Hepatoprotective Effect of Bauhinia variegata (Linn.) Whole Stem against Carbon Tetrachloride-Induced Hepatopathy in Rats

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ABSTRACT

The hepatoprotective activity of the ethanolic extract of Bauhinia variegata (Linn.) whole stem (BV) against carbon tetrachloride (CCl4) induced hepatic failure was investigated by in vitro and in vivo methods. The in vitro method resulted in a significant (p<0.001) increase in enzyme levels (AST, 36.21 ± 2.78 IU/l; ALT, 23.14 ± 1.91 IU/l and ALP, 37.3 ± 2.35 IU/l) following CCl4 treatment of liver explant cultures (toxic control) as compared to the normal control (AST, 11.55 ± 2.27 IU/l; ALT, 2.32 ± 1.17 IU/l and ALP, 12.1 ± 1.5 IU/l). BV extract at a dose of 3.3 mg/ml significantly lowered the levels of the enzymes, AST (20.99 ± 2.12 IU/l; p<0.01) and ALP (21.79 ± 1.82 IU/l; p<0.001). Acute hepatotoxicity (in vivo) was induced by intra-peritoneal (i.p.) injection of CCl4 (CCl4 + olive oil in a 1 : 1 ratio; 2 ml/kg). Administration of BV extract at a dose of 200 and 400 mg/kg of body weight to CCl4-treated rats for 7 days attenuated the marker enzyme level of liver, in a dose-dependent manner. BV extract at 400 mg/kg decreased the level of marker enzymes (AST, 272.77 ± 24.08 IU/l; ALT, 189.15 ± 7.16 IU/l and ALP, 97.15 ± 6.54 IU/l) significantly (p<0.001) with a significant (p<0.001) increase in body weight (6.16 ± 1.01 g) as compared to the toxic control group. The ethanolic extract of BV (400 mg/kg) exhibited significant and comparable hepatoprotective potential as that of the standard polyherbal drug Liv-52. The statistically significant results obtained in this study thus suggests a protective function of B. variegata whole stem against CCl4-induced hepatopathy both in vitro and in vivo.

Keywords: hepatotoxicity, hepatoprotectin, kanchnara, orchid tree, marker enzymes, oxidative stress, lipid peroxidation

Abbreviations: ALP, Alkaline phosphatase; AST, Asparate transaminersase; BV, Bauhinia variegata; CMC, Carboxy methyl cellulose; DMEM, Dulbeccos modified Egale’s medium; i.p, intra peritoneal; OECD, Organization for Economic Co-operation and Development

INTRODUCTION

The mammalian liver is not only critical for the overall health and metabolic activity of an individual but also functions as an important site for cellular detoxification. Thus, any dysfunction of the liver (hepatopathy) can potentially lead to multi-organ failure and death eventually. Hepatopathy, of varied etiology, is encountered globally irrespective of age, ethnic, racial backgrounds or environmental and geographical diversity. The reasons of hepatopathy incidence and associated fatalities are multifaceted which range from chemicals, drugs or substance induced hepatic anomalies to various metabolic and physiological disturbances causing hepatotoxicity and leading to liver damage. This places hepatopathy as one of the leading cause of death across the world (Friedman et al. 2003; Bernal et al. 2010).

Carbon tetrachloride (CCl4) is a widely used industrial chemical and is also a potent hepatotoxin. It induces hepatotoxicity due to free radical production thereby inducing oxidative stress and lipid peroxidation in liver tissue and consequently, causing irreversible liver damage by necrosis (Ram et al. 1999; Weber et al. 2003; Maddrey 2005). Bauhinia variegata Linn. (BV, family Caesalpiniaceae) is a deciduous plant widely distributed throughout tropical and subtropical regions of the world including India and China. It is commonly known as ‘orchid-tree’, ‘mountain ebony’ and its local Indian names are ‘kanchnara’ and ‘kanchana’ (Chopra et al. 1956). This plant species has been used as a medicinal plant and cattle feed by the tribes of India (Nadkarni 1954; Kurikar et al. 1999) and has also been used in various indigenous systems of medicine like Ayurveda and Unani (The Ayurvedic Pharmacopoeia of India 1990). Different parts of this plant are used traditionally for various ailments: as a liver tonic, antibacterial and to suppress the edema arising because of kidney failure (Mali et al. 2007). This plant has undergone vast pharmacological screening in the past decade and has been observed to have beneficial effects against many ailments in various experimental animal models. The bark extracts of BV have been shown to have concentration-dependent anti-bacterial activity with more sensitivity to Gram-negative than Gram-positive bacteria (Parenk et al. 2006). The chemopreventive and cytotoxic effects of BV were evaluated in N-nitrosodimethylamine (DEN)-induced experimental liver tumor in rats and human cancer cell lines and was found to be cytotoxic against human epithelial larynx cancer (HeP2) and human breast cancer (HBL-100) cells (Rajkapoor et al. 2006). The ethanolic extract of BV at a dose of 250 mg/kg for 15 days has been demonstrated to have a significant inhibitory action on Complete Freund’s adjuvant-induced arthritis in Wistar rats (Rajkapoor et al. 2007). More recently, the ethanolic and aqueous extracts from stem, bark and roots of BV have been demonstrated to possess antioxidant and antihyperlipidemic property both in vitro and in vivo, respectively (Rajani et al. 2009). Taken together the evidence mentioned above strongly demonstrates a significant medicinal value of BV. However, the protective effect of BV whole stem against CCl4-induced hepatotoxicity in rats has not been substantially explored. Thus, the present study was aimed to evaluate the hepatoprotective potential of the ethanolic extract of BV whole stem in a conventional model (in vivo and in vitro) of CCl4-induced hepatopathy.
MATERIALS AND METHODS

The whole stem of BV was collected from young matured plant from Bharatpur Forest Range, Bhujawaswar, Orissa, India during Nov-Dec and identified by Dr. B. K. Jaysingh, Gopabandhu Ayurvedic College, Puri, Orissa, India. A plant specimen (vide Voucher No: 081) was deposited in the herbarium of the University Department of Pharmaceutical Sciences, Utkal University, Bhubaneswar, Orissa, India. After authentication, fresh plant materials were collected in bulk, washed under running tap water to remove adherents, shade dried and pulverized in a mechanical grinder to produce coarse powdered plant material. The coarse BV whole stem powder was used for solvent extraction.

All animals used in the study were healthy male albino rats of Wistar strain weighing between 150-200 g (purchased from Ghosh Enterprises, Kolkata, India). The animals were maintained under standard laboratory conditions (12 ± 1 hr, day-night schedule; temperature maintained between 11-20 ± 2°C; housed in large spacious hygienic cages with access to food and water ad libitum) and were acclimatized for one week prior to the experiments. The experimental protocol was approved by the Institutional Animal Ethics Committee (Vide Registration No.: 990/c/06/CPSCSEA).

Chemicals used in the study were of analytical grade and were procured from Merck Specialties Pvt. Ltd., Mumbai, India. Dulbecco Modified Eagle’s Medium (DMEM) was procured from Sigma, USA. Liv-52 (standard polyherbal drug ‘Liv-52’ (5 ml/kg; p.o.) (Sandhir et al. 1999) with single i.p. dose of CCl4 on 1st and 7th day. Group IV and V (BV extract treated groups) received BV extract 200 and 400 mg/kg of body weight once a day for 7 days together with a single i.p. dose of CCl4 on day 1 and day 7, as mentioned above.

Following completion of experimental period, on day 8, rats were anesthetized, using phenobarbitone sodium (40 mg/kg; i.p.) and the blood samples along with liver tissues were collected from each test group. Blood samples were collected from the test animals under anesthesia by direct cardiac puncture at ventricle site before sacrifice and serum parameters of liver function including: AST, ALT, ALP, albumin and total protein were estimated as per reported procedures (Doumas et al. 1971; Tietz 1999; Cheshbrough 2003). The biochemical estimations were done in a Biochemical-semi-auto analyzer (EBRA-Chem-5 Plus. V2, West-Germany) by standard procedures using commercial kits.

RESULTS AND DISCUSSION

The in vitro hepatoprotective activity of BV against CCl4 was analyzed on rat liver explant cultures grown for 48 hr in vitro. To evaluate injury to the hepatic cells, the supernatant collected from the culture sets were processed to estimate the level of marker enzymes- AST, ALT and ALP. As shown in the Table 1, the toxic control set (CCl4-treated explant cultures) resulted in a significant (p<0.001) increase in AST (36.21 ± 2.78 IU/l), ALT (23.14 ± 1.91 IU) and ALP (37.3 ± 2.35 IU/l) level as compared to normal control set (AST, 11.55 ± 2.27 IU/l; ALT, 2.32 ± 1.17 IU/l and ALP, 12.1 ± 1.5 IU/l). Interestingly, in BV extract treated sets a dose-dependant decrease in the levels of all the estimated enzymes was observed. Treatment of the BV extract at a higher concentration (3.3 mg/ml) significantly lowered the AST (20.99 ± 2.12 IU/l; p<0.01) and ALP (21.79 ± 1.82 IU/l; p<0.001) level as compared to toxic control set. However, no significant change was observed in ALT level (16.94 ± 1.17 IU/l; p>0.05) on comparison with toxic set. The standard set (explant culture incubated with CCl4 + silymarin) showed an enhanced hepatoprotective efficacy as AST (15.08 ± 1.83 IU/l), ALT (10.02 ± 1.06 IU/l) and ALP (14.85 ± 2.18 IU/l) levels were decreased significantly (p<0.001) as compared to CCl4 treated explants culture set.

The results obtained from our in vivo hepatoprotective studies are outlined in Table 2. Analogous to the findings from our in vitro studies, we found a dose-dependant hepatoprotective effect of BV extract in whole animals. Change in body weight and serum parameters of liver function including AST, ALT, ALP, albumin and total protein were analyzed on 8th day of experiment. As compared to the controls, the CCl4 administered animals (toxic control...
Table 1 Parameters studied for in vitro hepatoprotective activity of Bauhinia variegata whole stem extract on rat liver explant cultures.

<table>
<thead>
<tr>
<th>Group</th>
<th>Change in body weight (g)</th>
<th>Albumin (g/dl)</th>
<th>Total protein (g/dl)</th>
<th>AST (IU/l)</th>
<th>ALT (IU/l)</th>
<th>ALP (IU/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Set 1 (control)</td>
<td>10.66 ± 1.28</td>
<td>2.83 ± 0.17</td>
<td>5.70 ± 0.35</td>
<td>75.04 ± 7.23</td>
<td>49.21 ± 4.90</td>
<td>80.30 ± 3.58</td>
</tr>
<tr>
<td>Set 2 [CCl4 (83.3 μl/ml)]</td>
<td>-12.83 ± 1.13</td>
<td>1.40 ± 0.10</td>
<td>3.11 ± 0.16</td>
<td>840.37 ± 28.26</td>
<td>746.75 ± 18.94</td>
<td>133.65 ± 3.47</td>
</tr>
<tr>
<td>Set 3 [CCl4 (83.3 μl/ml) + Silymarin (0.5 ml; 1.9 mg/ml)]</td>
<td>6.83 ± 0.70</td>
<td>2.71 ± 0.27</td>
<td>5.09 ± 0.20</td>
<td>246.23 ± 16.93</td>
<td>186.38 ± 14.60</td>
<td>86.49 ± 7.17</td>
</tr>
<tr>
<td>Set 4 [CCl4 (83.3 μl/ml) + BV extract (0.825 mg/ml)]</td>
<td>3.33 ± 1.90</td>
<td>2.55 ± 0.16</td>
<td>4.70 ± 0.36</td>
<td>393.08 ± 12.29</td>
<td>241.07 ± 11.73</td>
<td>112.58 ± 6.82</td>
</tr>
<tr>
<td>Set 5 [CCl4 (83.3 μl/ml) + BV extract (1.65 mg/ml)]</td>
<td>6.16 ± 1.01</td>
<td>2.61 ± 0.18</td>
<td>4.96 ± 0.31</td>
<td>272.77 ± 24.08</td>
<td>189.15 ± 7.16</td>
<td>97.15 ± 6.54</td>
</tr>
<tr>
<td>Set 6 [CCl4 (83.3 μl/ml) + BV extract (3.3 mg/ml)]</td>
<td>20.99 ± 2.12</td>
<td>16.94 ± 1.17</td>
<td>21.79 ± 1.82</td>
<td>393.08 ± 12.29</td>
<td>241.07 ± 11.73</td>
<td>112.58 ± 6.82</td>
</tr>
</tbody>
</table>

Values are given in mean ± SEM.

Table 2 Parameters studied for in vivo hepatoprotective activity of Bauhinia variegata whole stem extract on rats (8th day).

<table>
<thead>
<tr>
<th>Group</th>
<th>Change in body weight (g)</th>
<th>Albumin (g/dl)</th>
<th>Total protein (g/dl)</th>
<th>AST (IU/l)</th>
<th>ALT (IU/l)</th>
<th>ALP (IU/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (Normal control)</td>
<td>10.66 ± 1.28</td>
<td>2.83 ± 0.17</td>
<td>5.70 ± 0.35</td>
<td>75.04 ± 7.23</td>
<td>49.21 ± 4.90</td>
<td>80.30 ± 3.58</td>
</tr>
<tr>
<td>Group II (Toxic control; CCl4 toxicated)</td>
<td>-12.83 ± 1.13</td>
<td>1.40 ± 0.10</td>
<td>3.11 ± 0.16</td>
<td>840.37 ± 28.26</td>
<td>746.75 ± 18.94</td>
<td>133.65 ± 3.47</td>
</tr>
<tr>
<td>Group III (Standard; Liv-52 treated)</td>
<td>6.83 ± 0.70</td>
<td>2.71 ± 0.27</td>
<td>5.09 ± 0.20</td>
<td>246.23 ± 16.93</td>
<td>186.38 ± 14.60</td>
<td>86.49 ± 7.17</td>
</tr>
<tr>
<td>Group IV (BV, 200 mg/kg treated)</td>
<td>3.33 ± 1.90</td>
<td>2.55 ± 0.16</td>
<td>4.70 ± 0.36</td>
<td>393.08 ± 12.29</td>
<td>241.07 ± 11.73</td>
<td>112.58 ± 6.82</td>
</tr>
<tr>
<td>Group V (BV extract 400 mg/kg treated rats)</td>
<td>6.16 ± 1.01</td>
<td>2.61 ± 0.18</td>
<td>4.96 ± 0.31</td>
<td>272.77 ± 24.08</td>
<td>189.15 ± 7.16</td>
<td>97.15 ± 6.54</td>
</tr>
</tbody>
</table>

Values are given in mean ± SEM.

Fig. 1 Transverse sections of liver. (A) Group I (Normal control) section shows central vein surrounded by hepatic cord of cells. (B) Group II (Toxic control; CCl4 treated) section shows patches of liver cell necrosis with inflammatory collections around the central vein. (C) Group III (Standard; Liv-52 + CCl4 treated) induction almost near normal. (D) Group IV (BV extract, 400 mg/kg + CCl4) in vivo and in vitro. The BV extract treatments rendered resistance to the hepatic cells against CCl4 as evident from the improved metabolic functions (at the level of biochemical marker enzymes) and hepato-cellular architecture (at the level of tissue histology) as compared to the toxic group. Moreover, the results obtained in our treatment groups are comparable with the previously reported hepatoprotective effects of other plant species like C. angustifolia (Ilavarasan et al., 2001), Bacopa monnieri (Ghosh et al., 2007), Pterocarpus santalinus (Manjunath 2006), Eucalyptus maculata (Abdel-Fattah et al. 2001), and many other herbal preparations, Pricoliv (Dwivedi et al. 1990). It is well documented that the BV whole stem contains a variety of active components such as, octacosanol, stigmasterol (Prakash et al. 1976), 7-dihydroxy flavonone-4-O-α-L rhamnopyranosyl-β-D-glucopyranoside – a flavone glycoside (Gupta et al. 1979), β-sitosterol, lupeol, kaempferol-3-glucoside – a flavone glycoside (Gupta et al. 1984) and many other alkaloids, flavonoids and terpenoids (Duret et al. 1977; Gupta et al. 1980). Thus, we suggest that the hepatoprotective potential of BV whole stem, is because of the active phytochemicals present in it. It was mentioned earlier that CCl4 is a potent hepato-toxin which induces lipid peroxidation and consequently promotes liver damage or

In vivo and in vitro.
hepatopathy in vivo. Moreover, it has been evident from numerous studies on screening and evaluation of pharmacological effects of phytochemicals that, several phytochemicals such as flavonols, flavonoids, triterpenoids, sterols and alkaloids have the ability to inhibit lipid peroxidation induced by CCl4 (Baek et al. 1996; Mehta et al. 1999). Thus, we presume that the active phytochemicals present in BV whole stem (either single or complex molecular components all together) would be responsible for the hepatoprotective activity, exhibited by BV whole stem in our experimental setup. Thus, identification of the precise phytochemicals present in BV whole stem that have the hepatoprotective property will be necessary to advance our understanding in this order.

CONCLUSION

Present study was undertaken to investigate the hepatoprotective potential of Bauhinia variegata Linn. (whole stem) against chemical (CCl4) induced hepatopathy. The result obtained from this study suggested the hepatoprotective activity of BV whole stem against CCl4-induced hepatotoxicity. The hepatoprotective potential of ethanolic extract of BV whole stem was a dose-dependent effect. Thus, in conclusion our study puts forward the BV whole stem was a dose-dependant effect. Thus, in conclusion our study puts forward the BV whole stem was a dose-dependant effect. Thus, in conclusion our study puts forward the BV whole stem was a dose-dependant effect. Thus, in conclusion our study puts forward the BV whole stem was a dose-dependant effect. Thus, in conclusion our study puts forward the BV whole stem was a dose-dependant effect. Thus, in conclusion our study puts forward the BV whole stem was a dose-dependant effect. Thus, in conclusion our study puts forward the BV whole stem was a dose-dependant effect. Thus, in conclusion our study puts forward the BV whole stem was a dose-dependant effect. Thus, in conclusion our study puts forward the BV whole stem was a dose-dependant effect. Thus, in conclusion our study puts forward the BV whole stem was a dose-dependant effect. Thus, in conclusion our study puts forward the BV whole stem was a dose-dependant effect. Thus, in conclusion our study puts forward the BV whole stem was a dose-dependant effect. Thus, in conclusion our study puts forward the BV whole stem was a dose-dependant effect. Thus, in conclusion our study puts forward the BV whole stem was a dose-dependant effect. Thus, in conclusion our study puts forward the BV whole stem was a dose-dependant effect. Thus, in conclusion our study puts forward the BV whole stem was a dose-dependant effect. Thus, in conclusion our study puts forward the BV whole stem was a dose-dependant effect. Thus, in conclusion our study puts forward the BV whole stem was a dose-dependant effect. Thus, in conclusion our study puts forward the BV whole stem was a dose-dependant effect.

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