



Evaluation of Hepatoprotective Activity of Ethanolic Extract of *Sida Rhombifolia* Against CCl₄ Induced Hepatotoxicity in Rats

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Received: 14 Jan 2019 / Accepted: 12 Mar 2019 / Published online: 1 Apr 2019
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Abstract

Sida rhombifolia is a valuable herb belonging to the family Malvaceae, and widely distributed in world. It is popularly known as “Arrow leaf” and various parts of the plant are used in the treatment of skin problem, bronchitis, antifungal, eye-disease, cancer, diabetes, digestive, diuretic, emetic, expectorant, laxative, stimulant, stomachic, uterine tonic and urinogenital infection etc. Here in this study an attempt was made to evaluate the Hepatoprotective activity of ethanolic extract of leaves of *Sida rhombifolia* (200, 400mg/kg). Hepatotoxicity was induced in albino rats using Carbon tetrachloride (CCl₄ - 0.5ml/kg in 1:1 ratio with Olive oil), and Silymarin (50mg/kg) was taken as the Standard Drug. The biological markers like Aspartate Transaminase, Alanine Transaminase, Alkaline Phosphatase, Total bilirubin and Total Protein are monitored for changes caused due to CCl₄ induced Hepatotoxicity. It was observed that the leaf extract of *Sida rhombifolia* decreased the elevated levels of the hepatic enzymes significantly when compared to the standard (p<0.001). Thus the results suggest that the ethanolic extract of leaf of *Sida rhombifolia* is a reliable Hepatoprotective agent.

Keywords

Hepatoprotective activity, Silymarin, CCL₄, *Sida rhombifolia*, Ethanolic extract.

INTRODUCTION

The liver is a vital organ in the body and is primarily responsible for the metabolism of endogenous and

exogenous agents¹. It plays an important role in drug elimination and detoxification and liver may be damaged by xenobiotics, alcohol consumption,

malnutrition, infection, etc.,². Hepatotoxicity is defined as injury to the liver that is associated with impaired liver function caused by exposure to a drug or another non-infectious agent³. Number of studies shown that the plant extract protect against CCl₄ hepatotoxicity by inhibiting lipid peroxidation and enhancing antioxidant enzyme activity^{4,5}. Presently only a few Hepatoprotective drugs available for the treatment of liver diseases⁶.

Sida rhombifolia is a valuable herb belonging to the family Malvaceae, and widely distributed in India. *S. rhombifolia* is a potent Anti-inflammatory plant and used in The roots and leaves are sweetish, aphrodisiac, tonic, remove "tridosha", good in urinary complaints, discharges and strangury. These are also useful in fever, heart diseases, burning sensations, piles and all kind of inflammations (Ayurveda). The plant in combination with other drugs is prescribed as an antidote to snake venom (Charaka) and scorpion venom (Charaka, Sushruta). The root is held in great repute in the treatment of rheumatism. The Mundas apply the pounded leaves on swellings. In Assam the roots are taken internally to help child birth. The herb is also tied round the abdomen for the same purpose. The stems are abounding in mucilage and are employed as demulcent and emollients both for external and internal use. In Europe, the plant has been regarded as a valuable remedy in pulmonary tuberculosis and rheumatism. In Madagascar the plant is mostly used as an emollient and the infusion of the root is given in dysentery. The leaves are pounded and applied to tumors or chewed and applied to boils. Antimicrobial Activity, Anti-inflammatory Activity, Antispasmodic Activity, Hypotensive Activity, Uterine Stimulant Effect, CNS Effect and Antimalarial Activity.

MATERIALS AND METHODS

Chemicals: Silymarin (Sigma), carbon tetrachloride and all other reagents used were of analytical grade. Diagnostic kits used in this study were procured from Citron scientific., India and Finar chemicals., India.

Instruments: Centrifuge, Soxhlet apparatus, Rotatory flash evaporator, Autoanalyser.

Preparation of the Plant powder and extraction: *Sida rhombifolia* aerial parts were collected from East Godavari district, Yeleswaram, Andhra Pradesh. The plant was identified and authenticated by Prof. Dr. M. Prayaga Murthy Pragada, Department of Botany, Govt Degree College, Yeleswaram, East Godavari district, Andhra Pradesh, India. After authentication, *Sida rhombifolia* leaves (2kgs) were extracted with 95% ethanol using a soxhlet apparatus. The Ethanolic extract was filtered and concentrated by distillation

process. A brownish green colored residue was obtained (yield 6.79% w/w) and was kept in desiccators. This Ethanolic extract of *Sida rhombifolia* (SREE) was used for further experiments.

Animals: Male albino rats were used for the study. The animals were housed in groups of six and maintained under standard conditions (27±2°C, relative humidity 44 - 56% and light and dark cycles of 10 and 14 hours respectively) and fed with standard rat diet and purified drinking water ad libitum for 1 week before and during the experiments. All experiments and protocols described in present study were approved by the Institutional Animal Ethical Committee (IAEC) of MAM college of pharmacy with permission from Committee for the purpose of Control and Supervision of Experiments on Animals. All the experiments were performed in the morning according to current guidelines for the care of laboratory animals and the ethical guidelines for the investigation of experimental pain in conscious animals.

EXPERIMENTAL DESIGN

Hepatoprotective activity against CCL₄: After acclimatization for a week the animals are divided into 5 groups each with 6 animals. Group-I serve as the control and was administered with water daily. Group-II, III, IV, V were all treated with CCl₄ (0.5ml/kg i.p.1:1 in olive oil) on the 7th, 9th, 11th, 13th day of the experiment. Group-II acts as disease control as it is only administered with CCl₄. Group-III served as standard as it was administered Silymarin (50mg/kg p.o.) daily. Group-IV served as the Test- I as it was administered low dose of *Sida rhombifolia* ethanolic leaf extract (200mg/kg p.o.) dissolved in water daily all along 14 days. Group-V served as Test-II as it was administered high dose of *Sida rhombifolia* ethanolic leaf extract (400mg/kg p.o.) dissolved in water daily all along 14 days. On the 14th day 1hour after the treatment blood was collected from all animals by Retro orbital puncture for serum analysis. The blood is centrifuged for 10 mins at 1000 rpm and then serum is used to estimate the AST, ALT, ALP, TB and TP using commercially available test kits.

Experimental group design

Group-1: Control.

Group-2: Hepatotoxic rats with CCl₄+ Olive Oil (1:1ratio).

Group-3: standard Silymarin (50mg/kg p.o.)

Group-4: Rats having hepatotoxicity treating with extract of *sida rhombifolia* Low dose (200mg/kg p.o.).

Group-5: Rats having hepatotoxicity treating with extract of *sida rhombifolia* High dose (400mg/kg p.o.).

RESULTS:

Effect of ethanolic extract of *Sida rhombifolia* on biochemical parameters in Carbon tetrachloride induced hepatotoxic rats: Rats treated with Carbon tetrachloride developed a significant hepatic damage observed as elevated serum levels of hepatospecific enzymes like ALT, AST, ALP and TB when compared to normal control. Pretreatment with Silymarin, ethanolic extract had showed good protection against CCl₄ induced toxicity to liver. Test indicates a

significant reduction in elevated serum enzyme levels with extract treated animals compared to toxic control animals which are evident in table 1 and figure 1, 2, 3,4. Carbon tetrachloride treatment has considerably reduced serum total protein levels. Pretreatment with Silymarin and ethanolic extract of *Sida rhombifolia* showed a significant increase in total protein levels as compared with toxicant group. This is evident by table no.1 and figure 5.

Table - 1: Effect of ethanolic Extract of *Sida rhombifolia* on ALT, AST, ALP, TB & TP.

GROU P	TREATME NT	ALT(SGPT) (U/L)	AST(SGOT) (U/L)	ALP (U/L)	TOTAL BILIRUBIN(gm/ dl)	TOTAL PROTEIN (gm/dl)
I	Control	51.79±0.215	53.48±0.09	61.93±0.65	0.71±0.04	2.56±0.87
II	Toxic	115.75±0.70*	112.73±0.21*	121.63±0.77*	1.75±0.17	0.91±0.01**
III	Standard	63.24±0.023*	58.25±0.14**	71.85±0.81**	0.83±0.009	2.02±0.07**
IV	Test-1	73.51±0.22**	79.19±0.04**	83.84±0.82**	0.99±0.07	1.56±0.006*
V	Test-2	55.20±0.27**	52.38±0.11**	65.97±19.34*	0.85±0.004	2.12±0.02**

Values are expressed as mean±S.E.M, n=6, *P<0.05, **P<0.01, ***P<0.001 considered for significance (ANOVA followed by tukey's test).

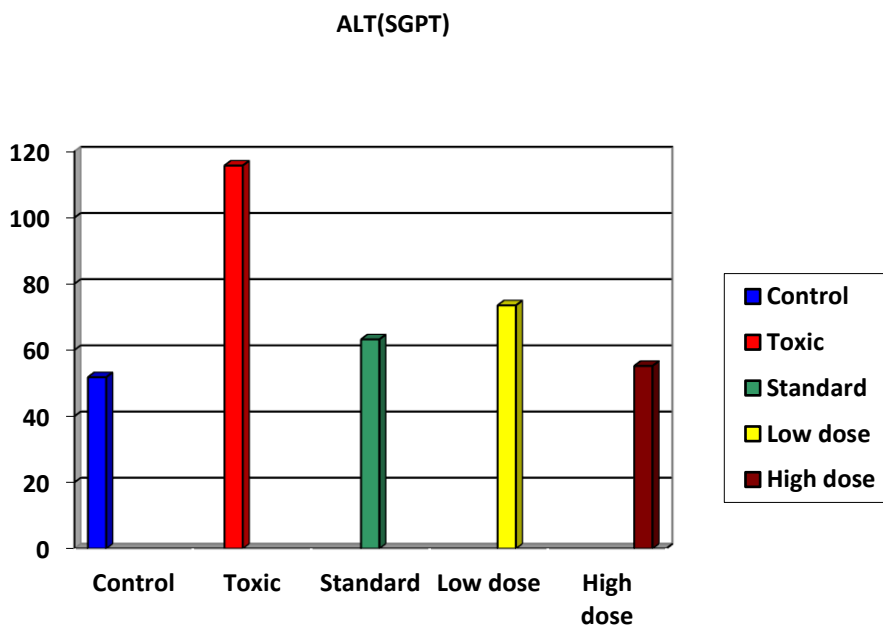


Fig.1: Effect of Ethanolic Extract of *Sida rhombifolia* on ALT levels in CCl₄ induced hepatotoxic rats.

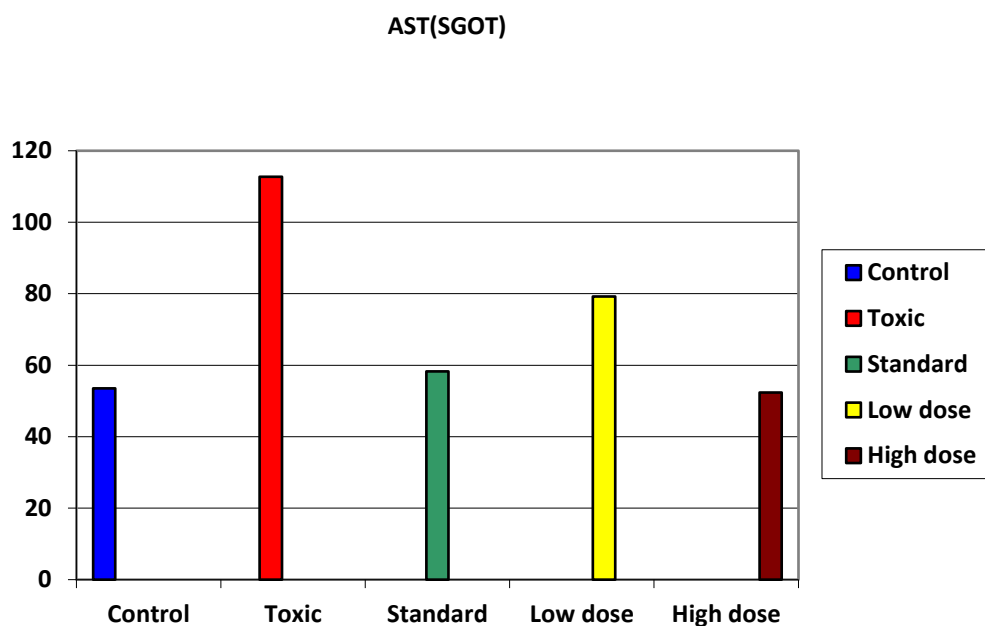


Fig.2: Effect of Ethanolic Extract of *Sida rhombifolia* on AST levels in CCl₄ induced hepatotoxic rats

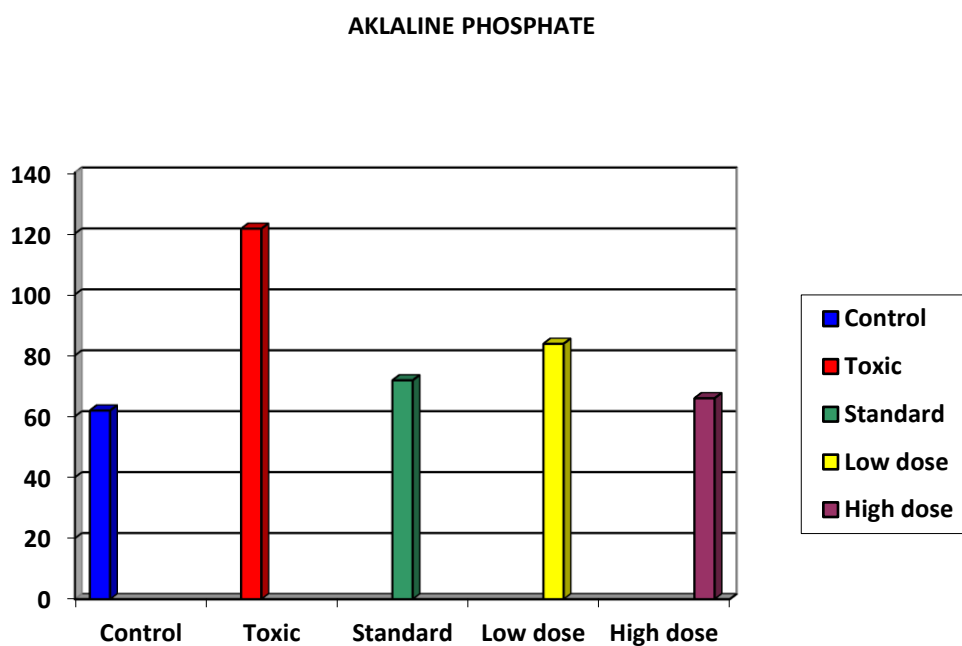


Fig.3: Effect of Ethanolic Extract of *Sida rhombifolia* on ALP levels in CCl₄ induced hepatotoxic rats.

TOTAL BILIRUBIN

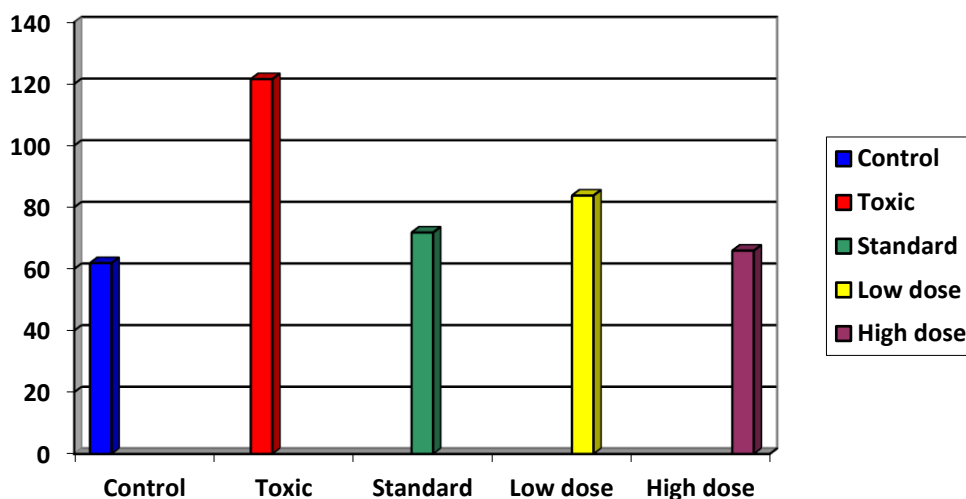


Fig.4: Effect of Ethanolic Extract of *Sida rhombifolia* on BILIRUBIN TOTAL levels in CCl₄ induced hepatotoxic rats.

TOTAL PROTEIN

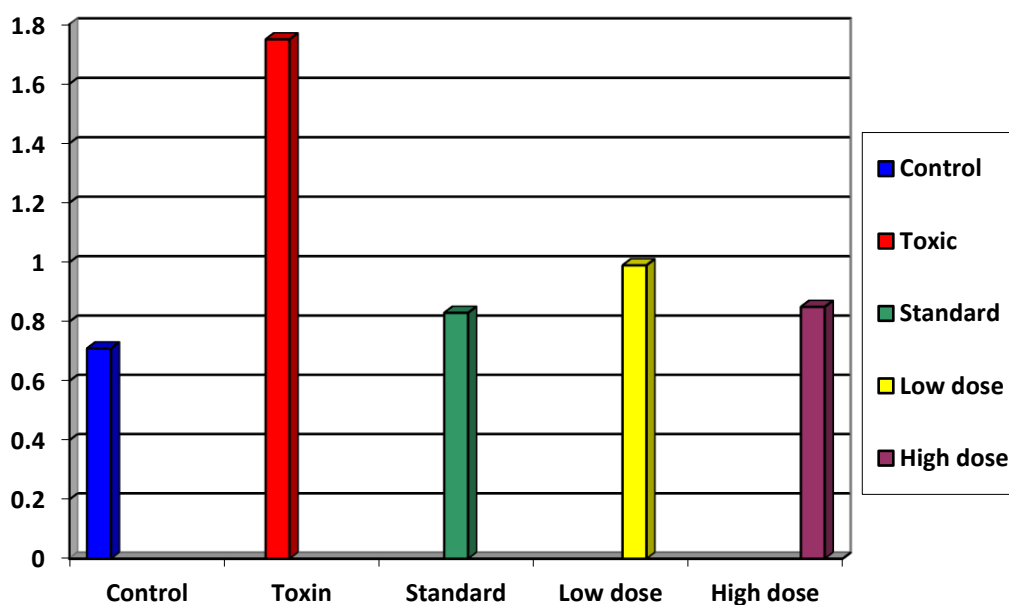


Fig.5: Effect of Ethanolic Extract of *Sida rhombifolia* on TOTAL PROTIEN levels in CCl₄ induced hepatotoxic rats.

DISCUSSION

Sida rhombifolia was selected for the present investigation. The *Sida rhombifolia* is traditionally

used in the treatment of inflammation, fever, hepatitis, tight chest, bronchitis, asthma, dysentery. In the present investigation, the ethanolic extract of

leaves of *Sida rhombifolia* was screened for hepatoprotective activity in rats. In the acute toxicity study no mortality occurred within 14 days up to a dose level of 2000 mg/kg b.w.p.o with ethanolic extract of plant. The LD₅₀ was, greater than 2000 mg/kg in mice. Hence, the pharmacological studies of this extract was carried out with 200 and 400 mg/kg.b.w.p.o

In recent years, many studies have been undertaken with Indian medicinal plant, in an attempt to develop safe and effective herbal drug for the treatment of liver disorders. In the present study, carbon tetrachloride was used for inducing liver damage, to investigate whether these plant extract could decrease efficiently. The results of the studies demonstrate that the various biochemical changes produced in serum as well as histological changes of liver by CCl₄ toxicity was prevented or reversed by administration of *Sida rhombifolia* extract. The CCl₄ administration to rats leads to marked elevation in the levels of serum ALT, AST and ALP. This might be due to the release of these enzymes from the cytoplasm of hepatic cells, into the blood circulation rapidly after rupture of the plasma membrane and cellular damage resulting from the CCl₄ - induced lipid peroxidation. Treatment with 200, 400 mg/kg.b.w.p.o significantly reduced the levels of these marker enzymes in CCl₄ treated rats. The decrease in the levels of these enzymes may be a consequence of the stabilization of plasma membrane as well as repair of the hepatic tissue damage caused by CCl₄.

In CCl₄ induced hepatotoxicity, elevated serum TB level is due to defective excretion of bile by the liver indicates the loss of integrity of the liver and necrosis. This leads to increase in the binding, conjugating and excretory capacity of hepatocytes, which is proportional to the erythrocyte degradation rate. At both the test doses showed a significant depletion in the serum bilirubin levels suggesting the possibility of the extract ability to stabilize. Biliary dysfunction of rat liver during injury with CCl₄ in prophylactic and curative studies. In CCl₄ hepatotoxicity, a depression in total protein occurs due to the disruption and dissociation of polyribosome on endoplasmic reticulum leading to defective protein biosynthesis. In hepatoprotective studies with 200, 400 mg/kg doses increased the serum TP and ALB levels with varying degree of significance. This may be due to the promotion of the assembly of ribosomes on endoplasmic reticulum to facilitate uninterrupted protein biosynthesis. The CCl₄ intoxication in rats causes excessive liver damage and oxidative stress.

The overall observation made on the biochemical parameters revealed that the extract has better hepatoprotective activity with the higher dose that is 400 mg/kg b.w.p.o., comparable to that of reference drug, Silymarin (50 mg/kg). It is evident from the results that the biochemical observation of the studies was complementing each other. This confirms that the extract of the selected plant has hepatoprotective effect in CCl₄ induced hepatotoxicity in rats by their ability to stabilize cell membranes, scavenge free radicals and Hepatoprotective properties. The results of the study substantiate the role of hepatoprotective properties of the phytochemicals in showing their hepatoprotective effect in CCl₄-induced hepatotoxicity in rats. It is well knowing that plant extract contains a number of chemicals belonging to the different classes, most of this is of pharmacological importance such as flavonoids, phytosterols, saponins, terpenoids and phenolic compounds, etc.

CONCLUSION

Sida rhombifolia was found to be safe and no toxicity was exhibited in mice up to 2g/kg.b.w.p.o Plant *Sida rhombifolia* was found to have potential protective effects at a dose 400 mg/kg b.w.p.o against CCl₄-induced hepatotoxicity in rats. The hepatoprotective effect of extract was well comparable to that of a known standard hepatoprotective drug, Silymarin (50 mg/kg). The plant extract was found to possess liver protection, which strongly supports their hepatoprotective effect. Thus, to conclude, the plant *Sida rhombifolia* was endowed with hepatoprotective properties. Since plant extract possess promising protective effect against various hepatotoxins. It needs comprehensive investigations for developing it as safe and effective liver protecting drugs.

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