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ANTI-HEPATOTOXIC ACTIVITY OF *CLERODENDRUM PHLOMIDIS*

AMITA VERMA[1]* and BAHAR AHMED[2]
[1]Department of Pharmaceutical sciences, Faculty of Health and Medical sciences, Allahabad Agriculture Institute- Deemed University, Naini, Allahabad.India.
[2]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Jamia Hamdard, New Delhi.India.
***Corres author:amitaverma.dr@gmail.com, vermaamita.dr@gmail.com**
Phone No.- 9415389171

ABSTRACT: In the present study, we evaluated the antihepatotoxic activity of the different fractions of *Clerodendrum phlomidis* 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 CCl_4 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.
KEY WORDS: Carbon tetrachloride, wistar rats, per oral, *Clerodendrum phlomidis*.

INTRODUCTION

In the present investigation CCl_4 was used to produce liver damage, the toxic effect of CCl_4 is due to its conversion by P_{450} to the highly reactive toxic free radicals $CCl_3^{\bullet}$ ($CCl_4 + e^- \rightarrow CCl_3^{\bullet} + Cl^-$).

Clerodendrum phlomidis (Verbenaceae) known as Arni in hindi. It is distributed More or less throughout India, Ceylon, Malay Peninsula. The sandals rub the plant over their bodies in dropsy and also give it to their cattle to cure them of diarrhea and worms, or when the stomach swells[1]. The decoction of roots is used as a demulcent in gonorrhea. The juice of leaves is used as bitter tonic[2] and also given in neglected syphilitic complaints[3]. The plant has been found to possess hypoglycemic activity[4]. The methanolic extract of C. phlomidis Linn. (MECP) leaves shows most of the pharmacological activities characteristic of minor tranquilizers[5]. It is used in Amrit nectar tablets (Amrit nectar tablets containing 38 herbs) The effect of aqueous & alcoholic extract of Amrit nectar tablet on rat liver microsomal lipid peroxidation are good[6]. Methanolic extract of leaves of C. phlomidis Linn (MECP) showed significant inhibitory activity against castor oil induced diarrhoea and PGE_2 induced enteropooling in rats. The extract also showed a significant reduction in gastrointestinal motility in charcoal meal test in rats[7]. 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[8].

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 (1.0 kg) was dried 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 ^oC), chloroform and methanol. The different fractions were dried under reduced pressure to get the crude dried fractions of petroleum ether, chloroform and methanol 10.0, 15.0, and 40.0 gm respectively.

Experimental animals and treatments

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*. 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 CCl_4 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 CCl_4 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 CCl_4 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 30-40 min. at room temperature. Serum was separated by centrifugation at 2500 rpm for 15 min. 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)[9], Alkaline phosphatase (ALP)[10], and Total protein (TP)[11] were carried out by reported methods.

Statical 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[12].

RESULTS

Group – I: **(**This group was given neither CCl_4 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 CCl_4).

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 centrizonal 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 (128.10 ± 1.77**), SGPT (100.14 ± 6.56**), ALP (63.11 ± 1.41^{ns}) units/ml and TP (4.59 ± $.0.16^{ns}$) gm/dl. and liver sections showed a mild sinusoidal dilation and a prominent centrilobular fatty change. There is no periportal inflammation.

Group – V: (Treated with chloroform fraction).

There was a decrease in the values of SGOT (90.50 ± 1.40**), SGPT (79.05 ± 1.78**), ALP (56.02 ± 2.74**) units/ml and TP (5.57 ± 0.15*) gm/dl. and liver section showed moderate sinusoidal dilatation around the central vein. Fine vacuolar change is also seen in scattered hepatocytes.

Group – VI: (Treated with methanol fraction).

There was a decrease in the values of SGOT (85.16 ± 2.23**), SGPT (76.85 ± 1.67**), ALP (50.65 ± 2.19**) units/ml and TP (5.48 ± 0.14*) 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.

DISCUSSION

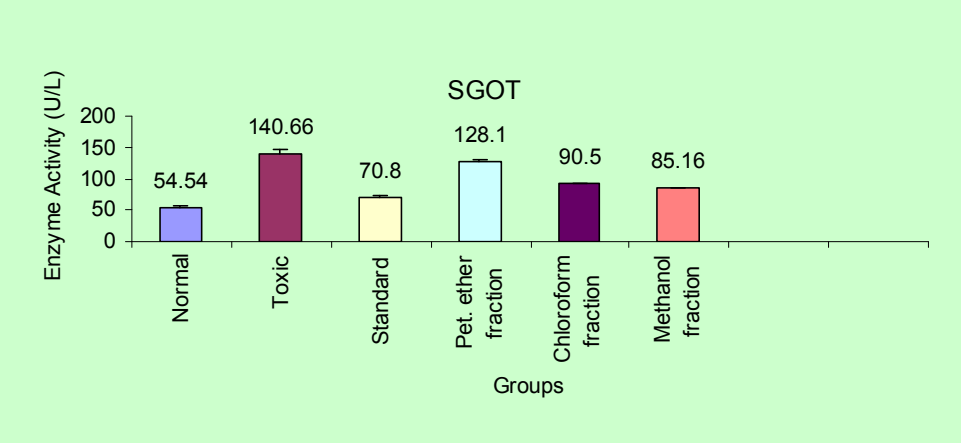
The table-I shows that the animals of group II, which received only CCl_4 were found to develop significant hepatic damage as was observed from elevated levels of SGOT, SGPT and ALP and the decrease in TP levels as compared to group I normal animals. The treatment with petroleum ether, chloroform, and methanolic fractions of alcoholic extract at a dose of 500-mg/kg b.w, p.o in groups IV, V and VI significantly prevented CCl_4 induced elevation of liver enzymes such as SGOT by 128.10, 90.50, 85.16 units/ml, SGPT by 100.14, 79.05, 76.85 units/ml, while ALP by 63.11, 56.02, and 50.65 units/ml respectively. On the other hand, TP levels were increased by 4.59, 5.57 and 5.48 gm/dl respectively, as compared to standard drug silymarin, which, decreased SGOT by 70.80, SGPT by 63.80, ALKP by 46.69 and increased TP levels by 6.26 against CCl_4 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.

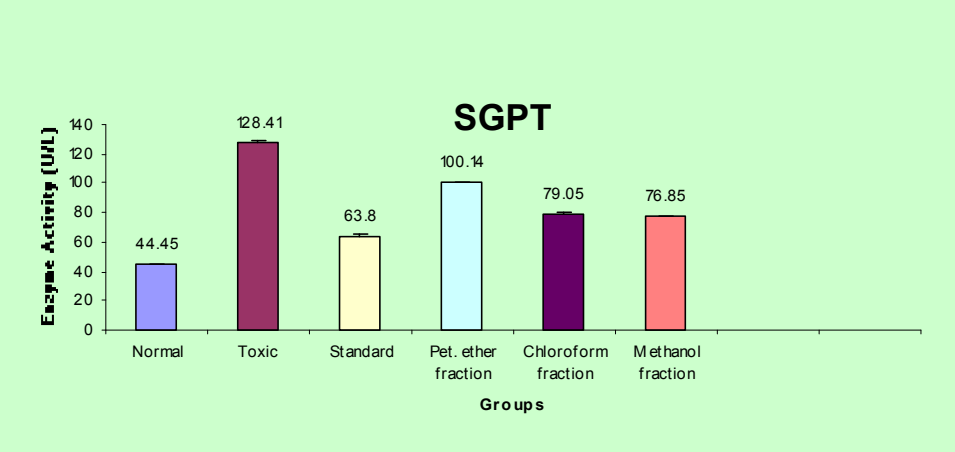
Table-I Effect of various extracts of *Clerodendrum Phlomidis aerial parts* on serum enzymatic activity in CCl_4 induced live damage in rats (n = 6)

Groups n=6	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 (p.o.)	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	Petroleum ether fraction	500 mg/kg (p.o.)	128.10 ± 1.77**	100.14 ± 6.56**	63.11 ± 1.41^{ns}	4.59 ± $.0.16^{ns}$
V	Chloroform fraction	500 mg/kg (p.o.)	90.50 ± 1.40**	79.05 ± 1.78**	56.02 ± 2.74**	5.57 ± 0.15*
VI	Methanolic fraction	500 mg/kg (p.o.)	85.16 ± 2.23**	76.85 ± 1.67**	50.65 ± 2.19**	5.48 ± 0.14*

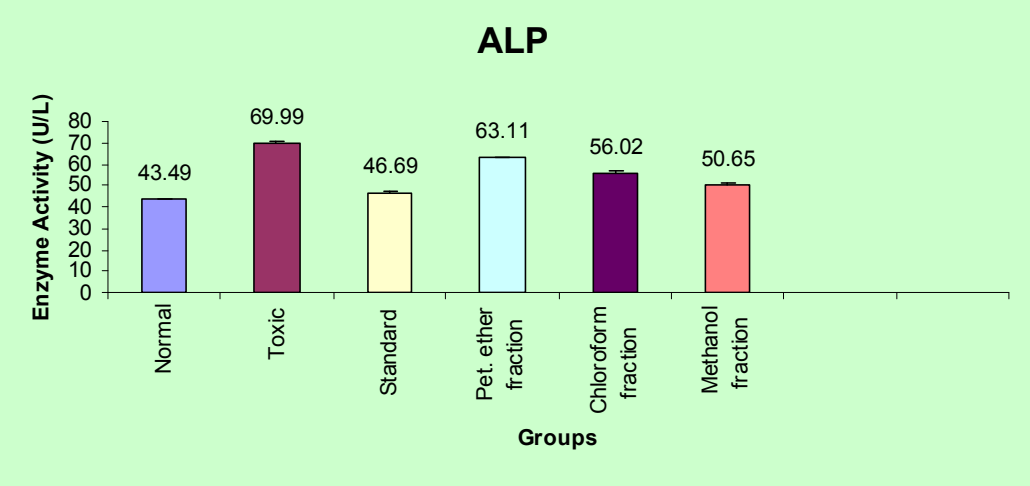
SGOT, serum glutamate oxaloacetate transaminase; SGPT, serum glutamate pyruvate transaminase; ALKP, alkaline posphatase: TP, total protein; p.o., per oral. ** $P < 0.01$;
*$P < 0.05$ vs CCl_4 of (Dunnett's test). Values are mean ± S.E. of six animals.



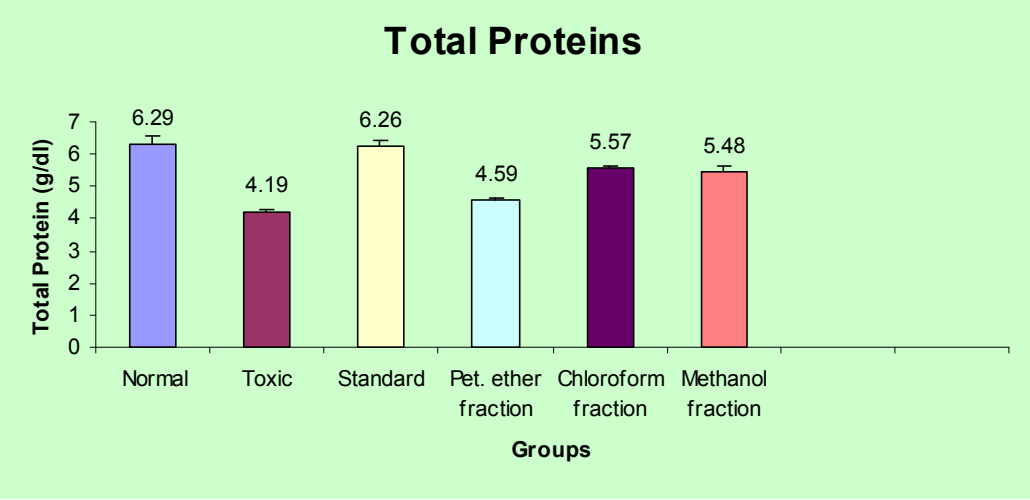
Graph-1: SGOT values after treatment with different fractions of *Clerodendrum Phlomidis* against toxic control.



Graph-2: SGPT values after treatment with different fractions of *Clerodendrum Phlomidis* against toxic control.



Graph-3: ALP values after treatment with different fractions of *Clerodendrum Phlomidis* against toxic control.



Graph-4: Total Protein values after treatment with different fractions of *Clerodendrum Phlomidis* against toxic control.

REFERENCES

1 Kirtikar K.R. and Basu B.D., Indian Medicinal Plants, International Book Distributers, Dehradun, 1999, 1947.
2 Nandkarni A.K., Indian Materia Medica, Popular Prakashan, Bombay, 2002, 353.
3 Chopra R.N., Nayar S.L. and Chopra I.C., Glossary of Indian Medicinal Plants, 1996, 71.
4 Pande M.C., Journal of natural Integrated Med Ass, 1978, 20 (8), 295.
5 Murugesan T., Saravanan K.S., Lakshmi S., Ramya G. and Thenmozhi K., Phytomedicine, 2001, 8(6), 472.
6 Dwivedi C., Agrawal P., Natarajan K. and Sharma H., J. of Alternative and Complementary Medicine, 2005, 11, 143.
7 Rani S., Ahamed N., Rajaram S. and Saluja R., Thenmozhi S., Murugesan T., J. Ethanopharmacol., 1999, 68, 315.
8 Anita R. and Kannan P., Turk. L. Biol., 2006, 30, 139.
9 Reitman S. and Frankel S., American J. Clin. Path., 1957, 28, 56.
10 Kind P.R.N. and King E.J., J. Clin. Path., 1954, 7, 332.
11 Wooton I.D., Microanalysis in Medical Biochemistry, J and A Churchill Ltd, London, 1964, 4th ed, 138.
12 Dunnet C.W., Biometrics, 1964, 20(3), 482.

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