PROTECTIVE EFFECT OF ETHANOL EXTRACT OF CENTAUREA BEHEN LINN IN CARBON TETRA CHLORIDE-INDUCED HEPATITIS IN RATS

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ABSTRACT

Objectives: To evaluate the hepatoprotective activity of Ethanolic extract of root of Centaurea behen Linn were studied against Wister rats with liver damage induced by carbon tetrachloride (CCl4)

Methods: The hepatoprotective effect of the ethanolic extract is measured against Wister rats with liver damage induced by carbon tetrachloride (CCl4) through measuring the serum levels of Aspartate aminotransaminase (AST), Alanine aminotransaminase (ALT) and alkaline phosphatase (ALP) and Bilirubin to a significant extent. The Ethanolic extract was screened for toxicity by oral toxicity studies according to OECD guidelines 423.

Results: Administration of Centaurea behen Linn (Dose 250mg/kg, 500mg/kg s.c) significantly prevented carbon tetrachloride induced elevation of serum ALT, AST, ALP and bilirubin level. Histological examination of the liver section revealed hepatic regeneration, after administration of various doses of Centaurea behen Linn.

Conclusion: The Ethanolic extracts of roots of Centaurea behen Linn showed significant decrease in the levels in serum markers, indicating the protection of hepatic cells, the extract of root of the plant could afford significant dose-dependent protection against CCl4 induced hepatocellular injury.

Keywords: Hepatoprotective, Hepatocytes, Centaurea behen, Silymarin.

INTRODUCTION

Cirrhosis is the damage of liver cells and their gradual replacement with scar tissue that impairs blood flow through the liver causing hepatoocyte death and loss of liver function [1]. Hepatic fibrosis occurs in response to liver damage and generates apoptotic cells after repeated injury [2]. This inflammatory response is accompanied by limited deposition of extra cellular matrix (ECM), so that if the regeneration of dying cells fails during persistent liver injury, hepatocytes are replaced by abundant ECM, including fibrillar collagen, depending on the origin of injury [3]. Treatment options for common liver disease such as cirrhosis, fatty liver and chronic hepatitis are problematic.

The effectiveness of treatments such as interferons, colchicines, penicilamine and corticosteroids are inconsistent at best and the incidence of side-effects profound [4]. Because of the role of oxidative stress in liver cirrhosis, antioxidants have been proposed as a treatment for cirrhosis [5]. Several studies have demonstrated the protective effects of antioxidants against induced liver injury by reducing oxidative stress in cells. [6, 7]

Carbon tetrachloride (CCl4) is one of the most common hepatotoxins that has been reported to show many metabolic and morphologic aberrations in the liver of the experimental animals similar to those observed in human viral hepatitis. It was found that chronic administration of CCl4 induces liver cirrhosis by a multiple step mechanism. CCl4, biotransformed in the liver to trichloromethyl radicals (-CCl3) which reacts with excess O2 forming reactive free radicals (-CCl3OO). These free radicals initiate peroxidation of membrane polyunsaturated fatty acids and covalently bind microsomal lipids and proteins forming lipid peroxides followed by cellular disorders and pathological changes [8]. To prevent and reduce the potential mutation in the cell, reactive oxygen species should be scavenged properly.

A number of herbas show promising activity, including Silymarin for liver cirrhosis, glycyrhrizin for chronic viral hepatitis, and herbal combinations from China and Japan that have been proven for treatment of liver diseases [9]. Silymarin, a reference drug, is a flavonolignan from "milkthistle" Silybum marianum, and widely used for the treatment of hepatitis and liver cirrhosis [10].

Centaurea behen L. is a root belongs to the family Asteraceae, native to South Asia and is commonly known as Safed Behman. In traditional medicine, several plants and herbs have been used experimentally to treat liver disorders, including liver cirrhosis, [11], [12]. Centaurea behen L. Possesses antioxidant [13], Anti anxiety [14], anti fungal [15], activities. In this study, we assessed the hepatoprotective effect of the ethanolic extract of Centaurea behen roots against Carbon tetrachloride -induced liver cirrhosis in Male wistar rats.

MATERIAL AND METHODS

Plant material collection and extraction

Plants sample were collected from Davabajar Mumbai. Collected roots were dried, powdered with mechanical grinder. The powder was passed through sieve and store in container. The powdered material was then extracted using solvent ethanol using soxhlet apparatus. After extracting all coloring matter, the solvent was removed by heating on water bath which give rise to a solid mass of material with volatiles. Then it was ground using a mechanical grinder to get powder and stored in airtight container. The plant material was then extracted with 95% ethanolic solvent using soxhlet apparatus for 24 hours. The fixed to the extraction apparatus was then heated to 95°C and the alcohol was distilled off until the material was completely dry. It was then extracted with methanol using soxhlet apparatus for 24 hours. The fixed to the extraction apparatus was then heated to 95°C and the alcohol was distilled off until the material was completely dry. It was then extracted with water using soxhlet apparatus for 24 hours. The fixed to the extraction apparatus was then heated to 95°C and the alcohol was distilled off until the material was completely dry. Anhydrous sodium sulfate was added to the extract, and the mixture was heated with a water bath until dry. The ethanolic extract was then filtered off and concentrated in a vacuo to afford a yellow solid.

Keywords: Hepatoprotective, Hepatocytes, Centaurea behen, Silymarin.
Acute toxicity

The ethanol extract of *Centaurea behen* L. was screened for acute toxicity, following the standard method (OECD No: 423). Albino mice of female sex weighing 20-25 gm were used in the study. Animals were maintained on normal diet and water prior to and during the course of experiments [16].

Experimental protocol [17]

**Group I**- Vehicle control: received single dose of vehicle once a day orally for 8 days and olive oil (0.5 ml/kg) s.c on day 7, 30 minutes after administration of the vehicle.

**Group II**- Disease control: received single dose of vehicle once a day orally for 8 days and CCL₄ (1ml/kg) in olive oil (1:1) s.c on day 7, 30 minutes after administration of the vehicle.

**Group III**- Test1: received 250mg/kg of extract once a day orally for 8 days and CCL₄ (1ml/kg) in olive oil s.c on day 7, 30 minutes after administration of the extract.

**Group IV**- Test 2: Received 500 mg/kg of the extract once a day orally for 8 days CCL₄ (1ml/kg) in olive oil (1:1) s.c. on day 7, 30 minutes after administration of the extract.

**Group V**- Standard control: received 25mg/kg of Silymarin once a day orally for 8 days and CCL₄ (1ml/kg) in olive oil (1:1) s.c. on day 7, 30 minutes after administration of silymarin.

Biochemical assessment

At the end of the experimental period, the animals were deprived of standard diet for 20 hr and anesthetized with diethyl ether. Blood samples of each animal were collected by puncturing retro orbital plexus in separate tubes without anticoagulant. It was kept at room temperature for 1 hr then serum was separated by centrifugation at 3000 rpm for 5 min for assessment of biochemical parameters. Alanine Aminotransferase (ALT) [18], Alkaline Phosphatase (ALP) [19] as well as Total Bilirubin [20] were estimated.

**Histopathological assessment**

For Histopathological examination, the rats were sacrificed by cervical decapitation and liver from each animal was excised then immersed in neutral buffered formalin for 24 hr. Liver tissues were cleaned and embedded in paraffin, cut in 5μm sections, stained with the haematoxylin and eosin and examined microscopically.

Statistical analysis

All data were expressed as mean±SD and were analyzed by one-way ANOVA to evaluate differences between groups. If significance was observed between groups, Duncan Multiple Range Test was used to compare the means of specific groups with p<0.05 considered as significant.

**RESULTS**

**Estimation of biochemical parameters**

The effect of ethanol extract of *Centaurea behen* L on serum transaminases, Aspartate aminotransaminase (AST), and Alanine aminotransaminase (ALT), alkaline phosphatase, bilirubin in CCL₄ intoxicated rats were summarized in Table 1. There was a significant increase (p< 0.05) in serum marker enzymes AST, ALT, ALP and bilirubin levels in group II (CCL₄ intoxicated rats). Treatment with ethanol extract of *Centaurea behen* L either simultaneously or after 8 weeks of CCL₄ administration (groups III and IV), and silymarin (group V) (p< 0.05) significantly decreased the elevated serum marker enzymes levels to almost normal.

**Table 1:** Effect of ethanolic extract of *Centaurea behen* L. on different biochemical parameters in Carbon tetra chloride induced hepatotoxicity.

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>AST (IU/L)</th>
<th>ALT (IU/L)</th>
<th>ALP (IU/L)</th>
<th>Bilirubin (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>55.300±17.321cd</td>
<td>21±6.00abc</td>
<td>250.33±54.647b</td>
<td>0.21±0.110b</td>
</tr>
<tr>
<td>II</td>
<td>140.00±27.105a</td>
<td>45±0.00ab</td>
<td>729.67±63.516a</td>
<td>0.80±0.411a</td>
</tr>
<tr>
<td>III</td>
<td>69.067±7.852bc</td>
<td>29±12.00abc</td>
<td>375.33±19.035bc</td>
<td>0.28±0.064b</td>
</tr>
<tr>
<td>IV</td>
<td>80.867±7.915bc</td>
<td>30±3.46abc</td>
<td>400.67±94.342b</td>
<td>0.36±0.168b</td>
</tr>
<tr>
<td>V</td>
<td>90.333±7.506b</td>
<td>34±6.93ab</td>
<td>435.00±27.221b</td>
<td>0.28±0.127b</td>
</tr>
</tbody>
</table>

All results are mean±SD for 5 animals. Values that have a different superscript letter (a, b, c, d) differ significantly with each other (p<0.05; Duncan’s Multiple Range Test).

**Histopathology**

Histological observation of liver tissue of the normal animal (group I) showed a normal liver architecture of hepatocytes since they were well arranged without any alteration at central vein. In (group II) hepatocytes showed severe and diffuse degenerative changes mainly hydropic and fat degeneration.

In addition, focal areas of necrosis and extensive intralobular fibrosis of the two forms portoportal and porto-central bridging fibrosis were observed.
In (group III) fibrous tissue proliferation was observed around portal tracts along with mild mononuclear cell infiltration mainly lymphocytes. In (group IV) the portal tracts demonstrated moderate infiltration with lymphocytes and few macrophages. Fibrous tissue proliferation around the portal areas and incomplete bridging of the hepatic parenchyma were observed. In (group V) the portal tracts displayed moderate infiltration with lymphocytes in addition to the presence of congested portal blood vessels and hepatic sinusoids.

**DISCUSSION**

The experiment of the present work was designed to study the in vivo effect of Ethanolic extract of *Centaurea Behen* L. on some Biomarker enzymes and Histopathological changes of hepatic lesions indicating hepatocellular injury induced by CCl₄ in male Wistar rats. Serum enzymes AST, ALT and ALP are sensitive markers of liver injury and their elevated levels are indicative of cellular leakage and loss of Functional integrity of cell membrane in liver that was initiated by hepatocellular damage caused by drug toxicity and xenobiotics [21],[22]. In the present study, significant increased levels of aminotransferases, with rise in the levels of ALT in CCl₄ intoxicated rats (Group II) indicated hepatic damage.

The results of the present study demonstrated that coadministration of ethanol extract of *Centaurea Behen* L. on some Biomarker enzymes and Histopathological changes of hepatic lesions indicating hepatocellular injury induced by CCl₄ in male Wistar rats. Serum enzymes AST, ALT and ALP are sensitive markers of liver injury and their elevated levels are indicative of cellular leakage and loss of Functional integrity of cell membrane in liver that was initiated by hepatocellular damage caused by drug toxicity and xenobiotics [21],[22]. In the present study, significant increased levels of aminotransferases, with rise in the levels of ALT in CCl₄ intoxicated rats (Group II) indicated hepatic damage.

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