

**HEPATOPROTECTIVE ACTIVITY OF *BERBERIS CORIACEAE* ON LIVER DAMAGE INDUCED BY CCL<sub>4</sub> IN RATS**

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**Summary**

Hepatoprotective efficacy of leaves extract of *Berberis coriaceae* was evaluated against biochemical and histopathological changes, induced by carbon tetrachloride in male wistar albino rats. Silymarin, a known hepatoprotective drug was used as positive control. Liver damage was studied by assessing parameters such as serum glutamate oxaloacetate transaminase, glutamate pyruvate transaminase, alkaline phosphatase, acid phosphatase and bilirubin in serum. The effect of administration of ethanol extract at dose of 100 mg/kg and 200 mg/kg on the above parameter was further investigated. Results of this study revealed that the extract showed significant hepatoprotective activity (P<0.05) by reducing the levels of the biochemical parameters in experimental animals. Histopathological study of the liver in experimental animals was also performed. These biochemical observations were supplemented by histopathological examination of liver sections. Furthermore, the acute toxicity of the extracts showed no signs of toxicity up to a dose level of 2000 mg/kg. The results suggest that the ethanol extract of *B. coriaceae* exhibits significant hepatoprotective properties.

**Key words** Carbon tetrachloride, Marker enzymes, *Berberis coriaceae*

**Introduction**

Liver is the largest organ in the vertebrate body and the site for intense metabolism. Liver diseases remain one of the serious health problems and are mainly caused by toxic chemicals. In spite of the tremendous advances made in allopathic medicine, no effective hepatoprotective medicine is available. Plant drugs are known to play a vital role in the management of liver diseases. The Indian traditional system of medicine, especially Ayurveda have put forward a number of medicinal plants and their formulations for liver disorders[1,2]. About 600 commercial preparations with claimed liver protecting activity are available all over the world. About 100 Indian medicinal plants belonging to 40 families are used for herbal formulation[3,4].

*Berberis coriaceae* (Berberidaceae) known in Hindi as Kashmal is an erect spiny shrub, ranging between 2 and 3 meters in height wood, hard and yellow; leaves, yellow to brown from outside and deep yellow from inside, removable in longitudinal strips by hand; spines (which, in fact, are modified leaves), three-branched and 1.5 cm long. Berberene hydrochloride, an alkaloid isolated from *Berberis coriaceae* was found to have significant anti-inflammatory activity on acute, subacute and chronic types of inflammation produced by immunological and non immunological methods.

Conical oral (20mg/kg) and intramuscular (2mg/kg) administration of berberine sulphate to rats increased the time duration of pentobarbitone –induced sleeping time and decreased serum cholesterol levels[5]. The plant was found to contain various triterpenes and flavonoids compounds. The present study was undertaken to scientifically prove the folklore use of the plant *B. coriaceae* against liver disorders.

### **Materials and methods**

**Plant materials:** The proposed study of *Berberis coriaceae* leaves were collected from the Sunder Nagar, Mandi, Himachal Pradesh, with the help of local tribal and field botanist. Care was taken to selected healthy plant and for normal leaves. The leaves were shade dried, reduced to coarse powder and stored in airtight container till further use.

**Preparation of extracts:** 1 Kg of powdered drug was packed in soxhlet apparatus and extracted with petroleum ether (60-80°C) to defat the drug. Defatted powdered drug was then extracted with chloroform. The chloroform extract was separated and the marc was further extracted with ethanol (95%). The solvents were removed by distillation and the last traces of solvent being removed under reduced pressure. Suspension of ethanol extract was prepared and was divided in two doses 100 mg/kg and 200 mg/ kg body weight and subjected for hepatoprotective activity.

**Experimental animals:** Male wistar albino rats having weight 180-230gm were kept in quarantine for 10 days under standard husbandry conditions (27.3°C, Relative humidity 65 ±10%) for 12 hrs in dark and light cycle respectively and were given standard food and water *ad libitum*. The project proposal was approved by the Institutional Animal Ethical Committee.

**Acute oral toxicity study:** Acute oral toxicity was performed by following OECD guideline – 420 fixed dose procedure for chloroform and ethanol extract and it was found that dose increasing up to 2000 mg/kg body wt. shown no toxicity or mortality in experimental rats. The LD<sub>50</sub> of the chloroform and ethanol extract as per OECD guidelines – 420 is greater than 2000 mg/kg[7,8].

**Experimental design:** Assessment of hepatoprotective activity was carried out on wistar albino rats. The animals were segregated into five groups of six animals each. Group I served as normal control receiving 5% CMC (10ml/kg). All other groups received CCl<sub>4</sub> (1ml /kg i.p.) with equal volume of olive oil (50% v/v) for two successive days. Group II animals were maintained as CCl<sub>4</sub> group, while group III and IV animals were treated orally for seven days with suspension of ethanol extract (100 mg/kg) and ethanol extract (200 mg/kg) respectively. Group V animals were treated with standard drug silymarin (25mg/kg). After the drug treatment all the animals were sacrificed by cervical dislocation. Blood was collected from the carotid artery and was allowed to clot for 45 min at room temperature; serum was separated by centrifugation at 2500 rpm for 15 min, used for the estimation of various biochemical parameters.

**Biochemical estimation:** Biochemical parameters such as serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP), acid phosphatase (ACP) and serum bilirubin were determined[8-10].

**Histopathology :** Liver were excised quickly fixed in a 10% buffered neutral formalin and proceeded for histopathology, they were processed for paraffin embedding following the standard microtechnique. Sections of liver stained with alum-haematoxylin and eosin were observed microscopically for histopathological changes. A few photomicrographs of representative types were also taken[11].

**Statistical analysis:** The results are expressed as mean  $\pm$  SEM of six animals from each group. The data were evaluated by one-way ANOVA followed by Turkey's multiple comparison tests. P values  $<0.05$  were considered statistically significant.

## Results

The result of acute toxicity study of ethanol extracts of *B. coriaceae* on laboratory animals showed that the animals were safe up to a maximum dose of 2000 mg/kg body weight. Hepatoprotective activity of ethanol extracts at the dose of 100 mg/kg and 200 mg/kg body weight were determined in CCl<sub>4</sub> induced hepatotoxicity model. The rats treated with CCl<sub>4</sub>, developed significantly liver damage, were observed from the alteration in the activities of serum enzyme (SGOT, SGPT, ALP and ACP), total bilirubin and direct bilirubin in serum (Table 1). The level of values of the SGOT, SGPT, ALP, ACP, total bilirubin and direct bilirubin were significantly increased in the CCl<sub>4</sub> treated rats as compared with the normal control group (P $<0.05$ ).

Treatment with ethanol extract of *B. coriaceae* at the different doses (100 mg/kg and 200 mg/kg) and also silymarin decreased the activity of serum transaminase, ALP, ACP and bilirubin in CCl<sub>4</sub> induced liver damaged rats compared to that of CCl<sub>4</sub> treated groups (P $<0.05$ ). It was found that the test samples offer protection against toxin as evidenced by remarkable reduction in all serum enzyme (P $<0.05$ ), and depicted that ethanol extract has strong hepatoprotective action.

In the histopathological study of liver from group I animals showed a normal hepatic architecture. In CCl<sub>4</sub> causes focal necrosis, portal infiltration, fatty changes, kupfer cell hyperplasia, hypdropic change. In group III animals, the necrosis which is more severe form of injury is markedly prevented and also shown fatty change. In group IV animals, the necrosis which is markedly prevented. Milder form of injury like fatty change and reduced necrosis persisted by the extract. The toxin mediated histological changes in the liver section of rats of test groups were much less intensity than those observed in the rats of CCl<sub>4</sub> treated group. In group V animals, the ballooning degeneration, and fatty changes of hepatocyte necrosis which is more severe form of injury is markedly prevented.

## Discussion

In the assessment of liver damage by CCl<sub>4</sub> hepatotoxin, the determination of enzyme level such as SGPT and SGOT are largely used. Necrosis or membrane damage release the enzyme in to circulation, therefore it can be measured in serum. Higher level of SGOT indicates the liver damage, due to active metabolite trichloromethyl free radical produced from carbon tetrachloride during metabolism by hepatic microsomes which in turn cause peroxidation of lipid of cellular membrane. SGPT catalyses the conversion of alanine to pyruvate and glutamate and is released in similar manner.

**Table 1: Effect of ethanol extract of *Berberis coriaceae* on CCl<sub>4</sub> induced hepatotoxicity in rats**

Treatment	SGOT (U/L)	SGPT (U/L)	ALP (U/L)	ACP (U/L)	Bilirubin (mg/100 ml of blood)	
					Direct	Total
Normal Control	62.7	102.3	182.4	115.9	0.31	0.59
CMC 10ml/kg	± 5.29	± 4.38	± 6.72	± 5.16	± 0.03	± 0.05
CCl <sub>4</sub>	221.3 <sup>a</sup>	292.5 <sup>a</sup>	357.2 <sup>a</sup>	199.8 <sup>a</sup>	1.79 <sup>a</sup>	2.27 <sup>a</sup>
1ml/kg i.p	± 4.31	± 6.24	± 4.58	± 5.37	± 0.19	± 0.13
Ethanol extract (100 mg/kg oral)	159.2 <sup>b</sup> ± 6.29	214.7 <sup>b</sup> ± 5.76	288.3 <sup>b</sup> ± 6.54	169.8 <sup>b</sup> ± 4.37	0.98 <sup>b</sup> ± 0.12	1.65 <sup>b</sup> ± 0.14
Ethanol extract (200 mg/kg oral)	80.6 <sup>b</sup> ± 7.31	140.7 <sup>b</sup> ± 5.83	179.5 <sup>b</sup> ± 4.60	110.2 <sup>b</sup> ± 6.42	0.28 <sup>b</sup> ± 0.11	0.61 <sup>b</sup> ± 0.05
Silymarin (25 mg/kg)	65.4 <sup>b</sup> ± 6.53	113.1 <sup>b</sup> ± 7.41	175.8 <sup>b</sup> ± 5.37	120.3 <sup>b</sup> ± 6.91	0.29 <sup>b</sup> ± 0.15	0.55 <sup>b</sup> ± 0.07

Values are expressed as mean ± SEM, n = 6 in each group. <sup>a</sup>P<0.05 when compared with normal control group, <sup>b</sup>P<0.05 when compared with CCl<sub>4</sub> treated group.

Therefore SGPT is more specific to the liver, and is thus a better parameter for detecting liver injury. Our results using the model of CCl<sub>4</sub>-induced hepatotoxicity in the rats demonstrated that ethanol extracts of *B. coriaceae* caused significant inhibition of SGPT and SGOT levels. Serum ALP, ACP and bilirubin levels on other hand related to the function of hepatic cells. Increase in serum level of ALP ACP and bilirubin is due to increased synthesis, in presence of increasing biliary pressure. Our results using the model of CCl<sub>4</sub>-induced hepatotoxicity in rats demonstrated that *B. coriaceae* extracts caused significant inhibition of ALP, ACP and bilirubin levels. Effective control of bilirubin level and alkaline phosphatase activity point towards on early improvement in the secretory mechanism of the hepatic cell.

Histopathological studies showed that CCl<sub>4</sub> caused focal necrosis, portal infiltration, fatty changes, Kupfer's cells hyperplasia and hydropic changes of the liver tissue. After administration of chloroform and ethanol extracts exhibited protection, this confirmed the results of biochemical studies[12]. All the effects of extracts were comparable with those of silymarin, a proven hepatoprotective.

The administration of ethanol extract of leaves of *B. coriaceae* decrease the CCl<sub>4</sub> induced elevated enzyme levels suggest the protection of structural integrity of hepatocyte cell membrane or regeneration of damaged liver cell by the extracts. The effectiveness of the normal functional conditions of the liver is indicated by the decreased level of serum bilirubin. Hence it confirms the hepatoprotective efficacy of leaves of *B. coriaceae* against CCl<sub>4</sub> induced hepatotoxicity.

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