Evaluation of the antioxidant activity of *Trianthema portulacastrum* L.

Trianthema portulacastrum L. (Aizoaceae) is historically valued as a green vegetable by poor people on the Indian Subcontinent. The plant is used in the treatment of edema in the liver and spleen,[1] uteralgia, and cough. The plant is lithotriptic for the kidney and bladder. In the Indian traditional medicine system, the plant is considered as a diuretic. It has significant hepatoprotective activity against paracetamol and thio-acetamide intoxication in rats.[2] The present investigation is to determine the antioxidant activities of the plant extract in relation to paracetamol and thioacetamide intoxication in rats.

Leaves of *T. portulacastrum* were collected during March–April 2002 from Namakkal, Tamilnadu, India and authenticated by Fr. K.M. Matthew, Director, Rapinat Herbarium, St. Joseph's College, Tiruchirapalli, Tamilnadu, India. Voucher herbarium specimens were deposited in the herbarium for future references. Coarse powder from the shade-dried leaves of *T. portulacastrum* (500 g) was extracted to exhaustion with ethanol using a soxhlet apparatus. The ethanolic extract thus obtained was dried under reduced pressure at a room temperature not exceeding 40 °C.

Paracetamol was obtained from the National Medical Company, Amritsar and thioacetamide was purchased from Loba Chemie, Mumbai. 5, 5'- Dithiobis- (2-nitro benzoic acid) (DTNB), oxidized (GSSG) and reduced (GSH) glutathione, 1-chloro-2, 4-dinitrobenzene (CDNB), ß-nicotinamide adenine dinucleotide phosphate, reduced form (NADPH), ß-nicotinamide adenine dinucleotide, reduced form (NADH), glutathione reductase, thiobarbituric acid (TBA) and trichloroacetic acid (TCA) were purchased from Sigma Chemical Co., St. Louis. MO, USA. All the other chemicals used were either of analytical grade or of the highest purity commercially available.

Male Wistar albino rats were purchased from Perundurai Medical College, Erode and kept for a week on a commercial diet under controlled conditions (temperature 22 ± 3 °C, relative humidity 60–80%, 12 h light/dark cycle with free access to food and water). Rats weighing 150-200 g were used for the experiments.

Four groups of rats, each containing six animals were used for paracetamol intoxication experiment. Group I served as control, Group II received the hepatotoxin in 50% w/v sucrose, Group III and IV received the hepatotoxin and the ethanolic extracts of *T. portulacastrum* (100 and 200 mg/kg, p.o) in 50% w/v sucrose, respectively.

The ethanolic extract of *T. portulacastrum* (100 and 200 mg/kg, p.o) which was found to be the working dose in our previous experiments,[2] was given daily once for 10 days in succession, followed by a single administration of paracetamol (3 g/kg, p.o.) on the 10th day 1 h after the extract administration. Similarly, another set was used for thioacetamide intoxication study. Thioacetamide was administered in 2% aqueous solution (w/v) (100 mg/kg, s.c).

The animals of the toxin control groups received vehicle daily for 10 days, followed by a single oral administration of toxicants. The normal control group received vehicle alone.

After 24 h of toxin administration, the rats of each group were sacrificed by cervical dislocation; blood was collected and used for estimation of reduced glutathione. The livers were dissected immediately, weighed, and homogenized. The homogenates were then centrifuged at 3200 X for 20 min at 4 °C and the supernatant that was obtained was used for the assay of various enzymes.

The blood glutathione, superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) were assayed by the method of Rotruck *et al*.[3] Glutathione-S-tranferase (GST), glutathione reductase (GSH-R), and liver Na-K-ATPase activity were measured by the method of James *et al*.[4] The concentration of TBARS (TBA reactive substances) in liver was measured using the method of Ohkawa *et al*.[5]

All experimental data were expressed as mean±SD and statistically assessed by one-way analysis of variance (ANOVA). The difference between test animals and controls was analyzed using Dunnett's test. $P < 0.05$ was considered significant.

The results show that pretreatment of rats with 100 mg or 200 mg/kg, p.o., of ethanolic extracts of *T. portulacastrum* prevented significantly the paracetamol- and thioacetamide-induced reduction of blood and liver glutathione, liver Na-K-ATPase level (Table 1). TBARS of toxicants-treated animals were significantly higher than the control animals. Administration of the ethanolic extract markedly decreased the level of TBARS. The degree of protection was more with the higher dose of the extract.

The effect of the ethanolic extract on GSH-R, GST, GPX, SOD, and CAT are shown in Table 2. Levels of these antioxidant enzymes were decreased significantly in toxicants-treated rats when compared with those of normal control animals. Treatment of rats with the extract (100 and 200 mg/kg, p.o.) prevented these reductions to a large extent ($P < 0.001$).

Thioacetamide is a potent hepatotoxin and carcinogen in rats.[6] In our study, thioacetamide produced the elevation in the levels of TBARS and depletion in GSH. Pretreatment of rats with *T. portulacastrum* extract significantly reduced the elevated levels of TBARS and increased the concentration of hepatic and blood GSH. These results suggest that a higher content of GSH in blood and liver would afford the tissue a better protection against an oxidative stress.

Because the detoxification of paracetamol can be mediated by GST-catalyzed conjugation with GSH in the liver,[7] the increased hepatic GST activity induced by the extract treatment can, therefore, reduce the acute paracetamol hepatotoxicity.

Table 1

Effect of ethanolic extract of *T. portulacastrum* on glutathione levels in blood and liver and liver Na^+K^+ATPase, and TBARS in paracetamol and thioacetamide intoxicated rats.

Groups		*Glutathione*		*Liver Na^+-K^+-ATP ase*	*TBARS (nmol MDA/g of*
		Blood (mg %)	*Liver (mmol/g of wet liver)*	*(U/g protein)*	*wet tissue/h)*
Normal control		2.05±0.04	10.70±0.68	10.20±0.82	345.3±10.4
Paracetamol control		0.85±0.02[a]	7.90±0.22[b]	7.30±0.41[b]	468.7±12.3[a]
Paracetamol + 100 mg/kg extract		1.44±0.03[c] (49.1)	8.80±0.31[d] (32.1)	8.96±0.63[c] (57.2)	393.5±8.6[c] (60.9)
Paracetamol + 200 mg/kg extract		1.82±0.02[c] (80.8)	9.90±0.27[c] (71.4)	9.74±0.54[c] (84.1)	367.8±9.7[c] (81.7)
Thioacetamide control		1.02±0.03[a]	8.20±0.41[b]	7.82±0.37[b]	454.5±8.42[a]
Thioacetamide +100 mg/kg extract		1.64±0.08[c] (60.1)	9.10±0.38[d] (36)	8.75±0.68[c] (39)	394.7±9.15[c] (54.7)
Thioacetamide + 200 mg/kg extract		1.98±0.06[c] (93.2)	9.80±0.31[c] (64)	9.54±0.74[c] (72.2)	369.5±10.28[c] (77.8)
One-way	F	60.2	43.5	42.8	25.2
ANOVA	df	6,36	6,36	6,36	6,36
	P	0.01	0.01	0.01	0.01

Values are mean ± SD; n=6 animals per group. Values in the parenthesis indicate percentage protection provided by the extract in individual biochemical parameters from their elevated values. The percentage of protection is calculated as 100 X (values of toxicant control values of sample) / (values of toxicant control values of control). [a]P <0.001 when compared with normal control; [b]P <0.01 when compared with normal control. [c]P <0.001 when compared with paracetamol control; [d]P <0.01 when compared with paracetamol control.

Table 2

Effect of ethanolic extract of *T. portulacastrum* on GSH-R, GSH-PX, GST, SOD and CAT in paracetamol and thioacetamide intoxicated rats.

Group		*GSH-R (µmol NADPH/ min/g of wet liver)*	*GSH-PX (U/mg protein)*	*GST (U/g of wet liver)*	*SOD (U/mg protein)*	*CAT (H_2O_2 decomposed/ min/mg protein)*
Normal Control		165.7 ±3.8	10.2±0.82	104.5±1.9	9.82 ±0.32	72.6±2.5
Paracetamol control		102.3 ±4.5[a]	6.80±0.64[b]	89.6±2.1[b]	5.56 ±1.04[a]	39.87±2.42[a]
Paracetamol + 100 mg/kg extract		145.8 ±5.4[c] (68.2)	8.43±0.54[d] (47.2)	94.3±0.9[c] (31.5)	8.21 ±0.78[c] (62.2)	58.72±3.18[c] (57.5)
Paracetamol + 200 mg/kg extract		160.5 ±7.2[c] (91.7)	9.20±0.38[c] (69.5)	98.1±1.7[c] (63.7)	9.15 ±0.72[c] (84.2)	64.52±2.74$_c$ (75.1)
Thioacetamide control		107.8 ±6.2[a]	7.2±0.72[b]	84.8±1.8[b]	5.92 ±0.75[a]	42.8±2.1[a]
Thioacetamide + 100 mg/kg extract		148.5 ±4.7[d] (70.2)	8.8±0.86[d] (52.4)	90.9±2.1[d] (30.9)	8.20 ±0.64[d] (58.4)	60.8±2.9[c] (60.3)
Thioacetamide + 200 mg/kg extract		159.3 ±5.2[c] (88.9)	9.6±0.65[c] (78.6)	98.4±1.9[d] (69)	9.30 ±0.27[c] (86.6)	69.1±2.4[c] (88.1)
One-way	F	78.5	69.5	43.2	35.6	14.5
ANOVA	df	6,36	6,36	6,36	6,36	6,36
	P	0.01	0.01	0.01	0.01	0.01

Values are mean ± S.D; n=6 animals per group. Values in the parenthesis indicate percentage protection provided by the extract in individual biochemical parameters from their elevated values caused by the hepatoprotection. The percentage of protection is calculated as in Table 1. [a]P <0.001 when compared with normal control; [b]P <0.01 when compared with normal control. [c]P <0.001 when compared with thioacetamide control; [d]P <0.01 when compared with thioacetamide control.

GSH-R is a family of cytosolic hepatic enzymes involved in the detoxification of a range of xenobiotic compounds by their conjugation with GSH.[8] It has been reported that paracetamol produces an increased blood GSH-R activity, which occurs at a maximum of 24 h after administration. Pretreatment of *T. Portulacastrum* significantly reduced GSH-R in toxicant-administered rats

The activities of Na^+ K^+ ATPase, SOD, and CAT were decreased ($P < 0.001$)in hepatotoxin-treated animals.[9] The extract may be useful for the prevention of hepatotoxin-induced liver damage. These findings show that the antioxidant capacity of the liver decreased leading to the generation of lipid peroxides resulting in liver damage. Ethanolic extract of the plant increases the activity of SOD and CAT and it scavenges free radicals and reduces hepatic damage. These results suggest that the hepatoprotective action of the extract might be due to its antioxidant activity.

In conclusion, the hepatoprotective effect of *T. portulacastrum* alcohol extract against paracetamol- and thioacetamide-induced hepatotoxicity in rats appears to be related to the inhibition of lipid peroxidative processes and to the prevention of GSH depletion.

G. Kumar, G.S. Banu*, M.R. Pandian*
Department of Biochemistry, Selvam Arts & Science College, Namakkal and *Centre for Biotechnology, Muthayammal College of Arts and Sciences, Kakkaveri, Rasipuram, Namakkal, Tamilnadu, India.
E-mail: rajamoses@yahoo.com.

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