



HEPATOPROTECTIVE ACTIVITY OF *PORTULACA PILOSA* LINN AGAINST CCL₄ INDUCED HEPATOTOXICITY IN RATS

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ABSTRACT

Key Words

Portulacopilosa; ethanolic extract; hepatoprotective; CCl₄ induced



Hepatoprotective activity of the ethanolic extract of the shade dried aerial parts of *Portulaca pilosa* Linn. was studied in wister rats using the CCl₄ induced hepatotoxicity. The activity was evaluated by using biochemical parameters such as serum aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP), total bilirubin and gamma glutamate transpeptidase (GGTP). The extract exhibited significant hepatoprotective activity at 200 mg/kg, p.o. body weight, which was comparable to the control and activity exhibited by the reference standard Silymarin in carbon tetrachloride induced hepatotoxicity model.

INTRODUCTION

Portulaca pilosa is a species of flowering succulent plant in the purslane family, Portulacaceae that is native to the Americas. This plant is somewhat unusual in that it can grow under a range of different environments, including dry soils, beaches, and disturbed habitats. This plant also grows on roadsides and railroads, on limestone, granitic, and sandstone outcrops. The leaf blades are linear to oblong-lanceolate, which is intermediate of the two. They can also be terete to hemispheric, with a range of size (5-20 x 1-3 mm). The plant has an acute apex with involucre-like bract of about six to nine. Flowers range from .5 to 1.2 cm in

diameter. The color of the petals can range from darkish pink to purple. The flower shape is obovate with 3-5 x 1.5-3 mm in size. The stamens in size of 5-12 mm but can reach up to 37 mm. It has been said that the species of *Portulaca pilosa* in Brazil has been used as a traditional remedy to cause diuresis, antipyresis and analgesia. Studies have shown that its extracts have renal effects. Its urinary sodium and potassium excretion^[1], anti-tyrosinase activity^[2], urinary problems and diabetes mellitus^[3] activities have also been reported. A review of literature afforded no information on the hepatoprotective aspects of this plant. So

the present study is therefore an attempt to assess the efficacy of this indigenous herb for its hepatoprotective activity against CCl₄ induced toxicity model^[4] in rats.

MATERIALS AND METHODS

Plant material: The aerial parts of the plant were collected from the foothill of Yercaud, Salem, in the month of Aug 2017 and cleaned to remove the debris. The collected plant was identified and authenticated by a botanist Dr. A. Marimuthu. A voucher specimen (PPM-1) has been kept in our museum for future reference. The plant parts were dried at room temperature for 10 d and coarsely powdered with the help of a hand-grinding mill and the powder was passed through sieve No. 60.

Preparation of the extract: The powder of aerial parts of *P. pilosa* was extracted separately by continuous hot extraction process using soxhlet apparatus with different solvents in increasing order of polarity from petroleum ether, chloroform, acetone, alcohol, to finally chloroform:water^[5]. After extraction, the extracts were concentrated under reduced pressure in tared vessel. The marc of crude drug powder was then once again subjected to successive extraction with other solvents and the extractive values were calculated with reference to the air-dried drug. The dry extracts were subjected to various chemical tests to detect the presence of different phytoconstituents.

Test animals: Wister rats of either sex and of approximately the same age, weighing about 150-175 g were used for the study. They were housed in polypropylene cages and fed with standard chow diet and water *ad libitum*. The animals were exposed to alternate cycle of 12 h of darkness and light each. Before each test, the animals were fasted for atleast 12 h. Male mice weighing about 20-25 g each were used for acute toxicity studies. The experimental protocols were subjected to the scrutinization of the Institutional Animal

Ethics Committee and were cleared by the same.

Hepatoprotective study: For determining the hepatoprotective activity animals were divided into six groups containing 6 animals each. Group I served as normal control and received orally 1 ml of propylene glycol daily for 7 consecutive days. Group II was served as positive control and received CCl₄ followed by 1 ml of propylene glycol. Group III, IV and V were treated with ethanolic extract of *P. pilosa*, 100, 200, 400 mg/kg, p.o., respectively for 7 consecutive days. Group VI treated with the reference compound Silymarin 200 mg/kg, p.o., for 7 consecutive days. On the seventh day 2 ml/kg, p.o. of CCl₄^[6] was administered 30 min of the last dose to all the rats except in group I. After 36 h, blood samples were withdrawn from all groups by cardiac puncture of non-anaesthetized rats. The biochemical parameters such as ALT^[7], AST^[8], ALP^[9], total bilirubin^[10], total protein^[11, 12] and GGTP^[13] were estimated as reported earlier. A small portion of liver was cut from the animals from each group and preserved in neutral buffered formalin and was processed for paraffin embedding, following the standard microtechnique^[14]. 5 μ section of the livers stained with alum haemotoxylin and eosin and studied for degenerative and necrotic changes. Statistical analysis^[15] was performed using student's t-test. The values are represented as mean \pm SEM. Level of significance was set at P<0.001.

RESULTS

The plant *P. pilosa* was collected from the foothill of Yercaud, Salem, air-dried and extracted by continuous hot extraction process using soxhlet apparatus. The average percentage yield of ethanolic extract of *P. pilosa* was found to be 3.4 % w/w. The LD₅₀ was found to be 2000 mg/kg for ethanolic extract of *P. pilosa*.

The ethanolic extract did not exhibit and toxic effects up to 1000 mg/kg when administered to mice as a single i.p. dose.

Table 1: Effect of ethanolic extract of *P. pilosa* on CCl₄ induced hepatotoxicity in rats

Treatment	Dose mg/kg, p.o.	AST U/L	ALT U/L	ALP U/L	GGTP U/L	Total Protein mg/dl	Total bilirubin mg/dl
Normal	1 ml	112.50±4.98	40.67±1.98	174.67±6.2	111.50±4.5	5.77±0.1	0.46±0.003
Control (CCl ₄)	1.25 ml/kg	185.17±4.49	110.47±3.3	269.33±5.5	240.60±3.4	2.93±0.0	0.99±0.02
Ethanolic extract of <i>P. pilosa</i>	100	145.73± 4.50*	71.33±3.92 *	217.80±9.1 1*	173.33± 7.32*	3.12± 0.15*	0.79±0.003 *
Ethanolic extract of <i>P. pilosa</i>	200	132.67± 4.51*	51.50±3.21 *	203.33±9.2 9*	141.33±8.2 3*	3.75±0.1 2*	0.83±0.002 *
Ethanolic extract of <i>P. pilosa</i>	400	125.35± 7.23*	49.50±3.39 *	192.67±7.2 3*	129.33±4.9 8*	3.98± 0.10*	0.75± 0.003*
Silymarin	200	120.37±4.72*	45.50±1.37 *	181.83±8.4 9*	123.50±5.1 1*	4.52±0.1 3*	0.66±0.003 *

***P<0.001 when compared with control. Number of individuals used=6 in each group. Days of drug treatment=7. Values are expressed as mean±SEM**

The results of biochemical parameters revealed the elevation of enzyme level in CCl₄ treated group indicating that CCl₄ induces damage to the liver. Liver tissue rich in both transaminase increased in patients with acute and hepatic diseases. AST, which is slightly elevated by cardiac necrosis is a more specific indicator of liver disease^[16]. A significant reduction was observed in AST, ALT, ALP, GGTP, total bilirubin and total protein levels in the animals treated with ethanolic extract of *P. pilosa*. The enzyme levels were almost restored to the normal. So the animals treated with ethanolic extract of *P. pilosa* in all the three doses exhibited statistically significant (P<0.001) and it shows dose dependent protection against CCl₄ induced hepatotoxicity in rats, which is comparable to the reference compound Silymarin. The histopathological studies support the biochemical findings. Hepatotoxicity induced by CCl₄ manifested itself by the 8th d with the liver showing massive degeneration enveloping the not so visible necrotic areas as compared to the normal. The liver sections of rats treated with the ethanolic extract were similar to liver sections of group IV and showed micro vesicular changes with

mild congestion and widening of the sinusoids. There was no evidence of necrosis.

DISCUSSION

Carbon tetrachloride is one of the most commonly used hepatotoxins in the experimental study of liver disease. The hepatotoxic effects of CCl₄ are largely due to its active metabolite, trichloromethyl radical^[16, 17]. These activated radicals bind covalently to the macromolecules and induce peroxidative degradation of membrane lipids of endoplasmic reticulum rich in polyunsaturated fatty acids. This leads to the formation of lipid peroxides. This lipid peroxidative degradation of biomembranes is one of the principal causes of hepatotoxicity of CCl₄^[18]. This is evidenced by an elevation in the serum marker enzymes namely AST, ALT, ALP, GGTP, total bilirubin and total protein. Estimation of serum transaminase levels gives a fairly good idea about the functional study of liver. The efficacy of any hepatoprotective drug is dependent on its capacity of either reducing the harmful effect or maintaining the normal hepatic physiology, which has been disturbed by a hepatotoxin. The extracts decreased CCl₄ induced elevated levels of the enzymes in

groups III and IV, indicates the production of structural integrity of hepatocytic cell membrane or regeneration of damaged liver cells by the extracts. Histopathological examination of the liver section of the rats treated with toxicant showed intense centrilobular necrosis and vasculolisation. The rats treated with extracts alone with toxicant showed sign of protection against these toxicants to considerable extent as evident from formation of normal hepatic cords and absence of necrosis and vasculoles.

Decrease in serum bilirubin after treatment with extract in liver damage indicated the effectiveness of the extracts in normal functional status of the liver. So, the result of present investigation indicates that the ethanolic extract of *P. pilosa* possess good hepatoprotective activity. Further investigations are required to characterize the active hepatoprotective principle and its mechanism of action.

Ethical issue: All experimental process of animals was approved by the Institutional Animal Ethical Committee of Vinayaka Mission's College of Pharmacy, Salem.

Conflict of interest

The authors declare that there are no conflicts of interest.

REFERENCES

1. M.J. Rocha, S.F. Fulgencio, A.C. Rabetti, M. Nicolau, A. Poli, C.M. Simões and R.M. Ribeiro-do-Vall. Effects of hydroalcoholic extracts of *Portulaca pilosa* Linn and *Achyroclinesatureioides* on urinary sodium and potassium excretion. J Ethnopharmacol, Jul 22; vol. 43, no. 3, pp. 179-83, 1994.
2. N. Baurin, E. Arnoult, T. Scior, Q.T. Do and P. Bernard. Preliminary screening of some tropical plants for anti-tyrosinase activity. J Ethnopharmacol, Oct; vol. 82, no.23, pp. 155-8, 2002.
3. C.A. Lans, Ethnomedicines used in Trinidad and Tobago for urinary problems and diabetes mellitus. J Ethnobiol Ethnomed, Oct vol. 13, no. 2, pp.45, 2006.
4. A.E.M. Maclean. Models of Liver diseases. Karger, Tiffenia, Italy. pp. 2-8, 1975.
5. C.K. Kokate, Practical Pharmacognosy, 3rdEdn., Vallabh Prakashan, New Delhi. pp. 107-109, 1994.
6. S. Mohideen, R. Ilavarasan, E. Sasikala and R. Thirumalai kumaran, Hepatoprotective activity of *Nigella sativa* Linn. Ind. J. Pharm. Sci., vol. 65, no.5, pp. 550-551, 2003.
7. S. Reitman and S. Frankel. A colorimetric method for the determination of serum glutamate oxaloacetic acid and glutamic pyruvate transaminases. Am. J. Cl. Path., vol. 28, no.4, pp. 56-63, 1957.
8. Armitage and G. Berry, Statistical methods of Medical Research, 2ndEdn. Blackwell Scientific Publications, Oxford. Pp.186-192, 1985.
9. P.R.N. Kind and E.J. King. Determination of serum alkaline phosphatase, Clin. Path., vol. 7, pp.322-326, 1954.
10. C.A. Burtis and E.R. Ashwood. Tietz Fundamentals of Clinical Chemistry, WB Saunders and company, Philadelphia. Pp.539-552, 1996.
11. J.G. Reinhold. Standard methods of Clinical chemistry. M. Reiner, 1stEdn, Academic Press, New York. pp.88-96, 1953.
12. R.J. Henry, D.C. Cannon and J.W. Winkelman. Clinical Chemistry 2ndEdn. Harper and Row, New York. pp. 881-890, 1974.
13. G. Szasz. A kinetic photometric method for serum gamma-glutamyltranspeptidase, Clin. Chem., vol. 15, no.2, pp. 124-36, 1969.

14. A.E. Galigher, E.N. Kozloff. Essential Practical Microtechnique, 2ndEdn., Lea and Febiger, Philadelphia. pp. 197-210, 1971.
15. R.F. Woodson, Statistical methods for the analysis of biomedical data- Probability and Mathematical Statistics, Wiley, Chichester. pp. 315-324, 1989.
16. Sherlock. Biochemical Assessment of Liver Function, Blackwell Scientific Publications, Oxford. pp. 14-21, 1981.
17. T.F. Slater. Necrogenic action of CCl₄ in the rat: A speculative mechanism based on activation. Nature. vol. 209, no.18, pp. 36-40, 1966.
18. N. Kaplowitz, T.Y. Aw, F.R. Simon and A. Stolz. Drug induced hepatotoxicity, Ann. Int. Med., vol104, no. 3, pp. 826-39, 1986.