Effect on blood protein content

Protein forms a major source for the production of other organic components such as carbohydrates which in turn are closely associated with diabetes. Hence, estimation of protein content of tissues has been made and the results are given in table 1.

The protein values for control mice varied between 8.04 ± 0.02 mg / 100 ml and 9.16 ± 0.02 mg / 100 ml. when alloxan was administered to normal animals, it paved way for the onset of diabetes. The blood protein content of the diabetic mice rapidly increased from 8.32 ± 0.02 mg / 100 ml to 10.17 ± 0.03 mg / 100 ml in the 1st day to 11.03 ± 0.04 mg / 100 ml in the 3rd day to 9.85 ± 0.11 mg / 100 ml in the 5th day and 9.83 ± 0.02 mg / 100 ml in the 7th day experimental animals.

Table 1. Blood protein content of male mice

Groups	Blood protein (mg / 100 ml) Experimental periods			
	1st day	3rd day	5th day	7thday
I Control (Received saline water)	8.31±0.02	9.16±0.02	8.61±0.02	8.04±0.02
II *Trigonella foenm- graecum* only	8.13*±0.02	8.66*±0.02	8.11*±0.03	7.91*±0.06
Change in %	2.17	5.46	5.81	1.62
III Alloxan administered	10.17**±0.03	11.03**±0.04	9.85**±0.11	9.83**±0.02
Change in %	22.38	20.41	14.40	22.26
IV Alloxan + *Trigonella foenm-graecum*	8.94**±0.04	8.36**±0.04	8.13**±0.04	8.09**±0.01
Change in %	7.58	8.73	5.57	0.62

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

All the values were significantly higher than that of the control animals. A point of interest was that when *T. foenm – graecum* seeds extract given to diabetic mice the blood protein values ranged between 8.09 ± 0.01 mg / 100 ml and 8.94 ± 0.04 mg / 100 ml, the values being closer to those of normal control animals. When *T. foenm – graecum* seeds extract alone was given to control animals the blood protein values ranged between 7.91 ± 0.06 mg / 100 ml and 8.66 ± 0.02 mg / 100 ml. the values were seemed to be nearer to the control and statistically insignificant.

Effect on liver protein content

The results on liver protein content are given in table 2. When alloxan was administered to the control animals, the liver protein content of the 1st day experimental animals rose higher up to 13.98 ± 0.01 mg / 100 mg of wet tissue. The same trend was noticed in the 3rd, 5th and 7th day of experimental animals. However, the administration of *T. foenm – graecum* seeds extract to diabetic mice caused a decrease the liver protein content ranged between 11.56 ± 0.01 and 12.31 ± 0.01 mg / 100 mg of wet tissue indicating the revival of the liver protein content to its normal value. When *T. foenm – graecum* seeds extract was alone given to the normal animals the liver protein values ranged between 10.07 ± 0.01 and 11.96 ± 0.06 mg / 100 mg of wet tissue. Therefore, it was clear that the *T. foenm – graecum* seeds extract administration to the control animals did not show any significance.

Table 2. Liver protein content of male mice

Groups	Liver protein (mg / 100mg of wet tissue) Experimental periods			
	1st day	3rd day	5th day	7thday
I Control (Received saline water)	11.91±0.02	11.88±0.03	11.67±0.02	12.89±0.02
II *Trigonella foenm- graecum* only	11.03*±0.07	10.03*±.07	11.06*±0.02	11.96*±0.06
Change in %	7.39	15.57	5.23	7.21
III Alloxan administered	13.98**±0.01	15.13**±0.02	14.57**±0.01	15.83**±0.03
Change in %	17.38	27.36	24.85	22.81
IV Alloxan + *Trigonella foenm-graecum*	12.13**±0.01	11.56**±0.01	12.31**±0.01	11.96**±0.02
Change in %	1.85	2.69	5.48	7.21

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

Effect on pancreatic protein content

Table 3 clearly show the results obtained on the pancreatic protein content. When alloxan was given to cause diabetes in mice, the values of pancreatic protein rapidly increased from 1st day to 7th day experimental animals. However, when *T. foenm – graecum* seeds extract was given to diabetic animals the pancreatic protein content in the 1st day experimental animals was gradually reduced the pancreatic protein to normal level.

Table 3. Pancreatic protein content of male mice

Groups	Pancreatic protein (mg / 100mg of wet tissue)			
	Experimental periods			
	1st day	3rd day	5th day	7thday
I Control (Received saline water)	4.94±0.01	4.61±0.02	4.53±0.01	4.70±0.02
II *Trigonella foenm- graecum* only	4.16*±0.01	4.03*±0.01	4.17*±0.01	4.03*±0.01
Change in %	15.79	12.58	7.95	14.26
III Alloxan administered	5.31**±0.03	5.58**±0.03	5.96**±0.03	5.77**±0.03
Change in %	7.49	21.04	31.57	22.77
IV Alloxan + *Trigonella foenm-graecum*	4.96**±0.03	4.85**±0.02	4.13**±0.02	4.51**±0.02
Change in %	0.40	5.21	8.83	4.04

DISCUSSION

The work of Funabiki *et al*., (1986) is relevant here. They reported that alloxan triggered the synthesis of tissue proteins thereby increasing the level of protein. Another factor for observed increase might be the inhibition of proteolysis. Such a possibility has been indicated in the work of Mortimore and Mondon (1970) who observed inhibition of proteolysis in liver tissue of alloxan induced diabetic rats. In the light of these observations, it is clear that the increase in liver protein content might be due to both the factors.

The increase in protein content of liver and blood is also reflected in pancreatic protein content. An increase in protein content of pancreas has been observed in the present investigation. It is possible that alloxan or its products would have caused the increase in protein synthesis. The observation of Akimoto *et al*., (2000) lends support to this suggestion. These authors observed increase in O-GlcNAc Transferase enzyme activity consequent to alloxan administration might have caused increase in the protein content.

If the increase in protein content is a factor then it would be reasonable to find ways to control such increase. The application of medicinal plants is one of the ways that can control the biochemical parameters. When the medicinal plants extract was given to alloxan induced diabetic animals, there was reduction in the protein contents of all the three tissues indicating the impact of *T. foenm – graecum* seeds extract. The fact that the medicinal plants can control protein is derived from the observation of Prakash *et al*., (1995). If *T. foenm – graecum* seeds extract could have such impact, then it would be of interest to understand the nature of the substance present in the *T. foenm – graecum* seeds extract, which might be control the biochemical parameters. The work of Anuratha and Ravikumar (2001) indicate that some of the soluble particles of *T. foenm – graecum* seeds could be responsible for the antioxidant property when administered to alloxan induced diabetic rats. Further studies on this aspect may shed light on the nature of such soluble particles of *T. foenm – graecum* seeds.

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REFERENCES

Akimoto Y, Kreppel LK, Hirano H, Hart GW (2000). Increased O-GlcNAc transferase in pancreas of rats with streptozotocin-induced diabetes. Diabetologia 43(10) : 1239-1247.

Anuradha CV, Ravikumar P (2001). Restoration of tissue antioxidants by Fenugreek seeds (*Trigonella foenumgraecum*) in alloxan-diabetic rats. Indian Journal of Physiology and Pharmacology 45: 408–420.

Barcelo A, RajaPathak. S (2001). Incidence and prevalence of diabetes mellitus in the Americas, American journal of public health. 10: 300-308.

Funabiki R., Nyumura N, Hara H, Yagasaki K (1986). Protein synthesis rate in brain, liver, skeletal muscle and intestines by starved or diabetic mice. Rep. Res. Commun. Essen. Amino Acids 112: 17–19.

Ivorra MD, Paya M, Villar A (1989). A review of natural products and plants as potential antidiabetic drugs. J Ethnopharmacol 27: 243-75.

Kumar GS, Shetty AK, Sambaiah K, Salimath PV (2005) Antidiabetic property of fenugreek seed mucilage and spent turmeric in streptozotocin-induced diabetic rats. Nutr Res 25 : 1021-1028.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951). Protein measurement with the folin phenol reagent. J Biol Chem 193: 265-75.

Mahendra P, Bisht S (2011). Diagnostic criteria in different population for diabetes mellitus. Journal of Pharmacy Research 4(4): 1189-1192.

Maritim AC, Sanders RA, Watkins JB (2003). III. Diabetes, oxidative stress and antioxidants: a review. J Biochem Mol Toxicol 17: 24-38.

Mortimore G E, Mondon CE (1970). Inhibition by insulin of valine turnover in the liver. J Biol Chem 245: 2375-2383.

Pandey M, Khan A (2002). Hypoglycaemic effect of defatted seeds and water soluble fiber from the seeds of *Syzygium cumini* (Linn.) Skeels in alloxan diabetic rats. Indian J of Expt Biol 40: 1178 – 1182.

Prakash AO, Dehadrai S, Jonathan S (1995). Toxicological studies on the ethanolic extract of *Crotalaria juncea* seeds in rats. J Ethnopharmacol 45: 167-176.

Puri D, Prabhu KM, Murthy PS (2002). Mechanism of action of a hypoglycemic principle isolated from fenugreek seeds. Indian J Physiol Pharmacol 4: 457-462.

Sasaki T, Natsy S, Sonae A (1972). Effect of acetic acid concentration on the colour reaction in the O – toluidine boric acid method for blood glucose estimation. Rinkshokagaku 1 : 346- 353.

Sharma AK (1993). Diabetes mellitus and its complications: An update, 1ed. Macmillan India Ltd, New Delhi: Sharma AK (ed), 92- 205.