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Der Pharmacia Lettre, 2013, 5 (2):333-337
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ISSN 0975-5071
USA CODEN: DPLEB4

Evaluation of hepatoprotective and lipid peroxidation activity of the leaves of the plant *Crataeva magna* Buch Ham (Family Capparidaceae)

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ABSTRACT

The current study compares the two extraction method and evaluates the hepatoprotective and lipid peroxidation study of Crataeva magna Buch Ham (family Capparidaceae). The finding of the results reveals that the sequential extraction method was significantly reduced the serum level of liver enzyme asparate aminotransferase (AST), alanine aminotransferase (ALP) when compared with the maceration method which were assessed against carbon tetrachloride induced hepatotoxicity rats. Further the lipid peroxidation activity of the methanolic extract obtained from sequential method was analyzed, which show significant result with IC_{50} = 0.176 mg/ml. Hence the methanolic extract obtained from the sequential extraction process represents a source hepatoprotective effect, probably acting by promoting the antioxidant defense system.

Keywords: *Crateva magna*, Hepatoprotective effect, lipid peroxidation activity, IC_{50}

INTRODUCTION

Hepatic dysfunctions due to ingestion or inhalation of hepatotoxins (acetaminophen, cadmium chloride, ethanol, carbon tetrachloride, ally alcohol) are increasing worldwide (1). There are hardly any proven remedies for the prevalent liver disorders among people. No drug has been developed in the modern system of medicine, which may stimulate the liver functions, protect it from damage or help in the regeneration of hepatic cells. Natural products have been shown to be a tremendous and consistent resource for the development of new drugs (2). However, there are more evidence suggesting that photochemical having antioxidant properties are associated with a lower risk of mortality from many of the diseases.

The occurrence of acute viral hepatitis is similar to CCl_4-induced liver damage in hepatotoxicity model for which investigation of the hepatoprotective effects of drugs and plant extracts need to be carried out (3).

Crateva magna is a multipurpose tree of Capparidaceae family that is also used to increase appetite & for the treatment of various diseases e.g. rheumatism and nephrotoxicity, arthritis, urinary disorders (4, 5). Crateva magna is a deciduous tree and grows throughout India, well-known for its handsome foliage and beautiful cream color flower. Its bark is used in Ayurveda for treating urinary disorder (6). However it is best known for its action on urinary calculi and it has official status in the Indian herbal pharmacopeia---2002. A wide variety of medicinally important compounds including friedelin, diosgenin, sitosterol, butulic acid, and betulin aldehyde have been reported from Crateva magna (7, 8). It has been reported that leaf extract of *C. magna* had significant free radical scavenging property which may attribute to its phenolics, flavonoid and proanthocyanidine contents (9). In folk medicine, its stem pith in the tribal peoples of Kandhamal district of Orissa known as Eastern Ghats of India that the bark is used for lactation after child birth, the cooked leaves of this plant for the treatment of jaundice, fever, vomiting and gastric irritation (10). There are no scientific studies in support of this traditional claim for its hepatoprotective

activity. Hence, an attempt was made to investigate the hepatoprotective activity and lipid peroxydation study of the leaves of the plant *C. magna*.

MATERIALS AND METHODS

Plant material

The plant was collected from the eastern part of Odisha, India, taxonomically identified and approved by the department of botany Utkal University, Bhubaneswar (voucher number : 1121, deposited in : Herbarium of Utkal University, Odisha, India).

The leaves of the plant *C. magna* were shaded dried and powered. The powdered of the plant (100g) was left to macerate in dark for 5 days with frequent agitation and the resulting liquid is filtered with what man filter paper, the filtered methanol extract was dried in an oven at 40°c for about 36 hr the resulting dried mass of the plant was then powered packed in to a glass vial properly labeled and stored in a dacicator over silica gel until further use.

Preparation of extraction method

Maceration extraction method

The leaves of the plant *C. magna* were shaded dried and powered. The powdered of the plant (100g) was left to macerate in dark for 5 days with frequent agitation and the resulting liquid is filtered with what man filter paper, the filtered methanol extract was dried in an oven at 40°c for about 36 hr the resulting dried mass of the plant was then powered packed in to a glass vial properly labeled and stored in a dacicator over silica gel until further use.

Sequential extraction method

Air dried *C. magna* leaves powered into a soxhelt apparatus and was extracted sequentially with petroleum ether (PE), chloroform (CF) and methanol (MA). The solvent were evaporated under reduced pressure and the fraction were then placed in a vacuum oven at 35°c for about 24 hr to remove any residual solvent .the resulting semisolid mass of each fraction were stored in a dacicator until subjected to further analysis and all the assay were done in triplicates.

Laboratory animals:

Adult albino Wister rats (150-200gm) of either sex (OUAT, Bhubaneswar) used in the experiment were allowed to acclimatize to the laboratory conditions for 7 days in acrylic cages prior to commencement of the experiment with 12hr day & night schedule at a temperature of 26±4 c. The animals were maintained with standard pellet diet & water ad libitum.

Acute toxic study

Thirty five Wister albino rats were divided in seven groups for five animals each. Group 1 received distilled water 10ml/kg orally and animal from groups 2 to 4 were given macerate extracts of *C. magna* at dose 200, 400, 500, 1000, 2000 mg/kg orally, respectively. Symptoms of toxicity and mortality were observed for 24hr, the behavioral and CNS profiles such as spontaneous rearing and grooming evidence of calmness and sedation and loss of writhing reflex were also observed.

In vivo hepatoprotective activity

In vivo hepatoprotective activity was evaluated on the basis of CCl_4 induced liver damaged in rats as previously describe by (11). The rats were divided into four groups experimental of six animals (table1). Group 1-was kept on normal diet and sever as normal control and received distilled water (10ml/kg)daily for 5 days and then given olive oil (3ml/kg; i.p.) on day 5, 30 min after receiving distilled water. Group 2 serve as CCl_4 control group and received distilled water (10 ml/kg; p.o.) daily for five day and then received CCl_4 diluted olive oil (1:1, 3ml/kg, i.p.) on day 5, 30 min after administration of distilled water. Group3 and group 4 were treated with methanolic extract obtained by maceration process of study plant at a dose of 200mg/kg body weight daily for 5 days. Group 3 received olive oil (3ml/kg, i.p.) on day 5, 30 min after receiving extract where as group 4 received CCl_4 diluted with olive oil (1:1, 3ml/kg i.p.) on day five , 30 min after administration of the extract. All of the animals were restricted to water after administration of CCl_4 or olive oil on day 5. Twenty four hours after administration CCl_4 in olive oil or olive oil alone blood was collected from all group by cardiac puncture and serum was separated by centrifugation at 3500 rpm (Eppendorf 5403) at 4°c for 15 min and analyze for various biochemical parameters. The result depicted in table no 1.

Assessment of liver function
Asparate aminotransferase (AST) (12) , alanine amino transferase (ALT) (13) and alkaline phosphate (ALP) (14) were analyzed at the department of biochemistry, pathology, laboratory , Sparsh hospital (Bhubaneswar, Odisha) using commercially available test kit and automatic bioanalyzer 912 (Hitachi).

The PE, CF and MA extracts obtained form the soxhelt extraction of the plant at a dose of 200mg/kg and Silymarin (group VI) (standard drug) at a dose of 50mg/kg were tested for hepatoprotective activity following the above procedure depicted in table no 2.

Histological condition of liver
Immediately after sacrifice of rats the small pieces of livers .the liver tissue were fixed in 10% formalin and proceed for embedding in paraffin sections of histopathological changes which include all necrosis fatty changes and infiltration of kuffer cell and lymphocytes.

Lipid peroxidation inhibition assay
Adult male Wister albino rats (180-220g) were used in this study. The animals were terminally anesthetized with diethyl ether. The liver was removed via abdominal dissection and 5%(w/v) homogenate of the liver in 0.15 m KCl was prepared using a homogenizer (homogenizer MSE England) under ice cold (0-4°c) condition the homogenate was then centrifuge (using Ependerf 5403, Germany) at 3000 rpm for 15 min at 4°c and the supernatant was used for analysis. Firstly, 100µL of MA extract of the plant C.M (0.125, 0.25, 0.5, 1.0, 2.0 mg/ml distilled water) were dispensed in to different test tube followed by 0.5 ml of supernatant and 1ml of 0.15M KCl. Peroxidation was initiated by the addition of 100µL of 0.2 mm $FeCl_3$. The mixture was included at 37°c for 30 min and the reaction was stopped by adding 2ml of ice-cold TBA-TCA-HCl-BHT solution. The TBA-TCA HCl solution was prepared by dissolving 1.68g TCA and 41.60 mg TBA in 10ml of 0.125 M HCl. One ml BHT solution (1.5 mg/ml ethanol) was added to 10 ml TBA-TCA HCl solution. The reaction mixture was heated for 60 min at 90°C and then cooled on ice and centrifuged at 3000 rpm. The supernatants were removed and absorbance was measured on a spectrophotometer at 532 nm wavelength. A control experiments was performed in the presence of distilled water without the extract. The percentage of lipid peroxidation in the samples was calculated using the following formula:

Percentage of lipid peroxidation = $[(A_0 – A_1)/A_0] \times 100$

Where A_0 is the absorbance of the control (100 µl of distilled water), and A_1 is the absorbance of samples contain methanolic fraction obtained from the soxhelt extraction of *C. magna*.

Statistical analysis
All the experiments were carried out in triplicates and the results are reported as mean ± standard error. The data were analyzed by one–way analysis of variance (ANOVA) and Tukey post test. P-values < 0.05 were considered significant.

RESULTS AND DISCUSSION

The results of acute oral toxicity studies in rats indicated that the methanolic extract obtained from maceration process of *C. magna* causes no death or sign of toxicity up to a dose of 2 g/kg of body weight. For solvent extraction, we first investigated extracting the leaves of the plant using maceration method, with 80% methanol as a solvent, but narrow significant reduction in the level of AST & ALT enzymes was observed (table no-1).

Table 1. Effects of the methanolic extract obtained from maceration method of *C. magna* at a dose of 200 mg/kg on activities of serum enzymes in rats injected with CCl_4 in olive oil (3ml/kg), i.p.

Treatment	Serum enzyme activities of (U/L) (Mean ± SEM)	
	AST	ALT
Group1 (Vehicle control)	40.8±1.3	30.2±2.2
Group2 (CCl_4 control)	160.7±9.6	103.1±12.3
Group3 (maceration extract of *C. magna*)	49.1±1.8	37.4±2.8
Group4 (maceration extract of *C. magna* +CCl_4)	109.1±4.2	67.4±6.2

We then investigated the sequential extraction method using three different solvent, in order of increasing polarity viz. petroleum ether, chloroform & methanol. The methanolic extract obtained from sequential extraction showed significantly suppressed the plasma AST, ALT & ALP activities when compared with CCl_4- intoxicated control. Nonpolar solvents like petroleum ether, and chloroform showed little activity in our study (table-2). In the study, three solvents were employed to determine the most effective extraction solvent having active principle. Thus the

non polar solvent used in the sequential extract adds the removal of most of the interfering substance there by the active principle being concentrated the methanolic extract.

Table 2. Effects of the different extract obtained from soxhelt extraction method of *C. magna* at a dose of 200 mg/kg on activities of serum enzymes in rats injected with CCl_4 in olive oil (3ml/kg), i.p.

Group	Serum enzyme activities of (U/L) (Mean ± SEM)			
	Dose	AST	ALT	ALP
Normal control	Solvent	43.05±1.7	31.2±4.3	49.7± 5.4
CCl_4 control	1ml/kg	155.4±8.1	203.4±38.1	171±5.4
PE	200mg/kg	143.8±13.4	196.1±17.1	146.4±11.2
CL	200mg/kg	133.4±6.2	195.4±6.7	149.1±17.2
MA	200mg/kg	80.8±11.8	92.4±8.1	94.5±11.6
Silymarin	50mg/kg	67.8±12.5	87.6±9.8	88.3±10.5

Histopathological condition of liver.

Fig (1A) shows liver section of normal control rats showed normal hepatic cell with well preserved cytoplasm, prominent nuclei & nucleoli and well brought out central vein. CCl_4 administration induced liver necrosis in the centribular region, sinusoidal congestion, broad infiltration of kupffer cells and loss of boundaries were observed in fig (1B). Though the extent of cellular necrosis was less pronounced compared to the CCl_4 group, increase in liver size, disorganization of the lobules, reactive inflammatory processes and necrosis with acidophilic cells, were observed in animals of administrating PE and CL were observed in fig (1C) and fig (1F) respectively. As is apparent in fig (1E), the severe hepatic lesions induced by CCl_4 were remarkable lowered by the administration of MA which is good agreement with the results of the biochemical analysis which is comparable to standard Silymarin treated liver section in fig (1F).

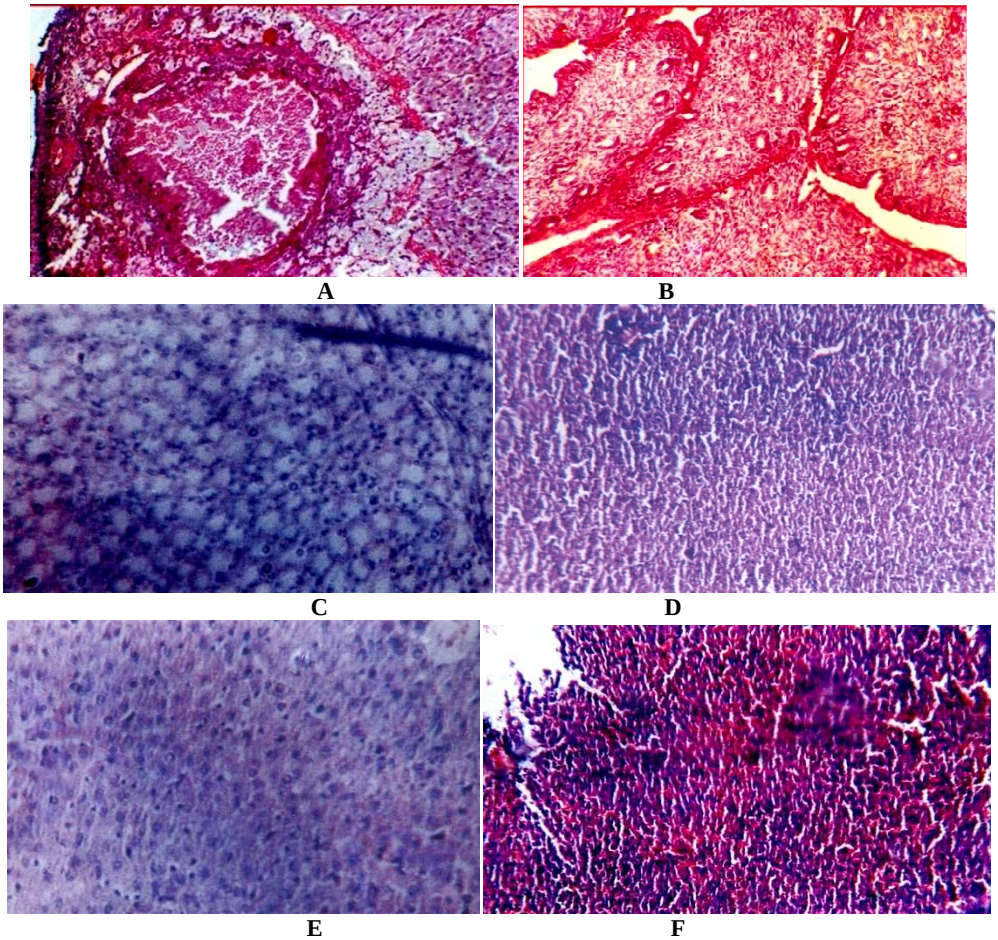

Figure 1. Representative microphotographs of H&E (×10)-stained histological sections of liver (1A) normal control; (1B) rats treated with CCl_4 (25 µL/kg, i.p.); (1C) rats treated with PE (200 mg/kg) and CCl_4 (25 µL/kg, i.p.); (1D) rats treated with CL (200mg/kg) and CCl_4 (25 µL/kg, i.p.); (1E) rats treated with MA (200mg/kg) and CCl_4 (25 µL/kg, i.p.); (1F) rats treated with Silymarin 50 mg/kg, p.o. and CCl_4 (25 µL/kg, i.p.);

Lipid peroxidation inhibition

The generation of trichloromethylperoxy radical due to administration of CCl_4 resulting lipid peroxidation reaction and destruction of microsomal cytochrome P-450 enzyme by abstracting hydrogen from PUFA (15,16) . The liver generates hydroxyl radical by Fenton reaction. Which could be scavenged by antioxidant compound present in the extract. Thus the natural compounds that reduced the chemical activating enzymes, or that scavenge free radicals generated might be good candidates for protection against chemical induced liver toxicity (17). The pretreatment of rats with MA from *C. magna* reduced lipid peroxidation as seen from result of lipid peroxidation assay. The result of the study finds that the extract inhibited lipid peroxidation in concentration dependent manner. IC_{50} of MA from *C. magna* is 0.176 mg/ml.

Table 3. Effect of inhibition of lipid peroxidation in vitro by MA extract of *C. magna*

Concentration(mg/ml)	Absorbance(h)	%of inhibition
0.03125	0.75 ± 0.41	9%
0.0625	0.67 ± 0.13	18.5%
0.125	0.512 ± 0.45	38%
0.25	0.241 ± 0.17	71%
0.5	0.127 ± 0.88	84.5%
1.0	0.0965 ± 1.2	89%
2.0	0.0724 ± 5.7	91%

CONCLUSION

The result of the present study would certainly help to ascertain that sequential extraction method was better than maceration method by concentrating the active principles and by removing interfering substance of the plants and further help to assured that the potency of the methanolic extract obtained from sequential process of leaves of *C. magna* as a potential source of hepatoprotective protective effect against the acute liver damage and hepatoprotective mechanisms of this extract on CCl_4-induced acute liver damage might be due to the decreased lipid peroxidation. However, further research is required to determine forming the hepatoprotective system and develop their application for pharmaceutical and food industries.

Acknowledgements

The authors are grateful to the authorities of School of Pharmaceutical Sciences, Siksha 'O'Anusandhan University, Bhubaneswar Orissa, India for providing the necessary facility, and encouragement and to carry out the research work.

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