Antihepatotoxic activity of seeds of *Cichorium intybus*

Bahar Ahmed a,b,∗, Tawfeq A. Al-Howiriny b, Abu B. Siddiqui a

a Antihepatotoxic Research Laboratory, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Jamia Hamdard, Hamdard Nagar New Delhi 110062, India
b Department of Pharmacognosy, Medicinal, Aromatic and Poisonous Plants Research Center, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia

Received 5 June 2002; received in revised form 2 April 2003; accepted 17 April 2003

Abstract

The different fractions of alcoholic extract and one phenolic compound AB-IV of seeds of *Cichorium intybus* Linn were screened for antihepatotoxic activity on carbon tetrachloride (CCl4)-induced liver damage in albino rats. The degree of protection was measured using biochemical parameters like aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALKP), and total protein (TP). The methanol fraction and compound AB-IV were found to possess a potent antihepatotoxic activity comparable to the standard drug Silymarin (Silybon-70). The histopathological study of the liver was also carried out, wherein the methanolic fraction and compound AB-IV showed almost complete normalization of the tissues as neither fatty accumulation nor necrosis was observed.

© 2003 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: *Cichorium intybus*; Antihepatotoxic activity; Histopathology; Silymarin

1. Introduction

The crude extracts of about 100 Indian medicinal plants belonging to 40 families are used in the herbal formulations for the treatment of various diseases of the liver. In addition, about 600 commercial preparations, mainly plant crude extracts with claimed liver-protecting activity, are available all over the world (Handa et al., 1986). The plant *Cichorium intybus* Linn (Family: Compositae, Asteraceae) commonly known as *Chicory* or *Kasni* is also used as liver tonic, cardiotonic, diuretic, stomachic, cholagogue, depurative, emmenagogue, hepatomegaly, cephalalgia, inflammations, anorexia, dyspepsia, flatulence, colic, jaundice, splenomegaly, amenorrhea dysmenorrhea, and asthma, etc. (*The Wealth of India*, 1992; *Sala*, 1994). The seed of the plant is also one of the main ingredients of *Jigrine*, a commercial product of Hamdard (Waqf) Dawkahna, New Delhi, which is used for the treatment of various diseases of liver.

The literature survey revealed that neither the systematic assessment of antihepatotoxic activity nor phytochemical investigation of the seeds of the plant have been done so far. We have recently reported one new and one rare sterol from ethyl acetate fraction of the seeds, namely cichosterol, characterized as 13,14-seco-stigma 5(6),14(15)-diene-3β-ol and stigma 5(6)-ene-3-α-O-β-D-glucopyranoside, respectively (Ahmed et al., 2002).

We now report a thorough pharmacological screening for the antihepatotoxic activity of different fractions of the seeds of the plant on carbon tetrachloride (CCl4)-induced liver damage in rats. The study showed different degrees of activity on measuring the different biochemical parameters like aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALKP), and total protein (TP), wherein the methanol fraction and compound AB-IV were found to be most active. The histopathological study of the liver of the methanolic fraction and compound AB-IV (isolated from methanol fraction) also showed almost complete normalization of the liver tissues as neither fatty accumulation nor necrosis was observed. The central vein appeared clearly indicating a potent antihepatotoxic activity.

2. Materials and methods

2.1. Plant material

The seeds of *Cichorium intybus* L. were procured from Khari Bawli market, Delhi, India and were identified by a taxonomist, Department of Botany, Hamdard University,
New Delhi, where a voucher specimen no. 765 has been deposited for future reference.

2.2. Preparation of the plant extracts

The dried seeds were crushed to a coarse powder (9.0 kg) and were exhaustively extracted with ethanol by cold percolation. The crude alcoholic extract was concentrated under reduced pressure to get a viscous mass (950 g). It was dissolved in boiling methanol and kept at room temperature, solidified fats (250 g) were removed by suction, and the filtrate so obtained was concentrated to get a viscous solid, and then subsequently fractionated into petroleum ether (60–80 °C) (200 g), ethyl acetate (250 g), and methanol soluble (150 g) fractions. The petroleum ether fraction could not be analyzed for its chemical components, since it was an oily material. The ethyl acetate fraction afforded a new steroid named as cichosterol and a rare steroid namely stigma (Ahmed et al., 2002). The methanol fraction (100 g) was deposited for future reference.

2.3. Experimental animals

The study was carried out on Wistar albino rats (150–200 g) of either sex as reported in the literature (Handa and Singh, 1995). The rats were bred in a colony in the Central Animal House of Jamia Hamdard. They were fed with a standard pellet diet (Gold Mohar, Lipton India Ltd., Kolkata) and water ad libitum. Before their use in the experiment, the rats were kept in standard environmental conditions (25–28 °C, 60–70% relative humidity and 12/12h light/dark cycle). Eight animals in each group were used in all sets of experiments.

2.4. Testing of antihepatotoxic activity

Animals were divided into eight groups of six rats in each for all the experiment. The first group served as vehicle control and received normal saline only. The second group served as CCl4-intoxicated control and received by gavage vehicle (normal saline) and CCl4 diluted with liquid paraffin (1:1, single dose). The third group was given standard drug Silymarin at the dose of 70 mg/kg body weight and the remaining groups were given different extracts at the dose of 500 mg/kg body weight (group 8 received 250 mg/kg) and CCl4. The vehicle (1% gum acacia in distilled water) or test drugs were administered orally for 5 days. CCl4 diluted with liquid paraffin (1:1) was administered in a dose of 1.5 ml/kg by oral route. After 24 h of CCl4 administration, blood of rats was obtained by puncturing retro-orbital plexus. The livers of animals from each group were taken for histopathological studies. The blood samples of each animal were taken and allowed to clot for 45 min at room temperature. Serum was separated by centrifugation at 2500 rpm at 30 °C for 15 min and analyzed for various biochemical parameters.

2.5. Assessment of the liver function

The biochemical parameters such as AST, ALT, and AKLP were estimated by reported methods (Reitman and Frankel, 1957; Kind and King, 1954). The TP was also measured according to the reported methods (Wootton, 1964; Dumas et al., 1971).

2.6. Statistical analysis

The results of the biochemical estimations are reported as mean ± S.E. Total variation present in a set of data was estimated by one-way ANOVA, student’s t-test and Dennett’s test were used for determining the significance (Woolson, 1987; Dennett, 1964).

2.7. Histopathological studies of the liver

The histopathological studies were carried out by reported method (Luna, 1986). The rats were sacrificed under light ether anesthesia after 24 h of the last dosage, and the livers removed and washed with normal saline. Small pieces of liver tissues were processed and embedded in paraffin wax. Sections of 5–6 μm in thickness were cut, stained with hematoxylin and eosin, and then studied under an electron microscope.

3. Results and discussions

As shown in Table 1, activities of liver enzymes, ALT, AST, and ALKP were markedly elevated, while the TP level was decreased in CCl4-treated animals in comparison to normal values. Administration of different extracts of the seeds of Cichorium intybus markedly prevented CCl4-induced elevation of AST, ALT and ALKP, and diminution of TP. The petroleum ether, ethyl acetate, methanol, total alcoholic extracts, and compound AB-IV decreased the levels of ALT, AST, and ALKP, while the level of TP increased against CCl4-intoxicated control. The methanol fraction and compound AB-IV were found to be most active at the dose levels of 500 and 250 mg/kg, respectively, exhibiting a decrease in ALT, AST, and ALKP as compared to standard drug Silymarin against intoxicated control in comparison to normal values. The level of TP was increased by all fractions in different proportions, wherein methanol and compound AB-IV were found to be most active as compared to
Effect of different fractions and the isolated compound of the total alcoholic extract of the seeds of *Cichorium intybus* on biochemical parameters in albino rats intoxicated with CCl₄

### Table 1

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>ALT (units/l)</th>
<th>AST (units/l)</th>
<th>ALKP (units/l)</th>
<th>TP (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (control)</td>
<td>Normal saline only</td>
<td>65.5 ± 17.3</td>
<td>76.6 ± 5.77</td>
<td>31.75 ± 2.05</td>
<td>9.41 ± 0.36</td>
</tr>
<tr>
<td>CCl₄ (toxicity control)</td>
<td>1.5 ml/kg (p.o.)</td>
<td>248.16 ± 16.17</td>
<td>180.83 ± 17.81</td>
<td>59.33 ± 4.04</td>
<td>5.66 ± 0.141</td>
</tr>
<tr>
<td>Silymarin (standard drug)</td>
<td>70 mg/kg (p.o.)</td>
<td>38.88 ± 21.17</td>
<td>39.66 ± 4.04</td>
<td>31.25 ± 2.16</td>
<td>7.48 ± 0.29</td>
</tr>
<tr>
<td>Petroleum ether fraction</td>
<td>500 mg/kg (p.o.)</td>
<td>105.55 ± 8.89</td>
<td>39.66 ± 4.04</td>
<td>30.77 ± 3.35</td>
<td>6.55 ± 0.32</td>
</tr>
<tr>
<td>Ethyl acetate fraction</td>
<td>500 mg/kg (p.o.)</td>
<td>99.99 ± 12.61</td>
<td>26.83 ± 2.02</td>
<td>32.30 ± 3.84</td>
<td>6.64 ± 0.26</td>
</tr>
<tr>
<td>Methanol fraction</td>
<td>500 mg/kg (p.o.)</td>
<td>24.0 ± 3.77</td>
<td>35.0 ± 7.17</td>
<td>31.25 ± 2.17</td>
<td>8.89 ± 0.66</td>
</tr>
<tr>
<td>Total alcoholic extract</td>
<td>500 mg/kg (p.o.)</td>
<td>108.88 ± 21.17</td>
<td>32.66 ± 4.04</td>
<td>35.12 ± 3.83</td>
<td>6.72 ± 0.40</td>
</tr>
<tr>
<td>Compound AB-IV</td>
<td>250 mg/kg (p.o.)</td>
<td>29.99 ± 5.77</td>
<td>35.10 ± 4.04</td>
<td>27.53 ± 5.40</td>
<td>8.59 ± 1.35</td>
</tr>
</tbody>
</table>

Values are mean ± S.E. of six rats. ALT, alanine transaminase; AST, aspartate transaminase; ALKP, alkaline phosphatase; TP, total protein; p.o., per oral.

* Single dose of CCl₄ on first day and daily dose of drug/extract were given for 5 days.
* * P < 0.001.
** P < 0.01.
*** P < 0.05.

#### Table 2

Histopathological studies of liver tissues

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (control)</td>
<td>On first day: The section of its liver showed normal hepatocytes without any necrosis and fatty depositions.</td>
<td>On fifth day: Neither the change nor any sign of degeneration was observed in the liver sections.</td>
</tr>
<tr>
<td>CCl₄ (toxicity control)</td>
<td>On second day of CCl₄ administration: The liver showed centrilobular necrosis with prominent and enlarged central vein. Fatty depositions were also seen. The section showed a classic view of degenerating liver.</td>
<td>After 5 days: The liver did not show any significant recovery. The section showed focal and centrilobular fatty change with necrosis.</td>
</tr>
<tr>
<td>Silymarin (standard drug)</td>
<td>The section showed good recovery with absence of necrosis and fatty depositions. The central vein was clear.</td>
<td>The liver section showed partial disappearance of fatty deposits and necrosis. Hepatic cells in focal areas showed various degrees of degenerative changes like cloudy swelling and hydropic degeneration. However, other areas showed hepatic cell with prominent nucleus and nucleolus indicating mild antineautical activity.</td>
</tr>
<tr>
<td>Petroleum ether fraction</td>
<td>The liver section showed partial disappearance of fatty deposits and necrosis. Hepatic cells in focal areas showed various degrees of degenerative changes like cloudy swelling and hydropic degeneration. However, other areas showed hepatic cell with prominent nucleus and nucleolus indicating mild antineautical activity.</td>
<td>It showed significant recovery with disappearance of fatty deposition and necrosis. The central vein appeared clearly indicating a potent antineautical activity.</td>
</tr>
<tr>
<td>Ethyl acetate fraction</td>
<td>The liver section did not show any significant activity.</td>
<td>It showed significant recovery with disappearance of fatty deposits and necrosis. The central vein appeared clearly indicating a potent antineautical activity.</td>
</tr>
<tr>
<td>Methanol fraction</td>
<td>The liver section showed partial disappearance of fatty deposits and necrosis. Hepatic cells in focal areas showed various degrees of degenerative changes like cloudy swelling and hydropic degeneration. However, other areas showed hepatic cell with prominent nucleus and nucleolus indicating mild antineautical activity.</td>
<td>There was a significant recovery except mild fatty change.</td>
</tr>
<tr>
<td>Total alcoholic extract</td>
<td>The section revealed a tremendous progress with disappearance of fatty deposits and necrosis. It showed superiority as compared with other groups.</td>
<td>The section revealed a tremendous progress with disappearance of fatty deposits and necrosis. It showed superiority as compared with other groups.</td>
</tr>
</tbody>
</table>

The above observations lead to the conclusion that the methanolic fraction possessed most active chemical component, which has also been isolated and named as AB-IV. The preliminary identification has shown it to be a pheno-locoid compound. Further elucidation of its structure is under progress in the laboratory.

Acknowledgements

The authors are thankful to the Head, Department of Pharmaceutical Chemistry for providing necessary research facilities, and to the in-charge, Central Animal Facility, Jamia Hamdard, New Delhi for providing rats and related

standard drug Silymarin against CCl₄-intoxicated control in comparison to normal values. In addition, decrease in liver enzymes and increase in TP was also observed by other fractions (Table 1).

The histopathological studies of the liver showed swelling and necrosis in hepatocytes in CCl₄-treated rats in comparison to normal control rats. Administration of different extracts of the plant exhibited a significant recovery of hepatocytes in different sections of the liver, wherein the methanolic fraction and compound AB-IV showed almost complete normalization of the tissues as neither fatty accumulation nor necrosis was observed. The central vein appeared clearly indicating a potent antineautical activity. The compound AB-IV showed superiority as compared with other groups. Other fractions also showed a considerable recovery of the liver tissues (Table 2).

Thus, the different extracts/fractions of the seeds of *Cichorium intybus* have various degrees of antineautical activity. The methanol fraction and compound AB-IV possessed better antineautical activity. Further, they did not produce any gross behavioral changes or mortality. It can, therefore, be said to be non-toxic and may be used as a safe drug.

4. Conclusion

The above observations lead to the conclusion that the methanolic fraction possessed most active chemical component, which has also been isolated and named as AB-IV. The preliminary identification has shown it to be a pheno-locoid compound. Further elucidation of its structure is under progress in the laboratory.

Acknowledgements

The authors are thankful to the Head, Department of Pharmaceutical Chemistry for providing necessary research facilities, and to the in-charge, Central Animal Facility, Jamia Hamdard, New Delhi for providing rats and related
facilities. One of the authors (A.B.S.) is also thankful to UGC, New Delhi for awarding GATE Scholarship.

References


