Hepatoprotective Activity of *Sida rhombifolia ssp. retusa* Against Thioacetamide and Allyl Alcohol Intoxication in Rats

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Summary

Sida rhombifolia ssp. *retusa* is a medically important herb of Indian origin commonly known as Mahabala and is used in *Ayurvedic* systems of medicine for the treatment of various ailments. In the present paper, aqueous extract of *Sida rhombifolia* ssp. *retusa* roots is shown to possess hepatoprotective activity. Pretreatment of rats with aqueous extract at two dose levels (400mg/kg and 800mg/kg body wt, orally) significantly ameliorated the liver damage in rats exposed to the hepatotoxic compound thioacetamide and allyl alcohol. The substantially elevated levels of serum enzymatic activities serum transaminases viz alanine transaminase (sALT), and aspartate transaminase (sAST), due to thioacetamide treatment were significantly lowered in extract pretreated rats. In other study with allyl alcohol, indicated that changes in body weight and in necrosis index were restored to normal by treatment with aqueous extract, the results, indicated that physical and biochemical changes along with change in necrosis index were restored to normal by treatment with aqueous extract. Thus the study clearly indicates that roots of *Sida rhombifolia* ssp. *retusa* have a potent hepatoprotective action against thioacetamide and allyl alcohol -induced hepatic damage in rats.

Key words: *Sida rhombifolia* ssp. *retusa*, Hepatoprotective, Thioacetamide, Allyl alcohol.

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Introduction

Liver is the most important organ concerned with the biochemical activities in the human body. It has great capacity to detoxicate toxic substances and synthesize useful principles. Therefore, damage to the liver inflicted by hepatotoxic agents is of grave consequences. There is an ever increasing need of an agent which could protect it from such damage. In view of severe undesirable side effects of synthetic agents, there is growing focus to follow systematic research methodology and to evaluate scientific basis for the traditional herbal medicines which are claimed to possess hepatoprotective activity[1]. Since lay people or peasants generally could not distinguish and define an activity related to an antihepatotoxic effect, a wide variety of parameters should be evaluated for the selection of remedies related to the disorders associated with this organ.

Some species of malvaceae family are popular for their effects on liver diseases. The aqueous extract of *sida cordifolia* shows hepatoprotective activity against carbon tetrachloride, paracetamol and rifampicin induced hepatotoxicity[2]. *Sida rhombifolia* L. ssp. *retusa* (L.) (Malvaceae) is distributed throughout the warmer parts of India commonly known as Mahabala. In *Ayurveda* (Indian System of Medicine), whole plants of *sida retusa* are used as a tonic, aphrodiasiac, diuretic and in the treatment of fever and inflammation. Renowned Ayurvedic physician *Charaka*[3] has categorized *bala* as *brmhaniya*- a bulk- promoting herb and as *balya*- tonic & *prajasthapana*- which promote reproduction. It also mentioned *bala* as rejuvenative (*Rasayana*) to muscle tissue and muscular system. The infusion made from the roots of this plant is used in the treatment of rheumatism and in neurological complaints including epilepsy[4]. Crude extracts of *S. retusa* roots produced sedative effect and also produced significant potentiation of pentobarbitone sleeping time in mice[5].

In the present communication, we report that aqueous extract prepared from the roots of *Sida retusa* has hepatoprotective activity. The study provides biochemical evidence to validate the use of *Sida retusa* as a component of some hepatotonic preparations used by Ayurvedic physicians in India.

Material and Methods

***Collection of plant material**: Sida retusa* plant was collected from the academy of development sciences, Kashele post, Karjat, Maharashtra during August 2005, and authenticated by Department of Botany, Pune University. The identified and authenticated voucher specimen was deposited in the herbarium.

Chemicals: The chemicals used in this experiment were of highest purity grade available commercially in India.

Preparation of* aqueous *extract: The roots of the herb were washed, dried and crushed in an iron mortar. Crushed material was subjected to extraction by hot maceration at 60–70°C for 6 h continuously in distilled water three times. The extract was filtered, combined and evaporated to dryness under reduced pressure (40–45°C).

The yield of the material was 10.590 %. The dried crude extract was suspended in distilled water using 1% gum acacia as suspending agent. The suspended material was administered to rats orally at a dose of 400 and 800 mg/kg body wt.

Test animals: Female Wistar rats with initial body weights from 140 to 175g were used throughout the experiment. They were housed in polypropylene cages under conditions of constant temperature (21°C), humidity (40%), and a standard 12-h light, 12-h dark cycle. They were provided with pellet diet and deionised water ad libitum. Animals received human care and the experiment was carried out following the guidelines set by the institute's ethical committee.

Induction of hepatotoxicity and treatment protocol: The method of Ahmed et al was used for thioacetamide induced liver damage[6]. Rats were divided into following four groups (n= 6). Group- I received the vehicle (water, 1 ml/kg p.o.) for 7 days and serves as control. The rats of group II also received vehicle for 7 days and served as toxic control. Rats of group III and IV were prophylactically treated with aqueous extract at two dose levels (400 and 800 mg/ kg p. o.) for seven day respectively. On 6th day, rats of group II, III and IV group were given a single dose of thioacetamide (100 mg/kg s. c.) as 2 % w/v solution in double distilled water. On 8th day blood was collected by cardiac puncture of rats under light ether anaesthesia. It was allowed to clot in centrifuged tubes at 4°C and then centrifuged at 3000 rpm for 10 min to separate the serum. Enzyme assays were performed on the serum prepared from the freshly collected blood from each individual rat. Livers were isolated to determine liver weight and volume, which were preserved in 10 % formalin solution.

Assay of serum transaminase (s) activities: Activities of serum transaminases viz alanine transaminase (sALT), and aspartate transaminase (sAST) were measured by the methods described by Reitman and Frankel[7]. For sALT, each 1 ml reaction mixture contained 0.1 M phosphate buffer (pH 7.2), 0.2 M DL-alanine, and 2 mM of 2-oxoglutarate. The reaction was initiated by adding 0.2 ml serum. The mixture was incubated for exactly 30 min at 37°C in a water bath. After incubation, 1 ml colouring reagent (20 mg of 2, 4-dinitrophenylhydrazine in 1 N HCl, and make up to 100 ml) was added and the mixture was allowed to stand for 20 min at room temperature. Finally, 10 ml of 0.4 N NaOH was added and the extinction was read at 505 nm after 5 min against reagent blank.

To measure the activity of sAST, the assay system, containing 1.0 ml of buffer–substrate solution (0.1 M phosphate buffer, pH 7.4; 0.1 M aspartate and 2 mM 2-oxoglutarate) and 0.2 ml serum, was incubated for 60 min at 37°C in a water bath. After incubation, 1 ml of colouring reagent (20 mg 2,4-dinitrophenylhydrazine in 1 N HCl, make up to 100 ml) was added and then the mixture was allowed to stand for 20 min at room temperature before adding 10 ml of 0.4 N NaOH. The extinction was read at 505 nm after 5 min against reagent blank. Allyl alcohol induced liver necrosis in rats.

Allyl alcohol induced liver necrosis: The method described by Vogel and Vogel[8] for allyl alcohol induced liver necrosis was used. Rats were divided into following four groups (n= 6). Group I received the vehicle (water, 1 ml/kg p. o.) for 2 days and serves as control. The rats of group II also received vehicle for 2 days and served as toxic control. After 7 hours of fasting rats

of group III and IV were treated with aqueous extract at two dose level (400 and 800 mg/ kg) respectively. One hour later, the animal in group II, III and IV were dosed orally with 0.4 ml/ kg of 1.25% solution of allyl alcohol in water. On 2nd day after 16 hours of previous dosing treatment with aqueous extract was retreated. Of 3 rd day after 24 hours, liver were isolated. The necrosis area was measured using Zeiss stereomicroscope and necrosis index was determined by adding the necrosis area for each animal. For all the 3 days changes in body weight was recorded.

Acute oral toxicity: Acute oral toxicity study was performed using nulliparous female rats as per OECD (organization for economic co-operation and development) guideline 425. The rats were observed for mortality as per OECD guideline 425. LD_{50} was determined using AOT 425 software programme.

Statistical analysis: All the data obtained during the investigation are expressed as mean ± S.D. of total number of animals used in each group. Data were subjected to one-way analysis of variance (ANOVA) followed by Turkey test to find out the difference between each experimental group and the control group. Student's *t*-test was used to compare the difference between hepatotoxin alone treated group and the group given extract prior to the hepatotoxin. A level of significance of $P < 0.05$ was regarded as statistically significant.

Results

Thioacetamide induced liver damage in rats.

Effect on liver weight and liver volume: A significant increase in liver weight and liver volume was observed in thioacetamide treated groups as compared to control group. Treatment with aqueous extract (400 and 800 mg/ kg p. o) significantly decreases the physical parameter like liver weight and liver volume to normal value as compared to toxic control (Table 1).

Table 1: Effect of aqueous extract of *Sida retusa* roots (400 & 800 mg/kg) on liver weight and volume

Group	Treatment	Liver weight* (g)	Liver volume*(ml)
1	Control (Distilled water p. o.)	6.297 ± 0.07	7.93 ± 0.07
2	Thioacetamide (100 mg/kg s.c.) (toxic control)	8.621 ± 0.06#	11.00 ± 0.09#
3	Aqueous extract (400 mg/kg p.o.)	7.577 ± 0.13[a]	8.45 ± 0.10[a]
4	Aqueous extract (800 mg/kg p.o.)	6.840 ± 0.52[a]	7.50 ± 0.25[a]

*Values are mean ± S.D (n = 6). #$P < 0.05$ compared with control group. [a]$P < 0.05$ compared with toxic control group

Biochemical changes: A significant increase in serum ALT and AST level were observed in thioacetamide treated group as compared to control group. Pretreatment of rats with aqueous extract (400 and 800 mg/ kg p. o.) for 7 days significantly prevented thioacetamide induced increase in serum ALT and AST levels (Table 2).

Table 2: Effect of aqueous extract of *Sida retusa* roots (400 & 800 mg/kg) on serum ALT and AST levels

Group	Treatment	ALT (IU/L)*	AST (IU/L)*
1	Control (Distilled water p. o.)	50.97 ± 4.07	148.93 ± 6.07
2	Thioacetamide (100 mg/kg s.c.) (Toxic control)	115.0 ± 6.61#	248.93 ± 9.89#
3	Aqueous extract (400 mg/kg p.o.)	51.57 ± 5.93a	154.45 ± 4.50a
4	Aqueous extract (800 mg/kg p.o.)	47.84 ± 4.52a	144.50 ± 5.25a

*Values are mean ± S.D (n = 6). #$P< 0.05$ compared with control group. a$P< 0.05$ compared with toxic control group

Allyl alcohol induced liver necrosis in rats

Effect on body weight: A significant decrease in body weight was observed in allyl alcohol treated group as compared to control group. Treatment of rats with aqueous extract (400 and 800 mg/ kg p. o.) showed significant increase in body weight as compared to allyl alcohol treated group (Table 3).

Table 3: Effect of aqueous extract of *Sida retusa* roots (400 & 800 mg/kg) and allyl alcohol on body weight

Group	Treatment	Body weight (g))*		
		1st Day	2nd Day	3rd Day
1	Control (Distilled water p. o.)	135.9 ± 3.7	136.50 ± 4.01	136.93 ± 6.07
2	Allyl alcohol (1.25%, 0.4 ml/kg p.o.) (toxic control)	130.0 ± 6.4#	119.72 ± 7.2#	112.93 ± 9.89#
3	Aqueous extract (400 mg/kg p.o.)	133.7 ± 5.4a	128.51 ± 6.78 a	130.45 ± 4.50a
4	Aqueous extract (800 mg/kg p.o.)	136.4 ± 4.1a	136.23 ± 5.21a	137.50 ± 5.25a

*Values are mean ± S.D (n = 6). #$P< 0.05$ compared with control group. a$P< 0.05$ compared with toxic control group

Necrosis index: The necrosis index of group treated with allyl alcohol alone was found to be 2.94 ± 0.41. Treatment of rats with aqueous extract (400 and 800 mg/ kg) showed significant dose dependant decrease in the necrosis index as compared to toxic control group (Table 4).

Table 4: Effect of aqueous extract of *Sida retusa* roots (400 & 800 mg/kg) on necrosis index

Group	Treatment	Necrosis index*	% Decrease in necrosis index
1	Allyl alcohol (1.25%, 0.4 ml/kg p.o.) (Toxic control)	2.94 ± 0.41	
2	Aqueous extract (400 mg/kg p.o.)	1.37 ± 0.93[a]	47.45
3	Aqueous extract (800 mg/kg p.o.)	0.71 ± 0.52[a]	71.50

*Values are mean ± S.D (n = 6). [a]$P < 0.05$ compared with toxic control group

Acute oral toxicity of Extract: No mortality was observed with aqueous extract of *Sida retusa* (175, 550, 1750 and 5000 mg/kg p.o.). The LD_{50} was found to be greater than 5000 mg/ kg (Table 5).

Table 5: Acute oral toxicity of aqueous extract of *Sida retusa* roots

Group	Dose (mg/kg)	Short term results	Long term results
1	175	0	0
2	550	0	0
3	1750	0	0
4	5000	0	0
5	5000	0	0
6	5000	0	0

0= survived

Discussion

In the present investigation thioacetamide and allyl alcohol hepatotoxicity model were used. Both the methods were reproducible and reliable in evaluating the hepatoprotective action of plants[9]. Thioacetamide is a potent hepatotoxin and carcinogen in rats. Thioacetamide is known to produce marked liver damage in exposed animals. This is evidenced by enzymatic changes, which include elevation in the levels of serum ALT, AST and ASP. These findings were in agreement with those of earlier workers who reported similar biochemical changes[10].

Toxicity experienced by liver during thioacetamide poisoning results from the production of metabolite, thioacetamide S- oxide, which is direct hepatotoxin[11]. It has also been observed that thioacetamide causes changes in nucleolus and increased synthesis of guanine and cytosine rich RNA, with concomitant decrease in ribosomal RNA in the cytoplasm[12].

Physical parameter like liver weight and liver volume were restored to normal by treatment with aqueous extract (400 and 800 mg/ kg) dose dependently. In the present study pretreatment with aqueous extract was found to significantly reverse the thioacetamide rise in the biochemical parameter like AST and ALT level, there by demonstrating the membrane stabilizing activity of the extract. As per the previous reports, thioacetamide caused centrilobular necrosis with some degenerative changes[13]. As shown in Table 2, extract pretreatment lowered the activities of serum ALT and AST. The activities of sALT and sAST were almost brought down to normal suggesting the membrane stabilizing effect of the extract. The difference between group II (thioacetamide alone) and group IV (extract pretreated followed by thioacetamide) was found to be statistically highly significant. The level of sALP, which was elevated in group II, was also brought down in the rats pretreated with the extract followed by thioacetamide. The mechanism of observed hepatoprotective action of the plant extract against thioacetamide- induced liver damage is unclear.

Allyl alcohol induced hepatic toxicity is due to formation of a toxic metabolite acrolein by the activity of alcohol dehydrogenase, acrolein then react with cellular glutathione to form an aldehyde- GSH adducts which is metabolized to acrylic acid. It leads to structural and functional dearrangement, necrosis and irreversible injury to the liver tissue[14].

Significant dose dependent decrease in the necrosis index was observed by treatment of rats by aqueous extract (400 and 800 mg/ kg p. o.). Physical parameter observed i.e. change in body weight (restored to normal) by treatment with the extract. Thus physical changes and necrosis index were restored to normal by the treatment of rats with aqueous extract (400 and 800 mg/ kg p. o.) dose dependently in allyl alcohol intoxicated rats. From the above experimental data, it is evident that aqueous extract protection by acting through a mechanism a mechanism non specific to thioacetamide and allyl alcohol induced hepatotoxicity.

It is thus apparent that the extract (400 and 800 mg/ kg p. o.) of roots has a great potential for further evaluation as the LD_{50} of the extract is greater than 5000mg/ kg. However sub acute toxicity studies and chronic studies shall be required to prove the safety of drug. All the above result represents that *Sida retusa* has hepatoprotective effect against thioacetamide and allyl alcohol treated rats.

Conclusion

The present communication reports that an aqueous extract prepared from the roots of *Sida retusa* can protect against liver damage induced by thioacetamide and allyl alcohol. The hepatoprotective activity is shown by the normalization of various serum enzymes elevated in response to thioacetamide- induced liver damage. The investigation provides biochemical

evidence to validate the use of *Sida retusa* as a component of some hepatotonic preparations used by Ayuvedic physicians.

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