

Effect of *Trigonella foenum-graecum* seed powder on the antioxidant levels of high fat diet and low dose streptozotocin induced type II diabetic rats

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Abstract. – BACKGROUND AND OBJECTIVES, Type II diabetes is a disease characterized by chronic hyperglycaemia and oxidative stress. Among the natural products, *Trigonella foenum-graecum* (Fenugreek) is found to have many active bio molecules. It is used traditionally in Indian folk medicine to treat diabetes.

MATERIALS AND METHODS, In the present study, the antioxidative potential of *Trigonella foenum-graecum* seed powder was assessed in high fat diet and low dose streptozotocin (35 mg/kg body weight) induced type II diabetic rats. Male Sprague Dawley rats were used for the study. Lipid peroxidation and the antioxidant activities (catalase, superoxide dismutase, glutathione peroxidase, glutathione reductase and reduced glutathione) were measured in pancreas and liver tissues of normal, diabetic and diabetic + *Trigonella foenum-graecum* treated rats. The diabetic + glibenclamide treated rats served as positive control.

RESULTS, Treatment of diabetic rats with *Trigonella foenum-graecum* significantly ($p \leq 0.001$) improved the fasting blood glucose levels to near normal blood glucose levels. The levels of thiobarbituric acid reactive substances (TBARS) were significantly higher and the activities of antioxidants were found to be lowered in diabetic rats, as compared to the normal rats. Improved activities of antioxidants and a significant decline in the levels of TBARS were observed in both *Trigonella foenum-graecum* treated and glibenclamide treated diabetic rats.

CONCLUSIONS, *Trigonella foenum-graecum*, apart from controlling the blood glucose levels, also has antioxidant potential to protect the organs such as liver and pancreas against the oxidative damage induced by diabetes.

Key Words:

Oxidative stress, Diabetes, Fenugreek, Antioxidants, Streptozotocin, Free radicals.

Introduction

Type II diabetes is a disease resulting from insulin deficiency and resistance to peripheral insulin action that causes a chronic hyperglycaemic state. In a pre-diabetic state, β -cells can overcome deficiency of insulin action by increasing insulin secretion. However, β -cell function increasingly deteriorates, and because the cells are unable to compensate for insulin resistance, hyperglycaemia ensues. Chronic hyperglycaemia can cause oxidative stress, leading to defective insulin secretion¹. Several lines of evidences suggest that type II diabetes is characterized by peripheral insulin resistance, pancreatic β -cell dysfunction, and decreased β -cell mass²⁻⁴ associated with increased rate of β -cell apoptosis. Obesity, especially abdominal obesity, appears to be one of the most important determinants of the risk of developing insulin resistance and type II diabetes. An increasing body of evidence suggests that free radical formation and oxidative stress are involved in the pathogenesis of diabetes and the development of diabetic complications^{5,6}. The reactive oxygen species (ROS) generation is increased in diabetes due to prolonged exposure to hyperglycaemia. Insufficient antioxidant defence mechanisms have been reported in diabetes⁷.

The pathogenesis of diabetes mellitus and the possibility of its management by the oral administration of hypoglycaemic agents have stimulated greater interest in recent years⁸. The drugs currently available for type II diabetes have a number of limitations, such as significant side effects including haematological, gastro-intestinal reactions, hypoglycaemic coma, and disturbances in liver and kidney metabolisms and high rates of secondary failure. In addition, these preparations

are not ideal for use during pregnancy⁹. As the knowledge of the heterogeneity of this disorder increases, there is a need to look for more effective agents with fewer side effects¹⁰.

Plant drugs are frequently considered to be less toxic and freer from side-effects than synthetic ones¹¹. One such traditional plant is *Trigonella foenum-graecum* commonly called fenugreek. India is the largest producer of fenugreek in the world. It is well known for its hypoglycaemic properties. Fenugreek seeds contain lysine and L-tryptophan rich proteins, mucilaginous fibre and other rare chemical constituents such as saponins (viz. diosgenin, yamogenin, gitogenin, tigogenin, and neotigogens), coumarin, fenugreekine, nicotinic acid, saponin, phytic acid, scopoletin and trigonelline, which are thought to account for many of its presumed therapeutic effects¹². The antihyperglycaemic effects of fenugreek seeds and its sub fractions are demonstrated in diabetic rats¹³ and also in humans¹⁴. The oxidative damage to tissues caused by diabetes may be a contributory factor to several diabetic complications. The combination of high fat diet and low dose streptozotocin treated rat serves as an animal model that replicates the natural history and metabolic characteristics of human type II diabetes and is also suitable for testing anti-diabetic agents for the treatment of type II diabetes¹⁵. Hence, the present study is focused on the effect of fenugreek on the antioxidant status of high fat diet and low dose streptozotocin induced type II diabetic rats.

Materials and Methods

Animals

Male Sprague Dawley rats of body weight 250-300 g supplied by NCLAS, National Institute of Nutrition, Hyderabad, Andhra Pradesh, India were used in this study. The animals were bred in the Central Animal House, Pondicherry University, Puducherry, India and fed on the standard pellet diet (Hindustan Lever Limited, Mumbai, India) for acclimatization. Water was given *ad libitum*. The standard pellet diet comprised 21% protein, 5% lipids, 4% crude fiber, 8% ash, 1% calcium, 0.6% phosphorus, 3.4% glucose, 2% vitamins and 55% nitrogen free extract (carbohydrates). It produces a metabolisable energy of 3600 KCal.

The animals were housed in plastic cages under controlled condition of 12h light/12h dark

cycles, 50% humidity and at $30 \pm 2^\circ\text{C}$. The animals used in the present study were maintained in accordance with the guidelines of the National Institute of Nutrition, Indian Council for Medical Research, Hyderabad, India and approved by the Institutional Animal Ethical Committee (IAEC), Pondicherry University.

Chemicals

Trigonella foenum-graecum seeds were purchased from local market in Puducherry, India and the seed powder was prepared. Streptozotocin and glibenclamide were purchased from Sigma-Aldrich (Bangalore, India). All chemicals and solvents used were of high purity and analytical grade.

Preparation of High Fat Diet

High fat diet was freshly prepared every day according to Srinivasan et al¹⁵.

Induction of Experimental Diabetes

Diabetes was induced in male Sprague Dawley rats by feeding them with high fat diet (HFD) for two weeks followed by a single intraperitoneal injection of streptozotocin (STZ) in 0.2 M citrate buffer (pH 4.0) at a dose of 35 mg/kg body weight¹⁵. The diabetic rats were fed with high fat diet until sacrifice.

Experimental Design

The animals were divided into 6 groups of 4 rats each.

Group I: (Normal). Control rats

Group II: (Diabetic). Rats were given HFD for two weeks followed by STZ dose and continued with HFD for two weeks

Group III: (Diabetic + 100 mg seed powder). Rats were given HFD for two weeks followed by STZ dose and continued with HFD mixed with 100 mg/kg body weight *T. foenum-graecum* seed powder for two weeks

Group IV: (Diabetic + 250 mg seed powder). Rats were given HFD for two weeks followed by STZ dose and continued with HFD mixed with 250 mg/kg body weight *T. foenum-graecum* seed powder for two weeks

Group V: (Diabetic + 500 mg seed powder). Rats were given HFD for two weeks followed by STZ dose and continued with HFD mixed with 500 mg/kg body weight *T.*

foenum-graecum seed powder for two weeks

Group VI: (Diabetic + Glibenclamide). Rats were given HFD for two weeks followed by STZ dose and continued with HFD mixed with 0.5 mg/kg body weight glibenclamide for two weeks

At the end of the experimental period, rats were weighed, killed after an overnight fast by cervical dislocation. Liver and pancreas were removed, cleared off blood and immediately transferred to ice cold containers containing phosphate buffer saline (pH 7.4). Blood was collected in heparinised tubes.

Biochemical Parameters

The liver and pancreas were weighed and homogenized in appropriate buffer (10%) for the estimation of various biochemical parameters. Blood glucose was estimated by ortho-toluidine method¹⁶. The markers of oxidative stress viz. lipid peroxidation and reduced glutathione were estimated by the method of Ohkawa et al¹⁷ and Ellman¹⁸ respectively. The free radical scavenging property of *T. foenum-graecum* was assayed by following the levels of antioxidant enzymes. The activity of catalase was assayed by the method described by Claiborne¹⁹ and that of superoxide dismutase by the methods of Marklund and Marklund²⁰. The activity of glutathione peroxidase was assayed by Rotruck et al²¹ and that of glutathione reductase was assayed by Carlberg and Mannervik²². Protein was estimated by the method of Lowry et al²³ using bovine serum albumin (BSA) as standard.

Statistical Analysis

Data were expressed as Mean \pm S.D. All the data were analyzed using the SPSS 7.5 –Windows Students version software (SPSS Inc., Chicago, IL, USA). One-way ANOVA followed by Tukey's test was used to assess the statistical significance between groups. $p \leq 0.05$ was considered to be statistically significant.

Results

Levels of Blood Glucose

The levels of blood glucose in diabetic group increased significantly when compared to control group. Treatment with *T. foenum-graecum* seed powder caused a significant reduction in the glu-

cose levels at all the three concentrations used. The change in the glucose level was significantly lower in both 100 mg and 250 mg *T. foenum-graecum* treated groups when compared to diabetic group. The glucose levels in the 500 mg *T. foenum-graecum* treated group was restored to normal range and this effect was comparable to that of the standard hypoglycaemic agent 'glibenclamide' used in this study (Figure 1).

Levels of Thiobarbituric Acid Reactive Substances (TBARS)

The lipid peroxidation (LPO) level in diabetic animals increased as could be seen from significantly elevated levels of TBARS in the pancreas and liver when compared to that of control group. Administration of *T. foenum-graecum* and glibenclamide decreased the TBARS significantly in diabetic rats. At the dose of 500 mg, *T. foenum-graecum* was found to be more effective when compared to the other doses used in this study and the effect was comparable to that of glibenclamide (Figures 2, 3).

Levels of Reduced Glutathione

There was a significant decrease in the levels of reduced glutathione (GSH) in pancreas and liver of diabetic rats when compared to control group. Administration of *T. foenum-graecum* and glibenclamide increased the levels of GSH in diabetic rats. The GSH levels in pancreas and liver increased significantly at all the 3 doses of *T. foenum-graecum* used in this study. The effect of 500 mg *T. foenum-graecum* is comparable to that of glibenclamide (Figures 2, 3).

Activity of Catalase

The activity of catalase (CAT) in pancreas as well as in liver was found to be decreased in diabetic animals when compared to control group. In the *T. foenum-graecum* treated groups the activity of catalase increased significantly compared to that of diabetic rats. At the dose of 500 mg, the effect of *T. foenum-graecum* was comparable to that of glibenclamide (Figures 4, 5).

Activity of Superoxide Dismutase

Significant reduction in the activity of superoxide dismutase (SOD) was observed in pancreas and liver of diabetic rats when compared to that of control group. The SOD activity improved in 100 mg *T. foenum-graecum* treated animals as compared to diabetic group but not significantly. The plasma SOD activity increased

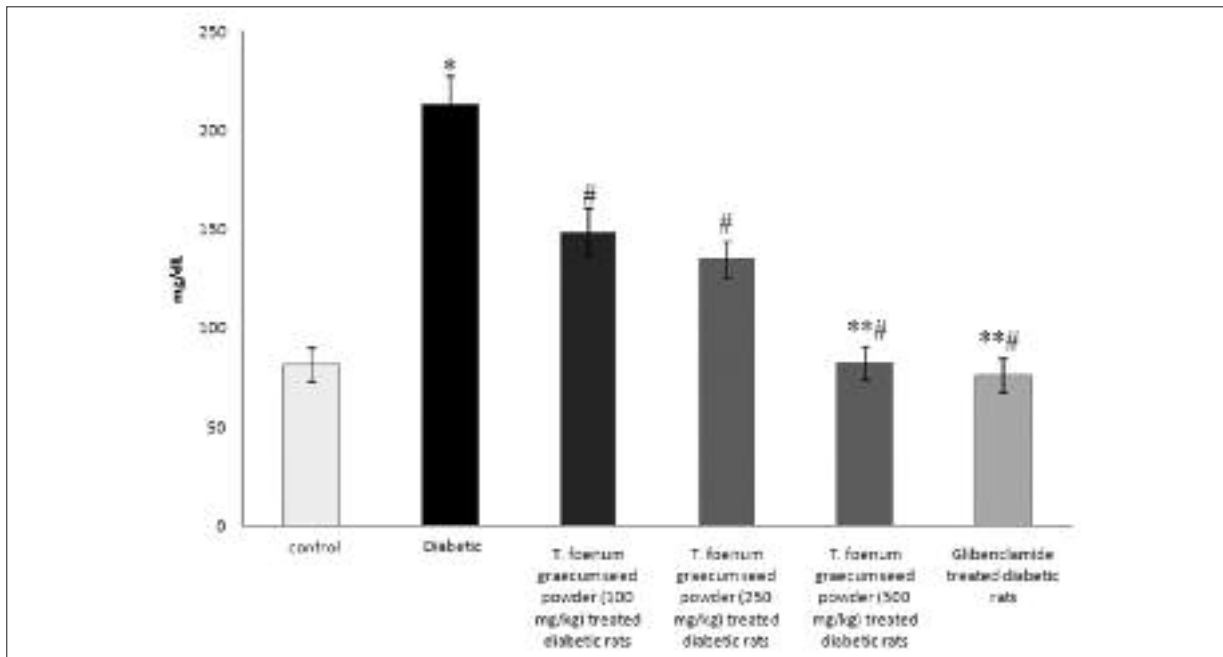


Figure 1. Shows the levels of blood glucose. Values are Mean \pm SD from 4 rats in each group. ANOVA followed by Tukey's test was used to assess the statistical significance between groups. * $p \leq 0.05$, Significance relative to control; # $p \leq 0.05$, Significance relative to diabetes; ** $p \leq 0.001$, Significance relative to diabetes.

significantly in 250 mg and 500 mg *T. foenum-graecum* treated groups when compared to the diabetic group. There was a significant increase in the activity of SOD in glibenclamide treated group (Figures 4, 5).

Activity of Glutathione Peroxidase

There was a significant reduction in the activity of glutathione peroxidase (GPx) in pancreas and liver of diabetic rats when compared to control group. In both pancreas and liver, significant

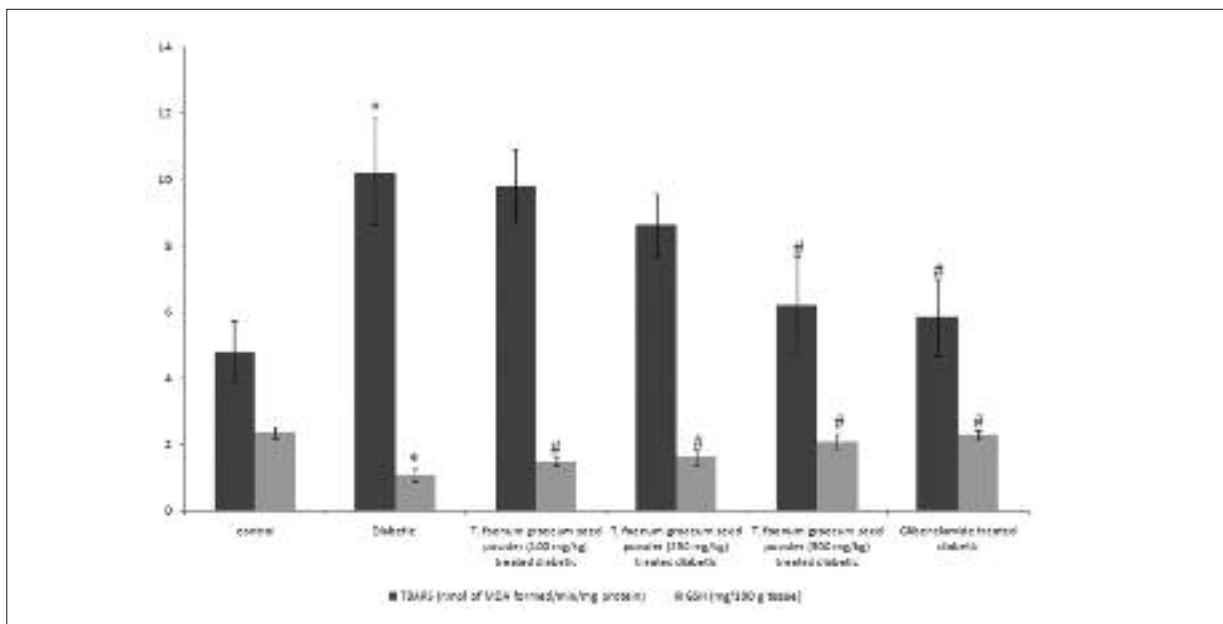


Figure 2. Shows levels of TBARS and GSH in pancreas. Values are Mean \pm SD from 4 rats in each group. ANOVA followed by Tukey's test was used to assess the statistical significance between groups. * $p \leq 0.05$, Significance relative to control; # $p \leq 0.05$, Significance relative to diabetes.

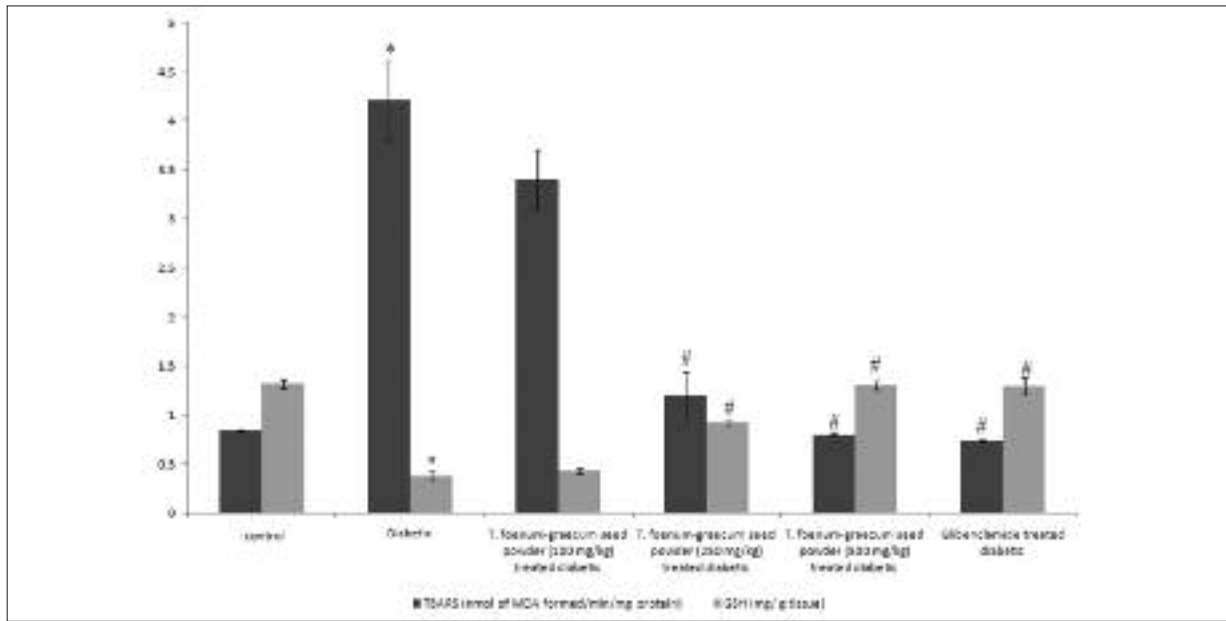


Figure 3. Shows levels of TBARS and GSH in liver. Values are Mean \pm SD from 4 rats in each group. ANOVA followed by Tukey's test was used to assess the statistical significance between groups. * $p \leq 0.05$, Significance relative to control; # $p \leq 0.05$, Significance relative to diabetes.

increase in the enzyme activity was observed at all the three doses of *T. foenum-graecum* seed powder used in the study when compared to the diabetic. The effect of *T. foenum-graecum* seed powder at 500 mg level was comparable to that of glibenclamide (Figures 4, 5).

Activity of Glutathione Reductase

There was a significant reduction in the activity of glutathione reductase (GR) in both pancreas and liver of diabetic rats when compared to control group. The activity improved in 100 mg and 250 mg *T. foenum-graecum* treated

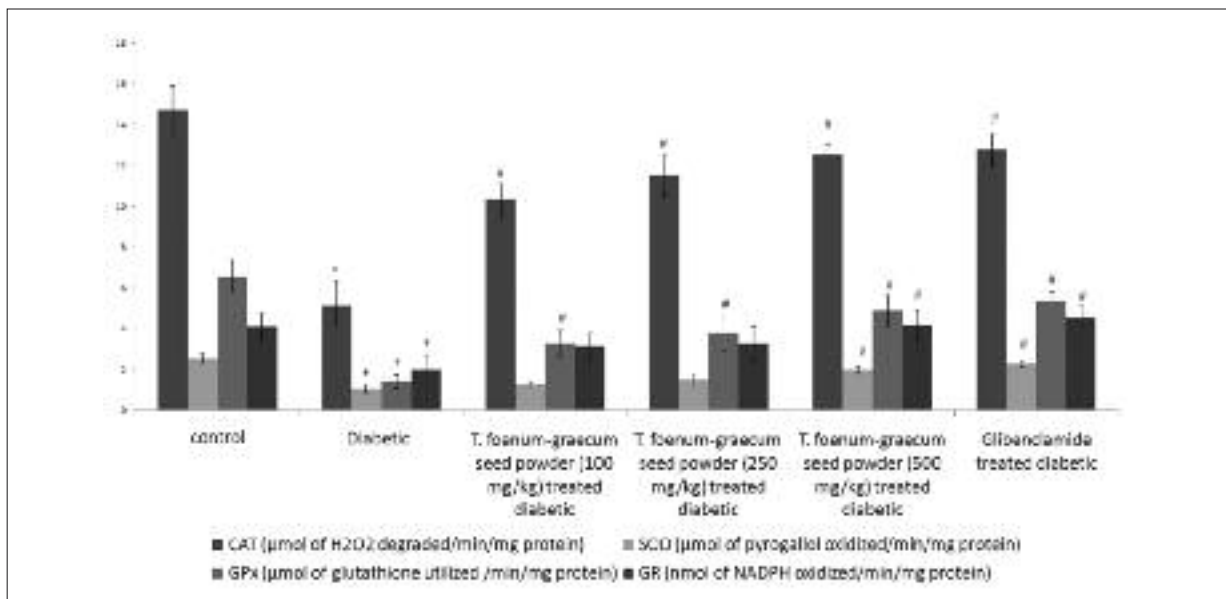


Figure 4. Shows the activities of antioxidant enzymes in pancreas. Values are Mean \pm SD from 4 rats in each group. ANOVA followed by Tukey's test was used to assess the statistical significance between groups. * $p \leq 0.05$, Significance relative to control; # $p \leq 0.05$, Significance relative to diabetes.

groups when compared to diabetic group but significant difference was not observed. There was a significant increase in the enzyme activity in 500 mg *T. foenum-graecum* treated group and the effect was comparable to that of glibenclamide (Figures 4, 5).

Discussion

Hyperglycaemia can lead to both a rise in reactive oxygen species (ROS) production and to the attenuation of free radical scavenging compounds²⁴. There are many ways by which hyperglycaemia may increase free radical generation, such as glycooxidation, polyol pathway, prostanoid biosynthesis, and protein glycation²⁵. There is also ample evidence that elevation in glucose concentration may depress natural antioxidant defence such as GSH²⁶. The imbalance between the generation of oxygen free radicals and an antioxidant defence system may increase the oxidative stress and lead to the damage of macromolecules such as DNA, proteins, or lipids.

In the present study, alterations in the antioxidant enzyme activities during diabetes were observed in both liver and pancreas. It seems that pancreas is vulnerable for attack by free radicals because decreased activity of antioxidant enzymes were found during diabetes. The decrease

in antioxidant enzymes in some tissues during diabetes may be due to the inactivation or inhibition of the enzymes by the increased production of oxygen free radicals during diabetes^{20,27}.

Lipid peroxidation is a free radical mediated process leading to oxidative deterioration of polyunsaturated lipids. Under normal physiological conditions, low concentrations of lipid peroxide are found in plasma and tissues. The possible source of oxidative stress in diabetes includes shifts in redox balance resulting from altered carbohydrate and lipid metabolism, increased generation of ROS, and decreased level of antioxidant defences such as GSH²⁴. In the present study, an increase in the level of TBARS and a decrease in the enzymatic and non-enzymatic antioxidants were observed in liver and pancreas of HFD+STZ induced diabetic rats.

The results of this study demonstrate the occurrence of oxidative damage in both liver and pancreas during experimental diabetes. The increase in lipid peroxidation levels in the pancreas are in agreement with similar findings in other tissues in earlier studies²⁸. Lipid peroxidation may bring about protein damage and inactivation of membrane bound enzymes either through direct attack by free radicals or through chemical modification by its end products, malondialdehyde and 4-hydroxynonenal²⁹. Glucose is known to induce lipid peroxidation through activation of the lipoxygenase enzymes³⁰. An

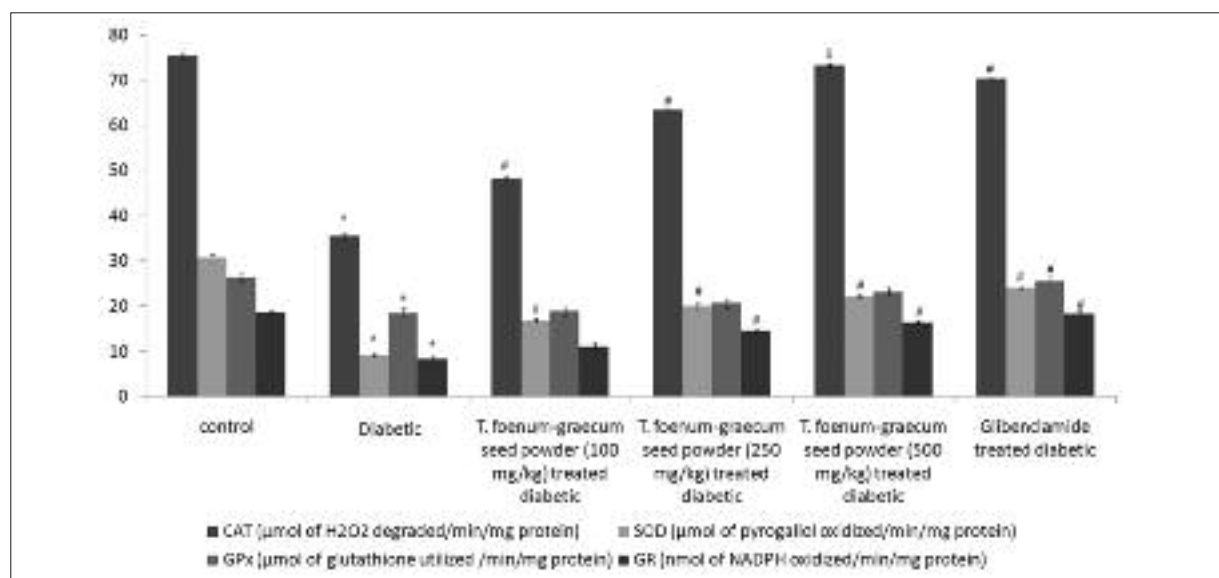


Figure 5. Shows the activities of antioxidant enzymes in liver. Values are Mean \pm SD from 4 rats in each group. ANOVA followed by Tukey's test was used to assess the statistical significance between groups. * $p \leq 0.05$, Significance relative to control; # $p \leq 0.05$, Significance relative to diabetes.

increased level of TBARS is an index of lipid peroxidation. The present investigation shows that *T. foenum-graecum* seed powder tends to bring liver and pancreas TBARS back to near normal. Increased lipid peroxidation under diabetic condition can be due to the increased oxidative stress in the cells as a result of depletion of antioxidants scavenger systems as reported by Anuradha et al³¹. It is also shown that supplementation of *T. foenum-graecum* seeds in the diet enhances the antioxidant potential in control and in diabetic rats³².

GSH is known to protect the cellular system against the toxic effects of lipid peroxidation³³. The decline in total glutathione concentrations in liver and pancreas of diabetic rats observed in this study may be due to utilization of nonprotein thiols by increased oxygen-free radicals produced in hyperglycaemic conditions associated with diabetes mellitus. *T. foenum-graecum* treatment at the three doses in this study caused significant reversal of glutathione content back to normal. The activities of the antioxidant enzymes, SOD, CAT, GPx and GR were observed to decrease in liver and pancreas of diabetic rats when compared with control animals. Higher levels of lipid peroxides and low SOD and CAT activity are indicative of an oxidative stress condition. The decreased activity of CAT and GPx in liver and pancreas of HFD+STZ induced diabetic rats could be due to the increased endogenous production of superoxide anions³⁴. Also the lipid peroxidation product 4-hydroxynonenal inhibits GPx, whereas GSH has been shown to protect GPx from this inhibition³⁵. Thus, an increase in the levels of TBARS and reduced GSH concentrations could explain the reduced GPx concentrations in these tissues under diabetic conditions. Improvement in the activities of SOD to normal levels were observed in *T. foenum-graecum* treated diabetic rats. The inactivation of GPx and CAT by superoxide anion would be protected by an increase in the activity of SOD³⁶. This could be the reason for the improvement in the activities of CAT and GPx in *T. foenum-graecum* treated diabetic rats.

All this suggests the antioxidant potential of *T. foenum-graecum* seed powder which may involve some mechanism related to ROS scavenging activity. *T. foenum-graecum* seed powder has a hypoglycaemic effect³⁷, which is a necessary and sufficient requirement for the control of the complications arising from glycation and glycooxidation of proteins and membranes. *T.*

foenum-graecum seeds may have possible antioxidant properties as a result of reducing blood glucose levels and play a crucial role in the defence against oxygen free radicals.

Conclusions

From the present study it can be inferred that *T. foenum-graecum* seed powder has potent antioxidant properties. Hence, *T. foenum-graecum* can be used to treat diabetic complications arising due to oxidative stress in type II diabetes mellitus.

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