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Effect of *Trigonella foenum graecum* (Fenugreek) on diabetes induced oxidative stress and tissue antioxidants in streptozotic diabetic rats

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ABSTRACT

The aim of the present study was to evaluate the effect of aqueous extracts of *Trigonella foenum graecum* seeds and leaves and compare their effects on lipid peroxidation and the levels of antioxidant enzymes in STZ – diabetic rats. Oral administration of the extracts (both seed and leaf) was given to two different groups of rats, for 25 days. We observed that the seed extract was more efficient than the leaf extract in combating oxidative stress in the heart, pancreas and liver of the diabetic rats and it is also augmented the antioxidant enzymes – superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) which were decreased in the STZ diabetic rats. The treatments of TFG seed extract (TFGSEt) and leaf extract (TFGLEt) were compared with that of treatment with glibenclaminde (300 µg kg^{-1}). This study clearly demonstrates that the TFGSEt is better than TFGLEt, and it seems to have more antiperoxidative activity.

Key words: *Trigonella foenum graecum*, Reactive oxygen species, Diabetes mellitus, Antioxidant enzymes.

INTRODUCTION

Diabetes currently is a major health problem for the people around the world. It is a chronic disorder of carbohydrate, fat and protein metabolism characterized by elevation of both fasting and postprandial blood sugar levels.

Increased generation of reactive oxygen species (ROS) and lipid peroxidation has been recognized in several diseases including diabetes mellitus. Oxidative stress and poor metabolic control enhance lipid peroxidation in diabetes (Costagliola *et al.*, 1998). Under physiological conditions autoxidation of glucose leads to H_2O_2, ROS and reactive ketoaldehydes which modify cellular proteins leading to their fragmentation by free radical mechanism (Hunt and Wolff, 1991). The levels of these ROS are controlled by various cellular defense mechanisms consisting of enzymatic [superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx)] and non-enzymatic [glutathione (GSH), vitamins A, E and C] scavenging components (Wohaieb and Godin,1987). An imbalance between the generation of ROS and the scavenging system results in uncontrolled and excessive production of ROS thereby leading to tissue damage.

The management of diabetes mellitus is a global problem, modern drugs including insulin and oral hypoglycemic agents control blood sugar levels with a number of undesirable side effects (Akhtar and Iqbal, 1991; Holman and Turner, 1991). The recommendations made by WHO on diabetes mellitus investigations on hypoglycemic agents from

medicinal plants have become important (WHO, 1980). The present study, therefore, aims at using *Trigonella foenum graecum* seeds and leaves in the treatment of diabetes mellitus.

Administration of TFG seed powder to diabetic animals has been shown to lower blood glucose levels (Shani *et al*, 1974) and shown to lower blood glucose levels and partially restore the activities of key enzymes of carbohydrate and lipid metabolism (Vats *et al*., 2003; Raju, *et al*., 2001). The hypoglycemic effect of *Trigonella* seeds and their major alkaloid trigonellin was first described by Fournier (1948) and saponin compounds diasgenin by Al Habori *et al* (2001). The chemical constituents of *Trigonella* seeds include volatile oils, alkaloids, saponins, sapogenins, flavonoids and mucilage (Duke, 1992). The seeds are therefore widely recommended for non – insulin dependent diabetes mellitus patients (Puri *et al*., 2002) and have also shown to have antioxidant property.

However, most of the studies were concentrated on *Trigonella* seeds and no effort was taken to study the comparative effects of leaves of *Trigonella foenum graecum.* Therefore it was considered worthwhile to evaluate and assess the antioxidant effect of the aqueous extracts of TFG seeds and leaves in STZ induced diabetes mellitus.

MATERIAL AND METHODS

Animals

Wistar male rats weighing 150 – 180g were obtained from Center for Animal Health Studies, Chennai, India.

Drugs and Chemicals

Streptozotocin and thiobarbituric acid (TBA) were purchased from Sigma Chemicals Co (St. Louis. MO USA). All other chemicals used were of analytical grade.

Induction of Diabetes

The animals were fasted for 18h then injected intravenously with 42mg STZ/Kg body weight in saline solution. Between 4 to 10 days after the treatment with STZ, the development of diabetes could be verified by an increase in the concentration of blood glucose in rats with a fasting blood glucose range 250 – 300 mg dl^{-1} were considered diabetic and included in the study.

Plant extract

Trigonella foenum seeds and leaves were dried in shade at 25°C and powdered with a blender. 50 g of powdered seed and leaves were mixed with 250 ml of distilled water were stirred magnetically overnight. The residue was removed by filtration and the aqueous extract was concentrated under vacuum and used in the study.

Experimental design

A total of 30 rats (6 normal and 24 STZ – Diabetic) were divided into four groups of 6 rats each.

Group I - Normal untreated rats
Group II - STZ – diabetic rats
Group III - STZ diabetic rats treated with TFGSEt 500mg / kg body wt/ twice a day.
Group IV - STZ diabetic rats treated with TFGLEt 500mg/ kg body wt / twice a day.
Group V - STZ diabetic rats treated with glibenclaminde (300µg/kgbody weight) twice a day

The TFGSEt and TFGLEt (500/ kg body weight) and glibenclamide (300µg/kg body weight) were administered in distilled water using an intragastric tube twice a day for 25 days. After 25 days of treatment, all the rats were sacrificied after an overnight fast. Blood was collected in potassium oxalate and sodium fluoride containing tubes for estimation of fasting blood glucose. Heart, pancreas and liver were removed, rinsed in ice cold water, weighed and homogenized for the study.

Biochemical estimation

Fasting blood glucose (Sasaki and Matsui, 1972), thiobarbituric acid reactive substances (TBARS) (Yagi, K. 1976), superoxide dismutase (SOD) (Misra and Fridovich, 1972), glutathione peroxidase (GPx) (Rotruck *et al*., 1973) catalase (CAT) (Sinha, 1972) and reduced glutathione (GSH) (Beutler, and Kelly, 1963) were assayed.

Statistical Analysis

Statistical analysis of the data was done

using students '*t*' test. Results are expressed as mean ± S.D.

RESULTS

In streptozotocin induced diabetic rats there was a significant ($p<0.001$) increase in fasting blood glucose of diabetic rats over the normal as shown in Table 1. Administration of TFGSEt and TFGLEt brought about a marked decrease in the blood glucose levels comparable to that of glibenclamide treated rats.

Table 2 shows a statistically significant increase in lipid peroxide levels ($p<0.001$) in streptozotocin induced diabetic rats with respect to normal controls and there was a significant decrease in lipid peroxide levels in diabetic rats treated with both *Trigonella* seed and leaf extract ($p<0.001$). Reduced glutathione was nearly halved in the STZ – diabetic rats when compared to normal controls in all the tissues studied. The levels improved on administration of the TFGSEt and TFGLEt

Table 3 shows the activities of the antioxidant enzymes SOD, CAT and GPx. In our study we report a significant reduction of all the three enzymes in the diabetic rats, while a dramatic recovery is observed on administration of the seed and leaf extracts of Trigonella foenum which restored them to near normal levels.

DISCUSSION

A scientific investigation of herbal remedies for diabetes may provide valuable leads for the development of alternative drug and therapeutic strategies. Diabetes is possibly the world's fastest growing metabolic disease, and as knowledge of heterogeneity of this disorder increases, so does the need for more appropriate therapies (Baily and Flatt, 1986). Traditional plant medicines as antidiabetics have been reviewed by Grover *et a.,l* (2002). Appropriate nutritional management is essential for restoring and maintaining a normal metabolic state. Therefore, diet remains a cornerstone in diabetic management. Spices like fenugreek have been shown to be beneficial.

In the present investigation we have compared the effects of TFGSEt and TFGLEt in the treatment of diabetes mellitus. Although a number of studies have reported using the TFGSEt there is lacunae in understanding if the leaf could also be used for treatment.

The results of the present study showed that the extracts both of the seeds and leaves produce a marked decrease in blood glucose levels of STZ induced diabetic rats. The hypoglycemic effect increased gradually at 25 days it was found to be maximum (data not supplied). The effect was observed to be slow but sustained for both extracts, without any risk of developing hypoglycemia.

Table 1: Effect of TFGSEt and TFGLEt on blood glucose in STZ – diabetic rats as compared to glibenclamide treated

	Blood glucose mg/dl	
	Initial	**Final**
Normal	80.25 ± 5.21	84.75 ± 3.21
STZ treated diabetic	295.2 ± 14.1*	327.2 ± 24.1*
STZ treated diabetic + TFGSEt	290.1 ± 16.2 *	98.1 ± 4.4
STZ treated diabetic + TFGLEt	292.5 ± 14.3*	99.2 ± 5.2
STZ treated diabetic + glibenclamide	292.4 ± 19.2*	142 ± 9.1*

Values are mean ± S.D for 6 rats in each group

Statistically significant differences are expressed as *$p<0.001$, when compared with normal rats.

Table 2: Effect of TFGSEt and TFGLEt on thiobarbituric reactive substance (TBARS) and reduced glutathione in heart, pancreas and liver of STZ – diabetic rats (n = 6).

Groups	Lipid peroxides(mmol / 100g fresh tissue)			GSH (mg / 100 fresh tissue)		
	Heart	Pancreas	Liver	Heart	Pancreas	Liver
Normal	0.40 ± 0.03	24.2 ± 2.26	0.59 ± 0.16	42.2 ± 6.2	25.4 ± 1.5	49.8 ± 6.5
STZ treated	0.70 ± 0.05*	48.8 ± 5.27*	0.83 ± 0.20*	25.1 ± 5.1	16.8 ± 0.2	28.2 ± 5.5
STZ treated + TFGSEt	0.53 ± 0.02*	31.1 ± 4.25*	0.48 ± 0.13*	36.3 ± 4.8*	23.6 ± 1.3*	46.2 ± 6.1*
STZ treated + TFGLEt	0.59 ± 0.04*	35.3 ± 5.45*	0.62 ± 0.24*	33.5 ± 5.2*	21.0 ± 1.5*	42.3 ± 5.2*
STZ treated + glibenclamide	0.54 ± 0.01*	34.2 ± 5.11*	0.44 ± 0.11*	35.2 ± 1.7*	22.5 ± 0.89*	38.7 ± 4.2*

Statistically significant differences are expressed as *p<0.001, when groups III, IV and V were compared with STZ induced diabetic rats.

Table 3: Effect of TFGSEt and TFGLEt compared to glibenclamide on superoxide dismutase, catalase and glutathione peroxidase in heart, pancreas and liver of diabetic rats (n = 6).

Groups	SOD (Units mg^{-1} protein)			Catalase (µ moles of H_2O_2 consumed min^{-1} mg^{-1} protein)			GPx (µg of GSH consumed min^{-1} mg^{-1} protein)		
	Heart	Pancreas	Liver	Heart	Pancreas	Liver	Heart	Pancreas	Liver
Normal	42.2 ± 6.2	25.4 ± 1.47	49.8 ± 6.5	7.48 ± 0.45	18.2 ± 0.91	52.8 ± 9.2	0.95 ± 0.06	32.1 ± 2.85	43.2 ± 1.82
STZ treated	25.1 ± 5.1	16.8 ± 0.20	28.2 ± 5.5	4.21 ± 0.21	8.05 ± 0.45	25.4 ± 4.5	0.42 ± 0.03	16.1 ± 1.05	21.1 ± 0.6
STZ treated + TFGSEt	36.3 ± 4.8*	23.6 ± 1.29*	46.2 ± 6.1*	7.25 ± 0.51*	17.2 ± 0.80*	51.8 ± 8.5*	0.88 ± 0.11*	29.1 ± 3.01*	42.1 ± 1.8*
STZ treated + TFGLEt	32.1 ± 2.9*	20.2 ± 1.20*	33.5 ± 6.2*	6.02 ± 0.58*	13.2 ± 0.65*	47.6 ± 5.3*	0.76 ± 0.23*	24 ± 2.04*	37.6 ± 2.3*
STZ treated + glibenclamide	35.2 ± 1.7*	22.5 ± 0.89*	38.7 ± 4.2*	6.05 ± 0.44*	16.0 ± 0.45*	50.3 ± 9.5*	0.80 ± 0.08*	30.1 ± 2.11*	41.2 ± 0.9*

Statistically significant differences are expressed as *p<0.001 when groups III, IV and V were compared with STZ diabetic rats.

The important parameter in evaluating the effect of an anti-diabetic compound, apart from being hypoglycemic in nature is in the restoration of the antioxidant status. We have studied the three major antioxidant enzymes SOD, CAT and GPx and reduced glutathione in the tissues after the supplementation of TFGSEt and TFGLEt and compared it with the diabetic control. An increase in the antioxidants after the administration of the extract has been reported in this study, which is in agreement with earlier published reports (Dixit *et al*., 2005).

In vitro and *in vivo* studies have shown that in a variety of tissues hyperglycemia results in the generation of oxygen free radicals and considerably increase oxidative stress. Lipid peroxidation is a marker of cellular oxidative damage initiated by reactive oxygen species (Farber *et al*., 1990). It was reported that diabetics are highly sensitive to oxidative stress (Baynes, 1991; Urano, 1991). In STZ-diabetic rats there is an elevated lipid peroxide levels as compared to the controls while the plant extract was able to reduce the lipid peroxides, although the seed extract is better than the leaf extract. The data included in this work suggested that the extracts (TFGSEt & TFGLEt) prevents cellular damage induced by STZ *via* inhibition of lipid peroxidation possibly because of the very high content of alkaloids, saponins and flavonoid in accordance with the data provided by Duke (1992).

Fowden *et al.,* (1973) was the first to isolate and identify the unusual amino acid, 4-hydroxy isoleucine in the TFGSEt. It was estimated that this amino acid accounted for 80% of the total amino acid within the seeds and it has the capacity to stimulate insulin secretion by direct effect on pancreatic β cells in rats and humans.

It would be of interest to assess if the leaf extract also contains 4-hydroxy isoleucine or some other compound which is able to bring about the insulin secretion. Further studies are required to confirm whether the extract apart from acting as a hypoglycemic agent also act by either directly scavenging the reactive oxygen metabolites, due to the presence of various antioxidant compounds (Gupta *et al*., 2002), or by increasing the synthesis of antioxidant molecules.

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