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Hepatoprotective activity of leaves of *Neptunia Oleracea* lour in Carbon tetrachloride induced rats.

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ABSTRACT

The ethanol and aqueous extracts of leaves of Neptunia Oleracea lour (Mimosaceae) were tested for their efficacy against carbon tetrachloride (CCl_4) induced hepatotoxicity in Wistar albino rats. The different groups of animals were administered ethanol and aqueous extracts to carbon tetrachloride treated rats. In the present study ethanol extract ($p<0.01$) and aqueous extract ($p<0.05$) significantly decreases the level of alanine aminotransferase, aspartase aminotransferase, alkaline phosphatatase, total bilirubin and direct bilirubin in blood whereas total protein level is elevated. The phytochemical screening revealed the presence of active phytoconstituents i.e. flavonoids, triterpinoids and tannins, which may offer hepatoprotection. The present work support the traditional claim of plant in the treatment of liver injury, may provide a new drug against a war with liver diseases.

Keywords: Neptunia Oleracea, Hepatoprotective effect, Carbon tetrachloride, Ethanol and Aqueous extracts.

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INTRODUCTION

Liver regulates various important metabolic functions. Hepatic damage is associated with distortion of these metabolic functions. Liver disease is still a worldwide health problem. Unfortunately, conventional or synthetic drugs used in the treatment of liver diseases are inadequate and sometimes can have serious side effects whereas herbs play a role in the management of various liver diseases. Many folk remedies from plant origin have been long used for the treatment of liver diseases. This is one of the reasons for many people in the world over including those in developed countries turning complementary and alternative medicine. Many traditional remedies employ herbal drugs for the treatment of liver ailments [1].

The plant Neptunia oleracea lour, (Mimosaceae) is an annual floating marine plant usually distributed in tanks and lakes all over India and Ceylon [2]. It is reported to possess astringent, antimicrobial and anticancer properties. The roots of the plant are used in late stages of syphilis [3, 4]. The preliminary phytochemical study revealed the presence of flavonoids, carbohydrates, anthroquinones, tannins and triterpenes in alcoholic extract [5]. The flavonoids, triterpinoids and tannins were known to possess the hepatoprotective activity [6]. Since there are no particular reports on hepatoprotective activity of leaves of the plant, it was considered worthwhile to evaluate the leaves for hepatoprotective activity.

Carbon tetrachloride (CCl_4) is one of the most commonly used hepatotoxins in the experimental study of liver diseases. The hepatotoxic effect of CCl_4 is largely due to its active metabolite, trichloromethyl radical [7]. The administration of CCl_4 in rats enhances hepatic protein oxidation and results in the accumulation of CCl_4 oxidized proteins in the liver [8]. The present study was conducted to evaluate the hepatoprotective effect of the extracts of leaves of Neptunia olercea on carbon tetrachloride induced liver damage in experimental rats.

MATERIALS AND METHODS

Plant material

The leaves of *Neptunia oleracea* were collected from the pond of Malayabad forest, Raichur (Karnataka) and authenticated at the Department of Botany, L.V.D. College, Raichur. The collected leaves were shade dried and pulverized to fine powdered of particle size (#) 40. Dried leaves powder 200 gm was defatted with petroleum ether ($60\text{-}80^0$ C) and further separately extracted with 95 % ethanol and water by Soxhelt extractor for 48 h. The extracts were concentrated to dryness under reduced pressure *in vacuo*. The yield of ethanol and aqueous extracts were found to be 15 gm and 10 gm (30 %, 20 % w/w) respectively. Both extracts were kept in a dessicator till experimentation.

The preliminary phytochemical screening of ethanol and aqueous extracts were performed to identify the presence of triterpinoids, flavonoids and tannins.

Animals

Albino rats of either sex weighing between 150 gm - 200 gm were selected for hepatoprotective activity. Animals were kept in polypropylene cages and fed on standard laboratory diet and water *ad libitum*, maintained at an ambient temperature of 25 ± 2^{o} C and exposing them to 12 h light/dark cycle. The ethical clearance obtained by the institutional animal ethics committee (Reg.No. 727 / 02 / C / CPCSEA) before the experiment.

Acute toxicity study

The acute toxicity of ethanol extract and aqueous extract was evaluated in mice. The animals were fasted prior to the acute toxicity study. Different groups containing two mice in each were orally administered with ethanol and aqueous extract at 0.5, 1.0, 1.5 and 2.0 gm/kg p.o. respectively. Control group received only propylene glycol (vehicle). Drug treated and control groups were placed in polypropylene cages with free access of food and water. Mortality and general behavior of the animals were observed continuously for initial 4 h and intermittently for next 6 h and then again at 24 h and 48 h after dosing. The parameters observed and recorded were sedation, hyperactivity, grooming, loss of rightening reflex, respiratory rate and convulsions. 1/10th of the lethal dose was taken as the screening dose [9].

Evaluation of hepatoprotective activity

Wistar albino rats were divided into five groups of six animals each. The carbon tetrachloride (1ml/kg) was administered to all groups of animals by subcutaneous injection except Group-I. Group-I served as control received normal saline (10 ml/kg i.p) only. Group-II received CCl_4 (1ml/kg. i.p). Group-III received the reference drug, silymarin (25 mg/kg i.p). Group-IV and Group-V received aqueous and ethanolic extract respectively in a dose of 200 mg/kg daily once for 15 days after CCl_4 administration. All the animals were dissected at the end of 15th day [6]. The blood sample of each animal was collected separately by carotid bleeding into sterilized dry centrifuge tubes and allowed to coagulate for 30 min at 37° C. The clear serum was separated at 3000 rpm for 10 min and was subjected to biochemical investigation viz., total and direct bilirubin [10], total protein [11], serum alanine transaminase, aspartate transaminase [12] and alkaline phosphatase [13]. Results of biochemical estimations were reported as mean ± S.E of six animals in each group. The data was subjected to one way ANOVA followed by Post-hoc Dunnett's test. $p < 0.05$ was considered as statistically significant [14].

Histological studies

The liver from each animal was removed after dissection; washed with ice cold saline, the liver sections were taken from each lobe of the liver and fixed with 10 % neutral formalin solution and embedded in paraffin by employing the standard technique, 5 µ in thick section were cut and stained with hematoxylin-eosin. Stained liver tissue were used for the preparation of histopathological slides by using microtome and observed under microscope for architectural changes seen during CCl_4 challenge in extracts of *Neptunia oleracea* treated and control groups.

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RESULTS AND DISCUSSION

The acute toxicity evaluation of extracts of leaves of *Neptunia oleracea* revealed no mortality when administered orally up to a maximum dose 2 gm/kg. At this dose there were no gross behavioral changes. The $1/10^{th}$ of lethal dose was taken as the screening dose.

Reports of preliminary phytochemical analysis indicated the presence of anthroquinones, carbohydrate, flavonoids and tannins in both ethanolic and aqueous extracts. Where as triterpenoids in ethanolic extract only.

Table1. Effect of ethanolic and aqueous leaves extracts of *Neptunia oleracea lour* on CCl_4 induced hepatotoxicity in rats.

Gr	Treatment	AST (U/L)	ALT (U/L)	ALP (U/L)	Total protein (gm%)	Bilirubin(mg/100 ml of blood) Direct	Total
1.	Control 10ml/kg	60.2±1.08	55.08±2.31	105.52±5.32	9.25±0.42	0.38±0.14	0.85±0.35
2.	CCl4 1ml/kg i.p	156.1±11.62	163.8±11.04	265.3±7.57	5.51±0.25	2.52±0.44	3.45±0.86
3	Silymarin (25 mg/kg)	70.2±5.60**	52.6±2.23**	125.6±3.94**	8.25±0.37**	0.46±0.20**	1.14±0.23**
4.	Aqueous Extract 200mg/kg	128.4±4.45*	139.5±1.84*	229.5±12.85*	6.73±0.24*	1.12±0.51*	1.74±0.28*
5.	Ethanol extract 200mg/kg	96.9±4.78**	79.8±2.91**	140.2±7.86**	8.02±0.32**	0.69±0.41**	1.28±0.13**

Data are expressed as mean + S.E., n =6.
*P<0.05 Vs Control, **P<0.01 Vs Control (one way ANOVA followed by Post-hoc Dunnett's test).

CCl_4 induced hepatic injury is the common model used for hepatoprotective drug screening. The extent of hepatic damage is assessed by the elevated level of biochemical parameters which is attributed to the generation of trichloromethyl free radical which in turn causes peroxidation of lipids of cellular membrane [15]. Effect of ethanol and aqueous extracts of leaves of *Neptunia oleracea* on CCl_4 induced liver damage in rats with reference to biochemical changes in serum is shown in the table No.1. At the end of 15 days treatment,

blood samples of CCl_4 treated animals showed significant increase in the levels of total and direct bilirubin, alanine transaminase, aspartate transaminase and alkaline phosphatase compared to normal control groups but the total protein level decreased reflecting the liver injury caused by CCl_4. Whereas blood samples from the animals treated with ethanol and aqueous extracts of *Neptunia oleracea* leaves showed significant decrease in the levels of serum markers and significant increase in protein level to the near normal which are comparable to the standard drug treated group of animals, indicating the protection of hepatic cells.

Histopatholgical studies demonstrated that carbon tetrachloride causes focal necrosis, portal infiltration, fatty change, kupffer cell hyperplasia and hydropic change. In the treated groups, necrosis which is more severe form of injury is markedly prevented; milder form of injury like fatty change and reduced necrosis persisted in both the extracts.

Flavonoids, triterpenoids and tannins are well known for their hepatoprotective activities [16-18]. In this study ethanol and aqueous extracts showed protective effect against toxicity induced by CCl_4, which may be attributed to the individual or combined effect of hepatoprotective activity of phytoconstituents present in it. So among the two extracts ethanol extract ($p<0.01$) showed more significant protection against CCl_4 induced hepatic damage over aqueous extract ($p<0.05$). Based on the above results of the pharmacological screening, it can be concluded that the ethanol and aqueous extracts of *Neptunia oleracea* leaves possesses significant hepatoprotective activity. However, detailed phytochemical

& pharmacological investigation of the leaves is under progress in our laboratory to elucidate the exact mechanism of action responsible for the hepatoprotective activity.

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