Hepatoprotective potential of *Trigonella foenum graecum* in deltamethrin induced albino rats

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**ABSTRACT**

**Objective:** Aim of investigation focuses attention on hepatoprotective and antioxidative effect of aqueous extract of *Trigonella foenum graecum* (TFG) in hepatic tissue of deltamethrin fed rats. **Methods:** In a 45 days treatment, rats were divided into six groups (I-VI) of six animals in each, experiments were repeated thrice. Group I served as control rats; Group II received TFG dose 1 (9 g seed powder/kg b. wt./day); Group III received TFG dose 2 (45 g seed powder/kg b. wt./day); Group IV received deltamethrin; Group V received both deltamethrin and TFG (9 g seed powder/kg b. wt./day) and Group VI received both deltamethrin and TFG (9 g seed powder/kg b. wt./day). **Results:** In the present study, higher dose of TGF did not affect the levels of hepatic marker enzymes, which suggests that this dose had no toxic effect on normal rats. Significant increases in the serum levels of hepatic markers enzymes (alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (AP) were observed in deltamethrin treated rats. Furthermore, antioxidant enzymes (superoxide dismutase, catalase and glutathione S-transferase) activity and reduced glutathione (GSH) content were decreased in hepatic tissue of deltamethrin treated rats. Additionally, serum cholesterol and hepatic lipid peroxidation were significantly enhanced. Co-administration of TFG and vitamin C to the group V and VI restored all the parameters cited above to near-normal values. **Conclusion:** The result obtained from present study revealed that TFG appeared to be a promising agent for protection against deltamethrin induced hepatotoxicity.

**Introduction**

Wide spread use of pesticide in public health programme and agriculture has caused a severe environmental pollution and potential health hazards. Among all the pesticides used, pyrethroids are most common. Among all the pyrethroids in use, deltamethrin (DM) is commonly used as an ectoparasiticide in animals and as insecticides in crop protection [1]. It is used as an alternative pesticide in malaria control programs dealing with developing countries [2]. At the beginning DM was believed to be less toxic. However, some reports have demonstrated its damage in mammalian and non-mammalian laboratory and wildlife animal species [3,4].

Oxidative stress and reactive oxygen species (ROS) mediated toxicity has long been considered as the responsible mechanisms for DM induced organ injury in mammals [5]. It has been reported to alter the activities of glutathione S-transferase, catalase etc [6] and glutathione contents [7], changes in extent of LPO [8], which indicate the possibility of free radical induced oxidative damage. Free radicals are defined as atoms or molecules that contain one or more unpaired electrons. Under normal conditions, free radicals are generated and detoxified by antioxidants present in the body and there is equilibrium between the generated free radical and present antioxidants. In stress condition these highly reactive free radicals cause oxidative damage to various biomolecules including protein, lipid, carbohydrate and nucleic acid [9-10]. Increased quantities of ROS initiate lipid LPO in the cellular, mitochondrial, and nuclear membranes, along with

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Seeds were cleaned, dried and finally powdered. 500 g of TFG seeds were purchased from a local market in Lucknow.

Plant material and preparation of extract

Materials and Methods

digestive stimulant action etc [15-16] antihyperglycemic, hypocholesterolemic, antioxidant potency, several beneficial effects on human health viz. antidiabetic, Fabaceae) in DM induced toxicity. TFG is known to have for 30 min. The decoction was cooled for 30 min at room temperature 25±2°C in the animal room in the department. In Institute (CDRI), Lucknow, India, for study and housed at 150-175 grams were purchased from Central Drug Research Ltd, Mumbai, India and had free access to water. Prior permission for animal use and approval of the protocol were obtained from the CPCSEA, Animal Ethics Committee of University of Lucknow, Lucknow. After 10 days acclimatization, animals were divided into six groups. Group I (control group): Rats were fed slandered pelleted diet and water ad libitum. Group II: Rats were fed with TFG 9 g/kg body weight/day. Group III: Rats were fed with TFG 45 g/kg body weight /day. Group IV (DM group): Rats were fed with DM (1.3 mg /kg body weight/day). Group V (combination group of DM and TFG): Rats were fed with DM (1.3 mg/kg body weight/day) and TFG extract (9 gram seed powder/kg body weight/day). Group VI (combination group of DM and vitamin C): Rats were fed with DM (1.3 mg /kg body weight/day) and vitamin C (150 mg/kg body weight/day).

After 45 days of treatment, the animals were dissected under ether anesthesia. Blood from each rat was withdrawn from carotid artery at the neck and collected in previously labeled centrifuging tubes and allowed to clot for 30 min at room temperature. Serum was separated by centrifugation at 3000 rpm for 15 minutes. Liver tissue was collected in separate tube and stored at -20°C until analysis. The separated serum was used for the estimation liver function marker enzymes i.e. Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline phosphatase (AP) and cholesterol levels.

Preparation of homogenate (10% w/v)

For the preparation of homogenate (10% w/v), hepatic tissues were washed thoroughly with isotonic ice cold solution and homogenized in a homogenizer (Potter-Elvenhjem type) using ice-cold 50mM phosphate buffer (pH 7.4) containing mammalian protease inhibitor cocktail from Sigma Chemical company [containing 4-(2-Aminomethyl) benzenesulfonyl fluoride or AEBSF, pepstatin A, E-64, bestatin, leupeptin and aprotinin]. The homogenate was centrifuged at 12,000 x g for 30 min at 4°C and supernatant was used for the assay of antioxidant enzyme activities/levels and lipid peroxidation.

Chemicals

All the drugs and chemicals used in this were purchased from Sigma Chemical Company Inc., St Louis, Mo, USA. All other chemicals were of analytical grade.

Biochemical Estimations

To evaluate the possible liver function, serum alkaline phosphatase (AP) activity was estimated by Moog’s method [18] as modified by King [19] using disodium phenyl phosphate as substrate. Serum Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) were estimated by King’s method [19]. Marker of lipid peroxidation viz. malonaldehyde (MDA) was estimated by method of Ohkawa et al [20] using thiobarbituric acid reagent. The results were expressed as nmoles MDA/gm tissue using 1, 1, 3, 3 tetraethoxyxpropane (TEP) as reference. GSH was determined by the method of Chandra et al [21] based on the development of a yellow color when 5,5’-Dithio-bis 2-nitrobenzoic acid (DTNB) is added to compounds containing sulphydryl groups. Activity of superoxide dismutase (SOD) was estimated according to method Misra and Fridovich [22]. One unit of the enzyme activity was expressed as 50% inhibition of auto-oxidation of epinephrine per minute. GST was determined using 1-chloro-2, 4-dinitrobenzene (CDNB) as a substrate. The activity of GST is expressed as µmoles of GSH-CDNB conjugate formed/min/mg protein [23]. Activity of catalase was assayed were by Sinha’s method [24]. One unit of catalase activity was defined as µmoles of H2O2 decomposed per min. Protein was estimated by the method of Lowry et al [25] using bovine serum albumin as standard. Total serum cholesterol was estimated by the method of Zak [26].

Statistical analysis

The values were expressed as mean ± S.E.M. The data were subjected to one way Analysis-of-Variance (ANOVA) followed by Newman-Keuls Multiple Comparison Test for comparison between groups and values having P<0.05 were considered significant.

Results

Effects on body and liver weight

All the rats in, all the five groups, chosen in the present study were having almost same body weight. The minimum dose of TFG extract was 9.0 gram seed powder/kg/body weight/day and maximum dose chosen was 45.0 gram seed powder/kg/body weight/day. Normal treated and DM treated groups (with TFG and vitamin C) showed no morbidity and the rats of all the groups showed normal growth as observed by similar gain in body and liver weights at the end of experiment. However, rats fed with DM showed only 11.7% gain (P<0.001) in body weight and significant (P<0.01) lower weight in liver organ. (Table 1)

Effects on hepatic function marker enzymes and total protein content

Effect of TFG at two doses was observed on liver function. No significant deviations from control values were observed in serum ALT, AST and AP levels of group II and III when compared with normal rats. Deltramethrin fed group showed significant (p<0.001) increase in liver function marker enzymes. (Table 2) Administration of TFG to deltamethrin fed rats showed significantly (P<0.001) lower level of liver function enzymes when compared with the DM group. The hepatoprotective effects shown by TFG were comparable with vitamin C. DM fed group showed significant (P<0.01) decrease in total protein content. However DM fed group treated with TFG and vitamin C showed significant (P<0.05) in total protein content.

Effects on levels of MDA, GSH and serum cholesterol

No significant alteration was observed in MDA levels in normal rats treated with different dose of TFG. MDA levels were significantly increased (P<0.001) in hepatic tissue of DM fed rats when compared with normal rats. Administration of TFG extract with same dose of DM significantly decrease in MDA levels (P<0.001). Similarly, vitamin C treatment to the DM fed rats resulted in significant decrease (P<0.001) in MDA levels. (Table 2) GSH contents were significantly decreased (P<0.001) in hepatic tissue of DM fed rats when compared with normal rats. Treatment of DM fed rats with TFG extract resulted in significant increase (P<0.001) in GSH content when compared with DM fed rats. Similarly, vitamin C treatment to the DM fed rats resulted in significant increase (P<0.001) in GSH contents (Table 2).

Effects on hepatic antioxidant activities

As seen in figure 1 and 2 the activities of hepatic SOD, catalase and GST were significantly (P<0.001) decreased in DM induced animals by 41.61%, 47.8% and 46.62%, respectively, as compared to normal rats. The DM fed group that received TFG extract showed a significant (P<0.001) improvement in hepatic SOD, catalase and GST activities by 33.81%, 45.29% and 34.4%, respectively, as compared to DM fed group. Similarly, vitamin C (non-enzymetic antioxidant) treatment resulted in significant increase (P<0.001) antioxidants activities in hepatic tissues when compared with DM induced animals.

Discussion

The present study includes experiments which were conducted to establish the antioxidative, hepatoprotective effect of *Trigonella foenum graecum* (TFG) in DM induced rats. Previous studies have been undertaken with traditional medicines in an attempt to develop new hepatoprotective molecules [27]. In the present study, we observed a reduction of body weight gain (%) and a decrease in liver weight in DM fed group. The reduction in body weight is probably attributed to toxic effects of DM in treated rats. These findings are in accordance with previous report where animals exposed to different pyrethroid compounds (deltamethrin, fenvalerate and diazinon) [28-30]. In the present study, TFG and vitamin C significantly mitigated the effect of DM on body and liver weights (Table 1).

Few toxicological studies have been reported related to natural product showing that lectins present in *Momordica charantia* seeds inhibit protein synthesis in human intestinal mucosa cells [31], vicine, a glycosidic compound may cause hemolysis in patients with G6PD deficiency [32]. In present study two doses of TFG extracts (9 and 45 g/kg b.w./day), were given to normal rats (group II &III) for 45 days. There was no morbidity and all the rats showed normal growth (gain in body weight), similar to that of normal rats. Effects of TFG extracts were observed on liver function enzymes. No significant deviation from control values were observed serum ALT, AST and AP levels (Table 2). The data suggest that prolong use of these extracts is safe.

The evaluation of serum AST, ALT and AP activities and total proteins bears significance with respect to the evaluation of the effects of pesticides on the liver, therefore their toxicity [33]. The disturbance in the transport function of the hepatocytes as a result of hepatic injury causes the leakage of enzymes from cells due to altered permeability of membrane [34]. Our results showed significant (P<0.001) increase in serum ALT, AST and AP activities (Table 2). The increase in activity of these enzymes in serum is indicative for liver damage and thus causes alteration in liver function [35-36]. The increased levels of serum enzymes indicate an enhancement of permeability, damage or necrosis of hepatocytes. However, TFG treated group prevent the increase in the activities of these enzymes. The recovery towards normalization of liver function marker enzymes by TFG was
almost similar to that caused by vitamin C in the present study. Similar results have been found in DM fed animals treated with vitamin C [37,28]. The decrement of serum total protein (Table 2) comes in agree with Eraslan et al., 2007 [33] and Saoudi et al 2011 [28]. This decrease in serum protein could be attributed to changes in protein and free amino acids metabolism and their synthesis in liver. In present study, after administration of TFG extract found to significantly (P<0.05) increase in total protein content (table 2).

Lipid peroxidation may be due to oxidation of molecular oxygen to produce superoxide radicals. This reaction is also the source of H2O2, which causes the production of malondialdehyde (MDA) by initiating the peroxidation of unsaturated fatty acids in the membrane. The hydroxyl radical can initiate LPO which is a free radical chain leading to loss of membrane structure and function [38-39]. The result of present study have been shown that DM exposure at dose (1.3 mg/kg body weight/day) through oral route for 45 day caused significant increase in LPO in liver tissue. The data suggested that the significantly elevated MDA level in liver in turn produced reactive oxygen species (ROS), which caused oxidative stress in these organs [40]. After administration of TFG extract found to significantly (P<0.001) decrease hepatic MDA levels (table 3). GSH is an important antioxidant system of most aerobic cells [41]. It plays a key role as a cofactor with a variety of enzymes including GPx. GSH depletion has been shown to intensify LPO and predispose cells to oxidant damage [42]. Present investigation demonstrates that GSH plays an important role in modulating the DM induced oxidative damage in rats. Our data showed TFG extract and vitamin C significantly increased (P<0.001) GSH levels in liver of DM fed rats (table 3). Pesticides can cause changes in blood cholesterol levels by altering the permeability of hepatic cells and disrupting lipid metabolism [36] and this can be indicated by elevated cholesterol levels in the serum. TFG in combination with DM reduced the elevation in serum cholesterol and minimized the toxic effects of LPO (table 3).

The antioxidant enzymes (SOD and catalase) constitute the first line of defense against oxidative stress. Both are the most important defense mechanisms against toxic effects of oxygen metabolism. SOD accelerates the dismutation of H2O2, also termed as a primary defense, as it prevents further generation of free radicals. Catalase helps in the removal of H2O2 formed during the reaction catalyzed by SOD. Many by-products of oxygen metabolism initiate different outcomes at the subcellular level. The superoxide radical has been shown to inhibit the activity glutathione peroxidase and catalase activities [38]; moreover; singlet oxygen and peroxyl radicals can inhibit SOD and catalase activities [39]. The data obtained from the present investigation demonstrated the decrease in SOD activity due to the increased production of ROS as evident from the increased LPO levels due to DM exposure. After treatment SOD and catalase activity were significantly increased (P<0.001) in liver tissue of DM exposed animals.

GST mediated conjugation is involved in the detoxification of many xenobiotics, which play an important role in protecting tissues from oxidative stress [43]. In the present study, LPO treatments affect GST activity in rats. These results suggest that GST is involved in the detoxification of these compounds.

### Table 1: Effects of *Trigonella foenum graecum* on body & liver weight of experimental rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Body Weight (g)</th>
<th>Liver weight (g) % change</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>45th day</td>
<td>% gain</td>
</tr>
<tr>
<td>Normal</td>
<td>186.6±4.5</td>
<td>21.9</td>
</tr>
<tr>
<td>Normal+TFG (9g)</td>
<td>194.4±5.1</td>
<td>21.5</td>
</tr>
<tr>
<td>Normal+TFG (45g)</td>
<td>181.4±9.0</td>
<td>24.0</td>
</tr>
<tr>
<td>Deltamethrin</td>
<td>168.6±10.7</td>
<td>11.7</td>
</tr>
<tr>
<td>Deltamethrin+TFG</td>
<td>175.6±5.5      **</td>
<td>20.3</td>
</tr>
<tr>
<td>Deltamethrin+Vit C</td>
<td>185.0±6.3      ***</td>
<td>19.3</td>
</tr>
</tbody>
</table>

All values are expressed as mean ± SEM, (n =6). Normal treated groups and deltamethrin treated group were compared with Normal group; Deltamethrin fed rats treated with TFG and vitamin C were compared with deltamethrin fed rats. *p<0.001, **p<0.01, ***p<0.05
**Table 2: Effects of *Trigonella foenum graecum* on hepatic function marker enzymes and total protein content of experimental rats**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Hepatic function marker enzymes</th>
<th>Total protein (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ALT (IU/l)</td>
<td>AST (IU/l)</td>
</tr>
<tr>
<td>Normal</td>
<td>40.3±3.1</td>
<td>133.5±6.7</td>
</tr>
<tr>
<td>Normal+TFG (9g)</td>
<td>42.5±2.9</td>
<td>141.2±9.3</td>
</tr>
<tr>
<td>Normal+TFG (45g)</td>
<td>45.1±4.3</td>
<td>147.7±11.2</td>
</tr>
<tr>
<td>Deltamethrin</td>
<td>79.3±7.4*</td>
<td>241.1±16.3*</td>
</tr>
<tr>
<td>Deltamethrin+TFG</td>
<td>51.8±5.1*</td>
<td>151.8±11.7*</td>
</tr>
<tr>
<td>Deltamethrin+Vitamin C</td>
<td>49.9±4.5*</td>
<td>153.7±10.3*</td>
</tr>
</tbody>
</table>

All values are expressed as mean ± SEM, (n = 6). Normal treated groups and deltamethrin treated group were compared with Normal group; Deltamethrin fed rats treated with TFG and vitamin C were compared with deltamethrin fed rats. *p<0.001, **p<0.01

**Table 3: Effects of *Trigonella foenum graecum* on hepatic MDA, GSH contents and serum cholesterol levels in experimental rats**

<table>
<thead>
<tr>
<th>Groups</th>
<th>MDA levels (nM/g tissue)</th>
<th>GSH contents (µM/mg protein)/(mg/dl)</th>
<th>Serum cholesterol (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>183.0±4.49</td>
<td>1.35±0.06</td>
<td>86.34±2.4</td>
</tr>
<tr>
<td>Normal+TFG (9g)</td>
<td>187.7±7.20</td>
<td>1.42±0.09</td>
<td>91.67±4.6</td>
</tr>
<tr>
<td>Normal+TFG (45g)</td>
<td>193.2±9.86</td>
<td>1.31±0.11</td>
<td>94.14±5.1</td>
</tr>
<tr>
<td>Deltamethrin</td>
<td>389.52±15.92*</td>
<td>0.62±0.05*</td>
<td>121.45±11.1*</td>
</tr>
<tr>
<td>Deltamethrin+TFG</td>
<td>204.7±6.37*</td>
<td>1.17±0.07*</td>
<td>99.30±7.2*</td>
</tr>
<tr>
<td>Deltamethrin+Vitamin C</td>
<td>197.42±3.71*</td>
<td>1.26±0.06*</td>
<td>95.53±6.2*</td>
</tr>
</tbody>
</table>

All values are expressed as mean ± SEM, (n = 6). Normal treated groups and deltamethrin treated group were compared with Normal group; Deltamethrin fed rats treated with TFG and vitamin C were compared with deltamethrin fed rats. *p<0.001

![Figure 1: Effects of *Trigonella foenum graecum* on hepatic SOD and catalase activities in experimental rats](image-url)
Normal rats fed with DMVit C; rats treated with deltamethrin and vitamin C. *p<0.001. 1U of SOD= 50% inhibition of auto-oxidation of epinephrine/ min. 1U of catalase= µmoles H₂O₂ decomposed/ min.

Figure 2: All values are expressed as mean ± SEM, (n=6). Normal treated groups and deltamethrin treated group were compared with Normal group; Deltamethrin fed rats treated with TFG and vitamin C were compared with deltamethrin fed rats. NC; normal control, NCTFG-1; Normal rats fed with TFG (9 g/kg body weight/day), NCTFG-2; Normal rats fed with TFG (45 g/kg body weight/day), DM; normal rats fed with deltamethrin, DMTFG; rats treated with deltamethrin and Trigonella foenum graecum, DMVit C; rats treated with deltamethrin and vitamin C. *p<0.001. 1U of SOD= 50% inhibition of auto-oxidation of epinephrine/ min. 1U of catalase= µmoles H₂O₂ decomposed/ min.

Conflict of interest statement
We declare that we have no conflict of interest.

Acknowledgements
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References
6. Monarca S., Pasquini R., Crea M. G., Leonerdis C., Deltamethrin, DMTFG; rats treated with deltamethrin and vitamin C. GSH contents and lowered activities of antioxidants viz. SOD, GST and catalase. The exposure with TFG resulted in significant (P<0.001) recovery in altered levels of these parameters. Therefore, it may be suggested that use of natural products TFG is non toxic, antioxidative, antilipidemic and also have beneficial effects in pesticide induced hepatotoxicity.

Conclusion
According to the results obtained from present study clearly indicate antioxidative potential of TFG protects liver tissue in against DM induced toxicity. DM induces oxidative stress in rat liver as evidenced by increased levels of LPO, decreased GSH contents and lowered activities of antioxidants viz. SOD, GST and catalase. The exposure with TFG resulted in significant (P<0.001) recovery in altered levels of these parameters. Therefore, it may be suggested that use of natural products TFG is non toxic, antioxidative, antilipidemic and also have beneficial effects in pesticide induced hepatotoxicity.
35. Yousef M. I., Awad T. I., Mohamed H. E., Deltamethrin-induced oxidative damage and