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ANTIHEPATOTOXIC EFFECT OF *PORTULACA OLERACEA* LINN. LEAVES AGAINST CCl_4 INDUCED HEPATOTOXICITY IN RATS

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ABSTRACT

Different extracts of *Portulaca oleracea* Linn. (Portulacaceae) were tested for their antihepatotoxic effect against CCl_4 induced hepatotoxicity in wistar albino rats. The degree of antihepatotoxicity was measured by using biochemical parameters like serum transamiases (SGOT and SGPT), alkaline phosphatase, total protein and total bilirubin in the treated groups, compared to the control. The alcoholic, aqueous and petroleum ether extracts showed the significant activity as comparable with standard drug silymarin. Other extract than chloroform did not exhibit a potent activity as compared to standard drug silymarin. Thus, the present study provides a significant rationale for the traditional use of this plant in the management of liver diseases.

Key words : *Portulaca olaracea,* Hepatoprotective activity, Silymarin, CCl_4

INTRODUCTION

Portulaca olaracea Linn. (Portulacaceae family) is a prostrate, succulent, branched often purplish stem herb. This is a very common weed of cultivated and undistributed land and native to the Old World Tropics. The *Portulaca olaracea* have several therapeutic effects including diuretic, anti-ascorbic, antipyretic, anti-asthma, anti-inflammatory and antitussive effect[1]. Previous studies have shown different pharmacological effects of this plant including analgesic, anti-inflammatory[2], hypoglycemic[3], skeletal muscle relaxant[4-6], skeletal muscle stimulant[7], smooth muscle relaxant[6], spasmolytic[8], uterine stimulant[9], diuretic, wound healing[10], gastric antiulcerogenic[11], antiasthmatic[1], antioxidant activity and

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an effect on the blood pressure[12, 13] and potassium channel opening effect[14].

The liver is exposed to drugs in higher concentrations than are most organs because most drugs are administered orally and are absorbed from the GIT. Thus, the whole dose must pass through the liver to reach the systemic circulation. Because of this, the liver is a vulnerable target for injury from chemicals and drugs and disordered hepatic functions is an important cause of abnormal drug handling and response[15].

The functional reserve of the liver masks to some extent the clinical impact of early damage. Regardless of cause, five general responses are seen. These processes and the morphologic terms used to describe them are inflammation, degeneration, cell death, fibrosis and cirrhosis[16]. Overall mortality from hepatic failure without liver transplantation is 70-95%. Liver disease accounts for over 44000 deaths per year in US (1.9% of all deaths), placing it as the eight leading cause of death, ranking between diabetes and suicide[17].

Despite extensive research in medical field, no drug in modern system of medicine can be claimed to cure liver disorder, which many times becomes fatal. Some plants extract viz. *Picrorrhiza kurroa; Andrographics paniculata* and *Eclipta alba* have been reported to possess clinically useful hepatoprotective activity. However, many plants remains unexplored in this regard[18].

At the present day, the herb *Protulaca oleracea* is mainly valuable as a refrigerant and alternative pot herb, particularly useful as an article of diet in scurvy and liver disease[19]. Hot water extract of dried leaves is taken orally as a diuretic and for liver disease[20]. Therefore, in the present study, the antihepatotoxic effect of successive extracts from *Portulaca oleracea* on CCl_4 was examined.

EXPERIMANTAL

Plant material

Portulaca oleracea was collected from in and around Shimoga district and identified by Professor S. B. Kamalakar HOD of Botany Sayadri College, Shimoga. A voucher specimen was preserved in the Pharmacognosy Department of National College of Pharmacy. Fresh leaves of the plant were separated, dried thoroughly under shade at room temperature and coarse powder was made in grinder at slow speed. Coarse powder was extracted successively with petroleum ether, chloroform, alcohol and distilled water, respectively. The yields of the successive extraction were pet. ether (4.39%), chloroform

(2.19%), alcohol (17.76%) and aqueous (25.40%). The marc after each extraction was dried at room temperature and every extract were concentrated under reduced pressure in distillation recovery unit.

Animals

Randomly selected Wistar albino rats of both sex weighing between 120-200 g and Swiss albino mice (20-25 g) were obtained from the Central Animal House, National College of Pharmacy, Shimoga, Karnataka. They were housed in clean metabolic cages and maintained in controlled temperature 25 ± 2 °C with 12 h light and dark cycle with relative humidity of approximately 60%. They received free standard pellet diet and tap water and acclimatized for 7 days before the experiment. Experimental protocol was ethically approved by Ethical Committee of National College of Pharmacy Shimoga. (NCP/IAEC : Clear/051/2007-2008, Dated 28/11/2007).

Toxicity study

Portulaca oleracea extracts in pet. ether and chloroform were subjected for toxicity study remaining alcoholic and aqueous extracts dose referred form Chan *et al*.[2] Extracts (Pet. ether and chloroform) were dissolved in tween-80 (0.5%) and emulsion was prepared in water for oral administration to different groups of mice in dose ranging from 1000-4000 mg /kg for the LD_{50} study using the up and down or staircase method[21]. chloroform extract of leaves did not show any mortality up to a dose of 3000 mg/kg b. wt. but for pet. Ether, it was 400 mg/kg b. wt. There was no lethality in any of the groups after 48 hours of treatment.

CCl_4 induced hepatotoxicity studies

The method of Swamy *et al.*[22] was used in the study. Rats were divided into seven groups of six rats in each group. Group 1 served as normal control group and received 0.5% Tween-80 (1 mL/kg, p. o.) on all 5 days and received olive oil (1 mL/kg, s. c.) on days 2 and 3.Group 2 served as CCl_4 control and were administered a single daily dose of 0.5% Tween-80 (1 mL/kg, p. o.) on all 5 days and on the 2nd and 3rd day, they were administered s. c., CCl_4 : olive oil (1 : 1), 1 mL/kg calculated as CCl_4. Group 3 received silymarin, the known hepatoprotective compound at a dose of 100 mg/kg/day, p. o., on all 5 days and a single dose of CCl_4 (1 mL/kg) s. c., on days 2nd and 3rd days, 30 min after silymarin administration. Group 4 received pet. ether extract of *Portulaca oleracea* (400 mg/kg/day, p. o.) on all 5 days and a single dose of CCl_4 (1 mL/kg) s. c., on days 2nd and 3rd days, 30 min after *Portulaca oleracea* administration. Group 5 received chloroform extract of *Portulaca oleracea* (300 mg/kg/day, p. o.) on all 5 days and a single dose of

CCl_4 (1 mL/kg) s. c., on days 2nd and 3rd days, 30 min after *Portulaca oleracea* administration. Group 6 received alcoholic extract of *Portulaca oleracea* (400 mg/kg/ day, p. o.*)* on all 5 days and a single dose of CCl_4 (1 mL/kg) s. c., on days 2nd and 3rd days, 30 min after *Portulaca oleracea* administration. Group 7 received aqueous extract of *Portulaca oleracea* (400 mg/kg/day, p. o.) on all 5 days and a single dose of CCl_4 (1 mL/kg) s. c., on days 2nd and 3rd days, 30 min after Portulaca *oleracea* administration. On the fifth day, all the animals were sacrificed by mild ether anesthesia. Blood samples were collected for evaluating the biochemical parameters.

Biochemical estimations (Liver function tests)

All blood samples were centrifuged after keeping for coagulation for 30 min at room temperature. The clear serum was separated at 3000 rpm for 10 min and biochemical investigations were carried out to asses liver functions viz., serum alkaline phosphatase (ALP), serum aspartate aminotransferase (AST), serum alanine aminotransferase (ALT), serum bilirubin and total protein according to the standard methods. [23-26]

Statistical analysis

All the values are expressed as mean ± S. D. The results were analyzed statistically by one way ANOVA followed by student's t-test. *P*-values < 0.05 were considered significant.

RESULTS AND DISCUSSION

The effect of different extracts of *Portulaca olaracea* on serum enzymes were studied and results were given in Table 1.Administration of CCl_4 to rats caused significant ($p \leq 0.05$) increase in serum enzymes like ALP, AST, ALT and serum bilirubin compared to normal control rats. Treatment with different extracts of *Portulaca olaracea* reversed these biochemical parameters significantly towards normal. Petroleum ether, alcoholic and aqueous extract exhibited hepatoprotection almost equivalent to silymarin 100 mg/kg/day p. o. Several mechanisms underlying this toxicity have been suggested. However, it is possible that beta-sitosterol, quercetin constituents of *Portulaca oleracea* may be responsible for the protective activity against CCl_4 hepatotoxicity[28]. CCl_4, the inactive metabolite is transformed to a free radical through the microsomal cytochrome P-450, dependent enzyme, resulting in activation of CCl_4, toxicity. An additional and important factor in the antihepatotoxic activity of any drug is the ability of its constituents to inhibit the aromatase activity of cytochrome P-450; thereby favoring liver regeneration. On that basis, it is suggested that flavonoids in *Portulaca olaracea* could be a factor contributing to

its antihepatotoxic ability through inhibition of cytochrome P-450 aromatase[29, 30].

Table 1 : Effect of *Portulaca olaracea* extracts on rat serum enzymes after CCl_4 administration (Biochemical estimation)

Groups	ALP (IU/L)	AST (IU/L)	ALT (IU/L)	Total Protein (mg/dL)	Serum bilirubin (IU/L)
CCl_4	74.07 ± 5.6	119.62 ± 6.95	137.92 ± 7.44	7.66 ± 0.40	0.51 ± 0.03
Silymarin	99.72 ± 7.51	357.18 ± 1.41	371 ± 3.41	6.77 ± 0.47	0.90 ± 0.017
Pet. ether	76.11 ± 3.33*	188.43 ± 3.02*	206.73 ± 4.37*	6.97 ± 0.20	0.63 ± 0.082*
Chloroform	68.24 ± 2.39*	193.07 ± 3.62*	209.3 ± 1.77*	6.99 ± 0.20	0.70 ± 0.02*
Alcohol	102 ± 0.87	348.8 ± 3.68	382.12 ± 1.82*	6.27 ± 0.03***	0.91 ± 0.02
Aqueous	59.17 ± 0.75*	228.7 ± 1.06*	243.48 ± 1.78*	6.77 ± 0.46	0.87 ± 0.08
	64.85 ± 0.43*	217.7 ± 1.51*	235.15 ± 1.39*	5.98 ± 0.10**	0.89 ± 0.02

Values are the mean ± S. D., n = 6.

* p≤ 0.001, **p≤ 0.01 and *** p≤ 0.05 in comparison to CCl_4-treated group

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