Comparison Hepatoprotective Effects of Crude and Germinated Fenugreek Seeds (Hulabah) in Carbon Tetrachloride Induced Hepatotoxicity Rats

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Abstract: Fenugreek (in Arabic, Hulabah) has medicinal properties such as appetite stimulation, anti-bacterial, antinociceptive, antidiabetic, antileukemic and hypocholesterolemic effects. The present study was designed to comparison hepatoprotective effects of crude fenugreek seeds (CFS) and germinated fenugreek seeds (GFS) against carbon tetrachloride (CCL) induced hepatotoxicity rats. Types and concentration of phenolic and flavonoid compounds in CFS and GFS were identified. Twenty eight male albino rats were divided randomly into four groups, comprising seven rats in each group. Group I served as normal control group, Groups II, III and IV were injected subcutaneously with CCL (2 ml/kg b. wt) to induced liver toxicity in rats. Group II represented positive control group, groups III and IV were fed on supplemented diet with CFS and GFS, respectively. Food intake (FI), body weights gain (BWG) and % change in body weight was calculated. Serum activity of gamma-glutamyltransferase (GGT), aspartate transaminase (AST), alanine aminotransferase (ALT), alkaline phosphates (ALP), superoxide dismutase (SOD) and catalase (CAT) enzymes, level of reduced glutathione (GSH), total bilirubin (TB), total protein (TP), albumin (Alb), malondialdehyde (MDA), urea nitrogen (UN), creatinine (Cr) and uric acid (UA) were assayed in all rats. Liver MDA and GSH level and antioxidant enzymes (SOD and CAT) as well as hisopathological changes were assayed. Results discovered that GFS have higher contents of phenolic (coumaric, gallic, caffic and sinapic acids) and flavonoids (pigenin, kamferol, luteolin and quercetin) compounds, compared to CFS. Liver injury induced by CCl, was characterized by a significant decrease in FI, BWG, serum level of TP, Alb and GSH and activity of SOD and CAT enzymes; and increase in GGT, AST, ALT and ALP activities and levels of TB, MDA, UN, Cr and UA. In addition to, a significant increase in liver MDA level and decrease in GSH level and activity of SOD and CAT enzymes, in positive rats compare to normal rats. Histopathological examination showed congestion of central vein and hepatic sinusoids and fatty changes in hepatocytes. The current results recognized that CFS and GFS caused significant improvement in all of the biochemical parameters and liver structure. The most improvements in all of biochemical parameters and liver structure which were tending toward normal results were showed in treated rats with GFS. In conclusion, the present findings suggested that fenugreek seeds have hepatoprotective effects against CCl-induced hepatotoxicity rats. GFS exhibited stronger antioxidant activity and hepatoprotective properties compared to CFS.

Key words: Liver Toxicity - CCL - Liver Functions - Antioxidant Enzymes - Fenugreek Seeds

INTRODUCTION

Fenugreek (Trigonellafoenum-graecum L.) is a member of the leguminous family [1] which is an annual plant in India, Middle East and Mediterranean countries [2]. Fenugreek (in Arabic, Hulabah) has been announced to be a medicinal plant with a large number of medicinal properties such as antifertility [3], anti-bacterial [4], anti-nociceptive effects [5], appetite stimulation, antidiabetic, antileukemic, hypocholesterolemic and antimicrobial effects [6].

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Fenugreek leaves are edible and often used as a vegetable dish. It helps in blood formation while seeds are valuable to treat anemia, being rich in iron [7]. Fenugreek leaves are source of saponins, protein, fat, carbohydrates, fiber, minerals (calcium, zinc iron and phosphorous) and vitamins (riboflavin, carotene, thiamine, niacin and vitamin C) [8].

Fenugreek seeds are the most valuable plant part that is fibrous, sticky and gummy in nature [9]. Dried seeds are an important spice and have wide applications in food and beverages [2, 6]. It contains a substantial amount of glycolipids, phospholipids, oleic, linoleic, linolenic acids [10, 11], vitamins as A, C, B, B, niacinic acid and niacin [12], fiber and many other functional nutrients [6, 13]. Germinated fenugreek seeds are rich in bioactive antioxidant substances and extensively used as a substantial ingredient in food preparations and/or herbal formulations [14]. Germinated fenugreek seeds had significantly higher contents of total protein and total lysine compared to ungerminated seeds. Additionally, germination decreased dietary fiber and starch thereby raising the level of sugars [15].

Liver is the largest organ in human body and regulates several important functions including metabolism [16] and detoxification of the most components that enter the body [17]. Carbon tetrachloride (CCl) is a highly toxic chemical agent and the most famous agent used to induce liver damage in animal study [18, 19]. The toxic effect of CCl is related to trichloromethyl radical (CCl) produced during oxidative stress [20]. It induces activation of liver macrophages and/or chemoattraction of extrhepatic cells such as neutrophils and lymphocytes [21]. The activated macrophages are released and lead to liver inflammation, fibrosis and injury [22]. Medicinal plants, herbs and spices have received much awareness as important source of biologically active compounds. The most important of these active components are vitamins, polyphenolic compounds, tannins, alkaloids, terpenoids and essential oils. Therefore, the present study was conduct to comparison hepatoprotective effects of crude and germinated fenugreek seeds and their effects on some biochemical and antioxidant biomarkers as well as histological changes in CCl4 induced hepatotoxicity rats. In addition to, identify types and concentrations of total phenolic and flavonoids compounds in crude and germinated fenugreek seeds.

MATERIALS AND METHODS

Materials

Fenugreek Seeds: Dried fenugreek seeds were purchased from the local market (Haraz market for herbs and medicinal plants), Cairo, Egypt.

Rats and Diet: Twenty eight male adult albino rats of Sprague-Dawley strain weighing 200 ± 5 g were purchased from the Laboratory Animal Colony, Ministry of Health and Population, Helwan, Egypt. Basal diet constituents were purchased from El-Gomhorya Company for Pharmaceutical and Chemical, Cairo, Egypt.

Chemicals and Kits: Carbon tetrachloride (CCl4), formalin, diethyl ether and other chemicals were purchased from El-Gomhorya Company for Chemical and Pharmaceutical, Cairo, Egypt. Kits for biochemical analysis of gamma-glutamyl transferase (GGT), aspartate transaminase (AST), alanine aminotransferase (ALT), alkaline phosphates (ALP), total protein (TP), total bilirubin (TB), albumin (Alb), malondialdehyde (MDA), reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), urea nitrogen (UN), creatinine (Cr) and uric acid (UA) were purchased from the Gamma Trade Company for Pharmaceutical and Chemicals, Dokki, Egypt.

Methods

Preparation of Fenugreek Seeds: Dried fenugreek seeds were first cleaned from broken seeds, dust and other foreign materials and washed with tap water to remove possible potential dust. Afterwards, it was dried by cotton cloth to remove the excess liquid prior to drying. The amount of cleaned seeds were divided into two parts, the first part was ground to fine powder particle size of less than 0.2mm. The final dried powder was stored in plastic containers for further use. The second parts subjected to germination process.

Germination of Fenugreek Seeds: Germination of fenugreek seeds was done according the method of Amany et al. [23]. In briefly, cleaned fenugreek seeds were soaked in tap water for 12 hr. at room temperature. The soaked seeds were allowed to germinate in strainer with covering by wet cotton cloth for 72 hr. at room temperature with frequent watering. After ending the germination process, the germinated seeds were dried at 50-55°C using oven vacuum. Then, a grinder mill and
sieves were used to obtain a powder particle size of less than 0.2mm. The final dried powder was stored in plastic containers for further use.

**Identification Types and Amount of Phenolic and Flavonoid Compounds:** Separation and determination of phenolic compounds in crude and germinated fenugreek seeds were carried out as described by Goupy et al. [24] using HPLC. Identification types and concentrations of flavonoids compounds were completed using HPLC as described by Mattila et al. [25].

**Preparation of Basal Diet:** The basal diet (AIN-93M) was prepared according to Reeves et al. [26]. Diet was formulated to meet the recommended nutrients levels for maintaining healthy rats. It consists of casein 20%, soybean oil 5%, sucrose 10%, choline chloride 0.20%, mineral mixture 4.0%, vitamin mixture 1.0%, fibers 5%, L-Cystine 0.18% and the remainder was corn starch.

**Induction of Hepatotoxicity:** Induction of liver toxicity in rats encouraged by the subcutaneous injection of CCL4 at a dose of 2 ml/kg according to the method described by Sundaresan and Subramanian [27].

**Experimental Groups:** All rats were acclimatized at the animal house conditions (25°C and 55% humidity with 12-hr light/12-hr dark schedule) for one week before starting experiment and fed with a basal diet and water **ad libitum**. Twenty eight male albino rats were divided randomly into four groups comprising seven rats in each group and fed on the basal diet with water **ad libitum** during experimental period (4 weeks). After acclimatization period animals were grouped as follows:

- **Group I:** Rats were injected subcutaneously with paraffin oil and served as normal control group.
- **Group II:** Rats were injected subcutaneously with CCL4 (2 ml/kg b. wt) and served as positive control group.
- **Group III:** Rats were injected subcutaneously with CCL4 (2 ml/kg b. wt) and feed on supplemented basal diet with crude fenugreek seeds (CFS) at level of 15% of basal diet.
- **Group IV:** Rats were injected subcutaneously with CCL4 (2 ml/kg b. wt) and feed on supplemented basal diet with germinated fenugreek seeds (GFS) at level of 15% of basal diet.

At the end of the experimental period (4 weeks), diets were withheld from all animals for 12-hr. (except of water), final body weights were recorded. Then, all animals were sacrificed under light diethyl ether. Blood samples were collected by cardiac puncture in clean centrifuge tubes and centrifuged for 15 minutes at 3000 rpm for serum separation. Then, serum was carefully pick out, transfers into dry clean test tubes and frozen at -20°C until use for biochemical analysis. Liver of all animals were cut into two parts, one was immediately prepared for determination of biochemical parameters. The other part of liver of all animals was immersed in neutral buffered formalin 10% for histopathology examination.

**Body Weight Gain and Percent Change in Body Weight Assay:** The changes in body weight were determined by weighing the animals on a balance seals prior the experiment (IBW) and after four weeks (FBW). The biological value of the different diets was assessed by the determination of its effect on body weight gain (BWG) and percent change of body weight which were calculated using the following formula:

\[
\text{BWG} = \frac{\text{Final Body Weight} - \text{Initial Body Weight}}{\text{Initial Body Weight}} \times 100
\]

**Biochemical Analysis:** Serum activity of GGT was determined according to method of Tietz [28] as described by kits (Diamond Co, Hannover, Germany) instructions. Serum AST, ALT activities were assayed colorimetric using kits instruction (Diamond Co, Hannover, Germany) as described by Reitman and Frankel [29]. Serum level of ALP was determined by the method of King and King [30] using kits (Diamond Co, Hannover, Germany) instructions.

Assay of serum TP, Alb and TB levels were determined colorimetric as described by Young [31], Young [32] and Tietz [33], respectively.

Serum concentrations of UN and Cr were determined using colorimetric kinetic as described by Waiker and Bonventre [34]. Serum uric acid (UA) was determined using the enzymatic colorimetric method as described by Fossati et al. [35].

Serum concentration of MDA was determined according to described methods by Draper and Hadley [36]. The principle of method is spectrophotometric measurements of the color produced by the reaction of thiobarbituric acid (TBA) with MDA. The concentration of MDA was then calculated as expressed as µmoles/dL.
Serum SOD, GSH and CAT activities were determined by Autoanalyzer (Roche-Hitachi, Japan) using commercial kits according to the methods described by Kakkor et al. [37], Hissin and Hiff [38] and Sinha [39], respectively.

**Preparation of Liver Tissue Homogenate:** Liver of all animals were cut into small pieces and immediately homogenized in 5-10 ml ice-cold medium containing buffer (50 mm potassium phosphate, PH 7.5, containing 2mM EDTA) per gram tissue using tissue homogenizer (Sonicator, model 4710, Cole-Parmer Instrument Company, USA). The homogenates tissues were centrifuged at 4000 rpm for 15 min at 4°C [40]. Then, supernatant was carefully separate for the determinations of for biochemical analysis.

**Determination of Liver MDA, GSH and Antioxidant Enzymes:** The liver content of MDA and GSH was determined as described by Ruiz-Larea et al. [41] and Bulaj et al. [42], respectively. The liver activity of SOD and CAT enzymes was chemically determined according to the methods by Kakkar et al. [37] and Sinha [39], respectively.

**Histopathological Examinations:** Liver of all rats was immersed in neutral buffered formalin (10%) for 24 hr. The fixed tissues were processed routinely, embedded in paraffin, sectioned, deparaffinized and rehydrated using the standard techniques according to the method of Bancroft and Gamble [43]. The extent of CCl₄-induced damage was evaluated by assessing the morphological changes in the liver sections stained with hematoxylin and eosin (H and E).

**Statistical Analysis:** The obtained results were expressed as Mean ± SD. Data were evaluated statistically with computerized SPSS package program (SPSS 20.00 software for Windows). Data of biochemical analysis were evaluated statistically using one-way analysis of variance (ANOVA). Significant difference among means was estimated at p<0.05.

**RESULTS**

Figure 1 presents the findings upon some of the identifications types and amount of phenolic compounds in CFS and GFS. The present data revealed that CFS and GFS have coumaric, gallic, caffic and sinapic acids. Caffeic and sinapic acids (0.933±0.02, 3.75± 0.03, 0.840±0.02 and 1.257±0.01 mg/100g, respectively) of CFS were lower than that of GFS (1.73±0.02, 5.523±0.03, 1.133±0.03 and 2.29±0.04, mg/100g, respectively). In CFS and GFS, gallic and sinapic acids were the most and moderate abundant of phenolic compounds, respectively, while the lowest abundant was coumaric and caffic acids.

Figure 2 shows some of the identification types and amount of flavonoid compounds in CFS and GFS. It obvious that GFS have the higher content of pigenin, kamferol, luteolin and quercetin (63.967±0.50, 12.433±0.12, 22.733±0.29 and 19.80±.20 mg/100g, respectively) compared to that of CFS (59.5±0.30, 10.47±0.35, 20.667±0.21 and 15.667±0.21 mg/100g, respectively). Pigenin and luteolin were the most abundant flavonoids, the moderate and the lowest abundant were quercetin and kamferol, respectively in CFS and GFS.

Results of food intake (FI), initial body weight (IBW), final body weight (FBW), body weight gain (BWG) and % change of body weight of normal rats, CCl₄ treated rats and CCl₄ treated rats with feeding on supplemented diet with CFS or GFS are presenting in Table 1. It showed that FI, FBW, BWG and % change of body weight of CCl₄ treated rats (positive rats) were significant decreased (p<0.05), compared with that of normal rats. On the other hand, CCl₄ treated rats with feeding on supplemented basal diet with CFS or GFS have significant increase in FI, FBW, BWG and % change of body weight, compared with that of positive rats. Whereas, CCl₄ treated rats with feeding on supplemented basal diet with GFS have significant increase (p<0.05) in FI, FBW, BWG and % change of body weight, compared with that of feeding on supplemented basal diet with CFS.

Tables 2 shows the means and standard deviations values for serum activity of GGT, AST, ALT and ALP enzymes of normal rats, CCl₄ treated rats and rats of co-administrated CCl₄ with feeding on supplemented diet with CFS or GFS. The present results revealed that serum GGT, AST, ALT and ALP activities were significant elevated (p< 0.05) in CCl₄ treated rats compared with that of the normal rats. The activities of these enzymes in the co-administration of CCl₄ with feeding on supplemented diet with CFS or GFS were significantly decreased (p<0.05) compared with that of the CCl₄ treated rats and feed only on basal diet. The CCl₄ treated rats and fed on supplemented diet with GFS have significant decrease (p<0.05) in the serum activities of these enzymes and were tend toward levels of normal rats, compared with that fed on supplemented diet with CFS.
Fig. 1: Identification types and amount of phenolic compounds in CFS and FFS

Fig. 2: Identification types and amount of flavonoid compounds in CFS and GFS

Table 1: Effect of CFS and GFS on FI, FBW, BWG and % change of BW in CCl₄ induced hepatotoxicity rats

<table>
<thead>
<tr>
<th>Parameters as Mean ± SD</th>
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<tr>
<td>Groups</td>
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<tr>
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<tr>
<td>G I: Normal control rats</td>
</tr>
<tr>
<td>GII: Positive control rats (CCl₄ treated)</td>
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<tr>
<td>G III: CFS + CCl₄</td>
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<tr>
<td>G IV: GFS + CCl₄</td>
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Means with different letters in each row are significantly differs at p<0.05.

CFS: Crude fenugreek seeds
GFS: Germinated fenugreek seeds

Table 2: Effect of CFS and GFS on serum GGT, AST, ALT and ALP activities in CCl₄ induced hepatotoxicity rats

<table>
<thead>
<tr>
<th>Parameters as Mean ± SD</th>
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<tr>
<td>Groups</td>
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<tr>
<td>-------------------------</td>
</tr>
<tr>
<td>G I: Normal control rats</td>
</tr>
<tr>
<td>GII: Positive control rats (CCl₄ treated)</td>
</tr>
<tr>
<td>G III: CFS + CCl₄</td>
</tr>
<tr>
<td>G IV: GFS + CCl₄</td>
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</table>

Means with different letters in each row are significantly differs at p<0.05.

CFS: Crude fenugreek seeds
GFS: Germinated fenugreek seeds
Table 3: Effect of CFS and GFS on TP, TB and Alb in CCl<sub>4</sub> induced hepatotoxicity rats

<table>
<thead>
<tr>
<th>Parameters as Mean ± SD</th>
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<tr>
<td>Parameters as Mean ± SD</td>
</tr>
<tr>
<td>Groups</td>
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<tr>
<td>---------</td>
</tr>
<tr>
<td>GI: Normal control rats</td>
</tr>
<tr>
<td>GII: Positive control rats (CCl&lt;sub&gt;4&lt;/sub&gt; treated)</td>
</tr>
<tr>
<td>GIII: CFS + CCl&lt;sub&gt;4&lt;/sub&gt;</td>
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<tr>
<td>GIV: GFS + CCl&lt;sub&gt;4&lt;/sub&gt;</td>
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Means with different letters in each row are significantly differs at p< 0.05.

SD: Standard Division of mean
CFS: Crude fenugreek seeds
GFS: Germinated fenugreek seeds

Table 3 illustrates serum levels of total protein (TP), Albumin (Alb) and total bilirubin (TB) in normal control group, CCl<sub>4</sub> treated group and CCL<sub>4</sub> treated groups and fed on supplemented diet with CFS or GFS. The present results revealed significant decrease (p<0.05) in serum level of TP and Alb and increase in serum TB level in hepatotoxicity rats, compared with that of the normal rats. Treated hepatotoxicity rats with CFS or GFS have significant increase in serum TP and Alb level and decrease in serum TB level compared with CCL<sub>4</sub> treated rats (positive rats). Treated hepatotoxicity rats with GFS have significant elevation in serum TP and Alb level and lowering in serum TB level, compared with that treated with CFS.

Table 4 represents results of serum lipid peroxidation (MAD) and GSH level and activities of antioxidant enzymes (SOD and CAT) of normal control group, CCL<sub>4</sub> treated group and CCL<sub>4</sub> treated groups with feeding on supplemented diet with CFS and GFS. It showed that CCL<sub>4</sub> treated group had significant increase in serum level of MDA and GSH and decrease in SOD and CAT activities, compared with normal group. On the other hand, CCL<sub>4</sub> treated rats with feeding on supplemented basal diet with CFS or GFS have significant decrease in serum MDA level and increase in serum GSH level and activity of SOD and CAT enzymes, compared with that of the CCL<sub>4</sub> treated rats (positive rats). CCL<sub>4</sub> treated rats with feeding on supplemented diet with GFS have significant decrease in serum MDA level and increase in serum GSH level and SOD and CAT activities, which were tend toward levels of normal rats, compared with that treated with CFS.

Table 5 shows the liver concentrations of MDA and GSH and activity of SOD and CAT enzymes of normal control group, CCL<sub>4</sub> treated control group and CCL<sub>4</sub> treated groups with feeding on supplemented diet with CFS or GFS. The present results showed significant increase in liver MDA level and decrease in GSH level and SOD and CAT activity were decreased in CCL<sub>4</sub> treated rats, compared to that of normal rats. Co-administration of CCL<sub>4</sub> with feeding on supplemented diet with CFS or GFS caused significant decrease (p<0.05) in liver MDA level and increased GSH level and SOD and CAT activities, compared with that of CCL<sub>4</sub> treated group alone. Treated hepatotoxicity rats with feed on GFS have significant decrease in liver MDA level and increase in GSH level and SOD and CAT activities, compared with that treated with CFS.

As shown in Figures 3, 4 and 5, the present results revealed that injected rats with CCL<sub>4</sub> (positive rats) have significant increase in serum urea nitrogen (UN), creatinine (Cr) and uric acid (UA) concentrations, respectively. CCL<sub>4</sub> treated groups with feeding on supplemented diet with CFS or GFS have significant decrease in serum level of UN, Cr and UA, compared with that of positive rats. The serum level of UN, Cr and UA were significant decreased (p<0.05) in treated hepatotoxicity rats with GFS, compared with that treated with CFS.

Microscopic histological examination of liver sections of normal rats showed no histological changes and the hepatocytes arranged in cell strands radiating from the central vein with intervening blood sinusoids which appeared to be lined by Kupffer cells as shown in Fig. 6. Liver sections of CCL<sub>4</sub> treated animal's revealed severe structural damage with congestion of central vein and hepatic sinusoids as well as fatty change of hepatocytes as shown in Fig. 7. In CCL<sub>4</sub> treated rats with feeding on supplemented diet with CFS did not completely regain the hepatocytes to normal. The liver sections revealed some fatty change of hepatocytes as shown in Fig. 8. Co-administration of GFS to the CCL<sub>4</sub> treated animals, the central vein appears normal and the hepatocytes regained their normal structure as shown in Fig. 9.
Table 4: Effect of CFS and GFS on serum MDA and GSH levels and SOD and CAT activities in CCl_4 induced hepatotoxicity rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>MDA (µmol/dl)</th>
<th>GSH (nmol/l)</th>
<th>SOD (mmol/dl)</th>
<th>CAT (mmol/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G I: Normal control rats</td>
<td>20.23±0.47</td>
<td>65.57±0.53</td>
<td>96.09±0.43</td>
<td>76.03±0.25</td>
</tr>
<tr>
<td>G II: Positive control rats</td>
<td>71.76±0.87</td>
<td>32.83±0.48</td>
<td>25.97±1.07</td>
<td>41.60±5.29</td>
</tr>
<tr>
<td>G III: CFS + CCl_4</td>
<td>35.86±0.45</td>
<td>55.99±0.75</td>
<td>79.20±0.57</td>
<td>56.97±0.95</td>
</tr>
<tr>
<td>G IV: GFS + CCl_4</td>
<td>21.61±0.46</td>
<td>60.04±0.54</td>
<td>94.84±0.51</td>
<td>74.56±0.47</td>
</tr>
</tbody>
</table>

Means with different letters in each row are significantly differs at p< 0.05.
SD: Standard Division of mean
CFS: Crude fenugreek seeds
GFS: Germinated fenugreek seeds

Table 5: Effect of CFS and GFS on liver MDA and GSH levels and SOD and CAT activities in CCl_4 induced hepatotoxicity rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>MDA (µ/mg tissues)</th>
<th>GSH (nmol/mg tissues)</th>
<th>SOD (µ/mg tissues)</th>
<th>CAT (µ/mg tissues)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G I: Normal control rats</td>
<td>2.13±0.20</td>
<td>54.28±1.95</td>
<td>58.01±0.56</td>
<td>68.13±1.24</td>
</tr>
<tr>
<td>G II: Positive control rats</td>
<td>9.43±0.39</td>
<td>26.59±0.87</td>
<td>23.04±0.58</td>
<td>27.93±0.83</td>
</tr>
<tr>
<td>G III: CFS + CCl_4</td>
<td>4.60±0.27</td>
<td>45.06±0.90</td>
<td>46.40±0.98</td>
<td>55.84±1.41</td>
</tr>
<tr>
<td>G IV: GFS + CCl_4</td>
<td>2.07±0.11</td>
<td>52.71±0.59</td>
<td>57.09±0.85</td>
<td>67.50±1.15</td>
</tr>
</tbody>
</table>

Means with different letters in each row are significantly differs at p< 0.05.
SD: Standard Division of mean
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Fig. 3: Effect of CFS and GFS on serum level of UN in CCl_4 induced hepatotoxicity rats

Fig. 4: Effect of CFS and GFS on serum Cr level in CCl_4 induced hepatotoxicity rats
DISCUSSION

Some of medicinal agents may cause liver injury and called hepatotoxins when taken in overdoses and/or even when introduced within therapeutic ranges [44]. A number of plants and their extracts have hepatoprotective effect by improving antioxidant status and play a major role in the management of liver diseases. Therefore, the present study was conduct to comparison hepatoprotective effects of CFS and GFS in CCl4 induced hepatotoxicity rats. In addition identify types and amount of phenolic and flavonoid compounds of CFS and GFS.

The identifications types of phenolic compounds in CFS and GFS were coumaric, gallic, caffic and sinapic acids. Gallic acid was the most abundant, followed by sinapic acid, coumaric and caffic acids, respectively. A pigenin, kamferol, luteolin and querectin were identified flavonoid compounds. The most abundant flavonoid compounds were pigenin and luteolin, followed by querectin and kamferol, respectively. As clear from the present results GFS have the higher content of the identified types of phenolic and flavonoids compounds. Previous researches revealed that fenugreek seeds contain quercetin and kaempferol [11, 45], coumarin compounds [46, 47]. Moreover, Benayad et al. [48] reported that the major diagnostic flavonoid in fenugreek seeds were a pigenin, kaempferol, luteolin and other derivatives of hydroxycinnamic acids (caffeic, dihydrogallic, sinapic, gallic, coumaric acids) with a predominance of caffeic acid derivatives. Predominate quantitative was pigenin, followed by luteolin and kaempferol which represents the major compounds of the total identified compounds. Germination (sprouting) has been suggested as an inexpensive and effective way to improve the quality of legumes. Sprouts are believed to be rich in health-promoting phytochemicals [49].
Fenugreek sprouts are rich in polyphenols, reducing sugars and minerals (K, Zn and Fe). Sprouts fenugreek seeds are rich in polyphenols compared with ungerminated seeds [50]. Khole et al. [14] reported that germinated fenugreek seeds are rich in bioactive antioxidant substances and used extensively as an important ingredient in daily food preparations and herbal formulations. Recently, Norziah et al. [51] indicated that germinated fenugreek seeds extract had significantly highest phenolics and flavonoids compared to extracts from row seeds.

Carbon tetrachloride (CCL\textsubscript{4}) is generally used for free radical induced liver injury [52]. It causes liver damage by initiating the process of lipid peroxidation by the formation of lipid peroxyl radicals [53] through bioactivation of CCL\textsubscript{4} into trichloromethyl free radical (CCL\textsubscript{3}) by cytochrome P450 system in liver microsomes and consequently causes lipid peroxidation of membranes that leads to liver damage [54]. This process also affects several other organs of the body such as lungs, hearts, testes, kidneys and brain [52].

The effect of administration CCL\textsubscript{4} to rats, the present results revealed that CCL\textsubscript{4} treated rats had significant decrease in FI, BWG and % change of body weight. These results were in convention with the obtained finding by Eidi et al. [55] and Ezejindu et al. [56] who indicated that CCL4 induced hepatotoxicity rats have significant decrease in final body weight and body weight gain. These results may be attributed to loss of appetite in the treated animals as confirmed by the decrease in food intake. Okamoto and Okabe [57] demonstrated that CCL\textsubscript{4} to rats induced oxidative stress and results in anorexia independently of hepatitis.

The manner of liver injuries caused by CCL\textsubscript{4} was observed from the significant increments in serum activities of GGT, AST, ALT and ALP enzymes. The obtained results were in agreement with Sturgill and Lambert [58] and Zeashan et al. [59] who announced that serum activities of liver enzymes are increased in all types of liver damage including CCL\textsubscript{4} induced hepatotoxicity. Austin [60] reported that liver disease is constantly associated with cellular necrosis, elevated in serum levels of SGOT, SGPT and ALP. Moreover, Naik and Panda [61] mentioned that the increase in serum AST, ALT and ALP levels in CCL\textsubscript{4}-treated animals is an indicator of liver damage as these enzymes leak out from liver into the blood at the instance of tissue damage, which is always associated with hepatonecrosis. Recently Sahreen et al. [62] reported that CCL\textsubscript{4} treated rats have significant increase in serum AST, ALT, ALP and γ GT levels. Das [63] indicated that liver damaged are monitored by raised biochemical marker enzymes (SGOT, SGPT and ALP).

The effect of CCL\textsubscript{4} on developed hepatocellular damage was evident from a significant lower in serum levels of TP and Alb and higher in TB, compared to normal rats. These results were in agreement with Zeashan et al. [59] and Khan and Al-Zohairy [64] who indicated that CCL\textsubscript{4} induced hepatic damage in experimental animals and reduced serum TP and Alb level. This indicate to the impair in liver functions or poor synthesis, either primary as in liver cells damage or secondary to loss protein in urine, due to nephritic syndrome and chronic glomerulonephritis induced by CCL\textsubscript{4} toxicity [65]. Bilirubin is a breakdown product of hemoglobin, which is transported from the spleen to the liver and excreted into bile. However, hyper-bilirubinemia is a direct result of hepatocellular damage [63]. The remarkable elevation of bilirubin in CCL\textsubscript{4}, administered rats was in agreement with Patrick- iwuanyanwu et al. [66]. These results were in accordance with Zeashan et al. [59]; Sunilson et al.[67] and Nafiu et al.[68] who indicated that CCL\textsubscript{4} induced hepatic damage in experimental animals and increased serum total and direct bilirubin level.

The damage in liver tissues induced by CCL\textsubscript{4} injection was characterized by the significant increase in serum and liver MDA concentrations and decrease in GSH level and activity of SOD and CAT enzymes compared to normal rats. Previous researchers reported that liver disease is constantly associated with cellular necrosis, elevated in tissue lipid peroxidation (MDA) and depletion in tissue GSH levels [60], reduces tissue CAT and SOD activities [69]. Administration of CCL\textsubscript{4} caused significant elevate in liver MDA as a product of lipid peroxidation [70, 62] and decrease in the level of SOD, CAT, GPx and GSH [71]. Mahmood and Rezq [72] showed significant increase in serum MDA level and decrease in GSH level and activity of antioxidant enzyme (SOD, GPx and CAT). Recently, researches revealed that liver of CCL\textsubscript{4} - intoxicated rats have significant increase in lipid peroxidation (MDA) and decrease in the level GSH and activities of antioxidant enzymes (SOD, catalase, GPx, GR and GST) compared to the normal control group [73].

Results revealed that serum levels of UN, Cr and UA were significant increased in CCL\textsubscript{4} treated rats. The present result was in agreement with Stephen et al. [74] and Muhammad et al. [75] who showed significant increase of serum BUN and Cr level of which are possible indicators of hepatic and/or kidney injuries induced
through CCl<sub>4</sub> treatment. Recently, Haghi et al. [76] revealed that serum level of UN, UA and Cr were significantly increased in CCl<sub>4</sub>-treated rats. These elevations are indicative of renal injury by CCl<sub>4</sub>.

The present results were supported by the histopathological examination of liver which showed severe structural damage including congestion of central vein and hepatic sinusoids and fatty changes in hepatocytes. This result was in agreement with Junnila et al. [18] and Karakus et al. [19] who observed that CCl<sub>4</sub> induced several changes in liver structure as fibrosis, cirrhosis and hepatocarcinoma. Adewale et al. [77] showed defect ranging from massive tissue necrosis, congested central vein, fatty degeneration and infiltration by inflammatory cells in rats treated with CCl<sub>4</sub>. Recently, Zhen et al. [78] observed hepatolobular injury with centrilobular necrosis, balloon cells and lipids accumulation in CCl<sub>4</sub>, treated rats.

Co-administration of CCl<sub>4</sub> with CFS or GFS induced significant improvement in FI and BWG, reduction in serum activity of GGT, AST, ALT, ALP enzymes, levels of TB, MDA, UN, Cr and UA and elevations in serum level of TP, Alb and GSH and activity of SOD and CAT enzymes, compared to CCl<sub>4</sub>, treated rats alone. Moreover, CFS and GFS significantly reduced liver lipid peroxidation (MDA) and elevated GSH level and activity of antioxidant enzymes (SOD and CAT). The present results were supported by the obtained observation of histopathological examination which showed improvement of liver structure in CCl<sub>4</sub>, treated rats and fed on supplemented diet with CFS or GFS. Germinated fenugreek seeds induced significant higher improvement in all study parameters and histopathological examination of liver which were tend toward normal level followed by CFS. These findings were in accordance with Anuradha and Ravikumar [79] who showed fenugreek seed normalize the elevated lipid peroxidation and improved susceptibility to oxidative stress associated with depletion of antioxidants in liver, kidney and pancreas in diabetic rats. In addition to, Thakran et al. [80] demonstrated that fenugreek seeds partially prevented the abnormalities in the liver structure (cytoplasm degeneration) and changes in neuropathic diabetic rats. These results were confirmed by the results of Preet et al. [81] who found that fenugreek seed reduced histopathological and biochemical abnormalities associated with type-1 diabetes. Recently, Das [63] reported that fenugreek seeds possess significant hepatoprotection against CCl<sub>4</sub> induced hepatotoxicity in albino rats.

Body has ability to counter the effect of free radicals induced by CCL<sub>4</sub> through antioxidants. These antioxidants are produced either endogenously or received from exogenous sources and include glutathione peroxidase, superoxide dismutase, catalase and glutathione reductase enzymes and other compounds with antioxidant activity include glutathione, flavonoids [82] which protect cells against oxidative stress [83]. The hepatoprotective effect of fenugreek seeds perhaps related to its antioxidant properties. The antioxidant properties of fenugreek seeds might be relate to its content of phenolic and flavonoid compounds. Acharya et al. [2] reported that medicinal properties of fenugreek seeds are associated with its phytochemicals such as galactomannans, phenolic compounds, alkaloids, proteins, vitamins (A, B1, C and nicotinic acid) and volatile oils. Phenolic compounds of fenugreek seeds can be considered cytoprotective during ethanol-induced liver damage [84]. Moreover, Kaviarasan et al. [85] found that administration of fenugreek seed polyphenol extract to ethanol-fed rats significantly reduced the levels of lipid peroxidation products and carbonyl content, increased the activities of antioxidant enzymes and restored the level of thiol groups. Fenugreek seed polyphenol extract ameliorates the pathological liver changes; lipid accumulation and collagen fibres induced by chronic ethanol feeding. Vishnu et al. [86] reported that fenugreek seeds have antioxidant activity and produce beneficial effects such as neutralization of free radicals and enhancement of antioxidant properties. Previous studies reported that the antioxidant activity is very well correlated with the content of phenolic components [87, 88]. Phenolic compounds are efficient scavenger of free radicals as well as transition metal ion chelating agents. Flavonoids possess a chemical structure with particular hydroxyl position in the molecule that is considered to be involved in proton donating and radical scavenging mechanism [89]. In this situation, many studies showed that phenolics and flavonoids have positive correlation with antioxidant capacities in plant extracts [90, 91]. Fenugreek seeds extract exhibits antioxidant activity by 1,1-diphenyl-2-picryl-hydrazyl (DPPH•) scavenging activity, total phenolic contents (TPCs), flavonoid content, chelating activity and reducing power. Therefore fenugreek seeds could act as an effective source of antioxidants [92]. The higher content of total phenolic and flavonoid compounds in the fenugreek seeds extracts means the greater it's the antioxidant capacity [51]. Germinated fenugreek seeds were shown to be rich in polyphenols [50]. Dixit et al. [93]
elucidated that GFS are considered to be more beneficial which may be related to the presence of flavonoids and polyphenols. So, the germinated fenugreek seeds have a significant antioxidant activity which induced significant improvements of liver and kidney function. Kumar et al. [94] observed strong correlation between total phenol and flavonoid with antioxidant activities such as metal chelating and β-carotene protective activity of the sprouts. Sushma and Devasena [95] found that treatment of GFS extract to rats with cypermethrin induced hepatic and renal toxicity, can restored antioxidant status and enzymatic activities to near normal levels. Norziah et al. [51] reported that extract from GFS exhibited stronger antioxidant activity compared to CFS, which could be attributed to its higher contents of phenolic and flavonoid. These results highlighted that higher contents of phenolic and/or flavonoid led to higher antioxidant activity. Thus, germination can lead to the development of such functional foods that have a positive effect on the human organism and that help in maintaining the health [96].

CONCLUSION

The present study concluded that fenugreek seeds effectively improved liver functions and protected against liver tissues damage induced by CCL4. Fenugreek seeds have protective effect against the loss of antioxidant activities as result of oxidative process caused by CCl4 injection due to its content of phenolic and flavonoids compounds. Germinated seeds exhibited stronger antioxidant activity and hepatoprotective properties compared to dry fenugreek seeds. These hepatoprotective properties of fenugreek seeds suggested that regular consumption of it, especially germinated seeds, may protect against liver disease and imbalanced antioxidant. Thus, the possibility that crude and germinated fenugreek seeds reduce the risk of liver disease and oxidation process remains open and further longitudinal studies are needed to confirm the importance of it in the prevention of liver disease.

REFERENCES


