



Preliminary evaluation of hepatoprotective activity of *Trianthema portulacastrum* Linn.

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Abstract

Objective: To study hepatoprotective effect of aerial parts of *Trianthema portulacastrum*. **Materials and methods:** Hepatoprotective activity of *T. portulacastrum* was evaluated using paracetamol and rifampicin as liver toxicants in rats. The potency of alcoholic and aqueous extracts of aerial parts was compared with that of silymarin at a dose of 100 mg/kg, P.O. The SGOT, SGPT and bilirubin levels of blood were measured spectrophotometrically. **Results:** Alcoholic extract of aerial parts caused significant fall in the enzyme levels in serum in rats. **Conclusion:** *T. portulacastrum* has shown significant activity in rats toxicated with paracetamol and rifampicin and can be recommended for further studies.

Key Words: *Trianthema portulacastrum*, Hepatoprotective activity, Paracetamol, Rifampicin, Silymarin.

1. Introduction

T. portulacastrum Linn. (Ficoidacea) is known as Satodo in Gujarati[1]. The roots has been advocated for inflammation and general tonic in Ayurvedic medicine and powder of aerial parts is used for the treatment of jaundice[2,3]. The drug is also reported as bitter, hot, alexiteric, analgesic, stomachic, laxative, alterative, cures “kapha”, bronchitis, heart diseases, anaemia, “vata”, piles, ascites[4] and hepatoprotective [5].

There is only one report in the literature regarding its hepatoprotective activity[2]. Our previous findings showed significant

hepatoprotective effect against carbon-tetrachloride-induced liver damage in rats [6]. To our knowledge, this is probably the first report to validate its utility in liver disease using other toxicants and give a rational basis to exploit further.

2. Material and methods

2.1 Plant material

The plant *T. portulacastrum* authenticated by comparison with herbarium preserved in the Department of Pharmacognosy, A.R. College

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of Pharmacy, Vallabh Vidyanagar (Gujarat) and aerial parts were collected from the campus in the month of September.

2.2 Preparation of extracts

The plant material was dried, in shade and then dried in oven at 40-50°C for 5 hrs. The dried material was then subjected to size reduction to coarse powder using grinding mill and soxhlet extracted successively with petroleum ether (40-60°C), benzene, chloroform, ethyl acetate, alcohol and water. All these extracts were dried. The alcoholic and aqueous extracts [6] were formulated as suspension in 4% aqueous gum acacia mucilage since gum acacia has negligible effect on blood serum parameters. The strength of the suspension was according to dose administered and expressed as weight of dried extract.

2.3 Preparation of standard drug

Silymarin [7] was used as the standard drug for evaluating hepatoprotective activity. Gift sample of silymarin was obtained from M/s. Ranbaxy Laboratories, Dewas, and made into suspension using 4% w/v gum acacia as suspending agent. The strength of suspension was adjusted to 50 mg/ml of silymarin. 4% w/v aqueous acacia mucilage was used as vehicle for both the paracetamol and rifampicin models.

2.4 Animals

Wistar strain albino rats (150-200 g.) of either sex maintained under standard environmental conditions (temp. 25-28°C and 12 h light/dark cycle) were used. They were allowed standard laboratory feed and water *ad libitum*. Groups of 5 animals each were used in all sets of experiments.

2.5 Hepatoprotective activity

2.5.1 Paracetamol induced liver damage [8]

Suspension of test extracts (alcohol and aqueous) and silymarin were administered by gavage at 100 mg/kg once daily for three days. On the

third day of treatment, paracetamol (3 g/kg, P.O.) [9] was administered 30 minutes after the administration of test suspension. Control animals received 1 ml/kg, P.O. of vehicle.

After 48 h of paracetamol administration, blood was collected from all the groups of rats by puncturing retro-orbital plexus. The blood samples were allowed to clot for 45 min at room temperature. Serum was separated by centrifugation at 2500 rpm at 37°C for 10 min and analysed for various biochemical parameters.

2.5.2 Rifampicin-induced liver damage [10]

Suspension of test extracts (alcohol and aqueous) and silymarin were administered by gavage at 100 mg/kg four times at 12 h intervals for the period of 36 h. Rifampicin (1 g/kg, P.O.) [11] was administered 30 min after first dose of test suspensions. Control animals received 1 g/kg, P.O. of vehicle. After 48 h of rifampicin administration, blood was collected from all groups of rats and serum was analyzed for various parameters as in case of paracetamol-induced liver damage.

2.5.3 Assessment of liver functions

Biochemical parameters, i.e. serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) [12] were analyzed using SGOT and SGPT kits manufactured by Span diagnostics Pvt. Ltd, Surat, whereas total bilirubin (T Bil) and direct bilirubin (D Bil) were analyzed according to reported methods [13]. The mean value \pm SEM was calculated for each parameter. Percent reduction against hepatotoxin by test sample was calculated by considering the enzyme level difference between the hepatotoxin treated and the control groups as 100% level of reduction and expressed by the formula

$$H = \{1 - (T_c - V / V_c - V)\} \times 100,$$

Where H is % hepatoprotective activity, T_c , V_c and V are the parameters measured in test drug + toxicant, vehicle + toxicant and vehicle treated animals respectively.

2.5.4 Histology

One animal from each group was sacrificed and the liver was collected and processed for histopathology by routine methods.

2.5.5 Statistical analysis

For determination of significant inter group differences each parameter was analyzed separately and one way analysis of variance was carried out, Dunnett's test was used for individual comparisons [14,15].

3. Results

As shown in Table 1 and 2, activities of serum GPT, GOT, T Bil. and D. Bil were markedly elevated in paracetamol and rifampicin treated animals respectively compared to normal

control rats, indicating liver damage. Administration of alcoholic extract of aerial parts of *T. portulacastrum* at dose of 100 mg/kg P.O. remarkably prevented paracetamol-induced (Table 1) and rifampicin-induced (Table 2) elevation on the serum GPT, GOT except bilirubin in case of rifampicin.

These results were comparable with silymarin. The aqueous extract of the plant was not as significant as alcoholic extract in providing hepatoprotection against paracetamol-induced liver damage (Table 1) and rifampicin-induced liver damage (Table 2).

4. Discussion

The present study indicates significant results regarding hepatoprotective activity of alcoholic extract of *T. portulacastrum*.

Paracetamol is a well known antipyretic and analgesic agent which produces hepatic necrosis in higher doses [16,17]. Paracetamol damages

Table 1
Effect of *T. portulacastrum* aerial parts on paracetamol induced hepatotoxicity in rats.

Treatment	Dose (mg/kg P.O.)	SGPT (U/ml)	SGOT (U/ml)	T. Bil. (mg/dl)	D. Bil. (mg/dl)
Control	-	44±0.8	122±2.6	1.10±0.04	0.29±0.03
Paracetamol	3 g -	198±7.6 ^{#@}	284±5.6 ^{#@}	2.64±0.08 ^{#@}	0.98±0.02 ^{#@}
Alc. Ext.	100	56±1.6 ^{*#} (92.3)	140±3.6 ^{*#} (88.9)	1.00±0.02 [*] (106.49)	0.28±0.01 ^{*#} (101.44)
Aq. Ext.	100	174±6.3 ^{*#} (15.60)	237±5.9 ^{*#} (29.10)	2.66±0.06 [#] (1.20)	0.80±0.02 ^{*#} (26.10)
Silymarin	100	48±2.1 [*] (97.40)	116±3.5 [*] (103.70)	1.08±0.02 [*] (101.20)	0.42±0.03 [*] (81.20)

Values are mean ± S. E. (n=5)

*p<0.01 significantly different from paracetamol.

p<0.01 significantly different from silymarin.

@ p<0.01 significantly different from control

One way analysis of variance coupled with Dunnett's test. P value less than 0.05 was taken as significant.

Figures in brackets indicate percentage change in hepatoprotective activity.

SGOT: Serum Glutamate Oxaloacetate Transaminase; SGPT: Serum Glutamate Pyruvate transaminase;

T. Bil: Total bilirubin; D. Bil: Direct bilirubin.

Alc. Ext. : Alcoholic Extract, Aq. Ext. : Aqueous Extract.

Table 2.

Effect of *T. portulacastrum* aerial parts on rifampicin induced hepatotoxicity in rats.

Treatment	Dose (mg/kg P.O.)	SGPT (U/ml)	SGOT (U/ml)	T. Bil. (mg/dl)	D. Bil. (mg/dl)
Control	-	64±1.2	112±2.4	1.20±0.04	0.24±0.03
Rifampicin	1g	162±6.5 ^{#@}	304±5.3 ^{#@}	3.26±0.08 ^{#@}	1.45±0.06 ^{#@}
Alc. Ext.	100	70±1.9 ^{*#} (93.90)	152±1.7 [*] (79.20)	1.24±0.04 [*] (98.10)	0.63±0.01 ^{*#} (67.80)
Aq. Ext.	100	164±3.8 [#] (2.00)	227±4.7 ^{*#} (40.20)	3.12±0.08 [#] (6.8)	1.42±0.02 [#] (2.50)
Silymarin	100	54±1.5 [*] (110.20)	158±2.4 [*] (76.04)	1.29±0.05 [*] (95.70)	1.00±0.03 [*] (37.20)

Values are mean ± S. E. (n=5)

*p<0.01 significantly different from rifampicin.

p<0.01 significantly different from silymarin.

@ p<0.01 significantly different from control

One way analysis of variance coupled with Dunnett's test. P value less than 0.05 was taken as significant.

Figures in brackets indicate percentage change in hepatoprotective activity.

SGOT: Serum Glutamate Oxaloacetate Transaminase; SGPT: Serum Glutamate Pyruvate transaminase;

T. Bil: Total bilirubin; D. Bil: Direct bilirubin.

Alc. Ext. : Alcoholic Extract, Aq. Ext. : Aqueous Extract.

liver by covalent binding of its toxic metabolite N-acetyl-p-benzoquinone-imine to sulfhydryl groups of proteins, resulting in lipid peroxidation induced by a decrease in glutathione in the liver [18]. Damage induced in the liver are accompanied by increases in the activity of some serum enzymes. The hepatoprotective actions was shown by lowering in the serum biochemical parameters in the paracetamol intoxicated rats pretreated with alcoholic extract.

Rifampicin is a broad spectrum antibiotic most commonly used as antitubercular drug particularly in combination with Isoniazide. It is largely metabolized to desacetyl rifampicin which undergo enterohepatic circulation. Since it actively and specifically binds to RNA polymerase, it inhibits the synthesis of all forms of RNA. Thus by inhibiting nucleic acid and protein synthesis it induces fatty liver and finally cirrhosis.

It causes fatal liver damage and acute hepatic failure which is accompanied by increase in the activity of some serum enzymes [19]. The hepatoprotective actions was shown by lowering in the serum biochemical parameters in the rifampicin intoxicated rats pretreated with alcoholic extract.

5. Conclusion

In conclusion, the alcoholic extract of the aerial parts of *T. portulacastrum* showed some protective effects on the experimental model of paracetamol-induced and rifampicin-induced hepatic injury. The prophylactic effect is fairly comparable to that of silymarin, suggesting the alcoholic extract could be potential source of hepatoprotective agents. Detailed investigations on the constituents responsible for the activity are under progress.

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