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EVALUATION OF ANTI-HEPATOTOXIC ACTIVITY OF *CLERODENDRUM PHLOMIDIS* L. ON CARBON TETRACHLORIDE INDUCED HEPATIC INJURY IN RATS

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ABSTRACT

Methanol extract of the stem of *Clerodendrum phlomidis* L. was investigated for anti-hepatotoxic activity against CCl_4 induced hepatic damage in male Wister rats. The extracts in dose of 500mg/kg b.w p.o for 7 days were compared with the standard silymarin (Silybon–70, 10mg/kg-b.w p.o). The methanol extract of the plant have shown anti-hepatotoxic activity by reducing the elevated levels of serum enzymes such as serum glutamate oxaloacetate transaminase (SGOT) by 29.88 %, serum glutamate pyruvate transaminase (SGPT) by 25.86 % while alkaline phosphatase (ALP) 14.21 %. On the other hand, total protein (TP) were increased by 27.20 %, as compared to standard drug silymarin which decreased SGOT by 49.66 %, SGPT by 50.31 %, ALKP by 33.29 % and increased TP levels by 49.40 %. These biochemical observations were also supplemented by histopathological examinations of the liver sections

Keywords: *Antihepatotoxic; Carbon tetrachloride; Clerodendrum phlomidis; Silymarin.*

INTRODUCTION

Herbal medicines have been used in the form of vegetables, drugs or their extracts for the treatment of diseases and maintaining health. Various commercial preparations available from the crude plant extracts are available as formulations for the treatment of liver ailments[1]. However, exposure to various therapeutic agents and environmental pollution leads to various disorders of organs, especially of liver[2]. *Clerodendrum phlomidis* L. (Verbenaceae), commonly known as 'Arni' a shrub 0.9-2.4 m. high, scarcely woody, not much branched; stems bluntly quadrangular; young parts usually glabrous, leaves often ternate as well as opposite, oblong. The plant has been used in dropsy[3]. The decoction of roots is slightly aromatic and astringent and is used as a demulcent in gonorrhea. It is also given to children during convalescence of measles. The juice of leaves is used as bitter tonic[4] and also given in neglected syphilitic complaints[5]. Psychopharmacological activity[6], antidiarrhoeal activity[7], antimutagenic activity[8] and antifungal activity[9] are some of its reported biological activities. The present article reports the anti-hepatotoxic activity of methanol extract. The ethyl acetate and hexane extracts of leaves and stems of *C. phlomidis* showed antifungal activity against plant and human pathogens but it is more effective in plants. It was tested by poison plate technique[9].

EXPERIMENTAL

Plant Material

The stem of *Clerodendrum phlomidis* were obtained from the market of Khari Bavli, Chandi Chauk, Old Delhi. A voucher specimen (CP-FP-32) of the plant has been kept in the herbarium of Jamia Hamdard University for further reference.

Preparation of plant extract

The plant material (1.5 kg) was dried and crushed to coarse powder and extracted with ethanol using cold percolation method till completely exhausted. The ethanol extract was then dried under reduced pressure to get the crude dried fraction of methanol 120.0 gm.

Experimental animals

Male Albino Wistar rats weighing 150–200 gm 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 libitium*. The animals were maintained at 25 to 28 °C with 40-70% RH and 12 h light/dark cycles and were fasted for 12 hours prior to the experiment.

Antihepatotoxic activity

The animals were divided into four groups consisting (5 each). The first group (I) served as normal control which received normal saline only. The second group (II) served as toxic control and received CCl_4 diluted

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with liquid paraffin in a ratio of (1:1) (1.5 ml/kg b.w, i.p.) on the first day to produce toxicity in the liver[10]. The third group (III) was given a single dose of CCl_4 on the first day (1.5 ml/kg b.w, i.p.) and then silymarin (Slybon-70, 10 mg/kg b.w, p.o.) was given for 7 days in the form of suspension using 1% Tween - 80). Group (IV) received a single dose of CCl_4 on the first day (1.5 ml/kg b.w, i.p.) and then methanol extract at the dose of (500 mg/kg b.w, p.o.) for 7 days in the form of suspension using 1% Tween - 80)[11]. On the day 8 the blood samples were withdrawn by puncturing the orbital plexus. The blood samples were allowed to clot for 30-40 min at room temperature. Serum was separated by centrifugation at 2500 rpm for 15 min. Serum from 5 animals was taken for biochemical evaluation. Two rats from each group were sacrificed by decapitation and their livers were studied for histopathology.

Assessment of liver function

Various biochemical parameters like serum SGOT, SGPT[12], ALP[13], and TP[14] were carried out by reported methods.

Statistical analysis

The data of biochemical estimations were reported as mean of the data standard error (□S.E), where n = 5. For determining the statistical significance one way analysis of variance (ANOVA) and Dunnett's test was employed. P–values of less than 0.05 were considered significant[15].

Histopathological studies

For histopathological study, the livers were quickly removed after autopsy and fixed in 10% formalin[16]. The rats were sacrificed and the livers removed were washed with normal saline. Small pieces of tissues were embedded in paraffin wax. The sections of about 5-6 □m were cut, stained and then observed under microscope for histopathological changes in liver and their pictographs were taken.

RESULTS AND DISCUSSION

Results in Table-1 show that the animals of group (II), who received only CCl_4 were found to develop significant hepatic damage as was observed from elevated levels of SGOT, SGPT and ALP and decrease in TP levels as compared to group (I) normal animals. However, in group (IV) treated with methanol extract of *C. phlomidis* significantly reduced CCl_4 induced elevation of liver enzymes such as SGOT by 98.62 units/ml, SGPT by 95.20 units/ml, while ALP by 60.04 units/ml respectively and TP level was increased by 5.33 gm/dl respectively. Standard drug silymarin (Slybon) also decreased SGOT by 70.80 units/ml, SGPT by 63.80 units/ml, ALKP by 46.69 units/ml and increased TP levels by 6.26 gm/dl against CCl_4 intoxicated rats. The above results indicated that the methanol extract of *C. phlomidis* was possessing antihepatotoxic activity.

Table 1: *Effect of methanol fraction of Clerodendrum phlomidis stem on serum enzymatic activity in CCl_4 induced liver damage in rats.*

Groups n=5	Treatment	Dose	SGOT units/ml	SGPT units/ml	ALKP units/ml	TP Gm/dl
I	Normal control	--	54.54± 1.26**	44.45± 1.36**	43.49± 1.73**	6.29± 0.40**
II	Toxic control	1.5 ml/kg (i.p.)	140.66± 1.81	128.41± 2.48	69.99± 2.21	4.19± 0.38
III	Silymarin (Standard drug)	10 mg/kg (p.o)	70.80± 1.62**	63.80± 2.09**	46.69± 1.12**	6.26± 0.46**
IV	Methanol extract	500 mg/kg (p.o)	98.62± 2.01**	95.20± 1.79**	60.04± 1.80*	5.33± 0.15**

SGOT, serum glutamyl oxaloacetate transaminase; SGPT, serum glutamate pyruvate transaminase; ALKP, alkaline phosphate: TP, total protein; i.p. intraperitonally; p.o. per oral. ** P < 0.01; *P < 0.05 vs CCl_4. Values are mean ± S.E. of five animals. One way analysis and Dunnett's test.

Similarly histopathological studies in Table 2 reveal that in animals of group (I) normal control, liver samples show normal architecture (Fig.1). In toxic control group (II), CCl_4 caused fatty deposition and necrosis of hepatocytes (Fig.2). In group (III) treated with standard drug silymarin, liver samples showed a good recovery with absence of necrosis and fatty depositions (Fig.3). In group (IV) treated with methanol extract of *C. phlomidis* showed a significant recovery of hepatocytes and liver histology was almost normal in them (Fig.4), which were in accordance with the results obtained from biochemical parameters (Table 1). The results thus indicate that methanol extract of *C. phlomidis* (stem) possessed antihepatotoxic activity. Further work needs to be carried out to isolate the active principle responsible for antihepatotoxic activity.

Table 2: *Histopathological changes.*

Groups	Treatment	Microscopic observations
I	Normal control	Liver samples show normal architecture without any degeneration, necrosis or inflammation.
II	Toxic control (CCl_4)	Prominent centrilobular fatty change with prominent and enlarged central vein along with significant periportal inflammation reflecting liver damage.
III	Silymarin (Standard drug)	Samples showed a significant reduction in portal inflammation and in the sinusoidal dilatation. The central vein was clearly visible. Liver samples also showed good recovery with absence of necrosis and fatty depositions.
VI	CCl_4 + Methanol extract	Liver histology was almost normal with only very little sinusoidal dilatation seen in some hepatic lobules. Central vein appeared clear with the disappearance of necrosis.

Histopathology Slides

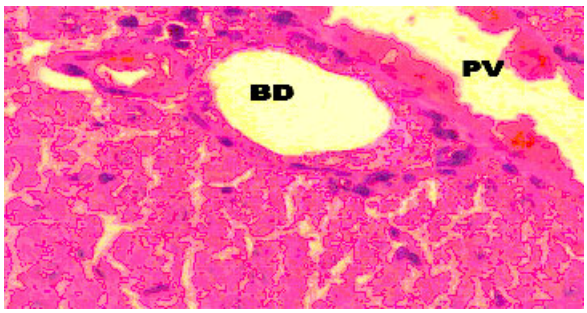

Fig. 1 *Group I. High power photomicrograph of normal control rat liver on 8th day (HE x 40X).*

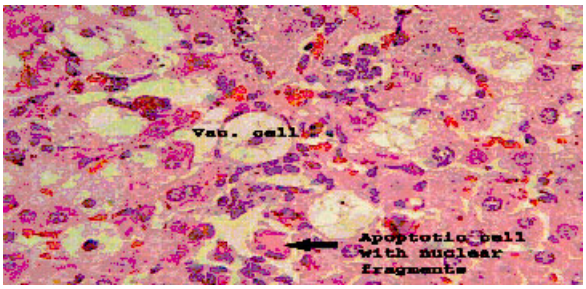

Fig. 2 *Group II. High power photomicrograph of toxic control rat liver on 8th day (HE x 40X).*

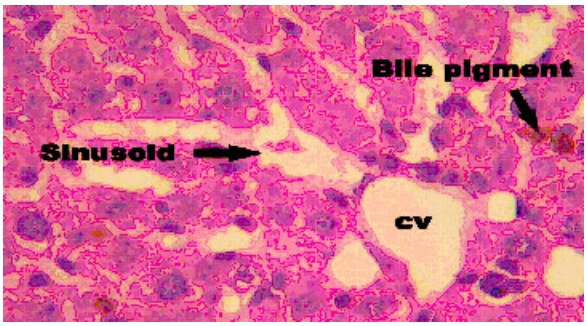

Fig. 3 *Group III. High power photomicrograph of silymarin treated liver on 8th day (HE x 40X).*

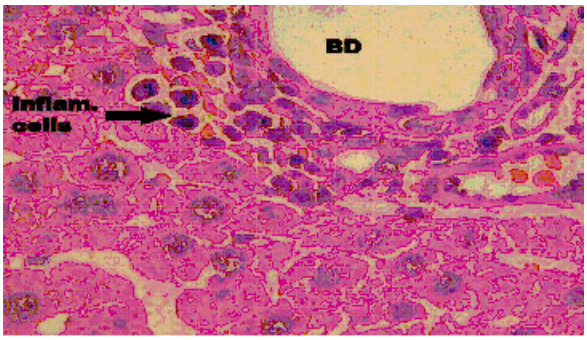

Fig. 4 *Group IV. High power photomicrograph of Clerodendrum phlomidis methanol fraction treated rat liver on 8th day (HE x 40X).*

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Corrigendum

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