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Research Article

PHYTOCHEMICAL AND PHARMACOLOGICAL SCREENING OF *MALLOTUS PHILIPPENSIS* AGAINST CCL_4- AND ATT-INDUCED HEPATOTOXICITY IN RATS

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ABSTRACT

Objective: *Mallotus philippensis* (*Mp*) is locally known as kamala and is a large woody multipurpose medicinal tree belonging to the family of Euphorbiaceae. *Mp* possess a wide variety of activities such as skin problem, bronchitis, antifungal, worm infestation (tapeworm) eye disease, cancer, diabetes, and diarrhea. Hence, the present study was intended to evaluate methanolic fruits extract of *Mp* for hepatoprotective activities.

Methods: The hepatoprotective activity was studied by CCl_4 at the dose of 1 ml/kg of body weight in liquid olive oil in the ratio of 1:1 and ATT (isoniazid – 7.5 mg/kg, rifampicin – 10 mg/kg, and pyrazinamide – 35 mg/kg b.w.) induced models. Acute toxicity study and preliminary phytochemical screening were also studied to evaluate the toxicity.

Results: No toxicity profile was observed in rats after oral administration of the methanolic fruits extract at the dose of 2 g/kg body weight. The different dose of 300 mg/kg and 500 mg/kg administered with the extract of *Mp*. There was a significant ($p<0.001$) reduction in biochemical parameters with respect to control. Phytochemical screening of the fruits extract revealed the presence of tannins, alkaloids, flavonoids and saponins, and terpenoids.

Conclusion: It can be concluded that the hepatoprotective activity elucidated by *Mallotus philippensis* could be mainly due to the presences of high-value class of compound like the phenolic group as the major content in the plant.

Keywords: *Mallotus philippensis*, CCl_4, ATT, Biochemical parameters and histopathological studies.

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INTRODUCTION

India has a rich culture of medicinal herbs and spices, which includes more than 2000 species and has a vast geographical area with high potential abilities for the Ayurvedic, Unani, and Siddha traditional medicines, but only very few have been studied chemically and pharmacologically for their potential medicinal value [1,2]. Annual incidence of drug-induced liver injury (DILI) has been estimated between 10 and 15/10,000 and 100,000 persons taking prescribed medicines [3]. Hence, natural products from medicinal plants need to be investigated by scientific methods for their hepatoprotective activity. The fruits of *Mallotus philippensis* (*Mp*) belonging to the family of *Euphorbiaceae* possess a wide variety of activities such as skin problem, bronchitis, antifungal, worm infestation (tapeworm) eye disease, cancer, diabetes, and diarrhea [4].

METHODS

Collection, identification, and authentication of the fruit

The fruits of *Mp* were purchased from local herbal dealer in Aligarh, India. It was identified by Dr. Athar Ali Khan, Taxonomist, Department of Botany, Aligarh. A voucher specimen bearing the number 433 was deposited in the herbarium of the Department of Botany, A.M.U, Aligarh, India.

Preparation of extract

The granulated fruits of *Mallotus philippensis* (100 g) were packed in a Soxhlet apparatus and subjected to continuous hot percolation for 8 h using 450 ml of methanol (95% v/v) as a solvent. The extract was concentrated to dryness under reduced pressure and controlled temperature and dried in a desiccator (yield 45.6 g, 15.20% w/w). The extract was suspended in 5% gum acacia and used for further experiments.

Preliminary phytochemical screening

The extract was screened qualitatively for the presence of various groups of phytoconstituents using different chemical tests [5].

Procurement of experimental animals

Animals were selected as per the OECD guidelines. Healthy young and nulliparous, non-pregnant Sprague Dawley female rats weighing from 160 to 180 mg of 8–12 weeks old were selected because literature survey of lethal dose 50% test shows that usually there is little difference in sensitivity between sexes, but generally females were found slightly more sensitive, were procured from listed suppliers of Sri Venkateswara Enterprises, Bengaluru, India. The animals were fed with standard pellet diet (Hindustan Lever Ltd., Bengaluru) and water *ad libitum*. All the animals were housed in polypropylene cages. The animals were kept under the alternate cycle of 12 h of darkness and light. The animals were acclimatized to the laboratory conditions for 1 week before starting the experiment. The animals fasted for at least 12 h before the onset of each activity. The experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC No. P. Col/02/1686//09/2016/IAEC/JSPC) after scrutinization. The animals received the drug treatments by oral routs.

OBSERVATIONS

Animals were observed individually for 48 h after dosing at the first 30 min, periodically and during the first 24 h, with special attention given during the first 4 h and daily thereafter, for a total of 14 days. Additional observations were also made if the animals continue to display signs of toxicity. Observations included were changes in skin, fur, eyes, mucous membranes, respiratory, circulatory, autonomic and central nervous systems, somatomotor activity, and behavior pattern. Observations were also made and checked for tremors, convulsions, salivation, diarrhea, lethargy, sleep, and coma. Results are tabulated in Table 1.

Experimental design [6]

The rats were divided into the seven groups of each containing six rats.

- Group I: Control rats, which fed normal diet and water.
- Group II: Rats treated with CCl_4 (1 ml/kg) once daily for 7 days.
- Group III: Rats treated with silymarin (50 mg/kg) + CCl_4 (1 ml/kg) once daily for 7 days.
- Group IV: Rats treated with *Mp* (300 mg/kg, i.p.) + CCl_4 (1 ml/kg) once daily for 7 days.
- Group V: Rats treated with *Mp* (500 mg/kg, i.p.) + CCl_4 (1 ml/kg) once daily for 7 days.
- Group IV: Rats treated with *Mp* (300 mg/kg, i.p.) + ATT (1 ml/kg) once daily for 35 days.
- Group V: Rats treated with *Mp* (500 mg/kg, i.p.) + ATT (1 ml/kg) once daily for 35 days.

Table 1: Acute toxicity study of methanolic extract of fruits of *Mallotus philippensis* based on OECD guidelines 423

S. No.	Number of animals	Dose in mg/kg	Report
1	3	5	No death
2	3	50	No death
3	3	300	No death
4	3	2000	No death

Statistical analysis

Values were represented as mean±standard error of mean of three parallel data's.

RESULTS

Preliminary phytochemical screening

The preliminary phytochemical analysis of methanolic fractions of *M. philippensis* shows the presence of steroids, alkaloids, flavonoids, glycosides, saponins, tannin, and carbohydrate.

DISCUSSION

The present study reveals the hepatoprotective activity of *M. philippensis* against CCl_4- and ATT-induced hepatic damage in rats. Liver damage is assessed by measuring the levels of serum transaminases such as AST, ALT, and ALP, which are released into the blood from damaged liver cells. They are also the indicators of liver damage (Shah and Gupte, 2004). The normalization of the elevated markers after administration of drugs is an indicator of their efficacy for regeneration of liver cells. It has been reported that serum transaminases return to normal level with the healing of liver parenchyma and hepatocytes [7]. We found that administration of CCl_4 with or without fractions did increase the weight and volume of the liver relative to the body weight of the rats as compared to the normal control group, but the increase was

Table 2: Results of gross behavioral studies in rats on administration of *Mallotus philippensis* 2000 mg/kg/p.o

Observation	Effects								
Gross activity	**Up to 3 h**	**3½ h**	**4 h**	**4½ h**	**5 h**	**5½ h**	**6 h**	**12 h**	**24 h**
Respiration	+	+	+	+	+	+	+	+	+
Writhing	-	-	-	-	-	-	-	-	-
Tremor	-	-	-	-	-	-	-	-	-
Convulsions	-	-	-	-	-	-	-	-	-
Hindlimb paralysis	-	-	-	-	-	-	-	-	-
Sense of touch and sound	+	+	+	+	+	+	+	+	+
Salivation	+	+	+	+	+	+	+	+	+
Diarrhea	-	-	-	-	-	-	-	-	-
Mortality	-	-	-	-	-	-	-	-	-

+: Normal, -: No effect

Table 3: Effect of *M. philippensis* fractions on liver function test in CCl_4-induced liver toxicity

Groups	AST (IU/ml)	ALT (IU/ml)	ALP (KAU/dl)	Bilirubin (mg/ml)
Group I	28.8±1.8	31.3±2.9	42.7±1.7	0.67±0.03
Group II	180.8±1.9***	166.8±6.0***	80.7±4.3***	0.73±0.05
Group III	55.2±3.3***	57.1±2.9***	52.3±2.2**	0.67±0.03
Group IV	144.3±8.8*	140.3±10.3	69.8±6.4	0.72±0.04
Group V	111.0±12.3***	123.3±10.7**	56.0±6.4**	0.67±0.05
Group VI	134.7±15.0**	127.3±15.2	60.0±4.4*	0.70±0.05
Group VII	88.0±7.4***	91.7±6.1***	56.7±5.5**	0.67±0.05

n=6; values were expressed mean±SEM; Group II was compared to Group I. Groups III to VII were compared to Group II. *p<0.01 versus CCl_4 group: Significant; **p<0.001 versus CCl_4 group: Highly significant data were analyzed by oneway ANOVA followed by Dunnett's ttest

Table 4: Effect of *M. philippensis* fractions on liver function test in ATT-induced liver toxicity

Groups	AST (IU/ml)	ALT (IU/ml)	ALP (KAU/dl)	Bilirubin (mg/ml)
Group I	28.5±0.98	31.4±2.34	43.3±1.2	0.73±0.04
Group II	169.0±6.7***	170.1±5.6***	80.2±2.7***	3.47±0.49***
Group III	56.4±3.3***	58.3±2.9***	51.4±2.2***	1.37± 0.03***
Group IV	124.0±9.5**	119.7±6.7***	67.3±4.8	2.82±0.14*
Group V	106.2±10.5***	106.5±9.6***	62.5±3.6**	2.1±0.28**
Group VI	122.2± 9.6**	130.5±8.9**	58.3±2.9**	2.22±0.21**
Group VII	104.2±11.2***	99.2±10.0***	56.2±1.5***	2.0±0.21**

n=6; values were expressed mean±SEM; Group II was compared to Group I. Groups III to VII were compared to Group II. *p<0.01 versus ATT group: Significant; **p<0.001 versus ATT group: Highly significant data were analyzed by oneway ANOVA followed by Dunnett's ttest

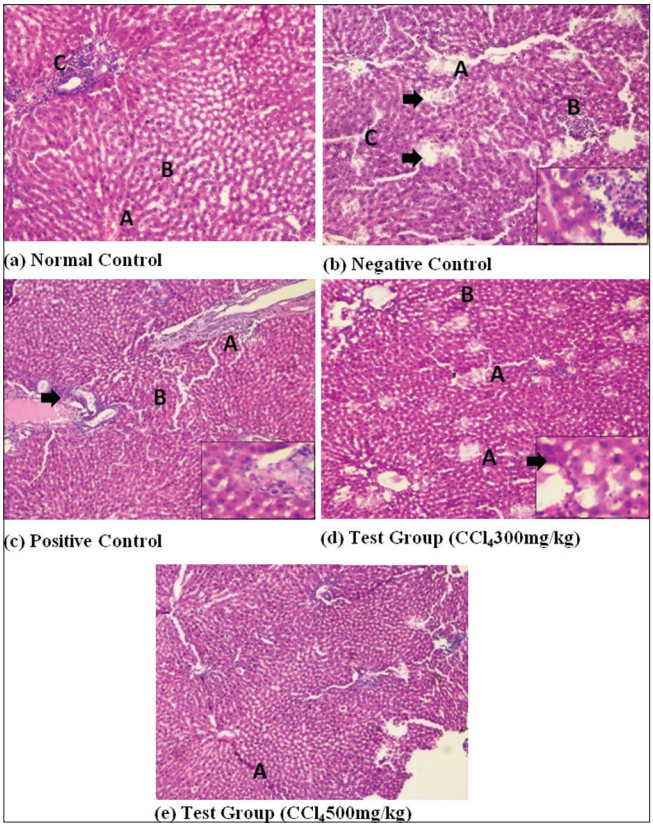

Fig. 1: Histopathological studies of liver (CCl_4 induced), (a) Group I, (b) Group II, (c) Group III, (d) Group IV, (e) Group V

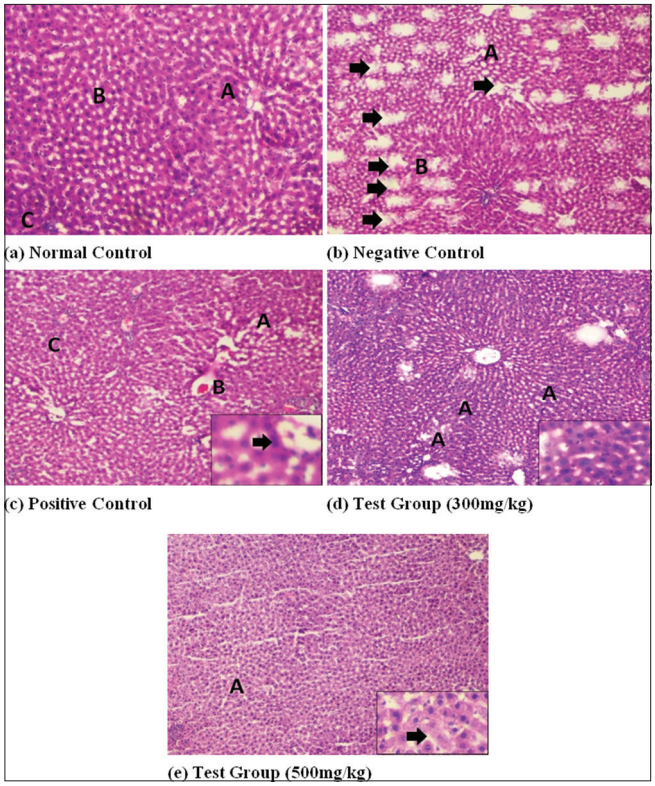

Fig. 2: Histopathological studies of liver (ATT induced), (a) Group I, (b) Group II, (c) Group III, (d) Group IV, (e) Group V

not statistically significant. The weight and volume of the liver are increased in inflammation due to extravasations of fluid in extracellular compartment. The methanol fractions of *M. philippensis* demonstrated significant hepatoprotective activity as shown by its ability to control the rise of serum transaminases. Although the effect was more at dose of 500 mg/kg for methanol fraction 123.3±10.7 IU/ml, with 300 mg/kg dose, no significant hepatoprotective activity was observed for liver specific enzyme ALT. There was no significant rise in the bilirubin levels of negative control as compared to normal control group or any significant decrease in the levels of bilirubin in test groups as compared to negative control. The percentage hepatoprotection was good at 500 mg/kg b.w 32.1% for ALT. Our findings are in accordance with the observations [8]. Similarly, the methanol and ethyl acetate fractions of *F. parviflora* showed significant hepatoprotective activity as shown by its ability of control the rise of serum transaminases. Although the effect was observed more at doses of 500 mg/kg of methanol fraction (124.2±9.9 IU/ml). There was no significant rise in the bilirubin levels of negative control as compared to normal control group or any significant decrease in the levels of bilirubin in test groups as compared to negative control. Methanol fraction of *M. philippensis* demonstrated significant hepatoprotective activity in ATT-induced liver injury, as shown by its ability of limit the rise of serum transaminases. Highly significant decrease was observed in liver-specific ALT levels with the fraction at a dose of 500 mg/kg (106.5±9.6 IU/ml). There was an increase in the bilirubin levels in the negative control that was significantly prevented in all test groups as compared to negative control methanol 300 mg/kg 2.82±0.14 IU/ml and 500 mg/kg 2.1±0.28 IU/ml, *M. philippensis* test groups the methanol fraction in the dose of 300 and 500 mg/kg b.w. offered a protection of 36.3% and 45.9% for ALT.

CONCLUSION

From the present work, we conclude that species of *M. philippensis* are highly potential in biological activity. The preliminary screening of the samples revealed the presences of high-value class of compound like the phenolic group as the major content in the fruits.

AUTHORS' CONTRIBUTIONS

Dr. Muthu ramu T: Concept, design, collection of data, laboratory and animal investigations, interpretation of data, drafting a final report, and approval of the article to be published. Dr. Mujeeb Ur Rahman: Concept, design, collection of data, laboratory and animal investigations, interpretation of data, revising of article, and approval of the article to be published. Mr. Abdurohman Mengesha Yessu: Laboratory investigations, interpretation of data, and revising of article.

CONFLICTS OF INTEREST

All authors report no conflicts of interest regarding this manuscript.

AUTHORS' FUNDING

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