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Research Article

HEPATOPROTECTIVE ACTIVITY OF AERIAL PARTS OF *PLUMBAGO ZEYLANICA* LINN AGAINST CARBON TETRACHLORIDE-INDUCED HEPATOTOXICITY IN RATS

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ABSTRACT

The present study was conducted to evaluate the hepatoprotective activity of methanolic extract of aerial parts of *Plumbago zeylanica* in CCl_4-induced hepatotoxicity in wistar rats. Silymarin (100mg/kg, p.o.) was given as reference drug. The extract of aerial parts of *Plumbago zeylanica* have shown very significant hepatoprotection against CCl_4-induced hepatotoxicity in wistar rats by reducing serum total bilirubin, SGPT ,SGOT and ALP levels. Histopathological studies also confirmed the hepatoprotective nature of the extract.

Keywords: Flavonoids, Triterpenes, Histopathological Studies, Biochemical Parameter

INTRODUCTION

Herbal drugs are prescribed widely even when their biologically active components are unknown because of their effectiveness, fewer side effects and relatively low cost[1]. *Plumbago zeylanica* Linn (Plumbaginaceae) is a perennial herb commonly distributed in forest of the Uttarakhand, India, and cultivated in the gardens throughout India. The plant is commonly known as Ceylon leadwort (English), Chita, Chitra (Hindi) and Chitramoolam (Tamil). The root is used as laxative, expectorant, astringent, abortifacient, and in dysentery[2-4]. Tincture of root bark is used as antiperiodic. The leaves are used as aphrodisiac and in scabies[5].

Earlier chemical examination of this plant revealed that the root contains plumbagin, 3-chloroplumbagin, 2,3-biplumbagin, 6,6-biplumbagin, zeylinone, isozeylinone, chitranone, droserone, plumbagic acid, plumbazeylanone, glucose, fructose, enzymes as protease and invertase. The leaves and stem contains little or no plumbagin. The aerial parts contain naphthoquinones, sitosterol, lupeol, lupenylacetate, hentriacontane, and amino acids[6]. Literature review indicated that the hepatoprotective activity of aerial parts of *P. zeylanica* have not been clinically evaluated so far. An active and safe drug is needed for the treatment of hepatitis. In view of this, the present study was aimed at evaluating the hepatoprotective activity of the aerial parts of *P. zeylanica* against carbon tetrachloride (CCl_4) induced hepatotoxicity in Wistar rats.

MATERIALS AND METHODS

Plant materials

The aerial parts of *P. zeylanica* were collected in December 2006, from the kaladhungi forest, Nainital, Uttrakhand (India). The plant was authenticated by Dr. V.K. Lal, Professor, College of Pharmacy, IFTM, Moradabad and a voucher specimen has been deposited in the departmental laboratory for further references.

Preparation of extract

The air dried plant materials were extracted successively with hexane, petroleum ether, chloroform and methanol using soxhlet apparatus. The extracts were concentrated

using rotary vacuum evaporator. The dried extracts were stored in airtight container and placed in refrigerator.

Animals

Wistar rats (150-200 g) were used in this study. They were maintained at standard housing conditions and fed with commercial diet and provided with water *ad libitum* during the experiment. The experiment was approved by institutional animal ethical committee (Reg. No. 837/ac/04/ CPCSEA/2007).

Chemicals

Carbon tetrachloride was procured from S.D. Fine Chemicals Ltd. (India), silymarin was obtained as gift sample from Ranbaxy (Devas, India), standard kit of SGPT, SGOT, ALP and bilirubin was obtained from Jain Scientific Industries, Moradabad, India. All other reagents used were of analytical grade.

Preliminary phytochemical screening

Hexane, petroleum ether, chloroform and methanolic extracts of aerial parts of *P. zeylanica* were screened for different phytochemical constituents viz. alkaloids, triterpenes, steroids, carbohydrates, tannins and flavonoids[7-8].

Acute toxicity study

The acute toxicity studies were carried out as per stair case method[9]. Sixty male rats were divided into six groups of 10 each and were administered with aliquot doses of the methanolic extract orally (100, 150, 200, 250, 300 and 400 mg/kg). Mortality was not noticed up to 300 mg/kg, whereas, 100% mortality was noticed in the dose of 400 mg/kg. The LD_{50} of the extracts was found to be 350 mg/kg body weight. One-fifth and one-tenth of this dose were selected as the therapeutic dose for the evaluation[10].

Assessment of hepatoprotective activity

Five groups of animals containing six each were used for the study. Group I served as control and received the vehicle (5% gum acacia; 1 ml/kg/day; p.o. for 14 days). Group II received 0.1 ml/kg/day, i.p. of CCl_4 for 10 days. The standard drug, silymarin was administered to Group III animals in the dose of 100 mg/kg/day, p.o. for 14 days. While, group IV and V were treated with methanolic extract at the doses of 35 & 70 mg/kg/day, p.o. (as per acute toxicity studies) for 14 days respectively. The CCl_4, silymarin and the extracts were administered concomitantly to the respective group of animals. On 14^{th} day the blood was collected by carotid artery under mild ether anesthesia, serum was collected by allowing the blood samples to coagulate for 30 min at 37 ^{0}C followed by centrifugation (3000 rpm for 15 min) and subjected for determination of biochemical parameters like total bilirubin[11], SGPT, SGOT[12] and ALP [13].

Histopathological observation

Liver tissue collected were used for the preparation of histopathological slides by using microtome, were suitably stained and observed under light microscope for architectural changes seen during CCl_4 challenge in methanolic extract of *P. zeylanica* treated and control groups.

Statistical analysis

The results are expressed as Mean ± SEM of six animals from each group. The data were evaluated by one-way ANOVA followed by Tukey's multiple comparison tests. P values <0.05 was considered statistically significant.

RESULTS

Preliminary phytochemical screening

The various phytoconstituents present in different extracts were furnished in table 1.

Biochemical parameters

Rats treated with carbon tetrachloride showed a significant hepatic damage as observed from elevated serum level of hepatospecific enzymes as well as severe alteration in different liver parameters (Table 2). SGPT, SGOT, ALP, and total bilirubin in serum were increased in carbon tetrachloride intoxicated control animals (Table 2). Treatment with the methanolic extract of P. *zeylanica* caused significant protection against CCl_4–induced increase in serum enzyme level and bilirubin in a dose responsive manner (Table 2). The degree of protection observed was maximum with higher dose of the extract (70 mg/kg).

Table 1: Preliminary phytochemical screening of different extracts of *Plumbago zeylanica*

Type of constituents	Hexane Extract	Petroleum Ether Extract	Chloroform Extract	Methanol Extract
Triterpenes	+	+	+	+
Steroids	+	+	+	-
Carbohydrates	-	-	-	+
Tannins	-	-	-	+
Flavonoids	-	-	-	+
Alkaloids	-	-	-	-

+ indicates present and – indicates absent

Table 2 : Effect of methanolic extract of *Plumbago zeylanica* on biochemical parameters in CCl_4 induced hepatic toxicity

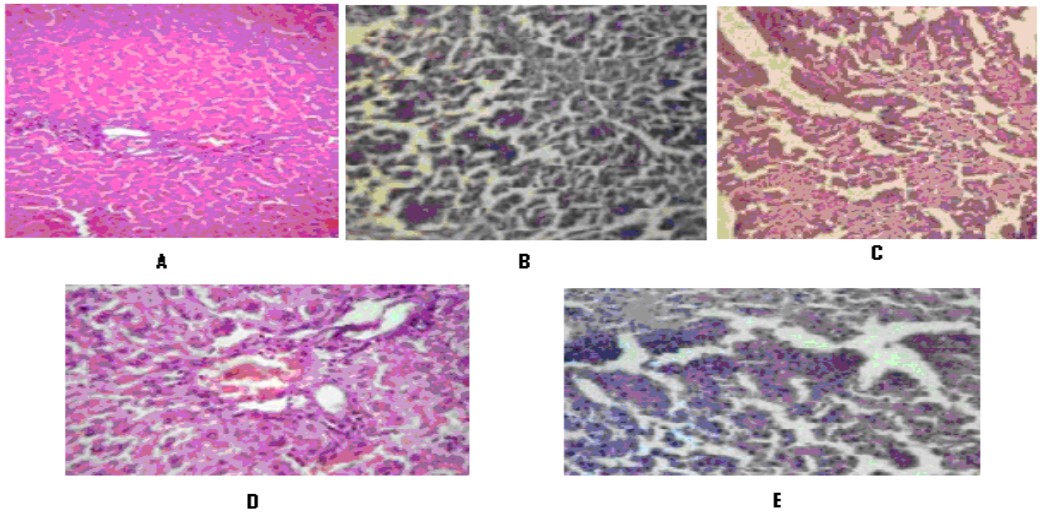
Treatment	Dose (mg/kg)	SGPT (IU/L)	SGOT (IU/L)	Total Bilirubin (mg/dl)	ALP (mg/dl)
Normal Control	-	45.16 ± 1.25	39.61±0.59	0.70 ± 0.03	160 ± 3.79
CCl_4 treated	-	217.30 ± 4.5	341 ± 3.8	0.87 ± 0.07	191.50 ±7.5
Silymarin + CCl_4	100	42.64 ± 0.33*	43.24 ± 0.30	0.50 ± 0.01*	181.60 ± 0.52*
MeOH extract + CCl_4	35	131.95 ± 0.46*	151.35 ± 0.40*	0.83 ± 0.01*	187.30 ± 0.31*
MeOH extract + CCl_4	70	115.30 ± 1.16*	127.36 ± 0.42*	0.51 ± 0.10*	185.20 ± 0.30*

Values are expressed as Mean ± SEM (n = 6). *P < 0.05 vs. CCl_4 treated group.

Histopathological studies

Histhopatological examination of liver sections of control group showed normal cellular architecture with distinct hepatic cells, sinusoidal spaces and central veins (Fig.1. A). Disarrangement of normal hepatic cells with centrilobular necrosis, vacuolization of cytoplasm and fatty degeneration were observed in CCl_4 intoxicated animals (Fig.1. B). The liver sections of the rats treated with methanolic extract of *P. zeylanica* and silymarin followed by CCl_4 intoxication showed a sign of protection as it was evident the absence of necrosis and vacuoles (Fig.1. C, D and E).

Fig. 1: Microphotographs of liver section taken from rats



Microphotographs (10 x 40) of liver section taken from rats. **A**, Normal control group; **B**, CCl_4 control group (0.1 ml/kg); **C**, methanolic extract (35mg/kg) +CCl_4; **D**, methanolic extract (70mg/kg) +CCl_4; **E**, Silymarin (100mg/kg) + CCl_4

DISCUSSION

Liver damage induced by CCl_4 is commonly used model for the screening of hepatoprotective drugs[14]. The CCl_4 is converted into reactive metabolite, halogenated free radical by hepatic cytochrome P450s[15] which in turn covalently binds to cell membrane and organelles to elicit lipid peroxidation with subsequent tissue injury. The measurement of lipid peroxide is also a marker of hepatocellular damage[16-17].The methanolic extract of aerial parts of *Plumbago zeylanica* administered prophylactically exhibited significant protection against CCl_4-induced liver injury as manifested by the reduction in toxin mediated rise in serum level of SGPT, SGOT, ALP and total bilirubin in rats.

The qualitative phytochemical investigation on the different extract of *P. zeylanica* showed positive test for carbohydrate, triterpenes, steroids, tannins and flavonoids. The methanolic extract has significant hepatoprotective activity. This may be probably due to the higher content of the triterpenes, tannins and flavonoids. Hence further work is necessary to elucidate the constituent responsible for hepatoprotective activity along with their mechanism of action.

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