EVALUATION OF HEPATOPROTECTIVE EFFECT OF HUGONIA MYSTAX LEAVES IN CARBON TETRACHLORIDE INDUCED LIVER DAMAGE IN RATS

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Abstract

The investigation was aimed to evaluate the hepatoprotective activity of ethanol extract of Hugonia mystax leaves (HMEE) against CCl4 induced liver damage in rats by determining different in vivo antioxidant effect (tissue GSH and lipid peroxidation), physical (wet liver weight and liver volume) and biochemical parameters (SGOT, SGPT, ALP and Bilirubin). The extract at the dose of 200mg/kg and 400 mg/kg produced significant effect by decreasing the activity of biochemical parameters (SGOT, SGPT, ALP and bilirubin) and tissue lipid peroxidation while it significantly increased levels of tissue GSH in a dose dependent manner. Histopathology observation and physical parameters (wet liver weight and liver volume) also confirm these findings. The effects of extract were comparable to that of standard drug silymarin. These results suggest that HMEE possess hepatoprotective activity against CCl4 induced liver damage in rats.

Key words : Hugonia mystax, hepatoprotective, carbon tetrachloride.

Introduction

Liver plays a major role in intense metabolic activities like detoxification and excretion of many exogenous and endogenous compounds (Reddy et al., 1993). Liver toxicity is a major health problem of worldwide proportions. Modern medicine has little to offer to alleviate hepatic diseases and there are not many drugs available to treat liver disorders. Herbal drugs are playing an important role in health care programmes worldwide and different vegetal species have been described for providing active principles that protect against liver damage in experimental animal models (Liu et al., 1993, 1995 and Scott, 1999).

Hugonia mystax, family Linaceae is a rambling scandent scrub with yellow tomentose twigs and branchlets horizontal provided with a pair of strong hooks. Leaves are simple, alternate, elliptic-obovate glabrous and penninerved (Kirtikar and Basu, 1999). Literature review mentioned that the roots are astringent, bitter, sweet, febrifuge and anthelmintic. They are useful in fevers, verminosis and vitiated conditions of vata, externally as a paste for inflammation (Vaidyaratnum, 1995). Bark of the root is also employed as an antidote to poison (Nadkami, 2002). The modern literature revealed that the plant is reported to possess antimicrobial activity (Vimalavadhy et al., 2012), anti-inflammatory activity (Rajeswari et al., 2013), in vitro cytotoxic effect (Anandakumar et al., 2011), in vitro anthelmintic activity (Mohankumar et al., 2015).

Preliminary phytochemicals analysis of HMEE revealed the presence of flavonoids, tannins and saponins. There are reports that the polyphenolic compounds are possessing antioxidant and hepatoprotective effects (Tiwari, 2001). Hence, the objectives of the present investigation were to study the effect of ethanol extract of Hugonia mystax leaves (HMEE) on physical, biochemical, in vivo antioxidants and histopathological parameters against CCl4 induced liver damage in rats.

Materials and Methods

Plant material

The leaves of plant Hugonia mystax were collected from fields of Tirupati, Andhra Pradesh, India. It was identified and authenticated by Dr. K. Madhava Chetty,
plant taxonomist, Department of Botany, Sri Venkateswara University, Tirupati, Andhra Pradesh (India). A herbarium specimen was preserved in the college herbal museum. The leaves were shade dried at room temperature and pulverized. The ethanol extract was prepared by using 70% ethanol in a soxhlet apparatus after de-fatting with petroleum ether and chloroform. Preliminary phytochemical investigation showed the presence of flavonoid, tannin and saponins in 70% ethanol extract of *Hugonia mystax* leaves (HMEE). So, HMEE was selected for the study of hepatoprotective activity.

**Animals**

Wistar albino rats (150-220 g) and mice (18-25 g) of either sex were used for the study. Approval from the institutional animal ethical committee (1555/PO/a/11/ CPCSEA) for usage of animal in the experiment was obtained as per the Indian CPCSEA guidelines.

**Acute toxicity studies**

The acute toxicity was determined on albino mice by fixed dose method of OECD Guide line no 423 given by CPCSEA.

**CCl₄ induced hepatotoxicity** (Pradeep *et al.*, 2005 and Suja *et al.*, 2004)

Healthy wistar albino rats were divided into 5 groups of 6 animals each. Group-I and Group II received distilled water (1 ml/kg) for 5 days. Group III received 100 mg/kg silymarin (standard drug) orally for 5 days. Group IV and Group V received 200 mg/kg and 400 mg/kg HMEE (orally), respectively for 5 days. Group-I received liquid paraffin (1ml/kg) s.c., on 2nd and 3rd day. Group-II, III, IV and V received CCl₄; liquid paraffin (1:1) at a dose of 2ml/kg s.c., on 2nd and 3rd day, after 30 min of vehicle, 100 mg/kg silymarin, 200 mg/kg and 400 mg/kg of HMEE administration. On 6th day, blood samples were collected by retro-orbital plexus method. Rats were sacrificed by cervical dislocation under mild ether anaesthesia and liver tissue was collected.

**Biochemical studies**

The blood was obtained from all animals by puncturing retro-orbital plexus. Collected blood was centrifuged (2000 rpm for 10 mins) to get clear serum and various biochemical studies like SGPT (Bradley *et al.*, 2003), SGOT (Rej *et al.*, 1973), ALP (McComb *et al.*, 1972), Bilirubin (total and direct), (Pearlman *et al.*, 1974) were estimated.

**Histopathology**

The liver was dissected out and stored in 10% formalin solution. The liver was processed for histopathological investigations.

**In vivo tissue GSH estimation**

Tissue Glutathione (GSH) measurements were performed using the modification of Ellamn procedure (Aykae *et al.*, 1985). Liver tissue samples were homogenized in ice cold trichloroacetic acid (1gm tissue in 10 ml 10% TCA) in an ultra trux tissue homogenizer. The mixture was centrifuged at 3000 rpm for 10 mins. Then 0.5 ml of supernatant was added to 2ml of (0.3M) disodium hydrogen phosphate solution. A 0.2 ml solution of dithiobisnitrobenzoate (0.4mg/ml in 1% sodium acetate) was added and absorbance was measured at 412 nm.

**In vivo lipid peroxidation estimation**

The degree of lipid peroxide formation was assayed by monitoring thiobarbituric reactive substance formation (Buege *et al.*, 1978). Take 1.0 ml of biological sample (0.1-2.0 mg of membrane protein or 0.1-2.0 µmol of lipid phosphate) to this adds 2.0 ml of TCA-TBA-HCL solution and mixed thoroughly. Solution was heated for 1 hr and cooled. Then precipitate was removed by centrifugation at 1000 rpm for 10 mins and absorbance of sample was determined at 535 nm against a blank that contain all the reagents minus lipid.

**Statistical analysis**

Results were expressed as mean ± SEM (n = 6). Statistical analysis was performed with one way ANOVA followed by Turkey-Kramer multiple comparisons test. P values less than 0.05 was considered to be statistically significant (p<0.05).

**Results**

**Acute toxicity**

The acute toxicity studies showed that the HMEE is safe at 2000 mg/kg. Therefore as per CPCSEA guideline 423, 1/10th (200 mg/kg) and 1/5th dose (400 mg/kg) were selected for further hepatoprotective studies.

**In vivo GSH**

There was a marked depletion of GSH level in CCl₄ treated group. Silymarin 100 mg/kg increased tissue GSH by 96.81%. Treatment with HMEE showed dose dependent increase in the levels of GSH. However, both doses of HMEE have shown lesser increase GSH level than standard silymarin (fig. 3).

**In vivo lipid per oxidation**

CCl₄ enhanced the lipid peroxidation. The treatment with HMEE significantly reduced the lipid per-oxidation in a dose dependant manner. Silymarin 100 mg/kg showed 50.10% inhibition, whereas 400 mg/kg of HMEE showed 47.40% inhibition, which was almost near to standard silymarin (fig. 4).
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Increased levels of liver weight (6.03 gm/100gm) (fig. 2), liver volume (5.85 ml/100gm) (fig. 1), SGPT (312.6 IU/l), SGOT (423.51 IU/l), ALP (452.03 IU/l), total bilirubin (2.48 mg/dl) and direct bilirubin (1.33 mg/dl) was observed in CCl$_4$ treated group. The pretreatment with HMEE (200mg/kg and 400mg/kg p.o.) has brought back the elevated levels of biomarker enzymes of hepatitis in a dose dependant manner (table 1). Treatment with 400 mg/kg of HMEE produced the hepatoprotective activity comparable to that of silymarin 100 mg/kg p.o.

### Histopathology

CCl$_4$ treatment showed congested sinusoids and extensive degeneration of hepatocytes. Treatment with HMEE had shown dose dependant improvement in the liver architecture as indicated by the histopathological observations that there was mild congested sinusoids and fatty change to a lesser extent.

### Table 1: Effects of HMEE on biochemical markers in CCl$_4$ induced hepatotoxicity in rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>SGOT IU/l</th>
<th>SGPT IU/l</th>
<th>ALP IU/l</th>
<th>Total bilirubin (mg/dl)</th>
<th>Direct bilirubin (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control (1ml dist. Water p.o.)</td>
<td>143.8 ± 11.03</td>
<td>53.98 ± 3.89</td>
<td>172.28 ± 12.135</td>
<td>0.74 ± 0.067</td>
<td>0.19 ± 0.04</td>
</tr>
<tr>
<td>Intoxicated control CCl$_4$ + Liq. Paraffin (1:1) (2 ml/kg s.c.)</td>
<td>423.51 ± 5.33</td>
<td>312.6 ± 9.67</td>
<td>452.03 ± 23.928</td>
<td>2.48 ± 0.16</td>
<td>1.33 ± 0.1294</td>
</tr>
<tr>
<td>CCl$_4$ + Standard (Silymarin) (2 ml/kg s.c.+100 mg/kg p.o.)</td>
<td>166.1 ± 7.02***</td>
<td>56.80 ± 5.81***</td>
<td>195.36 ± 10.34***</td>
<td>1.07 ± 0.08***</td>
<td>0.244 ± 0.031***</td>
</tr>
<tr>
<td>CCl$_4$+HMEE (2 ml/kg s.c.+200 mg/kg p.o.)</td>
<td>365.75 ± 11.878**</td>
<td>235± 22.51**</td>
<td>269± 17.71***</td>
<td>1.54 ± 0.079***</td>
<td>0.34 ± 0.013***</td>
</tr>
<tr>
<td>CCl$_4$+ HMEE (2 ml/kg s.c + 400 mg/kg.p.o.)</td>
<td>351.5 ± 22.583*</td>
<td>219± 23.140**</td>
<td>203.17± 3.198***</td>
<td>1.495 ± 0.035***</td>
<td>0.245 ± 0.129***</td>
</tr>
</tbody>
</table>

Values are the mean ± S.E.M. of six rats/treatment. Significance ***P<0.001, **P<0.01, *P<0.05, nsP>0.05, compared to CCl$_4$ treatment.
The results of physical, biochemical, in vivo antioxidant and histopathological studies revealed that the HMEE possess hepatoprotective activity against CCl₄ induced hepatotoxicity in albino rats.

**Discussion**

Subcutaneous administration of CCl₄ for two days elevated the SGPT, SGOT, ALP, total and direct bilirubin. These findings of intoxicated control are in conformity with the earlier reports (Singh et al., 1998). Pretreatment with HMEE (200 mg/kg and 400 mg/kg p.o.) for 5 days significantly reduced the elevated biochemical markers in a dose dependent manner. Treatment with 400 mg/kg of HMEE produced the hepatoprotective activity comparable to that of silymarin 100 mg/kg p.o.

CCl₄ is metabolized to trichloromethyl CCl₃⁺ radical due to the catalytic activity of CYP 450 2E₁ enzyme,
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which is further converted to trichloromethyl peroxide radical. This trichloro methyl peroxide radical is the main culprit in causing hepatotoxicity. This particular radical forms a covalent bond with sulphhydryl group of membrane GSH, protein thiols and unsaturated fats or lipids. This covalent bonding of free radicals with cellular macro molecules initiates the cascade of reactions leading to lipid peroxidation (Kyung et al., 2004; Jeong et al., 1999 and Wang et al., 2004). The lipid peroxidation inturn alter the membrane permeability and initiates chain of reaction leading to tissue damage and necrosis.

It was observed that the pretreatment with HMEE may be preventing the formation of trichloro methyl peroxo radical. It may be due to tissue GSH levels were not depleted and lipid peroxidation was minimized, this may be the possible mechanism of hepatoprotective offered by HMEE. However, our studies do not confirm whether test extract block CYP 450 2E1 enzyme and thereby inhibit the formation trichloromethyl CCl$_3^\cdot$ radical.

In conclusion, the present study demonstrates that HMEE possess in vivo antioxidant and hepatoprotective activity. In addition, the hepatoprotective property may be attributed to the polyphenolic compounds of plant, namely tannins, flavonoids and saponins. Further investigation is going on to isolate, characterize and screen the active principles that possess antioxidant and hepatoprotective property.

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**References**


