Hepatoprotective activity of methanol extracts of *Glinus oppositifolius* and *Trianthema decandra* against paracetamol induced liver damage

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SUMMARY

The plants, *Trianthema decandra* and *Glinus oppositifolius* are commonly used by tribal people in India for the treatment of liver diseases. Hepatoprotective activity of methanol extracts of *Glinus oppositifolius* and *Trianthema decandra* at the dose of 250 and 500 mg/kg body weight administered orally was evaluated against paracetamol induced liver damage in rats. Biochemical parameters such as serum glutamate oxaloacetate transaminase, serum glutamate pyruvate transaminase, serum alkaline phosphatase, bilirubin, total serum protein, lipid peroxide and glutathione content of the liver were estimated to determine liver function and metabolism. From the biochemical observations, it was concluded that methanol extracts of *Glinus oppositifolius* and *Trianthema decandra* significantly restored the altered biochemical parameters towards normal condition in paracetamol induced liver damage.

Key words: *Trianthema decandra*; *Glinus oppositifolius*; Hepatoprotective; Paracetamol

INTRODUCTION

The plant, *Trianthema decandra* (Family: Ficoidaceae) is commonly known as 'Gadabani', which is found in Deccan peninsula, on dry-soil, a roadside weed. In traditional medicinal system, the plant is widely used in the treatments of various ailments. Its decoction is used in the treatment of asthma, hepatitis, cancer and suppression of menses. Tribes normally consume root powder with milk in orchitis (Kirtikar and Basu, 1975). The another plant, *Glinus oppositifolius* (Family: Ficoidaceae) is commonly known as gima. It is present in the greater part of India, especially in Assam, West Bengal and Deccan peninsula. The plant is used as stomachic, aperient, antiseptic, anticancer and suppressive agent of the lochia, bitter tonic for liver disorders. (Kirtikar and Basu, 1975; Chatterjee and Pakrashi, 1991). The present study was carried out to evaluate the hepatoprotective activity of methanol extract of *Glinus oppositifolius* (MEGO) and methanol extract of *Trianthema decandra* (METD). Biochemical parameters such as serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), serum alkaline phosphatase (ALP), bilirubin, total serum protein, lipid peroxide and glutathione content of the liver were estimated to determine liver function and metabolism.

MATERIALS AND METHODS

Preparation of plant extracts

The whole plant material of *Glinus oppositifolius* was collected from Midnapore, West Bengal, during

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June-August when the plant was in full leaf and another plant, *Trianthema decandra* were collected from Kolli Hills Tamilnadu. The plant materials were taxonomically identified by the Botanical Survey of India, Shibpur, Howrah and the voucher specimen (GMC-1 and GMC-2) were retained in our laboratory for future reference. The collected plant material were dried under shade and then powdered with a mechanical grinder. The powder was passed through sieve number 40 and stored in an airtight container for further use. The air dried powered material of *Glinus oppositifolius* was defatted by extracting with petroleum ether in Soxhlet extraction apparatus. The defatted material was then extracted with methanol (80%). The solvent was completely removed under reduced pressure to obtain a dry mass and stored in vacuum dessicator. The yield of petroleum ether extract and methanol extracts were found to be 4.6% and 14.8% w/w respectively. The air dried powered material of *Trianthema decandra* was also extracted with petroleum ether and methanol (80%) successively in Soxhlet apparatus. The solvent was also completely removed under reduced pressure and stored in vacuum dessicator. The yield of petroleum ether extract and methanol extracts were found to be 7.4% and 13.8% w/w respectively.

Reagents for estimation of biochemical markers were obtained from Sigma Chemical Co. and solvents were obtained from E-Merck, Mumbai.

Treatment protocol
Adult male albino rats weighing 150 - 180 g were used for the present investigation. They were housed in clean polypropylene cages 72 h before use for acclimatization at a controlled condition of temperature with a 12 h light and 12 h dark cycle before the experiment. The rats had free access to feed with standard pellet diet and water *ad libitum*. The rats were divided into seven groups (n = 6). The rats of group 1 served as vehicle control and treated with normal saline (0.9% w/v sodium

chloride). Paracetamol (640 mg/kg suspended in 1% methylcellulose) was administered orally in animals of group two to group seven. Group 3, 4, 5 and 6 received MEGO and METD at the doses of 250 and 500 mg/kg body weight suspended in 1% methylcellulose for 16 days. The standard hepatoprotective drug, silymarin, at the dose of 25 mg/kg body weight was administered orally in group 7 for 16 days (Mukherjee *et al.*, 1997). After 24 h of the last dose and 18 h fasting, all animals were sacrificed. The blood was collected for biochemical estimation and the liver tissue was kept in the frozen condition for biochemical examination.

Biochemical estimation
SGOT (Oser, 1965), SGPT (Oser, 1965), ALP (Bergmeyer, 1974), bilirubin (Oser, 1965) and total serum protein (Lowry *et al.*, 1951) were estimated in serum of rats. Lipid peroxide (Ohakawa *et al.*, 1979) and glutathione (Mulder *et al.*, 1995) content of the liver were measured in liver tissue to determine liver function and metabolism.

Statistical analysis
Results are expressed as mean ± S.E.M. and the test of significance of the results were evaluated by student's '*t*' test.

RESULTS AND DISCUSSION

Rats treated with paracetamol alone developed significant liver cell damage was evident from a significant increase in serum enzyme levels of SGOT, SGPT, ALP and bilirubin when compared with vehicle control. The treatment of rats with MEGO and METD at the doses of 250 and 500 mg/kg body weight significantly reduced the elevated serum levels of these hepatospecific enzymes and bilirubin levels in paracetamol intoxicated rats in a dose dependent manner (Table 1).

Paracetamol was also markedly increased the formation malondialdehyde (MDA) in the liver that ascertains the status of lipid peroxidation.

Table 1. Effect MEGO and METD and silymarin on serum enzymes (SGOT, SGPT and ALP), bilirubin and total protein in paracetamol intoxicated rats

Groups	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)	Bilirubin (mg/dl)	Total Protein (mg/dl)
Vehicle control	75.82 ± 1.81	63.25 ± 1.56	35.57 ± 0.56	1.15 ± 0.20	7.05 ± 0.10
Paracetamol treated	858.85 ± 51.22	625.54 ± 35.72	72.44 ± 0.98	2.74 ± 0.22	3.10 ± 0.15
Paracetamol + MEGO (250 mg/kg)	242.52 ± 6.53	135.79 ± 6.58	55.85 ± 0.89*	1.98 ± 0.24	4.70 ± 0.19
Paracetamol + MEGO (500 mg/kg)	140.75 ± 5.85*	81.63 ± 4.57	44.58 ± 0.78*	1.43 ± 0.18	5.98 ± 0.24
Paracetamol + METD (250 mg/kg)	250.34 ± 6.37	145.87 ± 7.96*	56.72 ± 0.85	2.05 ± 0.25	4.74 ± 0.18
Paracetamol + METD (500 mg/kg)	175.85 ± 8.76*	85.85 ± 3.63	45.25 ± 0.75	1.45 ± 0.15*	5.57 ± 0.25*
Paracetamol + silymarin (25 mg/kg)	125.84 ± 3.84	75.86 ± 4.12	39.12 ± 0.70	1.27 ± 0.09	6.50 ± 0.15

P values calculated by ANOVA analysis followed by student's 't' test of significance with six animals in comparison with paracetamol treated group. *P < 0.05.

Both MEGO and METD decreased the elevated MDA formation in a dose dependent manner as compared with paracetamol control (Table 2). The administration of paracetamol caused a reduction in hepatic GSH levels. But both MEGO and METD significantly increased the reduced hepatic GSH levels in paracetamol treated rats as compared with only paracetamol treated group (Table 2).

Paracetamol (N-acetyl-p-aminophenol, Acetaminophen), a widely used antipyretic and analgesic drug is known to cause liver cell damage in animals and humans at high doses (Prescott *et al.*, 1971; Mitchell, 1988; Kuma and Rex, 1991; Eriksson *et al.*, 1992; Thompsen *et al.*, 1995). It is established that an oral therapeutic dose, a fraction of paracetamol is converted *via* the cytochrome P450 pathway to

form a highly toxic metabolite, N-acetyl-p-benzoquinone imine (NAPQI) (Dahlin *et al.*, 1984), which is normally conjugated with glutathione and excreated in the urine as conjugates. Overdoses of paracetamol reduce glutathione strores, leading to accumulation of NAPQI, mitochondrial dysfunction (Parmar *et al.*, 1995) and the development of hepatic necrosis. The decrease of GSH by acetaminophen intoxicated mice is elevated by the treatment of medicinal plant extract (Ye *et al.*, 2001). The MEGO and METD increased the GSH level to normal level in paracetamol intoxicated rats. MEGO and METD restored GSH level probably by reducing NAPQI level.

Paracetamol induced hepatotoxicity in rodents is a widely used animal model to assess the

Table 2. Effect of MEGO and METD and silymarin on lipid peroxidation and glutathione in paracetamol intoxicated rats

Groups	Lipid peroxidation (mM of MDA / mg of protein)	Glutathione (mg/mg of protein)
Vehicle control	225.5 ± 6.5	458.3 ± 15.2
Paracetamol treated	550.6 ± 16.8	135.4 ± 5.4
Paracetamol + MEGO (250 mg/kg)	307.8 ± 5.6	315.6 ± 6.1
Paracetamol + MEGO (500 mg/kg)	250.3 ± 5.6*	425.6 ± 11.5*
Paracetamol + METD (250 mg/kg)	310.5 ± 5.8	308.6 ± 5.3
Paracetamol + METD (500 mg/kg)	257.8 ± 5.1*	405.6 ± 12.1
Paracetamol + silymarin (25 mg/kg)	241.5 ± 5.2*	430.5 ± 12.3*

P values calculated by ANOVA analysis followed by student's 't' test of significance with six animals in comparison with paracetamol treated group. *P < 0.05.

hepatoprotective activity of new compounds (Handa *et al.*, 1986). The rise in serum levels of SGOT, SGPT and ALP has been attributed to the damaged structural integrity of the liver. Pretreatment with plant extracts prior to the administration of paracetamol significantly prevented the increase in ALP and aspartate aminotransferase activity in a dose dependent manner (Kyung *et al.*, 2001). The reversal of increased serum enzymes in paracetamol induced liver by these plant extracts may be due to prevention of leakage of the intracellular enzymes by its membrane stabilizing activity (Thabrew *et al.*, 1987). A substantial increase in serum enzymes (SGOT, SGPT and ALP) induced by paracetamol and its suppression to nearly normal levels by both MEGO and METD at the dose of 250 and 500 mg/ kg body weight was noted. MEGO and METD also reduced the elevated serum bilirubin level in paracetamol intoxicated rats. MEGO and METD reduced marker enzymes and bilirubin in paracetamol intoxicated rats, so these can be considered as hepatoprotective agents.

The protective role of GSH against cellular lipid peroxidation has been proven (Burk, 1983; Muriel *et al.*, 1992). The increase in hepatic lipid peroxidation by paracetamol intoxication is due to elevated MDA level in liver homogenate. The suppression of MDA level to nearly normal values by the treatment of METD and MEGO was noted. METD and MEGO probably by lowering MDA level reduce the level of lipid peroxidation and elevate the reduced level of GSH.

Our present study suggested that MEGO and METD restored the altered biochemical parameters and these were shown to be potent hepatoprotective agents.

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