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Hepatoprotective Activity of *Cyperus tegetum* Rhizome Against Paracetamol-Induced Liver Damage in Rats

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Abstract

In present study the methanol extract of *Cyperus tegetum* rhizome (MECT) was evaluated for its effect on paracetamol-induced liver damage in Wistar rats. Serum biochemical parameters viz. serum glutamine oxaloacetate transaminase (SGOT), serum glutamine pyruvate transaminase (SGPT), serum alkaline phosphatase (ALP), total serum protein, total bilirubin content and liver biochemical parameters such as thiobarbituric acid reactive substances (TBARS) and reduced glutathione content were estimated. Biochemical and histopathological observations indicated that MECT had remarkable hepatoprotective effect against paracetamol-induced liver damage in rats.

KEYWORDS: lipid peroxidation, glutathione, biochemical, silymarin

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1. Background

Traditional use: *Cyperus tegetum* Roxb. (Cyperaceae), commonly known as mat grass or mat stick, *Madur-kathi* in Bengali, is a robust perennial sedge growing naturally in marshy land in the humid tropics including India up to an altitude of 1800 m. The stem of the plant is used for making mats for floor covering as well as decoration, and cultivated commercially (Padhye & Moharir, 1958; Bhaduri et al., 1998). It has come to the author's notice that the plant has been traditionally used by the tribal people of West Bengal state, India, for the treatment of cachexia, atrophy and snake bite.

Known pharmacological or biological activity: Not reported.

Rationale for the study: Thorough search of scientific literature revealed that no pharmacological investigation is still reported on *C. tegetum*. Present investigation was therefore aimed to evaluate the possible hepatoprotective potential of *C. tegetum* rhizome against paracetamol-induced liver damage in Wistar rats in pursuit of newer liver protectants.

2. Materials and methods

Raw herb: The rhizomes of *C. tegetum* was used in the present study.

Type of extract: Methanolic.

Methodology for:

Authentication: The rhizomes of *C. tegetum* were collected during June-July 2007 from the cultivated land of Paschim Medinipur district, West Bengal state, India. The species was identified at Central National Herbarium, Botanical Survey of India, Kolkata, India and the voucher specimen [CNH/I-I/(198)/2007/Tech.II/162] was retained in Pharmacology Research Laboratory, Jadavpur University, Kolkata, India for future reference. Just after collection the rhizomes were washed thoroughly with tap water, cut into small pieces, shade dried at room temperature (24-26^{o}C) and ground mechanically into a coarse powder.

Extraction: The powdered plant material (263 g) was extracted successively with petroleum ether and 80 % aqueous methanol in Soxhlet extraction apparatus. The methanol extract was filtered and evaporated to dryness *in vacuo* (at 35 ºC

and 0.8 MPa) in a Buchi evaporator, R-114. The dry extract (MECT, yield 6.65 %) was kept in a vacuum desiccator until use. Preliminary phytochemical analysis (Harborne, 1998) of MECT revealed the presence of triterpenes, steroids, flavonoids and saponins in MECT.

3. Biological activity examined

Experimental animals: Adult male Wistar albino rats weighing 170-200 g were used for the present investigation. They were housed in clean polyacrylic cages (38×23×10 cm) with not more than four animals per cage and maintained under standard laboratory conditions (temperature 25 ± 2ºC, relative humidity 55-65%, with dark/light cycle 12/12 h). They were allowed free access to standard pellet diet (Hindustan Lever, Kolkata, India) and water *ad libitum*. The animals were acclimatized to laboratory condition for one week prior to experiment. All experimental procedures described were reviewed and approved by the University Animal Ethical Committee, Jadavpur University.

Drugs and chemicals: Paracetamol and Bovine serum albumin: Sigma Chemical Co., St. Louis, USA; Trichloroacetic acid (TCA): Merck Ltd. Mumbai, India; Thiobarbituric acid (TBA) and Reduced glutathione (GSH): SISCO Research Laboratory, Mumbai, India. All the other reagents used were of analytical reagent grade obtained commercially.

Acute toxicity: MECT was administered orally in graded doses to adult male Swiss albino mice to evaluate the acute toxicity.

Treatment schedule: The rats were divided into five groups (n = 8). A single dose of 640 mg/kg paracetamol in 1 % methyl cellulose was administered orally to each animals in group II, III, IV and V. After administration of paracetamol suspension, MECT was administered orally (p. o.) at the doses of 250 and 500 mg/kg body weight (b. w.) to groups III and IV respectively daily for 16 days. Group V received reference drug silymarin (25 mg/kg b.w; p.o.) daily for 16 days (Mukherjee et al., 1997). Group I served as normal (vehicle) control and group II served as paracetamol control and received normal saline (5 ml/kg b.w., p.o.) similarly for 16 days. After 24 h of last dose, blood was collected from overnight fasted rats of each group by cardiac puncture for estimation of serum biochemical parameters. Then the rats were sacrificed by cervical dislocation for the study of liver biochemical and histopathological parameters.

Serum biochemical parameters: Serum glutamine oxaloacetate transaminase (SGOT), serum glutamine pyruvate transaminase (SGPT), serum alkaline phosphatase (ALP) and total bilirubin content were estimated by using commercially available kits (Span Diagnostic Ltd., Surat, India). Serum total protein was estimated according to the reported method (Lowry et al., 1951).

Liver biochemical parameters: The levels of lipid peroxidation i. e. thiobarbituric acid reactive substances (TBARS) in the liver tissue were measured as per reported method (Okhawa et al., 1979). The levels of lipid peroxides were expressed as μmoles of malondialdehyde (MDA)/g of liver tissue. The reduced glutathione (GSH) level of liver tissue was determined as per reported method (Ellman, 1959) and expressed as μg/g of liver tissue.

Histopathological studies: For histopathological study the fresh liver tissues were collected and immediately fixed in 10% formalin, dehydrated in gradual ethanol (50-100%), cleaned in xylene and embedded in paraffin. Sections (4-5 μm) were prepared and then stained with hematoxylin-eosin dye for photomicroscopic observations.

Statistical analysis: All results were expressed as the mean ± standard error of mean (SEM). The results were analyzed for statistical significance by one-way ANOVA followed by Dunnett's *post hoc* test of significance. $P < 0.05$ was considered as statistically significant.

4. Research findings

Results: When administered orally, MECT was found to be non-toxic up to the maximum dose of 5000 mg/kg body weight in Swiss mice.

The effect of MECT on SGOT, SGPT, ALP, total bilirubin, and total protein is summarized in Table 1, and thiobarbituric acid reactive substances (TBARS), reduced glutathione (GSH) levels are summarized in Table 2. There were significant ($p < 0.001$) increase in SGOT, SGPT, ALP and total bilirubin levels and decrease ($p < 0.01$) in protein content in paracetamol control group when compared with those of saline control group. The MECT significantly ($p < 0.001$) and dose dependently restored the all altered serum and liver biochemical parameters to normal values which are comparable with that of reference drug silymarin treated group.

Histopathological study of livers of saline control group showed normal hepatocellular architecture (Fig.1). Livers challenged with paracetamol showed disarrangement of normal hepatic cells with massive centrilobular necrosis, inflammatory infiltration of lymphocytes and fatty changes (Fig. 2). The MECT

(500 mg/kg) treated rats exhibited significant protection against paracetamol intoxication as evident by presence of normal hepatic cords and absence of necrosis with minimal inflammatory conditions around the central vein (Fig. 4). However, moderate protection was observed in case of low dose group animals (Fig. 3).

Discussion: Paracetamol is a widely used antipyretic and analgesic drug which is safe in therapeutic doses but can cause hepatic damage in human and animals at higher doses (Kuma & Rex, 1991). The covalent binding of N-acetyl-p-benzoquinone imine (NAPQI), a highly toxic metabolite of paracetamol, to sulfhydryl groups of protein results in cell necrosis and lipid peroxidation (Jollow et al., 1973). NAPQI is normally conjugated with glutathione and excreated in the urine as conjugates. Toxic dose of paracetamol depletes hepatic reduced glutathione (GSH) level so that free NAPQI binds covalently to cellular macromolecules causing acute hepatocellular necrosis (Davis et al., 1974). Glutathione is the endogenous non-enzymatic antioxidant in our body system and it is protective against chemically induced hepatic damage and oxidative stress (Videla & Valenzula, 1972). Depleted GSH level with elevated level of lipid peroxidation in paracetamol-induced rats indicated that the dose of paracetamol 640 mg/kg was highly hepatotoxic. It was confirmed from the present study that the MECT significantly restored hepatic GSH content towards normal in paracetamol intoxicated rats indicating decreased free NAPQI level in the blood.

Lipid peroxidation is a phenomenon involved in peroxidative loss at unsaturated lipids, thus bringing about cellular lipid degradation and membrane disordering. Lipid peroxidation is usually measured through its catabolite malondialdehyde (MDA) as a marker of oxidative stress (Janero, 1990). MECT showed ability to prevent paracetamol induced increment of MDA level, suggesting that MECT inhibited hepatic lipid peroxidation in paracetamol intoxicated rats.

It has been well established that elevated levels of SGOT and SGPT are indicative of cellular leakage and loss of functional integrity of the hepatic cell membranes (Mukherjee et al., 1997). The MECT decreased the elevated serum enzyme levels and bilirubin level in the paracetamol treated rats which are comparable to the saline control group. It appears that the extract preserved the structural integrity of the hepatocellular membrane which is evident from the significant reduction in paracetamol induced rise in serum enzymes in rats. It was further supported by the histopathological studies showing recovery of hepatocellular lesions by MECT .

Preliminary phytochemical analysis of MECT indicated the presence of flavonoids and tannins and these types of polyphenols are well known natural antioxidants due to their electron donating property which either scavenge the

principal propagating free radicals or halt the radical chain (Sugihara et al., 1999). Thus the hepatoprotective activity of MECT may be due to the presence of polyphenolic compounds.

5. Conclusion

From the present investigation, it can be concluded that *Cyperus tegetum* rhizome demonstrated very effective hepatoprotective potential against paracetamol-induced liver damage in Wistar rats.

6. Significance, applications and implications

In the absence of reliable liver protective drugs in allopathic medical practices, naturally occurring compounds have been found to have major role in the management of various liver diseases. So search for effective hepatoprotective agents are continued in the plant kingdom. From present study it can be inferred that the methanol extract of *C. tegetum* rhizome dose dependently offered significant hepatoprotection against paracetamol-induced liver damage, normalizing biochemical parameters and liver histology in rats. To the best of our knowledge, present study is the first report of any pharmacological investigation on *C. tegetum*. Outcome of the present study was encouraging enough to carry out further pharmacological and phytochemical studies on *C. tegetum*.

Table 1. Effects of MECT and silymarin on serum biochemical parameters of normal and paracetamol intoxicated rats.

Treatment	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)	Total Bilirubin (mg/dl)	Total Protein (mg/dl)
Normal Control (5 ml/kg)	24.96 ±3 .22	20.66 ± 5.20	9.82 ± 0.73	0.52 ± 0.07	7.8 ± 0.23
PCM Control (0.5 ml/kg)	85.55 ± 2.37 §	84.66 ±10.72 §	22.46 ± 3.10 §	2.04 ± 0.34*	4.93 ± 0.35 §
PCM + MECT (250 mg/kg)	43.46 ± 4.79**	43.33 ± 5.6 ¶	13.03 ± 0.29 ¶	0.52 ± 0.12**	6.73 ± 0.29¤
PCM + MECT (500 mg/kg)	19.96 ± 5.4**	18.00 ± 3.0**	11.81±0.51**	0.33 ± 0.02**	7.13 ± 0.37 ¶
PCM + Silymarin (25 mg/kg)	31.62 ± 1.51**	34.66 ± 5.81 ¶	11.53 ± 0.44 ¶	0.59 ± 0.15 ¶	7.53 ± 0.29**

Values are mean ± SEM (n = 8). PCM: Paracetamol.
Paracetamol control group *vs.* normal control group, § $p < 0.001$, * $p < 0.01$.
Treated groups *vs.* paracetamol control group, ** $p < 0.001$; ¶ $p < 0.01$; ¤ $p < 0.05$ where the significance was assessed by one-way analysis of variance (ANOVA) followed by Dunnett's test.

Table 2. Effect of MECT and silymarin on liver biochemical parameters in normal and paracetamol intoxicated rats.

Treatment	MDA (µM/g wet tissue)	GSH (µg/g wet tissue)
Normal control (5 ml/kg)	40.42 ± 3.29	23.87 ± 1.72
PCM Control (0.5 ml/kg)	92.65 ± 1.90***	11.8 ± 0.69**
PCM + MECT (250 mg/kg)	51.95 ± 2.90 ¶	21.57 ± 1.42*
PCM + MECT (500 mg/kg)	38.57 ± 1.57 ¶	22.83 ± 2.08 ¶
PCM + Silymarin (25 mg/kg)	39.37 ± 2.58 ¶	22.15 ± 3.01*

Values are mean ± SEM (n = 8). PCM: Paracetamol.
Paracetamol control group *vs.* normal control group, **$p<0.01$, ***$p<0.001$. Treated groups *vs.* paracetamol control group,*$p < 0.05$, ¶ $p < 0.001$ where the significance was assessed by one-way analysis of variance (ANOVA) followed by Dunnett's test.

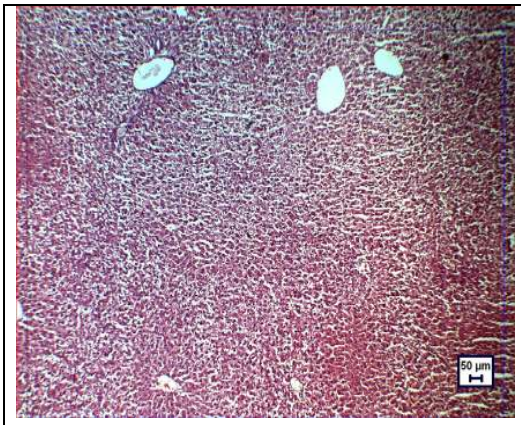

Fig.1. Normal control

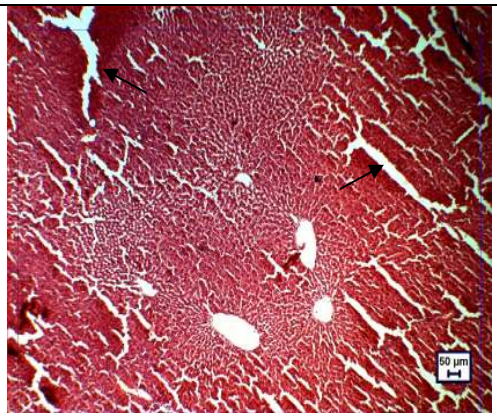

Fig.2. Paracetamol control

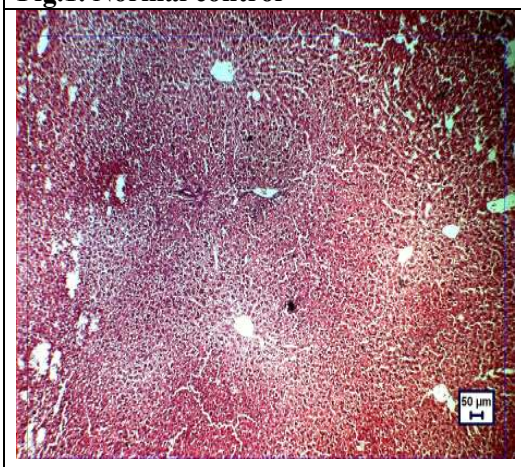

Fig.3 MECT (250 mg/kg) treated

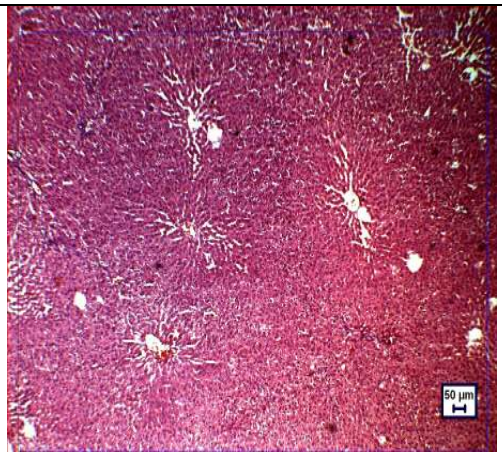

Fig.4. MECT (500 mg/kg) treated

Fig. 1. Liver section of normal rat (control), **Fig. 2.** Liver section of paracetamol intoxicated rat showing large necrosis, **Fig. 3.** Liver section of MECT (250 mg/kg) treated rat, showing reduction in necrosis, **Fig. 4.** Liver section of MECT (500 mg/kg) treated rat, showing signs of recovery.

7. References

1. Bhaduri S.K., S. Chanda, and P. Majumdar. 1998. Chemical characterization of the stem of *Cyperus tegetum* : A semi-aquatic plant of economic importance. *Bioresource Tech*. 63 (3): 279-281.
2. Davis, D.C., W.Z. Potter, D.J. Jollow, and J.R. Mitchell. 1974. Species differences in hepatic glutathione depletion, covalent binding and hepatic necrosis after acetaminophen. *Life Sci*. 14: 2099-2109.
3. Ellman, G.L. 1959. Tissue sulphydryl groups. *Arch. Biochem. Biophys.* 82 (1): 70-77.
4. Harborne, J.B. 1998. Phytochemical methods, A Guide to Modern Techniques of Plant Analysis, Springer (India) Pvt. Ltd., New Delhi.
5. Janero, D.R. 1990. Malondialdehyde and thiobarbituric acid-reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury. *Free Rad. Biol. Med.* 9: 515-540.
6. Jollow, D.J., J.R. Mitchell, W.Z. Potter, D.C. Davis, J.R. Gillete and B.B. Brodie. 1973. Acetaminophen induced hepatic necrosis. II. Role of covalent binding *in vivo*. *J. Pharmacol. Exp. Ther.* 187: 195-202.
7. Kuma, S., and D. Rex. 1991. Failure of physicians to recognize acetaminophen hepato-toxicity in chronic alcoholics. *Arch. Internal Med.* 151: 1189-1191.
8. Lowry, O.H., N.J. Rosebrough, A.L. Far, and R.J. Randall. 1951. Protein measurement with Folin phenol reagent. *J. Biol. Chem.* 193: 265-275.
9. Mukherjee, S., A. Sur, and B.R. Maiti. 1997. Hepatoprotective effect of *Swertia chirata* on rat. *Indian J. Exp. Biol.* 35: 384-388.
10. Okhawa, H., N. Oishi, and K. Yagi. 1979. Assay for lipid peroxides in animal tissue by thiobarbituric acid reaction. *Anal. Biochem.* 95: 351-358.
11. Padhye, M.D., and S.K. Moharir. 1958. Studies in embryology of *Cyperus tegetum* Roxb. Proc. Plant Sci. 48 (2): 89-95.
12. Sugihara, N., Arakawa, T., Ohnishi, M., Furunko, K., 1999. Anti and pro-oxidative effects of flavonoids as meta-induced lipid hydroperoxide-dependent lipid peroxidation in cultured hepatocytes loaded with alpha-linoleic acid. *Free Rad. Biol. Med.* 27: 1313-1323.
13. Videla, L.A., and A. Valenzula. 1982. Alcohol ingestion, liver glutathione and lipid peroxidation, metabolic interaction and parameters implication. *Life Sci.* 31: 2395-2407.