

HEPATOPROTECTIVE EFFECT OF ACETONE EXTRACT OF *LUFFA ECHINATA* ROOT AGAINST CARBON TETRACHLORIDE INDUCED LIVER INJURY IN RATS

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Summary

Chronic liver diseases are leading cause of death in many countries. Herbal drugs are considered and are of particular value in the treatment of chronic diseases requiring prolonged therapy. Keeping in view these points an attempt has been done to evaluate the possible consequence of acetone extract of root of *Luffa echinata* Roxb on the induced hepatotoxicity in the albino rats. Root of *Luffa echinata* Roxb was extracted with acetone and extract was subjected to preliminary phytochemical screening for the presence of different groups of compound. The hepatotoxicity was induced by Carbon tetrachloride (CCl₄) in the albino rats and then animals were treated with acetone extract. Level of tribute was assessed by employing biochemical parameters such as total bilirubin, direct bilirubin, Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and total protein in serum. The histopathological study was also done to evaluate the effect of root extract on the liver tissues. The results showed that acetone extract possess significant hepatoprotective activity.

Keywords: Hepatotoxicity; Carbon tetrachloride; *Luffa echinata*; Sylimarin.

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Introduction

The World Health Organization (WHO) estimates that about 80% of the populations living in the developing countries rely almost exclusively on traditional medicines for their primary health care needs. In almost all the traditional medicines, the medicinal plants play a major role and constitute the backbone of traditional systems [1]. Liver disease is a leading cause of death in many countries. While the principal causative factor in Europe and America is increasing alcohol consumption, malnutrition, anaemia, hepatotoxic drugs and infection are the most frequent causes for its incidence in Asia. The treatment requires prolonged therapy and if very strong drugs are given for prolonged periods extending several months, these may overburden the diseased liver and produce harmful effects. Paradoxically some of the modern drugs, which are given to treat liver diseases, may themselves cause liver damage [2].

Luffa echinata Roxb (Family cucurbitaceae), a climbing shrub, commonly known as Bindal, is widely distributed in Bihar, Uttar Pradesh, Bengal and Gujarat, Tropical Africa and Burma. It is commonly used in the indigenous system of medicine. The fruit is intensely bitter and fibrous. It is regarded as purgative and is also used in dropsy, nephritis, chronic bronchitis and lung complaints. An infusion of the fruit is given in biliary and intestinal colic. It is applied to the body in fever and jaundice [3].

Toxic necrosis leads to changes in the blood and to clinical features similar to those of acute viral hepatitis. Toxic steatosis leads to quantitatively similar but quantitatively more modest abnormalities [4-5]. CCl₄, paracetamol, halothane, methyldopa etc. induced hepatocellular necrosis produces in blood high levels of enzymes released from the damaged liver [6-8]. AST is a mitochondrial enzyme present in large quantities in heart, liver, skeletal muscle and kidney. ALT is a cytosolic enzyme present in liver. Transaminase determinations are useful in the early diagnosis of viral hepatitis. A high ratio of AST to ALT (Greater than two) may be useful in diagnosing alcoholic hepatitis and cirrhosis [9-10].

In the present study we evaluate the hepatoprotective action of *luffa echinata* on the CCl₄ induced hepatotoxicity in the albino rats by estimating the altered level of different hepatic biochemical markers and by histopathology of the liver.

Materials

The root of *luffa echinata* Roxb were collected from different locations around Ranchi Forest Division in the month of July and identified by Dr. Kaushal Kumar of State Medicinal Plants Board, Ranchi, Jharkhand India. All the drugs and chemicals used in the study were of analytical grade. Carbon tetrachloride was obtained from Merck Limited, India. Silymarin was obtained from Ranbaxy Laboratories Limited, India. Kits used for the estimation of AST and ALT levels were purchased from Centronic GmbH, Germany.

Methods

Extraction

About 1 Kg of root was coarsely ground in a mortar. Thereafter, it was gradually, but continuously, sun-dried for one day. The dried material was further crushed into fine powder using mortar and pestle. Finally powdered material was then extracted with acetone in a soxhlet apparatus. Subsequently, it was filtered using Whatman filter paper No. 1. For determining the yield from the extraction procedure, the residue was then dried to a constant weight under vacuum. The pH of this filtrate was determined with a SE962- P, Simatronics pH meter and after which it was stored in a stoppered brown bottle for subsequent evaluation.

Phytochemical Screening

Acetone extract from root of *luffa echinata* was screened for alkaloids, sterols, triterpenes, reducing sugar, tannins, glycosides, flavones and saponin according to the reported methods.

Animals and Experimental Protocol

Female Wistar albino rats, 4–6 weeks, 180–230 g, were divided into 6 groups of 8 animals each. They were housed in an air-conditioned room at 23 ± 3 °C, $55 \pm 5\%$ humidity, 12-h light and were supplied with standard diet and water *ad libitum*, one week before and during the experimental period.

All drugs were injected into animal's *i.p.* once daily. Group 1 served as a control group, receiving normal saline only (10 mL kg^{-1}) for 7 days; group 2 received CCl_4 (3 mL kg^{-1} 50% in olive oil) on the 7th day; group 3 received sylimarin (25 mg kg^{-1} in 50% ethanol) during 7 days, group 4 received sylimarin (25 mg kg^{-1} in 50% ethanol) during 7 days and CCl_4 on the 7th day in the above mentioned dose; group 5 received acetone extract of *luffa echinata* (200 mg kg^{-1}) once daily for 7 days and CCl_4 on the 7th day (3 mL kg^{-1} 50% in olive oil); group 6 received acetone extract of *luffa echinata* (300 mg kg^{-1}) once daily for 7 days and CCl_4 on the 7th day in above mentioned doses.

On the 8th day, *i.e.*, 24 hours after CCl_4 administration, the animals were sacrificed. Before sacrificing, about 3-5ml of blood was collected in a sterile centrifuge tube and left undisturbed at 37 °C, till the formation of clot, for one hour. The clot was dislodged using a sterile loop and refrigerated at 2-8°C for 3-4 h. During this period serum exuded and the clot retracted. The serum was aspirated using a sterile pipette after centrifugation at 3000 rpm for 15min. Serum collected was analyzed for enzyme levels immediately or within 24 h after storing at 0-4 °C. While liver tissues were processed for homogenate preparation and histopatological examination. All procedures described were reviewed and approved by the Institutional Animal Ethical Committee.

Determination of serum bilirubin

This was performed based on the modified method [11]. 0.4 ml of serum and 3.6 ml of water were added to each of two test tubes labeled A and B. 1.0 ml of diazo reagent was added to the tube A and 1.0 ml of blank solution (15 ml of Conc. H_2SO_4 in 1 L H_2O) was added to tube B. Both tubes were mixed immediately. After five min, the absorbance of A was measured spectrophotometrically at 540 nm using B as blank. This absorbance was termed E1. Absolute methanol (5.0 ml) was then added to each tube and mixed. After a further 30 min, the absorbance of A was read using B as a blank. This was termed E2 (for total bilirubin). The absorbance of a standard bilirubin solution ($0.016 \text{ mg}/100 \text{ ml}$) was read at 540 nm using water as the blank.

This was termed Es. Total serum bilirubin (expressed as mg/100 ml) was calculated as follows: $(E2 - A_{blank}) \times 0.016 \times 100 / 0.4$ Es. Similar procedure was applied for determination of direct bilirubin except addition of methanol. Distilled water was added finally in place of methanol in the both tubes.

Determination of total serum proteins

5.0 ml of Biuret reagent was added to 0.1 ml of serum. This was mixed and warmed at 37 °C for 10 min followed by cooling. Similarly, 0.1 ml of the protein standard (BSA) was added to 5.0 ml of Biuret reagent. Absorbance of both the standard and test mixtures was taken at 540 nm against a blank containing 5.0 ml Biuret reagent in 0.1 ml of water. Total protein content of the serum was determined using the formula: $\text{Absorbance of Test} \times \text{Concentration of Standard} / \text{Absorbance of Standard}$.

Determination of AST and ALT

The AST and ALT levels in the serum were estimated using commercially available kits.

Histopatological Examination

Animals were sacrificed on the day of blood sampling; the liver was removed, sliced and washed in saline. Liver pieces were preserved in 10% formaldehyde solution for the histopatological study. The liver pieces were processed and embedded in paraffin wax. Slices, 4–6 mm thick, were stained with hematoxylin and eosin and photographed.

Statistical Analysis

Results of biochemical parameters are reported as Mean \pm SD. Total variation ($P < 0.05$) present in a set of data was estimated by One Way Analysis (ANOVA). The F-ratio was also calculated. Calculations were performed in GraphPad prism v5 software (GraphPad prism software Inc, San Diego, CA).

Result and discussion

The preliminary phytochemical screening of *luffa echinata* acetone extract indicated the presence of sterols, triterpenes, reducing sugar, glycosides, flavone and tannins. The final pH of the extract was 6.71 and the extraction yield was 93%.

The levels of serum AST, ALT, bilirubin and total protein in the normal and treated groups of rats are shown in Tables 1. The levels of AST, ALT and bilirubin increased significantly in the CCl₄ treated rats, while the content of protein decreased significantly when compared to the control. Treatment with different doses of *luffa echinata* acetone extract was found to reverse this effect, i.e., to reduce the activities of AST, ALT and bilirubin and increase the protein content. Especially, 300 mg/kg of *luffa echinata* acetone extract was very effective, as the activities of those enzymes returned to normal.

Table: 1 Influence of *Luffa echinata* (root) extract on biochemical parameters of the rats intoxicated with CCl₄

Group	Biochemical parameters				
	ALT (U/ml)	AST (U/ml)	Total bilirubin (mg/100ml)	Direct bilirubin (mg/100ml)	Total protein (g/100ml)
1.	34 ± 2.38*	46.23 ± 4.28*	0.68 ± 0.30*	0.20 ± 0.05*	7.42 ± 0.36*
2.	264.2 ± 11.01	268.39 ± 9.70	3.36 ± 1.50	2.28 ± 0.19	2.16 ± 0.25
3	33 ± 1.26*	44.45 ± 2.67*	0.65 ± 0.56*	0.19 ± 0.18*	7.98 ± 0.76*
4.	42.4 ± 1.43*	53.06 ± 3.94*	1.03 ± 0.46*	0.37 ± 0.02*	6.82 ± 0.48*
5.	105.42 ± 2.23*	93.91 ± 4.25*	1.61 ± 0.72*	0.97 ± 0.11*	4.87 ± 0.36*
6.	80.25 ± 2.79*	73.84 ± 2.54*	1.28 ± 0.57*	0.7 ± 0.13*	5.77 ± 0.35*

* P < 0.05 vs respective toxic control group

The extent of CCl₄ induced hepatotoxic effect was assessed by the levels of released cytoplasmic enzymes such as AST and ALT in circulation. Bilirubin increased in the blood because of regurgitation of bile due to obstruction within the liver by the damage or inflammation [12]. The observation that the levels of bilirubin was brought down to their normal levels in the CCl₄-treated rats indicates that the liver is restored to its normal activity by the hepatoprotective action of acetone extract. Effective control of bilirubin content due to extract administration points towards an early improvement in the secretory mechanisms of the hepatic cell. Elevated level of AST is known to indicate a liver damage due to viral hepatitis, cardiac infarction and muscle injury. ALT, a better bio-indicator of liver injury than AST, catalyses the conversion of alanine to pyruvate and glutamate, and is released in a similar manner. Bilirubin levels conversely are related to the function of the hepatic cells.

Hypoproteinaemia is the deficiency of protein in the plasma, partly due to dietary insufficiency or excessive excretion. The extract of *luffa echinata* has displayed such effect, as indicated by the significant decrease (p < 0.05) in the total serum proteins of all the treatment groups when compared to the control group. Decrease in total plasma proteins is the major sign of massive hepatic necrosis, chronic cirrhosis and other disorders with significant destruction or replacement of liver cells. Inadequate amino acid supply as in protein caloric malnutrition or malabsorption can also lead to hypoproteinaemia. The observed decrease in the total serum proteins might have resulted from the first-pass febrile shock experienced by the experimental animals following administration of the extract [13-15].

This protective effect of *luffa echinata* acetone extract was confirmed by histological examination (Figure. 1). Hystopathological study of the control group liver (Figure. 1a) showed a normal hepatic architecture. Massive fatty changes, gross necrosis, broad infiltration of lymphocytes and of Kupffer cells around the central vein and loss of cellular boundary (Figure. 1b) were observed in the livers of CCl₄-treated rats. In the groups pre-treated with sylimarin or *luffa echinata* acetone extract (Figure. 1c and 1d), the livers exhibited an almost normal architecture, with the presence of double nucleus hepatocytes, except for a slight deformity of hepatocytes with pyknosis and clearing of cytoplasm.

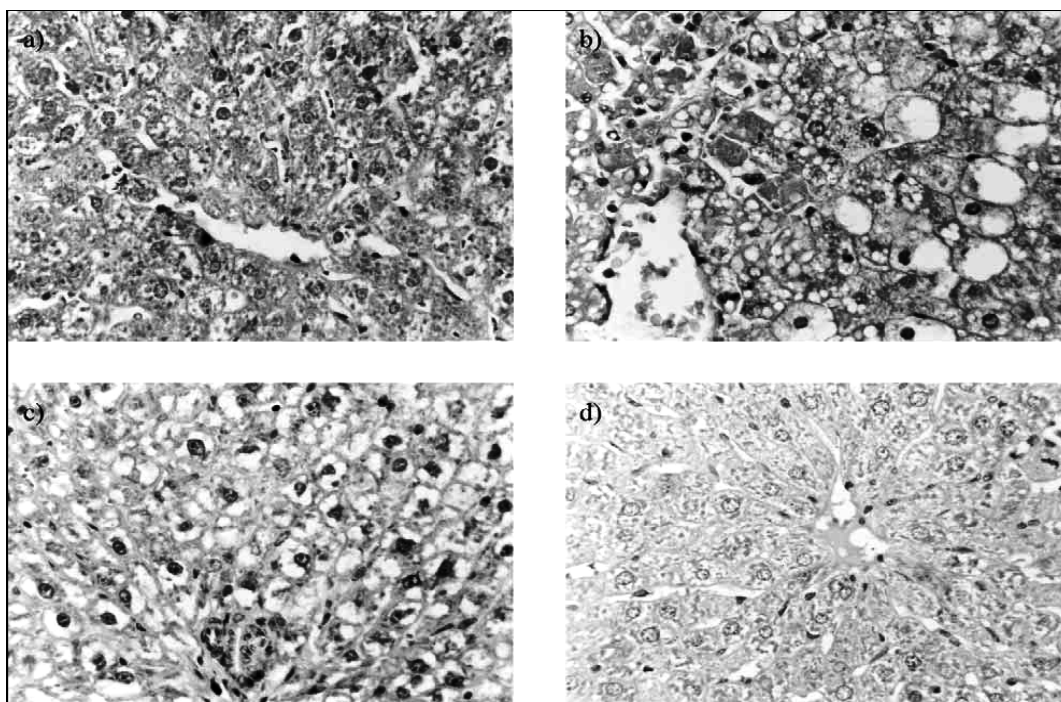


Figure 1. Photomicrographs of rat liver slices. Groups of rats: a) group 1, control; b) group 2, CCl₄-olive oil (3 mL kg⁻¹ on the 7th day); c) group 4, sylimarin (25 mg kg⁻¹ for 7 days, CCl₄, as above on the 7th day); d) group 6, *luffa echinata* root extract (300 mg kg⁻¹ for 7 days, CCl₄, as above on the 7th day).

Our study shows the increased activities of AST, ALT and bilirubin in the rats treated with CCl₄ are due to extensive liver damage. CCl₄ inflicts wide-ranging effects on liver metabolism, including adverse reaction on DNA, RNA, protein synthesis, necrosis of hepatocytes and cirrhosis. CCl₄ induced liver injury is initiated by formation of a reactive metabolite, trichloromethyl radical CCl₃ by microsomal mixed function oxidase system (MFOS). This biotransformation is catalysed by a cytochrome P- 450 dependent monooxygenase. The activated CCl₃ radicals binds covalently to the macromolecules and induces peroxidative degeneration of the biomembranes is one of the principle causes of CCl₄ induced hepatotoxicity. Treatments with *luffa echinata* acetone extract on the other hand, decreased the serum AST, ALT and bilirubin and restored them to nearly normal condition, indicating the stabilization of plasma membrane as well as the repairing of the hepatic tissue damaged by CCl₄. The above positive changes can be considered as an expression of the functional improvement of the hepatocytes, resulted from an acceleration of cell regeneration.

Conclusion

Hypoproteinaemic effects and improved serum biochemical parameters such as AST, ALT and bilirubin were indications that the crude extract of *luffa echinata* root extract has a significant effect in liver injuries. Further research is necessary to determine the possible phytoconstituents responsible for hepatoprotective activity and their pharmacological mechanism of action.

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