



Anti-hepatotoxic activity of *Nymphaea stellata* seeds in carbon tetrachloride induced toxicity

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Received: 9th August, 2009 ; Accepted: 19th August, 2009

ABSTRACT

In the present study, we evaluated the antihepatotoxic activity of the different fractions of *Nymphaea stellata* 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;
Nymphaea stellata;
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⁻).

Nymphaea Stellata (Nymphaeaceae) known as milkamal in hindi and blue water lily in english. It is distributed throughout warmer parts of India, tropical Asia and Africa^[1]. It has been mentioned for the treatment of liver disorders in Ayurveda, an ancient system of medicine. In Indo-China the seeds are considered a powerful stomachic and restorative. In Guinea the infusion of the roots and the stem is considered emollient and diuretic; it is taken against blennorrhagia and diseases of the urinary tract. A decoction of the flowers is considered narcotic and antiaphrodisiac. In Madagascar the leaves are applied topically in erysipelas^[1]. Flowers are said to be

refrigerant and alleviative of cough, bile, vomiting, giddiness, worms and burning of the skin. Syrup of the flowers is useful in remittent and other high fevers, heat apoplexy and inflammatory diseases of the brain. Filaments of this plant are astringent, bleeding piles and menorrhagia and seeds are used in diabetes^[2]. The extract of flowers afforded the good hepatoprotection against CCl₄ induced hepatotoxicity^[3]. The ethanolic extract was given by oral route to diabetic rats at the doses of 100 and 200mg/kg/day for seven days have reduced significantly the plasma glucose level increased by intraperitoneal injection of 120mg/day of alloxan^[4].

EXPERIMENTAL

Chemicals and plant fractions

Carbon tetrachloride was used as toxicant in this

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study. The seeds of *Nymphaea stellata* were collected from Chandi Chauk, Old Delhi and authenticated by Dr. M.P. Sharma, Reader and taxonomist, Department of Botany, Faculty of Science, Jamia Hamdard. A voucher specimen No. NS-FP-21 has been deposited in herbarium for future reference. The plant material (1.0kg) 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°C), 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 40.0gm 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) (1.5ml/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 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)^[5]. Alkaline phosphatase (ALP)^[6] and Total protein (TP)^[7] 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^[8].

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

(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.

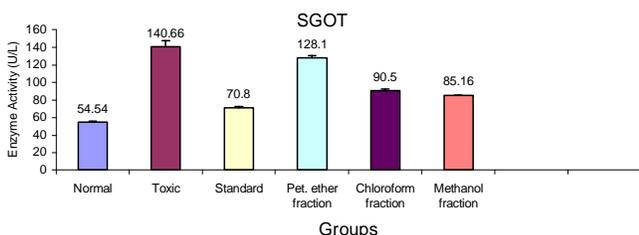
Group-V: (Treated with chloroform fraction)

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

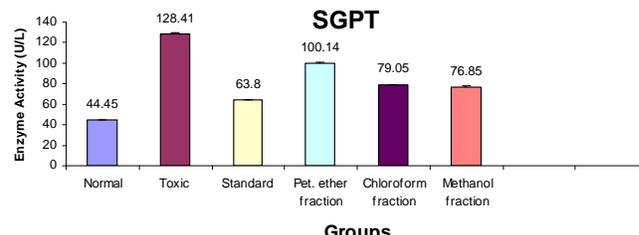
around the central vein. Fine vacuolar change is also seen in scattered hepatocytes.

Group-VI: (Treated with methanol fraction)

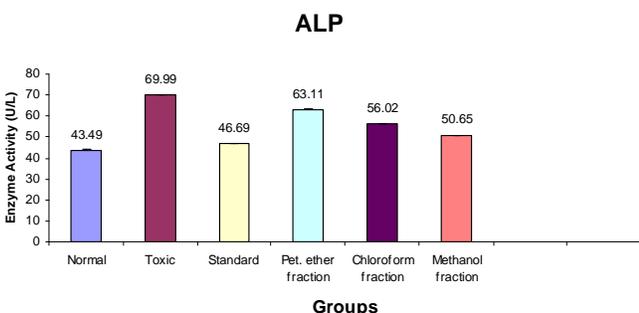
There was a decrease in the values of SGOT (85.16±2.23**), SGPT (76.85±1.67**), ALP (50.65±2.19**)units/ml and TP (5.48±0.14*)gm/dl. and liver sections showed a significant reduction in periportal inflammation and in the sinusoidal dilatation. Samples also showed good recovery with absence of necrosis, the central vein and the portal vein were both clearly visible.



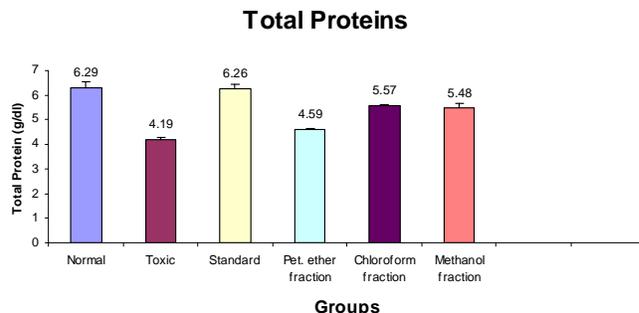
Graph 1 : SGOT values after treatment with different fractions of *Nymphaea stellata* against toxic control



Graph 2 : SGPT values after treatment with different fractions of *Nymphaea stellata* against toxic control



Graph 3 : ALP values after treatment with different fractions of *Nymphaea stellata* against toxic control



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

TABLE 1 : Effect of various fractions of *Nymphaea stellata* seeds 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
I	Normal control	-	54.54±1.26**	44.45±1.36**	43.49±1.73**	6.29±0.40**
II	Toxic control	1.5ml/kg (p.o.)	140.66±1.81	128.41±2.48	69.99±2.21	4.19±0.38
III	Silymarin (standard drug)	10mg/kg (p.o.)	70.80±1.62**	63.80±2.09**	46.69±1.12**	6.26±0.46**
IV	Petroleum ether fraction	500mg/kg (p.o.)	128.10±1.77**	100.14±6.56**	63.11±1.41 ^{ns}	4.59±0.16 ^{ns}
V	Chloroform fraction	500mg/kg (p.o.)	90.50±1.40**	79.05±1.78**	56.02±2.74**	5.57±0.15*
VI	Methanol fraction	500mg/kg (p.o.)	85.16±2.23**	76.85±1.67**	50.65±2.19**	5.48±0.14*

SGOT, serum glutamic oxaloacetic transaminase; SGPT, serum glutamic pyruvate transaminase; ALP, alkaline phosphatase; TP, total protein; p.o., per oral, ** P<0.01; *P<0.05 vs CCl₄; P>0.05 ns, Values are mean±S.E. of five animals, One way analysis and dunnett's test

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

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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 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 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.

ACKNOWLEDGEMENTS

The authors are thankful to the Head Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Jamia Hamdard for providing necessary research facilities and to the in-charge Central Animal Facility, Jamia Hamdard, New Delhi for providing rats and other related facilities for pharmacological activities.

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