Research Article

ANTIOXIDANT AND HEPATOPROTECTIVE ACTIVITIES OF SOME SAUDI MEDICINAL PLANTS

Essam Abdel-Sattar1*, Naglaa Shehab1, Fathalla Harraz2, Salah Ghareib3

1Department of Pharmacognosy, Faculty of Pharmacy, Cairo University, 11562 Cairo, Egypt
2Department of Pharmacognosy, Faculty of Pharmacy, Alexandria University, Alexandria, Egypt
3Department of Pharmacology and Toxicology, Faculty of Pharmacy, King Abdulaziz University, Jeddah 21589, Saudi Arabia

*Corresponding Author Email: abdelsattar@yahoo.com

INTRODUCTION

Liver diseases are considered as one of the serious health problems, as it is an important organ for the detoxification and deposition of endogenous and exogenous substances. A number of pharmacological and chemical agents act as hepatotoxins and produce a variety of liver ailments [1]. Steroids, vaccines and antiviral drugs which have been employed as a therapy for liver diseases, have potential adverse effects especially when administered for long terms. Also no effective drugs are available, which stimulate liver functions and offers protection to the liver from the damage or help to regenerate hepatic cells [2]. In absence of reliable liver-protective drugs in modern medicine, hepatoprotective drugs from plant sources seem to be attractive alternative. The Kingdom of Saudi Arabia is characterized by its wide area showing variations in the climate which results in wide variation of its flora [3, 4]. Many plants of the Saudi flora are highly reputed for their uses in folk and traditional medicine eg. Cucumis prophetarum are used by local people to control hepatotoxicity [5, 6]. However the extract of the fruit decreased significantly the ALT activity and bilirubin level [7]. Mothana et al. [8] reported the antioxidant activity of Chrozophora oblongifolia, Capparis spinosa and Hypoestes forsskalei of selected Yemeni medicinal plants which could be of value in liver diseases. Van Puyvelde et al.[9] reported the isolation of the hepatoprotective principle of Hypoestes triflora leaves. Gadgoli and Mishra [10] studied the antihepatotoxic activity of p-methoxy benzoic acid isolated from Capparis spinosa. In addition, two Rumex species were investigated for their antioxidant and antihepatotoxicity [11, 12].

ABSTRACT

The methanolic extracts of seven Saudi medicinal plants were evaluated for their hepatoprotective and antihepatotoxic activities against carbon tetrachloride (CCl4) induced hepatic damage in rats (500 mg/kg each). The activities were assessed by measuring their effects on plasma marker enzymes such as alanine transaminase (ALT), alkaline phosphatase (ALP) and aspartate transaminase (AST). Plants’ extracts with significant hepatoprotective activities were: Cluytia myricoides, Cucumis prophetarum, Hypoestes forsskalei and Blepharis ciliaris respectively. While rise in the ALT was observed by Rumex nervosus (165.9%), with no hepatoprotective effect. The antihepatotoxic effect of R. nervosus was shown by the significant reduction of AST activity and non-significantly decreasing of ALT and ALP activities, despite of its good antioxidant activity. H. forsskalei, C. myricoides, and C. oblongifolia extracts showed strong antihepatotoxic activity which was higher than that of silymarin (150 mg/Kg). All the plants’ extracts significantly decreased glutathione peroxidase (GPx) and malondialdehyde (MDA) level. The tissue content of reduced glutathione and glutathione-S transferase were significantly increased by all plant extracts except Capparis spinosa and B. ciliaris. C. myricoides and C. oblongifolia extracts showed highest antioxidant activity. The histopathological findings are to a high extent, supporting the biochemical findings of the fore-mentioned results.

KEYWORDS: C. myricoides; C. oblongifolia; Antihepatotoxic; Hepatoprotective; Antioxidant.
Therefore, it was of interest that the present study was designed to find an effective plant-based herbal medicine from the Saudi flora which can be used as hepatoprotective and antihepatotoxic agent [5, 6].

**MATERIALS AND METHODS**

**Plants material and extraction**

Plants belonging to different families were collected from Western areas of Saudi Arabia, during March till May (2009) (Table 1). The plants were kindly identified by the staff members of Department of Biology, Faculty of Science, King Abdulaziz University. Vouchers specimens were kept at the Herbarium of Department of Natural Products and Alternative Medicine, Faculty of Pharmacy, King Abdulaziz University, Jeddah, Saudi Arabia. The air-dried powdered plants (500 g each) were separately extracted with methanol (3 X 2L) using Ultra turrax T50 homogenizer (Janke and Kunkel, IKA Laborteknik, Stauten, Germany). The methanolic extracts were filtered and evaporated under reduced pressure by using rotary evaporator. The residues were kept at 4°C for the biological study. The percentage yields of the dried extracts was determined and presented in Table 1.

**Chemicals and kits**

Carboxymethylcellulose-sodium (CMC-Na) was purchased from Acros Organics (NJ, USA), liquid paraffin, carbon tetrachloride and heparin sodium were purchased from Merck (Dramstadt, Germany). Biochemical parameters like liver enzymes: Biodiagnostic Kits for determination of ALT, AST and ALP and antioxidant: SOD, GPx, GR, GST, MDA and GSH were purchased from Diagnostic and Research Reagents, Dokki, Giza, Egypt. Silymarin was purchased from Sigma Chemical Co., St. Louis, MO., USA.

**Animals.** Male Wister rats, weighing 140-180 g were purchased from King Fahed Medical Research Center, King Abdulaziz University. The animals were kept in a special well-equipped atmosphere under standard conditions (temperature 22 ±2 ºC, relative humidity 50-60 %, with 12 h day/night lighting cycle) and fed with normal rat chow and water provided ad libitum. They were left for a period of one week for accommodation before performing the experiments. All animals investigations were performed in accordance with the guidelines of the Biochemical and Research Ethical committee at the King Abdalaziz University, Jeddah, Saudi Arabia (which in accordance of the NIH guidelines).

**Hepatoprotective effect**

The plant residues were suspended in 1% CMC-Na. Male Wister rats were distributed between 10 groups (8 animals each), group 1 received liquid paraffin (1ml/kg b.w., s.c.) as control, group 2 received CCl₄ and left without treatment, group 3 received standard silymarin (150 mg/kg in 1 % CMC-Na) and groups 4-10 received the plant extracts in a dose of 500 mg/kg. The rats were received either silymarin or the different extracts by oral gavage once daily for 7 days before induction of liver injury by CCl₄. Animals of groups 1 and 2 received 1 % CMC-Na once daily for 7 days.

**Induction of liver injury**

Liver injury was induced by subcutaneously (sc) injection of CCl₄ in a dose of 35 μg/100 g diluted in liquid paraffin (0.3 ml/kg) [13]. Animals of all groups received CCl₄ in liquid paraffin at day 8, except the control one. Twenty four hours, after injecting CCl₄, blood samples were collected, plasma was separated by centrifugation at 3500 rpm and the liver enzymes namely ALT, AST and ALP activities were determined. The differences between the extract-treated rats and the untreated ones showed the protective activity of the given extracts (Table 2). These results were the leading to the subsequent studies (antihepatotoxic) of the given extracts.

**Antihepatotoxic effect**

Hepatotoxicity in Male Wister rats was induced by s.c. injection of CCl₄ dissolved in liquid paraffin (30% solution), in a dose of 1 ml/kg b.w. for 4 weeks. The rats were distributed between 10 groups (8 animals each), as follow: group 1, control, received only liquid paraffin (1
ml/kg b.w., s.c.), without s.c. injection of CCl₄, each other day for 4 weeks.

Group 2: Left untreated and served as a negative control group, starting from day 29 (5th week), rats were given 1% CMC-Na solution in a dose of 5 ml/kg body weight/day once daily by oral gavage.

Group 3: received silymarin, as standard, in a dose of 150 mg/kg/day, suspended in 1%CMC-Na, starting from day 29 (5th week), by oral gavage once daily for 4 weeks and served as a positive control. The other seven groups received the assigned plant extracts suspended in 1%CMC-Na by oral gavage in a dose of 500 mg/kg/day for 4 weeks. At the end of the experiment (8 weeks), blood samples were collected from the orbital sinus of each rat and plasma were separated by centrifugation at 3500 rpm. Plasma samples were kept under -20 ºC and were used for determination of the other parameters. Animals were anesthetized with diethyl ether and sacrificed by cervical dislocation for separation of the liver. Livers were dissected out, divided into two parts. One part was kept in liquid nitrogen for determination of antioxidant status by measuring the levels of tissue contents of reduced glutathione (GSH), malondialdehyde (MDA), the enzyme activities of superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione –S-transferase (GST) and glutathione reductase (GR). The other part was immediately fixed in buffered formalin 10% and was used for histopathological studies.

**Histopathological analysis**
For histological studies, liver tissues were fixed with 10% phosphate-buffered neutral formalin, dehydrated in graded (50–100%) alcohol and embedded in paraffin. Thin sections of 0.5 mm thickness were cut and stained with hematoxylin and eosin stain for microscopic assessment. The initial examination was qualitative, with the purpose of determining histopathological lesions in liver tissue.

**Statistical analysis**
Data are expressed as mean ± standard error (SE) of mean. Unless otherwise indicated, statistical analyses were performed using one-way analysis of variance (ANOVA). If the overall F-value was found statistically significant (p<0.05), further comparisons among groups were made according post hoc Tuckey’s test. All statistical analyses were performed using GraphPad InStat 3 (GraphPad Software, Inc. La Jolla, CA, USA) software.

**RESULTS AND DISCUSSION**
Table 1 showed the percentage yields of the dried extracts of the different plants under investigation.

**Hepatoprotective activity**
Results in Table 2, showed that CCl₄ induced sharp and significant elevation in the liver enzymes, AST, ALP and ALT (104.9 %, 31.9 % and 103.2 %, respectively). These findings are in accordance with that of Bhattacharyya et al. [18, 19]. C. myricoides, B. ciliaris, C. prophetarum, sylimarin and H. forsskalei significantly reduced the plasma enzymes of both AST and ALP (51.9, 39.9, 38.2, 32.7, 28.1% and 27.0, 37.8, 60.5, 38.0, 50.2% respectively). The same plants extracts, except B. ciliaris, as well as sylimarin also showed significant reduction on the plasma ALT (33.7, 37.9, 32.7 and 22.5 % respectively). ALT was significantly increased by R. nervosus, C. spinosa and C. oblongifolia (165.9, 42.4 and 26.3% respectively). C. oblongifolia extract produced significant reduction of liver enzyme AST by 36.6% in the CCl₄-treated rats. C. spinosa extract increased the ALP by 64.8%.
According to these findings, plants’ extracts with significant hepatoprotective activity can be arranged in a descending order as follows: C. myricoides, C. prophetarum, H. forsskalei and B. ciliaris. The plant extract of C. oblongifolia showed mild hepatoprotective activity. The extract of R. nervosus extract did not produce significant hepatoprotective activity.

Table 1: List of Plants collected, their parts used and percentage of their methanolic extracts.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>AST (U/L)</th>
<th>% change</th>
<th>ALT (U/L)</th>
<th>% change</th>
<th>ALP (U/L)</th>
<th>% change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>70.66 ± 2.31</td>
<td></td>
<td>34.12 ± 2.31</td>
<td></td>
<td>135.57 ± 24.923</td>
<td></td>
</tr>
<tr>
<td>CCl4 control</td>
<td>144.81 ± 12.03</td>
<td>+ 104.9</td>
<td>45.01 ± 2.02</td>
<td>+ 31.9</td>
<td>275.47 ± 16.24</td>
<td>+ 103.2</td>
</tr>
<tr>
<td>Silymarin + CCl4</td>
<td>97.47 ± 11.81</td>
<td>- 32.7</td>
<td>34.86 ± 3.58</td>
<td>- 22.5</td>
<td>170.77 ± 20.13</td>
<td>- 38.0</td>
</tr>
<tr>
<td>B. ciliaris + CCl4</td>
<td>94.32 ± 7.85</td>
<td>- 39.9</td>
<td>52.65 ± 4.49</td>
<td>+ 17.0</td>
<td>171.27 ± 23.144</td>
<td>- 37.8</td>
</tr>
<tr>
<td>C. myricoides + CCl4</td>
<td>69.65 ± 8.58</td>
<td>- 51.9</td>
<td>29.86 ± 2.21</td>
<td>- 33.7</td>
<td>200.97 ± 19.90</td>
<td>- 27.0</td>
</tr>
<tr>
<td>H. forsskalii + CCl4</td>
<td>104.12 ± 10.18</td>
<td>- 28.1</td>
<td>30.30 ± 4.06</td>
<td>- 32.7</td>
<td>127.05 ± 19.94</td>
<td>- 50.2</td>
</tr>
<tr>
<td>C. prophetarum + CCl4</td>
<td>88.72 ± 7.52</td>
<td>- 38.2</td>
<td>27.94 ± 7.51</td>
<td>- 37.9</td>
<td>108.90 ± 29.54</td>
<td>- 60.5</td>
</tr>
<tr>
<td>CCl4</td>
<td>91.78 ± 7.48</td>
<td>- 36.6</td>
<td>56.83 ± 1.43</td>
<td>+ 26.3</td>
<td>334.62 ± 41.35</td>
<td>+ 21.0</td>
</tr>
<tr>
<td>R. nervosus + CCl4</td>
<td>136.05 ± 21.26</td>
<td>- 6.08</td>
<td>119.0 ± 4.01</td>
<td>+ 165.9</td>
<td>233.30 ± 13.14</td>
<td>- 15.0</td>
</tr>
<tr>
<td>C. spinosa + CCl4</td>
<td>126.84 ± 8.97</td>
<td>- 13.0</td>
<td>64.06 ± 2.34</td>
<td>+ 42.4</td>
<td>454.02 ± 34.15</td>
<td>+ 64.8</td>
</tr>
</tbody>
</table>

Abb.: Abbreviation; SN: specimen number; PC: place of collection; HR: Al-Hadda road; T: Taif; J: Jeddah; JHR: Jeddah-Al-Hadda road.

Table 2: Evaluation of the protective effect of the plant extracts on the biochemical parameters of liver in CCl4-induced hepatic damage in rats

<table>
<thead>
<tr>
<th>Plant</th>
<th>Family name</th>
<th>Abb</th>
<th>PC</th>
<th>SN</th>
<th>Parts used</th>
<th>% yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cucumis prophetarum</td>
<td>Cucurbitaceae</td>
<td>CP</td>
<td>HR</td>
<td>CP1075</td>
<td>Aerial parts</td>
<td>7.44</td>
</tr>
<tr>
<td>Cluertia myricoides</td>
<td>Euphorbiaceae</td>
<td>CM</td>
<td>T</td>
<td>CM1083B</td>
<td>Aerial parts</td>
<td>13.64</td>
</tr>
<tr>
<td>Chrozophora oblongifolia</td>
<td>Euphorbiaceae</td>
<td>CO</td>
<td>JHR</td>
<td>CO1080</td>
<td>Aerial parts</td>
<td>6.67</td>
</tr>
<tr>
<td>Blepharis ciliaris</td>
<td>Acanthaceae</td>
<td>BC</td>
<td>J</td>
<td>BC1171</td>
<td>Aerial parts</td>
<td>7.50</td>
</tr>
<tr>
<td>Hypoestes forsskalei</td>
<td>Acanthaceae</td>
<td>HF</td>
<td>El-Shefaa, T</td>
<td>HF1004</td>
<td>Whole plant</td>
<td>22.69</td>
</tr>
<tr>
<td>Capparis spinosa</td>
<td>Capparaceae</td>
<td>CS</td>
<td>HR</td>
<td>CS1035</td>
<td>Aerial parts</td>
<td>7.17</td>
</tr>
<tr>
<td>Rumex nervosus</td>
<td>Polygonaceae</td>
<td>RN</td>
<td>Waddi Kama, T</td>
<td>RN1129</td>
<td>Aerial parts</td>
<td>7.59</td>
</tr>
</tbody>
</table>

The values are expressed as the mean ± SE of the mean.

* Significantly different from the values of the normal rats at P<0.05.

b significantly different from the control values of CCl4 at P<0.05

Antihapatotoxicity activity

Results in Table 3 showed that CCl4 induced a significant increase in liver enzymes, AST, ALT and ALP (92.4%, 72.2% and 134.7% of normal rats, respectively). Silymarin induced significant reduction of AST and ALT by 32.0% and 51.3% compared to the control value, respectively, but not significantly affect the ALP. These results are in agreement with that reported by Favari and Pérez-Alvarez [20]. Oral administration of the plants’ extracts of C.
oblongifolia, H. forsskali and C. myricoides extracts induced significant reduction in AST and ALT (48.7, 47.2, 42.5% and 52.3, 84.6, 75.6%, respectively) of the control cirrhotic rats. On the other hand, B. ciliaris and C. prophetarum extracts did not significantly reduce the AST and ALT. In addition, the administration of C. oblongifolia and C. prophetarum extracts induced a significant reduction in ALP by 33.4 and 33.2% respectively. R. nervosus and C. spinosa extracts affect AST by reduction of 49.7% and 36.9%, respectively, although the ALT and ALP did not significantly affected.

The results of H. forsskali, C. myricoides, and C. oblongifolia extracts also showed higher antihepatotoxic activities comparable to that of silymarin. Both C. spinosa and R. nervosus showed lower activity than silymarin. On other hand, B. ciliaris and C. prophetarum did not have antihepatotoxic activities.

Table 3: Evaluation of the curative effect of the plant extracts on the biochemical parameters of liver in CCl4 induced hepatic damage in rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>AST (U/L)</th>
<th>% change</th>
<th>ALT (U/L)</th>
<th>% change</th>
<th>ALP (U/L)</th>
<th>% change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>106.25 ± 12.25</td>
<td>+ 92.4</td>
<td>28.53 ± 2.16</td>
<td>+ 722.4</td>
<td>216.97 ± 28.31</td>
<td>+ 134.7</td>
</tr>
<tr>
<td>CCl4 control</td>
<td>204.44 ± 9.53</td>
<td>+ 11.83</td>
<td>234.39 ± 4.17</td>
<td>+ 50.90</td>
<td>509.02 ± 52.44</td>
<td>+ 134.7</td>
</tr>
<tr>
<td>Silymarin + CCl4</td>
<td>138.98 ± 17.12</td>
<td>- 32.0</td>
<td>114.13 ± 27.34</td>
<td>- 51.3</td>
<td>463.93 ± 77.81</td>
<td>- 8.9</td>
</tr>
<tr>
<td>B. ciliaris + CCl4</td>
<td>154.58 ± 32.14</td>
<td>- 24.4</td>
<td>144.36 ± 30.72</td>
<td>- 38.4</td>
<td>549.45 ± 83.04</td>
<td>+ 7.9</td>
</tr>
<tr>
<td>C. myricoides + CCl4</td>
<td>117.55 ± 15.52</td>
<td>- 42.5</td>
<td>57.21 ± 30.28</td>
<td>- 75.6</td>
<td>583.82 ± 58.67</td>
<td>+ 14.7</td>
</tr>
<tr>
<td>H. forsskali + CCl4</td>
<td>108.78 ± 26.66</td>
<td>- 47.2</td>
<td>35.96 ± 14.26</td>
<td>- 84.6</td>
<td>399.3 ± 58.03</td>
<td>- 21.5</td>
</tr>
<tr>
<td>C. prophetarum + CCl4</td>
<td>171.55 ± 10.95</td>
<td>- 16.4</td>
<td>190.36 ± 1.98</td>
<td>- 18.8</td>
<td>340.23 ± 109.92</td>
<td>- 33.2</td>
</tr>
<tr>
<td>C. oblongifolia + CCl4</td>
<td>108.93 ± 16.64</td>
<td>- 48.7</td>
<td>119.98 ± 24.33</td>
<td>- 52.3</td>
<td>339.07 ± 32.72</td>
<td>- 33.4</td>
</tr>
<tr>
<td>R. nervosus + CCl4</td>
<td>103.98 ± 13.68</td>
<td>- 49.7</td>
<td>137.52 ± 22.65</td>
<td>- 45.5</td>
<td>446.8 ± 39.1</td>
<td>- 12.2</td>
</tr>
<tr>
<td>C. spinosa + CCl4</td>
<td>129.64 ± 11.83</td>
<td>- 36.9</td>
<td>174.43 ± 23.16</td>
<td>- 25.5</td>
<td>427.35 ± 47.46</td>
<td>- 16.0</td>
</tr>
</tbody>
</table>

The values are expressed as the mean ± SE of the mean.

* significantly different from the values of the normal rats at P<0.05.

Antioxidant activity

Results presented in Table 4 showed that CCl4 induced significant increase in the activities of GPx and MDA by 320.8 and 351.1%, respectively, while it decreased the contents of GSH, GR and GST in liver tissues by 56.8, 59.3 and 64.8%, respectively, compared to normal control. However, CCl4 did not significantly affect the activity of SOD in liver tissues. Similar observations were reported by Pandit et al., [21]. Silymarin significantly increased the GSH, GR and GST tissue contents by 177.4, 27.7 and 24.8 %, respectively, while it decreased the GPx and MDA activities in liver tissues by 47.6 and 59.9%, respectively. It did not affect the SOD activity in liver tissues. These findings were in accordance with those reported by Pradeep et al., [22]. The results of the present study also showed that both C. myricoides and C. oblongifolia extracts induced a significant increase in the activity of SOD by 104.9% and 103.7% of the CCl4 treated control rats, respectively. All the plant extracts significantly decreased the GPx and the MDA levels comparing to the CCl4 control group. However, they significantly increased the tissue...
contents of reduced glutathione (GSH), except *C. spinosa* and *B. ciliaris*, compared to the control cirrhotic rats. Also all the plant extracts significantly increased the GST levels in liver tissue. Only *B. ciliaris*, *C. myricoides* and *C. oblongifolia* significantly increased the tissue contents of glutathione reductase (GR) by 16.1, 19.0 and 16.1, respectively. *C. myricoides* and *C. oblongifolia* extracts have a potent antioxidant activity as they increased the enzyme activity of SOD while they decreased that of GPx and MDA, hence increasing the tissue contents of GSH.
Table 4: The effect of the plant extracts on hepatic lipid peroxidation levels and antioxidant enzymes activities in CCl₄-induced hepatic damage in rats

<table>
<thead>
<tr>
<th>Plant Extracts</th>
<th>SOD (U/mg protein)</th>
<th>GPx (nmol/mg protein)</th>
<th>GR (U/mg protein)</th>
<th>GST (nmol/mg protein)</th>
<th>MDA (U/mg protein)</th>
<th>GSH (mg/g protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>45.8675 ± 14.03</td>
<td>323.56 ± 40.80</td>
<td>84.82 ± 8.62</td>
<td>1004.8 ± 72.3</td>
<td>3.33 ± 0.3</td>
<td>3.33 ± 0.298</td>
</tr>
<tr>
<td>CCl₄ control</td>
<td>63.4075 ± 13.68</td>
<td>1361.74 ± 213.10 ⁹</td>
<td>34.53 ± 3.24 ⁹</td>
<td>353.5 ± 59.4 ⁹</td>
<td>15.02 ± 1.4 ⁹</td>
<td>1.44 ± 0.198 ⁹</td>
</tr>
<tr>
<td>Silymarin + CCl₄</td>
<td>61.4925 ± 7.24</td>
<td>713.29 ± 147.87 b</td>
<td>44.10 ± 4.30 b</td>
<td>441.3 ± 39.5 b</td>
<td>6.03 ± 0.5 b</td>
<td>3.995 ± 0.309 b</td>
</tr>
<tr>
<td>B. ciliaris + CCl₄</td>
<td>71.3625 ± 12.5</td>
<td>632.24 ± 108.74 b</td>
<td>40.10 ± 4.31 b</td>
<td>535.2 ± 39.5 b</td>
<td>9.43 ± 0.6 b</td>
<td>2.065 ± 0.56 b</td>
</tr>
<tr>
<td>C. myricoides + CCl₄</td>
<td>129.943 ± 7.73 b</td>
<td>389.0775 ± 127.35 b</td>
<td>41.10 ± 4.34 b</td>
<td>476.4 ± 39.4 b</td>
<td>7.78 ± 0.7 b</td>
<td>4.043 ± 0.49 b</td>
</tr>
<tr>
<td>H. forsskalii + CCl₄</td>
<td>68.905 ± 20.99</td>
<td>294.64 ± 37.96 b</td>
<td>38.10 ± 4.44</td>
<td>498.2 ± 39.3 b</td>
<td>8.66 ± 0.6 b</td>
<td>3.466 ± 0.132 b</td>
</tr>
<tr>
<td>C. prophetarum + CCl₄</td>
<td>40.76 ± 8.89</td>
<td>583.63 ± 142.06 b</td>
<td>39.10 ± 4.34</td>
<td>654.8 ± 39.5 b</td>
<td>6.34 ± 0.4 b</td>
<td>2.866 ± 0.47 b</td>
</tr>
<tr>
<td>C. oblongifolia + CCl₄</td>
<td>129.215 ± 13.93 b</td>
<td>323.68 ± 128.50 b</td>
<td>40.10 ± 4.38 b</td>
<td>490.7 ± 39.8 b</td>
<td>8.55 ± 0.6 b</td>
<td>4.83 ± 0.17 b</td>
</tr>
<tr>
<td>R. nervosus + CCl₄</td>
<td>52.46 ± 24.55</td>
<td>535.357 ± 94.01 b</td>
<td>37.10 ± 4.29</td>
<td>466.0 ± 39.6 b</td>
<td>7.76 ± 0.7 b</td>
<td>3.63 ± 0.56 b</td>
</tr>
<tr>
<td>C. spinosa + CCl₄</td>
<td>41.495 ± 16.06</td>
<td>292.11 ± 97.66 b</td>
<td>39.10 ± 4.38</td>
<td>488.6 ± 39.7 b</td>
<td>8.86 ± 0.7 b</td>
<td>2.012 ± 0.31 b</td>
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SOD, superoxide dismutase; GPx, glutathione peroxidase; GR, glutathione reductase; GST, glutathione-S-transferase; MDA, malondialdehyde and GSH, reduced glutathione.

The values are expressed as the mean ± SE of the mean of 8 rats.

* Significantly different from the values of the normal rats at p < 0.05.

b Significantly different from the control values of CCl₄-induced hepatotoxic rats at p < 0.05.
Evaluation of histopathological changes

Histopathological examination of the liver sections from normal rats showed normal parenchymal architecture; no significant lesions were observed (Fig. 1A). In CCl₄-treated group, diffuse central and peripheral necrosis, destruction of the lobular architecture and the formation of septa with sinusoidal dilation was seen (Fig. 1B). Silymarin+CCl₄-treated group showed normal liver section with mild dilated blood sinusoids and very little effect on liver tissues (Fig. 1C). In addition C. myricoides, H. forsskalei and C. oblongifolia extracts (Fig. 2 A, B and Fig. 3 A respectively) produced normal parenchymal cells, portal system and blood sinusoids. Histopathological findings indicated that administration of these extracts improved the hepatocytes damaging which induced by CCl₄, with mild fatty changes in the hepatic parenchymal cells, which corroborated the changes observed in the hepatic enzymes and antioxidant activities. Bleplaris ciliaris extract (Fig. 1D) showed a peripheral zonal degeneration and necrosis. Cucumis prophetarum, Rumex nervosus, and C. spinosa extracts (Fig. 2 C and Fig. 3 B and C respectively) showed severe diffuse liver cell degeneration with central and peripheral cell necrosis.

Fig.1: Histopathological examination of Liver sections from rats of the control group rats(A), rats treated with CCl₄(B), silymarin + CCl₄ (C) and extract of B. ciliaris + CCl₄ (D) (H&E X 250)
Fig 2: Histopathological examination of liver sections from rats treated with extracts of *Cluytia myricoides*, *Hypoestes forsskali* and *Cucumis prophetarum* + CCl$_4$ (A-C, H&E X 250).

Fig 3: Histopathological examination of liver sections from rats treated with extracts of *Chrozophora oblongifolia*, *Rumex nervosus*, and *Capparis spinosa* + CCl$_4$ (A-C, respectively H&E X 250).
Natural antioxidants, which neutralize free radicals, have been received great attention by nutritionists and medical researchers for their potential effects in the prevention of chronic and degenerative diseases [23]. In our study, we are focusing on the liver damage which always accompanied by cellular necrosis, increased in tissue ALP and diminution of reduced liver glutathione. In addition, elevated levels of hepatic serum enzymes are indicative of cellular leakage [24]. The hepatotoxic effect of CCl₄ are related to its active metabolite trichloromethyl radical, 'CCl₃, [25] this is observed by an elevation in the serum marker enzymes namely AST, ALT, ALP. Also these effects were coupled with a marked hepatic oxidative stress (decreased GSH, SOD, GPx, GR and GST activities in liver tissue and increased production of MDA) as well as histopathological changes indicating liver injury. Antioxidant is significantly delays or prevents oxidation of the substrate, including every type of molecules found in vivo. It was found that phenolic and polyphenolic compounds such as flavonoids are very efficient scavengers of free radicals [26] because of their molecular structures, which include an aromatic ring with hydroxyl groups containing mobile hydrogen. Silymarin, a mixture of flavonolignanes from milk thistle is a hepatoprotective herbal product with a potent antioxidant activity and has been used as a positive control drug in vivo animal models [27]. Administration of plant extracts of C. myricoides, C. prophetarum, H. forsskalei and B. ciliaris illustrated significant hepatoprotective actions against CCl₄ injuring. The hepatoprotective activity of the fore-mentioned plant extracts is designated by the reduction of the blood levels of AST, ALT and ALP while C. oblongifolia showed mild activity. In addition, treatment with C. myricoides, H. forsskalei, C. oblongifolia extracts as well as silymarin improved the liver function after CCl₄ injures (anti-hepatotoxicity), an effect that was evidenced by the significant reduction in AST and ALT. It was reported that the increased antioxidant activity or inhibition of the generation of free radicals is important in the protection against CCl₄-induced liver lesion [18]. The increased glutathione tissue content and SOD enzyme activity, in one hand, and the reduction of GPx enzyme activity on other hand, may act as a protective mechanism against lipid peroxidation in liver tissues. Our results showed that there were correlations between the ability of the given extracts to reduce the activity of GPx and the increased GSH tissue contents, in one hand and their antihepatotoxic effects on the other hand. Moreover, C. myricoides and C. oblongifolia extracts have a potent antioxidant activity as they increased the enzyme activity of SOD while they decreased that of GPx and MDA, hence increasing the tissue contents of GSH. The hepatoprotective and antihepatotoxic effects of these extracts may be due to their potent antioxidant activities. The histopathological findings of C. myricoides, H. forsskali, C. oblongifolia extracts are to a high extent, supporting the other biochemical findings in both their effects on liver enzymes, in one hand, and their antioxidant activities on the other hand. So C. myricoides, H. forsskali, C. oblongifolia extracts and silymarin have potent antioxidant activities. In addition, they were more potent than silymarin as a hepatoprotective and antihepatotoxic. Histopathological findings indicated that pretreatment with these extracts offered a protection to the hepatocytes from damage by CCl₄, which corroborated the changes observed in the hepatic enzymes. Complementing our findings, antioxidant activity was observed before for C. oblongifolia [8]. C. oblongifolia has been reported to be rich in the flavonoid compounds [28]. Flavonoids and tannins [29] were isolated from the aerial parts of H. rosea. The aerial part of C. prophetarum is used by traditional medicine practitioners as remedy for liver disorders [5]. However the extract of the fruit at higher dose decreased significantly the ALT activity and bilirubin level [7]. Cucurbitacins and flavonoidal compounds were isolated from C. prophetarum [30].
In the present study, the aerial parts of *C. prophetarum* showed hepatoprotective activity but devoid from antioxidant or antihepatotoxicity activity and this may be due to its content of triterponoidal constituents with a few flavonoidal components. Harraz et al., [31] reported the presence of flavonoids in *B. ciliaris*, they were mainly of flavones and flavanone types, in addition, El-Shanawany et al., [32], isolated a new isoflavone glycoside genistein-7-O-(6″-O-E-caffeoyl-β-d-glucopyranoside and seven known phenolic compounds. Therefore, the hepatoprotective activity of *B. ciliaris* may be attributed to its phenolic contents. Several phenolic acids from the fruit of *C. spinosa* were isolated [33]. In the present study the extract of the aerial parts of *C. spinosa* decreased the GPx and the MDA and increased the GST levels but has no effect on the tissue contents of GSH. The extract of *C. spinosa* showed no antioxidant activity and this result had been proved by histopathological examination which showed sever diffuse liver cell degeneration with central and peripheral cell necrosis.

Reviewing the available literature revealed that several *Rumex* species showed antioxidant activities [34]. The medicinal importance of *Rumex* spp is a reflection to their chemical composition, since they contain many bioactive substances such as flavonoids, anthraquinones and antioxidant constituents [35, 36]. In the present study, the extract of the aerial parts of *R. nervosus* significantly reduced plasma AST, however, ALT and ALP did not significantly affect the antihepatotoxicity activity and no effect was observed on plasma AST and ALP. Therefore, *R. nervosus* has neither hepatoprotective nor antihepatotoxic activity and also showed no antioxidant activity.

**CONCLUSION**

In conclusion, our results supported the possible hepatoprotective effect of *C. myricoides, C. prophetarum, H. forsskalei* and *B. ciliaris* in addition; treatment with *C. myricoides, H. forsskalei, C. oblongifolia* extracts improved the liver function against CCl₄-induced hepatotoxicity in rats. This antihepatotoxic effect may be attributed partially to their antioxidant activity.

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*Corresponding author
Email address: abdelsattar@yahoo.com