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Activity of Glutathione-s-transferase in *Trigonella foenum-graceum* Whole Seed Powder Treated Diabetic Murine Model System

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Abstract: ***Humans have highly developed antioxidant systems, which works well within the body and can easily fight with free radicals that causing oxidative stress. The increased oxidative stress can cause initial β cell damage in type I diabetes, or impaired insulin production, release or function in type II diabetes. Glutathione-S-transferases (GSTs) are a family of Phase II detoxification enzymes that catalyse the conjugation of glutathione (GSH) to a wide variety of endogenous and exogenous electrophilic compounds. GST catalyzes attachment of the thiol of glutathione to electrophiles, an important condition affecting GST expression is oxidative stress. Trigonella foenum-graceum is one of the oldest cultivated medicinal plants identified in written history, and many studies showed that the it's seeds acquire anti-oxidant properties. Our study evaluated that effect of different doses (5, 10 and 15 mg/kg) of Trigonella foenum-graceum whole seed powder show magnitude of elevation in GST activity of both liver and kidney. In liver when compared with normal control the specific activity of GST is significantly reduced by 0.45 folds in diabetic control group. When compared to normal control low dose treated group show significant decrease of 0.67 folds, medium dose group show decrease of only 0.72 folds but not significant while high dose treated group show minimum reduction of 0.87 folds. Similarly the specific activity of GST is significantly reduced by 0.40 folds in diabetic control group as compared to normal control. Low dose treated group shows significant reduction by 0.68 folds and in medium dose treated group decrease of 0.79 folds was observed. High dose treated group animal's shows minimum reduction by 0.81 folds but both medium and high dose had no significant change.***

Keywords: Diabetes, *Trigonella foenum –graceum*, Glutathione-s-transferase

1. Introduction

Humans have evolved highly complex antioxidant systems (enzymic and nonenzymic), which work synergistically, and in combination with each other to protect the cells and organ systems of the body against free radical damage (4). Under the normal conditions the antioxidant defense system within the body can easily handle free radicals that are produced. If there are too many free radicals produced and too few antioxidants, this may cause chronic damage and this condition when the level of ROS exceeds the defense mechanisms, a cell is said to be in a state of "oxidative stress"(7,14).The increased oxidative stress in noninsulin-dependent diabetes mellitus (NIDDM) is associated with an increased oxidative damage to DNA (2,6). It can cause initial β cell damage in type I diabetes, or impaired insulin production, release or function in type II diabetes(11). Glutathione S-transferase (GST) is a family of enzymes that plays an important role in detoxification of xenobiotics. GST catalyzes attachment of the thiol of glutathione to electrophiles (5).

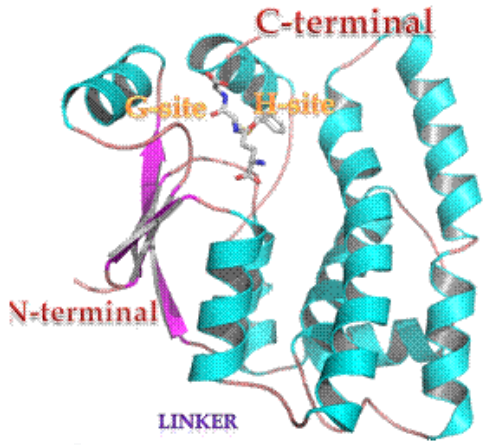

Figure 1: Crystallographic structure of glutathione S-transferase (http://www.decatbios.aua.gr)

An important condition affecting GST expression is oxidative stress, usually observed in diabetes **(13)** .Fenugreek is as one of the oldest cultivated medicinal plants identified in written history, and many studies showed that the seeds acquire anti-oxidant properties in seeds and leaves of fenugreek (8) Plants are found to be effective and their low cost and minimal side effects have increased the interest of scientists to develop plant based drugs for managing diabetes(12). Therefore here we evaluate the activity of GST enzyme in liver and kidney of diabetic murine model system.

2. Materials and Methodology

2.1 Preparartion of test diet

The test diets were prepared by using seeds of *Trigonella foneum-graceum*. Seeds were ground in mixer and resulting powder form of *Trigonella foneum-graceum* was mixed with the regular diet in appropriate quantity. The three different doses Trigonella foneum (5, 10 and15) mg/kg body weight are provided to different groups of mice for 15 days. .

2.2 Animals

All animals used in this work were male Swiss albino mice weighing 200 to 220 grams approx. They were housed individually in special clear sided cages at controlled temperature (20-25º C) with a 12:12-h light: dark cycle and had free access to water and chow diet over a 2-wk adaptation period. All experiments were performed as per the directives of the institutional animal ethics committee from which the project has been approved.

2.3 Induction of diabetes

The Diabetes was induced in the rats, after 12h fasting the rats were weighed and a solution of alloxan at 2% diluted in saline at 0.9% was administered to the animals in a single dose corresponding to 40 mg of alloxan per kg of animal weight injected into their penial vein. The same volume of 0.9% NaCl injectable solution was injected to the control rats. After 72 hours of alloxan injection; the diabetic rats (glucose level > 250 mg/dl) were separated and used for the study. The animals that were not above this established standard were discarded from the study. Fasting glycemia was measured by using a clinical glucometer. The diabetic state was ascertained in terms of loss of body weight, polyuria, glycosuria, polydipsia, polyphagia and blood glucose levels Symptoms of diabetes were observed within a week of alloxan injection.

2.4 Experimental setup

In the present experimental study, the mice were divided into five groups with six animals in each group. Body weight and fasting blood glucose levels of all the rats were determined before the start of the experiment. The study was done on the basis of following groups:-
Group 1 -Normal control, untreated
Group 2 - Diabetic control
Group 3 - Diabetic rats treated with 5 mg/kg of body weight whole seed powder
Group 4 - Diabetic rats treated with 10 mg/kg of body weight whole seed powder
Group 5 - Diabetic rats treated with 15 mg/kg of body weight whole seed powder

All groups were routinely observed and body weight of mice was recorded. At the end of 15 days treatment and after 12 h of fasting, rats were sacrificed by cervical dislocation, and blood samples were obtained from the retro-orbital sinus using glass capillary in glass tubes containing sodium citrate (3.5mg/ml) of blood. The blood was centrifuged for 5 min at 3,000 rpm in 4°C, where upon the plasma was separated carefully and stored at −80°C for further analysis.

2.5 Preparation of homogenate

Animals were sacrificed by cervical dislocation and the entire liver was then perfused immediately with ice-cold 0.85% NaCl and thereafter carefully removed, trimmed free of extraneous tissue and rinsed in chilled 0.15M Tris-KCl buffer (pH 7.4). Kidney was also removed quickly and rinsed with chilled 0.15M Tris-KCl buffer (pH 7.4). Both the organs were homogenized in ice-cold 0.15 M Tris-KCl buffer (pH 7.4) to yield 10% (w/v) homogenate. This homogenate was centrifuged at 15,000 rpm for 30 min. the resultant supernatant was transferred into pre-cooled ultracentrifugation tubes and centrifuge at 1,05,000 x g for 60 min. The cytosol fraction was used for assaying cytosolic glutathione S-transferase.

2.6 Assay of Glutathion-s-transferase

Glutathione S-transferase (GST) was assayed using method of Habig *et al.* (1).The assay is based on GSH-CDNB (1-chloro, 2,4-dinitrobenzene) conjugate formation which is catalyzed by GST .The reaction product, GS-DNB Conjugate, absorbs at 340 nm. The rate of increase in the absorption is directly proportional to the GST activity in the sample

$$\text{CDNB} + \text{GSH} \xrightarrow{\text{GST}} \text{CDNB-GSH conjugate}$$

Reaction volume (1 ml) contain 0.3M sodium phosphate buffer pH 6.5 (334 µl), 1 mM CDNB in 95% ethanol (33 µl), 1mM GSH (33 µl) and rest was sample and distilled water to made reaction volume 1ml. Whole mixture without enzyme was incubated at 37°C for 2 min. The reaction was initiated by the addition of enzyme sample and the enzyme activity was followed for 3 min at 340 nm.specific activity of GST was calculated using extinction coefficient 9.6 at 340 nm and expressed in terms of µmole CDNB-GSH conjugate formed/min/mg protein.

$$\text{Specific Activity} = \frac{\text{OD sample} \times V \times \text{dilution}}{E \times v \times \text{mg protein per ml}}$$

Where, OD sample = optical density with sample, V = volume of reaction mixture, E = extinction coefficient, V = volume of diluted sample taken

2.7 Chemicals

GST assay kit was obtained from Sigma Aldrich Chemicals Pvt. Ltd. All reagents and chemicals that were used in this work were of analytical grade.

2.8 Statistical analysis

Experimental values are expressed as mean ± SEM. Independent Sample t-test was carried out for statistical comparison. Statistical significance was considered to be indicated by a p value < 0.05 in all cases.

3. Results and Discussion

Activity of GST in liver

The specific activity of GST is significantly reduced by 0.45 folds in diabetic control group compared to normal control group. The magnitude of elevation in GST activity by the *Trigonella foenum* whole seed powder treatments was found as shown in figure 2. When compared to normal control low dose treated group3 show significant decrease of 0.67 folds, medium dose treated group 4 animals show no significant decrease of only 0.72 folds but high dose treated group5animals show minimum reduction of 0.87 folds.

Activity of GST in kidney

The specific activity of GST is significantly reduced by 0.40 folds in diabetic control group2 as compared normal control. The magnitude of elevation in GST activity by *Trigonella foenum* whole seed powder treatments was found when compared with diabetic control as seen in figure3. Low dose treated group3 animal's shows significant reduction by 0.68 folds and in medium dose treated group4 animal's decrease of 0.79 folds was observed. High dose treated group5 animal's shows minimum reduction by 0.81 folds but both medium and high dose had no significant change

The results of our study revealed that all doses of *Trigonella foenum* whole seed powder treatments show rise in GST concentration as compared to normal control group. Previous reported findings also indicate the activity of GST in relation to diabetes (2,9,10). The pathogenesis of the Diabetes Mellitus is of multifactorial nature and the functional trouble at the level of β-cells is manifest from its earliest stages of development (3). Hyperglycemia and free fatty acid intake are among the causes for oxidative stress conditions. Therefore Oxidative stress has also been proposed as a major participant in the patophysiology of diabetic complications(6).As antioxidants delay or inhibit cellular damage mainly through their free radical scavenging property. Hence we can say that antioxidant defense should be regarded important in diabetes and its complications.

Table1

Groups	GST① activity in liver	GST① activity in kidney
Group1	11.01±2.97 100	10.03±0.94 100
Group2	4.93±1.46[c] 44.78	3.97±2.16[a] 39.58
Group3	7.373±1.58[d] 66.97	6.82±0.24[a] 67.99
Group4	7.96±3.16[d] 72.00	7.96±3.16 79.36
Group5	9.54±1.11 86.65	8.14±3.11 81.16

Values are expressed as mean ± SD of 6-8 animals. Values in parentheses represent relative change in parameters assessed ($p<0.0001$)[a] , ($p<0.001$)[b] , ($p<0.01$)[c], ($p < 0.05$)[d] represent significant changes against normal control. ① Represents units as µmole CDNB-GSH conjugate formed/ min/ mg protein

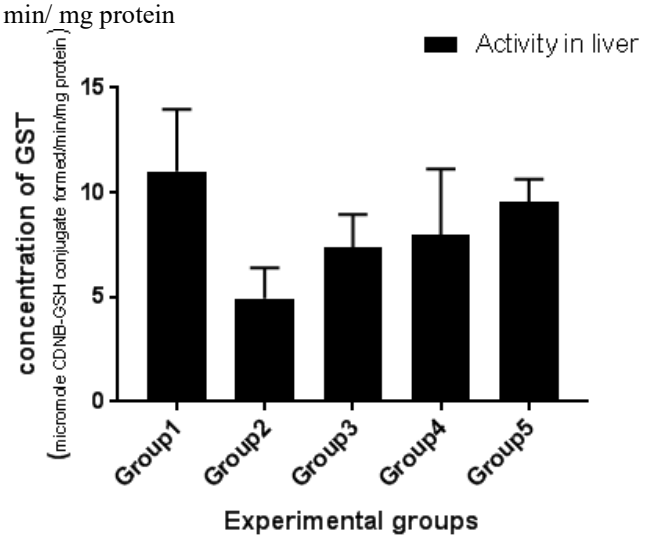

Figure 2: Activity of GST in liver

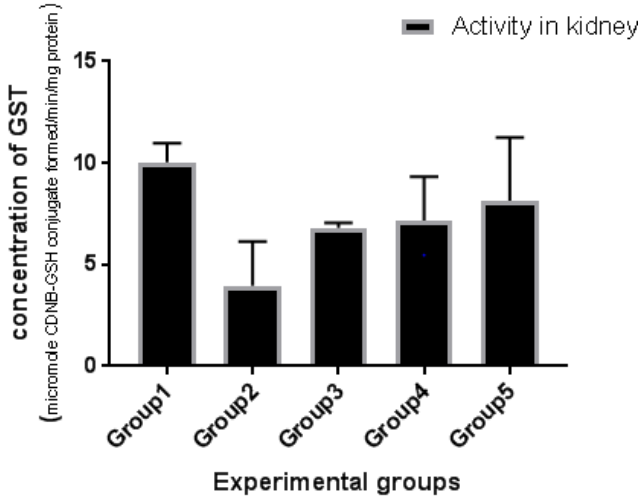

Figure 3: Activity of GST in kidey

4. Conclusion

Trigonella foenum-graceum is known to be for its antioxidative property. These antioxidative compounds from plant sources are effective to fight diseases with oxidative stress such as diabetes. The present investigation shows protective action of *Trigonella foneum –graceum* on diabetes by enhancing GST activity. Medications from plant sources are cheaper and of low or no side effects in comparison of allopathic medicines, so they must be utilized for welfare of human beings.

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