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Research Article

HEPATOPROTECTIVE ACTIVITY OF *ALSTONIA SCHOLARIS* FRUITS AGAINST CARBON TETRACHLORIDE-INDUCED HEPATOTOXICITY IN RATS

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ABSTRACT

The hepatoprotective activity of ethanolic extract of fruits of *Alstonia scholaris* was evaluated by carbon tetrachloride (CCL4)-induced hepatotoxicity in rats. The toxicant CCL4 was used to induce hepatotoxicity at a dose of 2 ml/kg as 1:1 mixture with olive oil. The ethanolic extract of fruits of *Alstonia scholaris* was administered in the dose of 150 and 300 mg/kg/day orally for 5 days. Silymarin (50 mg/kg) was used as standard drug. The hepatoprotective effect of the ethanolic extract was evaluated by the assessment of biochemical parameters such as SGOT, SGPT, SALKP, total bilirubin and histopathological studies of the liver. Treatment of animals with the ethanolic extract significantly reduced the liver damage and the symptoms of liver injury by restoration of architecture of liver as indicated by lower levels of serum bilirubin as compared with the normal and silymarin treated groups.

Keywords: Carbon tetrachloride, Hepatoprotective activity, *Alstonia scholaris*, Silymarin.

INTRODUCTION

The liver is an organ of paramount importance. Due to its unique and considerable regenerative capacity, even a moderate cell injury is not reflected by measurable change in its metabolic functions. However, some of its functions are so sensitive that abnormalities start appearing depending upon the nature and the degree of initial damage. The etiology of the liver disorders depends on various factors such as nutritional, biochemical, bacteriological, viral or environmental aberration. The liver plays a significant role not only in the metabolism and disposition of the chemicals to which it is exposed directly or indirectly, but also in the metabolism of fats, carbohydrates, proteins and immunomodulation. A slight alteration in hepatic structure and function may result in portal hypertension, ascites, jaundice and increased bleeding causing multiple metabolic changes affecting other organs as well. The magnitude of derangement of liver by disease or hepatotoxins is generally measured by level of glutamate pyruvate transaminase, glutamate oxaloacetate transaminase, alkaline phosphatase, bilirubin, albumin and whole liver homogenate.

Herbal drugs are playing an important role in health care programs worldwide, and there is a resurgence of interest in herbal medicines for treatment of various ailments including hepatopathy. India, the abode of ayurvedic system of medicine, assigns much importance to the pharmacological aspects of many plants. Hepatoprotective effect of some plants like *Spirulina maxima, Eclipta alba, Boehmeria nivea, Cichorium intybus*, and *Picrorhiza kurroa* has been well established. Nearly 150 phytoconstituents from 101 plants have been claimed to possess liver protecting activity. At the same time, surprisingly, we do not have satisfactory plant drugs/formulations to treat severe liver diseases. Most of the studies on hepatoprotective plants are carried out using chemically induced liver damage in rodents as models. Many folk remedies from plant origin have long been used for the treatment of liver disorder[1-4].

The plant *Alstonia scholaris* is found to have anti-inflammatory, analgesic[5], anti-arthritic, antioxidant[6], antibacterial[7], anti-tussive, anti-asthmatic, expectorant[8], antidiarrhoeal, spasmolytic[9], antidiabetic, antihyperlipidemic[10], broncho-vasodilatory[11] and antineoplastic activity[12]. The literature survey on *Alstonia scholaris* indicates no pharmacological work on this plant is

reported till date, keeping in view the hepatoprotective activity of *Alstonia* species we are interested in screening the possible activity of *Alstonia scholaris* fruit extract. Hence this work is studied for repairing and healing of adversely affected liver cells.

MATERIALS AND METHODS

Chemicals

All the chemicals used were of analytical grade. Carbon tetrachloride (E. Merck), silymarin (Sigma Chemical Co.,) and liquid paraffin were purchased from the local supplier. Diagnostic kits for the estimation of SGOT, SGPT, SALKP and total bilirubin were purchased from local supplier manufactured by Ranbaxy Diagnostics Ltd., New Delhi, India. Water represents the double distilled water, standard orogastric cannula was used for oral drug administration.

Plant Collection and Extraction

Alstonia scholaris fruit was collected from the Aditya Campus situated in Aditya Nagar, Surampalem, East Godavari District, Andhra Pradesh (India). The fruits of the plant *Alstonia scholaris* were removed and dried under shade and powdered in a mechanical grinder. The powder was extracted with ethanol and the ethanolic extract was used for pharmacological work. All chemicals and reagents used for the study of pharmacological activities were of analytical grade. The extraction was done for 72 hours. After extraction the extract was separated from marc by filtration through filter paper. The marc was pressed with muslin cloth to remove the solvent which is left in the marc after filtration. The filtrate was preserved in a well closed container. The marc left after extraction was further extracted by cold maceration with the same solvent. And the process was repeated one more time and the drug was extracted with a gap of 3 days.

Animal Studies

Albino rats of either sex weighing between 125-160 gm were obtained from National Institute of Nutrition, Hyderabad, Andhra Pradesh, India. The animals were housed under standard environmental conditions (temperature of 22 ± 1°C with an alteration 12 h light – dark cycle and relative humidity of 60 ±5%), one week before the start and also during the experiment as per the rules and regulations of the Institutional Animal Ethics Committee. They were fed with standard laboratory diet supplied by National Institute of Nutrition, Hyderabad, Andhra Pradesh, India and water *ad libitum.* Food was withdrawn 12 h before the experiment and water was allowed *ad libitum.*

The animals were divided into 5 groups (Six per group) Group I served as normal control received distilled water (1ml /kg, p.o.) daily for 5 days and receive olive oil (1ml/kg, S.C) on days 2 and 3. Group II served as CCl_4 control group animals were administered a single daily dose of 0.5% tween-80 (1ml) p.o., on all 5 days and on the second and third day they were administered SC CCl_4: olive oil (1:1). Group III animals were administered *Alstonia scholaris* fruit extract (150mg/kg) p.o. on all 5 days and a single dose of CCl_4 (2ml/kg) SC on days 2 and 3, 30 minutes after *Alstonia scholaris* fruit extract administration. Group IV animals were administered *Alstonia scholaris* fruit extract (300mg/kg) p.o. on all 5 days and a single dose CCl_4 (2ml/kg) SC on days 2 and 3, 30 minutes after *Alstonia scholaris* fruit extract administration. Group V animals were administered with silymarine (50mg/kg) p.o. the known hepatoprotective compound on all 5 days. After the course of treatment all the animals were sacrificed under ether anaesthesia. Blood samples were collected for evaluating the biochemical parameters and liver tissue samples were collected for histopathological studies[13-17].

Histopathological Studies

After draining the blood, liver samples were excised, washed with normal saline and processed separately for histological observations. 7-mm thick paraffin section of buffered formalin-fixed liver sample was stained with Haematoxylin-Eosin for photo microscopic observation of the liver histological architecture of the control and treated rats.

Statistical Analysis

All the results were expressed as mean ±SEM. One-way Analysis of Variance (ANOVA) was used for the statistical analysis of data. Dunnett's multiple comparision test[18] was used for determining the significance. A probability value of $P<0.05$ was considered as significant.

RESULTS

The administration of CCl_4 to the rats resulted in significant increase in serum enzymes like glutamate oxaloacetate transaminase, glutamate pyruvate transaminase, alkaline phosphatase compared to normal control rats. Treatment with *Alstonia scholaris* fruit extract caused significant reduction of these values and are expressed in [Table 1] dose dependently almost comparable to silymarin treated group. The toxic effect of CCl_4 was controlled in the animals treated with the extracts by way of

restoration of the levels of the liver function biochemistry similar to that of the standard drug silymarin. Among the extract-treated groups, significant hepatoprotective activity was observed.

The extract at dose of 150 and 300 mg/kg produced marked decrease in serum levels of SGOT, SGPT and total bilirubin and the levels are comparable with standard drug silymarin 50mg/kg. It was found that at dose of 300mg/kg could reverse CCl_4 induced hepatotoxicity. The histopathological changes induced by CCl_4 treatment as evidenced by centrilobular necrosis and bridging hepatic necrosis and its protection to normalcy by the treatment with the ethanolic extract of *Alstonia scholaris* fruit was indicative of the hepatoprotective action of the extract [Figures A -E].

In the histopathological studies, the liver sections of rats treated with vehicle showed normal hepatic architecture [Figure A], whereas that of CCl_4-treated group showed total loss of hepatic architecture with intense peripheral central vein necrosis, fatty changes, congestion of sinusoids, Kupffer cell hyperplasia, crowding of the central vein and apoptosis [Figure B]. In case of rats treated with *Alstonia scholaris* ethanolic extract 300 mg/kg [Figure C] and 150 mg/kg [Figure D] and with silymarin [Figure E] respectively, a normal hepatic architecture was seen with only moderate accumulation of fatty lobules and mild degree of cell necrosis, clearly indicating the protection offered by standard drug silymarin and the plant extract.

DISCUSSION

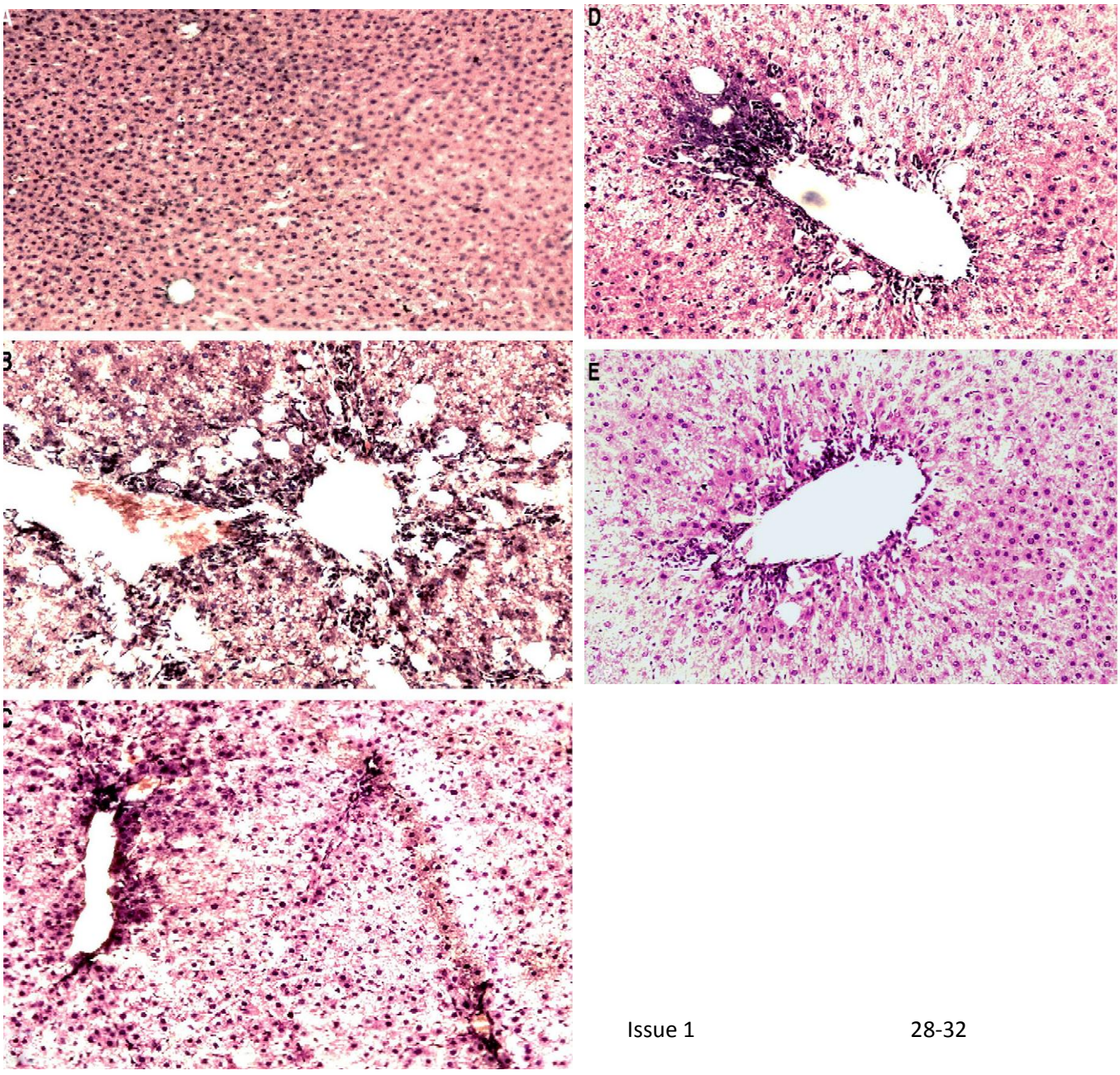
CCl_4 has been one of the most intensively studied hepatotoxicants to date. It consistently produces liver injury in many species. Acute poisoning with CCl_4 becomes manifested as a multisystem disorder involving the liver, kidney, brain, lungs, adrenal gland and myocardium. CCl_4 is a potent hepatotoxin and a single exposure can rapidly lead to severe centrizonal necrosis. Protection of hepatic damage induced by CCl_4 administration was observed by evaluating SGOT, SGPT and SALKP levels in treated, CCl_4 control and normal control rats. Since these enzymes are cytoplasmic in nature, upon liver injury these enzymes enter into the circulatory system due to altered permeability of membrane. Marked increase of SGOT, SGPT and SALKP indicate a severe damage to tissue membranes during CCl_4 induced liver damage [Table 1] similarly serum levels of SGOT, SGPT and total bilirubin were significantly increased in CCl_4 treated group rats**.** Administration of ethanolic extract of *Alstonia scholaris* fruits significantly prevented CCl_4 induced elevation of SGOT, SGPT and SALKP indicates the hepatoprotective activity of the herb.

Table 1: Effect of ethanolic extract of *Alstonia scholaris* fruits on CCl_4-induced hepatotoxicity in rats

Groups	SGOT (U/L)	SGPT (U/L)	SALKP (U/L)	TB (mg/dl)
I (Vehicle control)	165.83±1.31*	92.65±.23*	48.4±1.93	0.69±0.03
II (CCl_4 control)	283.21±2.45	249.72±2.83	98.8±2.20	1.83±0.02
III EE 150mg/kg	236.5±3.20***	207.16±1.83***	55.4±2.30	1.22±0.03
IV EE 300mg/kg	209.16±2.83**	168.34±2.20	34.1±1.96	0.90±0.02
V (Silymarin + CCl_4)	179.55±1.84***	115.77±1.83**	36.4±1.38	0.73±0.01***

Values are mean ± SEM for 6 animals; P: ≤ 0.001 compared to control group I; P<0.05; **<0.01 and ***<0.001 compared to respective control CCl_4 group; EE- Ethanolic extract; SGOT- serum glutamic oxaloacetic transaminase; SGPT- serum glutamate pyruvate transaminase; SALKP- serum alkaline phosphatase; TB- total bilirubin

Figures: (A – E) Effect of *Alstonia scholaris* extract on histopathological changes that occurred in rats during carbon tetrachloride intoxication (haematoxylin and eosin×200). (A) Normal control; (B) CCl4 control; (C) *Alstonia scholaris* ethanolic extract (300 mg/kg); (D) *Alstonia scholaris* ethanolic extract (150 mg/kg); (E) silymarin (50 mg/kg).



CONCLUSION

In the present study, it is proved that the ethanolic extract of *Alstonia scholaris* fruits was found to have significant hepatoprotective acivities, it showed dose dependent effects. Treatment of animals with the ethanolic extract significantly reduced the liver damage and the symptoms of liver injury by restoration of architecture of liver as indicated by lower levels of serum bilirubin as compared with the normal and silymarin treated groups. Histology of the liver sections confirmed that the ethanolic extract prevented hepatic damage induced by CCl_4 showing the presence of normal hepatic cords, absence of necrosis and fatty infiltration.

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