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Hepatoprotective Activity of Methanolic Extract of Stem Bark of *Alstonia Scholaris (*l.) R.br.

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ABSTRACT

The methanolic extract of *Alstonia scholaris* (L) R.Br. stem bark was screened for hepatoprotective activity against Swiss albino rats with liver damage induced by carbon tetrachloride. The results of hepatoprotective activity revealed that the methanolic extract of *Alstonia scholaris* significantly decreased the biochemical parameters (SGOT, SGPT, ALP, TP and TB). Silymarin (25 mg/kg), a known hepatoprotective drug, was used for comparison. The extract did not show any mortality up to a dose of 2000 mg/kg body weight. The findings indicated that the methanolic stem bark extract of *Alstonia scholaris* (L.) R.Br. (200 mg/kg) was effective in bringing the functional improvement of hepatocytes. The hepatoprotective activity was also supported by histopathological studies of liver tissues.

Key words: *Alstonia scholaris,* extract, hepatoprotective, carbon tetrachloride.

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INTRODUCTION

Liver, the most versatile but complex internal organ of human body, plays a vital role in metabolic activities. Its importance also lies in its impetus in management of internal environment and biochemical conversion of endogenous and exogenous chemical to harmless and excretable compounds. Therefore being a vital organ, its protection has a special status in therapeutics. Prolonged drug therapy, excessive use of the some of the commonly used medicines like paracetamol, diclofenac etc., alcoholism, exposure to certain xenobiotic, pollutants and certain disease state affect liver functioning to a varying degree. Despite of the excellent regeneration capacity of this organ, a slight injury or toxicity may lead to fatal complications. Therefore damage to the liver inflicted by hepatotoxic agents is of grave consequences. Unavailability of rational therapy in modern medicine and no or very less positive influence of synthetic drugs in liver damage have urged researchers in this field to look forward to herbal drugs with better hepatoprotective action[1].

Traditional medicines are effective in certain disease of liver and are based on their age-old use in folklore system of medicine. Natural products of plant origin with hepatoprotective and antioxidants properties play an important role in treatment of liver toxicity[1, 2].

Alstonia scholaris is a plant from the family apocynaseae, is extensively cultivated in most regions of the world and common avenue tree. The bark is bitter, astringent, acrid, thermogenic, digestive & laxative[3]. Literature survey revealed that the plant is useful in malaria, tumors, abdominal disorders, diarrhoea, leprosy, skin diseases, asthma, bronchitis, cardiopathy, helminthiasis, infections and snake-bite[3-13]. The milky exudate is bitter and good for ulcers, vitiated conditions of vata and otalgia[13]. *Alstonia scholaris* consists of flavonoidal glycosides[14], indole & other alkaloids[15-17], steroids[18], terpenoids[19], etc.

In the Indian system of medicine, certain herbs claimed to provide relief against liver disorders. The claimed therapeutic reputation has to be verified in a scientific manner. Scientists are working on this area of research and the hepatoprotective properties of a number of plants/herbs have been evaluated with exciting results[20-24]. In view of these observations and biologically active nature of *Alstonia scholaris*, it was thought worthwhile to study the hepatoprotective effect of *Alstonia scholaris* (L.) R.Br.

MATERIALS AND METHODS

Collection and identification of Crude drug: The stem bark of *Alstonia scholaris* (L.) R.Br. was collected from the surrounding areas of Dibrugarh University, Dibrugarh and was identified

and authenticated by Dr. M. Islam, Department of Life Sciences, Dibrugarh University. A herbarium specimen has also been deposited in the college museum.

Preparation of extracts

Shade dried powdered plant material (200 gm) was extracted in Soxhlet assembly successively with petroleum ether (60-80°C), chloroform (60-62°C), methanol (60-70°C) and water (100°C). Each time before extracting with the next solvent, the removed plant material was dried in hot air oven below 50°C. Each extract was concentrated by distilling off the solvent and then evaporated to dryness in a rota-vapor[25]. Methanolic extract was used for evaluating hepatoprotective activity since this extract showed the presence of maximum number of plant constituents.

Phytochemical Screening:

The different extracts obtained were tested separately for the presence of various plant constituents, such as, alkaloids, carbohydrates, glycosides, flavonoids, saponins, phenolic compounds and tannins[26].

Animal studies

Experimental animals

Swiss albino mice (20-25 g) and Swiss albino rats (120-160 g) of both sexes were obtained from animal house of Department of Pharmaceutical Science, Dibrugarh University, Dibrugarh, Assam. The study protocol was approved from the Institutional Animal Ethics Committee (IAEC). The animals maintained in standard laboratory conditions of temperature, humidity and 12 h light and dark cycles. The animals were fed with standard pellet diet and water *ad libitum*.

Acute toxicity studies

Albino mice were divided into groups of six animals each. The control group received saline and the other groups received 100 to 2000 mg/kg *p.o.* of test extracts. Immediately after dosing, the animals were observed continuously for the first 4 h and then for 48 h after extract administration to record the mortality[27].

Hepatoprotective studies

Hepatic injury was induced in rats by intraperitoneal administration of a single dose of 1.5 mL/kg Carbon tetrachloride (CCl_4)[28, 29.] Silymarin, a known hepatoprotective agent was used as reference standard[30]. Animals were grouped as follows:

Group-I: Served as control group, given normal food and water daily for 7 days.

Group-II: Toxicant group, given water daily for 7 day followed by single dose of CCl_4 (1.5 mL/kg *i.p.*) on day 7.

Group-III: Treated with *A. scholaris* methanolic extract (200 mg/kg *p.o.*) for 7 days followed by single dose of CCl_4 (1.5 mL/kg *i.p.*) on day 7.

Group-IV: Treated with Silymarin (25 mg/kg *p.o.*) for 7 days followed by single dose of CCl_4 (1.5 mL/kg *i.p.*) on day 7.

At the end of treatment, all the animals were sacrificed on the 8th day and blood was collected in sterile centrifuge tubes & allowed to clot. Serum was separated and used for the assay of marker enzymes viz. serum transaminases (SGPT & SGOT), serum alkaline phosphatase (ALP), total bilirubin (TB) and total protein (TP). The results are presented in Table-2.

Biochemical parameters estimated

Estimation of SGOT

SGOT catalyses the transfer of amino group from L-aspartate to 2-oxoglutarate forming oxaloacetate and L-glutamate. The rate of this reaction is monitored by an indicator reaction coupled with malate-dehydrogenase (MDH) in which the oxaloacetate formed is converted to malate in the presence of reduced nicotinamide adenine dinucleotide (NADH). The oxidation of NADH in this reaction is measured as a decrease in absorbance of NADH at 340 nm, which is proportional to SGOT activity[31,32] (Table-2).

Estimation of serum SGPT

SGPT catalyses the transfer of amino group from L-alanine to 2-oxoglutarate with the formation of pyruvate and L-glutamate. The pyruvate so formed is allowed to react with NADH to produce L-lactate. The rate of this reaction is monitored by an indicator reaction coupled with LDH in the presence of NADH (nicotinamide adenine dinucleotide). The oxidation of NADH in this reaction is measured as a decrease in the absorbance of NADH at 340 nm, which is proportional to SGPT activity[31, 32] (Table-2).

Estimation of Serum Alkaline Phosphate (ALP)

Serum alkaline phosphatase hydrolyses *p*-nitrophenyl phosphate into *p*-nitrophenol and phosphate in the presence of oxidizing agent Mg^{2+}. This reaction is measured as absorbance is proportional to the ALP activity[33] (Table-2).

Estimation of Total Bilirubin (TB)

Bilirubin reacts with diazotised sulphanilic acid in acidic medium to form pink coloured azobilirubin with absorbance directly proportional to bilirubin concentration. Direct bilirubin, being water soluble directly reacts in acidic medium. However, indirect or unconjugated bilirubin is solubilised using a surfactant and then it react similar to direct bilirubin[34] (Table-2).

Estimation of Total Protein (TP)

Proteins in an alkaline medium, binds with cupric ions present in the Biuret reagent to form a blue-violet colored complex. The intensity of the color formed is directly proportional to the amount of proteins present in the sample. The reagents are mixed well and incubated at 37°C for 5 min. The absorbance of the standard (Abs. S), and test sample (Abs. T) were measured against the blank at 578 nm[34] (Table-2).

Histopathological studies

The livers were removed immediately after rats scarification and fixed in 4% formalin. After processing, the tissues were embedded in paraffin wax and sectioned into 4-5 µm thickness using microtome. The sections were stained with hematoxylin and eosin (H&E) for microscopic observation, which includes fatty changes; hepatocytes disarrangements and necrosis symptoms[35] (Figure a-d).

RESULTS AND DISCUSSION

In Indian system of medicine, certain herbs claimed to provide relief against liver disorders. The claimed therapeutic reputation has to be verified in a scientific manner. In the present study one such extract *Alstonia scholaris* (L.) R.Br. was taken for the hepatoprotective study. Preliminary phytochemical screening of the petroleum ether extract, chloroform extract, methanolic extract & aqueous extract of *Alstonia scholaris* (L) R.Br. showed the presence of alkaloids, glycosides, flavonoids, phenolic compounds & carbohydrate in sufficient amounts in the Methanolic extract and in lesser amounts in aqueous extract of the stem bark powder (Table-1). Methanolic extract was used for evaluating hepatoprotective activity since this extract showed the presence of maximum number of plant constituents. In the toxicity study, no mortality occurred throughout the experiment with the doses upto 2000 mg/kg *p.o.* of the plant extract.

Table 1: Preliminary Phytochemical screening of different extracts of stem bark of *Alstonia scholaris* (L.) R.Br.

Test for	Petrolium ether extract	Chloroform extract	Methanolic extract	Aqueous extract
Alkaloids	--	--	++	+
Glycosides	--	--	++	+
Flavonoids	--	--	++	+
Phenolic compounds and tannins	--	--	++	+
Carbohydrate	--	--	++	+
Proteins and amino acids	--	--	--	--

+ Indicates presence; -- indicates absence

The functional integrity of hepatic mitochondria was altered, leading to liver damage by the hepatotoxin; CCl_4. Thereby levels of marker enzymes SGPT and SGOT were found to be elevated in cytoplasm and in blood as these were released into blood. The biochemical tests results revealed that the test group receiving dose (200 mg/kg body wt.) significantly reduced the toxicants effect (Table-2).

Table 2: Biochemical estimation of rats in the CCl_4 induced Hepatotoxicity experiment.

Group	Treatment	SGOT (U/L)	SGPT (U/L)	ALP (mg/dl)	TB (mg/dl)	TP (mg/dl)
Group I	Control	74.40 ± 1.32	23.39 ± 3.19	13.43 ± 0.59	0.62 ± 0.02	24.36 ± 0.62
Group II	Toxicant	131.62 ± 7.76*	153.78 ± 13.76*	55.95 ± 1.23*	1.69 ± 0.14*	18.15 ± 0.96*
Group III	Drug Extract	115.39 ± 4.08***	88.96 ± 6.88***	30.40 ± 0.98***	1.38 ±0.08*	20.1 ± 0.86***
Group IV	Standard	95.84 ± 3.99***	58.87 ± 2.90***	21.58 ± 1.12***	1.19 ± 0.08***	21.57 ± 0.33***

The values are presented as mean SEM, n=6, *p<0.05 and ***p<0.001; toxicant group was compared with control group while extract treated & standard group were compared with toxicant group.

The results of SGOT analysis reveled that CCl_4 (1.5 mL/kg *i.p.*) produced significant increase in SGOT level up to 131.62±7.76 as compared to control group 74.40±1.32. Extract (200 mg/kg *p.o*) decreased this level significantly (115.39±4.08) as compared to toxicant group. Silymarin also produced significant protection (95.84±3.99) against CCl_4 (Table-2).

In SGPT analysis, control group rats showed the level 23.39±3.19 which was very high in toxicant group 153.78±13.76. Extract treated rats (200 mg/kg *p.o*) showed significantly decreased SGPT values (88.96±6.88) as compared to toxicant group. Silymarin also produced significant protection (58.87±2.90) against CCl_4 induced hepatotoxicity (Table-2).

Serum alkaline phosphatase (ALP) level was found to be 13.43±0.59 in control group and this level was significantly higher in toxicant group 55.95±1.23. The extract dose of 200 mg/kg induced significant protection against (ALP level in extract group; 30.40±0.98) CCl_4 induced hepatotoxicity. Similar protection was observed in Silymarin group with ALP level 21.58±1.12.

The extract dose of 200 mg/kg produced significant protection against CCl_4 induced hepatotoxicity as shown by the levels of other parameters like total bilirubin (TB) & total protein (TP) in control, toxicant and extract treated rats (Table-2).

In histopathological examination of different groups showed- The control group (Group 1) animals showed normal architecture as the kupfer cell hyperplasia, portal area and central vein were prominent with normal hepatocytes (Fig. a); CCl_4 challenged animals (Group 2; Toxic

group) showed severe fatty changes in midzonal and periportal area, acute inflammatory changes, diffused fatty infiltration and focal area degeneration (Figure. b); the drug extract treated (200 mg/kg *p.o*) animal group (Group 3) showed mild fatty changes (Figure. c) which was comparable to that of the standard reference compound i.e. Silymarin; The animals were treated with the standard drug Silymarin (Group 4) and then treated with CCl_4 also showed mild degree of pathological changes of hepatic cells and fatty degeneration (Figure. d).

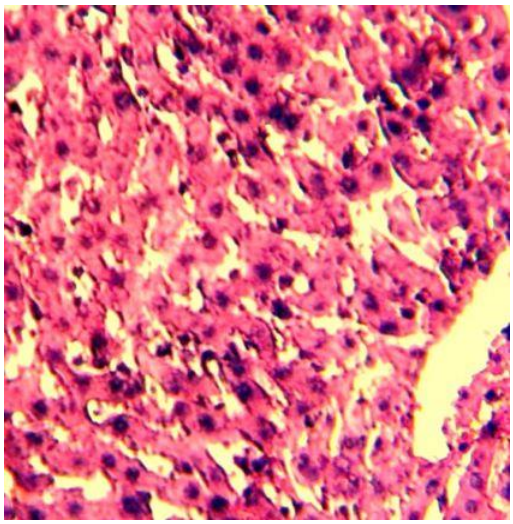

Figure (a): Control group

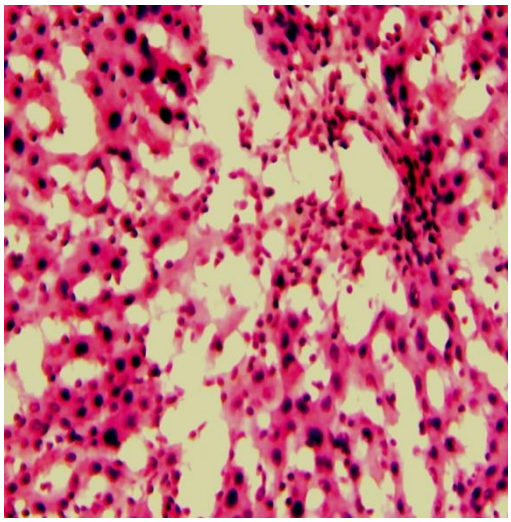

Figure (b): Toxic group

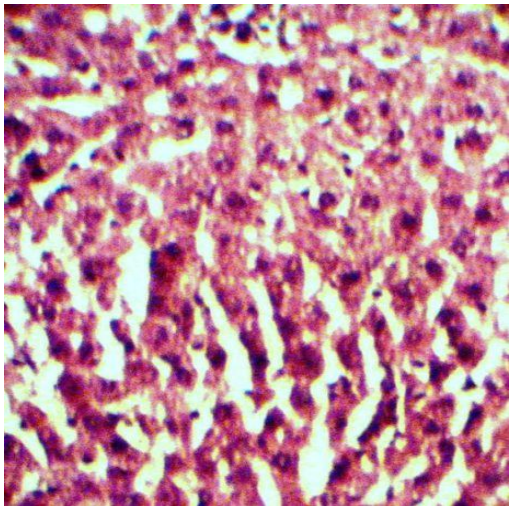

Figure (c): Drug extract group

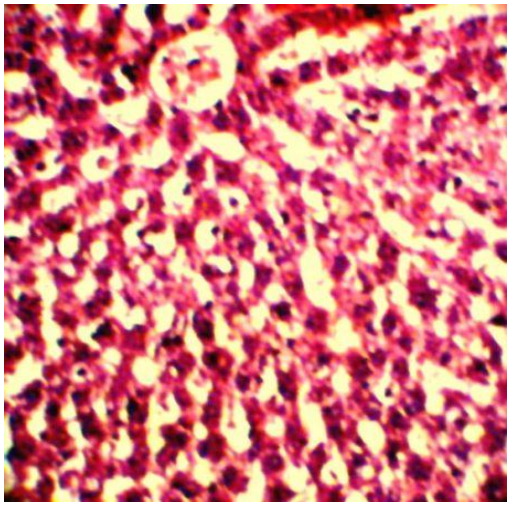

Figure (d): Standard group

Figure (a) showed normal architecture of liver cells, Figure (b) showed severe centrilobular fatty changes, Figure (c) showed varieties of mild fatty changes, Figure (d) also showed mild fatty changes but less than Figure (c).

The present investigations revealed that the given dose of CCl_4 (1.5 ml/kg, ip) produced significant elevation in SGPT, SGOT, ALP, bilirubin (direct and total), indicating all impaired liver function and these parameters have been reported to sensitive indicator of liver injury[36]. The massive production of reactive species may lead to depletion of protective physiological moieties and ensuing widespread propagation of the alkylation's as well as peroxidation, causing damage to the macromolecules in vital biomembranes.

In was noted that the administration of CCl_4 decreased the levels of total protein. The marked elevation of bilirubin in the serum of group II, CCl_4 intoxicated rats, were significantly decreased in the group III ASE (*Alstonia scholaris* methanolic extract) treated animals. These parameters were brought back to near normal levels in the group IV ASE treated animals and group IV standard group (Silymarin). ASE treatment showed a protection against the injurious effects of carbon tetrachloride that may result from the interference with CYP 450, resulting in the hindrance of the formation of hepatotoxic free radicals. The site-specific oxidative damage in some susceptible amino acids of proteins is now regarded as the major cause of metabolic dysfunction during pathogenesis.

The present study revealed that the effect of pretreatment of methanolic extract of bark of *Alstonia scholaris* had been effective in offering protection, alleviated the increased serum enzyme activity (SGOT & SGPT) induced by CCl_4, indicating improvement of the functional status of the liver, which was also supported by the histopathological findings, which is comparable to that of the standard reference Silymarin.

CONCLUSION

It is concluded that treatment with methanolic extract decreased the CCl_4 induced elevation in biochemical parameters (SGOT, SGPT, ALP, TB and TP). These findings suggest that the methanolic bark extract of *Alstonia scholaris* (L.) R.Br. (200 mg/kg) was effective in bringing about functional improvement of hepatocytes. The healing effect of this extract was also confirmed by histological observations. The study demonstrated that the bark extract of *Alstonia scholaris* (L.) R.Br. consisted of flavonoids and phenolic compounds which may have a potential therapeutic approach to hepatoprotective action. Further, a detailed phytochemical and pharmacological investigation should be carried out in order to find out active constituents present in the plant, which could be responsible for the above said activity and its exact mechanism of action.

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