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Hepatoprotective Effect of Stamen Extracts of *Mesua ferrea* L. against Oxidative Stress induced by CCl_4 in Liver Slice Culture Model

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Abstract – Stamens of *Mesua ferrea* L. are a well-known herbal drug used in Indian System of Traditional Medicine to treat various diseases. The claimed activity of this plant part is necessitated to investigate antioxidant and hepatoprotective activity. Authenticated plant sample was extracted with hexane, ethanol (EtOH) and water (aq.) using ASE 100 accelerated solvent extractor. Antioxidant activity was evaluated by means of different *in vitro* assays. Hepatoprotective effect was investigated on carbon tetrachloride induced oxidative stress in liver slice culture model. Cytotoxic marker lactate dehydrogenase (LDH) released in culture medium and the activity of lipid peroxidation along with antioxidant enzymes (AOEs) namely superoxide dismutase (SOD), catalase (CAT) and glutathione reductase (GR) were estimated. Hexane and EtOH extracts were significantly inhibited DPPH, NO, SOD and $ABTS^+$ radical in dose dependent manner. The trade of phenol content was: aq. extract < hexane extract < EtOH extract. A significant correlation was shown by total phenol content and free radical scavenging activity of extracts. The culture system treated with hexane extract, EtOH extract or ascorbic acid exhibited significant depletion in LDH, lipid peroxidation, antioxidative enzymes SOD, CAT and GR. Hexane extract and EtOH extracts of stamen of *M. ferrea* protected liver slice culture cells by alleviating oxidative stress induced damage to liver cells.
Keywords – *Mesua ferrea* extracts, Photochemiluminescence, CCl_4, Oxidative stress, Liver slice culture model

Introduction

Mesua ferrea L. (Family – Calophyllaceae) is a well known herbal drug commonly known as 'Indian Rose Chestnut' in English. The plant is distributed over India, Sri Lanka, Thailand, Cambodia, Malaysia, Myanmar, Philippines, Singapore and Vietnam. Flowers are terminal as well as axillary, bisexual, solitary or up to 9-flowered open panicle; pedicel with small paired bracts; stamens numerous, free or connate only at the base. Stamens of *M. ferrea* are used in Ayurvedic System of Medicine for treatment of fever, itching, nausea, leprosy, dysentery, skin diseases, bleeding piles, metrorrhagea, menorrhagea, excessive thirst, and sweating cough, dysentery and liver diseases (Dymock *et al*., 1890; Anonymous, 1962; Desai, 1975; Deshpande *et al*., 1989). It is a major constituent of many common household, ayurvedic medicinal formulations like *Samsarkara churna*, *Pushyanugachurna*, *Vyaghri-hareetaki - avaleha*, *Amritarishta*, *Lavanabhaaskarchurna*, *Kanakaasava* and *Khadirishta* (Desai, 1975; Deshpande *et al*., 1989; Joseph *et al*., 2010). Flavanoids (mesuaferrone A and B), cyclohexadiane derivatives (mesuaferrol, mesuanic acid) and other compounds (β-amyrin, β-sitosterol) have been reported from stamens (Banerji & Chowdhuary, 1993). Essential oil has been found to possess antihelminthic activity against hookworm and tapeworm (Banerji & Chowdhuary, 1993). Acetone extract of stamens has been reported to be non-toxic on mice at 1600 mg/kg (*p.o.*) (Sharma, 2002). The radical scavenging activity of methanolic extract of stamen has been evaluated (Bagul *et al*. 2006).

Previously, antioxidant and hepatoprotective activity has been reported on methanolic extract of flowers (Garge *et al*., 2009). Stamens of *M. ferrea* are recommended plant part by Ayurvedic literature (Dymock *et al*., 1890; Anonymous, 1962; Desai, 1975; Deshpande *et al*., 1989; Sharma, 2002). However, the therapeutic uses of stamens have not been widely studied. Hence, to investigate the hepatoprotective effects of this plant part, the work has been emphasized on evaluation of *in vitro* antioxidant and hepatoprotective activity of various extracts of *M. ferrea* stamen against CCl_4 induced oxidative stress in liver slice culture model employing photochemiluminescence and spectrophotometric methods.

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Experimental

Collection of plant material and extraction – The plant material was collected in bulk quantity from Sindhudurg, (Maharashtra, India) during post monsoon season of 2008. The plant sample was identified, authenticated and deposited in the crude drug repository of Agharkar Research Institute, Pune 411 004; vide voucher specimen number: I/F 029.

Stamens from flowers were shade dried, coarsely powdered and stored in an airtight container at 25 ± 4 °C. Powdered material (75 g) was extracted successively with hexane, ethanol and water using ASE 100 accelerated solvent extractor (Dionex, Vienna, Austria). Extraction was performed at 100 bar pressure and temperature of 60 °C for 20 min in five replicate cycles. The extracts were concentrated under vacuum using rotary evaporator and yields of hexane, ethanol (EtOH) and water extracts (aq.) were 3.95, 5.46 and 8.15 g respectively.

Radical-scavenging effect of extracts in DPPH radicals – DPPH radical-scavenging ability was assessed according to method of Jung *et al.* (2005). Briefly, to a methanolic solution of DPPH (60 mM, 2 ml), 50 µl of each test extract at different concentrations dissolved in methanol was added. Absorbance measurements of DPPH radical with/without extract and control commenced immediately at 515 nm. Decrease in absorbance was determined after 70 min when the absorbance stabilized. Ascorbic acid was used as a reference antioxidant. Percent inhibition of DPPH radical in samples was calculated according to the formula:

$$\% \text{ Inhibition} = [(A_{C(0)} - A_{A(t)}) / A_{C(0)}] \times 100$$

Where $A_{C(0)}$ is the absorbance of the control at $t = 0$ min and $A_{A(t)}$ is the absorbance in presence of antioxidant at $t = 70$ min.

Nitric oxide scavenging activity – Nitric oxide scavenging activity was measured according to method of Marcocci *et al.* (1994). Sodium nitroprusside (5 mM) in phosphate buffered saline was mixed with each test extracts of different concentrations and incubated at 25 °C for 30 min and 1.5 ml of the incubated solution was removed and diluted with 1.5 ml of Griess' reagent. Absorbance of the chromophore formed during diazotization of nitrite with sulfanilamide and subsequent coupling with naphthylethylene diamine was measured at 546 nm along with control. Ascorbic acid was used as a reference antioxidant. The percentage inhibition of nitric oxide generated was measured by comparing the absorbance values of control and test samples.

Superoxide scavenging activity – Superoxide scavenging activity was carried out by using alkaline DMSO method (Henry, 1976). Solid potassium superoxide was allowed to stand in contact with dry DMSO for at least 24 h and the solution was filtered immediately before use. Filtrate (200 ml) was added to 2.8 ml of an aqueous solution containing nitroblue tetrazolium (56 mM), EDTA (10 mM) and potassium phosphate buffer (10 mM, pH 7.4). Each test extract (1 ml) of different concentrations in water was added to the reaction mixture and the absorbance was recorded at 560 nm against a control in which pure DMSO has been added instead of alkaline DMSO.

Trolox equivalent antioxidant capacity (TEAC) assay – Total antioxidant activity of the extract was measured using the TEAC assay (Miller et al., 1995) with minor modifications. The TEAC value is based on the ability of the antioxidant to scavenge the blue-green 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonate) ($ABTS^{\cdot}+$) radical cation relative to the ABTS+ scavenging ability of the water soluble vitamin E analogue, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox). $ABTS^{\cdot}+$ radical cation was generated by the interaction of $ABTS^{\cdot}+$ (100 µM), H_2O_2 (50 µM) and horse raddish peroxidase enzyme (4.4 unit/ml). To measure antioxidants capacity, 0.25 ml of each extract (25 µg/ml) was mixed with an equal volume of $ABTS^{\cdot}+$, H_2O_2, peroxidase and deionized water. The reaction mixture was allowed to stand for 10 min at room temperature. Decrease in absorption at 734 nm after the addition of the reactant was used to calculate the TEAC value. The TEAC value is expressed as the milimolar concentration of Trolox solution having an antioxidant equivalent to a 1000 ppm solution of the sample under investigation. Higher the TEAC value of sample, stronger the antioxidant ability.

Photochemiluminescence Assay – Method of Photo-chemiluminescence (PCL) was used for determination of integral antioxidative capacity (AC) of methanol and water-soluble substances in extracts. Photochem® apparatus (Analitik jena AG, Germany) was used to determine the antioxidant capacity of the extracts against superoxide anion radicals generated from luminol which plays a double role of photosensitizer as well as the radical detecting agent. The activity was measured using standard kit ACW and ACL (Analitik jena AG, Germany). Each extract was measured at 10 µg/ml concentrations. A standard curve was plotted and the results were calculated for methanol soluble substance in trolox and for water soluble substance in ascorbic acid equivalents (nmol/g) (Govindrajan *et al.*, 2004).

Total phenolic content – Total phenolic content in each extract was determined with folin-ciocalteu reagent (Slinkar & Singleton, 1977) using pyrocatechol as a reference standard. About 0.1 ml extract was mixed thoroughly with 2 ml of 2% Na_2CO_3. After 5 min of incubation, 0.1 ml of 50% folin-ciocalteu reagent was added and allowed to stand for 2 h with intermitted shaking. The absorbance was measured as micrograms of pyrocatechol equivalent (PCE) by using an equation that was obtained from the standard graph.

Experimental animals – To assess the hepatoprotective activity, adult albino mice (6 - 8 weeks old) of either sex breed in the animal house of Agharkar Research Institute, Pune- 411 004, were used for the preparation of liver slices. The approval for this work, using animals, was taken from the Institutional Animal Ethical Committee of Agharkar Research Institute, Pune- 411 004.

Liver slice culture – Liver slice culture was maintained following the protocol developed by Wormser and Ben (1990) and Invittox protocol No. 42. (1992). The mice were dissected open after cervical dislocation; liver lobes were removed and transferred to pre-warmed Kred's Ringer Hepes (KRH) (2.5 mM Hepes, pH 7.4, 11 8 mM NaCl, 2.85 mM KCl, 2.5 mM $CaCl_2$, 1.5 mM KH_2PO_4, 1.18 mM $MgSO_4$, 5 mM β-hydroxy butarate and 4.0 mM glucose). The liver was cut into thin slices using sharp blade. The slices were weighed and the slices weighing between 4 and 6 mg were used for the experiment. Each experimental system contained 20 - 22 slices weighing together 100 - 120 mg. Slices were washed with 10 ml KRH medium, every 10 min over a period of 1 h. These were then pre-incubated for 60 min in small plugged beakers containing 2 ml KRH on a shaker water bath at 37 ºC. At the end of pre-incubation, the medium was replaced by 2 ml of fresh KRH and incubated for 2 h at 37 ºC.

Experiment design – The liver slices were further divided into individual cultures for the further respective treatments. Set 1, control, Set 2, 15.5 mM CCl_4; Set 3, 50 μg/ml hexane extract; Set 4, 50 μg/ml EtOH extract; Set 5, 50 μg/ml aq. extract; Set 6, 15.5 mM CCl_4 + different concentration (10, 25, 50 μg/ml) of hexane extract; Set 7, 15.5 mM CCl_4 + different concentration (10, 25, 50 μg/ml) of EtOH; Set 8, 15.5 mM CCl_4 + different concentration (10, 25, 50 μg/ml) of aq.extract; Set 9, 15.5 mM CCl_4 + 10 mM ascorbic acid; Set 10, 10 mM ascorbic acid. After the respective treatments, all the cultures were incubated in constant temperature water bath at 37 ºC for 2 h. At the end of incubation, each group of slices was homogenized in appropriate volume of chilled potassium phosphate buffer (100 mM, pH 7.8) in an ice bath to give a tissue concentration of 100 mg/ml. The culture medium was collected and used for estimation of lactate dehydrogenase (LDH), which was employed as a cytotoxicity marker. The homogenates were centrifuged at 10,000 rpm for 10 min at 4 ºC and the supernatants assayed for LDH, catalase, peroxidase and superoxide dismutase. Ascorbic acid (AA) was used as standard.

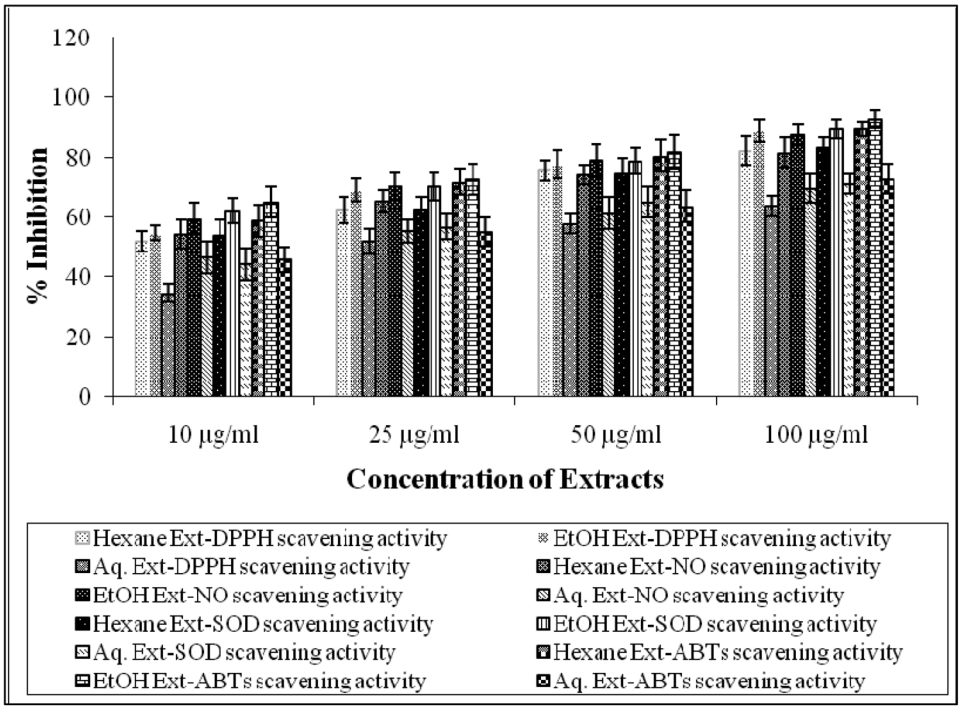
Measurement of lactate dehydrogenase activity – Lactate dehydrogenase (LDH; EC 1.1.1.27) activity was measured at 340 nm (Renner *et al*., 2003). Each unit of enzyme was calculated as 1 μmol of nicotinamide adenine phosphate (NAD) reduced per minute. Commercially available LDH was used as the standard.

Measurement of lipid peroxidation – Lipid peroxidation was estimated in terms of thiobarbaturic acid reactive substances (TBARS) formed in liver tissue homogenate, with some modification (McMillan *et al*., 1998). Tissue homogenate was prepared in 5% trichloroacetic acid (TCA). To 1 ml homogenate, 4 ml of 0.5% thiobarbituric acid in 20% TCA was added and incubated at 95 ºC for 30 min. The mixture was immediately cooled on ice, during which colour changed from orange to pink. Mixture was centrifuged at 4000 rpm for 10 min. Estimation of μmol of malondialdehyde (MDA) formed was done by calculating the difference in the absorbance of the supernatant at 532 nm (specific) and 600 nm (non-specific).

Measurement of antioxidant enzymes – The superoxide dismutase (SOD) activity was assayed spectrophotometrically by means of inhibition of pyrogallol autooxidation (Marklund & Marklund, 1974). Extent to which the enzyme decreases reduction of nitroblue-tetrazolium (NBT) by superoxide radical generated by riboflavin in the presence of light was monitored at 560 nm. One unit of enzyme was defined as the amount of enzyme causing 50% reduction in formazan formation under specified conditions. Catalase (CAT; EC 1.11.1.6) assay was carried out according to method of Aebi (1983). One unit was defined as amount of the enzyme that converts 1 μmol H_2O_2 to water in 1 min. Glutathione reductase (GR; EC 1.6.4.2) was estimated according to method by Ellman (1959) and Bulaj *et al*. (1998). One unit was defined as the amount of enzyme required for oxidization of 1 μmol of nicotinamide adenine dinucleotide phosphate (NADPH) to reduce of Nicotinamide adenine dinucleotide phosphate (NADP) per minute.

Estimation of proteins – Protein in the tissue homogenates were estimated according to the method of Bradford (1976).

Statistical analysis – Data were expressed as mean ±



Values are mean ± SD of three experiments

Fig. 1. Percentage inhibition of free radical by *M. ferrea* stamen extracts at various concentrations

Table 1. Integral Antioxidant Capacity (IAC) of *M. ferrea* stamen extracts – Water-soluble antioxidant capacity, corresponding to the activity expressed as nmol equivalents of ascorbic acid for each gram of tested extract; Lipid soluble antioxidant capacity, corresponding to the activity expressed as nmol equivalents of trolox for each gram of tested extract

Samples	Photochemiluminescence-ascorbic acid equivalent (nmol/g)	Photochemiluminescence-Trolox equivalent (nmol/g)
Hexane extract	2.713	12.322
EtOH extract	3.541	13.756
Aq. extract	2.541	7.890
AA	–	–

standard deviation. The results of treatment effects were analyzed using one-way ANOVA test (Graphpad Prism 4) and p values < 0.001 were considered as highly significant and p values < 0.05 were considered as significant.

Results and Discussion

Antioxidant potential was evaluated in terms of scavenging of DPPH, nitric oxide, SOD radical and TEAC, photochemiluminescence *in vitro* systems (Fig. 1 and Table 1). Hexane and EtOH extract exhibited high antioxidant activity followed aq. extract which significantly decreased the DPPH, NO, SOD and ABTS+ radical in dose dependent manner as compared to standard. IC_{50} values of hexane, EtOH, aq. extract and ascorbic acid were for DPPH 37.55 ± 1.45, 34.67 ± 1.67, 44.90 ± 1.34 and 4.1 µg/ml, respectively; for NO 38.33 ± 0.85, 34.66 ± 1.23, 45.34 ± 1.67 and 3.9 µg/ml, respectively; for superoxide radical, 39.33 ± 0.65, 34.66 ± 1.33, 48.34 ± 1.87 and 5.3 µg/ml, respectively; for $ABTS^{+}$ 37.89 ± 1.34, 35.78 ± 1.68, 50.87 ± 2.09 and 6.03 ± 0.98 µg/ml, respectively. Integral Antioxidant Capacity (IAC) represents the antioxidant capacity of hydrophilic and lipophilic antioxidants, calculated as nmol equivalents in activity of Trolox/ascorbic acid (Govindrajan *et al*., 2004). Sample extracts had distinctly varied ACW and ACL values. Hexane and EtOH extract exhibited high IAC than water extract (Table 1). The key role of phenolic compounds is the ability to scavenge free radicals and ROS such as singlet oxygen, superoxide free radical, and hydroxyl

Table 2. Effect of stamens extracts in protecting liver cells from CCl_4 induced cytotoxicity by ameliorating oxidative stress

Treatments	LDH Units/100 mg tissue wet wt.	SOD Units/100 mg tissue wet wt.	CAT Units/100 mg tissue wet wt.	GR Units/100 mg tissue wet wt.
Control	7.83±1.16	17.83±1.47	15.00±1.41	0.151±0.012
CCl_4	43.53±1.63	56.33±2.25	87.5±1.04	0.498±0.008
AA	7.53±1.47	17.5±2.14	15.33±1.75	0.150±0.007
Hexane extract	7.00±2.09	17.43±1.72	15.65±1.37	0.156±0.003
EtOH extract	8.33±1.16	17.23±1.47	15.83±1.22	0.159±0.011
Aq. extract	7.17±1.47	17.0±1.41	15.67±1.67	0.158±0.006
CCl_4 + Hexane extract 10 μg/ ml	34.33±1.04[a]	32.0±0.89[a]	44.67±1.03[a]	0.362±0.008[a]
CCl_4 +Hexane extract 25 μg/ml	27.5±1.09*	26.33±1.96*	35.5±1.04*	0.314±0.005*
CCl_4 + Hexane extract 50 μg/ ml	21.33±1.36*	20.67±0.81*	26.17±2.13*	0.261±0.007*
CCl_4 + EtOH extract 10 μg/ ml	27.83±0.75[a]	29.83±0.75[a]	39.5±1.37[a]	0.272±0.008[a]
CCl_4 + EtOH extract 25 μg/ ml	22.33±2.48*	24.5±0.54*	28.24±1.09*	0.236±0.008*
CCl_4 + EtOH extract 50 μg/ ml	18.83±1.21*	18.17±1.16*	22.74±2.28*	0.206±0.008*
CCl_4 + Aq. extract 10 μg/ ml	39.33±0.81[a]	48.67±0.81[a]	47.83±0.75[a]	0.386±0.055[a]
CCl_4 + Aq. extract 25 μg/ ml	31.0±0.89[a]	43.0±0.89[a]	39.5±0.54[a]	0.356±0.005[a]
CCl_4 + Aq. extract 50 μg/ ml	27.0±0.89*	38.17±1.94*	36.67±0.81*	0.298±0.008*
CCl_4 + 50 mM Ascorbic acid	13.67±1.03*	15.33±1.63*	19.83±1.83*	0.189±0.018*

Cytotoxicity was assessed in terms of % lactate dehydrogenase (LDH) released, and the response to oxidative stress was measured in terms of antioxidant enzymes SOD, superoxide dismutase; CAT, Catalase; GR, Glutathione reductase activity. Ascorbic acid was used as a standard. Values represent means of at least three experiments and their standard deviation.
[a] Significantly differ compared with respective CCl_4 treated group, $p < 0.05$
* Significantly differ compared with respective CCl_4 treated group, $p < 0.001$

radicals (Hall & Cuppett, 1997). Total phenol content, expressed as mg pyrocatechol equivalent (PCE)/g extracts. The trend of phenol content was as: aq. extract (104 mg PCE/g) < hexane extract (156 mg PCE/g) < EtOH extract (242 mg PCE/g). A significant correlation was shown by total phenol content and free radical scavenging activities of all extracts. The highest antioxidant activity of hexane extract and EtOH extract may be due to presence of phenolic compounds.

The liver slice culture is an *in vitro* technique consisting of highly organized cellular community in which different cell types are subject to mutual contact. It provides desirable complexity of structurally and functionally intact cells (Frazier, 1992; Naik *et al*., 2004). Oxidative stress was induced by adding cytotoxic CCl_4 to the liver slice culture. Release of LDH in the liver slice culture medium was used as cytotoxicity marker. CCl_4 was highly toxic to the treated cells as concentration of LDH was increased in the medium as compared to control. The hexane, EtOH and aq. extracts were found to be non-toxic at dose 50 μg/ml as it showed percentage release of LDH in the medium similar to that of control untreated slices. Therefore, in further experiments, all extracts at dose of 50 μg/ml were used.

LDH release in the culture system treated with CCl_4 was found to be 5 times more as compared to control. After addition of hexane and EtOH extracts along with CCl_4 cytotoxicant, the amount of LDH release in medium reduced highly significant ($p < 0.001$) in dose dependent manner. The activity was comparable with standard (Table 2). CCl_4 is known to generate oxidative stress in cells, which can be measured from the extent of lipid peroxidation in liver tissue. The lipid peroxidation levels in the liver slice culture medium were assessed by TBARS assay. Lipid peroxidation was measured in terms of thiobarbituric acid reactive substances and was expressed as μmol of malondialdehyde formed/100 mg tissue. The amount of lipid peroxidation increased folds in CCl_4 (Table 3) treated liver cells compared to respective control. The extent of lipid peroxidation was reduced to near control levels highly significant ($p < 0.001$) when liver cells were treated either with hexane and EtOH extracts than aq. extract along with CCl_4 (Table 3).

Time course of lipid peroxidation was assessed in the presence of cytotoxic agent alone and together with different extracts. CCl_4-treated cells showed increase in lipid peroxidation paralleled with the increase in LDH release by the cells. However, in presence of hexane and EtOH extracts along with cytotoxic agent the lipid peroxidation, like the LDH release, returned to the control

Table 3. Percentage release of LDH and extend of lipid peroxidation in liver slice culture in CCl_4 induced cytotoxicity

	Treatment	Time at which activity was measured			
		0.5 h	1 h	1.5 h	2 h
LDH release	Control	3.67 ± 0.97	4.33 ± 0.95	5.67 ±1.12	7.82 ± 1.23
	CCl_4	29.32 ± 1.34	34.55 ± 1.23	37.78 ± 1.56	43.98 ± 1.28
	AA	4.88 ± 0.98	6.09 ± 0.95	7.01 ± 1.16	7.34 ± 1.21
	Hexane ext	4.23 ± 0.87	5.13 ± 0.66	6.59 ± 1.23	7.67 ± 1.56
	EtOH ext	4.66 ± 0.97	4.91 ± 0.95	5.77 ± 1.12	7.55 ± 1.23
	Aq. ext	4.39 ± 1.34	5.60 ± 1.23	6.01 ± 1.56	6.93 ± 1.28
	CCl_4 +AA	18.58 ±0.97*	21.69 ±0.95*	15.27 ±1.12*	13.27 ±1.23*
	CCl_4 + Hexane ext	27.88± 0.98*	32.16 ± 0.95*	26.82 ± 1.12*	21.28 ± 1.24*
	CCl_4 + EtOH ext	21.64 ± 0.67*	30.37 ± 1.69*	24.13 ± 1.34*	20.27 ±1.56*
	CCl_4 + Aq. Ext	29.01 ±1.34[a]	38.08 ± 1.23[a]	31.66 ± 1.56[a]	27.02 ±1.28[a]
LPO release	Control	1.63 ±0.14	2.18 ± 0.15	2.23 ± 0.08	2.29 ± 0.18
	CCl_4	2.31± 0.17	2.89 ± 0.15	3.36 ± 0.15	3.91 ± 0.14
	AA	1.71 ± 0.18	2.02 ± 0.18	2.03 ± 0.16	2.16 ± 0.14
	Hexane ext	1.83 ± 0.28	2.01 ± 0.15	2.11 ± 0.17	2.18 ± 0.19
	EtOH ext	1.69 ± 0.14	2.09 ± 0.2	2.11 ± 0.19	2.16 ± 0.16
	Aq. ext	1.62 ± 0.13	2.00 ± 0.24	2.13 ± 0.22	2.21 ± 0.24
	CCl_4 + AA	1.72 ± 0.21*	2.21 ± 0.19*	2.53 ± 0.20*	2.25 ± 0.29*
	CCl_4 + Hexane ext	2.04 ±0.09*	2.36 ±0.11*	2.85 ±0.24*	2.47 ±0.28*
	CCl_4 + EtOH ext	2.11 ±0.15*	2.43 ±0.18*	2.94 ±0.09*	2.31 ±0.14*
	CCl_4 + Aq. Ext	2.34 ±0.22[a]	2.69 ±0.25[a]	3.02 ±0.14[a]	3.23 ±0.18[a]

Values are mean of three experiments; CCl_4 - Carbon tetrachloride; AA, Standard ascorbic acid, at 50 mM concentration; Ext, Extract; LPO, Lipid peroxidation

[a] Significantly differ compared with respective CCl_4 treated group, $p < 0.05$

* Significantly differ compared with respective CCl_4 treated group, $p < 0.001$

levels which was highly significant ($p < 0.001$) than aq. extract (Table 3). Since lipid peroxidation is caused by free radicals, all extracts appear to reduce the amount of free radicals substantially. CCl_4 induces oxidative stress in the cells by generation of ROS. Antioxidant enzymes (AOEs) SOD, CAT and GR protect cells from oxidative stress of highly reactive free radicals. Oxidative SOD and CAT are known enzymes to prevent damage by directly scavenging the harmful active oxygen species. GR plays a role in recycling the oxidized glutathione to reduced glutathione, which acts as an antioxidant (Naik *et al*., 2004). Activities of all three AOEs were checked in liver slice cultures treated with CCl_4 alone or CCl_4 and extracts. The activities of SOD, CAT and GR were increased in the liver tissue treated with CCl_4. The liver tissue treated with hexane and EtOH extracts along with CCl_4 showed highly significant ($p < 0.001$) reduced antioxidant enzymes activities when added along with the toxicants to the culture (Table 2).

The results in this study revealed that significant depletion was observed in the lipid peroxidation, antioxidative enzymes SOD, CAT, and GR on the administration of the hexane and EtOH extracts or ascorbic acid in the CCl_4 induced toxicity in the liver. In conclusion, the results of present study suggest that the hexane and EtOH extracts could prevent oxidative liver damage. Further comprehensive pharmacological investigations will be needed to validate the ethnobotanical claims and elucidate the mechanism of hepatoprotective effect.

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References

Aebi, H.E., Catalase. In: Bergmeyer, H.U., (eds.), Methods of Enzymatic Analysis, Vol. 3, Verlagchemie GmbH, Weinhein, 1983, pp. 277-282

Anonymous, The Wealth of India - A Dictionary of Indian Raw Material and Industrial Products, Raw Material Vol VI: L-M, New Delhi,

CSIR, 1962.
Bagul, M., Srinivasa, H., Anandjiwala, S. and Rajani, M., Phytochemical evaluation and free natural radical scavenging activity of stamen of *Mesua ferrea* Linn, var. *ferrea. Indian Drugs* **43**, 665-670 (2006).
Banerji, R. and Chowdhuary, A.R., *Mesua ferrea*: Chemical constituents and biological activity. *J. Chem. Soc. Pak.* **15**, 207-211 (1993).
Bradford, M.M., A rapid sensitive method for the quantitation of microgram quantities of protein utilizing the principle of dye binding. *Anal. Biochem.* **72**, 248-254 (1976).
Bulaj, G.T., Kortemme, T., and Goldenberg, D.P., Ionization-reactivity relationships for cysteine thiols in polypeptides. *Biochem.* **37**, 8965-8972 (1998).
Desai, V.G., Aushadhi Sangraha, Shri Gajanan Book Depo, Bombay, 1975.
Deshpande, A.P., Javalgekar, R.R., and Ranade, S., Dravyagunvidhyan, Anmola Prakhashan Pune, India, 1989.
Dymock, W., Warden, C.J., and David, H., Pharmacographica Indica Vol I., Ballimaran Jayyed Press Delhi, 1890.
Ellman, G.L., Tissue sulfhydryl groups. *Arch. Biochem. Biophys.* **82**, 70-77 (1959).
Frazier, J.M., *In vitro* toxicity testing: Application to safety evaluation, Marcel Dekker, Inc New York, 1992.
Garg, S., Sharma, K., Ranjan, R., Attri, P., and Mishra, P., In vivo Antioxidant activity and hepatoprotective effects of methanolic extract of *Mesua ferrea* Linn. *Int. J. PharmTech Res.* **1**, 1692-1696 (2009).
Govindrajan, R., Vijayakumar, M., Rao, C.V., Shirwaikar, A., Rawat, A.K.S., Mehrotra, S., and Pushpangadan, P., Antioxidant potential of *Anogeissus latifolia*. *Biol. Pharm. Bull.* **7**, 1266-1269 (2004).
Hall, C.A. and Cuppett, S.L., Structure-activities of natural antioxidants, In: Aruoma, O.I. and Cuppett, S.L., (eds.), Antioxidant methodology: *In vivo* and *in vitro* concepts, AOCS Press Champaign, IL, 1997, pp. 141-172.
Henry, L.E.A., Halliwell, B., and Hall, D.O., The Superoxide dismutase activity of various photosynthetic organisms measured by a new and rapid assay technique. *FEBS Lett.* **66**, 303-306 (1976).
Invittox Protocol No. 42., Liver slice hepatotoxicity screening system. The ERGATT/FRAME Data Bank of in vitro techniques in toxicology, INVITTOX, England, 1992.
Joseph, C.R., Ilanchezhian, R., Biswajyoti, P., and Harish, C.R., Pharmacognostic studies of Nagakeshar (*Mesua Ferrea* L.) - An ingredient in Vyaghrihareetaki Avaleha. *Int. J. Res. Ayurveda Pharm.* **1**, 264-272 (2010).
Jung, K.-A., Song, T.-C., Han, D., Kim, I.-H., Kim, Y.-E., and Lee, C.-H., Cardiovascular protective Properties of Kiwi fruit extracts *in vitro*. *Biol. Pharm. Bull.* **28**, 1782-1785 (2005).
Magalhaes, L.M., Segundo, M.A., Reis, S., and Lima, L.M.C., Methodological aspects about *in vitro* evaluation of antioxidant properties. *Anal. Chim. Acta.* **61**, 1-19 (2008).
Makker, H.P.S., Dawra, R.K., and Singh, B., Determination of both tannin and protein in tannin-protein complex. *J. Agric. Food Chem.* **36**, 523-525 (1988).
Marcocci, L., Maguire, J.J., Droy-Lefaix, M.T., and Parker, L., The nitric oxide-scavenging properties of *Ginkgo biloba* extract EGB 761. *Biochem. Biophys. Res. Comm.* **201**, 748-755 (1994).
Marklund, S. and Marklund, G., Involvement of superoxide anion radical in auto oxidation of pyrogallol and a convenient assay for superoxide dismutase. *Eur. J. Biochem.* **47**, 469-464 (1974).
McMillan, D.C., Charles, B.J. and Jollow, D.J., Role of lipid peroxidation in dapsone-induced hemolytic anemia. J. Pharmacol. Exp. Ther. **287**, 868-876 (1998).
Miller, N.J., Diplock, A.T., and Rice-Evans, C.A., Evaluation of the total antioxidant as a marker of the deterioration of apple juice on storage. *J. Agric. Food Chem.* **43**, 1794-1801 (1995).
Naik, R.S., Mujumdar, A.M., and Ghaskadbi, S., Protection of liver cells from ethanol cytotoxicity by curcumin in liver slice culture *in vitro*. *J. Ethnopharmacol.* **95**, 31-37 (2004).
Renner, K., Amberger, A., Konwalinka, G., Kofler, R., and Gnaiger, E., Changes of mitochondrial respiration, mitochondrial content and cell size after induction of apoptosis in leukemia cells. *Biochem. Biophys. Acta.* **1642**, 115-123 (2003).
Sharma, P.C., Yelne, M.B., and Dennis, T.J., Database on medicinal plants used in Ayurveda Vol 5, CCRS, Department of ISM & H, Ministry of Welfare, India, 2002.
Slinkard, K. and Singleton, V.L., Total phenol analysis: Automation and comparison with manual methods. *Am. J. Enol. Vitic.* **28**, 49-55 (1977).
Wormser, U. and Ben, Z.S., The liver slice system-an in vitro acute toxicity test for assessment of hepatotoxins and their antidotes. *Toxicol. In Vitro* **4**, 449-451 (1990).

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