Hepatoprotective Activity of Aristolochia bracteata Retz against Carbon Tetrachloride Induced Hepatic Damage

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ABSTRACT
Administration of ethanol extract of Aristolochia bracteata whole plant and standard drug silymarin in rats showed significant hepatoprotective action against CCl₄ induced hepatotoxicity. Elevated serum marker enzymes of SGOT, SGPT, ALP and serum bilirubin were significantly reduced to near normal level in whole plant ethanol extract of A. bracteata treated rats. Lipid peroxidation level was found to be decreased significantly in whole plant ethanol extract of A. bracteata at 250 and 500 mg/kg doses; similarly it increased the antioxidant enzyme levels of GPx, GRD, SOD, CAT and GSH. Thus, the results suggest that A. bracteata extract acts as a potent hepatoprotective agent against CCl₄ induced hepatotoxicity in rats.

Keywords: Aristolochia bracteata, carbon tetrachloride, bilirubin, antioxidant enzymes.

INTRODUCTION
Liver diseases remain as one of the serious health problems. It is the key organ of metabolism and excretion. It is often exposed to variety of xenobiotics and therapeutic agents. Conventional drugs used in the treatment of liver diseases are often inadequate. It is therefore necessary to search for alternative drugs for the treatment of liver diseases to replace the currently used drugs of doubtful efficacy and safety. More than 900 drugs have been implicated in causing liver injury and it is the most common reason for a drug to be withdrawn from the market. Drug induced liver injury is responsible for 5% of all hospital admissions and 50% of all acute liver failures.

Herbal drugs play a role in the management of various liver disorders most of which speed up the natural healing processes of the liver. Numerous medicinal plants and their formulations are used for liver disorders in ethnomedicinal practice as well as traditional system of medicine in India. However, the readily available hepatoprotective herbal drugs are not sufficiently active to effectively combat severe liver disorders. In view of lack of synthetic agents for the treatment of hepatic disorder, there is a growing focus to evaluate traditional herbal medicines for hepatoprotective activity. Therefore, there is a need to develop satisfactory hepatoprotective drugs.

Aristolochia bracteata Retz is a shrub distributed throughout India, belonging to the family Aristolochiaceae. A. bracteata is used in traditional medicine as gastric stimulant and in the treatment of cancer, lung inflammation, dysentery and snake bites. In Indigenous system of medicine, it is reported that the leaves were used for skin diseases, rheumatism and analgesic.

The whole plant was used as purgative and antihelmintic, antipyretic and antiinflammatory agents. Along with its use against health disorders, it was felt worthwhile to screen the hepatoprotective activity of A. bracteata so that its further role in natural hepatoprotective agent is explored.

However, no scientific information is available regarding the hepatoprotective effect of A. bracteata.

Therefore in the present study an attempt has been made to evaluate the hepatoprotective activity as well as antioxidant profile of A. bracteata whole plant extract with a view towards elucidating the probable mechanism of action by employing in vivo methods.

MATERIALS AND METHODS
Collection of Plant Sample
The whole plant of Aristolochia bracteata Retz was collected from Vadavalli, Coimbatore, Tamil Nadu.

The plant was identified and authenticated in Botanical Survey of India, Southern Circle, Coimbatore, Tamil Nadu, India. A voucher specimen was deposited in Ethnopharmacology Unit, Research Department of Botany, V.O. Chidambaram College, Tuticorin, Tamil Nadu.

Preparation of Plant Extract for Phytochemical Screening and Hepatoprotective Studies
The whole plant of A. bracteata was shade dried at room temperature and the dried whole plant was powdered in a Wiley mill.

Hundred grams of powdered A. bracteata whole plant was packed in a Soxhlet apparatus and extracted with ethanol.

The extract was subjected to qualitative test for the identification of various phytochemical constituents as per the standard procedures.

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The ethanol extract was concentrated in a rotary evaporator. The concentrated ethanol extract was used for hepatoprotective studies.

Animals

Normal healthy male Wistar albino rats (180-240g) were used for the present investigation. Animals were housed under standard environmental conditions at room temperature (25±2°C) and light and dark (12:12h). Rats were fed with standard pellet diet (Goldmohur brand, MS Hindustan lever Ltd., Mumbai, India) and water ad libitum.

Acute Toxicity Studies

Acute oral toxicity study was performed as per OECD-423 guidelines (acute toxic class method), albino rats (n=6) of either sex selected by random sampling were used for acute toxicity study. The animals were kept fasting for overnight and provided only with water, after which the extracts were administered orally at 5mg/kg body weight by gastric intubation and observed for 14 days. If mortality was observed in two out of three animals, then the dose administered was assigned as toxic dose. If mortality was observed in one animal, then the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for higher doses such as 50, 100 up to 2000 mg/kg body weight.

Experimental Design

In this investigation, a total of 25 rats (20 CCl₄ hepatic toxicity induced rats and 5 normal rats) were taken and divided into five groups of 5 rats each. Group I: Rats received normal saline was served as a normal control. Group II: CCl₄ hepatic toxicity induced control: Rats received 2.5ml/kg body weight of CCl₄ for 14 days. Group III: Liver injured rats received ethanol extract of whole plant of *A. bracteata* at the dose of 250mg/kg body weight for 14 days. Group IV: Liver injured rats received ethanol extract of whole plant of *A. bracteata* at the dose of 500mg/kg body weight for 14 days. Group V: Liver injured rats received standard drug silymarin at the dose of 100mg/kg body weight for 14 days.

Biochemical Analysis

The animals were sacrificed at the end of experimental period of 14 days by decapitation. Blood was collected, sera separated by centrifugation at 3000 g for 10 minutes. Serum protein and serum albumins were determined quantitatively by colorimetric method using bromocresol green. The total protein minus the albumin gives the globulin. Serum glutamate pyruvate transaminase (SGPT) and serum glutamate oxaloacetate transaminase (SGOT) were measured spectrophotometrically by following the method of Reitman and Frankel. Serum alkaline phosphatase (ALP) was measured by the method of King and Armstrong. Total bilirubin and conjugated bilirubin were determined as described by Balistre and Shaw. The unconjugated bilirubin concentrations were calculated as the difference between total and conjugated bilirubin concentrations. Gamma glutamyl transferase (GGT) was estimated by the method of Szasz.

Quantitative estimation of MDA formation was done by determining the concentration of thiobarbituric acid reactive substances (TBARS) in plasma by the method of Satoh. Enzymatic antioxidants, superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GRD), reduced glutathione (GSH) and glutathione peroxidase (GPx) were also assayed in erythrocytes.

Statistical Analysis

The data were expressed as the mean ± S.E.M. The difference among the means has been analyzed by one-way ANOVA. *p*<0.01 and *p*<0.05 were considered as statistical significance using SPSS Software.

RESULTS

Phytochemical Screening

Phytochemical screening of *A. bracteata* whole plant reveals the presence of alkaloids, coumarins, flavonoids, quinones, phenols, saponins, steroids, tannins, terpenoids, sugars, glycosides and xanthoproteins.

Acute Oral Toxicity Study

Ethanol extract of *A. bracteata* whole plant did not cause any mortality upto 2000 mg/kg dose level. Hence 1/₅th and 1/₁₀th of the maximum dose (i.e. 250 and 500 mg/kg p.o) were selected for the present study.

Effect on Body Weight

The effect of ethanol extract of *A. bracteata* whole plant on body weight of the normal control, CCl₄ intoxicated control and plant extract treated rat groups are shown in Table 1.

An increase in body weight was noticed in all groups except group II liver damaged control rats when compared with normal control. The body weight loss was higher in liver damaged control rats.

Effects on Serum Protein and Serum Enzymes

Table 2 shows the effect of ethanol extract of whole plant of *A. bracteata* on serum total protein, albumin, globulin, A/G ratio, serum transaminases and alkaline phosphatases in CCl₄ intoxicated rats. There was a significant (*p*<0.01) increase in serum GOT, GPT and ALP levels in CCl₄ intoxicated control group (Group II) when compared to the normal control group (Group I). The total protein level was significantly (*p*<0.05) decreased from 8.14 mg/dl in normal control to 6.74 mg/dl in CCl₄ intoxicated control.

The whole plant ethanol extract of *A. bracteata*, at a dose of 500 mg/kg, decreased the elevated serum marker enzymes significantly. Treatments with silymarin, the standard drug and plant extract reversed the altered total protein and albumin to almost near normal level.
Effects on Bilirubin and Gamma-Glutamyl Transferase

The effect of whole plant ethanol extract of *A. bracteata* on total, conjugated, unconjugated bilirubin and γ-glutamyl transferase are shown in Table 3.

There was a significant (*p*<0.01) elevation of total, conjugated, unconjugated bilirubin and γ-glutamyl transferase in the serum of CCl₄ intoxicated group (Group II) when compared to normal control (Group I).

Whole plant ethanol extract of *A. bracteata*, at a dose of 500mg/kg body weight, reduced the levels of total, conjugated and unconjugated bilirubin (Group IV).

Effects on Lipid peroxidation and Antioxidant Enzymes

The hepatoprotective effect of whole plant ethanol extract of *A. bracteata* on lipid peroxidation (LPO), glutathione peroxidase (GPx), glutathione reductase (GRD), superoxide dismutase (SOD) catalase (CAT) and reduced glutathione (GSH) activities are shown in Table 4. When compared to the normal control rats (Group I), there was a significant (*p*<0.01) increase in the level of lipid peroxidation and a significant decrease in the levels of glutathione peroxidase, glutathione reductase, superoxide dismutase and catalase in CCl₄ intoxicated control rats (Group II).

Treatment with the whole plant ethanol extract of *A. bracteata* at the dose of 500 mg/kg body weight, decreased the elevated lipid peroxidation level significantly and restored the altered glutathione peroxidase, glutathione reductase, superoxide dismutase, catalase and reduced glutathione levels towards normal in a dose dependent manner. The results were well comparable with that of silymarin, the standard drug treated rats.

**Table 1**: Effect of the whole plant ethanol extract of *A. bracteata* on the body weight of the normal, liver damaged and drug treated rats

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>Dose (mg/kg BW)</th>
<th>Initial Body weight (g)</th>
<th>Final Body weight (g)</th>
<th>Mean weight Gain (↑)/ Loss (↓) (g)</th>
<th>Percentage of Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>0.9% Saline</td>
<td>206.31±4.94</td>
<td>218.33±3.14</td>
<td>12.02↑</td>
<td>5.83</td>
</tr>
<tr>
<td>Group II</td>
<td>0.9% Saline</td>
<td>201.54±6.84</td>
<td>192.16±4.31**</td>
<td>9.38↓</td>
<td>4.65</td>
</tr>
<tr>
<td>Group III</td>
<td>250 (mg/kg)</td>
<td>194.38±4.13</td>
<td>201.83±5.68</td>
<td>7.45↑</td>
<td>3.83</td>
</tr>
<tr>
<td>Group IV</td>
<td>500 (mg/kg)</td>
<td>197.84±6.83</td>
<td>212.83±5.68aa</td>
<td>14.99↑</td>
<td>7.58</td>
</tr>
<tr>
<td>Group V</td>
<td>100 (mg/kg)</td>
<td>192.34±5.16</td>
<td>208.27±8.36a</td>
<td>15.93↑</td>
<td>8.28</td>
</tr>
</tbody>
</table>

Values are mean ± SD of 5 animals in each group. Statistical analysis ANOVA followed by Dunnett t-test * * * * p < 0.05; ** p <0.01 Compared normal control vs liver injured rats * p < 0.05; ** p<0.01 Compared liver injured rats vs drug treated.

**Table 2**: Effect of the whole plant ethanol extract of *A. bracteata* on the serum protein, albumin, Globulin concentration and serum GOT, GPT and ALP enzyme activity in the normal, liver damaged and drug treated rats

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>Dose (mg/kg)</th>
<th>T. Protein (mg/dl)</th>
<th>Albumin (g/dl)</th>
<th>Globulin (g/dl)</th>
<th>A/G Ratio</th>
<th>SGOT (U/L)</th>
<th>SGPT (U/L)</th>
<th>ALP (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>0.9% Saline</td>
<td>8.14±0.74</td>
<td>4.85±0.27</td>
<td>3.29±0.38</td>
<td>1.5:1</td>
<td>18.36±0.8</td>
<td>21.31±0.67</td>
<td>138.36±1.22</td>
</tr>
<tr>
<td>Group II</td>
<td>0.9% Saline</td>
<td>6.74±0.13*</td>
<td>3.91±0.23</td>
<td>2.83±0.12*</td>
<td>1.4:1</td>
<td>92.16±7.84**</td>
<td>104.16±2.19**</td>
<td>198.24±6.29**</td>
</tr>
<tr>
<td>Group III</td>
<td>250 (mg/kg)</td>
<td>7.86±0.31**</td>
<td>4.23±0.23</td>
<td>3.63±0.56</td>
<td>1.2:1</td>
<td>43.84±3.46**</td>
<td>48.16±2.16**</td>
<td>152.40±2.24**</td>
</tr>
<tr>
<td>Group IV</td>
<td>500 (mg/kg)</td>
<td>8.24±0.34a</td>
<td>4.88±0.35a</td>
<td>3.36±0.27</td>
<td>1.5:1</td>
<td>28.36±1.14aa</td>
<td>26.13±1.24aa</td>
<td>129.25±2.16aa</td>
</tr>
<tr>
<td>Group V</td>
<td>100 (mg/kg)</td>
<td>8.09±0.13</td>
<td>4.31±0.73</td>
<td>3.78±0.32</td>
<td>1.1:1</td>
<td>20.16±0.93aa</td>
<td>23.16±0.83aa</td>
<td>126.22±4.86aa</td>
</tr>
</tbody>
</table>

Values are mean ± SD of 5 animals in each group. Statistical analysis ANOVA followed by Dunnett t-test * * * * p < 0.05; ** p <0.01 Compared normal control vs liver injured rats * p < 0.05; ** * p<0.01 Compared liver injured rats vs drug treated; ns – not significant.© Copyright protected. Unauthorised republication, reproduction, distribution, dissemination and copying of this document in whole or in part is strictly prohibited.
Table 3: Effect of the whole plant ethanol extract of *A. bracteata* on the serum total bilirubin, conjugated and unconjugated bilirubin and GGTP levels in the normal control, liver injured control and drug treated rats

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>Dose (mg/kg BW)</th>
<th>Total Bilirubin (µmol/L)</th>
<th>Conjugated Bilirubin (µmol/L)</th>
<th>Unconjugated Bilirubin (µmol/L)</th>
<th>GGTP (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>0.9% Saline</td>
<td>0.93±0.041</td>
<td>0.23±0.068</td>
<td>0.70±0.026</td>
<td>7.34±0.64</td>
</tr>
<tr>
<td>Group II</td>
<td>0.9% Saline</td>
<td>4.08±0.36**</td>
<td>1.97±0.05**</td>
<td>2.11±0.36**</td>
<td>19.28±1.13**</td>
</tr>
<tr>
<td>Group III</td>
<td>250 (mg/kg)</td>
<td>1.04±0.05a</td>
<td>0.32±0.04**</td>
<td>0.72±0.02**</td>
<td>13.46±0.83*</td>
</tr>
<tr>
<td>Group IV</td>
<td>500 (mg/kg)</td>
<td>0.83±0.07aa</td>
<td>0.26±0.03**</td>
<td>0.57±0.07**</td>
<td>8.13±0.21*</td>
</tr>
<tr>
<td>Group V</td>
<td>100 (mg/kg)</td>
<td>0.88±0.04aa</td>
<td>0.26±0.02**</td>
<td>0.62±0.07**</td>
<td>8.13±0.31**</td>
</tr>
</tbody>
</table>

Values are mean ± SD of 5 animals in each group. Statistical analysis ANOVA followed by Dunnett t-test * p < 0.05; ** p<0.01 Compared normal control vs liver injured rats a p < 0.05; aa p<0.01 Compared liver injured rats vs drug treated; ns – not significant

Table 4: Effect of the whole plant ethanol extract of *A. bracteata* on the serum LPO, GPX, GRD, SOD, CAT and GSH activity in the normal control, liver injured control and drug treated rats

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>Dose (mg/kg)</th>
<th>Parameters</th>
<th>GPx (u/mgProtien)</th>
<th>GRD (u/mg)</th>
<th>SOD (u/mg)</th>
<th>CAT (u/mg)</th>
<th>GSH (u/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>0.9% Saline</td>
<td>LPO (µmol/L)</td>
<td>1.84±0.013</td>
<td>4.31±0.164</td>
<td>0.40±0.054</td>
<td>0.27±0.034</td>
<td>3.74±0.051</td>
</tr>
<tr>
<td>Group II</td>
<td>0.9% Saline</td>
<td></td>
<td>6.34±0.124**</td>
<td>2.84±0.103**</td>
<td>0.21±0.074**</td>
<td>0.11±0.083**</td>
<td>1.24±0.046**</td>
</tr>
<tr>
<td>Group III</td>
<td>250 (mg/kg)</td>
<td></td>
<td>2.93±0.161</td>
<td>3.78±0.056**</td>
<td>0.32±0.015**</td>
<td>0.20±0.056**</td>
<td>2.31±0.052**</td>
</tr>
<tr>
<td>Group IV</td>
<td>500 (mg/kg)</td>
<td></td>
<td>1.78±0.026aa</td>
<td>4.05±0.018**</td>
<td>0.39±0.023**</td>
<td>0.26±0.076**</td>
<td>3.68±0.018**</td>
</tr>
<tr>
<td>Group V</td>
<td>100 (mg/kg)</td>
<td></td>
<td>1.69±0.039aa</td>
<td>4.26±0.028**</td>
<td>0.42±0.016**</td>
<td>0.26±0.059**</td>
<td>3.69±0.084**</td>
</tr>
</tbody>
</table>

Values are mean ± SD of 5 animals in each group. Statistical analysis ANOVA followed by Dunnett t-test * p < 0.05; ** p<0.01 Compared normal control vs liver injured rats a p < 0.05; aa p<0.01 Compared liver injured rats vs drug treated; ns – not significant

**DISCUSSION**

The present study demonstrates the hepatoprotective and antioxidant effects of ethanol extract of *A. bracteata* whole plant against CCl4 induced liver injury in rats. The liver is one of the vital organs in our body responsible for detoxification of toxic chemicals and drugs. Thus it is the target organ for all toxic chemicals. Numerous studies noted that CCl4 is widely used to induce liver damage because it is metabolized in hepatocytes by cytochrome P450, generating a highly reactive carbon centered trichloromethyl radical, leading to initiating a chain of lipid peroxidation and thereby causing liver fibrosis.24-26

Hepatic damage induced by CCl4 causes instability of liver cell metabolism, inducing triacylglycerol accumulation, changes in serum transaminases activities, increased lipid peroxidation, membrane damage and depression of protein synthesis, these are the indicators of liver damage.27-28

The present study demonstrates a significant increase in the activities of SGOT, SGPT and ALP levels that indicates increased permeability, severe damage to tissue membrane and necrosis of hepatocytes after exposing the CCl4. Administration of whole plant ethanol extract of *A. bracteata* at different dose levels (250 and 500 mg/kg) prevented the rise in levels of above serum enzymes. It indicates a possible stabilization of plasma membranes as well as repair of hepatic tissue damages caused by CCl4 exposure.

Diminution of total protein and albumin induced by CCl4 is a further indication of liver damage.29

Administration of whole plant ethanol extract of *A. bracteata* has increased the levels of serum total protein towards the respective normal value, which indicates hepatoprotective activity.

Stimulation of protein synthesis has been advanced as a contributory hepatoprotective mechanism which accelerates the regeneration process and the production of liver cells.30
As a breakdown product of haeme in red blood cells, bilirubin is regarded as a clinical and pathophysiological indicator of necrosis of liver tissues. Pretreatment with *A. bracteata* whole plant extract in different groups (Group III & IV) resulted a significant decrease in total, conjugated and unconjugated bilirubin levels as compared to CCl₄ treated group (Group II).

γ-glutamyl transferase (GGT) is a microsomal enzyme, which is widely distributed in tissue including liver. The activity of serum γ-glutamyl transferase is generally elevated as a result of liver disease, since γ-glutamyl transferase is a hepatic microsomal enzyme. Serum γ-glutamyl transferase is most useful in the diagnosis of liver diseases. Change in γ-glutamyl transferase is parallel to those of amino transferase. The acute damage caused by CCl₄ increased the γ-glutamyl transferase level but the same attains the normal after the treatment with *A. bracteata* whole plant extract due to their antioxidant activity.

Hepatoprotective activity is associated with antioxidant activity, since it is a free radical mediated damage. Elevated level of MDA reflects an enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanisms to prevent formation of excessive free radical. The elevation of MDA level, which is one of the end products of lipid peroxidation in the liver tissue, and the reduction in hepatic GSH levels are important indicators in CCl₄ intoxicated rats. It was ascertained from this study that MDA levels have been suppressed and depletion of GSH by CCl₄ was prevented in the plant drug treated rat groups when compared to CCl₄ intoxicated group.

SOD, CAT enzymes are important scavengers of superoxide ion and hydrogen peroxide. These enzymes protect the cellular constituents from oxidative damage.

In the present study, it was observed that the whole plant ethanol extract of *A. bracteata* significantly increased the SOD and CAT activity in CCl₄ intoxicated rats thereby diminished CCl₄ induced oxidative damage.

Glutathione peroxidase (GPx) is a seleno-enzyme, two third of which (in liver) is present in the cytosol and one third in the mitochondria. It catalyses the reaction of hydroperoxide with reduced glutathione to form glutathione disulphide (GSSG) and the reduction product of the hydroperoxides. In the present study, treatment with whole plant ethanol extract of *A. bracteata* increased the activity of GPx in CCl₄ induced liver damaged rats.

This study finally confirms *A. bracteata* whole plant having hepatoprotective effect on hepatic damage induced by CCl₄. Thus it can be concluded that mechanism of hepatoprotective activity of *A. bracteata* whole plant may be due to its free radical scavenging activity and synergistic effect of phenolics and flavonoids present in the whole plant. The GC-MS analysis of the whole plant of *A. bracteata* confirmed the presence of 9, 12, 15 – Octadecatrienoic acid, methylester (Z, Z, Z) and Vitamin E. The presence of these compounds might be responsible for the hepatoprotective activity.

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