HEPATOPROTECTIVE ACTIVITY OF *ALSTONIA SCHOLARIS* (L.) R.Br. LEAVES AGAINST THIOACETAMIDE INDUCED HEPATOTOXICITY

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Summary

Objective: The present study was conducted to evaluate the hepatoprotective activity of methanolic extract of leaves of *Alstonia scholaris* using Thioacetamide-induced liver damage in Albino rats. **Methods:** Two doses of methanolic extract of *Alstonia scholaris* (100 and 200mg/kg) were administered orally to the animals for 9 days and hepatotoxicity was induced by Thioacetamide on the 9th day. Silymarin was used as the standard drug in the study. Blood samples were withdrawn thereafter and analysed for marker enzymes like SGPT, SGOT, ALP and serum (total) bilirubin. **Results:** The methanolic extracts showed significant ($p<0.01$) protective effect by lowering serum levels of various biochemical parameters such as serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), serum alkaline phosphatase (ALP), and total serum bilirubin; the higher dose producing more promising results. **Conclusion:** The present study demonstrated the hepatoprotective activity of methanolic extract of *Alstonia scholaris* validating the traditional claims made on the same.

Keywords: *Alstonia scholaris*, Thioacetamide, Hepatoprotective.

Introduction

Alstonia scholaris L. belonging to family Apocynaceae is commonly known as Saptparna. The plant is native to India and grows in deciduous and evergreen forests and also in plains. [1] The leaves Decoction of leaves is found efficacious in congestion of liver, also in dropsy. [2] 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. [3, 4] The bark extract has been reported to posse's antiplasmodial, immunostimulant, anticancer effect and is also hepatoprotective. [5, 6] The bark is bitter, astringent, digestive, laxative, anthelmintic, antipyretic, stomachic, cardiotonic and tonic. [7] Leaves contain different constituent's alschomine, isoalschomine, tubotaiwine, akuammidine, picraline, picrinine, picrarinal, areline etc. [8]

Materials and methods:

1. Plant material & Preparation of extracts: The plant material was collected from local areas of Lucknow, Uttar Pradesh and was authenticated from National Botanical Research Institute, Lucknow by depositing a herbarium (Voucher specimen Ref No. NBRI/CIF/180/2010) and was identified as *Alstonia scholaris* L. The shade dried leaves of *Alstonia scholaris* L. R.Br. were subjected to size reduction to a coarse powder by using dry grinder. The powdered material was subjected to extraction by decoction using methanol as solvent. The extract was concentrated under vacuum to obtain the residue. The residue was dried in vacuum dessicator and stored in air tight container until further use. Various concentrations of the leaves were prepared freshly everyday using distilled water for the period of 09 days.

2. Chemicals and equipments: Thioacetamide LR (SD Fine Chemicals, India) used as hepatotoxic agent, Silybon (Silymarin Suspension- Micro Labs, India) used as standard drug, and auto-sampler –analyzer STAT-FAX 3300 for estimation of marker enzymes.

3. Experimental Animals: Male albino rats weighing 150-250g maintained under standard husbandary conditions were used for all studies. They were fed standard rodent pellets diet and water *ad libitum.* The care and handling of rats were in accordance with the internationally accepted standard guidelines for use of animals, and the protocol was approved by our Institutional Animal Ethics Committee under the CPSCEA. (BBDGEI/IAEC/03/2011)

4. Acute toxicity study: The acute toxicity studies were performed in accordance with the OECD (Organization for Economic Co-operation and Development) guidelines no. 425 (Up and Down Procedure). [9] No death was observed till the end of the study. The test samples were found safe upto the dose of 2000mg/kg.

5. Evaluation of hepatoprotective activity: Hepatoprotective activity was evaluated using acute hepatic injury models induced by Thioacetamide, following the methods used by Roy et al (2006). [10]

Thioacetamide (TAA) induced liver toxicity:
The TAA was diluted with distilled water (1:1) before administration. The animals were divided into five groups of six each. The animals were then subjected to either one of the following treatments for 9 days.
Group I: distilled water (1ml, p.o.)
Group II: distilled water (1ml/day, p.o.) for 9 days + TAA (100mg/kg, i.p.) on ninth day
Group III: silymarin (100mg/kg/day, p.o.) for 9 days + TAA (100mg/kg, i.p.) on ninth day
Group IV: MEAS (100mg/kg/day, p.o.) for 9 days + TAA (100mg/kg, i.p.) on ninth day
Group V: MEAS (200mg/kg/day, p.o.) for 9 days + TAA (100mg/kg, i.p.) on ninth day

After dosing of TAA, blood was withdrawn from the retro-orbital plexus of rats in each group. Blood samples were collected and the serum was used for assay of the marker enzymes such as serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT), alkaline phosphatase (ALP) and serum (total) bilirubin.

6. Statistical Design: Results are expressed as mean ±S.E.M. Statistical analysis of the data was performed with one-way analysis of variance (ANOVA) followed by Dunnett's test (Graph Pad Prism version 3.00, USA)

Results and Conclusions

Group I (control) exhibited normal blood levels of all marker enzymes (SGPT, SGOT, ALP); however induction of hepatotoxicity by Thioacetamide caused a dramatic increase in the blood levels of all marker enzymes as well as serum bilirubin in the disease control group i.e. Group II (Table 1; Figure 1 and 2). The standard drug Silymarin; which is a proven hepatoprotective, facilitated successful reduction in the levels of marker enzymes and serum bilirubin (Group III). The test extracts dosed at 100 mg/kg and 200 mg/kg effectively reduced the blood levels of the marker enzymes and serum bilirubin; serving as an index of hepatoprotective potential; the higher dose eliciting a more prominent response. The lower dose of *Alstonia scholaris* methanolic extract (Group IV) reduced the SGPT, SGOT, ALP and serum bilirubin values; however a greater reduction was brought about by the higher dose of *Alstonia scholaris* methanolic extract (Group V); which closely approximated the response afforded by the standard drug (Table 1; Figure 1 and 2).
The methanolic extracts of *Alstonia scholaris* leaves afforded a decent hepatoprotective potential against Thioacetamide induced liver damage and produced dose dependent effects when administered at doses of 100mg/kg and 200mg/kg orally. Thus *Alstonia scholaris* can be explored as a useful herbal option in liver care.

Group	Drug	SGPT(U/L)	SGOT(U/L)	ALP(IU/L)	Serum bilirubin (Total) (mg/dl)
Group I normal control	Distilled water	42.750±1.750	46.000±2.799	217.500±7.773	0.615±0.020
Group II disease control	Thioacetamide 100mg/kg	141.000±3.536	237.750±6.860	519.500±11.034	2.550±0.165
Group III standard	Silymarin 50mg/kg	59.250±3.945*	90.500±3.279*	231.000±22.147*	0.878±0.040*
Group IV test-1	MEAS 100mg/kg	74.750±2.626*	92.250±1.750*	285.250±9.569*	0.949±0.274*
Group V test-2	MEAS 200mg/kg	63.250±2.394*	75.750±2.780*	229.750±4.029*	0.875±0.014*

* All values are expressed as mean ± SEM; N= 6; P<0.01 significant compared to diseased group.

Table 1.0: Effect of *Alstonia scholaris* extracts on Biochemical parameter of liver

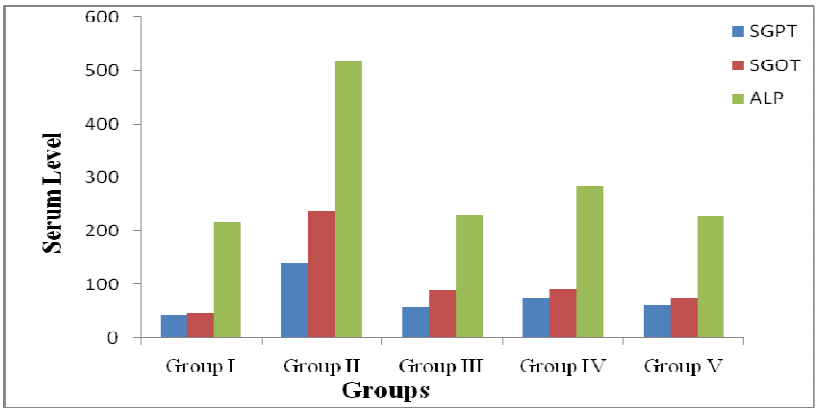

Fig 1: Effect of *Alstonia scholaris* extracts on SGOT, SGPT and ALP levels.

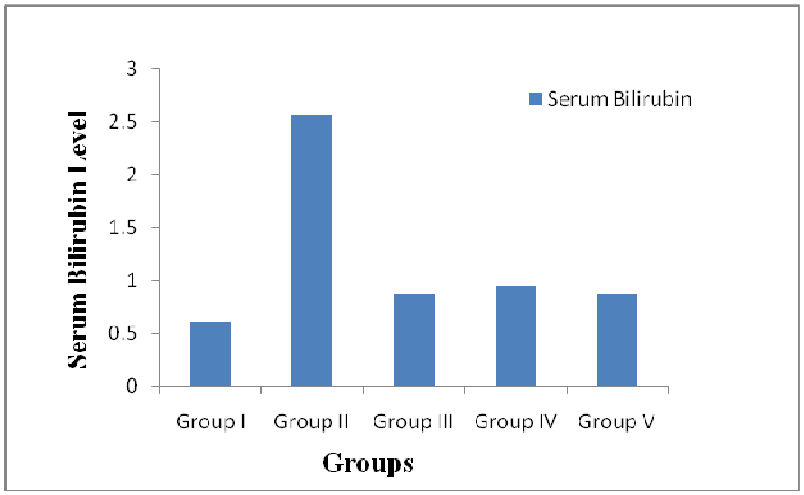

Fig 2: Effect of *Alstonia scholaris* extracts on serum bilirubin

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