HEPATOPROTECTIVE ACTIVITY OF FRUIT EXTRACTS OF PHYLLANTHUS ACIDUS LINN. IN CARBON TETRACHLORIDE INDUCED HEPATOTOXIC MODEL IN ALBINO RATS

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ABSTRACT

BACKGROUND

Phyllanthus acidus Linn. (PA) plant is widely used for treating jaundice in rural areas of North Eastern States of India, but there is lack of scientific evidence regarding its usage in liver disorders, although experiments with shrubs and herbs belonging to same species have been performed.

AIMS

To study the effect of aqueous fruit extract of Phyllanthus acidus Linn. (PA) in carbon tetrachloride (CCl_4) induced hepatotoxicity in albino rats.

SETTING AND DESIGN

Twenty four healthy albino rats of either sex weighing 100-125gm were utilized for the study. They were randomized into 4 groups (Normal, Control, Standard and Test) of 6 animals in each group.

METHODS AND MATERIAL

Hepatic injury was induced in all groups (Except normal group) by i.p. injection of 1:1v/v CCl_4 in olive oil (1ml/kg body weight) for 7 days simultaneously with control, standard and test drugs.

STATISTICAL ANALYSIS

Results were analysed by ANOVA followed by student 't' test.

RESULTS AND CONCLUSION

The administration of CCl_4 to the animals resulted in a marked increase in hepatic markers. Animals treated with PA exhibited a significant decrease in the hepatic markers in comparison to the control group. The present study shows that the aqueous fruit extracts of Phyllanthus acidus Linn. Has significant hepatoprotective activity in CCl_4 induced hepatotoxic model in albino rats.

KEYWORDS

Hepatoprotective, Phyllanthus Acidus, Carbon Tetrachloride.

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INTRODUCTION

The liver is the key organ regulating homeostasis in the body, which is involved in almost all the biochemical pathways related to growth and fight against disease.[1] In spite of tremendous scientific advancement in the field of hepatology in recent years, liver problems are on the rise. Jaundice and hepatitis are two major hepatic disorders that account for a high death rate.[2,3]

Phyllanthus acidus Linn. (PA) belongs to the family: Phyllanthaceae (Division: Magnoliophyta, Class: Magnoliophyta, Order: Malpighiales). They are native of South America; cultivated widely in tropical regions. The plant is intermediary between shrubs and tree, reaching 2 to 9 meters (6½ to 30 feet) in height and profusely branching.

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The branches bear alternate leaves that are ovate with pointed ends. The leaves are 2-7.5cm long and thin. They are green and smooth on the upper side and blue-green on the underside. The fruit makes a delicious preserve; green leaves are used as a vegetable. The bark is used for tanning.[4] The plant is widely used for treating jaundice in rural areas of North Eastern States of India, but there is lack of scientific evidence regarding its usage in liver disorders, although experiments with shrubs and herbs belonging to same species have been performed. Hence, the present study was aimed to investigate the hepatoprotective activity of fruit extract of P. acidus in CCl_4 induced hepatotoxic model in albino rats.

MATERIALS AND METHODS

Plant Materials and Extract

The fruits of P. acidus were collected during the month of June of 2014 from the localities of Agartala, Tripura and were authenticated by Dr. R. K. Sinha, Department of Botany, Tripura University. The material was air dried under shade and powdered mechanically. About 350gm of powdered material was boiled with 1000ml of distilled water for 30min and filtered through Whattman no.1 filter paper to obtain PA.

The extract was concentrated and dried using water bath.[5] The yield was 0.5%. All the experiments are done in the Department of Pharmacology, Tripura Medical College and Dr. B.R.A.M. Teaching Hospital, Agartala.

Animal

Albino rats of either sex weighing 100-125gm were obtained from Animal House, Tripura Medical College and Dr. B.R.A.M. Teaching Hospital, Hapania, Agartala. They were maintained at standard laboratory conditions and fed with standard rat diet and water ad-libitum during the period of experiment. They were habituated to laboratory conditions for 24 hours prior to the experimental procedure to minimize any stress.

Induction of Hepatotoxicity

Hepatic injury was induced by intra-peritoneal injection of 1:1v/v CCl_4 in olive oil at a dose of 1ml/kg/day for 7 days.[6]

EVALUATION OF HEPATOPROTECTIVE ACTIVITY

Twenty four healthy albino rats of either sex were randomized into 4 groups of 6 animals in each group. They are as follows:

I. **GROUP- A (NORMAL)**
Normal saline-1ml/kg/day intra-peritoneally for 7 days.

II. **GROUP- B (CONTROL)**
CCl_4 and normal saline- 5ml/kg/day orally for 7 days.

III. **GROUP- C (STANDARD)**
CCl_4 and Silymarin 100mg/kg/day orally for 7 days.

IV. **GROUP- D (TEST)**
CCl_4 and PA 500mg/kg/day orally for 7 days.

Control, Standard and Test drugs were administered orally with the help of stomach tube once daily for 7 days. Silymarin was chosen as standard and used at a dose of 100mg/kg/day.[6]

No adverse effect or mortality was detected in the albino rats with oral PA (2gm/kg) observed for 24 hours during preliminary toxicity test. The dose of the standard drug was calculated from human dose by extrapolation based on surface area.[7]

ASSESSMENT OF HEPATOPROTECTIVE ACTIVITY

After 7 days, blood samples from each rat were collected from orbital sinus with the help of capillary tube.[8] Serum was separated by centrifugation. The serum was used for the assay of hepatic markers viz., Aspartate transaminase (SGOT), Alanine transaminase (SGPT), alkaline phosphatase (ALP) and serum bilirubin by kinetic methods in a semi-autoanalyser.[9]

Ethics

The study was conducted with the approval of Institutional Animal Ethical Committee.

Statistical Analysis

Results were analysed by ANOVA followed by student 't' test.

RESULT

The administration of CCl_4 to the animals resulted in a marked increase in total bilirubin, serum SGOT, SGPT and ALP levels in control group indicating liver injury caused by CCl_4. This finding is in accordance with that of another study.[5] Animals treated with PA showed a significant lower levels in the hepatic markers in comparison to the control group ($p<0.001$). The prevention of raise of hepatic markers by the standard drug was more significant in comparison to control ($p<0.001$). PA was as effective as standard in preventing the increase of the hepatic markers ($p>0.5$). (Results are shown in Table-1 and Figure-1).

GROUP	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)	Total Biluribin (IU/L)
Normal(NS)	148.5±6.090	98±7.183	168±6.928	0.42±0.0424
Control (CCl_4)	544±10.373	188±12.987	684.5±7.661	2.51±0.2024
Standard (CCl_4)+ silymarin)	194*±7.823	128*±8.124	178*±8.342	0.58*±0.0834
Test (CCl_4 + PA).	197*#±8.694	104*^±8.438	208*^±9.338	0.82*^±0.0933

Table 1: Effect of Phyllanthus acidus Linn. on hepatic markers in CCl_4 induced hepatotoxicity in albino rats

One way F 2868.638 29.023 1009 382.7736
ANOVA df 3, 20 3, 20 3, 20 3, 20
P value <0.01 <0.01 <0.01 <0.01
Values are expressed as mean ± standard deviation.
* p<0.001 as compared to control value.
# p>0.5 as compared to standard value.
^ p<0.001 as compared to control value.

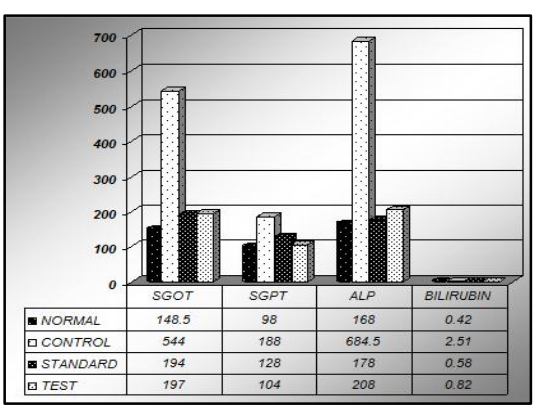

Fig. 1: Effect of Phyllanthus acidus Linn. On hepatic markers in CCl_4 induced hepatotoxicity in albino rats

DISCUSSION

The liver can be injured by many chemicals and drugs. CCl_4 is widely used as hepatotoxin in experimental studies. The CCl_4 is biotransformed by cytochrome p450 to produce the trichloromethyl free radicals, which in turn covalently binds to cell membranes and organelles to elicit lipid peroxidation. This causes loss of integrity of cell membranes and damage of hepatic tissue.[10] During hepatic damage, cellular enzymes like SGOT, SGPT, ALP present in the liver cells leak into the serum resulting in increased concentrations.[11] In the present study CCl_4 treated animals there is significant increase in SGOT, SGPT, ALP and bilirubin level in comparison to normal group. PA significantly prevents the increase of these hepatic markers due to CCl_4. The prevention of the increase in hepatic markers due to CCl_4 by PA is comparable to that of standard drug silymarin. The component(s) of the extract responsible for this effect however was not investigated. Further investigations are needed for identification of the active compounds of PA responsible for hepatoprotective activity.

CONCLUSION

The present study shows that the aqueous fruit extract of Phyllanthus acidus Linn. Has significant hepatoprotective activity in carbon tetrachloride induced hepatotoxicity in albino rats.

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