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Hepatoprotective activity of *Mentha arvensis* Linn. leaves against CCL_4 induced liver damage in rats

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ABSTRACT

Objective: To study the Hepatoprotective activity of ethanol, chloroform and aqueous extracts of *Mentha arvensis* leaves against CCL_4 induced liver damage in rats. **Methods:** Hepatotoxicity was induced by CCL_4 and the biochemical parameters such as serum glutamate pyruvate transminase (sGPT), serum glutamate oxaloacetate transaminase (sGOT), alkaline phosphatase (sALP), serum bilirubin (sB) and histopathological changes in liver were studied along with silymarin as standard Hepatoprotective agents. **Results:** The Phytochemical investigation of the extracts showed presence of flavonoids, steroids, triterpenoids, alkaloids, glycosides, carbohydrates, tannins, phenolic compounds. Treatment of the rats with chloroform, ethanol and aqueous extract with CCL_4 administration caused a significant reduction in the values of sGOT, sGPT, sALP and sB ($P<0.01$) almost comparable to the silymarin. The Hepatoprotective was confirmed by histopathological examination of the liver tissue of control and treated animals. **Conclusions:** From the results it can be concluded that *Mentha arvensis* possesses Hepatoprotective effect against CCL_4 induced liver damage in rats.

1. Introduction

Mentha arvensis Linn (Lamiaceae) is distributed throughout the Western Himalayas and is cultivated throughout world for use as vegetable[1]. It is used as a carminative, anti-spasmodic, anti-peptic ulcer agent[2]. It contains 70-90% menthol[3]. It is used in oral products such as toothpaste and mouth freshners due to its physiological cooling effects, as fragrance component in soaps, detergents, cosmetic and perfumes, toothpastes[4]. It contains monoterpenes such as menthone, menthofuran, sesquiterpenes, flavonoids, triterpenes, carotenoids, tannins and minerals[5].

Liver is the key organ of metabolism and excretion is continuously and variedly exposed to xenobiotics because of its strategic placement in the body. The toxins absorbed from the intestinal tract gain access first to the liver resulting in a variety of liver ailments. Thus liver diseases remain one of the serious health problems[6]. Conventional or synthetic drugs used in the treatment of liver diseases are sometimes inadequate and can have serious adverse effects[7].

Carbon tetrachloride (CCL_4) is a widely used hepatotoxic agent in rodents and its trichloromethyl radical (CCL_3) induced toxicity in rat's liver which closely resembles human cirrhosis. Hence, it is an acceptable animal model for analyzing hepatoprotective activity [8]. The survey of literature reveals that the *Mentha arvensis* are found to be used in the traditional system of medicine as a liver tonic. However Hepatoprotective activity of *Mentha arvensis* has not been scientifically investigated. Therefore, in the present study hepatoprotective effect of extracts of *Mentha arvensis* leaves have been evaluated against CCL_4 induced liver damage in the Wister albino rats.

2. Materials and Methods

2.1. Drugs and chemicals

Silymarin was obtained as a gift sample from Cadila Pharma Ltd, India. CCL_4 was obtained as gift sample from Thermo Fisher Scientific India Pvt. Ltd. Standard kit of serum glutamate pyruvate transaminase (sGPT), serum glutamate oxaloacetate trasaminase (sGOT) and alkaline phosphatase (sALP) were obtained from ERBA diagnostic Mannheim GmbH, Mallaustr, Mannheim/Germany. All other reagents used for the experiments were of high analytical grade.

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2.2. Plant material

The *Mentha arvensis* leaves were collected from the Miltops Exports, Jamnagar, Gujarat. The herb was authenticated by Dr. Harsha Hegde, Scientist B, Regional Medical Research Centre, ICMR, Belgaum. (Voucher No.RMRC-548).

2.3. Preparation of extracts

The fresh leaves were cleaned, shade dried and then coarse powdered. The dried coarse powder of the leaves was subjected to hot continuous extraction with chloroform, ethanol respectively and cold maceration with distilled water. After complete extraction, the extract was collected and the solvent was distilled off. It was concentrated to dry residue on water bath. The extracts were subjected to preliminary phytochemical investigation. Suspensions of each extract were prepared using 1% Tween 80. CCL_4 was mixed with olive oil in the ratio of 1:1 and subjected for Hepatoprotective activity against CCL_4 induced liver damage.

2.4. Experimental animals

Albino -Wistar rats weighing 150 to 180 g were housed in standard laboratory conditions of temperature [(25±2) ℃], 12 h light and dark places and with food and water ad libitum.

2.5. Acute oral Toxicity

The acute oral toxicity study was carried out as per the guidelines set by OECD, revised draft guidelines 423, received from CPCSEA, Ministry of Social Justice and empowerment, Govt. of India[9].

2.6. Evaluation of Hepatoprotective activity

The method used for evaluation of hepatoprotectivity was according to Ranawat[10] with some minor modifications. In the CCL_4 induced liver toxicity model, CCL_4 (0.5 ml/kg i.p.) was administered daily for 7 days to all animals except group 1. Silymarin (100 mg/kg, p.o) was used as standard. The animals were segregated in to six groups of six each. Group 1, which served as normal control and received 1% Tween 80. Group 2 received 1% Tween 80 p.o. for seven days and served as negative control. Group 3 received silymarin (100 mg/kg, p.o) for seven days. Group 4 received ethanolic extract (375 mg/kg, p.o.) for seven days. Group 5 received chloroform extract (375 mg/kg, p.o.) for seven days. Group 6 received aqueous extract (375 mg/kg, p.o.) for seven days.

The animals were sacrificed 24 h after last treatment under light anesthetic ether. Blood from each rat was withdrawn by retro orbital plexux under ether anesthesia for biochemical investigation i. e. sGOT, sGPT, sALT and bilirubin estimation. Blood was allowed to coagulate at 37℃ for 30 min and the serum was separated by centrifugation at 2500 rpm for 15 min. The liver of one animal from each group was removed and processed immediately for histopathological investigation.

2.7. Histopathological studies

One animal from the each group was utilized for this purpose. The liver specimens obtained from the control and treated groups of animals were fixed in 10% buffered formalin for 24 h. The formalin fixed liver samples were stained with haematoxylin-eosin for photomicroscopic observations of the liver histopathological architecture.

2.8. Statistical analysis

The data are presented as mean±SEM and analyzed by one way ANOVA, followed by Dunnett's 't' test. The results of all the extracts including the standard drug are comparted with the result produced by control. And it is considered as significant as $P<0.05$.

3. Results

Preliminary Phytochemical investigation revealed the presence of flavonoids, steroids, triterpenoids in chloroform extract, alkaloids, flavonoids, glycosides, carbohydrates, triterpenoids, tannins, phenolic compounds in ethanolic extract, alkaloids, flavonoids, glycosides, carbohydrates, triterpenoids in aqueous extract.

3.1. Acute toxicity

The extracts were found to be safe in the dose used and there was no mortality up to a dose of 3000 mg/kg b.w. for all extracts. Hence 375 mg/kg b. w. p. o. were selected for the activity.

3.2. Hepatoprotective activity

Administration of CCL_4 induced a marked increase in the serum hepatic levels, sGOT, sGPT, sALP and SB as compared to normal controls indicating liver damage (centrilobular necrosis). Pretreatment of the rats with chloroform, ethanol and aqueous extract prior to CCL_4 administration caused a significant reduction in the values of sGOT, sGPT,sALP and sB ($P<0.01$) almost comparable to the silymarin(Table 1).

3.3. Histopathologial results

The Hepatoprotective effect of *Mentha arvensis* leaves was confirmed by histopathological examination of the liver tissue of control and treated animals. The CCL_4 treated liver sections showed severe central vein congestion, sinus congestion, entrilobular degeneration and centrilobular necrosis along with moderate portal triaditis and inflammation. Fatty changes and ballooning of hepatocytes were also showed (Figure 1). The aqueous and chloroform extract treated group showed moderate

Table 1

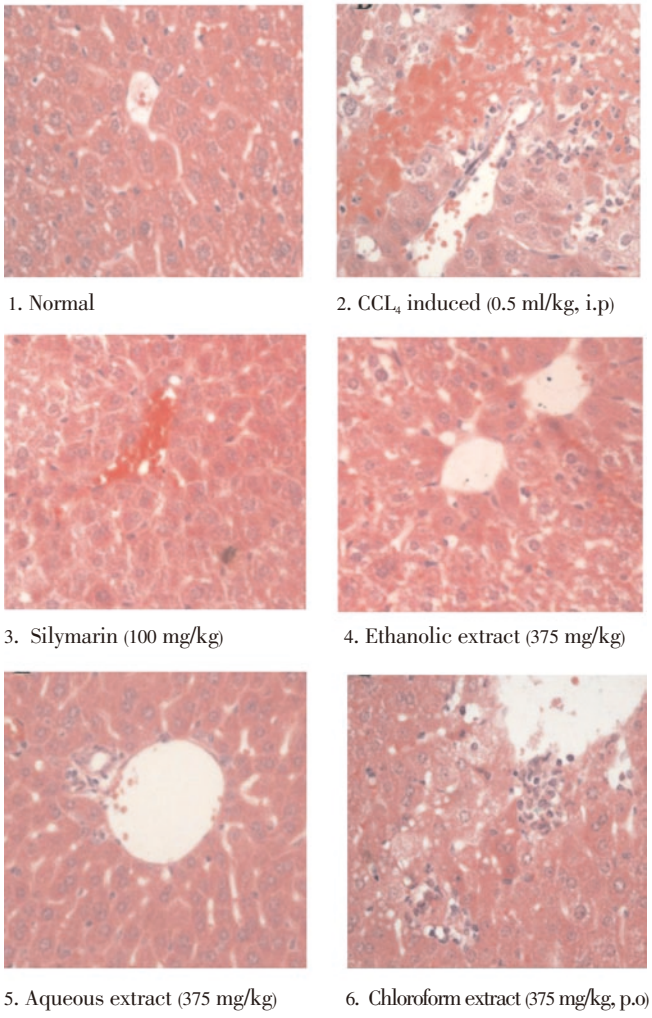
Biochemical assessment of CCL_4 induced liver injury.

Groups	SGPT (IU/L)	SGOT (IU/L)	ALP (IU/L)	Serum bilirubin (mg/dl)	Total bilirubin(mg/dl)
Normal	49.90±0.82	77.85±0.88	93.23±1.24	0.47±0.0045	1.46±0.0099
CCL_4 induced	225.00±1.34	268.80±0.92	309.4±2.74	1.06±0.0055	4.30±0.012
Standard drug	66.04±0.50***	92.96±0.82***	107.00±0.60***	0.52±0.0062***	1.61±0.0084***
EE treated	74.62±0.73***	103.00±0.62***	121.60±0.96**	0.59±0.0086***	1.74±0.0065***
AQE treated	99.52±0.90**	124.8±0.59**	138.70±0.84**	0.69±0.0048*	2.21±0.0087*
CHE treated	135.90±0.63**	161.4±0.63**	190.70±0.97*	0.79±0.0054	2.62±0.0089

Data are presented as means±SEM (n=6), one way Annova followed by Dunnett's t test.

**P<0.05, ***P<0.01 vs CCL_4 treated group

central vein congestion, sinus congestion, centrilobular degeneration, centrilobular necrosis and mild inflammation, portal triaditis. The ethanolic extract treated group showed mild central vein congestion, sinus congestion, centrilobular degeneration, centrilobular necrosis but moderate inflammation and portal triaditis. Silymarin treated group had maintained the normal histology with minimal damage.



1. Normal
2. CCL_4 induced (0.5 ml/kg, i.p)
3. Silymarin (100 mg/kg)
4. Ethanolic extract (375 mg/kg)
5. Aqueous extract (375 mg/kg)
6. Chloroform extract (375 mg/kg, p.o)

Figure 1. Histopathological monograph of extract and standard.

1:Normal; 2: CCL_4 (0.5ml/kg i.p) alone; 3:CCL_4+Silymarin(0.5ml/kg+100mg/kg)

4: CCL_4+ethanolic extract (0.5ml/kg+375mg/kg); 5: CCL_4+Aqueous extract (0.5ml/kg+375mg/kg); 6: CCL_4+Chloroform extract (0.5ml/kg+375mg/kg)

4. Discussion

CCL_4 metabolism begins with the trichloromethyl free redical (CCL_3) by the action of the mixed function of the cytochrome P450 oxygenase system. This free radical, which is initially formed as relatively unreactive, reacts very rapidly with oxygen to yield a highly reactive trichloromethyl peroxy radical (CCL_3O_2). Both radicals are capable of binding to proteins or lipids, or abstracting a hydrogen atom from an unsaturated lipid, thus, initiating lipid peroxidation[11-14]. Lipid peroxidation may cause peroxidative tissue damage in inflammation. Therefore, inhibition of the cytochrome P450 dependent oxygenase activity could cause a reduction in the level of toxic reactive metabolites and a decrease in tissue injury. On the other hand, an elevation of plasma sGOT, sGPT, sGALP and bilirubin activities could be regarded as a sign of damage to the liver cell membrane.

Many compounds known to beneficial against carbon tetrachloride-mediated liver injury exert their protective action by toxin mediated lipid peroxidation either via a decreased production of CCL_4 derived free radicals or through antioxidant activity of the protective agent themselves[15].

So in the present study, CCL_4 was employed as toxic agent and the protective effect of *Mentha arvensis* leaves against the CCL_4 induced hepatotoxicity was studied. The extent of toxicity was estimated by histopathological studies and biochemical enzyme markers like sGOT, sGPT, sALP and SB levels. The ethanol extract showed more significant result as compared to other extracts of *Mentha arvensis*. Report shows flavonoids and steroids are may be responsible for Hepatoprotective effect [16-19]. Perhaps flavonoids present in the *Mentha arvensis* leaves may be responsible for the marked Hepatoprotective effects, observed in the present study.

It can be concluded that *Mentha arvensis* leaves possess a protective effect against CCL_4 induced hepatotoxicity in rats as evidenced by the biochemical, histological parameters.

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Conflict of interest statement

We declare that we have no conflict of interest.

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