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Research Article

HEPATOPROTECTIVE ACTIVITY OF ETHYL ACETATE EXTRACT OF *IPOMOEA NIL* (L.) ROTH SEEDS ON RATS

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ABSTRACT

Objective: Hepatoprotective activity of ethyl acetate extract of seeds of *Ipomoea nil* has been evaluated by CCl_4 induced liver damage models in rats.

Methods: In the present study, ethyl acetate extract at the dose levels of 200 and 400 mg/kg b. wt. was used. CCl_4 was used as hepatotoxicant, and Silymarin, at the dose of 100 mg/kg b. wt. was used as the reference standard. Rats intoxicated with CCl_4 have shown increased levels of biochemical enzymes like, SGOT, SGPT, SALP and serum bilirubin. A section of liver was taken and subjected to histopathological studies.

Results: Two different doses employed were found to significantly inhibit the serum marker enzymes. The results are comparable with standard Silymarin.

Conclusion: Based on the above study, it is concluded that ethyl acetate extract of *Ipomoea nil* possess significant hepatoprotection against CCl_4 induced hepatotoxicity in albino rats.

Keywords: *Ipomoea nil,* Hepatotoxicity, CCl_4, Silymarin.

INTRODUCTION

Liver is the key organ in metabolism, detoxification and secretary function in the body. It also regulates important metabolic functions. It is involved with almost all the biochemical pathways to growth, fight against disease, nutrient supply, energy provision and reproduction. Therefore, maintenance of a healthy liver is essential for the overall well-being of an individual. In India, numerous medicinal plants and their formulations are used in ethno medical practices and traditional system of medicine for liver disorders. Therefore, searching for effective and safe drugs for liver disorders are continues to be an area of interest.

Herbal drugs are prescribed widely even when their biologically active components are unknown because of their effectiveness, fewer side effects and relatively low cost. In spite of the tremendous advances made in allopathic medicine, no effective antihepatotoxic medicine is available till date. Plant drugs are known to play a vital role in the management of liver diseases. A number of medicinal plants are used in traditional system of medicine for the management of liver disorders. However many of them have not investigated for their described effects. *Ipomoea nil* roth belonging to the family Convolvulaceae, is one such medicinal plant used in the treatment of liver disorders in folk medicine. It also has anticancer, antimicrobial, antiinflammatory, antimalarial, antidiabetic activities, etc. No investigation has been carried out on hepatoprotective activity of the plant *Ipomoea nil*. Hence the present investigation has been carried out to study the hepatoprotective activity of the ethyl acetate extract of the *Ipomoea nil* seeds on CCl_4-induced liver toxicity in albino rats [1].

MATERIALS AND METHODS

Plant material

Ipomoea nil was collected in the month of Sep 2012 and authenticated by Dr. A. K. Pradeep, Herbarium Curator, Department of Botany, Calicut University, Malappuram, Kerala, and a voucher specimen was deposited in the Department (Specimen No: 107885).

Plant extract preparation

Seeds of the plant was air-dried, protected from direct sunlight, and then powdered. The powdered plant material (500 g) was extracted with ethyl acetate in a Soxhlet apparatus, for 72 h, by continuous hot extraction method[1]. The each extracts were then concentrated under reduced pressure on a rotary evaporator to dryness to give the crude residue [2]. The crude residues were employed for further investigation. The extracts were subjected to qualitative chemical tests for the detection of various plant constituents like carbohydrates, glycosides, proteins and amino acids, fixed oils and fats, gums and mucilage, alkaloids, phytosterols, flavanoids, tannins and phenolic compounds, saponins, triterpenoids, etc [3].

Animals

Albino mice of Swiss strain and albino rats of Wistar strain were used for toxicological studies and pharmacological studies respectively. These animals were obtained from the animal house of Central Animal Facility, IISC, Bangalore, India. Female mice selected were nulliparus and non-pregnant. Female mice weighing 25 to 30 g and rats of either sex weighing 125 to 150 g were used for the study. Each animal, at the commencement of its dosing, was between 8 and 12 weeks old and their weight variation was within ± 20% of the mean weight of any previously dosed animals. The temperature in the experimental animal room was 22ºC (± 3ºC) and the relative humidity was between 50-60%. These animals were fed with pellet diet and drinking water *ad libitum*. They were kept in 12 h/12 h light/dark cycle. The animal experimental protocol has been approved by OACE of U win life science, Malappuram, where the animal studies were carried out (OACE No: ULSB/ DAEC/ KER/59/363/13).

Hepatoprotective activity

Albino rats of Wistar strain weighing 125-150 g of either sex were used for the study. They were housed in polypropylene cages with not more than six animals per cage and maintained under standard conditions. All the experiments were performed in the morning according to current guidelines for the care of the laboratory animals and the ethical guidelines for the investigation of experimental pain in conscious animals.

Liver damage was induced in rats by administering CCl_4 subcutaneously (s. c.) in the lower abdomen in a suspension of liquid paraffin (LP) in the ratio 1: 2 v/v at the dose of 1 ml CCl_4/kg b. wt. of each animal. CCl_4 was administered twice a week, on every first and fourth day of both the weeks [4].

Thirty rats were divided in to five groups. Group I served as control and received oral administration of normal saline at the dose of 1 ml/kg b. wt., once a day for 14 days [5]. Group II received s.c. administration of CCl_4 twice a week for a total of 14 days. Group III were the Silymarin [6] (the known hepatoprotective compound)

treated group, and received the same at a p.o. dose of 100 mg/kg b. wt. along with CCl_4. Group IV and V were treated with the extract at 200 and 400 mg/kg respectively along with CCl_4.

Upon completion of 14 days, blood samples were collected by making an incision on jugular vein in heparinized microfuge tubes. The blood samples thus collected were immediately centrifuged at 2200 rpm for 15 min. When serum clearly separated out, the serum was analyzed for serum glutamate oxaloacetic transminase (SGOT), serum glutamate pyruvate transaminase (SGPT), serum alkaline phosphatase (SALP), and total bilirubin levels [7,8]. The results thus obtained were subjected to statistical analysis using *student t-test* and analysis of variance. The livers were dissected out immediately, washed with ice cold saline, and fixed in 10 % formalin immediately to avoid decomposition. Embedding in paraffin wax was carried out by removal of water using alcohol from 30-100% and then stained with hemotoxylin, which has an aqueous base. The sections were dehydrated using increasing concentration of alcohol and then stained with eosin. They were treated with diphenyl xylene (DPX) and examined under the microscope [9].

Statistical analysis

Experimental results were grouped according to the treatment, and the arithmetic average was calculated for each group from the values for each individual with that group. This average was expressed as the mean ± the standard error of the mean (SEM) for six determinations. Experimental data were analyzed statistically by one-way analysis of variance (ANOVA). Tukey's multiple comparison tests was used to determine significant differences between means. The p value corresponding to the test statistic value was reported to denote the degree of significance. PRISM Instat software was used for statistical analysis.

RESULTS AND DISCUSSION

The present studies were performed to assess the hepatoprotective activity in rats against CCl_4 as hepatotoxin to prove its claims in folklore practice against liver disorders. CCl_4 induced hepatic injury is commonly used as an experimental method for the study of hepatoprotective effects of medicinal plants extracts and drugs. The extent of hepatic damage is assessed by the level of various biochemical parameters in circulation and histological evaluation.

Table 1: Effect of ethyl acetate extract of *Ipomoea nil* seeds on CCl_4 induced hepatic injury (Serum parameters)

Groups	Treatment and dose (mg/ml)	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)	Serum bilirubin (mg/dl)
Group A	Control	74.25±1.416	187.61±1.847	399.43±0.974	1.546±0.194
Group B	CCl_4	333.08±1.65	554.99±2.278	654.99±1.338	2.576±0.149
Group C	CCl_4 + Silymarin	60.109±2.990***	196.65±1.331***	396.70±0.542***	0.922±0.061***
Group D	CCl_4 + Ext (200 mg)	217.09±2.580***	277.09±3.480***	477.09±1.420***	1.159±1.518***
Group E	CCl_4 + Ext (400 mg)	69.219±1.895***	177.43±1.366***	357.45±5.581***	0.586±0.070***

Values are the mean ± SEM of six animals each.

*P<0.05, **P<0.01 and ***P<0.001, when compared with control.

It is well known that carbon tetrachloride is converted by cytochrome P_{450} mixed function oxygenases in smooth endoplasmic reticulum of liver into toxic metabolite, mainly trichloromethyl radical (CCl_3). This free radical in the presence of oxygen may cause peroxidation of lipids on target cell resulting in extensive damage to liver

The results of biochemical parameters revealed to the alteration of enzyme levels in CCl_4 treated group indicating that CCl_4 induces damage to the liver. Table 1 show that CCl_4 causes a significant increase in SGOT level from control 74.25 ± 1.416 IU/L to 333.08 ± 1.650 IU/L after CCl_4 intoxication. Administration of extract at the dose level of 200 and 400 mg/kg in CCl_4 intoxicated rats caused reduction in SGOT level to 217.09 ± 2.580 IU/L and 69.219 ± 1.895 IU/L respectively (P<0.001).

Further Table 1 reveals that CCl_4 causes a significant increase in SGPT level from control 187.61±1.847 IU/L to 554.99±2.278 IU/L after intoxication. Administration of extract at the dose level of 200 and 400 mg/kg in CCl_4 intoxicated rats led to reduction of SGPT level to 277.09±3.48 IU/L and 177.43±1.366 IU/L respectively (P<0.001).

SALP level in the control group increased from 399.43 ± 0.974 IU/L to 654.99 ± 1.338 IU/L in CCl_4 intoxicated rat as shown in Table 1. Administration of extract at the dose level of 200 and 400 mg/kg in CCl_4 intoxicated rats led to lowering of the SALP level to 477.09 ±1.42 IU/L and 357.45 ± 5.58 IU/L respectively (P<0.001).

Destruction of hemoglobin yields bilirubin which is conjugated in the liver to diglucoroxide and excreted in the bile. Bilirubin accumulates in plasma when liver insufficiency exists or biliary obstruction is present or rate of hemolysis increases. The serum bilirubin level increased from 1.546 ± 0.194 mg/dL in the control group to 2.576 ± 0.149 mg/dL after CCl_4 intoxication as shown in Table 1. Administration of extract at the dose of 200 and 400 mg/kg in CCl_4 intoxicated rats reduced the serum bilirubin to 1.159 ± 1.518 mg/dL and 0.586 ± 0.69 mg/dL (P<0.001).

In this study, CCl_4 administration to rats leads to a marked elevation in the levels of serum enzymes like SGOT, SGPT, SALP and serum bilirubin level. This might be due to release of these enzymes from the cytoplasm, into the blood stream rapidly after rupture of the plasma membrane and cellular damage [10]. Treatments with extract at the dose of 400 mg/kg significantly reduced the levels of these marker enzymes in CCl_4 treated rats. This implies that the extract tends to prevent liver damage, suppresses the leakage of enzymes through cellular membranes, preserves the integrity of the plasma membranes and hence restores these enzymes levels. This is in agreement with the commonly accepted view that serum levels of transaminases return to normal with the healing of hepatic parenchyma and the regeneration of hepatocytes [11]. Effective control of SALP and bilirubin, points towards an early improvement in the secretary mechanism of the hepatic cells. Decrease in serum bilirubin after treatment with the extract in liver damage indicated the effectiveness of the extract in normal functional status of the liver. The protective effect exhibited by extract at dose level of 400 mg/kg was comparable with the standard drug Silymarin.

The histological examination of the liver sections reveals that the normal liver architecture was disturbed by hepatotoxin intoxication. In the liver sections of the rats treated with extracts and intoxicated with CCl_4, the normal cellular architecture was retained significantly when compared to silymarin, thereby confirming the protective effect of the extract. In accordance with these results, it may be hypothesized that tannin, saponins and flavonoids, which are present in extracts, could be considered responsible for the hepatoprotective activity.

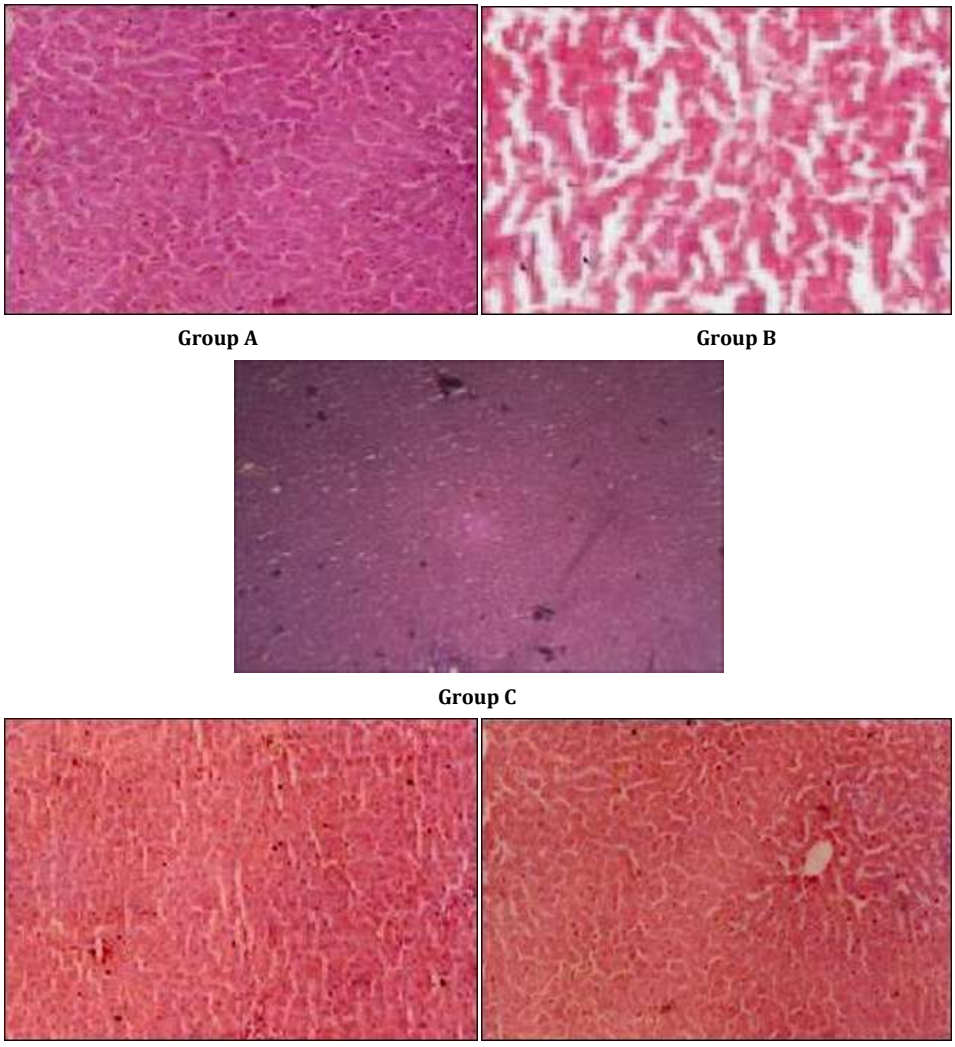

Fig. 1: Histopathological changes in liver of albino rats. Hematoxylin and Eosin (x100)

Group A: Normal structure of hepatic lobules is seen; white spots indicate the presence of vacuoles, a prominent blood vein with normal appearance.

Group B: Massive fatty changes are seen; necrosis, ballooning degeneration and broad infiltration of the lymphocytes are seen.

Group C: Drastic recovery of hepatic parenchyma, mild congestion and micro vesicular changes are seen.

Group D: Marked recovery of hepatic cells, mild congestion and micro vesicular changes are seen.

Group E: Drastic recovery of hepatic parenchyma, mild congestion and micro vesicular changes are seen.

CONCLUSION

Based on the results obtained, it may be concluded that the ethyl acetate extract of *Ipomoea nil* is non-toxic and is safe. As the results indicated, the extract possesses significant hepatoprotective activity. A study of effect of extract on immunological parameters, like TNF-alpha, interleukin, etc is required to be conducted. Also, a thorough study of clinical trials is required to be performed. After carrying out these studies, the plant may be considered as a low cost, potent, herbal medicine for liver disorders.

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