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Hepatoprotective And Antioxidant Activity Of Ethanol Extract Of Mentha arvensis Leaves Against Carbon Tetrachloride Induced Hepatic Damage In Rats

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Abstract: The aim of the study is to investigate the ethanol extract of *Mentha arvensis* leaves were screened for its hepatoprotective and antioxidant activities in CCl_4 (1 ml/kg bw) induced hepatic damage in rats. The extracts at doses of 100, 200 and 400 mg/kg bw were administered orally once daily for five days. The extract of *M. arvensis* decreases the elevated levels of serum biochemical markers aspartate aminotransferase (AST), alanine aminotransferase (ALT), Alkaline phosphatase (ALP), Total and direct bilirubin. The antioxidant enzymes catalase (CAT), superoxide dismutase (SOD), glutathione (GSH) significantly increased and MDA level significantly decreased in extract treated groups. Silymarin (50 mg/kg) was used as standard and exhibited significant hepatoprotective activity. The results of this study strongly indicate that *Mentha arvensis* leaves have potent hepatoprotective action at the dose of 400 mg/kg bw.
Keywords: *Mentha arvensis*, CCl_4, Hepatoprotective, Antioxidant activity.

Introduction

Conventional or synthetic drugs used in the treatment of liver diseases are sometimes inadequate and have serious adverse effects. So there is a worldwide trend to go back to natural products of herbal origin that are in use for the treatment of liver ailments, including hepatic parenchyma regeneration[1.] In the absence of reliable liver protective drugs in allopathic medical practices, herbs play a role in the management of various liver disorders. Most of the herbal drugs speed up the natural healing processes of liver [2].

Mentha arvensis Linn. (Family: Lamiaceae) known as pudina in Hindi, Sanskrit, Kannada. The plant is widely distributed throughout India and leaves of the plant are extensively used in traditional system of medicine for various ailments like carminative, digestive, expectorant, cardiotonic, diuretic, dentifrice, jaundice, hepatalgia, inflammation of liver, peptic ulcer, diarrhea, bronchitis and skin diseases [3,4,5,6]. Several medicinal property have been reported on it including postcoital antifertility[7], abortificient and antinidational properties in various

mammalian species[8]. The antifertility and reversible contraceptive efficacy of *M. arvensis* [9,10] were reported. The plant consist essential oils of monoterpenes like menthol, menthone, carvone and pulegone major constituents. This plant also possesses anti-candida[11] and it has shown radio protective activity against gamma radiation [12]. The literature survey revealed that so far no scientific studies carried out on hepatoprotective activity of the leaves of *M. arvensis*. Hence, in the present study we focused to evaluate the hepatoprotective activity of the ethanol extract of *M. arvensis* leaves against CCl_4 induced liver injury in rats.

Materials And Methods

Collection Of Plant Material

The fresh leaves of *M. arvensis* were collected from Harapanahalli, Davanagere (District), Karnataka, India was authenticated by Dr. Huddar, Head of Department of Botany, S.K. Arts and H.S.K. Science College, Vidyanagar, Hubli, Karnataka (Ref No. SKA-HSK/AUTH/2007-08).

Preparation Of Plant Extract

The air dried leaves of *M. arvensis* coarsely powdered and extracted with ethanol (40-60^{o}C) in a Soxhlet extractor for 18-20 h and solution was evaporated to dryness under reduced pressure and controlled temperature by using rotavapor. The extract was stored in a refrigerator at 4 °C until further use. The extract was subjected for preliminary qualitative phytochemical studies.

Animals

Wister albino rats (150-200 g) of either sex, procured from animal house KLE'S college of pharmacy, Hubli were used for the study. The animals were housed in standard conditions with natural light and dark cycle and provided with standardized pelleted feed and clean drinking water ad libitum. Approval from the institutional animal ethical committee for the usage of animals in the experiments was obtained (Ref No. KLESCOPH/IAEC.Clear/2006-2007/06).

Acute Toxicity Study

The acute toxicity tests for ethanol extracts of *M. arvensis* leaves were performed on Albino mice of either sex weighing between 20-30 gm following OECD Guidelines. The animals were fasted overnight prior to the experiment. The Up and Down method was adopted for toxicity studies. The maximum non lethal dose was found to be 2000 mg/kg body weight. Hence, the three dose was taken as effective dose (100, 200 and 400 mg/kg body weight) for the extract to evaluate hepatoprotective activity.

Hepatoprotective Activity

The rats were divided into six groups containing six rats in each group. Group I (normal control) received distilled water (1 ml/kg p.o.) daily for 5 days and received olive oil (1 ml/kg s.c.) on day 2 and 3. Group-II (CCl_4 control) animals received distilled water (1 ml/kg p.o.) daily for 5 days and administered CCl_4: olive oil (1:1, 2 ml/kg s.c.) on day 2 and 3. Group-III animals received standard drug silymarin (50 mg/kg p.o.) once daily for 5 days. Group IV-VI animals were administered orally at the doses of 100, 200 and 400 mg/kg of ethanol extract respectively. Groups III-VI animals were administered simultaneously CCl_4: olive oil (1:1, 2ml/kg s.c.) on day 2 and 3 after 30 min administration of the silymarin and extract[13].

On the sixth day blood samples were collected under mild ether anesthesia. The animals were sacrificed and liver was dissected out and 30% liver homogenate was prepared with phosphate buffer. The serum was obtained by centrifugation for evaluating the serum biochemical parameters of AST, ALT, ALP, Total and Direct bilirubin using assay kits. After centrifugation, the clear supernatant was used for assay of antioxidant enzymes MDA, glutathione [14], SOD[15], CAT[16].

Statistical Analysis

The results were expressed as the mean ± S.E.M. The study was analyzed by using one-way ANOVA followed by Dunnett's test.

Results

Phytochemical studies of ethanol extract of *M. arvensis* showed the presence of alkaloids, carbohydrates, glycosides, flavonoids, triterpenoids and tannins. In acute toxicity, no mortality was observed up to a dose level of 2000 mg/kg body weight.

The effects of ethanol extract of *M. arvensis* on CCl_4 intoxicated rats are shown in Table 1. In the CCl_4 treated group, serum AST, ALT, ALP, total and direct bilirubin levels were significantly increased when compared to normal control. The elevated levels of serum AST, ALT, ALP and total and direct bilirubin were significantly reduced in extract treated groups when compared to the CCl_4 treated control group. The antioxidant study revealed MDA level increased in CCl_4 treated group, whereas treatment with ethanol extract at 100, 200 and 400 mg/kg bw significantly reduced the MDA level. There is a marked depletion of GSH, SOD and CAT levels in CCl_4 treated group. The groups treated with 100, 200 and 400 mg/kg bw of ethanol extract of leaves was significantly increased the levels of GSH, SOD and CAT. Standard drug silymarin (50 mg/kg bw) also shown significant results. By observing the results, the ethanol extract of *M. arvensis* leaves has shown dose dependent activity.

Treatment	Biochemical parameters				
	AST U/L	ALT U/L	ALP IU/L	Total bilirubin mg/dl	Direct bilirubin mg/dl
Normal Control (1ml dist. water + 1 ml/kg olive oil s.c.)	58.99 ± 1.09	38.18 ± 1.67	121.11 ± 0.62	0.78 ± 0.04	0.26 ± 0.03
CCl_4 + olive oil control (1 ml + 1 ml/kg s.c.)	283.31 ± 1.43	167.76 ± 1.29	218.77 ± 0.75	2.85 ± 0.16	1.53 ± 0.07
CCl_4 + Silymarin (2 ml/kg s.c.+50 mg/kg p.o.)	95.06 ± 1.01[a]	55.19 ± 1.15[a]	126.11 ± 1.14[a]	1.41 ± 0.06[a]	0.41 ± 0.04[a]
CCl_4 + Ethanol extract (2 ml/kg s.c.+100 mg/kg p.o.)	223.51 ± 3.61[a]	146.73 ± 2.00[a]	196.90 ± 1.11[a]	1.88 ± 0.06[a]	0.75 ± 0.04[a]
CCl_4 +Ethanol extract (2 ml/kg s.c.+200 mg/kg p.o.)	160.84 ± 2.09[a]	108.42 ± 2.12[a]	150.49 ± 1.23[a]	1.68 ± 0.07[a]	0.63 ± 0.04[a]
CCl_4 +Ethanol extract (2 ml/kg s.c.+400 mg/kg p.o.)	113.72 ± 1.11[a]	74.75 ± 1.41[a]	137.84 ± 1.12[a]	1.46 ± 0.04[a]	0.48 ± 0.04[a]

Table 1: Effect of ethanol extract of *M. arvensis* leaves on AST,ALT,ALP, Total and direct bilirubin level in CCl_4 induced hepatotoxicity in rats.

Values are mean ï S.E.M.,n=6. [a] P<0.01 significance as compared to CCl_4 treated group.

Table 2 : Effect of ethanol extract of *M. arvensis* leaves on antioxidant enzymes in CCl_4 intoxicated rats

Treatment	GSH	SOD	CAT	MDA
Normal Control (1ml dist. water + 1 ml/kg olive oil s.c.)	0.85 ± 0.01	0.80 ± 0.01	0.90 ± 0.01	0.28 ± 0.00
CCl_4 + olive oil control (1 ml + 1 ml/kg s.c.)	0.45 ± 0.01	0.44 ± 0.01	0.49 ± 0.01	0.50 ± 0.00
CCl_4 + Silymarin (2 ml/kg s.c.+50 mg/kg p.o.)	0.80 ± 0.01[a]	0.76 ± 0.01[a]	0.84 ± 0.01[a]	0.17 ± 0.00[a]
CCl_4 + Ethanol extract (2 ml/kg s.c.+100 mg/kg p.o.)	0.61 ± 0.01[a]	0.62 ± 0.01[a]	0.65 ± 0.01[a]	0.24 ± 0.01[a]
CCl_4 +Ethanol extract (2 ml/kg s.c.+200 mg/kg p.o.)	0.72 ± 0.01[a]	0.68 ± 0.01[a]	0.73 ± 0.01[a]	0.21 ± 0.01[a]
CCl_4 +Ethanol extract (2 ml/kg s.c.+400 mg/kg p.o.)	0.77 ± 0.01[a]	0.73 ± 0.01[a]	0.79 ± 0.01[a]	0.19 ± 0.01[a]

Values are mean ± S.E.M.,n=6. [a]P<0.01, compared to CCl_4 treated group.

Discussion

Hepatoprotective effects of medicinal plants against drug models of hepatotoxicity remain poorly understood. Whereas most of the anti-hepatoprotective activities of plants have been investigated utilizing chemical models, this is the first report to study the hepatoprotective effect of ethanol extracts of *M.arvensis* against CCl_4 induced hepatotoxicity in rats.

CCl_4 produces an experimental damage that histologically resembles viral hepatitis. Toxicity begins with the change in endoplasmic reticulum, which results in the loss of metabolic enzymes located in the intracellular structures. The toxic metabolite $CCl_3^{\bullet}$ radical is produced which further reacts with oxygen to give trichloromethyl peroxy radical attack on polyunsaturated faty acids in the presence of oxygen to produce lipid peroxides leading to liver damage[17]. Hepatotoxicity leads to elevation of the serum marker enzymes which are released from the liver into blood[18]. The increased levels of AST, ALT, ALP and total and direct bilirubin are indicators of liver injury. In the present study revealed a significant increase in the levels of AST, ALT, ALP and total and direct bilirubin on exposure to CCl_4, indicating hepatic injury. The treatment with ethanol extract of *M. arvensis* leaves in all the dose levels attenuated the increased levels of serum enzymes and caused a subsequent recovery towards normalization[19].

The body has an effective defense mechanism to prevent and neutralize the free radical-induced damage. This is accomplished by a set of endogenous antioxidant enzymes such as SOD and CAT. These enzymes constitute a mutually supportive team of defense against ROS. In CCl_4-induced hepatotoxicity, the balance between ROS production and these antioxidant defenses may be lost, oxidative stress results leading to hepatic necrosis. The reduced activities of SOD and CAT observed the hepatic damage in the rats[20]. Pre-treatment with ethanol extract of leaves of *M. arvensis* was increased the depleted hepatic SOD and CAT levels in dose dependent manner. The non-enzymatic antioxidant, GSH is a critical determinant of tissue susceptibility to oxidative damage and the depletion of hepatic GSH was increased toxicity from the chemicals. A decrease in hepatic tissue GSH level was observed in the CCl_4-treated groups and increase in hepatic GSH level in the ethanol extract treated groups may be due to GSH regeneration[21].

The increase in MDA levels in liver suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanisms to prevent the formation of excessive free radicals. Hence, CCl_4 induced lipid peroxidation was also significantly reduced by the treatment of ethanol extract of *M. arvensis* leaves. On preliminary phytochemical analysis of the extract revealed the presence of flavonoids and phenolic compounds which have been known for its hepatoprotective and antioxidant Properties.

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