

Original Article

HEPATOPROTECTIVE ACTIVITY OF THE WHOLE PLANT OF *NEPTUNIA PROSTRATA* L. IN CARBON TETRACHLORIDE INDUCED RATS

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ABSTRACT

Objective: The aim of this study is to investigate the hepatoprotective activity of the whole plant of *Neptunia Prostrata* L.

Methods: The whole plant was collected and identified as *Neptunia Prostrata* L. The collected plants were shade dried and pulverized to fine powdered of particle size (#) 40. It was then defatted with petroleum ether for 24 hour and soaked with methanol and ethanol, respectively. The extracts was filtered and distilled off using a rotary evaporator. The phytochemical screening of the extracts was carried out and thin layer chromatography study was also done. Acute toxicity study and *in vivo* hepatoprotective activity of the methanolic extract using CCl₄ (carbon tetra chloride) induced model was investigated.

Results: The phytochemical screening revealed the presence of alkaloids, glycosides (saponins), flavonoids, tannins, carbohydrates, proteins, phenolic, steroids and terpenoids. Thin-layer chromatography of the methanolic and ethanolic extracts with their fractions using different solvents were performed by taking petroleum ether and ethyl acetate (2:8) as mobile phase system and were able to observe the presence of many spots. Oral administration of methanolic extract of *Neptunia prostrata* at doses till 2000 mg/kg was found safe and shows good hepatoprotective activity by showing decreased levels of serum SGOT (serum glutamate oxaloacetate transaminase), SGPT (serum glutamate pyruvate transaminase) and ALP (alkaline phosphatase) when compared with the standard drug silymarin.

Conclusion: The preliminary phytochemical screening of the methanol and ethanolic extract shows phytoconstituents such as flavonoids, triterpenoids, tannins, saponins, alkaloids and chromatographic studies indicates the presence of several components in varying abundance. The decrease of serum bilirubin level by the methanolic extract of the plant shows hepatoprotective activity. It has confirmed the traditional claim for its use in the treatment of jaundice.

Keywords: Phytochemical, Methanol, *Neptunia prostrata* L, Ethanol and hepatoprotective

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INTRODUCTION

Liver disorder is the main challenged faced by the world today. Herbal plants of liver protection contain a variety of chemical constituents like phenols, coumarins, monoterpenes, glycosides, alkaloids and xanthenes. Medicinal plants have fewer side effects and can be used irrespective of the age group [1]. Manifold number of plants and formulations have proved to have hepatoprotective activity; 160 phytoconstituents obtained from 101 plants have claimed liver protecting activity [2]. *Neptunia prostrata* is an annual floating aquatic herb distributed in lakes and marshy places all over India [3]. It is available in 25 different states of India including the north-eastern state such as Manipur, Tripura [4, 5]. It is grown wild and being cultivated as vegetable throughout Southeast Asia, particularly Thailand and Indo-China [6]. The tribals of North east India cultivate this plant as vegetable and medicinal plant purposes and also prepare various tasty delicacy dishes [7]. The whole plant has been used for different kinds of remedies like gastritis, acidity, constipation, dysentery [8] and also reported to possess antioxidant [9] anti-cancer [10] antimicrobial [11] anti-inflammatory, analgesic activities [12] refrigerant and astringent properties [13] laxative [14] and also for treatment of jaundice [15]. The roots of the plant are used in late stages of syphilis [16]. The preliminary phytochemical study revealed the presence of flavonoids, carbohydrates, anthroquinones, tannins and triterpenes in alcoholic extract [17]. Since there are no particular reports on hepatoprotective activity of leaves of the plant, it was considered worthwhile to evaluate hepatoprotective activity.

Carbon tetrachloride (CCl₄) is one of the most commonly used hepatotoxins in the experimental study of liver diseases. The hepatotoxic effect of CCl₄ is largely due to its active metabolite, trichloromethyl radical [18]. The administration of CCl₄ in rats enhances hepatic protein oxidation and results in the accumulation

of CCl₄ oxidized proteins in the liver [19]. The present study was conducted to evaluate the hepatoprotective effect of the extracts of whole plant of *Neptunia prostrata* on carbon tetrachloride induced liver damage in experimental rats.

MATERIALS AND METHODS

Plant sample collection and identification

The whole plant of *Neptunia prostrata* which was collected from Abhoynagar vegetable market. It was identified by a taxonomist at the Dept. of Botany, Tripura University, India.

Processing of plant materials and extraction

Fresh whole plant of *Neptunia prostrata* was washed thoroughly 2-3 times with running water and once with sterile distilled water. The collected whole plants were shade dried and pulverized to fine powdered of particle size (#) 40. The collected 400 gm of powdered plant material was defatted with petroleum ether for 24 h. and soaked with methanol and ethanol, respectively. The extracts was filtered and distilled off by using rotary evaporator. 18 gm of crude ethanolic extract was suspended into 100 ml of distilled water to make 95% aqueous solution and fractioned sequentially with dichloromethane, ethyl acetate and isobutanol, respectively (each solvent 100 ml). The extracts were filtered through Whatman filter paper no.1 and concentrated at 50 °C using rotary evaporator (Ika, Japan). The concentrated extracts were stored in airtight container at 4 °C refrigerator for further experiments [20, 21].

Thin layer chromatographic (TLC) analysis

Thin layer chromatography of the *Neptunia prostrata* methanolic extract NPME and *Neptunia prostrata* ethanolic extract NPEE was carried out. The NPEE and its fractions were also carried out for TLC

study such as (NPIF *Neptunia prostrata* isobutanol fraction, NPEF *Neptunia prostrata* ethylacetate fraction, NPDF *Neptunia prostrata* dichloromethane fraction). It was performed in a prepared silica gel G coated alumina plate cutted suitably into 6 cm in length and 2 cm in width. The extracted material was dissolved in a minimum amount of their respective solvent used and spotted on the pre-activated plates using capillary tube, TLC was run in petroleum ether: ethyl acetate (2:8) mobile phase system. Chromatogram was developed and dried in air. The extracts were chromatographed on silica-coated TLC plates (Sigma-Aldrich) [22].

Phytochemical screening

The methanolic extract and ethanolic extract were performed for phytochemical screening, which includes test for alkaloids [23], test for carbohydrates, tannins, phenols [24], test for glycosides, saponins [25], test for flavonoids, proteins, steroids, and terpenoids [26] were performed.

In vivo hepatoprotective study

Experimental animals: animal experimentation was carried out with minimum invasive procedures as per the guidelines of CPCSEA. The ethical clearance obtained was (IAEC/BCPSR/003/2016).

Acute toxicity study [27]: The acute toxicity study for methanolic extracts of *Neptunia Prostrata* whole plant was performed using albino rats. The animals were fasted overnight prior to the experiment and maintained under standard conditions. All the extracts were administered orally in the increasing dose and found safe up to the dose of 2000 mg/kg.

Evaluation of hepatoprotective activity [28]: Rats were divided into four groups of six animals each. All groups received CCL₄ (2 ml/kg/BW/s. c) except group A. Group A (Normal control) animals were administered with normal saline (10 ml/kg, i. p). Group B

experimental control received CCL₄: liquid paraffin (1:1, 2 ml/kg body weight, i. p). Group C received standard drug silymarin (100 mg/kg, i. p). Test groups animals Group D were administered 200 mg/kg of methanolic extracts in the form of aqueous suspension once daily after CCL₄ administration for 15 d. Animals were sacrificed 24 h after the last treatment. Blood was collected, allowed to clot and serum was separated at 2500 rpm (revolution per minute) for 15 min and biochemical investigations were carried out. Liver was dissected out and used for histopathological studies.

Estimation of serum biochemical parameters: Serum biochemical parameters were estimated according to standard methods. The activity of the enzymes ALT, AST, and ALP was measured using commercial enzymatic biochemical diagnostic kits.

RESULTS

Phytochemical screening

The methanolic and ethanolic extract of the plant shows many phytochemicals such as alkaloids, glycosides (saponins), flavonoids, tannins, carbohydrates, proteins, phenolic, steroids and terpenoids etc. (table 1).

TLC

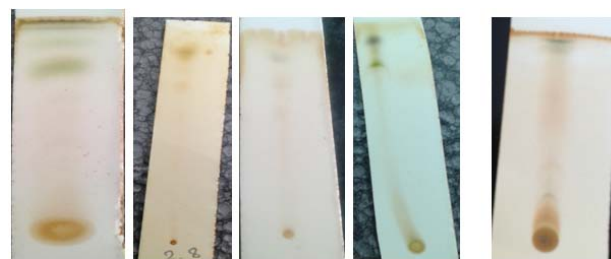
Thin-layer chromatography of the methanolic extracts and ethanolic extracts with their fractions was performed by taking petroleum ether and ethyl acetate (2:8) as mobile phase system and was able to observe the presence of many spots.

Acute toxicity studies

Oral administration of methanolic extract of *Neptunia prostrata* at doses till 2000 mg/kg body weight did not produce any significant behavioral change and during the experimental period, no death occurs. So, it was determined that the drug was safe up to 2000 mg/kg body weight.

Table 1: Phytochemical test for methanolic and ethanolic extract of *Neptunia Prostrata*

S. No.	Phytochemical test	Methanol extract	Ethanol extract
1.	Alkaloid	+	+
2.	Glycosides	+	+
3.	Saponin	+	+
4.	Anthraquinone	-	-
5.	Flavonoids	+	+
6.	Tannins	+	+
7.	Carbohydrates	+	+
8.	Protein	+	+
9.	Phenolic	+	+
10.	Steroids	+	+
11.	Terpenoids	+	+



a) NPEE b) NPDF c) NPEF d) NPIF e) NPME

Fig. 1: Ethanolic extract and its fractions TLC of the extracts methanolic extract

Hepatoprotective effect of NPME in CCL₄-induced liver damage

Effect of NPME on liver function parameters: The CCL₄ induced hepatic injury was confirmed by measuring the activity of hepatic marker enzymes ALT, AST, ALP and total bilirubin of rats. A

significant increase in the levels of ALT, AST, and ALP was observed in CCL₄ treated rats from those of the control group. Administration of NPME (200 mg kg⁻¹) significantly attenuated the elevation of these parameters. The hepatoprotective effect shown by NPME was almost comparable to that of silymarin (fig. 2).

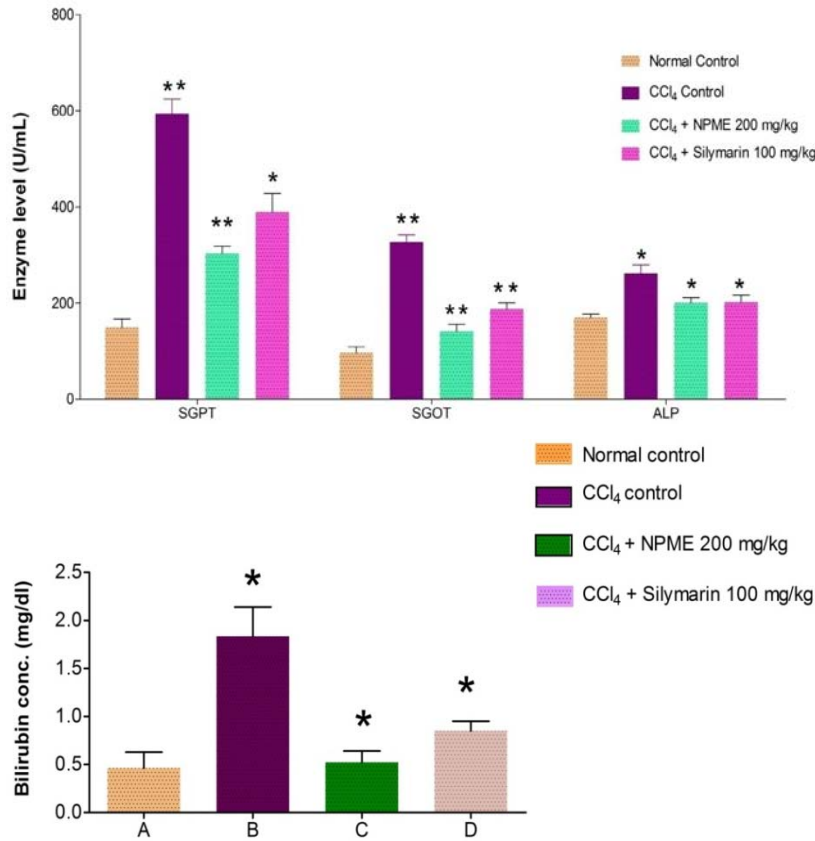


Fig. 2: Effect of NPME on liver function parameters

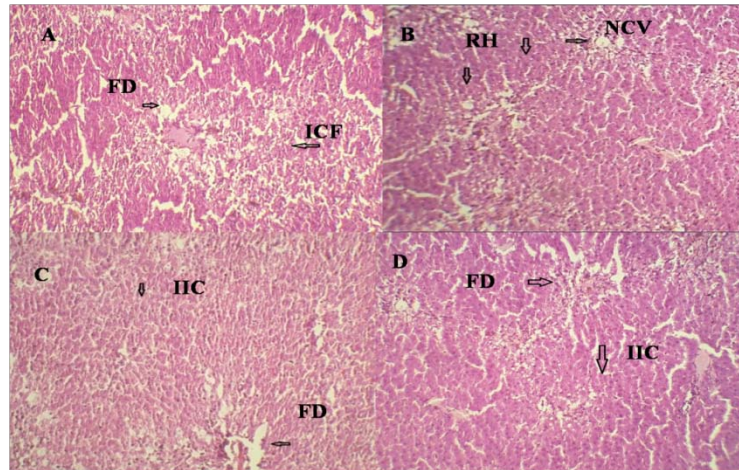


Fig. 3: Histopathological observations, a) Liver sections of CCl₄ induced liver toxicity, b) Left arrow indicates fatty degeneration and right arrow indicates inflammatory cell infiltration, c) Liver section of normal control: left arrow shows normal central vein and down arrow indicates radiating hepatocytes, d) Liver section treated with NPME+CCl₄: down arrow indicates infiltration of inflammatory cells and right arrow shows fatty degeneration, e) Liver section treated with Standard silymarin+CCl₄: left arrow indicates fatty degeneration and down arrow shows infiltration of inflammatory cell

DISCUSSION

The preliminary phytochemical investigations of methanol and ethanol extracts has been performed and found the presence of phytoconstituents such as alkaloids, glycosides (saponins), flavonoids, tannins, carbohydrates, proteins, phenolic, steroids and terpenoids etc. (table 1) Thin-layer chromatography of the extracts by taking petroleum ether and ethyl acetate (2:8) as mobile phase system has observed the presence of many spots. (fig. 1). Phytochemical screening and thin-layer chromatography studies indicate the presence of several components in varying abundance.

Administration of CCl₄ causes severe liver damage because it is metabolized by cytochrome P₄₅₀ in hepatocytes, producing a highly reactive carbon centered trichloromethyl radical, thereby initiating a chain of lipid peroxidation causing liver fibrosis. Increased levels of serum SGOT, SGPT and ALP of the CCl₄ treated animals shows liver damage as the enzymes leak out from the liver into the blood at the instance of tissue damage which is always associated with hepatonecrosis [29]. The strength of a hepatoprotective drug to reduce the injurious effects or to preserve the normal hepatic physiological mechanisms that have been disturbed by a hepatotoxin is the index of its protective effects [30]. The oral

administration of NPME at doses till 2000 mg/kg body weight did not produce any significant behavioural change and during the experimental period, no death occurs. So, it was determined that the plant extract was safe up to 2000 mg/kg body weight.

Biochemicals analysis: Serum levels of SGOT, SGPT, ALP and total bilirubin serve as hepatotoxicity indexes. CCL₄ administration produces significant elevations of serum as compared to the normal control group indicating liver damage. Treatment of rats with NPME (200 mg+CCL₄) caused a significant reduction in the values of SGOT, SGPT, ALP and total bilirubin (fig. 2).

Histological studies: The hepatoprotective effect of NPME was confirmed by histopathological examination of the liver tissue of control and treated animals. The histological architecture of CCL₄ liver sections showed severe fatty degeneration of hepatocytes. However, administration of NPME (200 mg/kg) almost normalized these defects in the histological architecture to the level of normal control groups. It also shows more activity than the standard silymarin-treated groups, showing its potent hepatoprotective effects (fig. 3). The presence of phytoconstituents like flavonoids, triterpenoids, tannins, saponins, alkaloids are known to possess hepatoprotective activity [31].

CONCLUSION

The preliminary phytochemical screening and chromatographic studies indicates the presence of several phytoconstituents in varying abundance. The decrease of serum bilirubin level by the methanolic extract of the plant shows hepatoprotective activity. Hence, from the experimental evidence, it has confirmed the traditional claim of it's used as hepatoprotective.

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AUTHORS CONTRIBUTIONS

All authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

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