Studies on Hepatoprotective Activity of Various Extracts of *Macaranga peltata* (Roxb.) on Paracetamol-Induced Hepatotoxicity Rats

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ABSTRACT

BACKGROUND

Liver disease is universal problem. In the absence of reliable liver-protective drugs in allopathic medical practices, herbal products take a role in the management of various liver diseases. Current investigation was to examine hepatoprotective potential of *Macaranga peltata* (Family *Euphorbiaceae*) on paracetamol-induced hepatotoxicity in rats.

METHODS

The aerial parts of *Macaranga peltata* dry powder was extracted with various solvents (PE, EA and methanol) through Soxhlet extractor and crude extract was utilized testing of hepatoprotective activity. Paracetamol (2 g/Kg b. wt.) given orally to rats induced hepatotoxicity on the 5th day of the investigational period.

RESULTS

Rats with paracetamol-induced hepatotoxicity exhibited elevated ($p < 0.001$) activities of liver enzymes [SGOT, SGPT, ALP, gamma-glutamyl transpeptidase (GGT)], creatinine, urea, total bilirubin, total cholesterol & TG and reduced total protein in serum. Furthermore, rats which were given oral methanolic concentrates of *Macaranga peltata* (200 mg/Kg b. wt.) showed major reduction in the level of SGOT, SGPT, ALP, gamma-glutamyl transpeptidase (GGT) creatinine, urea, total bilirubin, total cholesterol & triglycerides and also significantly elevated the concentration of total protein and albumin when compared to other concentrates.

CONCLUSION

Thus, results suggest that methanolic concentrates of *Macaranga peltata* could afford better hepatoprotective activity against paracetamol induced hepatotoxicity in rats.

KEY WORDS

Macaranga peltata, Paracetamol, Hepatic Markers Enzyme, Hepatotoxicity

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BACKGROUND

Liver disease is a universal problem. Conventional medicines utilized in the management of liver diseases are occasionally insufficient and can have serious side effects. Thus, it is essential to search for alternative medicine for the treatment of liver disease in order to replace presently used drugs of doubtful efficacy and safety.¹ Paracetamol-induced liver damage can be considered in animal models and most mechanisms are translatable to humans.² Paracetamol acts as an antipyretic and analgesic agent used in the clinical field. During beneficial doses, paracetamol is metabolized by glucuronidation or sulfation by the cytochrome p450 structure into the reactive metabolite NAPQI. Paracetamol-induced liver toxicity has been a most significant crisis for many years and various strategies have been investigated, together with the utilizing the natural bio active compounds with hepatoprotective activity.^{3,4} In the absence of reliable liver-protective drugs in allopathic medical practices, herbal take part in a role in the management of various liver diseases.

Macaranga peltata (Roxb.) is well known as Chandwar in the family of Euphorbiaceae. *Macaranga peltata* is a small tree commonly found in Indian forests.⁵ The *Macaranga peltata* powders were used in the treatment of venereal diseases. *Macaranga peltata* of leaves and bark decoction is used in the treatment of ulcers. The gum powder of *Macaranga peltata* bark is used to join fractured bones. The fruit is eaten during the periods of scarcity. The wood of *M. peltata* is pale brown with a mottled appearance and is reported to be suitable for making pencil, matches and paper pulp.⁶ This plant is used for antibacterial and antifungal activity.⁷ However, no data are available in the literature on the hepatoprotective activity of aerial parts of *Macaranga peltata* (Roxb.). Thus, the study to assess hepatoprotective activities of *Macaranga peltata* (Roxb.) in hepatotoxicity rats.

METHODS

Chemicals

Paracetamol was purchased from Sigma-Aldrich, the USA utilized in the experiment. All other chemicals and reagents were utilized AR grade.

Gathering and Identification of *Macaranga peltata*

The *Macaranga peltata* (Family Euphorbiaceae) were gathered from Kulasekaram, Kanyakumari District, Tamil Nadu, India. Plant identification was made from Survey of Medicinal Plant Unit (SMP), Govt. Siddha Medical College, Palayamkottai, Tirunelveli, Tamilnadu. The *Macaranga peltata* were desiccated under shadowy, segregate, crushed through grinder.⁸

Preparation of Plant Concentrates

The pulverized materials were progressively concentrated with PE (40-60 °C) through hot constant percolation method in Soxhlet equipment⁹ for twenty-four hours. At that moment the marc was used to EA (76-78°C) for twenty four hours &

then mark was subjected to methanol for twenty four hours. The concentrates were concentrated through drying and subjected to solidification through lyophilizer till dry powder was acquired.¹⁰

Animals

Male Wistar rats of 16-18 weeks age, weighing 150-160 g were collected from the Pharmacology department, MNR College of Pharmacy, Sangareddy, Hyderabad, Telangana, India. Rats were set aside in cages, 2 per cage, with twelve: twelve hours light and dark cycle at 25 ± 2°C. Rats were maintained on their particular diets and water ad libitum. Animal Ethical Committee's clearance was approved by the Ethical Committee of MNR College of Pharmacy, Sangareddy, Hyderabad, Telangana (CPCSEA/COP/10/16-09-2019).

Experimental Design

Acute Toxicity Test- Albino Wistar rats were separated in six groups and each group contained six animals (n = 6). Rats were fasted for four hours with free access to water only. The various concentrates of *Macaranga peltata* suspended in 0.5% CMC was administered orally at a dose of 5 mg/Kg at first and mortality was noted for three days. The acute toxicity study was carried out OECD guideline 423.

Hepatoprotective activity

Animals were separated in six groups and each group contain six animals.

Group I :	Animals served as Control group received with vehicle (0.5% CMC) for 7 days.
Group II :	Animals served as a negative control, received 7 days only 1 ml vehicle and paracetamol 2 g/Kg b. wt. given on the 5 th -day by orally
Group III :	Animals received Pet. Ether concentrates of <i>Macaranga peltata</i> 200 mg/Kg b. wt., orally for seven days. On 5 th day onwards paracetamol 2 g/Kg b. wt. administered by orally.
Group IV :	Animals received ethyl acetate concentrates of <i>Macaranga peltata</i> 200 mg/Kg b. wt., orally for seven days. On 5 th day onwards paracetamol 2 g/Kg b. wt. administered by orally.
Group V :	Animals received a methanolic concentrates of <i>Macaranga peltata</i> 200 mg/Kg b. wt., orally for seven days. On 5 th day onwards paracetamol 2 g/Kg b. wt. administered by orally.
Group VI :	Animals received 25 mg/Kg b. wt. of Silymarin by oral for seven days and on 5 th day onwards paracetamol 2 g/Kg b. wt. administered by orally. ¹¹

Groups III, IV and V rats were orally fed with the various concentrates of *Macaranga peltata* (PE, EA and methanol) and Group VI rats were fed with silymarin. Both the *Macaranga peltata* concentrates and silymarin were suspended in 0.5% CMC individually and fed to the particular rats through oral intubation. Next day of experiments 8th day, all the animals were sacrificed through cervical decapitation. Blood was collected in test tubes in dry condition and allowed to coagulate at ambient temperature for 30m. Serum was removed through centrifugation at 2000 rpm for 10m. The removed serum was utilized for the examination of liver enzymes.

Liver Marker Enzymes- Rietman and Frankel et al., 1975 method was used to determine the SGOT and SGPT¹² and Kind and King et al., 1954 method was to determine ALP.¹³ Lowry et al., 1951 method was to determine the total protein (TP) levels¹⁴ and Patton and Crouchet et al., 1977 method

was used to determination of Urea.¹⁵ The Jaffe's et al.,1986 method was used to determination of creatinine¹⁶ and Henry et al., 1974 method was utilized for estimation of TC.¹⁷ Mallay HT et al., 1936 method was used for determination of total bilirubin (TB)¹⁸ and Foster et al., 1973 method was used for determination of TG.¹⁹

Statistical Analysis

Statistical investigation was conducted by ANOVA and groups were compared through Duncan's Multiple Range Test (DMRT) using SPSS Software Package, version 10.0. Results were expressed as means $\pm$ standard Error for 6 rats in each group. A value of $P \leq 0.05$ was considered to be statistically significant.

RESULTS

Acute Toxicity

In the present study shown that lethal dose of various concentrates of *Macaranga peltata* were showed the safety of concentrates. Administration of various concentrates of *Macaranga peltata* in rats did not change any autonomic or behavioural reaction. There was no mortality of various concentrates of *Macaranga peltata* was recorded at 2000 mg/Kg. Acute toxicity results revealed that LD50 value of 2000 mg/Kg. Hence the therapeutic dose was considered as 1/10th (200 mg/Kg b.wt. *Macaranga peltata*) concentrates was taken for further investigation.

Activity of Various Concentrates of *Macaranga peltata* on Average Liver Weight Changes in Rats

Table 1 appeared activity of various concentrates of aerial parts of *Macaranga peltata* on average liver weight changes in normal and paracetamol-induced hepatotoxic rats. The paracetamol induced rats group showed gained liver weight. The administration of methanolic concentrates of aerial parts of *Macaranga peltata* attenuated the liver weight reduced and silymarin treated group restored liver weight.

Group	Final Liver Weight (g/100g)
Group I	4.32 $\pm$ 0.37
Group II	6.20 $\pm$ 0.44 ^{a*}
Group III	5.82 $\pm$ 0.24 ^{b**}
Group IV	5.27 $\pm$ 0.22 ^{b**}
Group V	4.62 $\pm$ 0.32 ^{b*}
Group VI	4.57 $\pm$ 0.84 ^{b*}

Table 1. Effect of Various Concentrates of *Macaranga peltata* on Average Liver Weight Changes in Rats

Data be articulated as mean $\pm$ SEM, n = six rats each group.
P values, * $P < 0.05$; ** $P < 0.01$; ns= not significant; compared to Paracetamol group.
One way ANOVA followed by Dunnett's test. a $\rightarrow$ Group II compared to Group I; b $\rightarrow$ Group II compared to Group III, IV, V and VI.

Effect of Various Concentrates of *Macaranga peltata* on Liver Enzymes and Other Parameters in Paracetamol-Induced Hepatotoxicity in Wistar Rats

Table 2 shows the effect of various concentrates of aerial parts of *Macaranga peltata* on hepatic marker enzymes in the serum from normal and paracetamol-induced hepatotoxic

rats. The paracetamol-induced rats were showed increment activities of SGPT (182.61 $\pm$ 0.78), SGOT (188.36 $\pm$ 0.56), ALP (268.59 $\pm$ 0.33) and GGT (2.04 $\pm$ 0.01). Administration of EA concentrates of *Macaranga peltata* showed considerably SGPT (144.75 $\pm$ 0.87), SGOT (165.03 $\pm$ 0.62), ALP (241.03 $\pm$ 0.57) and GGT (1.60 $\pm$ 0.02) in group IV rats. Administration of methanol concentrates of *Macaranga peltata* showed considerably SGPT (77.35 $\pm$ 0.47), SGOT (105.79 $\pm$ 0.58), ALP (204.03 $\pm$ 0.55) and GGT (1.36 $\pm$ 0.01) in group V rats. The administration of methanolic concentrates of *Macaranga peltata* attenuated the hepatic marker enzymes and silymarin restored enzyme activities to normal values.

Group	SGPT (IU.L ⁻¹)	SGOT (IU.L ⁻¹)	ALP (IU.L ⁻¹)	GGT (IU.L ⁻¹)
Group I	67.13 $\pm$ 0.25	96.29 $\pm$ 0.58	193.54 $\pm$ 0.55	1.31 $\pm$ 0.01
Group II	182.61 $\pm$ 0.78 ^{a**}	188.36 $\pm$ 0.56 ^{a**}	268.59 $\pm$ 0.33 ^{a**}	2.04 $\pm$ 0.01 ^{a**}
Group III	175.22 $\pm$ 0.77 ^{b**}	183.94 $\pm$ 0.90 ^{b**}	265.42 $\pm$ 0.49 ^{b**}	1.96 $\pm$ 0.01 ^{b**}
Group IV	144.75 $\pm$ 0.87 ^{b**}	165.03 $\pm$ 0.62 ^{b**}	241.03 $\pm$ 0.57 ^{b**}	1.60 $\pm$ 0.02 ^{b*}
Group V	77.35 $\pm$ 0.47 ^{b**}	105.79 $\pm$ 0.58 ^{b*}	204.03 $\pm$ 0.55 ^{b*}	1.39 $\pm$ 0.02 ^{b*}
Group VI	69.71 $\pm$ 0.41 ^{b**}	98.41 $\pm$ 0.29 ^{b**}	196.18 $\pm$ 0.92 ^{b**}	1.36 $\pm$ 0.01 ^{b**}

Table 2. Activity of Various Concentrates of *Macaranga peltata* on Serum Enzymes SGPT, SGOT, ALP and GGT in Paracetamol Induced Hepatotoxicity in Wistar Rats

Statistical information and particulars of group I-VI was similar as in Table 1

The effect of various concentrates of *Macaranga peltata* on Creatinine, Urea and Total Bilirubin in the serum from normal and hepatotoxic rats were summarized in Table 3. The hepatotoxic control group showed increased the level of Total Bilirubin when compared to control group of rats. Treatment of methanolic concentrates of *Macaranga peltata* had significant reduction in the Creatinine, Urea and Total Bilirubin and silymarin restored Creatinine, Urea and Total Bilirubin to normal values.

Group	Creatinine (mM ⁻¹)	Urea (mM ⁻¹)	Total Bilirubin (mg/dL)
Group I	55.39 $\pm$ 0.59	7.80 $\pm$ 0.03	1.46 $\pm$ 0.02
Group II	79.21 $\pm$ 0.60 ^{a**}	20.06 $\pm$ 0.03 ^{a**}	4.67 $\pm$ 0.03 ^{a**}
Group III	77.54 $\pm$ 0.44 ^{b**}	19.06 $\pm$ 0.06 ^{b**}	4.50 $\pm$ 0.02 ^{b**}
Group IV	63.72 $\pm$ 1.02 ^{b**}	14.35 $\pm$ 0.12 ^{b**}	3.53 $\pm$ 0.04 ^{b**}
Group V	59.11 $\pm$ 0.60 ^{b*}	9.10 $\pm$ 0.02 ^{b*}	1.70 $\pm$ 0.22 ^{b*}
Group VI	56.97 $\pm$ 0.45 ^{b**}	8.04 $\pm$ 0.02 ^{b**}	1.61 $\pm$ 0.02 ^{b**}

Table 3. Activity of Various Concentrates of *Macaranga peltata* on Creatinine, Urea and Total Bilirubin in Paracetamol Induced Hepatotoxicity in Wistar Rats

Statistical information and particulars of group I-VI was similar as in Table 1

The effect of various concentrates of *Macaranga peltata* on TG and TC in the serum from normal and hepatotoxic rats were shown in Table 4. The hepatotoxic control group showed increased the level of TG as 85.06 $\pm$ 0.84 and TC as 195.45 $\pm$ 1.01 that of group I rats. The levels of Triglyceride and Total cholesterol were elevated in treated with Paracetamol-induced rats as compared to normal rats (group I). Oral administration of methanol concentrates showed reduced the level of TG and TC in group V rats as 59.76 $\pm$ 1.33 and 107.41 $\pm$ 0.96. The administration of methanolic concentrates of *Macaranga peltata* and silymarin treated group of rat significant reduction in the level of TG and TC as compared PE and methanol concentrates. This effect could be presence of bio active compounds in the methanolic concentrates of *Macaranga peltata*.

Group	TG (mg/dl)	TC (mg/dl)
Group I	52.25 ± 0.43	97.48 ± 0.52
Group II	85.06 ± 0.84 ^{a**}	195.45 ± 1.01 ^{a**}
Group III	78.96 ± 0.79 ^{b**}	190.46 ± 0.95 ^{b**}
Group IV	68.61 ± 0.71 ^{b**}	170.21 ± 0.81 ^{b**}
Group V	59.76 ± 1.33 ^{b*}	107.41 ± 0.96 ^{b*}
Group VI	54.70 ± 0.23 ^{b**}	100.55 ± 0.43 ^{b**}

Table 4. Activity of Various Concentrates of *Macaranga peltata* on TG and TC in Paracetamol Induced Hepatotoxicity in Wistar Rats

Statistical information and particulars of group I-VI was similar as in Table 1

The various concentrates of *Macaranga peltata* on TP and albumin in the serum from normal and paracetamol-induced hepatotoxic rats were depicted in Table 5. The hepatotoxic control group showed reduction of TP as 4.87 ± 0.02 and albumin as 4.87 ± 0.02 . Oral administration of ethyl acetate concentrates showed marked reduction in TP and ALB levels in group IV rats as 5.53 ± 0.02 and 5.43 ± 0.02 . Oral administration of methanol concentrates showed reduced the level of TP and ALB in group V rats as 6.78 ± 0.02 and 6.15 ± 0.03 . The administration of methanolic concentrates of *Macaranga peltata* and silymarin treated rats were significantly elevated the level of TP and albumin as compared PE and ethyl acetate concentrates. The effective elevation of total protein and ALB can be attributed to an improvement in the hepatic cells secretory mechanisms.

Group	TP(mg/dl)	ALB (g/dl)
Group I	7.91 ± 0.05	6.57 ± 0.06
Group II	4.87 ± 0.02 ^{a**}	4.87 ± 0.02 ^{a*}
Group III	5.17 ± 0.03 ^{b**}	5.11 ± 0.03 ^{b*}
Group IV	5.53 ± 0.02 ^{b*}	5.43 ± 0.02 ^{b**}
Group V	6.78 ± 0.02 ^{b**}	6.15 ± 0.03 ^{b**}
Group VI	7.19 ± 0.05 ^{b**}	6.29 ± 0.04 ^{b**}

Table 5. Activity of Various Concentrates of *Macaranga peltata* on Serum (TP and ALB) in Paracetamol Induced Hepatotoxicity in Wistar Rats

Statistical information and particulars of group I-VI was similar as in Table 1

DISCUSSION

In the present study shown that lethal dose of various concentrates of *Macaranga peltata* were showed the safety of concentrates. Administration of various concentrates of *Macaranga peltata* in rats did not change any autonomic or behavioural reaction. There was no mortality of various concentrates of *Macaranga peltata* was recorded at 2000 mg/Kg. Therefore, we have calculated and selected for 200 mg/Kg b.wt. *Macaranga peltata* concentrates was taken for further investigation.

Paracetamol in larger doses produces liver necrosis after undergoing bio-activation to a toxic electrophile, N-acetyl-p-benzoquinone imine (NAPQI) by cytochrome P-450 monooxygenase.²⁰ The administration of methanolic concentrates of *Macaranga peltata* attenuated the hepatic marker enzymes and silymarin restored enzyme activities to normal values. These biochemical restorations may be due to the inhibitory effects on cytochrome P-450 or/and promotion of its glucuronidation.²¹ Increased levels of bilirubin reflect the depth of jaundice and increased aminotransferases and alkaline phosphatase was the clear manifestation of cellular leakage and loss of functional integrity of the cell.²²

The paracetamol treated group showed increased the level of Creatinine, Urea. Several metabolic disorders including urea and creatinine derangements are possible in

the presence of acetaminophen over dosage.²³ Liver is central to the ruling of cholesterol concentration in the body, does not synthesize cholesterol for goes to another cells but it also separate cholesterol from the body by changing to bile salts and eliminate to bile. The administration of methanolic concentrates of *Macaranga peltata* and silymarin treated rats were significantly elevated the level of TP and albumin as compared PE and ethyl acetate concentrates. The effective elevation of total protein and ALB can be attributed to an improvement in the hepatic cells secretory mechanisms.

CONCLUSIONS

Current study showed that methanolic concentrates of *Macaranga peltata* protect hepatocytes against paracetamol-induced hepatic tissue damage by reducing the activities of hepatic enzymes. From our results, methanolic concentrates of *Macaranga peltata* had a better hepatoprotective effect in rats with paracetamol-induced hepatotoxicity. However further studies are in progress to isolate the active constituents of methanolic concentrates of *Macaranga peltata*.

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