Prevention of carbon tetrachloride-induced hepatotoxicity in rats by *Adhatoda vasica* leaves

Sir,

The plant *Adhatoda vasica* Nees (AV) of the Acanthaceae family has been used for thousands of years in India. Extracts of the leaves of AV are extensively used in cough, asthma, bronchitis, tuberculosis, inflammation and allergy. Several active constituents have also been isolated from different parts of AV. Though the plant is traditionally used in the treatment of jaundice in Bengal, more evidence is needed to substantiate its pharmacological effects.

It is well known that the hepatotoxic effect of carbon tetrachloride is due to the oxidative damage by free radical generation, and antioxidant property is claimed to be one of the mechanisms of hepatoprotective drugs. Therefore, the aim of the present study was to evaluate the antioxidant effect of AV in carbon tetrachloride (CCl₄)-induced hepatotoxicity in rats.

Fresh leaves of AV were air-dried, powdered and soaked in 80% ethanol for 72 h. The extract was then filtered, concentrated under vacuum and lyophilized to obtain a solid mass (6.2%). The chemical constituents of the ethanolic extract were investigated. From preliminary phytochemical analysis it was found that the extract showed positive response for the presence of flavonoids, tannins, alkaloids, reducing sugars and saponins. In atomic absorption spectrophotometric (AAS) analysis, the extract revealed the presence of Mg, Co, Cu, Mn and Cr in trace amounts.

The hepatoprotective study was carried out in adult male Wistar rats (150-175 g). They were housed in clean polypropylene cages and fed with commercial rat chow and water *ad libitum*. Permission from the institutional ethical committee for laboratory use of animals was duly obtained. A total of 30 animals were equally divided into 5 groups (n = 6 in each group). Group I (normal control) and Group II (CCl₄-treated control) were given 0.9% saline (5 ml/kg, b.w.) for 9 days. Group III and Group IV were pretreated with AV (100 and 200 mg/kg, p.o., respectively) for 9 days, while Group V was pretreated with silymarin (25 mg/kg, p.o.) for 9 days. Liver damage was induced in these rats, except Group I, with 1:1 (v/v) mixture of CCl₄ and olive oil (1ml/kg, s.c.) at Day 7 while, olive oil (0.5 ml/kg, s.c.) was injected to Group I. The doses of the test drug were selected on the basis of earlier work in our laboratory. After 48 h of CCl₄ treatment, i.e., on the 9th day the animals were sacrificed under anesthesia and blood was collected for the assay of serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT) and serum alkaline phosphatase (ALP). The livers were removed immediately, washed with ice-cold saline and a 10% homogenate was prepared in phosphate buffer (pH 7.0). The homogenate was centrifuged at 3000 rpm for 15 min at 4°C and the supernatant was used for the estimation of thiobarbituric acid reacting substances (TBARS), superoxide dismutase (SOD), catalase and reduced glutathione (GSH) and protein. The pieces of liver were preserved in 10% formaldehyde solution for histological study. The results were statistically analyzed using one-way analysis of variance (ANOVA) followed by Student-Newman-Keuls test. Values <0.05 were considered significant.

It is well established that CCl₄ is metabolized in the liver to the highly reactive trichloromethyl radical and this free radical leads to auto-oxidation of the fatty acids present in the cytoplasmic membrane phospholipids and causes functional and morphological changes in the cell membrane. This is evi-

### Table 1

**Effect of *Adhatoda vasica* (AV) on serum GOT, GPT and ALP and liver SOD, catalase, GSH and TBARS in CCl₄-induced hepatotoxicity in rats**

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>SGOT (U/L)</th>
<th>SGPT (U/L)</th>
<th>ALP (KA U)</th>
<th>SOD (U)</th>
<th>Catalase (U)</th>
<th>GSH (mM/g tissue)</th>
<th>TBARS (nM MDA/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>42 ± 2.5</td>
<td>56 ± 1.2</td>
<td>35 ± 2.2</td>
<td>1.8 ± 0.1</td>
<td>2.4 ± 0.2</td>
<td>5.2 ± 0.5</td>
<td>3.2 ± 0.02</td>
</tr>
<tr>
<td>II</td>
<td>CCl₄</td>
<td>94 ± 6.8a</td>
<td>103 ± 6.9a</td>
<td>80 ± 4.6a</td>
<td>0.3 ± 0.03</td>
<td>0.8 ± 0.03a</td>
<td>1.2 ± 0.09</td>
<td>5.6 ± 0.06a</td>
</tr>
<tr>
<td>III</td>
<td>AV (100 mg/kg) + CCl₄</td>
<td>72 ± 5.2a,b</td>
<td>82 ± 8.7a,b</td>
<td>66 ± 8.2a,b</td>
<td>0.5 ± 0.02b</td>
<td>1.6 ± 0.03b,a</td>
<td>2.8 ± 0.03b</td>
<td>4.1 ± 0.03b,a</td>
</tr>
<tr>
<td>IV</td>
<td>AV (200 mg/kg) + CCl₄</td>
<td>56 ± 4.9a,c</td>
<td>66 ± 6.4a,c</td>
<td>49 ± 5.9a,c</td>
<td>1.1 ± 0.03b,c,a</td>
<td>2.2 ± 0.08b,c,a</td>
<td>3.4 ± 0.08b,c,a</td>
<td>3.7 ± 0.02b,c,a</td>
</tr>
<tr>
<td>V</td>
<td>Silymarin (25 mg/kg) + CCl₄</td>
<td>54 ± 5.6a,c</td>
<td>60 ± 5.8a,c</td>
<td>41 ± 4.6a,c</td>
<td>1.5 ± 0.02b,c,a</td>
<td>3.9 ± 0.03b,c,a</td>
<td>3.1 ± 0.02b,c,a</td>
<td>3.5 ± 0.01b,c,a</td>
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<tr>
<td></td>
<td>One-way</td>
<td>78 ± 5.08</td>
<td>94 ± 8.95</td>
<td>76 ± 4.95</td>
<td>2.1 ± 0.02</td>
<td>3.0 ± 0.03</td>
<td>4.2 ± 0.05</td>
<td>6.7 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>ANOVA</td>
<td>3.20</td>
<td>3.20</td>
<td>3.20</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
</tr>
</tbody>
</table>

*Values are mean±SEM; n=6 rats in each group; Statistical analysis by one-way ANOVA followed by Student-Newman-Keuls test; *P* values <0.01 as compared to Group I; *a* as compared to Group II and *b* as compared to Group III; CCl₄ was administered s.c. and other drugs were given orally.*
denced by an elevation of the serum marker enzymes namely SGOT, SGPT and ALP in CCl₄ treated rats. Pretreatment with the test drug AV in both doses as well as pretreatment with standard drug silymarin significantly (P<0.01) reduced these liver enzyme levels dose dependently, showing that AV has hepatoprotective action (Table 1). Histopathological findings indicated that pretreatment with AV (100 and 200 mg/kg) offered protection to the hepatocytes from damage induced by CCl₄, with mild fatty changes in the hepatic parenchymal cells, which corroborated the changes observed in the hepatic enzymes.

In our earlier work, it was reported that the antioxidant activity or inhibition of the generation of free radicals is important in the protection against CCl₄-induced liver lesion. At present, a significant elevation in the levels of end products of lipid peroxidation or MDA in the liver of rats treated with CCl₄ was observed when compared to normal control (Table 1). Pretreatment with AV (100 and 200 mg/kg) as well as silymarin significantly (P<0.01) reversed these changes. AV also enhanced significantly (P<0.01) the protective enzymes, SOD and catalase when examined in the liver homogenate. SOD and catalase act as protective enzymes against lipid peroxidation in liver tissues. In the present study, CCl₄ further depleted GSH concentration in the liver of rats (Table 1), whereas, AV as also silymarin pretreatment reversed this effect (P<0.01). Flavonoids, tannins and microelements have been suggested to act as antioxidants and exert their antioxidant activity by scavenging the lipid peroxidation. Therefore, the present work provides conclusive evidence for the hepatoprotective effect of AV against carbon tetrachloride-induced hepatotoxicity. The plausible mechanism of the hepatoprotective action of AV might be at least partly due to its antioxidant effect.

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References


Pharmaceutical drug advertisements in national and international journals

Sir,

Recent advances in research and clinical experiences keep on changing the drug therapy for many diseases. Therefore, scientific information on new drugs and therapeutics is undoubtedly of paramount importance. Industry drug advertisements are intended to be persuasive rather than educational and it is not meant for educating the physicians in the use of drugs. However, a busy doctor unable to find time to update himself may start relying on drug advertisements. The drug advertisements in journals usually give inadequate and substandard information. Although this aspect had been explored in the past by many authors, in the present study, a retrospective comparative evaluation was carried out to analyze the drug information provided in pharmaceutical advertisements in national and international journals. For this purpose 54 advertisements from 50 issues of national journals and 50 advertisements from 50 issues of international (USA and UK) journals were selected and reviewed. The list of titles of national and international journals from which these advertisements were selected is shown in Table 1. The journals selected were for the years 2001 to 2003 and included those from medicine and surgery. Only new drug advertisements were included while reminder/repeat advertisements were excluded in the present study. The information was collected using the format of drug information sheets suggested by the WHO’s ethical criteria for medical drug promotion, that included details about the brand name/non-proprietary name, pharmacological data, clinical information, pharmaceutical information and legal aspects. The percentage of advertisements providing information about each aspect was worked out. The data obtained from national and international journals were compared using the Chi-square test and P<0.05 was considered significant. The results are shown in Table 2.

The present study indicated differences in the drug information provided in the advertisements published by national and international journals. The advertisements in international journals provided more complete drug information as per the recommendation laid down by WHO in comparison to national journals. However, they were found to be deficient in providing information regarding some of the aspects like pharmacological effects (12%), mechanism of action (16%) and pharmacokinetic data (8%). Whereas, the information in the advertisements of the national journals was inadequate in nearly all aspects of pharmacological data, clinical information (0%), pharmaceutical information (0-33.3%) and legal aspects (0-11.1%). The main stress in national journals ap-