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Hepatoprotective Activity of *Portulaca quadrifida* Linn against CCl_4 Induced Hepatotoxicity in Rats.

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Research Article

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ABSTRACT

Hepatoprotective activity of the ethanolic extract of the shade dried aerial parts of *Portulaca quadrifida* Linn. was studied in wister rats using the CCl_4 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 quadrifida Linn. a prostrate fleshy annual or stoloniferous perennial herb with so mewhat base but sometimes with simple main stems, 5-40 cm tall and generally widespread in warm countries. *Portulaca quadrifida* Linn. belongs to the family portulacaceae. It is a small diffused, succulent, annual herb found throughout the tropical parts of India. It is used as a vegetable and also used for various curative purposes. It is said to be useful in asthma, cough, urinary discharges, inflammations and ulcers. A poultice of the plant is applied in abdominal complaints, erysipelas and haemorrhoids [1]. *Portulaca quadrifida* Linn. has been reported to possess antifungal activity against *Aspergillus fumigates* and *Candida albicans* [2] and the neuropharmacological activities were reported by Syed *et al* [3]. 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 CCl_4 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 June 2013 and cleaned to remove the debris. The collected plant was identified and authenticated by a botanist Dr. A. Marimuthu, Department of Botany, Government Arts College, Attur. A voucher specimen (PQM-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.

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Preparation of the extract

The powder of aerial parts of *P. quadrifida* 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.

Acute toxicity studies [6]

The animals were divided into control and test groups containing six animals each. The control group received the vehicle (1 % acacia) while the test groups got graded doses of different extracts orally and were observed for mortality till 48 h and the LD_{50} was calculated.

Hepatoprotective study

For determining the hepatoprotective activity animals were divided into four 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 CCl_4 followed by 1 ml of propylene glycol. Group III and IV were treated with ethanolic extract of *P. quadrifida* (200 mg/kg, p.o.) and reference compound Silymarin (200 mg/kg, p.o.), respectively for 7 consecutive days.

On the seventh day 2 ml/kg, p.o. of CCl_4 [7] 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 nonanaesthetized rats. The biochemical parameters such as ALT [8], AST [9], ALP [10], total bilirubin [11], total protein [12,13] and GGTP [14] 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 [15]. 5 µ section of the livers stained with alum haemotoxylin and eosin and studied for degenerative and necrotic changes. Statistical analysis [16] was performed using student's t-test. The values are represented as mean±SEM. Level of significance was set at $P<0.001$.

RESULTS

The plant *P. quadrifida* 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. quadrifida* was found to be 3.8 % w/w. The LD_{50} was found to be 2000 mg/kg for ethanolic extract of *P. quadrifida*.

Table 1: Effect of ethanolic extract of *P. quadrifida* on CCl_4 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± 6.22	111.50± 4.51	5.77± 0.10	0.46± 0.003
Control (CCl_4)	1.25 ml/kg	185.17± 4.49	110.47± 3.37	269.33± 5.53	240.60± 3.41	2.93± 0.06	0.99± 0.02
Ethanolic extract of *P. quadrifida*	300	130.67± 4.90*	51.33± 2.29*	197.50± 8.41*	131.33± 7.23*	3.93± 0.12*	0.77± 0.002*
Silymarin	200	120.37± 4.72*	45.50± 1.37*	181.83± 8.49*	123.50± 5.11*	4.52± 0.13*	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±S.E.

The ethanolic extract did not exhibit and toxic effects up to 1000 mg/kg when administered to mice as a single i.p. dose. The results of biochemical parameters revealed to the elevation of enzyme level in CCl_4 treated group indicating that CCl_4 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 [17]. 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. quadrifida*. The enzyme levels were almost restored to the normal. So the animals treated with ethanolic extract of *P. quadrifida* exhibited statistically significant ($P<0.001$) protection against CCl_4 induced hepatotoxicity in rats, which is comparable to the reference compound Silymarin. The histopathological studies support the biochemical findings. Hepatotoxicity induced by CCl_4 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 CCl_4 are largely due to its active metabolite, trichloromethyl radical [17, 18]. 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 CCl_4 [19]. 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 CCl_4 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 toxican showed intense centrilobular necrosis and vascuolisation. 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 cards and absence of necrosis and vascuoles.

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. quadrifida* possess good hepatoprotective activity. Further investigation are required o characterize the active hepatoprotective principle and its mechanism of action.

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