Hepato-Protective activity of the aqueous extract of *Launaea intybacea* (Jacq) Beauv against carbon tetrachloride-induced hepatic injury in Albino Rats

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ABSTRACT

The present study was conducted to evaluate the hepato-protective activity of aqueous extract of *Launaea intybacea* in carbon tetrachloride -induced hepatic injury in albino rats. Silymarin (200mg/kg) was given as reference standard. The hepatic injury of CCl4 and the hepatoprotective effect of aqueous extract of *Launaea intybacea* were estimated by their liver function tests. The aqueous extract of aerial parts of *Launaea intybacea* have shown very significant hepatoprotection against carbon tetrachloride -induced hepatic injury in albino rats in reducing serum total bilirubin, SALP, SGPT, SGOT levels and liver homogenates LPO, SOD, CAT, GPX, GST and GSH levels.

Key words: *Launaea intybacea*, hepatic injury, carbon tetrachloride and silymarin.

INTRODUCTION

The liver is one of the few organs of highly specialized function whose cells can undergo an astonishing degree of regeneration. Modern allopathic treatment does not hold promise to cure liver disease perfectly. A number of medicinal plants are used in traditional system of medicine for the management of liver disorders.1 However, many of them have not investigated for their described effects. *Launaea Intybacea* is one such medicinal plant used in the treatment of liver disorders in folk medicine. The plant *launaea intybacea* (Jacq) Beauv. is used against jaundice, hepatomegaly, dyspepsia, skin disease, dry cough and galactoriya.2, 3 The present study was conducted to evaluate the hepatoprotective activity of *Launaea Intybacea* plant powder aqueous extract against liver disorders induced by carbon tetrachloride in wistar albino rats. Plants extracts have been used by traditional medicinal practitioners for the treatment of liver disorders for centuries.4 The extract of plant powder were administered orally to the animals. Various biochemical parameters were studied to evaluate the hepato protective activity of aqueous extract. Serum bilirubin, serum alkaline phosphate, serum glutamic oxaloacetic transaminase and serum glutamate pyruvate transaminase and liver homogenate superoxide dismutase, catalase, glutathione peroxidase, lipid peroxidation, glutathione-reduced and glutathione-transferase were determined to assess the effect of the aqueous extract the carbon tetrachloride induced liver disorders. Liver damage is always associated with cellular necrosis, increase in tissue lipid peroxidation and depletion in the tissue GSH levels. In addition, serum levels of many biochemical markers like SGOT, SGPT, cholesterol, bilirubin, alkaline phosphate are elevated.5,6 The study revealed that aqueous extract significantly reduced serum bilirubin, SGOT, SGPT and SALP levels and liver homogenates LPO, SOD, CAT, GPX, GST and increases GSH levels. The present finding suggest that the plant *launaea intybacea* possess potential hepatoprotective activity. The alkaloids, saponins, tannins and phenolic compounds are responsible for the hepatoprotective activity. The present study was undertaken to evaluate the hepatoprotective ability of *launaea intybacea* plant in experimental
animals and scientifically validated the traditional use for liver disorders.

MATERIALS AND METHODS

Plant material
The plant material used in this study was collected during month of Oct-Nov. in Akole Dist-Ahmednagar(MH), India and authenticated from Department of Botanical Survey of India, Pune (India).

Preparation of the Extracts
The plants were washed thoroughly with tap water and air dried in shade at room temperature. They were mechanically powdered and sieved. The aqueous extract was prepared by cold maceration (72 h.). The liquid extract obtained was concentrated in vacuum at 40°C. The yield of extract was 30.12% and the extract was phytochemically investigated.

Animals
Albino rats (either sex) of Sprague dawley strain, weighing 150-200g were used. The animals were acclimatized to laboratory conditions (RT-25°C) for 4 days and given pelleted animal feed (Hindustan Lever) and drinking water. Diagnostic reagent kits (Enzopak) were used for the estimation of serum SALP, SGPT and SGOT levels and assay procedure was used for the estimation of liver homogenates LPO, SOD, CAT, GPX, GST and GSH.

Toxicity studies
The WHO has set guidelines for toxicity studies of herbal medicine. It supports appropriate usage of herbal medicines and encourage the remedies, which are proved to safe and efficacy. Acute toxicity study was performed for aqueous extract according to the acute toxic classic method as per OECD guidelines, albino rats were used for acute toxicity study. The animals were kept fasting for overnight providing only water, after which the extract was administered orally at the dose of 100, 200 and 400 mg/kg and observed for 16 days. If mortality was observed in 2 out of 3 animals, then the dose administered was assigned as toxic dose. If the mortality was observed in animal, then the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for further higher dose i.e., 400 mg/kg.

Hepatoprotective Activity
The animals were divided into four groups comprising of six albino rats in each group using randomization technique and treated with the extract for sixteen days to assess the hepato-protective potential of the plant. The first group (vehicle control) received vehicle for all the days. The second group was kept as toxin control and given only the carbon tetrachloride treatment. The third group received aqueous extract in the dose of 200mg/kg p.o. and the forth group received the silymarin in the dose of 200mg/kg p.o. as a reference material for the study. All the animals except the vehicle control received carbon tetrachloride all 16th day of the treatment. The animals were sacrificed by cervical dislocation after 48 hours of carbon tetrachloride administration. The blood samples were collected by cardiac puncture in heparinized microfuge tubes. The blood samples thus collected were immediately centrifuged at 2200rpm for 15 minutes. When serum clearly separated out, the serum was analyzed for SGPT, SGOT and SALP levels using enzopak reagent kits by the method proposed by Reitman and Frankel. The results thus obtained were subjected to statistical analysis using student t-test and analysis of variance. The livers were dissected out immediately, washed with ice cold saline and 10% homogenates in 1.15%(w/v) KCl were prepared. The homogenates were centrifuged at 7000xg for...
10 min at 4°C and the supernatants were used for the assays of LPO, SOD, CAT, GPX, GST and GSH. The results thus obtained were subjected to statistical analysis using student t-test and analysis of variance.10,11 (Table: 1 and Table: 2)

RESULTS AND DISCUSSION

The present studies were performed to assess the hepatoprotective activity in rats against carbon tetrachloride as hepatotoxin to prove its claims in folklore practice against liver disorders. A number of reports indicates that overdose of carbon tetrachloride can produce centrilobular hemorrhagic hepatic necrosis in humans and experimental animals.12,13 Carbon tetrachloride -induced hepatic injury is commonly used as an experimental method for the study of hepatoprotective effects of medicinal plants extracts and drugs. The extent of hepatic damage is assessed by histological evaluation and the level of various biochemical parameters in circulation. CCl₄ undergoes hepatic metabolism to give rise to trichloro methyl radicals, which upon reacting with reactive oxygen species yields trichloromethyl peroxide radicals, which forms covalent bond with membrane lipids and destroy the membrane integrity. It produces hepatotoxicity by altering liver microsomal membranes in experimental animals. From the Table 1 it was evident that extract was able to reduce all the elevated biochemical parameters due to the hepatotoxin intoxication. The levels of total proteins and albumin were reduced due to the carbon tetrachloride induced hepatotoxicity. The reduction is attributed to the initial damage produced and localized in the endoplasmic reticulum which results in the loss of P450 leading to its functional failure with a decrease in protein synthesis and accumulation of triglycerides leading to fatty liver. Reduction in the levels of SB, SALP, SGOT and SGPT towards the normal value is an indication of regeneration process. The protein and albumin levels were also raised suggesting the stabilization of endoplasmic reticulum leading to protein synthesis. The protective effect exhibited by extracted at dose level of 200 mg/kg was comparable with the standard drug silymarin. The histological examination of the liver sections reveals that the normal liver architecture was disturbed by hepatotoxin intoxication.

Table: 1 Effect of aqueous extract of *Launaea intybalcea* aerial parts on carbon tetrachloride-induced hepatotoxicity (Serum parameters).

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Groups</th>
<th>Total Bilirubin* (mg/dl)</th>
<th>SALP (Units/ml)*</th>
<th>SGPT (Units/ml)*</th>
<th>SGOT (Units/ml)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Control (propyle glycol) 1 ml</td>
<td>0.74 ± 0.06</td>
<td>233.12±1.21</td>
<td>79.21 ± 1.12</td>
<td>193.21 ± 1.20</td>
</tr>
<tr>
<td>2.</td>
<td>Carbon tetrachloride 3 ml / Kg</td>
<td>2.30± 0.05</td>
<td>426.13±1.32</td>
<td>354.50±1.10</td>
<td>330.24± 1.39</td>
</tr>
<tr>
<td>3.</td>
<td>Aqueous Extract (200mg/kg)</td>
<td>0.71± 0.12</td>
<td>231.03±18.13</td>
<td>78.78± 3.3</td>
<td>192.06± 1.07</td>
</tr>
<tr>
<td>4.</td>
<td>Silymarin (200mg/kg)</td>
<td>0.79± 0.04</td>
<td>230.05±14.30</td>
<td>79.31± 33.43</td>
<td>193.35 ±11.01</td>
</tr>
</tbody>
</table>

* Values of mean ± S.E.M. (n=6)
Table: 2 Effect of aqueous extract of *Launaea intybacea* aerial parts on carbon tetrachloride-induced hepatotoxicity (Liver homogenates)

<table>
<thead>
<tr>
<th>Groups</th>
<th>LPO nmoles/mg of protein</th>
<th>SOD Units/mg of protein</th>
<th>CAT Units/mg of protein</th>
<th>GPX (µg/mg)</th>
<th>GST µg/mg of protein</th>
<th>GSH µg/mg of protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.42 ± 0.5</td>
<td>131.4 ± 1.2</td>
<td>35.6 ± 1.2</td>
<td>4.36 ±0.03</td>
<td>2.06 ± 0.12</td>
<td>0.45 ± 0.07</td>
</tr>
<tr>
<td>Propylene glycol (1 ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carbon tetrachloride (3 ml/Kg)</td>
<td>5.05 ± 1.53</td>
<td>35.28 ± 1.19</td>
<td>21.42 ± 0.3</td>
<td>1.22 ±0.02</td>
<td>0.23 ± 0.02</td>
<td>0.12 ± 0.32</td>
</tr>
<tr>
<td>Water extract (200mg/kg)</td>
<td>0.42 ± 1.65</td>
<td>133.18 ± 1.35</td>
<td>35.40 ± 0.25</td>
<td>4.24 ±0.06</td>
<td>2.0 ± 0.04</td>
<td>0.45 ± 0.03</td>
</tr>
<tr>
<td>Silymarin (200mg/kg)</td>
<td>0.44 ± 0.01</td>
<td>131.32 ± 1.63</td>
<td>36.01 ± 2.5</td>
<td>4.32 ±0.01</td>
<td>2.01 ± 0.11</td>
<td>0.45 ± 0.01</td>
</tr>
</tbody>
</table>

*a Values of mean ± S.E.M. (n=6)*

In the liver sections of the rats treated with extracted and intoxicated with carbon tetrachloride, rats treated with aqueous extract and intoxicated with carbon tetrachloride, the normal cellular architecture was retained as compared to silymarin, there by confirming the protective effect of the extract. In accordance with these results, it may be hypothesized that tannin, saponins and flavonoids, which are present in extracts, could be considered responsible for the hepatoprotective activity.

The aqueous extract of aerial parts of *Launaea intybacea* (Jacq) Beauv have shown very significant hepatoprotection against carbon tetrachloride-induced hepatotoxicity in albino rats in reducing serum total bilirubin, SALP, SGPT and SGOT levels. It is also found that treatment with aqueous extract of plant have brought down the elevated level of LPO and also significantly enhanced the reduced levels of SOD, CAT, GPX, GST and GSH. Liver section of *Launaea intybacea* treated animal group clearly showed normal hepatic cells and central vein thereby confirming hepatoprotective activity. In conclusion the aqueous extract of *Launaea intybacea* could be an important source of hepatoprotective compounds.

REFERENCES: