



Anti-hepatotoxic activity of phyllanthus debelis

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ABSTRACT

In the present study, we evaluated the antihepatotoxic activity of the different fractions of *Phyllanthus debelis* by performing biochemical parameters and histopathological studies against toxicity caused by the carbon tetrachloride. The histopathological studies of the liver showed swelling and necrosis in hepatocytes in CCl₄ treated rats, treatment with different fractions have reduced significantly the necrosis and swelling of the hepatocytes. The biochemical parameters also showed the significant antihepatotoxic activity.

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KEYWORDS

Carbon tetrachloride;
Wistar rats;
Per oral;
Phyllanthus debelis;
Anti-hepatotoxic activity.

INTRODUCTION

In the present investigation CCl₄ was used to produce liver damage, the toxic effect of CCl₄ is due to its conversion by P₄₅₀ to the highly reactive toxic free radicals CCl₃• (CCl₄ + e⁻ → CCl₃• + Cl⁻).

Phyllanthus debelis (Euphorbiaceae) distributed in India, Sri Lanka, Burma, Indonesia, Pacific Islands and the West Indies. It commonly grows as a weed in rice fields, moist lands and muddy flats amidst grasses and restricted to the coastal regions. Aqueous extract of leaves showed strong anti complement effect on both the classical and alternate pathways of the human complement system in vitro. The effects were dose-dependent and most pronounced in the classical complement pathway assay. The extract also exhibited a direct dose-dependent inhibition of luminal-induced chemiluminescence of human polymorphonuclear leukocytes upon stimulation with zymosan^[1].

The petroleum ether extract of *Phyllanthus debelis*

Klein. ex Willd whole plant was subjected to analgesic and anti-inflammatory screening using various animal models. The extract exhibited significant anti-inflammatory activities in the acute carrageenan-induced rat paw edema and the chronic granuloma pouch models. However, it was devoid of analgesic activity in the tail-flick model^[2].

The literature survey has revealed that neither chemical investigation has been done nor any chemical compound has been isolated so far.

EXPERIMENTAL

Chemicals and plant fractions

Carbon tetrachloride was used as toxicant in this study. The whole plant of *Clerodendrum phlomidis* was collected from Chandi Chauk, Old Delhi and authenticated by Dr.M.P.Sharma, Reader and taxonomist, Department of Botany, Faculty of Science, Jamia Hamdard. The plant material (3.0kg) was dried

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and crushed to coarse powder and extracted with ethanol using cold percolation till completely exhausted. The ethanolic extract was dried and then successively fractionated into with petroleum ether (60-80°C), chloroform and methanol. The different fractions were dried under reduced pressure to get the crude dried fractions of petroleum ether, chloroform and methanol 30.0, 40.0, and 160.0gm respectively.

Experimental animals and treatments

Male Albino Wistar rats weighing 150-200gm were employed for assessing the antihepatotoxic activity. They were procured from the Central Animal House of Jamia Hamdard, New Delhi (173/CPCSEA), after approval under the project proposal number-326. They were fed with a standard pellet diet and water *ad libitum*. A set of five rats was kept in a plastic cage and maintained at 25°C to 28°C with 40-70% RH and 12 hr light/dark cycles and were fastened for 12 hours prior to the experiment. The six groups of 6 rats in each group, the first group served as normal control, which was untreated. The 2nd group as toxic control received CCl₄ diluted with liquid paraffin in a ratio of (1:1) (1.5 ml/kg b.w, p.o.) on the first day only to produce toxicity in liver and thereafter no treatment of fractions. 3rd group as standard control received CCl₄ on the first day and thereafter received treatment with standard drug silymarin (Silybon-70) at a dose of 10 mg/kg body weight, p.o for 7 days. The group 4th to 6th received CCl₄ on the first day and then treated with different fractions as petroleum ether, chloroform, methanol dose of 500 mg/kg body weight, p.o for 7 days. All dosing was started at the same time in the morning to avoid the effects of biological rhythm changes.

Method of analysis

On 8th day the blood samples of four rats from each group were withdrawn by puncturing the retro-orbital plexus under ether anesthesia. The blood samples were allowed to clot for 30-40min. at room temperature. Serum was separated by centrifugation at 2500rpm for 15min. and various biochemical parameters were estimated and the livers of the two animals were taken for the histopathology.

Biochemical parameters like serum Serum glutamic oxaloacetic transaminase (SGOT), Serum glutamic pyruvate transaminase (SGPT)^[3], Alkaline phosphatase (ALP)^[4], and Total protein (TP)^[5] were carried out by reported methods.

Statistical analysis

The data of biochemical estimations were reported as mean±standard error. For determining the statistical significance one-way analysis of variance (ANOVA), Dunnett's test was employed. P-values of less than 0.05 were considered significant^[6].

RESULTS

Group-I: (This group was given neither CCl₄ nor treatment)

They had normal values of SGOT (54.54±1.26**), SGPT (44.45±1.36**), ALP (43.49±1.73**)units/ml and TP (6.29±0.40**)gm/dl and liver section showed normal architecture without any degeneration, necrosis or inflammation seen.

Group-II: (The animals were given only CCl₄)

These rats were found to possess high values of SGOT (140.66±1.81), SGPT (128.41±2.48), ALP (69.99±2.21)units/ml and TP (4.19±0.38)gm/dl. and liver sections showed prominent centrilobular necrosis with fatty change throughout the liver with prominent and enlarged central vein. There is significant periportal inflammation.

Group-III: (Treated with standard drug-silymarin)

There was a drastic fall in the values of SGOT (70.80±1.62**), SGPT (63.80±2.09**), ALP (46.69±1.12**)units/ml and TP (6.26±0.46**)gm/dl. and liver sections showed only a mild dilatation of sinusoids in the centrilobular areas. A focal area of bile pigment deposits is seen near the central vein.

Group-IV: (Treated with petroleum ether fraction)

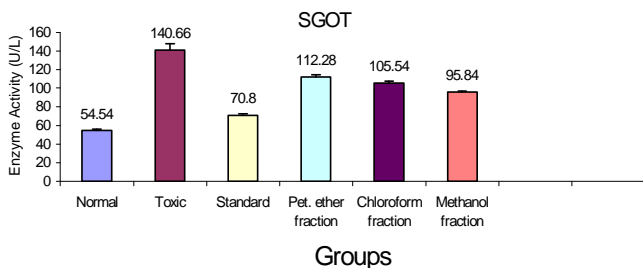
There was a slight fall in the values of SGOT (112.28±2.59**), SGPT (97.65±1.67**), ALP (60.164±1.589*)units/ml and TP (4.87±0.25^{ns})gm/dl. and liver sections showed a sinusoidal dilatation around the central vein. Fine vacuolar change is seen in scattered hepatocytes.

Group-V: (Treated with chloroform fraction)

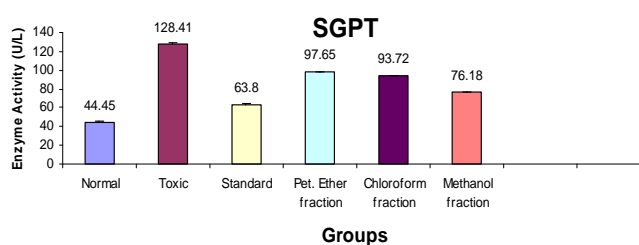
There was a decrease in the values of SGOT (105.54±2.45**), SGPT (93.72±1.58**), ALP (53.91±2.43**)units/ml and TP (5.1±0.17*)gm/dl. and liver section showed a mild sinusoidal dilatation is seen in the centrizonal hepatocytes. The remaining part of the liver parenchyma is within normal limits.

Group-VI: (Treated with methanol fraction)

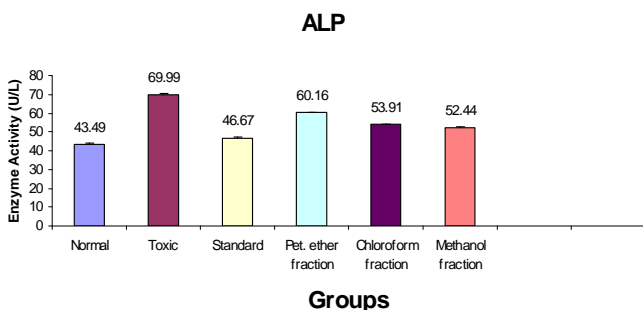
There was a decrease in the values of SGOT (95.84±2.48**), SGPT (76.18±1.98**), ALP (52.44±2.29**)units/ml and TP (5.6±0.15**)gm/dl. and liver sections showed a significant reduction in periportal inflammation and in the sinusoidal dilatation. Samples also showed good recovery with absence of necrosis, the central vein and the portal vein were both clearly visible.



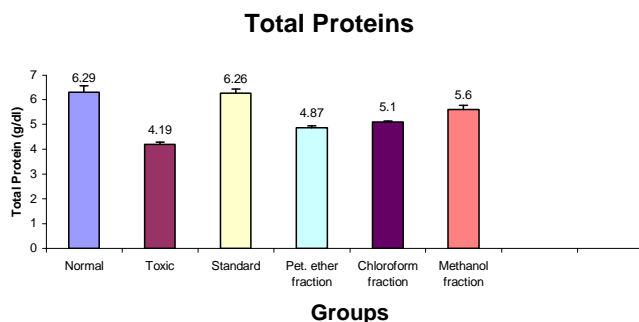
Graph 1 : SGOT values after treatment with different fractions of *Phyllanthus debelis* against toxic control



Graph 2 : SGPT values after treatment with different fractions of *Phyllanthus debelis* and compound PD-8 against toxic control



Graph 3 : ALP values after treatment with different fractions of *Phyllanthus debelis* against toxic control



Graph 4 : Total Protein values after treatment with different fractions of *Phyllanthus debelis* against toxic control

TABLE 1 : Effect of various fractions of *Phyllanthus debelis* whole plant on serum enzymatic activity in CCl₄ induced liver damage in rats

Groups (n=5)	Treatment	Dose	SGOT units/ml	SGPT units/ml	ALP units/ml	TP gm/dl
1	Normal (control)	-	54.54±1.26**	44.45±1.36**	43.49±1.73**	6.29±0.40**
2	Toxic (control)	1.5mg/kg (p.o.)	140.66±1.81	128.41±2.48	69.99±2.21	4.19±0.38
3	Silymarin (standard drug)	10mg/kg (p.o.)	70.80±1.62**	63.80±2.09**	46.67±1.12**	6.26±0.46**
4	Petroleum ether fraction	500mg/kg (p.o.)	112.28±2.59**	97.65±1.67**	60.16±1.589*	4.87±0.25 ^{ns}
5	Chloroform fraction	500mg/kg (p.o.)	105.54±2.45**	93.72±1.58**	53.91±2.43**	5.1±0.17*
6	Methanol fraction	500mg/kg (p.o.)	95.84±2.48**	76.18±1.98**	52.44±2.29**	5.6±0.15**

SGOT, serum glutamate oxaloacetate transaminase; SGPT, serum glutamate pyruvate transaminase; ALP, alkaline phosphatase; TP, total protein; p.o., per oral. ** P<0.01; *P<0.05 vs CCl₄ of (Dunnett's test). Values are mean±S.E. of six animals

DISCUSSION

The TABLE 1 shows that the animals of group II, which received only CCl₄ were found to develop significant hepatic damage as was observed from elevated

levels of SGOT, SGPT and ALP and the decrease in TP levels as compared to group I normal animals. The treatment with petroleum ether, chloroform, and methanolic fractions of alcoholic extract at a dose of 500mg/kg b.w, p.o in groups IV, V and VI significantly prevented CCl₄ induced elevation of liver enzymes such

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as SGOT by 112.28, 105.54, 95.84units/ml, SGPT by 97.65, 93.72, 76.18units/ml, while ALP by 60.16, 53.91, and 52.44units/ml respectively. On the other hand, TP levels were increased by 4.87, 5.1 and 5.6gm/dl respectively, as compared to standard drug silymarin, which, decreased SGOT by 70.80, SGPT by 63.80, ALP by 46.67 and increased TP levels by 6.26 against CCl₄ intoxicated rats in comparison to normal control. The above results indicated that the methanolic fraction was most active among the three fractions.

Histopathological studies have also revealed that rats treated with methanolic fraction had almost normal architecture of hepatocytes indicating significant recovery as compared to the standard Silymarin.

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