HEPATOPROTective activity of Eclipta Alba Hassk. AGAINST PARACetAMOL INDUCed HEpATOCellular DAMAGE IN MICE.

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ABSTRACT
The effect of Eclipta alba (EA) extract was studied on paracetamol induced hepatic damage in mice. Treatment with 50% ethanol extract of E. alba (100 & 250 mg/100 g body weight) was found to protect the mice from hepato-toxic action of paracetamol as evidenced by significant reduction in the elevated serum transaminase levels. Histopathological studies showed marked reduction in fatty degeneration and centrilobular necrosis in animals receiving different doses of E. alba along with paracetamol as compared to the control group.

The mice administered Liv-52, used for comparative evaluation, showed a significant reduction in serum enzyme activity and normal livers. It is stipulated that the extract treated groups were partially protected from hepatocellular damage caused by paracetamol.

JK - Practitioner 2004; 11(4):278-280

INTRODUCTION:
Eclipta alba Hassk. (Bhringaraja, Fam : Compositae) is a perennial shrub which grows widely in moist tropical countries. Different uses have been reported for this shrub. It is used as alterative, anthelmintic, expectorant, antipyretic, antiasthmatic, tonic, deobstruent in hepatic and spleen enlargement, in skin diseases and as a substitute for Taraxacum (a popular liver tonic)1,2. It is good for the diseases of spleen, stomatitis, toothache, hemicrania, fever, pain in liver and cures vertigo (Yunani). Its juice in combination with honey is administered for Catarrh and Jaundice1 (Chopra, 1996). In Gujarat district and Punjab, it is used externally for ulcers and as an antiseptic for wounds in cattle. Recently Chandra, 19873, have observed a significant anti-inflammatory activity of the powder in rats. It has been reported to be useful in liver ailments4 (Handa, 1986) & has been shown to possess hepatoprotective activity against carbon-tetrachloride induced liver cell damage in animals5. But systematic research on any possible effect of E-alba on paracetamol induced hepatotoxicity seems to be scarce. In the present study, 50% ethanol extract of the whole plant was screened for hepatoprotective activity in mice using paracetamol as hepatotoxin. Liv-52 syrup was used for comparative evaluation.

MATERIAL AND METHODS
Eclipta alba (EA) was purchased from a local market in Delhi and identified at the Central Council of Research in Unani medicine. The coarsely powdered drug was extracted with 50% ethanol by cold maceration (I.P 1985)6. The solvent was removed at low temperature & reduced pressure and extract stored in a refrigerator prior to pharmacological studies. The extract yield was 13.2% (w/w) in terms of starting material. The dried mass of the extract was macerated with 1% gum acacia & suspended in distilled water to be given orally with a catheter (EA 100 & 250 mg/100 g).

Animals and exposure conditions
Healthy albino mice (15 to 30 g) of either sex were housed under uniform husbandry conditions and given pelleted diet (Lipton's India Ltd.) and water ad libitum. The animals were housed at a temperature of 25 C ± 1 C with a 12 hr light /dark cycle.

Paracetamol induced hepatocellular damage.
Mice were randomly divided into five groups of 8 mice each. Group I served as normal control and received 1% gum acacia suspension only. All other groups received paracetamol once (500 mg/kg, p.o, aqueous solution)7 with Group II serving as paracetamol treated control. 48 hr. after paracetamol administration, groups III & IV also received EA extract 100& 250 mg/100 g respectively & Group V Liv-52 syrup (Himalaya Drug Company, India; 2ml/100g) p.o, once daily for 5 consecutive days. 16 hr. after administration of last dose of drugs, animals were anaesthetized with Ketamine & Xylazine and sacrificed.
ether and blood was collected from the retino-bulbar venous plexus and serum was separated by centrifugation. The serum was then estimated for alanine amino-transferase (ALT) levels, which has been shown to be elevated by paracetamol. Mice were killed, livers excised, rinsed clean in saline and preserved in 10% formalin for histopathological study [using 5 micrometer thick sections stained with haematoxylin eosin, (H&E)]. Results were statistically analysed using student's 't' test.

RESULTS

Table shows that serum ALT levels were significantly higher in animals receiving paracetamol and reduced significantly in III & IV Groups which received paracetamol & EA. The values returned to normal in animals of Group IV receiving 250mg/100g/day EA with paracetamol. Liv-52, which was used for comparative evaluation, produced a highly significant (p< 0.01) fall in the enzyme levels. Histopathological studies revealed centrilobular and focal necrosis and ballooning in livers of mice challenged with paracetamol (Fig 1). But only mild ballooning with sinusoidal dilatation and binucleate cells was observed in Group III, treated with EA (100mg/100g per day) (Fig2) while binucleate cells spread throughout the liver sections and slight fatty changes were observed at the dose of 250mg/100g/day of EA (Fig3). 50% of the livers in Group IV were normal. The livers treated with Liv-52 syrup appeared to be normal as in Group I (Fig 4).

DISCUSSION

Paracetamol causes acute centrilobular hepatic necrosis in rats, mice, guinea pigs, hamsters, rabbits, cats, dogs and pigs. Mice and hamsters are very sensitive while rat is very resistant & in present study, mice were chosen. Only serum ALT levels were estimated as it is the more specific index of liver cell damage. Centrilobular haemorrhagic necrosis is the characteristic lesion in humans and in experimental animals. In the present investigation it was observed that serum ALT levels were significantly reduced in animals receiving EA and paracetamol than those given paracetamol alone indicating that the degree of hepatic cell damage was of
lesser magnitude in treated Groups. EA (100 and 250mg/100gm/day) has been found to reduce serum ALT levels in a dose dependent manner (18.4% and 31.18% fall, respectively) and with the latter dose the values returned to normal levels (Table).

The attributivity of the observed alterations of serum ALT levels to hepatic damage on health was confirmed by histopathological studies of liver which have shown that livers challenged with paracetamol have centrizonal necrosis, focal necrosis and ballooning. In animals treated with EA there was no noticeable hepatocellular necrosis. Only mild ballooning & binucleate cells were observed in animals treated with 100mg /g /day of EA while binuclear cells were seen spread all over at the dose of 250mg/100g /day of EA (Fig 2 &3). Binucleate cells in liver are indication of hepatic cell regeneration.

These observations point towards a hepatoprotective activity of EA in the experimental model. Liv-52 syrup, claimed to be a hepatoprotective agent was found to be more effective as hepatoprotective compared to EA as evidenced by both biochemical (38% fall in ALT levels) and histopathological (normal livers) studies.

The results of this study are in corroboration with the earlier reports on the hepatoprotective activity of EA against allyl alcohol induced hepatic necrosis in rats and carbon-tetrachloride induced hepatotoxicity in guinea pigs and rationalise its use as a constituent of various herbal hepatoprotective formulations.

BIBLIOGRAPHY

EFFECT OF 50% ETHANOLIC EXTRACT OF E. ALBA AND LIV-52 ON ALANINE AMINO TRANSFERASE (ALT) LEVELS AGAINST PARACETAMOL INDUCED LIVER CELL DAMAGE IN MICE.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>ALT LEVELS I/U/L</th>
<th>STATISTICALLY Compared groups</th>
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<tbody>
<tr>
<td>I. 1% gum acacia Control</td>
<td>35.09±3.91 (n = 6)</td>
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<tr>
<td>II. Paracetamol (500 mg/kg)</td>
<td>50.31±6.81 (n = 6)</td>
<td>II Vs I p &gt; 0.05</td>
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<tr>
<td>III. Paracetamol (500 mg/kg) + Liv-52 (2ml / 100g/day)</td>
<td>31.82±2.78* (n = 6)</td>
<td>III Vs II p &lt; 0.05</td>
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<tr>
<td>IV. Paracetamol (500 mg/kg) + E.alba (100mg/100g/day)</td>
<td>41.01±6.82 (n = 6)</td>
<td>IV Vs II p &gt; 0.05</td>
</tr>
<tr>
<td>V. Paracetamol (500 mg/kg) + E.alba (250mg/100g/day)</td>
<td>34.62±3.86 (n = 5)</td>
<td>V Vs II p &gt; 0.05</td>
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Paracetamol administered in a single oral dose of 500mg/Kg 48 hours before administration of E.ALB A and LIV-52.