HEPATOPROTective ACTIVITY OF EXTRACTS OF BETA VULGARIS BULB

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ABSTRACT

Beta vulgaris (Family: Chenopodiaceae) is one of the medicinal plants used in traditional Indian medicine for the management of various liver disorders. B. vulgaris bulbs are also used as expectorant, diuretic and to cure mental disorders. However, no scientific report is available for the hepatoprotective potential of B. vulgaris bulbs. The present study was aimed to scientifically evaluate the hepatoprotective activity of B. vulgaris bulbs in CCl4 induced hepatotoxic rats. The dried, pulverized B. vulgaris bulbs were separately extracted with petroleum ether, chloroform, ethanol and water by cold maceration technique for 6 days. The active extract was fractionated using n-hexane, ethyl acetate and n-butanol. All the extracts (200 and 400mg/kg) and the fractions (100 and 200mg/kg) of active extract were evaluated for their hepatoprotective activity in hepatotoxic rats. Hepatotoxicity induced rats were orally treated with extracts and fractions for 7 days. biochemical analysis and histopathology studies were performed to support the hepatoprotective activity. Among all the extracts and fractions, ethanol extract of B. vulgaris bulbs (400mg/kg) and its ethyl acetate soluble fraction (200mg/kg) showed significant (p<0.001) hepatoprotective effect. The significant (p<0.001) restoration was observed in the biochemical parameters such as SGOT, SGPT, alkaline phosphate, cholesterol, total protein, albumin, total bilirubin and direct bilirubin. Histopathological examination also supported the hepatoprotective effect of the ethyl acetate soluble fraction. The hepatoprotective activity of the extract and fraction was well comparable to the standard drug, silymarin (100mg/kg). The present study validates the traditional use of B. vulgaris bulbs for the treatment of liver disorders.

Key words: Beta vulgaris, alcohol extract, fractionation, hepatoprotective

INTRODUCTION

Liver infections which are still a worldwide wellbeing issue may be classified acute or chronic hepatitis, hepatosis and cirrhosis. Metabolism of carbohydrate, protein and fat and detoxification are the primary functions of the liver1. Hepatic cells take an active part in the waste disposal processes, and other functions are found in the liver2. Because of the tremendous scientific advancement in the field of hematology in recent years, there is a huge chances for the liver problems3. Continuously and variably exceeds to environmental toxins, abused by alcohol and prescribed and over-the-counter drug lead to various liver ailments such as hepatitis, cirrhosis and alcoholic liver disease4.

There are a number of synthetic drugs available for the treatment of hepatic disorders, but still there is a need for the novel drug discovery due to their ineffectiveness, unwanted side effects etc. Scientific studies available on medicinal plants point out that phytoconstituents might be having a promising therapeutic response for the treatment of many health problems. Herbs and herbal formulations could significantly contribute to recovery processes of the intoxicated liver in the Malay traditional medicine. Due to their minimal side effects in terms of treatment as well as relatively low costs, herbal drugs are widely prescribed, even when their biologically active constituents are not fully identified. Despite the incredible advances in modern medicine, still there is no effective drug available that stimulates liver function, offer protection to the liver from damage or help to regenerate hepatic cells5. Beta vulgaris popularly known as beet root which belongs to Amaranthaceae family is known as “Shahya” among Arabs and Ubis Bi among Malays. The plant root is traditionally used by Arabs to treat spleen and liver diseases and inflammatory disorders6. Roots are expectorant, diuretic and used as a cure for mental troubles and liver diseases. Leaves are tonic, diuretic and useful in alleviating inflammation, paralysis and diseases of spleen and liver7. However, no scientific evidence is available for the hepatoprotective potential of B. vulgaris bulb. Hence the present study was undertaken to evaluate the hepatoprotective activity of Beta vulgaris bulbs to validate the traditional use.

MATERIALS AND METHODS

Plant material

The Beta vulgaris bulbs were collected from Kota Sriemas, Nilai, Malaysia in the month of November 2015 and identified by Pharmacognosist at KPJ Healthcare University College, Kota Sriemas, Nilai, Negeri Sembilan, Malaysia. A voucher specimen was deposited at School of Pharmacy, KPJUC/SP/H3/122B.

Preparation of extracts and fractionation

Beta vulgaris bulbs were washed thoroughly with water and dried under air circulation. The dried bulbs (1 kg) were ground into...
coarse powder. The powder was divided into four equal portions and macerated with solvents such as Petroleum ether, chloroform, ethanol and distilled water at room temperature for 7 days. The macerated bulbs were filtered and concentrated using Rotary vacuum evaporator under reduced pressure. The color, consistency and % yield of the extracts were noted (Table 1). The active extract was fractionated using n-hexane, ethyl acetate and n-butanol. Then the filtrate was collected and concentrated using Rotary vacuum evaporator under reduced pressure. The color, consistency and % yield of the extracts were noted.

Animals

Healthy adult male wistar albino rats were procured from KPJUC Vivarium, KPJ Healthcare University College, Malaysia and housed in groups of six animals, in standard cages, at room temperature (25 ± 3 °C), with 12 h dark/12 h light cycles, and food and water ad libitum. The animals weighing 150 - 200 g, were used for the experiment. 12 h prior to the experiments, they were transferred to the laboratory and given only water ad libitum. The protocol was approved by KPJ Healthcare University Research and Ethical Committee (KPJUC/IAEC/2014/4/21(06)).

Acute toxicity studies

Acute toxicity studies were carried on albino mice as per the guidelines (No. 423) given by the Organisation for Economic Co-operation and Development (OECD, 2001), Paris. The animals were fasted overnight prior to the acute experimental procedure. The pet. ether, chloroform, ethanol and water extracts of Beta vulgaris bulbs were suspended in CMC separately. The extracts were administered separately to all the three animals in each group at a starting single dose of 5 mg/kg. The animals were observed continuously for signs of intoxication, lethargy, behavioral modification and morbidity for a period of 2 h, then occasionally for 4 h for severity of any toxic signs and mortality. When no mortality was observed the same dose would be additionally administered to one more animal for each group. If no mortality is observed at this dose, the same procedure would be repeated for dose levels of 50, 500, 1000 and 2000 mg/kg of extracts on separate newer groups. The LD₅₀ was thus determined and 1/10⁶ of LD₅₀ value was taken as ED₅₀ value for this present animal study. The animals were kept under observation up to 14 days after drug administration to find out any delayed mortality.

Evaluation of Hepatoprotective activity

The hepatoprotective activity of B. vulgaris bulb extracts and the fractions of active extract was evaluated using Carbon tetrachloride (CCl₄) induced hepatotoxicated rats with small modifications by the method developed by Rekha et al., 2009. Rats were divided into eleven groups of six animals each. The rats in group I received single dose of 5% gumacacia mucilage (1ml/kg, p.o) and served as of control. The rats in group II received single dose and a single dose of CCl₄ (1.25 ml/kg i.p.) diluted in liquid paraffin (1:1) 30 min after the administration of 1st dose of vehicle to induce the hepatotoxicity and served as negative control. The rats in group III received single dose of Silymarin (100mg/kg) and served as standard. While the rats in test group (IV – XI) received single dose of respective test extract (200 and 400 mg/kg b.wt. p.o). 30 min after the first dose of silymarin and test extract, CCl₄ (1.25ml/kg i.p.) was administered to the rats in the groups III – XI.

The fractions of active ethanol extract were also evaluated for the hepatoprotective activity. Single dose of CCl₄ (1.5 ml/kg b.wt. i.p.) was administered on the 1st day to all the rats to induce the hepatotoxicity except group 1 (control rats). The rats in group III received three doses of Silymarin (100mg/kg) at 0 h, 12 h and 24 h and served as standard. The fractions (100 and 200 mg/kg b.wt. p.o.) of active ethanol extract were orally administered to the hepatotoxicated rats except groups I, II and III for 7 days.

Biochemical analysis

At day 8, blood sample of each animal was separately collected by orbital sinus puncture under mild ether anesthesia in Eppendorf’s tubes (1 ml) containing 50 μl of anticoagulant (10% trisodium citrate) and plasma was separated by centrifuging at 6000 rpm for 15 min. and serum was separated to determine the biochemical parameters such as SGOT, SGPT, alkaline phosphate, cholesterol, total protein, albumin, total bilirubin and direct bilirubin. Standard assay kits were used to estimate the serum biochemical parameters.

Histopathological studies

Slices of the liver from each of the six animals in all groups were preserved in 10% buffered neutral formalin (pH 7.4). The tissues were mounted by Peter Fi’s double embedding paraffin sections of 5–10μ size These sections were then stained with haemotoxylin–eosin dye and observed under a low power microscope for any pathological changes.

Statistical analysis

Results of the biochemical estimations are reported as mean S.E.M.Total variation, present in a setof data was estimated by one-way analysis of variance (ANOVA). Student’s t-test was used for determining significance. Minimum level of significance was fixed at 0.05.

RESULTS

The color, consistency, and percentage yield of B. vulgaris bulb extracts and fractions are tabulated in Table 1. Among the four extracts, alcohol extract had the highest percentage yield (18.94%) followed by water extract (14.62%), chloroform extract (5.28%) whereas pet. extract had the lowest percentage yield (4.24%). Also, among the three fractions, the ethyl acetate fraction of active alcohol extract had the highest percentage yield (9.46%) followed by n-butanol fraction (2.16%) and n-hexane fraction (5.28%). In acute toxicity studies, no mortality and no change in general behavior were observed in the animals treated with all the extracts up to a dose of 2000 mg/kg b.wt., p.o and all the fractions up to a dose of 1000 mg/kg b.wt. p.o. There was an increase in the biochemical parameters such as SGOT, SGPT, ALP, total bilirubin and direct bilirubin and there is a decrease in total cholesterol and total protein in the CCl₄ intoxicated rats. After the 7 days of treatment with ethanol extract (400 mg/kg b.wt.) and its ethyl acetate fraction (200 mg/kg b.wt.) significantly restored the altered biochemical parameters (P<0.001). The hepatoprotective activity was further supported by histopathological findings. Histopathological liver sections of control group showed normal cellular architecture with distinct hepatic cells. Disarrangement of normal hepatic cells with necrosis are observed in CCl₄ intoxicated liver. The liver sections of the rat treated with ethanol extract (400 mg/kg b.wt. p.o.) and its ethyl acetate fraction (200 mg/kg b.wt. p.o.) of B. vulgaris bulbs followed by CCl₄ intoxication showed absence of necrosis observed were comparable with standard Silymarin (100 mg/kg p.o.).
### Table 1: Effects of various extracts of *B. vulgaris* on serum biochemical parameters in CCl₄ intoxicated rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>SGOT (U/L)</th>
<th>SGPT (U/L)</th>
<th>ALP (K.A unit)</th>
<th>Total Bilirubin (mg/dl)</th>
<th>Direct Bilirubin (mg/dl)</th>
<th>Total Protein (g/dl)</th>
<th>Total Cholesterol (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>83.03 ± 7.60</td>
<td>124.64 ± 7.21</td>
<td>12.59 ± 0.16</td>
<td>0.07 ± 0.005</td>
<td>0.04 ± 0.001</td>
<td>3.73 ± 0.18</td>
<td>120.38 ± 3.25</td>
</tr>
<tr>
<td>CCl₄ (1.5 ml/kg)</td>
<td>150.42 ± 9.45</td>
<td>180.37 ± 6.53</td>
<td>12.51 ± 0.34</td>
<td>0.30 ± 0.03</td>
<td>0.23 ± 0.05</td>
<td>6.83 ± 0.27</td>
<td>178.37 ± 6.86</td>
</tr>
<tr>
<td>Pet ether extract (200 mg/kg)</td>
<td>151.24 ± 9.58</td>
<td>174.22 ± 6.24</td>
<td>16.12 ± 0.04</td>
<td>0.33 ± 0.04</td>
<td>0.28 ± 0.04</td>
<td>6.68 ± 0.44</td>
<td>179.14 ± 6.64</td>
</tr>
<tr>
<td>Pet ether extract (400 mg/kg)</td>
<td>150.66 ± 9.42</td>
<td>172.58 ± 8.16</td>
<td>15.98 ± 0.76</td>
<td>0.31 ± 1.02</td>
<td>0.29 ± 0.08</td>
<td>6.59 ± 0.92</td>
<td>179.56 ± 9.64</td>
</tr>
<tr>
<td>Chloroform extract (200 mg/kg)</td>
<td>149.12 ± 9.26</td>
<td>178.84 ± 8.62</td>
<td>15.56 ± 1.84</td>
<td>0.32 ± 0.04</td>
<td>0.26 ± 0.02</td>
<td>6.46 ± 0.22</td>
<td>176.82 ± 7.02</td>
</tr>
<tr>
<td>Chloroform extract (400 mg/kg)</td>
<td>148.56 ± 9.52</td>
<td>179.22 ± 5.38</td>
<td>15.62 ± 0.06</td>
<td>0.31 ± 0.02</td>
<td>0.25 ± 0.06</td>
<td>6.28 ± 0.56</td>
<td>174.16 ± 8.44</td>
</tr>
<tr>
<td>Ethanol extract (200 mg/kg)</td>
<td>116.30 ± 3.26</td>
<td>146.72 ± 0.44</td>
<td>14.22 ± 0.26</td>
<td>0.12 ± 0.02</td>
<td>0.08 ± 0.01</td>
<td>6.42 ± 0.14</td>
<td>136.24 ± 5.02</td>
</tr>
<tr>
<td>Ethanol extract (400 mg/kg)</td>
<td>101.97 ± 0.88</td>
<td>138.23 ± 4.19</td>
<td>13.31 ± 0.29</td>
<td>0.08 ± 0.03</td>
<td>0.05 ± 0.00</td>
<td>6.19 ± 0.28</td>
<td>128.36 ± 2.03</td>
</tr>
<tr>
<td>Water extract (200 mg/kg)</td>
<td>134.52 ± 7.24</td>
<td>162.12 ± 0.68</td>
<td>16.14 ± 0.32</td>
<td>0.28 ± 0.05</td>
<td>0.25 ± 0.00</td>
<td>5.94 ± 0.26</td>
<td>148.66 ± 6.48</td>
</tr>
<tr>
<td>Water extract (400 mg/kg)</td>
<td>128.14 ± 7.24</td>
<td>157.28 ± 0.68</td>
<td>15.74 ± 0.32</td>
<td>0.21 ± 0.05</td>
<td>0.18 ± 0.00</td>
<td>5.94 ± 0.26</td>
<td>148.66 ± 6.48</td>
</tr>
<tr>
<td>Standard drug (100 mg/kg)</td>
<td>97.25 ± 1.20</td>
<td>130.40 ± 7.40</td>
<td>13.69 ± 1.20</td>
<td>0.06 ± 0.03</td>
<td>0.04 ± 0.02</td>
<td>4.04 ± 0.20</td>
<td>126.33 ± 5.01</td>
</tr>
</tbody>
</table>

*P < 0.05, **P < 0.01, ***P < 0.001, CCl₄ Vs control. 1) P < 0.05, 2) P < 0.01, 3) P < 0.001, extract treated groups Vs CCl₄; Values are mean ± S.E.M.

### Table 2: Effects of various fractions of *B. vulgaris* on serum biochemical parameters in CCl₄ intoxicated rats

<table>
<thead>
<tr>
<th>Treatment /Groups</th>
<th>SGOT U/L</th>
<th>SGPT U/L</th>
<th>ALP U/L</th>
<th>LDH U/L</th>
<th>TC mg/dl</th>
<th>Total Protein g/l</th>
<th>Albumin g/l</th>
<th>Total Bilirubin mg/dl</th>
<th>Direct Bilirubin mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>70.2 ± 0.62</td>
<td>48.2 ± 0.38</td>
<td>342.01 ± 1.78</td>
<td>354.25 ± 2.37</td>
<td>76.28 ± 0.78</td>
<td>7.58 ± 0.13</td>
<td>3.38 ± 0.12</td>
<td>0.38 ± 0.01</td>
<td>0.158 ± 0.78</td>
</tr>
<tr>
<td>CCl₄ (1.5 kg/kg)</td>
<td>487.78 ± 0.72</td>
<td>352.01 ± 2.32</td>
<td>471.02 ± 1.08</td>
<td>588.23 ± 3.25</td>
<td>32.86 ± 0.23</td>
<td>4.78 ± 0.39</td>
<td>1.92 ± 0.09</td>
<td>2.62 ± 0.00</td>
<td>1.33 ± 0.03</td>
</tr>
<tr>
<td>n-butanol fraction (100 mg/kg)</td>
<td>465.14 ± 6.86</td>
<td>304.64 ± 5.26</td>
<td>466.66 ± 2.32</td>
<td>546.58 ± 2.74</td>
<td>40.24 ± 1.06</td>
<td>5.16 ± 0.64</td>
<td>1.96 ± 0.24</td>
<td>2.56 ± 0.08</td>
<td>1.48 ± 0.04</td>
</tr>
<tr>
<td>n-butanol fraction (200 mg/kg)</td>
<td>423.81 ± 7.23</td>
<td>297.72 ± 3.12</td>
<td>452.82 ± 2.07</td>
<td>523.35 ± 1.27</td>
<td>38.81 ± 0.72</td>
<td>4.97 ± 0.78</td>
<td>2.01 ± 0.12</td>
<td>2.32 ± 0.04</td>
<td>1.12 ± 0.02</td>
</tr>
<tr>
<td>Ethyl acetate fraction (100 mg/kg)</td>
<td>124.68 ± 1.64</td>
<td>90.26 ± 3.12</td>
<td>403.12 ± 4.66</td>
<td>365.52 ± 5.62</td>
<td>56.72 ± 0.96</td>
<td>7.24 ± 2.14</td>
<td>2.34 ± 0.86</td>
<td>0.62 ± 0.54</td>
<td>0.48 ± 0.06</td>
</tr>
<tr>
<td>Ethyl acetate fraction (200 mg/kg)</td>
<td>101.23 ± 0.32</td>
<td>87.38 ± 3.12</td>
<td>388.24 ± 2.42</td>
<td>323.13 ± 3.46</td>
<td>64.28 ± 1.72</td>
<td>6.01 ± 0.17</td>
<td>2.96 ± 0.17</td>
<td>0.48 ± 0.12</td>
<td>0.28 ± 0.04</td>
</tr>
<tr>
<td>n-hexane fraction (100 mg/kg)</td>
<td>454.22 ± 7.24</td>
<td>316.28 ± 6.62</td>
<td>450.82 ± 4.86</td>
<td>574.82 ± 5.02</td>
<td>44.54 ± 1.14</td>
<td>5.86 ± 0.98</td>
<td>1.76 ± 0.36</td>
<td>2.58 ± 0.12</td>
<td>1.34 ± 0.96</td>
</tr>
<tr>
<td>n-hexane fraction (200 mg/kg)</td>
<td>427.21 ± 3.17</td>
<td>307.52 ± 1.37</td>
<td>438.23 ± 2.39</td>
<td>554.25 ± 0.27</td>
<td>39.21 ± 0.27</td>
<td>4.89 ± 2.37</td>
<td>1.98 ± 0.02</td>
<td>2.46 ± 0.03</td>
<td>1.20 ± 0.02</td>
</tr>
<tr>
<td>Silymarin (100 mg/kg)</td>
<td>75.23 ± 0.43</td>
<td>53.32 ± 0.17</td>
<td>358.09 ± 2.01</td>
<td>362.11 ± 1.58</td>
<td>72.42 ± 0.38</td>
<td>6.49 ± 0.13</td>
<td>2.56 ± 0.02</td>
<td>0.40 ± 0.02</td>
<td>0.17 ± 0.04</td>
</tr>
</tbody>
</table>

*P < 0.05, **P < 0.01, ***P < 0.001, CCl₄ Vs control. 1) P < 0.05, 2) P < 0.01, 3) P < 0.001, extract treated groups Vs CCl₄; Values are mean ± S.E.M.

One Way ANOVA; n = 6
DISCUSSION

The present study indicates the potential hepatoprotective activity of bulbs of *B. vulgaris* extracts and its fractions against CCl_4 induced hepatotoxic rats to prove its folklore claim. CCl_4, an experimental hepatotoxicant has been widely used to induce hepatotoxicity in the experimental animals to produce liver damage including necrosis, liver cell proliferation and suppression of antioxidant system. CCl_4 is biotransformed by the cytochrome P-450 system to produce the trichloromethyl free radical, which in turn covalently binds to cell membranes and organelles to elicit lipid peroxidation, disturb Ca^{2+} haemostasis and finally result in cell death [10]. As the level of serum enzymes such as SGOT, SGPT, LDH, ALP, total and direct bilirubin are affected due to the liver damage, the analysis of such biochemical parameters in serum is important. The restoration of these enzyme level may be an indication of regeneration process and repair of hepatic tissue damage induced by CCl_4.

In this present study, a significant elevations in the levels of serum SGOT, SGPT, LDH, ALP and total bilirubin and direct bilirubin was found in CCl_4 induced hepatotoxic rats. As these enzymes get released into serum and leads the damage to the cell membrane of hepatocytes. Pretreatment with bulbs of *B. vulgaris* was found significantly reverse these changes. Hence a reduction in the levels of these enzymes explains the hepatoprotective activity of bulbs of *B. vulgaris*. All the results were well comparable with the standard hepatoprotective agent silymarin (100 mg/kg b.wt. *o.p.*) which contains flavonolignans [13]. The index of the protective effect of a hepatoprotective drug is determined by its ability to reduce the injurious effects or to preserve the normal hepatic physiological mechanisms which have been disturbed by a hepatotoxin [14].

The various phytoconstituents present in the extracts are responsible for the pharmacological effect. Hence, the hepatoprotective activity of *B. vulgaris* bulb might be due to the presence of phytoconstituents. Earlier works have reported the presence of alkaloids, glycosides, triterpenoids, flavonoids, sterols, saponins, phenolics and tannins in the different extracts of *B. vulgaris* leaves and the presence of flavanoids and phenolics might contribute the hepatoprotective activity of *B. vulgaris* leaves [1]. The hepatoprotective activity of flavonoids and phenolics was supported by a study on methanol extract of *Orthosiphon stamineus* [15]. Also the presence of flavonoids might be responsible for the hepatoprotective activity of leaves of *Tapinanthus bangwensis* [16], *Leptadenia reticulata* stems [17] and *Asparagus racemosus* roots [18]. The antioxidant and hepatoprotective activities of Cinnamon ethanolic extract is due to the presence of flavanoids [18]. And a report shows that steroids present in the *Amorphophallus paeonifolius* tubers may be responsible for the significant hepatoprotective effects [19]. Another one report stated that the hepatoprotective effect of *Raphanus sativus* is due to the presence of anthocyanins [20].

CONCLUSION

The findings of this present study demonstrates that *B. vulgaris* bulb has promising hepatoprotective action. The study scientifically validates the traditional use of *B. vulgaris* bulb for the treatment of liver diseases. Therefore to isolate the phytoconstituent responsible for the hepatoprotective activity of *B. vulgaris* bulbs is necessary to search for the novel herbal drug for the treatment of liver diseases with potent efficacy and safety. Further study on the isolation and structure determination of the hepatoprotective principle is in process. Moreover the exact mechanism of action of the active compound for the hepatoprotective effect is need to be clarified in future studies.
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