**Protective effect of *Trigonella foenum-graecum* on thioacetamide induced hepatotoxicity in rats**

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**Abstract** *Trigonella foenum-graecum* belongs to the family Fabaceae and is indigenous to countries that lie on the eastern shores of the Mediterranean Sea and is also cultivated in India, Egypt and Africa. This study provides the evidence indicating the therapeutic effect of the extract prepared from the dried seeds of *Trigonella foenum-graecum* on an animal model of hepatotoxicity and on cell proliferation. Rats were induced liver cirrhosis by thioacetamide (0.03% in water for 16 weeks). Transmission electron microscopy of the liver tissue was done to evaluate liver cirrhosis. The herbal extract was administered orally for 3 weeks after induction and biochemical estimations were done. After the administration of extract the oxidative stress and lipid peroxidation were reversed. The elevated levels of alkaline phosphatase, γ-glutamyl transferase and selected biochemical markers of liver cirrhosis including drug metabolizing enzymes were also reversed. The study has implications in finding a treatment for liver cirrhosis by a natural herbal drug with no side effects.

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**Keywords**

Therapeutic agent; Thioacetamide; Cirrhosis; Biochemical markers; *Trigonella foenum-graecum*; Electron microscopy; Herbal drugs; Drug metabolizing enzymes

**1. Introduction**

The liver is one of the most important organ in the body. The liver has important role in regulating various physiological processes. The liver is involved in most of the biochemical pathways for growth, fighting against disease, nutrient supply, energy provision and reproduction. It also plays a role in the metabolism of carbohydrate, protein and fat, detoxification, secretion of bile and storage of vitamins (Ahsan et al., 2009).

The liver helps in the removal of substances from the portal circulation and thus makes it susceptible to first and persistent attack by offending foreign compounds, culminating in liver dysfunction (Bodakhe and Ram, 2007). Liver diseases have become one of the major causes of morbidity and mortality all over the world. Among them, drug induced liver injury is one of the most common causative factor that poses a major clinical and regulatory challenge (Russmann et al., 2009). In spite of tremendous scientific advancement in the field of hepatology in recent years, liver problems are in rise. Jaundice and hepatitis are two major hepatic disorders that account for the high death rate (Nazeema and Brindha, 2009). There are potent indigenous herbal medicines available for the treatment of liver disorders in various parts of the world and most of them have not yet scientifically been validated. If they are validated, it could lead to the development of cost effective drugs (Jamal et al., 2009).
The use of herbal medicines for the treatment of liver diseases has a long history. The active chemical constituents of liver protective plants include phenols, coumarins, monoterpenes, glycosides, alkaloids and xanthones (Bhawna and Kumar, 2009). Flavonoids are phenolic compounds that are widely distributed in plants, and have been reported to exert multiple biological effects, including antioxidant and free radical scavenging abilities (Baeck et al., 1996). Since there is lack of modern medicine for liver disorders, a large number of medicinal preparations are recommended for the treatment of liver disorders (Chatterjee, 2000) and quite often claimed to offer significant relief. Attempts are being made globally to get scientific evidences for these traditionally reported herbal drugs.

Trigonella foenum-graecum Linn. is commonly known as fenugreek and belongs to the family leguminosae. Fenugreek is one of the most widely used plants in various indigenous systems of medicine for the treatment of different ailments. Trigonella foenum-graecum with common name Methi in India is used in medicine to tonify kidneys, disperse cold and alleviate pain. These seeds of herb when taken raw or toasted are a good remedy for hernia and pain in the groin. 2–3 g of raw fenugreek seeds early in the morning with warm water before brushing the teeth has healing effect on joint pains, without any side effects (Sharma et al., 1996; Warrier et al., 1995). Other medicinal uses of Trigonella foenum-graecum include its use as anti uleer (Jayawera, 1981; Al-meshal et al., 1985), wound healing (Taranalli and Kuppast, 1996), CNS stimulant (Natrjan et al., 2007, immunomodulatory (Bilal et al., 2003), antioxidant (Kavirasana et al., 2007), anti diabetic (Al-Habori and Raman, 1988; Ravikumar and Anuradha, 1999; Zia et al., 2001), anti-neoplastic (Sur et al., 2001), anti-inflammatory and anti-pyretic (Ahmadiani et al., 2001) drugs. The present study is aimed to evaluate the hepatoprotective activity extract of fenugreek seeds against thioacetamide (TAA) induced hepatotoxicity.

2. Materials and methods

2.1. Procurement of material and extraction

The dry seed of fenugreek, Trigonella foenum-graecum, were obtained from a local herbal market. The seeds were identified and authenticated by the Herbarium of Pharmacognosy Department, Faculty of Pharmacy, King Saud University. Trigonella foenum-graecum seeds were dried at 40 °C and finely powdered and extracted in a Soxhlet apparatus at 60 °C for 7 h in 50% ethanol. The extracted solution was dried by evaporation under reduced pressure in a rotary evaporator and collected for storage at 4 °C in a refrigerator until used for further analysis. The extract was suspended in 1% gum acacia for hepatoprotective studies.

2.2. Animals

Wistar rats (160–180 g, female), were used throughout this study. Rats were divided into groups randomly. Animals were provided the pellet diet and water ad libitum at temperature 22 ± 2 °C and humidity at >40% with a 12-h light/dark cycle. All animal procedures were performed in accordance with the standards set forth in the Guidelines for the Care and Use of Experimental Animals by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and the National Institutes of Health (NIH). The study protocol was approved by the Animal Ethics Committee, College of Science, King Saud University.

2.3. Experimental design

Rats were divided into four groups, each group consisted of six animals. Rats in group I and group II received normal drinking water where group III and group IV received 0.3% TAA dissolved in drinking water for 16 days as described by Oren et al. (1996) to induce hepatotoxicity. One week after exposure of TAA it was discontinued for Groups I and III and they received normal saline, whereas, groups II and IV received hydroalcoholic extract of Trigonella foenum-graecum seeds by oral route, (500 mg/kg body weight, p.o suspended in 1% gum acacia) daily for 3 weeks.

Evaluation of liver and other biochemical alterations was performed by transmission electron microscopy and biochemical parameters. At the end of experimental period, all the animals were sacrificed by cervical decapitation.

2.4. Evaluation of liver function

The liver was removed, weighed and morphological changes were observed. A 10% of liver homogenate was used for antioxidant studies such as lipid peroxidation (LPO), glutathione, glutathione reductase, glutathione peroxidase, xanthine oxidase (xod) and glutathione S transferase. Blood samples were collected and allowed to clot. Serum was separated by centrifuging at 2500 rpm for 15 min and analyzed for various biochemical parameters that is, alkaline phosphatase (ALP) and γ-glutamyl transferase (GGT) according to the reported methods. (Nishino et al., 2005; Mira et al., 1995; Luck, 1971).

2.4.1. Alkaline phosphatase

The serum alkaline phosphatase (ALP) activity was estimated using a diagnostic kit (Sigma) based on the spectroscopic procedure described by Kind and King (1954).

2.4.2. Serum gamma glutamyl transferase (GGT)

GGT was measured according to the method of Orlowski and Meister (1973). Briefly, 0.2 ml serum was mixed with 0.8 ml of substrate solution consisting of 0.528 g of 40 mM glycylglycine, 0.121 g of 40 mM gamma glutamyl p-nitroanilide and 0.223 g of 11 mM MgCl₂ in 100 ml of 185 mM Tris buffer at pH 8.25. The reaction mixture was incubated for 10 min at 37 °C and reaction was stopped by adding 1 ml of 25% trichloroacetic acid. The mixture was centrifuged at 1500 g and absorbance of the supernatant was taken at 405 nm. The enzyme activity was calculated in Eq unit/ml.

2.4.3. Lipid peroxidation (LPO)

Lipid peroxidation was determined by estimating the level of malondialdehyde according to the method of Bernheim et al. (1948). Briefly, trichloroacetic acid is used to eliminate interference caused by the malondialdehyde precursors. The reaction mixture containing 1.8 ml phosphate buffer (0.1 M, pH 7.4) and 0.2 ml tissue homogenate (10%, w/v) was incubated at
37 °C in a shaker water bath for 1 h. The reaction was stopped by adding 1.0 ml of 10% trichloroacetic acid followed by 1.0 ml of 0.67% thiobarbituric acid and kept in a boiling water bath for 20 min. Tubes containing the reaction mixture were cooled in ice and centrifuged at 2500g for 10 min. The absorbance of the supernatant was taken at 432 nm against a reagent blank. LPO was calculated and expressed as nmol malondialdehyde formed/mg protein at 37 °C using a molar extinction coefficient of 1.56 × 10^5 M^{-1} cm^{-1}.

2.4.4. Glutathione

Concentration of glutathione in the tissue sample was determined by the method of Jollow et al. (1974). Briefly, 1.0 ml of tissue homogenate was precipitated with 1.0 ml of 4% sulfosalicylic acid, and the samples were cooled for 1 h at 4 °C before centrifuging at 1200g for 15 min in a cooling centrifuge. Absorbance of the assay mixture, consisting of 0.1 ml of supernatant, 2.7 ml of phosphate buffer (0.01 M, pH 7.4) and 0.2 ml of freshly prepared 5′-dithiobis-2-nitrobenzene (40 mg/10 ml of 0.1 M of phosphate buffer, pH 7.4) in a total volume of 3.0 ml was taken at 412 nm. The concentration of glutathione was expressed as μmol/g liver.

2.4.5. Glutathione reductase and peroxidase

Enzymes involved in glutathione metabolism, glutathione reductase (GR) and glutathione peroxidase (GPx), were measured in the post mitochondrial fraction (PMF) of the tissue by the spectrophotometric procedure described previously (Carlberg and Mannervik, 1975). For determining the activity of GR, the reaction mixture consisted of 1.68 ml of phosphate buffer (0.1 M, pH 7.4), 0.1 ml of 0.1 mM NADPH, 0.1 ml of 0.5 mM EDTA, 0.05 ml of 1 mM oxidized glutathione and 70 μl of PMS (10% w/v) in a final volume of 2.0 ml. Enzyme activity was calculated using a molar extinction coefficient of 6.22 × 10^3 M^{-1} cm^{-1} at 340 nm and expressed as nmol of NADPH oxidized/min/mg protein. The composition of the reaction mixture for measuring the activity of GPx was: 1.53 ml phosphate buffer (0.05 M, pH 7.0), 0.1 ml 1 mM EDTA, 0.1 ml 1 mM NaN3, 0.1 ml 1 mM reduced glutathione, 0.1 ml 0.2 mM NADPH, 0.01 ml 0.25 mM H2O2 and 100 μl PMF in a final volume of 2.0 ml. Nmoll of NADPH oxidized/min/mg protein was calculated using a molar extinction coefficient 6.22 × 10^3 M^{-1} cm^{-1}.

2.4.6. Xanthine oxidase (XOD)

Xanthine oxidase (XOD) is a form of xanthine oxid-reductase, a type of enzyme that generates reactive oxygen species. These enzymes catalyze the oxidation of hypoxanthine to xanthine and can further catalyze the oxidation of xanthine to uric acid. The enzyme activity was measured by the spectrophotometric procedure described by Stirpe and Corte (1969). Briefly, the reaction mixture consisting of 0.2 ml of PMS (10% w/v) diluted to 1 ml with 0.1 M phosphate buffer (pH 7.4) was incubated at 37 °C for 5 min. The reaction was initiated by 0.1 ml of xanthine and the reaction mixture was again kept at 37 °C for 20 min. The reaction was stopped by the addition of 0.5 ml ice cold perchloric acid (10%) and diluted by adding 2.5 ml distilled water after 10 min. This mixture was centrifuged at 4000g for 10 min and the absorbance of the clear supernatant was read at 290 nm. The enzyme activity was expressed as μmol uric acid formed/mg protein.

2.4.7. Glutathione S transferase

GST is a phase-II drug-metabolizing enzyme. GST was measured by the method described by Habig et al. (1974). The assay mixture in a total volume of 3.0 ml consisted of phosphate buffer 0.1 M (pH 7.4), 1 mM GSH, 1 mM CDNB and the enzyme fraction (10% w/v). The reaction was read at 340 nm and nmol CDNB conjugate formed/min/mg protein was calculated using a molar extinction coefficient of 9.6 × 10^3 M^{-1} cm^{-1}.

2.5. Statistical analysis

All results are expressed as mean ± SEM. The level of significance between different groups was calculated on the basis of analysis of variance test (ANOVA) and the drug-treated group was compared with the rats with hepatotoxicity; P < 0.05 was considered to be statistically significant.

3. Results

We examined the gross organ morphology, transmission electron microscopy and biochemical changes in rats induced liver cirrhosis with TAA for 16 days and confirmed liver cirrhosis. Gross morphology of the tissue and transmission electron microscopy of respective samples showed the therapeutic effect of drug extract on liver cirrhosis (Figs. 1 and 2). The liver weight: body weight ratio was also calculated and was found to be substantially increased in cirrhotic rats (Table 1). Morphological observations showed an increased size and enlargement of the liver in TAA treated groups. These changes were reversed by treatment with Trigonella foenum-graecum seed extract at the doses tested. The levels of serum ALP and GTT were markedly elevated in TAA treated animals, indicating liver damage. ALP levels increased by 44% in the TAA treated group where as in the TAA treated group which received the Trigonella foenum-graecum seed extract the elevation of ALP was markedly reduced to only 23% (Table 2). Analysis of LPO levels by thiobarbituric acid reaction showed

Figure 1 The section of liver from cirrhotic rat treated with extract showed normal lobular architecture and, mitochondria are normal and scattered, vacoules are less and macrophages sparse.
a significant ($P < 0.0001$) increase in LPO in the TAA treated rats. Treatment with *Trigonella foenum-graecum* seed extract at the dose of 500 mg/kg significantly ($P < 0.0001$) prevented the increase in LPO level which was brought to near normal. TAA treatment caused a significant ($P < 0.0001$) decrease in the level of GSH in the liver tissue when compared with control group. Treatment with *Trigonella foenum-graecum* seed extract at the dose of 500 mg/kg resulted in a significant increase of GSH when compared to TAA treated rats (Table 3). TAA treatment caused a significant ($P < 0.0001$) decrease in the level of GR and GPx in the liver tissue when compared with control group. Treatment with *Trigonella foenum-graecum* seed extract at the dose of 500 mg/kg resulted in a significant increase of GR and GPx when compared to TAA treated rats (Table 4). The drug metabolizing enzymes XOD and GST in TAA treated group showed a significant ($P < 0.0001$) increase in their respective levels as compared to normal control. Following Treatment with *Trigonella foenum-graecum* seed extract at 500 mg/kg significantly ($P < 0.0001$) prevented the increase in xanthine oxidase and glutathione-S-transferase levels which were brought to near normal.

### 4. Discussion

The liver is one of the vital organs of the animal body and plays a central role in transforming and clearing the chemicals,

### Table 1 Effect of test drug on liver and body weight ratio.

<table>
<thead>
<tr>
<th>Group</th>
<th>Liver weight (g)</th>
<th>Body weight (g)</th>
<th>Liver/bodyweight ratio (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>06.24 ± 0.27</td>
<td>266.67 ± 15.08</td>
<td>2.33</td>
</tr>
<tr>
<td>II</td>
<td>05.90 ± 0.25</td>
<td>240.25 ± 9.08</td>
<td>2.45</td>
</tr>
<tr>
<td>III</td>
<td>08.92 ± 0.30</td>
<td>170 ± 16.82</td>
<td>5.24</td>
</tr>
<tr>
<td>IV</td>
<td>09.40 ± 0.45</td>
<td>198 ± 8.06*</td>
<td>4.74</td>
</tr>
</tbody>
</table>

Data represent mean ± SEM ($n = 6$). * $P < 0.01$ when compared to Group III. Group I: normal control, Group II: control rats receiving the extract alone, Group III: cirrhotic group and Group IV: treated with the extract after inducing with liver Cancer.

### Table 2 Effect of the test drug on serum biochemical markers of liver cirrhosis.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Alkaline phosphatase (ALP) Equivalent units/ml</th>
<th>Gamma glutamyl transferase (GTT) Nmof p-nitroanilide/mg protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>14.68 ± 0.86 (100.00)</td>
<td>302.00 ± 12.51 (100.00)</td>
</tr>
<tr>
<td>II</td>
<td>14.46 ± 0.73 (098.50)</td>
<td>302.94 ± 08.79 (100.31)</td>
</tr>
<tr>
<td>III</td>
<td>21.10 ± 0.93 (143.73)</td>
<td>359.93 ± 18.56 (119.18)</td>
</tr>
<tr>
<td>IV</td>
<td>18.15 ± 0.69 (123.43)</td>
<td>317.81 ± 07.65 (103.23)</td>
</tr>
</tbody>
</table>

Data represent mean ± SEM ($n = 6$). Values in bracket show percent values with respect to Group I. Group I: normal control, Group II: control rats receiving the extract alone, Group III: cirrhotic group and Group IV: treated with the extract after inducing liver cirrhosis. While there was about 44% increase in the activity of ALP in cirrhotic rats, increase in ALP activity was about 23% in treated animals. The activity of gamma-glutamyl transferase in treated rats was almost similar to the normal control value.

### Table 3 Effect of the test drug on the biochemical markers of oxidative stress in liver cancer.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Lipid peroxidation (LPO) (nmol MDA formed/mg protein)</th>
<th>Reduced glutathione (GSH) (µmol/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>482.34 ± 9.99</td>
<td>1382 ± 19.80</td>
</tr>
<tr>
<td>II</td>
<td>508.83 ± 4.35</td>
<td>1358 ± 20.05</td>
</tr>
<tr>
<td>III</td>
<td>600.89 ± 8.56*</td>
<td>1300 ± 13.09*</td>
</tr>
<tr>
<td>IV</td>
<td>544 ± 6.26*</td>
<td>1318 ± 10.62*</td>
</tr>
</tbody>
</table>

* $P < 0.0001$ compared with normal Group I.  
+ $P < 0.0001$ compared with group II.  
+ $P < 0.0001$ compared with TAA Group III. Data represent mean ± SEM ($n = 6$).
but it is susceptible to toxicity from other agents. Certain medicinal agents, like paracetamol, when taken in overdoses or sometimes even within therapeutic ranges, may damage the liver. Other chemical agents, such as those used in laboratories and industries, natural chemicals (e.g. microcystins) and herbal remedies can also induce hepatotoxins. More than 900 drugs have been implicated in causing liver injury and it is one of the most common reasons for a drug to be withdrawn from the market. Products of natural origin have been found to be effective in various types of liver disease. Present study provides much evidence of the therapeutic effect of the hydroalcoholic extract of the dried seed of *Trigonella foenum-graecum (Smart et al., 1986). Present study did not separate bioactive compounds because we wanted to check the effectiveness of the extract before. Future studies involve extraction of bioactive compound in drug extract. The pharmacologically active principles present in extract may act by inhibiting the activity of some specific enzymes and/or ameliorating oxidative stress.

### 4.1. Hepatocellular injury and degenerative changes

The improvement in the liver in rats treated with the test drug could be because of multiple mechanisms of action of the bioactive principles present in the hydroalcoholic drug extract. The present study did not separate bioactive compounds because we wanted to check the effectiveness of the extract before. Future studies involve extraction of bioactive compound in drug extract. The pharmacologically active principles present in extract may act by inhibiting the activity of some specific enzymes and/or ameliorating oxidative stress.

### Table 4

<table>
<thead>
<tr>
<th>Groups</th>
<th>Glutathione reductase (nmol NADPH oxidized/min/mg protein)</th>
<th>Glutathione peroxidase (nmol NADPH oxidized/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>527.92 ± 30.26</td>
<td>180.34 ± 10.33</td>
</tr>
<tr>
<td>II</td>
<td>523.23 ± 20.30</td>
<td>165.56 ± 12.34</td>
</tr>
<tr>
<td>III</td>
<td>416.17 ± 22.34(^a)</td>
<td>134.20 ± 13.32(^b)</td>
</tr>
<tr>
<td>IV</td>
<td>526.21 ± 30.36(^c)</td>
<td>160.33 ± 10.20(^a,b)</td>
</tr>
</tbody>
</table>

\(^a\) P < 0.0001 compared with normal Group I.
\(^b\) P < 0.0001 compared with TAA Group III. Data represent mean ± SEM (n = 6).

### Table 5

<table>
<thead>
<tr>
<th>Groups</th>
<th>Xanthine oxidase (nmol of uric acid/mg of protein)</th>
<th>Glutathione-S-transferase (nmol of CDNB conjugate/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>174.25 ± 05.06</td>
<td>653.31 ± 22.49</td>
</tr>
<tr>
<td>II</td>
<td>154.86 ± 09.15</td>
<td>640.11 ± 16.35</td>
</tr>
<tr>
<td>III</td>
<td>193.34 ± 04.46(^a)</td>
<td>707.83 ± 08.65(^c)</td>
</tr>
<tr>
<td>IV</td>
<td>158.54 ± 11.73(^a,b)</td>
<td>633.03 ± 22.16(^b)</td>
</tr>
</tbody>
</table>

\(^a\) P < 0.0001 compared with normal Group I.
\(^b\) P < 0.0001 compared with TAA Group III. Data represent mean ± SEM (n = 6).
although the level increased slightly. Enzymes involved in GSH metabolism were also measured in this study and it was found that there was a decrease in the activity of GPx and GR in cirrhotic rats, while the activity of these enzymes increased considerably in treated rats. GPx is a particularly important biomarker because of its ability to scavenge the effect of peroxides produced. The reaction of XOD and GST, showed an increase in cirrhotic rats and a decrease in treated ones (Table 5). XOD is a Mo–Fe–S flavin containing hydroxylase, and is reported to contribute in various forms of injury (Ali et al., 2008). These enzymes produce ROS in catalyzing the reaction, and therefore, an increase in its activity leads to the oxidative stress. ROS contributes to diseases that are associated with pulmonary vascular lesions in both relevant animal models and humans. The drug extract might contain a bioactive substance that affects the S phase of the cell cycle in some unknown mechanism.

5. Conclusion

The present study shows that the hydroalcoholic extract prepared from Trigonella foenum-graecum partially reduces liver cirrhosis in rats by inhibiting the activity of certain enzymes, mainly xanthine oxidise, and scavenging free radicals. Future studies involve exploring the effect of each bioactive principle separately.

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