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Hepatoprotective activity of *Baliospermum montanum* (willd) Muell.-Arg. in rats treated with carbon tetrachloride: In vivo and in vitro studies

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ABSTRACT

Rats and primary cultures of rat hepatocytes were used as the in vivo and in vitro models to evaluate the hepatoprotective activity of sub-fractions from total methanol extract of *Baliospermum montanum*. Carbon tetrachloride was selected as hepatotoxin. Silymarin was the reference hepatoprotective agent. In the in vivo study, serum transaminases, alkaline phosphatase, total bilirubin, total cholesterol, albumin together with total protein and histopathological examination were the criteria for the evidences of liver injury. Carbon tetrachloride caused the alterations in all the biochemical parameters and centrilobular necrosis. Among the Ethyl methyl ketone and methanol sub-fractions tested (50, 100 and 150 mg/kg), methanol sub-fraction (150 mg/kg) of the bio-active total methanol extract and silymarin (100 mg/kg) enhanced liver cell recovery by restoring all the altered biochemical parameters back to normal. In the in vitro study, release of transaminases, total protein together and hepatocyte viability were the criteria. Primary cultures of hepatocytes were treated with carbon tetra chloride (10 µl/ml), and various concentrations (100, 500 and 1000 µg/ml) of ethyl methyl ketone and methanol sub-fractions of total methanol extract and silymarin (100 µg/ml). Carbon tetrachloride reduced the hepatocyte viability and also altered the biochemical parameters, which were restored significantly ($P<0.05$) by ethyl methyl ketone (1000 µg/ml) and methanol (500 and 1000 µg/ml) sub-fractions. These results suggest that *Baliospermum montanum* possess the hepatoprotective activity against carbon tetrachloride induced liver injury in both rats and primary cultures of rat hepatocytes.

INTRODUCTION

Baliospermum montanum (willd) Muell.-Arg. (Euphorbiaceae) commonly known with the name of Danthi in India is a stout, monoecious undershrub with many shoots arising from the base. The roots are described as purgative, anthelmintic, and diuretic, and also used to treat pains, enlarged spleen, inflammations, and leukoderma (1) abdominal tumours and cancer (2). The ayurveda claims the usefulness of roots in the treatment of jaundice (1). Presence of steroids, terpenoids and flavonoids (3); phorbol esters (4) has been reported in the plant. Preliminary studies on roots of *B. montanum* showed significant hepatoprotective activity of total methanol extract at a dose level of 200 mg/kg, against carbon tetrachloride induced hepatotoxicity (unpublished data). In present study the bio-active total methanol extract has been further fractionated in an attempt to enrich hepatoprotective activity and to identify active constituents. The hepatoprotective activity of ethyl methyl ketone and methanol sub-fractions of methanol fraction obtained from total methanol extract was assessed at various dose levels using carbon tetrachloride as hepatotoxin, both *in vivo* and *in vitro* using primary cultured rat hepatocytes. Silymarin was used as a positive control.

MATERIALS AND METHODS

Plant material

The roots of the *B. montanum* were procured from Centre for Medicinal Plants Research, Arya Vaidya Sala, Kottakkal, Kerala state and their identity was confirmed at the same. The voucher specimen (HDT/CMPR/04/ SVSK/ 05–06) was deposited in the Herbarium of the Institute.

Preparation of extracts

450 g of powdered roots of the plant was completely extracted with methanol using soxhlet apparatus. The methanol extract (ME) concentrated in vacuum yielded solid mass (6.25%; w/w). The methanol extract (25 g) was adsorbed on to the 250 g of silica gel (60–120 mesh) and fractionated using chloroform (CFME) and methanol (MFME). The recovered fractions were finally evaporated to give yields of 12.06% and 68.86% of solid respectively. Further 40 g of the methanol fraction was adsorbed on to the 250 g of silica gel and fractionated using ethyl methyl ketone (EMKMFME) and methanol (MFMFME) with resultant yields of 8.47 and 73.44% respectively. The thin layer chromatographic (TLC) studies of ME revealed the presence of flavonoids, terpenoids, and steroids (5); the CFME showed the terpenoids and steroids with trace quantities of flavonoids; while MFME flavonoids with trace quantities of terpenoids. The sub-fractions EMKMFME showed flavonoids, while the MFMFME, flavonoids and traces of terpenoids. The sub-fractions, EMKMFME and MFMFME were selected at dose levels of 50, 100 and 150 mg/kg for *in vivo* and; at dose levels of 100, 500 and 1000 µg/ml for *in vitro* studies. The results were compared with a standard hepatoprotective drug silymarin (100 mg/kg *in vivo* and 100 µg/ml *in vitro*). All the test substances were suspended in vehicle (5 % acacia mucilage for *in vivo* and 30% DMSO for *in vitro* studies).

Animals

Wistar albino rats weighing 175–225 g of either sex, maintained under standard husbandry conditions (Temp 23 ± 2°C, relative humidity 55 ± 10% and 12 h light dark cycle) were used for all studies. Animals were allowed to take standard laboratory feed and tap water. The experiments were performed after the experimental protocols approved by the institutional animal ethics committee. Groups consisted of 6 rats each unless otherwise noted.

Hepatoprotective activity in vivo Toxicity studies

Acute toxicity studies were performed for EMKMFME and MFMFME according to the acute toxic classic method (6) described by OECD. Female albino rats were used for acute toxicity study. The animals were kept fasting for overnight providing only water. The rats were divided into two groups of 3 animals each. The group of rats were administered orally with appropriate extracts of B. montanum at a dose of 300 mg/kg. The animals were observed continuously after dosing during first 30 min, periodically for first 24 h with special attention given during first 4 h and daily thereafter, for a total of 14 days. As there was no mortality seen at this dose level, the procedure was repeated with further dose (2000 mg/kg) using fresh animals.

Carbon tetrachloride-induced hepatotoxicity

Rats were divided into 9 groups of 6 each, control, carbon tetrachloride, silymarin and test groups. The rats of control group received three doses of 5% acacia mucilage (1 ml/kg, p.o.) at 12 h intervals (0 h, 12 h and 24 h). The rats of carbon tetrachloride (CCl_4) group received three doses of vehicle at 12 h intervals and a single dose of CCl_4 (1.25 ml/kg i.p.) diluted in liquid paraffin (1:1) 30 min after the administration of first dose of vehicle.

The animals in silymarin group received three doses of silymarin (100 mg/kg) at 0 h, 12 h and 24 h. CCl_4 (1.25 ml/kg i.p.) was administered 30 min after the first dose of silymarin while the test groups were given first dose of extract in acacia mucilage at 0 h which was followed by a dose of carbon tetrachloride (1.25 ml/kg i.p.) after 30 min, while at 12 h, and 24 h the second and third dose of respective extracts (50, 100 and 150 mg/kg p.o.). After 36 h of administration of carbon tetrachloride, blood was collected and serum was separated and used for determination of biochemical parameters (7).

Assessment of liver function

Blood was collected from all the groups by puncturing the retro-orbital plexus and was allowed to clot at room temperature and serum was separated by centrifuging at 2500 rpm for 10 min. The serum was used for estimation of biochemical parameters to determine the functional state of the liver. Serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) were estimated by a UV kinetic method based on the reference method of International Federation of Clinical Chemistry (8) in which both SGOT and SGPT were assayed based on enzyme-coupled system; where keto acid formed by the aminotransferase reacts in a system using NADH. The coenzyme is oxidised to NAD and the decrease in absorbance at 340 nm is measured. For SGOT malate dehydrogenase is used to reduce oxaloacetate to malate where as for SGPT the

pyruvate formed in the reaction is converted to lactate by lactate dehydrogenase. Alkaline phosphatase (ALKP) was estimated by method described by Mac Comb and Bowers (9) involving hydrolysis of *p*-nitrophenylphosphate by alkaline phosphatase to give *p*-nitrophenol which gives strong yellow colour in alkaline solution. The increase in absorbance due to its formation is directly measured photometrically at 400 nm and is directly proportional to ALKP activity; while total bilirubin (TBL) by Jendrassik and Grof method (10) which involves the reaction of bilirubin with diazotized sulphanilic acid to form an azocompound, the color of which is measured at 546 nm. Total cholesterol (CHL) was determined by CHOD-PAP Method of Richmond (11) in which the free cholesterol is hydrolysed by cholesterol oxidase to cholestenone-4-en-3-one and hydrogen peroxide. Hydrogen peroxide by the action of peroxidase liberates oxygen which reacts with 4-amino antipyrine and phenol to form red coloured compound which is measured at 500 nm.

Total protein (TPTN) was estimated by Biuret method (12) where proteins produce a violet colour complex with copper ions in an alkali solution. The absorbance of the colour complex is directly proportional to the protein in the sample, while the albumin (ALB) was estimated by BCG (13) involving formation of blue-green complex with bromocresol green at slightly acidic pH which is measured photometrically. All the estimations were carried out using standard kits on auto analyser of Merck make (300 TX, E.Merck-Micro Labs, Mumbai).

Histopathological studies

Animals from control and treated groups were used for this purpose. The animals were sacrificed and the abdomen was cut open to remove the liver. The liver was fixed in Bouin's solution (mixture of 75 ml of saturated picric acid, 25 ml of 40% formaldehyde and 5ml of glacial acetic acid) for 12 h, then embedded in paraffin using conventional methods (14) and cut into 5 µm thick sections and stained using haematoxylineosin dye and finally mounted in diphenyl xylene. The sections were then observed under microscope for histopathological changes in liver architecture and their photomicrographs were taken.

Hepatoprotective activity in vitro Hepatotoxins and test substances

For *in vitro* studies CCL_4 (0.1 N), was used to produce sub maximal toxicity in isolated rat hepatocytes. The test solutions are tested at dose levels of 100, 500 and 1000 µg/ml. Silymarin was used as a positive control at a dose of 100 µg/ml. All the substances are dissolved in a vehicle 30% DMSO (15).

Isolation of rat hepatocytes

The method developed by Sarkar and Sil, (16) was used for the isolation of hepatocytes with slight modifications. The livers were isolated under aseptic conditions and placed in HEPES (*N-2-hydroxyethylpiperazine-N-2-ethanesulphonic acid*) buffer I containing HEPES (0.01 M), NaCl (0.142 M), and KCl (0.0067 M), pH 7.4. The livers were cut into small pieces and then incubated with a second buffer containing HEPES (0.1 M), NaCl (0.0667 M), KCl (0.0067 M), and 0.5% Collagenase type IV, pH 7.6 for about 45 min at 37°C in an incubator with constant shaking. Hepatocytes were obtained after filtration and cold centrifugation (4°C, 200 rpm/min for 2 min, and 3 times) and suspended in HEPES buffer I. The viability of the hepatocytes was assessed by trypan blue (0.2%) exclusion method.

Primary cultures of rat hepatocytes

The method of Tingstrom and Obrink (17) with slight modifications was used for the primary culturing of rat hepatocytes. The freshly isolated viable hepatocytes were suspended in culture medium RPMI-1640 supplemented with calf serum (10%), HEPES and gentamicin (1 µg/ml). These cells (approximately 1–1.2 X 10^6/ml) were then seeded into culture bottles and incubated at 37°C in atmosphere of 5% CO_2 in carbon dioxide incubator. Upon incubation for 24 h the hepatocytes formed a mono layer. The newly formed cells were round and most appeared as individual cells. These cells were 95–96% viable as confirmed by trypan blue exclusion test.

Hepatic cytotoxicity testing

The sub fractions, EMKMFME and MFMFME were tested for their hepatic cytotoxicity at 500, 1000 and 1500 µg/ml on isolated rat hepatocytes. After 24 h of incubation at 37°C in CO_2 incubator, the percentage viability of hepatocytes was tested using trypan blue exclusion (18) and by the estimation of total protein content.

Hepatoprotective activity

Twenty four hours after the establishment of the monolayers of hepatocytes, the medium was decanted and the culture was washed with HEPES buffer I and finally the hepatocytes were suspended in Buffer I. The hepatic cytotoxicity was induced with CCl_4 (0.1 N). Triplicate hepatocyte suspensions (0.1 ml) from different cultures were distributed into various culture tubes labelled as control, toxicant, standard (silymarin + toxicant) and test (test sample + toxicant). The control group received 0.1 ml of vehicle (30% DMSO) and toxicant groups received 0.1 ml of CCl_4, while the test groups received

0.1 ml of respective test solutions (100, 500 and 1000 µg/ml) followed by 0.1 ml of hepatotoxin. The standard groups received 0.1 ml of silymarin solution (100 µg/ml) followed by hepatotoxins. The content of the all culture tubes were made up to 1 ml with HEPES buffer I. The contents of all the tubes were mixed well and incubated in a CO_2 incubator for 24 h at 37°C. In test and standard groups the hepatocytes were pre incubated with respective solutions for 30 min and then exposed to hepatotoxins. After incubation hepatocyte suspensions were collected to assess cell damage. Cell viability was evaluated by trypan blue exclusion method. Hepatocytes suspensions were centrifuged at 200 rpm and leakage of the enzymes GOT, GPT and total proteins secreted outside the cells were determined from the supernatant (18).

Assessment of hepatoprotective activity

The effect of different extracts in liver protection was determined by measuring an increase in the percentage of viable cells in that group of cells incubated with extracts, compared with the control and toxicant groups. Reversal of toxin-induced elevations in the levels of enzymes and toxin-induced reductions in levels of proteins were also considered to be an important criterion of hepatoprotective activity. GOT and GPT were estimated by a UV-Kinetic method based on the reference method of International Federation of Clinical Chemistry (8). TPTN (12) was estimated by Biuret method.

Statistical analysis

The mean values±SEM are calculated for each parameter. For determining the significant inter group difference each parameter was analysed separately and one-way analysis of variance (19) was carried out and the individual comparisons of the group mean values were done using Dunnet's test (20).

RESULTS

Acute toxicity studies.

The EMKMFME and MFMFME did not cause any mortality up to 2000 mg/kg and were considered as safe.

When normal hepatocytes were treated with the extracts under test, there are no alterations in the values of % viable cells and TPTN content as compared to the control at the dose level up to 1500 µg/ml indicating that the extracts were not toxic to the cells.

Carbon tetrachloride-induced hepatotoxicity

The results of CCl_4-induced hepatotoxicity *in vivo* were represented in Table 1. CCl_4 intoxication in normal rats elevated the levels of GOT, GPT, ALKP, TBL, and CHL; where as decrease in the levels of TPTN and ALB were observed significantly indicating acute hepatocellular damage and biliary obstruction. The rats that have received 150 mg/kg of MFMFME showed a significant ($P<0.05$) decrease in all the elevated SGOT, SGPT, ALKP, TBL and CHL levels and significant ($P<0.05$) increase in reduced TPTN and ALB levels as observed with silymarin. The rats which received EMKMFME have also shown significant ($P<0.05$) changes in the levels of all biochemical parameters except ALB.

Histopathological examination of liver sections of control group showed normal cellular architecture with distinct hepatic cells, sinusoidal spaces and central vein (Fig.1a). Disarrangement of normal hepatic cells with necrosis and vacuolization are observed in CCl_4-intoxicated liver (Fig.1b). The liver sections of the rat treated with 150 mg/kg of MFMFME followed by CCl_4 intoxication (Fig.1d), showed less vacuole formation and absence of necrosis and overall less visible changes observed as observed with silymarin treated group (Fig.1c), supplementing the protective effect of the

Table 1: Effect of Baliospermum montanum on carbon tetrachloride-induced toxicity in rats.

GROUP	GOT (IU/L)	GPT (IU/L)	ALKP (IU/L)	TBL (mg/dl)	CHL (mg/dl)	TPTN (g/dl)	ALB (g/l)
Control	118.17 ± 04.87	104.67 ± 03.87	191.83 ± 08.99	1.48 ± 0.19	95.50 ± 05.50	6.53 ± 0.57	4.10 ± 0.41
CCl_4	299.50 ± 21.12	384.00 ± 15.45	436.50 ± 31.76	4.36 ± 0.57	205.50 ± 16.97	2.76 ± 0.45	2.01 ± 0.19
Silymarin (50 mg/kg)	120.33 ± 04.28*	120.50 ± 05.71*	188.17 ±10.14*	2.03 ± 0.20	100.83 ± 06.81	6.03 ± 0.58	4.13 ± 0.38
EMKMFME (50 mg/kg)	291.50 ± 10.47	331.83 ± 29.92	403.00 ± 35.49	3.50 ± 0.54	193.50 ± 19.13	3.26 ± 0.56	1.96 ± 0.27
EMKMFME (100 mg/kg)	271.33 ± 10.26	323.50 ± 18.03	387.83 ± 19.45	3.08 ± 0.61	185.81 ± 22.41	2.53 ± 0.47	2.48 ± 0.28
EMKMFME (150 g/kg)	176.50 ± 20.06*	175.17 ± 09.29*	273.50 ± 19.26*	2.43 ± 0.26*	134.17 ± 06.81*	5.18 ± 0.50**	2.95 ± 0.37
MFMFME (50 mg/kg)	260.67 ± 14.14	317.67 ± 20.26	372.83 ± 21.61	4.10 ± 0.69	184.67 ± 13.91	3.56 ± 0.48	2.67 ± 0.33
MFMFME (100 mg/kg)	206.50 ± 08.30*	258.17 ± 27.74*	350.66 ± 31.99*	2.88 ± 0.25	157.50 ± 16.91	4.35 ± 0.45	3.83 ± 0.24
MFMFME (150 mg/kg)	129.00 ± 08.85*	127.33 ± 07.18*	209.17 ± 14.57*	2.31 ± 0.23*	106.82 ± 06.95*	6.21 ± 0.45**	4.02 ± 0.56**

Data represents the mean±SEM of six animals. MFMFME: Methanol sub fraction of methanol fraction, EMKMFME: Ethyl methyl ketone sub fraction of methanol fraction.

*Significant reduction compared to CCl_4 ($P<0.05$).

**Significant increase compared to CCl_4 ($P<0.05$)

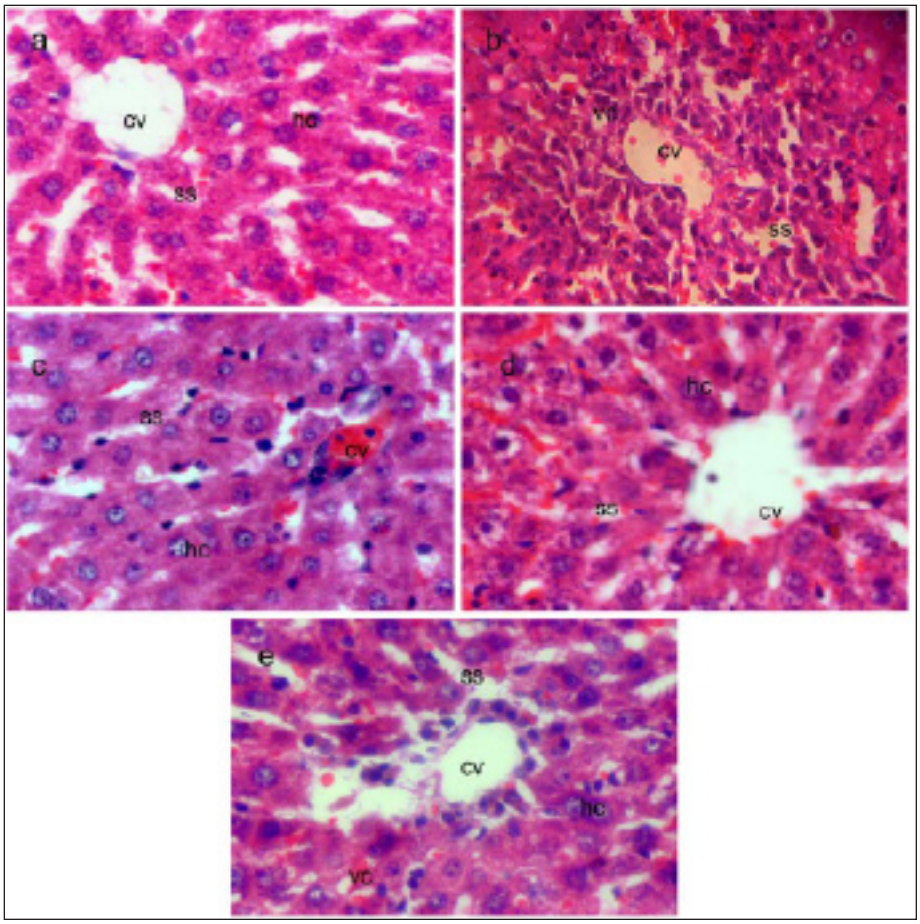

Figure 1: Representative photomicrographs of histopathological changes showing effect of the test material on the rats intoxicated with carbon tetrachloride.
Fig.1a Control
Fig.1b Carbon tetrachloride, 1.25 ml/kg i.p.
Fig.1c Silymarin, 100 mg/kg p.o.
Fig.1d MFMFME, 150 mg/kg p.o.
Fig.1e EMKMFME, 150 mg/kg. 400 X. Haematoxylin-eosin stain. cv: central vein, vc: vacuole, ss: sinusoidal spaces, hc: hepatocytes.

extract. Liver sections of the rat treated with 150 mg/kg of EMKMFME followed by CCl_4 intoxication (Fig.1e) also showed protective effect, but with less intensity.

Incubation of hepatocytes with 10 µl/ml solution of CCl_4 resulted in induction of significant ($P<0.05$) sub maximal toxicity which was indicated by 62.32% and 56.60% depletion in viability and TPTN content of hepatocytes respectively. Similarly an elevation about 150.02% and 146.92% of GOT and GPT levels are observed respectively upon intoxication with CCl_4.

Hepatocytes treated with MFMFME in concentrations of 500–1000 µg/ml showed a concentration dependant significant ($P<0.05$) protective effect by restoring the viability of hepatocytes (43.03–66.69%), TPTN content (47.57–.67%), GOT (71.82–79.65%) and GPT (45.63–64.18%). EMKMFME at a concentration of 1000 µg/ml also showed significant ($P<0.05$) protective effect by restoring the viability of cells (13.84%), TPTN (37.44%), GOT (38.03%) and GPT (34.07%), while the positive control silymarin showed good protective effect by restoring viability (86.35), TPTN (77.01), GOT (92.74) and GPT (89.52). The results obtained are shown in Table 2. MFMFME at dose level of 1000 µg/ml showed maximum protection though the activity was not similar ($P<0.05$) to that of silymarin.

DISCUSSION

The present studies were performed to assess the hepatoprotective activity in rats against carbon tetrachloride

Table 2: Effect of B. montanum against CCl4-induced toxicity on rat hepatocytes

Group	Viable cells (%)	GOT (IU/L)	GPT (IU/L)	TPTN (g/dl)
Control	95.84 ± 0.34	19.11 ± 0.08	20.67 ± 0.29	4.01 ± 0.02
CCl_4 10 µl/ml	36.11 ± 0.61	47.78 ± 0.27	51.04 ± 0.33	1.74 ± 0.12
Silymarin 100µg/ml	87.82 ± 0.90* (86.35)	21.13 ± 0.52* (92.74)	23.83 ± 0.17* (89.52)	3.42 ± 0.08** (77.01)
EMKMFME 100µg/ml	38.22 ± 0.60 (03.52)	46.21 ± 0.63 (05.46)	50.62 ± 0.25 (01.38)	1.79 ± 0.06 (02.20)
EMKMFME 500 µg/ml	39.11 ± 1.14 (05.01)	44.79 ± 0.90 (10.40)	47.22 ± 0.03 (12.56)	2.12 ± 0.04 (16.73)
EMKMFME 1000 µg/ml	44.40 ± 0.56* (13.84)	36.85 ± 0.35* (38.03)	40.53 ± 0.36* (34.57)	2.59 ± 0.12** (37.44)
MFMFME 100µg/ml	53.46 ± 0.28* (28.97)	44.67 ± 0.39 (10.82)	43.42 ± 0.27* (25.06)	2.62 ± 0.18** (38.76)
MFMFME 500 µg/ml	61.88 ± 0.82* (43.03)	27.14 ± 0.57* (71.82)	37.17 ± 0.05* (45.63)	2.82 ± 0.06** (47.57)
MFMFME 1000 µg/ml	76.05 ± 0.80* (66.69)	24.89 ± 0.37* (79.65)	31.53 ± 0.31* (64.18)	3.14 ± 0.04** (61.67)

Data represents the mean±SEM of three values, MFMFME: Methanol sub fraction of total methanol extract, EMKMFME: Etylmethyl ketone sub fraction of total methanol extract.

*Significant reduction compared to hepatotoxin ($P<0.05$).

**Significant increase compared to hepatotoxin ($P<0.05$).

as hepatotoxin to prove its claims in Ayurveda against liver disorders and also to develop an in vitro test system using primary cultured rat hepatocytes. CCl_4-induced hepatic injury is commonly used as an experimental method for the study of hepatoprotective effects of medicinal plants extracts and drugs. CCl_4 is metabolised by the microsomal cytochrome P_{450} to a highly reactive trichloromethyl free radical and trichloromethyl peroxy radical. These free radicals start a chain of free radical formation which attack membrane lipids and proteins there by causing the destruction of microsomes and liver cells causing cell lysis. Leakage of cytosolic enzymes out of the cells thus occurs due to increase in cell permeability, membrane damage and cell necrosis (21–22).

From the Table 1 it was evident that MFMFME was able to reduce all the elevated biochemical parameters due to the hepatotoxin intoxication. The levels of total proteins and albumin were reduced due to the CCl_4 induced hepatotoxicity. The reduction is attributed to the initial damage produced and localised in the endoplasmic reticulum leading to its functional failure with a decrease in protein synthesis and accumulation of triglycerides leading to fatty liver. Inhibition of bile acids synthesis from cholesterol which is synthesized in liver or derived from plasma lipids, leading to increase in cholesterol levels was also resulted due to CCl_4 intoxication. Suppression of cholesterol levels by the extracts suggest the bile acids synthesis inhibition was reversed. Reduction in the levels of SGOT and SGPT towards the normal value is an indication of regeneration process. Reduction of ALKP levels with concurrent depletion of raised bilirubin level suggests the stability of the biliary function during injury with CCl_4. The protein and albumin levels were also raised suggesting the stabilization of endoplasmic reticulum leading to protein synthesis. The protective effect exhibited by MFMFME at dose level of 150 mg/kg was comparable with the standard drug silymarin.

The histological examination of the liver sections reveals that the normal liver architecture was disturbed by hepatotoxin intoxication. In the liver sections of the rats treated with MFMFME and intoxicated with CCl_4 the normal cellular architecture was retained as that of silymarin, there by confirming the protective effect of the extract.

Isolated hepatocytes have become a useful model for pharmacological, toxicological, metabolic and transport studies of xenobiotics since the development of techniques for high yield isolation of rat hepatocytes (23). Freshly isolated rat hepatocytes are also very useful and common tool for study of cytotoxicity and metabolic studies in this area, as they keep enzymatic activity similar to in vivo for several hours (24). Various hepatotoxins viz. carbon tetrachloride, thioacetamide and paracetamol have been shown to result in the reduction of viability of hepatocytes, and leakage of enzymes which are considered to be the markers of cellular injury Similar changes in the present study confirm these changes and also indicate satisfactory standardisation of our isolation and culture procedures.

In the present study, the hepatotoxin employed reduced cell viability possibly due to injury of plasma membrane of hepatocytes resulting in the leakage of cellular enzymes. Incubation of hepatocytes with extracts significantly restored their viability as well as altered biochemical parameters induced by hepatotoxin (Table 2). In our study we have observed significant but sub maximal toxicity after 24 h of incubation with hepatotoxin. Though both the extracts were able to restore the changes caused by hepatotoxin significantly, the restoration exhibited by MFMFME at dose levels of 1000 µg/kg was high.

In accordance with these results, it may be hypothesized that flavonoids, which are present in MFMFME, could be considered responsible for the hepatoprotective activity. In conclusion this study underlines the therapeutic potential

of *Baliospermum montanum*. Further work is in progress in an attempt to isolate and estimate the flavonoids of bio active extract.

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