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HEPATOPROTECTIVE PROPERTIES OF *ALSTONIA SCHOLARSIS* AGAINST CARBON TETRA CHLORIDE INDUCED HEPATIC DAMAGE IN RATS

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ABSTRACT

Alstonia scholaris Linn (Apocynaceae) known as Saptaparna, is a medium sized tree known for its pharmacological activities like antimicrobial, antimalarial, antidote for snake bite and anticholeric properties etc. This evergreen tree is a native to the Indian sub-continent and South East Asian countries. In the present study carbon tetrachloride (CCl_4) induced liver damage model has been employed as a tool to assess the hepatoprotecitive activity of ethyl acetate, hexane and methanol extracts of *Alstonia scholaris*. Significant increase in the total serum protein is observed in the blood serum of animals treated with methanol, hexane and ethyl acetate extract of leaf of *Alstonia scholaris* indicating the protection of normal regulatory homeostatic function of liver. Thus the current study reveals that plant extracts from the plant *Alstonia scholaris* have significant hepatoprotective property.

INTRODUCTION

Alstonia (Family: Apocynaceae) also known as devil tree or Sapataparni genus of evergreen trees or shrubs with white funnel-shaped flowers and milky sap. Many higher plants accumulate extractable organic approaches, substances in quantities sufficient to be economically management of disease. About 1 to 10 % of the plants are used as food by man and other animals. 'Rigveda' is the oldest book containing the information regarding the medicinal plants. Hippocrates mentioned 300 to 400 medicinal plants. Plants have been a rich source of medicines because they produce wide array of bioactive molecules, most of which probably evolved as chemical defense against predation or infection. The Decoction of leaves is found efficacious in congestion of liver, also in dropsy [1]. It also stimulates the liver, hence useful in liver disorders. In current Ayurveda practice, a decoction of the bark is also considered efficacious in malaria, while a decoction of the leaves is used against liver ailments [2, 3]. The bark extract has been reported to posse's antiplasmodial, immunostimulant, anticancer effect and is also Hepatoprotective [4, 5]. The bark is bitter, astringent, digestive, laxative, anthelmintic, antipyretic, stomachic, cardiotonic and tonic [6]. Leaves contain different constituent's alschomine, isoalschomine, tubotaiwine, akuammidine, picraline, picrinine, picrarinal, areline etc [7].

The current investigation aimed to explore scientifically the hepatoprotective activity of *Alstonia scholarsis* in rats.

METHODS

Plant material and preparation of the extract

Fresh leaves of *Alstonia scholaris* free from disease were collected from local areas in Davanagere district, Karnataka. The plant material was shade dried and then powdered using a mechanical grinder. The powdered materials of plant were subjected to successive solvent extraction using Soxhlet apparatus and refluxed successively with ethyl acetate, hexane and methanol. The extracts were filtered and concentrated in vacuum using rotary flash evaporator (Buchi, flawil, Switzerland). The crude extracts were administered to the animals as aqueous solutions.

Phytochemical screening

The preliminary phytochemical analysis of ethyl acetate, hexane and methanol extracts was carried out using the methods as described in Harborne (1984) [8]; Trease and Evans (1989) [9]; Kokate *et al*., (1998) [10]; Khandelwal, (2005) [11].

The dry weight of the ethyl acetate, hexane and methanol extracts was obtained by allowing the solvent to evaporate and was used to determine concentration in mg/mL. (Methodology based on Betoni *et al*., (2006) [12] (Table 1).

Table 1: Characteristics of the Plant Extract

Scientific Name	Common Name	Part Of The Plant Used	Extract Dry Weight (mg/ml)
Alstonia scholaris	Dita dark	Leaf	70

Hepatoprotective activity:

For screening hepatoprotecitve activity Wistar strain animals of either sex, weighing about 150 – 200 g were selected and divided into five groups of six each. The animals of group I served as control and received 1 ml / kg / day of 1 % gum tragacanth orally. The animals of group II, III and IV received 0.1 ml / kg / day CCl_4 (E-Mark Mumbai, India) with 1:1 olive oil by intraperitoneal injection. The animals of group III served as standard and received 100 mg / kg/ day of Silymarin (Ranbaxy Laboratories, Dewas) orally. The methanol, hexane and ethyl acetate extracts of *Alstonia scholaris* leaf (group IV, V and VI) were administered orally to the respective groups .The drugs were administered concomitantly for 14 days in the doses mentioned below (Table 2). Institutional animal ethics committee (IAEC) approved the experimental protocol and care of the animals was taken as per guidelines of CPCSEA Department of animal welfare, Govt. of India.

Table 2: The Drugs Administration Concomitantly for 14 Days in the Dose.

Group	Extract/ Constituents	Modes of Drug Administration	Dose
I	Control.	Oral	1 ml / kg/ day of 1 % tragacanth
II	CCl_4 (E-Mark Mumbai, India).	Intraperitoneal	0.1 ml / kg/ day with 1 : 1 olive oil
III	Silymarin	Oral	100 mg / kg / day
IV	Methanol extract of the leaf of *Alstonia scholaris*	Oral	40 mg / kg/ day.
V	Hexane extract of the leaf of *Alstonia scholaris*	Oral	40 mg / kg /day.
VI	Ethyl acetate extract of the leaf of *Alstonia scholaris*	Oral	40 mg / kg / day

Liver function tests:

a. **Determination of total bilirubin in serum**

In this study bilirubin was estimated using Diazo reagent. The optical density was measured at 540 nm to get a quantitative measure of bilirubin present in the serum of the test animals [13].

b. **Determination of total serum protein by Biuret method**

Six test tubes were taken and labeled as blank, standard and TP1 to TP3 (Total Protein) for the serum of each group. To the test tube blank 3 ml of distilled water, to the test tube standard 3ml of standard protein solution, to the test tube TP1 to TP 3 0.1 ml of serum of respective group of animals and 2.9 ml of 0.9 % sodium chloride was added. The contents of these test tubes were mixed by gentle shaking and 3 ml of Biuret reagent was added to all the tubes. The test tubes were allowed to stand for 10 minutes. After mixing thoroughly Optical density was measured at 540 nm.

c. **Assay of serum aspartate transaminase (AST) and serum alanine transaminase (ALT) activity**

Aspartate transaminase converts L-Aspartate and α-Ketoglutaric acid to oxaloacetate and L-Glutamate. Oxaloacetate under basic conditions react with 2, 4 dinitro phenyl hydrazine to give 2, 4 dinitro phenyl hydrazone, brown coloured complex which has been colorimetrically estimated at 505nm. Similarly, Alkaline transaminase converts L- Alanine and α-Ketoglutaric acid to pyruvate and glutamate. Pyruvate then reacts with 2, 4 DNPH to give a brown color complex. The serum obtained from the test animals were used to convert L-Aspartatic acid and L-Glutamate and α-Ketoglutaric acid to give brown colored complex on treatment with 2, 4 DNPH. The optical densities of the reaction mixtures were noted at 505nm. These optical readings gave the quantitative measure of ASP and ALP [14].

d. Estimation of serum alkaline phosphatase activity (ALP)

Sodium β glycerophosphate was used as the substrate to estimate the alkaline phosphatase present in the serum of the test animals. This reaction was colorimetric ally assayed using amino naphtol sulphonic acid (ANSA) reagent [15].

Statistical analysis

The data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey'multiple pair wise comparison test to assess the statistical significance. $P \leq 0.05$ was considered statistically significant, using software ez ANOVA ver. 0.98. The data are presented in Table 3

RESULTS AND DISCUSSION

The phytochemical profiles of various solvent extracts from plant used in this study. The analysis revealed the presence of alkaloids, flavonoids, triterpenoids, sterols, tannins and glycosides.

Liver function tests:

a. Determination of total bilirubin in serum:

Liver damage can be assessed by determination of total bilirubin in blood serum. In the normal healthy adult animals, the concentration of direct bilirubin rages from 0.0 to 0.2 mg / dl. Whereas indirect bilirubin concentration is 0.2 to 0.8 mg/dl. An increased bilirubin concentration > 1.0 mg/dl results in the clinical condition of Jaundice, which can be due to rise in the levels of both direct and indirect bilirubin. Disorder of the excretory hepatic function due to CCl_4 leads to elevated concentrations of bilirubin in blood. The biochemical estimation of blood serum of CCl_4 treated animal groups showed elevated levels of serum total bilirubin (2.85± 0.77) indicating the liver damage due to CCl_4 absorption. The reference standard silymarin with CCl_4 treated animal groups showed almost normal level of bilirubin (0.75 ± 0.14) as similar to the control (0.58 ± 0.14) indicating the protection of hepatic cells. The animal groups treated with methanol extract of leaf of *Alstonia scholaris* (1.07 ± 0.14) and hexane extracts of leaf (0.96 ± 0.27) indicating the potency of tested drugs in suppressing the hepatic damage caused by CCl_4 (Table 3).

b. **Determination of serum total protein by Biuret method**;

The serum proteins like albumin, alpha and beta globulins, fibrinogen, prothrombin, choline esterase etc., are synthesized in the liver cells. Damage to the liver cells decreases the levels of these proteins. Thus the analysis of serum proteins may provide a helpful insight to know the extent of liver damage and the depth of jaundice.

In healthy adults, the normal value of total protein is 6.0 to 8.0 g/dl. The concentration of albumin fraction ranges from 3.5 to 5.0 g/dl. Whereas the value of globulin is 2.3 to 3.5 g/dl. Cirrhosis of liver is responsible for the decrease in the albumin level of serum, which is associated with decrease in globulin level. Thus serum protein level can be a diagnostic feature of liver disorder.

The CCl_4 treated animal groups showed drastic reduction in the total serum protein level (4.18 ± 0.45) indicating the severe disturbance in the regulatory homeostatic function of liver due to CCl_4 toxicity. Significant increase in the total serum protein is observed in the blood serum of animals treated with methanol extract of leaf (6.24 ± 0.43) of *Alstonia scholaris*, hexane extract

of the leaf (6.55 ± 0.52) and ethyl acetate extract (7.56 ± 0.45) indicating the protection of normal regulatory homeostatic function of liver (Table 3).

c. **Assay of serum aspartate transaminase (AST) and serum alanine transaminase activity (ALT):**

The blood serum has large number of enzymes but to assess the normal and pathological symptoms of liver, alanine transaminse (glutamate pyruvate transaminase) and aspartate transaminases (glutamate oxalate acetate transaminase) are used. The normal ranges of AST and ALT are from 6 to 38 IU/dl and 8 to 35 IU/ dl at 37^0 C respectively. The concentration of these enzymes increases in serum whenever the tissues are damaged. It is presumably due to release of enzyme from the destroyed cells. In hepatic necrosis the serum levels of AST and ALT could be expected to increase from 2 to 20 folds of the upper limit of normal depending upon intensity of liver damage. In the present investigation, it was noticed that in CCl_4 treated group of animals the AST and ALT enzymes levels were increased significantly due to severe hepatotoxicity (AST: 1217.52 ± 72.33 and ALT: 303.33± 28.44 IU/L) respectively. However, in the animal groups treated with standard drug Silymarin, methanol extracts of the leaf showed significant reduction in the levels of AST (200.73 ± 18.24 IU /L, 292.71 ± 36.52 IU/L) and ALT (99.83 ± 8.03 IU /L, 98.37 ± 8.98 IU/ L) respectively. In the animal groups treated with the hexane extracts of leaf also showed significant reduction in the AST and ALT (416.78 ± 57.06 IU /L, 139.21 ± 20.35 IU / L) respectively. The animals treated with ethyl acetate extracts of leaf also showed significant reduction in the AST and ALT (312. 22 ± 47. 57 IU / L, 99.92 ± 9.39 IU / L) respectively (Table 3).

d. **Estimation of serum alkaline phosphatase activity (ALP)**:

Serum alkaline phosphatase is a globulin enzyme of low molecular weight found in higher concentration in bones, hepatobiliary tract and kidney. The serum level of these enzymes was increased in both hepato cellular and obstructive Jaundice. But it was very less in chronic hepatitis.

In normal groups of animals the concentration of this enzyme is in the serum ranges from 15 – 112 IU /L. In CCl_4 treated group due to necrosis of hepato billiary tract the level of enzyme was significantly increased when compared to normal animals.

In the present study the CCl4 treated animal group showed significant rise in the ALP (407.32 ± 37.06). However, in the animal groups treated with standard drug silymarin, methanol extract of

leaf of Alstonia scholaris showed significant reduction in the levels of ALP (167.84 ± 20.78 IU/L, 156.45 ± 22.44 IU/L) respectively. In the animal group treated with hexane extract showed significant reduction in the ALP (216.04 ± 28.23 IU/L) respectively(Table: 3).Significant increase in the total serum protein is observed in the blood serum of animals treated with methanol extract of leaf of *Alstonia scholaris*, hexane extract of the leaf and ethyl acetate extract indicating the protection of normal regulatory homeostatic function of liver [16] showed the hepatoprotective activity of *Moringa oliefera* on antitubercular drug induced liver damage in rats, [17] showed the hepatoprotective activity of *Nyctanthes arbor-tristis* [18] showed the hepatoprotective activity of *Cleome viscose* [19]*Ficus carica* Linn [20] have shown the hepatoprotective activity of *Annona squamosa*, [21] have shown the hepatoprotective activity of *Silybum marianum* [22] showed the hepatoprotective activity of aqueous ethanolic extract of *Chamomile capitula* in paracetamol in toxicated albino rats.

Table 3: Hepatoprotective Effect of Crude Extracts of Leaf of *Alstonia scholaris*

Extract/ Constituents	**Bilirubin in serum (mg/dl)**	**Serum protein (g/dl)**	**AST (IU/L)**	**ALT (IU/L)**	**ALP (IU/L)**
Carbon tetra chloride	2.85±0.77	4.18±0.45	1217.52 ±72.33	303.33± 28.44	407.32±37.06
Silymarin	0.75±0.14	-	200.73± 18.24	99.83±8. 03	167.84±20.78
Methanol extract of the leaf of *Alstonia scholaris*	1.07±0.14	6.24±0.43	292.71± 36.52	98.37±8. 98	156.45±22.44
Hexane extract of the leaf of *Alstonia scholaris*	0.96±0.27	6.55±0.52	416.78± 57.06	139.21± 20.35	216.04±28.23
Ethyl acetate extract of the leaf of *Alstonia scholaris*	-	7.56±0.45	312.22± 47.571	99.42±9. 39	-

The values are the mean of three experiments ± S.E. $P \leq 0.05$ Vs Standard antioxidant (Tukey's pairwise comparison test).

CONCLUSION

The methanolic, hexane and ethyl acetate extracts of *Alstonia scholaris* show a significant hepatoprotective activity. The administration of CCl_4 in rats is known to cause centrilobular hepatic necrosis or toxic hepatitis and the injury caused by this toxic substance is similar to that of human infective hepatitis. Significant increase in the total serum protein is observed in the blood serum of animals treated with methanol extract of leaf of *Alstonia scholaris*, hexane extract of the leaf and ethyl acetate extract indicating the protection of normal regulatory homeostatic function of liver. Bilirubin content in serum decreases in animal treated with hexane extract as compare to methanol extract.

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