

The Liver Protective Activities of Some Iranian Medicinal Plants Against Liver Damage in Mice Induced by CCL₄ Intoxication

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ABSTRACT : The aim and objective of this study are to carry out the liver protective activities against the CCL₄ intoxication in mice with some Iranian medicinal plants traditionally used for liver injuries. The methanol extracts of *Cichorium intybus*, *Lactuca scariola*, *Eucalyptus camadulensis* were evaluated. With various doses of these plants, liver protective activities were performed after CCL₄ administration to mice. The serum aminotransferases activities, liver sizes, and histopatological examinations of liver were studied. At a dose of 50 mg/kg, all three plants were able to protect liver damages induced by CCL₄.

Key Words : *Cichorium intybus*, *Lactuca scariola*, *Eucalyptus camadulensis*, Hepato-protection

I. INTRODUCTION

Liver is a major target organ for injuries induced by chemicals and hepatitis viruses and many efforts have been made to develop and produce the therapeutic remedies for liver injury (Yoshinobu *et al.*, 1983; Chang *et al.*, 1978). The Iranian plant *Khar Maryam*, which belongs to the Compositae family, has been widely used as a therapeutic agent for liver injuries in Iran (Reynolds *et al.*, 1989). Therefore, we tried to evaluate some Iranian medicinal plants (*Cichorium intybus*, *Lactuca scariola* and *Eucalyptus camaldulensis*) which have been traditionally used for liver injuries, especially focused on Compositae family (Amin, 1992; Samsam and Moatar, 1992). *Eucalyptus camaldulensis* has been used for liver diseases and also it reduced the glucose contents in diabetic patient. It was reported that *Cichorium intybus* leaves contain sodium sulfate, sodium phosphate, potassium nitrate, and bitter glycosides called chicorine and root part contains inulin, and *Cichorium intybus* have been reported to be effective on liver failure and liver disorder (Tavakoli and Sedaght, 1991; Zargar, 1990). *Lactuca scariola*, which contains lactucarium, lactucerine, lactucique acid, and lactucopicrine, was reported to have diuretic, sedative and liver pro-

TECTIVE activities. The leaves of *Eucalyptus camaldulensis* contains volatile oil, rutine, tannin and eucalyptic acid.

II. MATERIALS AND METHODS

1. Materials

Carbon tetrachloride, formaldehyde and sodium hydroxide were purchased from Merck (Germany) and alanine transaminase (ALT) and aspartate transaminase (AST) kits were purchased from Zist Shimi Co. (Tehran, Iran). Plant materials were purchased from local market at Ahwaz (Iran). Out bred Albino Swiss white male mice were supplied from Razi Reserach Center in Hasarak Karaj Iran and used under proper light control. Centrifuge Beckman Model Tj-6, Microtom Model 2045, Dsakura Tissue Passage Model RH-12EP2 were used.

2. Preparations of Extracts

Methanol (80%) extraction of 300 grams of air-dried *Cichorium intybus*, *Lactuca scariola*, and *Eucalyptus camaldulensis* were carried out and the extracted materials were concentrated under reduced pressure in vacuum evaporator into complete dryness.

3. Acute Toxicity Test

In order to evaluate the toxic effects of plant materials, *Cichorium intybus* (700 mg/kg body weight), *Lactuca scariola* (700 mg/kg body weight), and *Eucalyptus camaldulensis* (200 mg/kg body weight) were administered p.o. by following the dose schedule listed in Table 1. according to the previously described methods (Chang and Yun, 1978).

4. Evaluation of Plants Against CCl₄-induced Hepatotoxicity

Doses of 50 mg/kg, 200 mg/kg, 300 mg/kg, 500 mg/kg, and 700 mg/kg of *Cichorium intybus*, dose of 50 mg/kg, 200 mg/kg and 700 mg/kg of *Lactuca scariola* and dose of 50 mg/kg and 200 mg/kg of *Eucalyptus camaldulensis* were orally administered to mice (male, 20-25 g, 10 in each group). The negative control group received saline and the positive control group received CCl₄ in olive oil as described in Table 2 (Chang *et al.*, 1989). On the 5th day, blood sample was collected from jugular vein for the determination of liver transaminases activities and weight changes of liv-

er were determined and liver were kept in 10% formaldehyde for histopathological examinations.

III. RESULTS AND DISCUSSION

Iranian traditional medicines, which have been used traditionally for the treatment of liver injuries, were evaluated for the their liver protective activities, especially focused on the Compositae family. Three plants were selected and evaluated for their acute toxicity in mice at high doses (Table 1). As shown in Table 3, *Cichorium intybus* and *Lactuca scariola* did not changed ALT and AST enzyme activities and liver sizes were not changed at doses of 700 mg/kg when compared with negative control group. However, *Eucalyptus camaldulensis* revealed toxic effects at a dose of 200 mg/kg.

In order to evaluate the liver protective activities of plant materials against CCl₄ intoxication, different doses of *Cichorium intybus* extracts were tested (Table 2). At a dose of 50 mg/kg, it revealed a significant decreases of ALT and AST activities induced by CCl₄ and also the liver size was significantly recovered as compared with CCl₄ treated positive control group (Table 4). The his-

Table 1. Dose schedule for the evaluation of hepato-toxicities in mice

Treatment*	Days					
	1	2	3	4	5	
Control	Saline	Saline	Saline	Saline	Saline	Serum and liver collection serum and liver collection
CCl ₄	Saline	CCl ₄ **	CCl ₄	Saline	Saline	
<i>Cichorium intybus</i> (700 mg/kg)	Extract***	Extract	Extract	Saline	Saline	Serum and liver collection
<i>Lactuca scariola</i> (700 mg/kg)	Extract	Extract	Extract	Saline	Saline	Serum and liver collection
<i>Eucalyptus camadulensis</i> (200 mg/kg)	Extract	Extract	Extract	Saline	Saline	Serum and liver collection

*Each group consists of 10 mice (male, 20-25 g).

**CCl₄ dissolved in olive oil was given orally (0.2 ml/kg/day/mouse).

***Each extract was dissolved in physiological saline was given orally.

Table 2. Dose schedule for the evaluation of plants against CCl₄-induced hepatotoxicity in mice

Treatment*	Days					
	1	2	3	4	5	
Control	Saline	Saline	Saline	Saline	Saline	Serum and liver collection serum and liver collection
CCl ₄	Saline	CCl ₄ **	CCl ₄	Saline	Saline	
<i>Cichorium intybus</i>	Extract***	CCl ₄ +Extract	CCl ₄ +Extract	Saline	Saline	Serum and liver collection
<i>Lactuca scariola</i>	Extract	CCl ₄ +Extract	CCl ₄ +Extract	Saline	Saline	Serum and liver collection
<i>Eucalyptus camadulensis</i>	Extract	CCl ₄ +Extract	CCl ₄ +Extract	Saline	Saline	Serum and liver collection

*Each group consists of 10 mice (male, 20-25 g).

**CCl₄ dissolved in olive oil was given orally (0.2 ml/kg/day/mouse).

***Different concentrations of each extract dissolved in physiological saline was given orally.

Table 3. Evaluation of hepato-toxicity of some Iranian plants using serum aminotransferases and liver sizes in mice

Treatment*	ALT activities (U/L)	AST activities (U/L)	Liver size (cm ³)
Control	39.0±1.9***	76.5±2.0***	1.21±0.07***
CCl ₄ **	75.0±2.8	145.0±5.7	1.47±0.03
<i>Cichorium intybus</i> (700 mg/kg)	32.5±2.1	115.5±6.0	1.24±0.07
<i>Lactuca scariola</i> (700 mg/kg)	27.0±2.5	87.5±2.9	1.21±0.04
<i>Eucalyptus camadulensis</i> (200 mg/kg)	77.9±3.6	142.0±4.7	1.56±0.06

*Plant extract dissolved in physiological saline was given orally.

**CCl₄ dissolved in olive oil was given orally (0.2 ml/kg/day/mouse).

***Each value represents the mean±S.D. for 10 mice (male, 20-25 g).

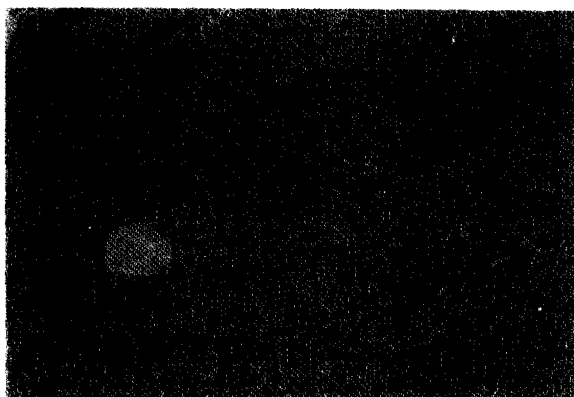


Fig. 1. Histopathological examination of liver of mouse stained with hematoxylin and eosin (Negative control group).

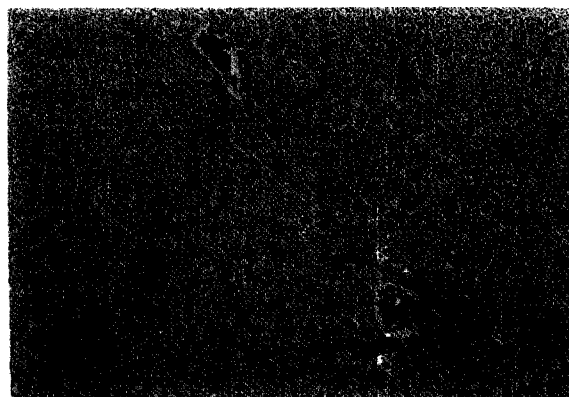


Fig. 2. Histopathological examination of liver of mouse stained with hematoxylin and eosin (CCl₄-treatment group).

topathological examination showed that there were not any abnormal fats, necroses in the liver cells and also liver structure was recovered to normal shape (Fig. 3) as compared to control groups (Fig. 1 and 2), suggesting this plants have a liver protective activity induced by CCl₄ at this low dose. However, the groups administered 200 mg/kg and 300 mg/kg of *Cichorium intybus* extract one hour after CCl₄ administration revealed somewhat reduced liver protective activities against CCl₄ intoxication. At a dose of 500 mg/kg, this plant materials did not show any liver protective activities induced by CCl₄. It is interesting to note that this plants enhanced the CCl₄ intoxication at a dose of 700 mg/kg (Table 4) and the histopathological examinations also showed an enhanced liver damage such as necrosis and fatty liver (data not shown).

In case of *Lactuca scariola*, the group received a dose of 50 mg/kg one hour after CCl₄ administration showed significant decrease in the transaminases activities (ALT and AST) as compared with the CCl₄ group and the liver sizes did not

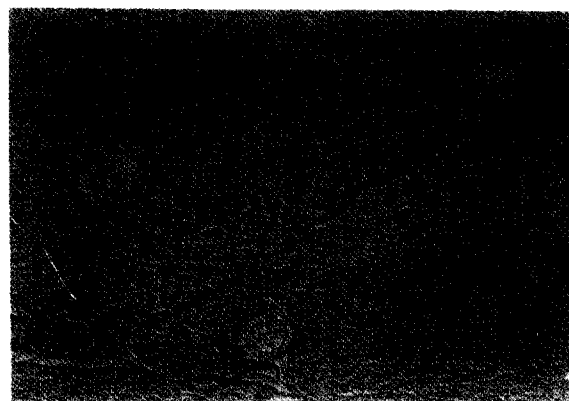


Fig. 3. Histopathological examination of liver of mouse stained with hematoxylin and eosin (CCl₄+*Cichorium intybus*, 50 mg/kg).

show any significant changes. At high doses (200 mg/kg, 700 mg/kg), liver protective activities were not observed (Table 5).

When *Eucalyptus camadulensis* was administered to mice after CCl₄ treatment, liver protective activity was observed at a dose of 50 mg/kg, however, no effect was observed at high dose (200

Table 4. Effects of *Cichorium intybus* methanol extracts on serum aminotransferases activities and liver sizes induced by CCl₄

Treatment*	ALT activities (U/L)	AST activities (U/L)	Liver size (cm ³)
Control	34.5±1.6***	78.0±3.6***	1.05±0.05***
CCl ₄ **	74.5±4.0	150.0±6.7	1.60±0.07
CCl ₄ + <i>Cichorium intybus</i> (50 mg/kg)	43.0±5.3	109.0±7.4	1.25±0.79
CCl ₄ + <i>Cichorium intybus</i> (200 mg/kg)	64.5±5.5	138.0±4.6	1.35±0.72
CCl ₄ + <i>Cichorium intybus</i> (300 mg/kg)	73.3±3.0	146.5±4.8	1.37±0.95
CCl ₄ + <i>Cichorium intybus</i> (500 mg/kg)	73.5±2.1	143.0±5.8	1.57±0.49
CCl ₄ + <i>Cichorium intybus</i> (700 mg/kg)	102.0±4.6	168.5±4.2	1.63±0.49

*Plant materials were administered orally.

**CCl₄ dissolved in olive oil was given orally (0.2 ml/kg/day/mouse).

***Each value represents the mean±S.D. for 10 mice (male, 20-25 g).

Table 5. Effects of *Lactuca scariola* methanol extracts on serum aminotransferases activities and liver sizes induced by CCl₄

Treatment*	ALT activities (U/L)	AST activities (U/L)	Liver size (cm ³)
Control	29.5±1.6***	76.5±2.5***	1.19±0.07***
CCl ₄ **	78.0±6.5	150.0±6.2	1.61±0.07
CCl ₄ + <i>Lactuca scariola</i> (50 mg/kg)	44.0±4.2	93.5±4.2	1.40±0.06
CCl ₄ + <i>Lactuca scariola</i> (200 mg/kg)	71.5±2.4	137.5±6.0	1.50±0.07
CCl ₄ + <i>Lactuca scariola</i> (700 mg/kg)	67.5±2.0	119.0±4.2	1.63±0.07

*Plant materials were administered orally.

**CCl₄ dissolved in olive oil was given orally (0.2 ml/kg/day/mouse).

***Each value represents the mean±S.D. for 10 mice (male, 20-25 g).

Table 6. Effects of *Eucalyptus camaldulensis* methanol extracts on serum aminotransferases activities and liver sizes induced by CCl₄

Treatment*	ALT activities (U/L)	AST activities (U/L)	Liver size (cm ³)
Control	35.5±1.9***	84.0±1.3***	1.05±0.05***
CCl ₄ **	75.0±4.2	154.0±6.0	1.60±0.07
CCl ₄ + <i>Eucalyptus camaldulensis</i> (50 mg/kg)	44.0±3.1	78.0±5.1	1.25±0.79
CCl ₄ + <i>Eucalyptus camaldulensis</i> (200 mg/kg)	77.5±5.3	140.0±4.4	1.35±0.72

*Plant materials were administered orally.

**CCl₄ dissolved in olive oil was given orally (0.2 mg/kg/day/mouse).

***Each value represents the mean±S.D. for 10 mice (male, 20-25 g).

mg/kg) as shown in Table 6.

IV. CONCLUSION

We investigated the Iranian medicinal plants for their hepatoprotective activities. The results show that *Cichorium intybus* and *Lactuca scariola* did not induced the liver damage at a dose of 700 mg/kg, however, *Eucalyptus camadulensis* induced the liver damaged at a dose of 200 mg/kg (Table 3). *Cichorium intybus* and *Eucalyptus camadulensis*, at a dose of 50 mg/kg, were significantly able to protect liver damage induced by CCl₄ in mice, however, *Lactuca scariola* demonstrated little liver

protective activity. It is interesting to note that liver protective activities were reduced as doses are increased. Therefore, it is worth to find the active principles from these traditionally used Iranian plants, especially focused on the Compositae family.

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