



## **Trigonella foenum graecum Seed Extract Enhances the Antiperoxidative and Antidiabetic Activities of Glibenclamide**

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### ABSTRACT

Seed of fenugreek, *Trigonella foenum graecum* (TFG) has been well investigated for its antidiabetic and antioxidative potential. In this study an attempt was made to reveal the possible synergistic effects of TFG seed extract and glibenclamide (GLB) in the regulation of diabetes mellitus and hepatic lipid peroxidation. The combined effects of TFG and GLB were compared with that of individual treatments for the regulation of alloxan induced hyperglycemia, hepatic LPO and to the changes in the status of antioxidants. High Performance Liquid Chromatography (HPLC) was also performed in order to find out the concentrations of major active compounds. Combined administration of TFG and GLB not only inhibited LPO and glucose to a great extent, but also ameliorated the alloxan induced decrease in insulin and antioxidants such as superoxide dismutase (SOD), catalase (CAT) and glutathione (GSH) as compared to the individual drugs. HPLC analyses of the test extract revealed the presence of trigonelline, quercetin and rutin; former one being in the highest proportion, suggesting its greater involvement in the TFG induced amelioration of diabetes mellitus. Our findings suggest that fenugreek seed extract, when added along with glibenclamide may ameliorate diabetes mellitus to a greater extent as compared to monotherapy. It also greatly improved the antioxidant defense system, clearly suggesting the use of fenugreek seed extract along with glibenclamide for better diabetic control.

**Keywords:** *Trigonella foenum graecum*, diabetes mellitus, glibenclamide, mice.

### INTRODUCTION

A common health problem, Diabetes mellitus (DM) is increasing throughout the world. Although most of the patients use conventional drugs for its treatment, plant based medicines are believed to be more effective, economic and safe. In recent years several plant parts have been evaluated in relation to the regulation of DM.<sup>1,2</sup> Seed of fenugreek, *Trigonella foenum graecum* (TFG) is one such botanical that is well investigated for its antidiabetic and antioxidative potential.<sup>3-5</sup>

Similarly, on the use of conventional medicines that control DM, glibenclamide (GLB) is very commonly prescribed. This has also been used as a standard hypoglycemic agent in animal experiments.<sup>6</sup> Patients suffering from DM normally use either synthetic drug or alternative medicine and not both the drugs at a time. In fact, until today no investigation was made to explore the synergistic effects of trigonella seed extract and a conventional antidiabetic drug, in the regulation of DM and/or tissue LPO. Therefore, it was thought to evaluate the synergistic effects of TFG in the augmentation of antiperoxidative and antidiabetic properties of glibenclamide, if any. For antiperoxidative study, hepatic tissues were used because of the fact that liver is considered as the primary target organ of a drug. Concentrations of the major active compounds trigonelline, quercetin and rutin that are reported to possess anti-hyperglycemic and antioxidative properties were also estimated in order to find out their possible involvement in the effects of the test extract.

### MATERIALS AND METHODS

#### Plant material

Good quality fenugreek seeds (100 g), purchased from local market (Indore, India) were finely powdered and dissolved in 1400 ml ethanol and then kept overnight at room temperature. The extract was vacuum dried to obtain the ethanol free powder (yield 17.2 %), which was dissolved in double distilled water (DDW) for experimental use.

#### Animals and diet

Swiss colony bred albino male mice weighing  $28 \pm 2$  g were housed in a room maintained at  $27 \pm 1^\circ\text{C}$  and on 10 h light and 14 h dark photo schedule with the provision of mice feed and water *ad libitum*. Animals were handled in accordance with the guidelines of the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA), Government of India, and India.

#### Chemicals

All most all the chemicals were purchased from E. Merck Ltd, Mumbai, India and the test drug, glibenclamide (Aventis, India) was procured from a registered local pharmacy shop. Radioimmunoassay (RIA) kit for the estimation of insulin was supplied by Bhabha Atomic Research Centre, Mumbai, India; while kit for glucose estimation was purchased from Ranbaxy Pvt. Ltd., Mumbai, India.



### HPLC fingerprint of the extract

Phytochemical analysis was done in HPLC (Jasco, PU-1580) with HSS-1500 monitoring system, using a Hypersil ODS (5 $\mu$ m) reversed phase C-18 column (250 x 4mm). The mobile phase consisted of methanol and water (7:3). The flow rate was 0.5 ml /min, while the injection volume and the monitoring wavelength were 20  $\mu$ l and 254 nm respectively. The quantity of the three compounds in the extract was calculated by interpolation of the peak area obtained by the sample in the calibration curve.

### In vivo study

Thirty five adult healthy male mice were divided into 5 groups of 7 each. Animals of group I receiving DW served as control, while that of group II, III, IV and V were treated (i.p) with single dose (150 mg/kg) of alloxan<sup>7</sup> to render them hyperglycemic. From fifth day of alloxan administration, group III, IV and V received TFG (100 mg / kg, p.o) as used earlier,<sup>8</sup> GLB (2 mg / kg, p. o.)<sup>9</sup> and equivalent doses of TFG as well as GLB respectively; whereas animals of group II received only the vehicle (0.1 ml DDW). The extract / vehicle was administered between 11.00 to 12.00 hour of the day to avoid circadian variation, if any. Experiment was continued for 14 days<sup>10</sup> and on 15<sup>th</sup> day all overnight fasted animals were sacrificed by cervical dislocation. Blood from each animal was collected and serum was isolated for the estimation of insulin and glucose concentrations. Liver was removed quickly, freed from blood clots, and processed for the biochemical estimations.

### Biochemical estimations

For the estimation of LPO, SOD, CAT, GSH and protein, liver tissues were homogenized in 10 % (w/v) ice cold phosphate buffered saline (pH 7.4, 0.1 mol / L), centrifuged at 15000 g for 30 min and the supernatant was processed for different assays as done before.<sup>11</sup> LPO was determined using the method of Ohkawa et. al.,<sup>12</sup> in which malondialdehyde (MDA), a product formed due to the peroxidation of lipids was measured and the values were expressed in nM of MDA formed / h / mg protein.

While CAT activity was studied following the method of Aebi<sup>13</sup> and was expressed as  $\mu$ m of H<sub>2</sub>O<sub>2</sub> decomposed / min / mg protein, the activity of SOD was estimated by the method of Marklund and Marklund<sup>14</sup> by measuring the % inhibition of pyrogallol autoxidation and was finally expressed as units / mg protein.

For the estimation of GSH content (reduced sulfhydryl group) the protocol of Ellman<sup>15</sup> was used in which the absorbance of the product formed by the reaction of Ellman's reagent with GSH was taken at 412 nm and was finally expressed as  $\mu$ m GSH / mg protein. Protein assay was carried out following the routine protocol of Lowry.<sup>16</sup>

For serum insulin estimation, protocol provided in RIA kit was used as done earlier in our laboratory,<sup>17</sup> where double antibodies (primary and secondary) and labeled insulin were used.<sup>125</sup> Serum glucose concentration was

measured by the routine glucose oxidase / peroxidase method.<sup>17</sup>

### Statistical evaluations

Statistical analysis of data (expressed as mean  $\pm$  SE) was done using analysis of variance (ANOVA), followed by Student's – t test.

## RESULTS AND DISCUSSION

While alloxan administration significantly increased serum glucose with a concomitant decrease in insulin concentration ( $p < 0.001$  for both) similar to our findings reported earlier,<sup>17, 18</sup> administration of either TFG or GLB reversed these changes (Table 3, 4 and Figure 1). Reports are available on the antioxidative and antidiabetic activity on both the drugs individually.<sup>19, 20</sup> Interestingly, on administration of TFG seed extract as well as glibenclamide, these positive changes were much greater suggesting a better control of hyperglycemia. Infact the coadministration of both the drugs significantly decreased the serum glucose and increased the insulin level ( $p < 0.001$  &  $p < 0.01$  respectively), but with a greater percent changes (53 % and 90 % respectively).

Alloxan has been found to be selectively toxic to pancreatic  $\beta$  cells as a glucose analogue.<sup>21</sup> In addition, the toxic action of alloxan is thought to be mediated by the generation of reactive oxygen species (ROS).<sup>22</sup> During the reduction process in the pancreatic beta cells, alloxan reacts with –SH group in sugar binding site of glucokinase resulting in the formation of disulphide bond and inactivation of the enzyme. The reduction process leads to the formation of dialuric acid. Autoxidation of dialuric acid involves the intermediate formation of alloxan radical establishing a redox cycle for generation of ROS.

Accordingly a significant increase in hepatic LPO ( $p < 0.001$ ) with a concomitant decrease in SOD, CAT and GSH ( $p < 0.01$  for all) was found in animals receiving alloxan as reported earlier by other workers.<sup>23,24</sup> In case of TFG, a significant decrease was noted in LPO ( $p < 0.001$ ) with a parallel increase in SOD, CAT and GSH as compared to their respective values in alloxan treated animals (Table 3 & 4). Although there was no significant increase in GSH and SOD activities following the administration of GLB, co-treatment of TFG and GLB not only decreased LPO with high percent but also enhanced the SOD, CAT and GSH (74%, 147% & 61% respectively). Here also the combined effect was much better than the effects exerted by TFG & GLB alone.

The generation of free radicals is known to induce LPO which has been associated with a number of diseases including diabetes mellitus.<sup>25</sup> The determination of MDA level provide a good measure of peroxidation,<sup>26</sup> which is one of the chief mechanisms of cell damage leading to necrosis or apoptosis.<sup>25</sup>

In the present investigation there was a significant elevation in liver MDA content of diabetic rats. The increase in MDA content in alloxan treated animals

suggests that peroxidative injury may be involved in the development of diabetic complications. In addition, the increase in LPO might be an indication of a decrease in enzymatic and non enzymatic antioxidants of defense mechanism like SOD, CAT and GSH.

GSH is mainly involved in the synthesis of important macromolecules and in the protection against reactive oxygen compounds.<sup>27</sup> In our study a marked decrease in hepatic GSH was observed in alloxan induced diabetic mice, as observed earlier by other workers.<sup>23,24</sup> The level of SOD and CAT also decreased in case of alloxan induced diabetic mice because these enzymes catalyze the removal of O<sub>2</sub> and H<sub>2</sub>O<sub>2</sub> with the help of which alloxan leads to the formation of hydroxyl radical (OH).<sup>28</sup>

The effects exerted by alloxan were reversed by the administration of GLB and TFG. GLB acts by inhibiting ATP sensitive potassium channels in pancreatic  $\beta$  cell which results in cell membrane depolarization and opening of voltage dependent calcium channel. This results in an increase in intracellular calcium in the  $\beta$  cells and subsequent stimulation of insulin release, which help to ameliorate diabetic condition induced by alloxan.<sup>29</sup>

The peaks in the HPLC spectrum matched with that of standard trigonelline, quercetin and rutin in the ethanolic extract of the test material with the retention time of 5.28, 5.02 and 4.42 min respectively (Figure 2). The concentration of trigonelline, quercetin and rutin in the extract was calculated to be 8.7, 2.55 and 2.73 $\mu$ g/ml respectively.

**Table 1:** Effects of fenugreek seed extract (TFG) and/or glibenclamide (GLB) in alloxan (ALX) induced diabetic mice with respect to the alterations in hepatic LPO (nM MDA/ h/ mg protein), SOD (units/ mg protein), CAT ( $\mu$ M H<sub>2</sub>O<sub>2</sub> decomposed / min/ mg protein) activity and GSH content ( $\mu$ M GSH/ mg protein).

| Treatment  | LPO                           | SOD                          | CAT                           | GSH                          |
|------------|-------------------------------|------------------------------|-------------------------------|------------------------------|
| Control    | 0.539 $\pm$ 0.05              | 5.17 $\pm$ 0.26              | 20.47 $\pm$ 2.75              | 3.11 $\pm$ 0.30              |
| ALX        | 0.892 $\pm$ 0.05*             | 3.40 $\pm$ 0.37**            | 10.98 $\pm$ 1.04**            | 1.76 $\pm$ 0.12**            |
| ALX+TFG    | 0.510 $\pm$ 0.04 <sup>†</sup> | 5.27 $\pm$ 0.26 <sup>†</sup> | 25.66 $\pm$ 2.29 <sup>†</sup> | 2.44 $\pm$ 0.15 <sup>†</sup> |
| ALX+GLB    | 0.603 $\pm$ 0.07 <sup>†</sup> | 4.16 $\pm$ 0.37              | 18.91 $\pm$ 1.14 <sup>†</sup> | 2.18 $\pm$ 0.22              |
| LX+TFG+GLB | 0.360 $\pm$ 0.02 <sup>†</sup> | 5.90 $\pm$ 0.30 <sup>†</sup> | 27.21 $\pm$ 2.06 <sup>†</sup> | 3.02 $\pm$ 0.32 <sup>†</sup> |

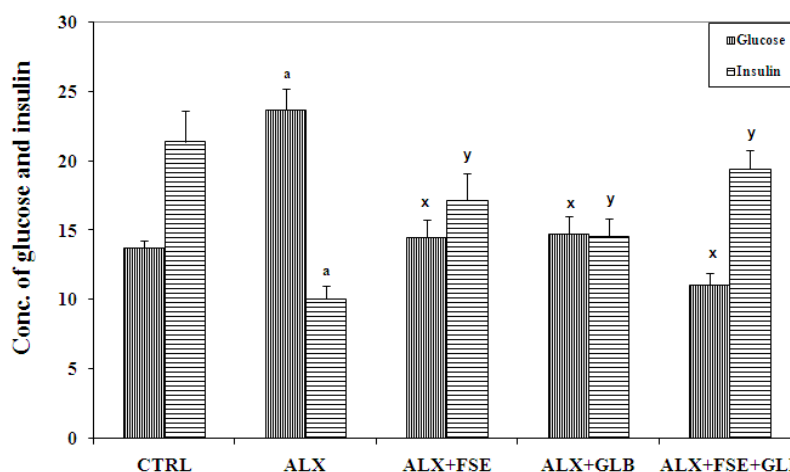
Data are in mean  $\pm$  SEM (n = 7), \* p < 0.001 and \*\*, p < 0.01 as compared to the respective control values. <sup>†</sup> p < 0.001 and <sup>†</sup> p < 0.01 compared with respective values in alloxan treated group. LPO, lipid peroxidation; SOD, superoxide dismutase; CAT, Catalase and GSH, reduced glutathione.

**Table 2:** Percent increase ( $\uparrow$ ) or decrease ( $\downarrow$ ) following the treatment of TFG and/ or GLB in alloxan induced diabetic mice.

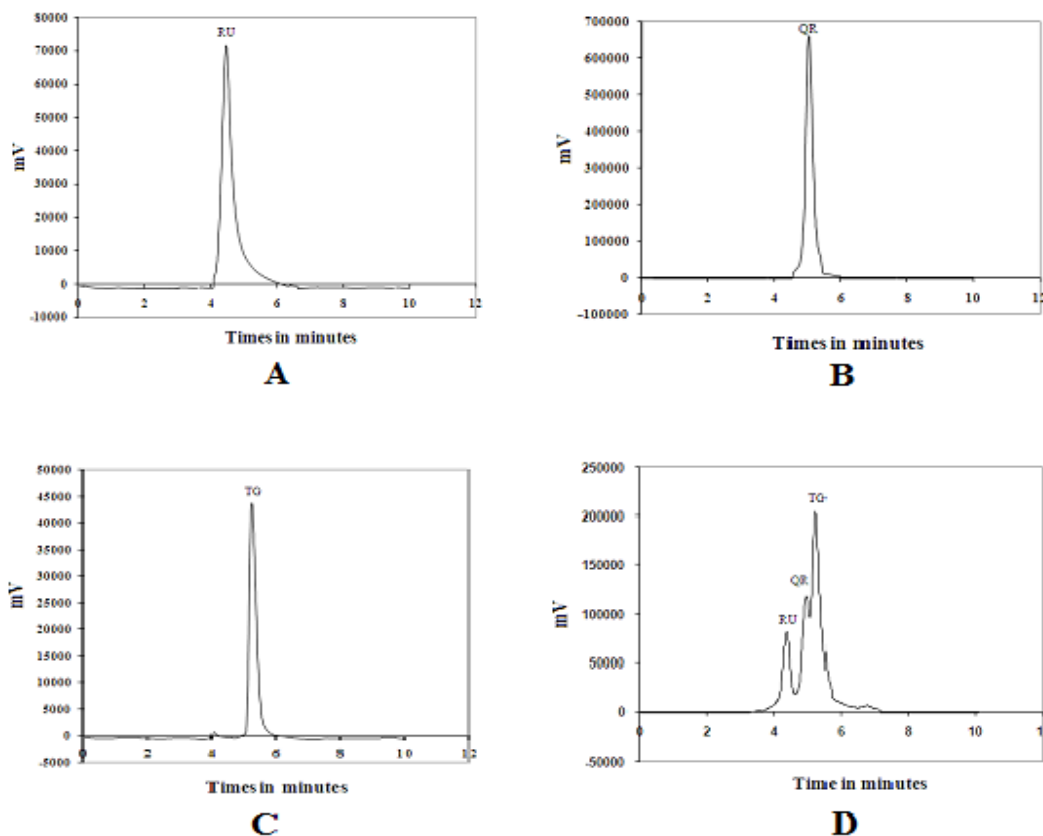
| Treatment   | Glucose         | Insulin       | LPO             | SOD           | CAT            | GSH           |
|-------------|-----------------|---------------|-----------------|---------------|----------------|---------------|
| ALX+TFG     | 39 $\downarrow$ | 70 $\uparrow$ | 43 $\downarrow$ | 55 $\uparrow$ | 134 $\uparrow$ | 39 $\uparrow$ |
| ALX+GLB     | 38 $\downarrow$ | 45 $\uparrow$ | 32 $\downarrow$ | 22 $\uparrow$ | 72 $\uparrow$  | 24 $\uparrow$ |
| ALX+TFG+GLB | 53 $\downarrow$ | 93 $\uparrow$ | 60 $\downarrow$ | 74 $\uparrow$ | 148 $\uparrow$ | 72 $\uparrow$ |

TFG, *Trigonella foenum graecum* and GLB, Glibenclamide.; Percent increase and decrease have been calculated from the respective values of alloxan treated group.

**Figure 1:** Changes in serum glucose (in mg % X 10) and insulin ( $\mu$ U /ml) concentrations following the treatment of *Trigonella foenum graecum* (TFG) seed extract or glibenclamide (GLB) alone or in combination of both in the alloxan (ALX) induced diabetic mice.



Data are in mean  $\pm$  SEM (n = 7). <sup>a</sup>, p < 0.001, as compared to the respective control values.; <sup>x</sup>, p < 0.001 and <sup>y</sup>, p < 0.01, as compared to the respective values in alloxan treated group.

**Figure 2:** HPLC chromatograms for different active components. (a) Rutin, (b) Quercetin, (c) Trigonelline and (d) All three in test seed extract.

As HPLC analysis of TFG seed extract showed the presence of alkaloids, flavonoids and polyphenols which are known to be naturally occurring antidiabetic agents,<sup>30, 31</sup> it is presumed that the antihyperglycemic effect of TFG might have been mediated through these phytochemicals. The mechanism may also involve the stimulation of glucose dependent insulin secretion from beta cells of pancreas.<sup>32</sup>

## CONCLUSION

In conclusion, the fenugreek seed extract, when added along with glibenclamide, not only inhibited serum glucose with parallel increase in insulin concentration, but also greatly improved antioxidant defense system, clearly suggesting the possible advantage for the diabetic patients receiving conventional antidiabetic drug as well as fenugreek seed extract.

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