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Hepatoprotective Activity of Methanolic Extract of *Mallotus philippensis* (Lam.) muell.-arg in Rats

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The main objective of the study is to investigate the hepatoprotective properties of the methanolic extract of the *Mallotus philippensis* (Euphorbiaceae) leaves, against ethanol induced liver damage in albino rats. The methanolic extract of *Mallotus phillipensis* was evaluated for potential hepatoprotective activity in albino rats by ethanol induced hepatotoxicity after pretreatment with two different doses (100 mg/kg and 200 mg/kg b.w, p.o) of methanolic extract of the *Mallotus philippensis*. Silymarin (25 mg/kg) served as standard hepatoprotective agent. Ethanol produced significant changes in physical (increased liver weight and volume), biochemical (increase in serum alanine transaminase, aspartate transaminase, alkaline phosphatase, direct bilirubin, total bilirubin, cholesterol, triglycerides and decrease in total protein level), antioxidant (catalase, superoxide dismutase and lipid peroxidation), histological (damage to hepatocytes) and functional (thiopentone-induced sleeping time) liver parameters. Pretreatment with methanolic extract of the *Mallotus philippensis* leaves significantly prevented the physical, biochemical, antioxidant, histological and functional changes induced by ethanol in the liver. The present study indicates that methanolic extract of the *Mallotus philippensis* leaves possessed hepatoprotective activity in the doses used.

Key Words: Ethanol, Hepatoprotective activity, *Mallotus philippensis*.

INTRODUCTION

The liver regulates many important metabolic functions. Hepatic injury is associated with distortion of these metabolic functions[1]. Additionally, it 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 disease remain one of the serious health problems. Modern medicines have little to offer for alleviation of hepatic diseases and it is chiefly the plant based preparations which are employed for the treatment of liver disorders. There are not much drugs available for the treatment of liver disorders[2]. Therefore, many folk remedies from plant origin are evaluated for its possible antioxidant and hepatoprotective effects against different chemical-induced liver damage in experimental animals.

Mallotus philippensis (kamala tree) is a small to medium-sized monoecious tree, up to 25 m tall of the family Euphorbiaceae. Kamala tree is wide spread, from the western Himalaya, through India, Srilanka, to Southern China, Taiwan and the Ryukyu Islands, Burma (Myanmar), Thailand and throughout Malaysia to Australia. It is commonly known as dyers rottlera, kamala dye tree, monkey face tree, orange kamala, red kamala, scarlet croton[3]. The plant has been traditionally used as a potent anti-allergic, antimicrobial and in liver disorders[4]. The poulticed leaves and bark are used for skin diseases, ring worms[5] and scabies. Based on the ethnopharmacological information of the plant the present study was aimed to investigate the hepatoprotective activity of methanolic extract of the *Mallotus philippensis* (MEMP) on ethanol induced hepatotoxic model in rats.

EXPERIMENTAL

The leaves of *Mallotus philippensis* were collected from Nilgiris, Ooty, Tamil Nadu and was authenticated by Field Botanist Dr. Rajan S. The leaves were shade-dried at room temperature and the methanolic extract of the *Mallotus philippensis* was obtained by extracting with 1500 mL of methanol for 7 days, using soxhlet apparatus. The extract was concentrated and dried using rotary flash evaporator in vacuum at < 40 ºC. It was stored in refrigerator at < 10 ºC. The yield of the extract was found to be 1.5 % (w/w).

Phytochemical screening: A preliminary phytochemical screening[6] was carried out to detect the presence of various phytoconstituents.

Swiss albino mice (18-20 g) and Wistar albino rats (100-150 g) of either sex were acclimatized for 10 days under standard housing conditions maintained at a room temperature of 24 ± 1 °C, relative humidity of 45-55 % with 12:12 h light/dark cycle. The animals had free access to rat food and water. The animals were habituated to laboratory conditions for 48 h prior to the experimental protocol to minimize any non-specific stress. The Institutional Animal Ethics Committee of Dayananda Sagar College of Pharmacy, Bengaluru, India, approved the experimental protocol in accordance with the guidelines provided by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) with approval number, DSCP/M Pharm Col/IAEC/24/09-10.

Acute toxicity studies: Acute oral toxicity (AOT) of methanolic extract of the *Mallotus philippensis* leaves were determined using nulliparous, non-pregnant female mice. The animals were fasted for 3 h prior to the experiment and were administered with single dose of extract dissolved in 2 % w\v Tween 80 and observed for mortality for up to 24 h (short-term toxicity). Based on the short-term toxicity, the dose of the next animal was determined as per OECD[7] guideline 425. All the animals were also observed for long-term toxicity (14 days) studies.

Hepatoprotective activity[8]

Ethanol induced liver toxicity: Albino rats of either sex (150-200 g) were selected and divided into five groups of six animals each. The animals were pretreated twice daily with vehicle (2 % v/v tween 80, p.o)/methanolic extract of the *Mallotus philippensis* (100 mg/kg, p.o)/methanolic extract of the *Mallotus philippensis* (200 mg/kg, p.o)/silymarin (100 mg/kg, p.o), 1 h before ethanol administration. All the animals, except normal control group, received ethanol (3.76 g/kg, p.o) twice daily for a period of 25 days. On day 26, thiopentone sodium (40 mg/kg, i.p) was injected and the sleeping time recorded in all the animals[9].

The same animals were then anesthetized using anesthetic ether, after 1 h complete recovery from thiopentone sodium effect and blood collected by retroorbital puncture and centrifuged (3000 rpm for 10 min) to obtain serum. The levels of serum alanine transaminase (ALT), aspartate transaminase (AST)[10], alkaline phosphatase (ALP)[11], direct and total bilirubin[12], cholesterol[13], triglycerides[14] and total proteins[15] were estimated as per the standard procedures prescribed by the manufacturer (Coral Clinical Systems, Verna Goa, India). Immediately after the collection of blood the animals were euthanized with an over dosage of ether and their livers were removed, washed with ice-cold saline and weighed. The wet liver volume was measured. Small piece of liver tissue was collected and preserved in 10 % formalin solution for histopathological studies. Livers of animals were homogenized with ice-chilled 10 % KCl solution and centrifuged at 2000 rpm for 10 min. The supernatant was collected and the lipid peroxidation was assessed in tissue by measuring the levels of catalase (CAT)[16], thiobarbituric acid reactive substances (TBARS) like malondialdehyde[17] and tissue antioxidant enzymes superoxide-dismutase (SOD)[18].

RESULTS AND DISCUSSION

Preliminary phytochemical studies revealed the presence of alkaloids, carbohydrates, steroids, saponins, triterpenes and flavonoids in methanolic extract of the *Mallotus philippensis* leaves. The methanolic extract of the *Mallotus philippensis* was found to be nontoxic up to a dose of 2000 mg/kg.

Treatment of rats with toxicant (ethanol) produced an increase in the weight and volume of wet liver. Rats pretreated with silymarin and methanolic extract of the *Mallotus philippensis* leaves showed significant decrease in wet-liver weight and volume compared to control (toxic) group (Tables 1 and 2).

Toxicant administration resulted in significant elevation of aspartate transaminase, alanine transaminase, SALP, triglycerides, cholesterol, direct bilirubin and total bilirubin levels, while total protein levels were found to be decreased compared to normal control group. Pre-treatment with silymarin and methanolic extract of the *Mallotus philippensis* leaves significantly prevented the biochemical changes induced by toxicant. The hepatoprotective effect offered by methanolic extract of the *Mallotus philippensis* was found to be significant (Table-3).

A significant reduction in thiopentone-induced sleep time was observed with methanolic extract of the *Mallotus philippensis* leaves compared to the toxicant-treated control group (Table-2).

Hepatocytes of the normal control group showed a normal intact architecture of the liver. In the toxicant-treated group the liver showed conjestion of sinusoids. Some of the hepatocytes showed hemorrhage, necrosis, steatosis and degenerative changes. Moderate mononuclear inflammatory infiltrations were seen within all the zones. Silymarin, methanolic extract of the *Mallotus philippensis* 100 mg/kg and 200 mg/kg pretreated groups showed minimal congestions in sinusoids, mild infiltrations, regeneration of hepatocytes (Fig. 1) and their

TABLE-1
EFFECT OF THE *Mallotus philippensis* EXTRACT ON FUNCTIONAL AND PHYSICAL PARAMETERS IN ETHANOL TREATED ALBINO MICE

Groups	Dose	Thiopentone sleeping		Mean liver (wt. g/100 g)	Mean liver (volume mL/100 g)
		Onset (s)	Duration (min)		
I. Normal control	10 mL/kg, p.o.	168.5 ± 3.5	119.16 ± 4.485	2.535 ± 0.535	2.535 ± 0.535
II. Toxicant control	Ethanol-37.6 mg/kg, p.o.	85.0 ± 5.5	189.50 ± 3.033	4.190 ± 0.040	4.345 ± 0.095
III. Standard (Silymarin)	100 mg/kg, p.o. + ethanol	140.0 ± 1.0**	163.80 ± 2.427*	2.973 ± 0.076*	2.733 ± 0.1202*
IV. MEMP	100 mg/kg, p.o. + ethanol	156.0 ± 2.0**	140.30 ± 1.216**	2.770 ± 0.110**	2.366 ± 0.2728**
V. MEMP	200 mg/kg, p.o. + ethanol	152.5 ± 1.0**	138.40 ± 0.871**	2.780 ± 0.230**	2.610 ± 0.110*

Results are expressed as mean ± SEM; n = 6 rats in each group. *p < 0.05; **p < 0.001 are considered significant compared with ethanol intoxicated group using one-way ANOVA followed by Tukey Kramers post hoc test; MEMP = Methanolic extract of *Mallotus phillipensis*.

TABLE-2
EFFECT OF THE *Mallotus philippensis* EXTRACT ON ETHANOL INDUCED BIOCHEMICAL CHANGES IN ALBINO RATS

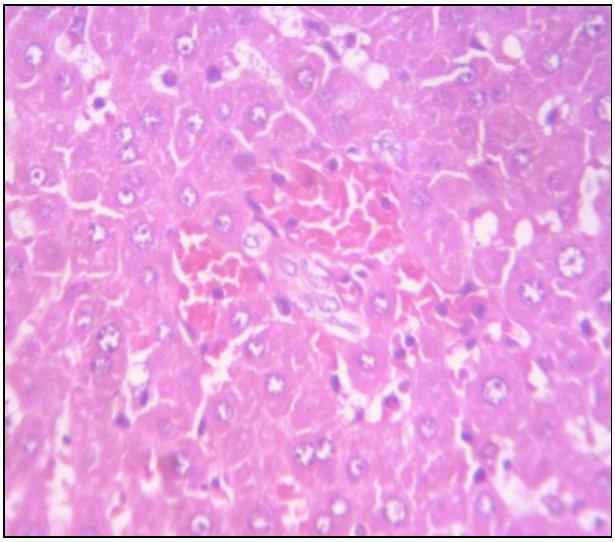
Groups	Mean ± SEM							
	SGOT (U/L)	SGPT (U/L)	ALP (mg/dl)	TBR (mg/dl)	DBR (mg/dl)	TG (mg/dl)	TC (mg/dl)	TP (mg/dl)
I. Normal control	34.90±1.400	28.35±0.90	28.15±1.141	0.19±0.01	0.186±0.0081	24.67±0.623	5.775±0.178	6.78±0.06
II. Ethanol control	166.95±1.450	115.8±1.480	85.88±1.388	1.62±0.09	0.82±0.0202	145.78±3.732	29.56±0.458	3.47±0.02
III. 100 mg/kg of MEMP	111.55±0.850	84.7±0.55**	64.0±2.05**	1.02±0.09**	0.63±0.01**	99.94±0.88**	18.65±0.96**	4.81±0.145**
IV. 200 mg/kg of MEMP	103.30±0.400	68.4±1.20**	38.6±0.97**	0.72±0.05**	0.46±0.02**	56.11±0.94**	13.55±0.581**	5.15±0.095**
V. Positive control	79.86±0.803	48.97±0.05**	30.8±2.05**	0.47±0.05**	0.31±0.018**	48.55±0.85**	8.88±0.347**	5.82±0.155**

Results are expressed as Mean ± SEM; n = 6 rats in each group. *p < 0.05; ** p < 0.001 are considered significant compared with ethanol intoxicated group using one-way ANOVA followed by Tukey Kramers post hoc test; MEMP = Methanolic extract of *Mallotus phillipensis*.

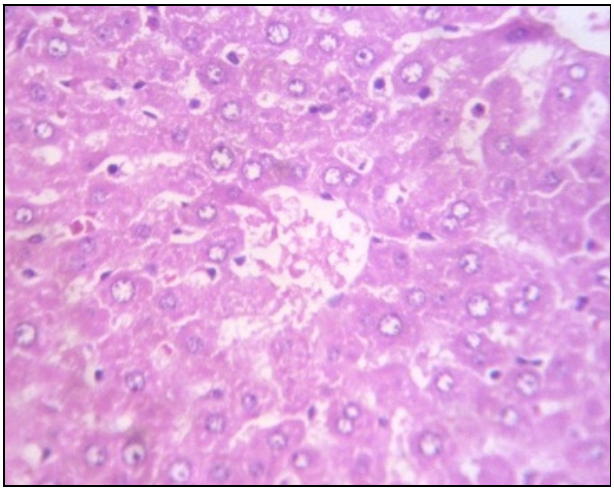
TABLE-3
EFFECT OF METHANOLIC EXTRACT OF *Mallotus phillipensis* (MEMP) LEAVES ON CATALASE, SOD AND LIPID PEROXIDATION IN ETHANOL INDUCED HEPATOTOXIC RATS

Group	Treatment	Dose	Catalase (Mean ± SEM)	SOD (Mean ± SEM)	LPO (Mean ± SEM)
I	Normal control	10 mL/kg p.o.	92.00 ± 1.732	11.86 ± 0.9130	3.63 ± 0.4784
II	Toxicant control	Ethanol 3.76 mg/kg, p.o.	27.97 ± 1.204	3.82 ± 0.0850	8.81 ± 0.1460
III	Standard	100 mg/kg, p.o. + Ethanol	83.90 ± 0.820***	8.95 ± 0.1607***	5.83 ± 0.1764***
IV	MEMP	100 mg/kg, p.o. + Ethanol	35.91 ± 0.850**	6.40 ± 0.3210**	7.50 ± 0.2082**
V	MEMP	200 mg/kg, p.o. + Ethanol	54.60 ± 1.172***	7.50 ± 0.2082***	6.80 ± 0.1528***

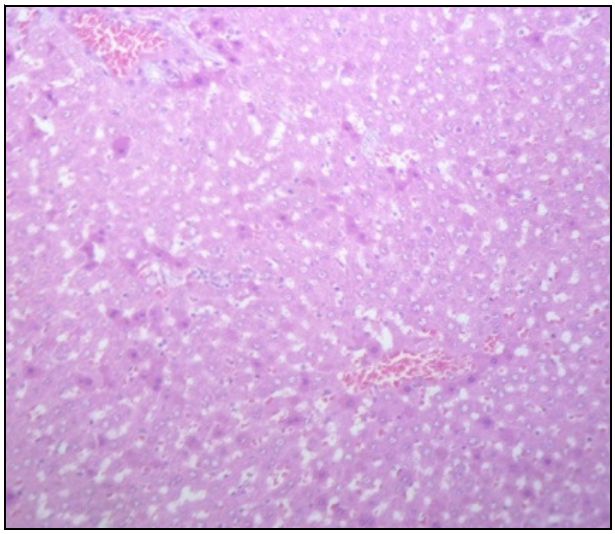
a = nmol of MDA/mg of protein; b = Units/mg of protein; c = µmol of H_2O_2 consumed/min/mg of protein, d = µg/mg of protein. Values are mean ± SEM (n = 6) one-way ANOVA followed by Tukey-Kramer's test. *p < 0.05, **p < 0.01, ***p < 0.001.



Ethanol control



Silymarin control



Methanolic extract of the *Mallotus philippensis* 200mg/kg

Fig. 1. Histopathological studies of the rat liver in ethanol induced hepatotoxicity

lobular architecture was normal indicating the hepatoprotective effect of these extracts.

Elevated levels of thiobarbituric acid reactive substances and a significant reduced activity level of superoxide-dismutase and catalase were recorded in toxicant administered animals. The methanolic extract of the *Mallotus philippensis* leaves and silymarin administered animals revealed significant depletion in the levels of thiobarbituric acid reactive substances and elevation in the activity levels of tissue superoxide-dismutase and catalase (Table-3).

The liver can be injured by many chemicals and drugs. In the present study ethanol was selected as a hepatotoxicant to induce liver damage, since it is clinically relevant[19]. Ethanol produces a constellation of dose-related deleterious effects in

the liver. In chronic alcoholics, hepatomegaly occurs due to accumulation of lipids and proteins in hepatocytes, with an impaired protein secretion by hepatocytes. Water is retained in the cytoplasm of hepatocytes leading to enlargement of liver cells, resulting in increased total liver mass and volume[18] as observed in the present study. This alcohol-induced increase in total wet-liver weight and volume was prevented by pre-treatment with methanolic extract of the *Mallotus philippensis* leaves, thus indicating a hepatoprotective effect.

During hepatic damage, cellular enzymes like aspartate transaminase, alanine transaminase and alkaline phosphatase present in the liver cells leak into the serum, resulting in increased concentrations. Ethanol administration for 25 days significantly increased all these serum enzymes, whereas the methanolic extract of the *Mallotus philippensis* leaves pretreated animals had significantly reduced aspartate transaminase, alanine transaminase and SALP levels and increased total protein levels, indicating their hepatoprotective effect against alcohol -induced liver cell damage.

Ethanol induces hypercholesteremia and hypertriglyceridemia, which may be due to the activation of enzyme HMG CoA reductase, the rate-limiting step in cholesterol biosynthesis[19]. The increased serum triglyceride level in ethanol-treated rats may be due to the decreased activity of lipoprotein lipase, which is involved in the uptake of triglyceride-rich lipoprotein by the extrahepatic tissues[22]. Pretreatment with methanolic extract of the *Mallotus philippensis* leaves reduced the elevated cholesterol and triglyceride levels, suggesting that the extracts prevented ethanol-induced hyperlipidemia probably due to their hepatoprotective activity.

Ethanol also alters the metabolic activity of hepatocytes, thereby inducing hepatic damage. Barbiturates are a class of xenobiotics that are extensively metabolized in the liver. Derranged liver function leads to delay in the clearance of barbiturates, resulting in a longer duration of hypnotic effect. In the present study, administration of thiopentone sodium to rats pretreated chronically with alcohol resulted in an increased duration of thiopentone sleep time. Pre-treatment with methanolic extract of the *Mallotus philippensis* leaves decreased thiopentone-induced sleep time, an indirect evidence of their hepatoprotective effect.

Intake of ethanol results in excessive generation of free radicals. Free radicals are the reactive oxygen species (ROS) are known to cause oxidative damage to number of molecules in cell, including membrane-lipids, proteins and nucleic acids. In the present study the hepatic cellular injury in ethanol administered animals might be increased oxidative stress leading to lipid per oxidation. Then the significant increase of the tissue thiobarbituric acid reactive substances levels in the ethanol administered animals revealed lipid peroxidation in the liver. It is decreased by the administration of methanolic extract of the *Mallotus philippensis* leaves. Similar studies reported that significant decrease in the activity of liver superoxide-dismutase, catalase in alcohol intoxicated rat was observed and the therapeutic treatment with methanolic extract of the *Mallotus philippensis* leaves herbal drug promoted the hepatoprotection by elevating free radical scavenging activity.

Histological changes such as steatosis (fatty changes in hepatocytes), inflammatory infiltrations and perivenular fibrosis were observed in ethanol-treated (toxic) control group. Both the extracts prevented these histological changes, further indicating their hepatoprotective activity. All the histological changes observed were in correlation with the physical, biochemical, antioxidant and functional parameters of the liver. Thus it can be concluded that methanolic extract of the *Mallotus philippensis* leaves possess a protective effect against ethanol-induced hepatotoxicity in rats, as evidenced by the physical, biochemical, functional, antioxidant and histological parameters.

REFERENCES

1. P.L. Wolf, *Indian J. Clin. Biochem.*, **14**, 1999 (1999).
2. M. Karan, K. Vasisht and S.S. Handa, *Phytother. Res.*, **13**, 24 (1999).
3. P. Oudhia, Medicinal Herbs of Chhattisgarh, India Having Less Known Traditional Uses XXVII, Sinduri (*Mallotus philippensis*, Family Euphorbiaceae) (2001).
4. http://www.nadanherbcomp.com.au/herb-s.html Access date:07/07/2009
5. K.R. Kirtikar and B.D. Basu, Indian Medecinal Plants, International Book Distributor, Dehradun, Vol. 2, p. 238 (1987).
6. K.R. Khandelwal, Practical Pharmacognosy, Techniques and Experiments, Nirali Prakashan, Pune, India, edn. 9 (2000).
7. OECD 2001-Gudeline on Acute Oral Toxicity (AOT), Environmental Health and Safety Monograph Series on Testing and Adjustment, No. 425 (2001).
8. V. Shukla, R.K. Tiwari and D.P. Agarwal, *Adv. Pharmacol. Toxicol.*, **2**, 9 (2001).
9. V. Gujrati, N. Patel, N.V. Rao, K. Nandakumar, T.S. Gouda, Md. Shalam and S.M. Shanta Kumar, *Indian J. Pharmacol.*, **39**, 43 (2007).
10. IFCC Methods for the Measurement of Catalytic Concentration of Enzymes, *J. Clin. Chem. Clin. Biochem.*, **24**, 481 (1986).
11. G.N. Bowers and R.B. McCommb, *J. Clin. Chem. Bio.*, **10**, 182 (1972).
12. L. Jendrassik and P. Grof, *Biochemistry*, **2**, 281 (1938).
13. P.N.M. Demacker, H.E. Vos-Janssen and A.G.M. Hijmans, *Clin. Chem.*, **26**, 1775 (1980).
14. P. Trinder, *Ann. Clin. Biochem.*, **6**, 24 (1969).
15. A.G. Gornall, C.J. Baradawill and M.M. David, *Biol. Chem.*, **177**, 751 (1949).
16. H. Aebi, Catalase in Methods of Enzymatic Analysis, Verlag Chemie Weinheim, Academic Press, Inc. New York and London, edn. 2, p. 673 (1974).
17. M.A. Leo and M. Arai, *Gastroenterology*, **82**, 194 (1982).
18. C.D. Blancho and S. Gentil, *Diab. Res. Clin. Prac.*, **8**, 29 (1990).
19. M. Younes and O. Strubelt, *Free Radic Res. Commun.*, **3**, 19 (1987).