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Evaluation of Hepato-Protective Activity in the Ethanolic Extract of *Sida rhombifolia* Linn. against Paracetamol - Induced Hepatic Injury in Albino Rats

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ABSTRACT

The scientific evaluation of medicinal plants used in the preparation of folk remedies has provided modern medicine with effective pharmaceuticals for the treatment of diseases. The ethanolic extract of *Sida rhombifolia* Linn (Malvaceae) whole plants were investigated for its hepatoprotective effect on paracetamol (2g/kg/b.wt/p.o suspended in 0.5% CMC) induced acute liver damage in Wistar albino rats. Hepatoprotective activities were measured by diagnostic marker enzymes such as AST, ALT, ALP, bilirubin, albumin and total protein in serum. The ethanolic extract of *Sida rhombifolia* (SRE) at the dose of (100 & 200mg/kg/p.o) produced significant hepatoprotective effect by decreasing the activity of serum enzymes, bilirubin and proteins. The effects of ethanolic extract of *Sida rhombifolia* were comparable to that of standard drug Silymarin. These results suggest that ethanolic extract of *Sida rhombifolia* have potential therapeutic value in the treatment of some liver disorders in albino (Wistar) male rats.

Keywords: *Sida rhombifolia*, Hepatoprotective effect, Paracetamol

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INTRODUCTION

Liver is the most important organ, which plays a pivotal role in regulating various physiological processes in the body. It is involved in several vital functions such as metabolism, secretion and storage. It has great capacity to purify toxic substances and synthesize useful principles; therefore, damage to the liver inflicted by hepatotoxic agents is of grave consequences [1].

Liver diseases are mainly caused by toxic chemicals, excessive consumption of alcohol, infections and autoimmune disorders. Now a day's drug-induced liver toxicity is a common cause of liver injury. It accounts for approximately one-half of the cases of acute liver failure and mimics all forms of acute and chronic liver disease [2].Different types of drugs such as acetaminophen, Chloroquine, Rifampicin and Isoniazid are inducing hepatotoxicity in world [3]. The rate of hepatotoxicity has been reported to be much higher in developing countries like India (8% - 30%) compared to that in advanced countries (2% - 3%) with a similar dose schedule [4].

Herbal medicines derived from plant extracts are being increasingly utilized to treat a wide variety of clinical disease [5]. More attention has been paid to the protective effects of natural antioxidants against drug-induced toxicities especially whenever free radical generation is involved. Plant drugs are known to play a vital role in the management of liver diseases. There are numerous herbal formulations (Liv 42, Liv-52, Liver cure, Livol, Livomyn, Livfit, Livogen and Livactine) claimed to have hepatoprotective activities. In India, more than 87 medicinal plants were used in different combinations in the preparation of 33 patented herbal formulations [6-9]. In spite of tremendous advances in modern medicine, there are no effective drugs available that stimulate liver function/offer protection to the liver from damage or help to regenerate hepatic cells. Aiming these factors the present of investigation was undertaken to evaluate the hepatoprotective activity in ethanolic extract of *Sida rhombifolia* Linn. (SRE) in paracetamol induced hepatotoxicity in albino (Wistar) male rats model.

MATERIALS AND METHODS

Plant material

Sida rhombifolia Linn (Malvaceae) is 50-120cm, small gregarious shrub, the leaves are dark green, diamond-shaped, arranged alternately along the stem, 4-8cm long, ovate or ovate-oblong, obtuse or sub acute at apex. The flowers are moderately delicate, flowers occur singly on flower stalks (peduncles) that arise from the area between the stems and leaf petioles, fruits are ribbed capsule, which breaks up into 8-10 segments. The seeds are trigonous, glabrous and tufted-pubescent near the hilum.

The whole plant of *Sida rhombifolia* (young matured) were collected from the rural belt of Tirunelvelli District in Tamilnadu, during the month of September (2011) and identified by

the botanist of Department of Botany, Annamalai University, Tamilnadu by comparing with the voucher specimen present in the herbarium. After authentification fresh plant materials were collected in bulk, washed under running tap water to remove adhering dust, dried under shade and pulverized in a mechanical grinder. The coarse powders were extracted with 70%v/v of ethanol in a soxhlet apparatus, evaporated to dryness and stored in desiccators were used for further studies.

Experimental animal:

The institutional animal ethics committee (Register No.160/1999/CPCSEA), Annamalai University, Annamalai Nagar, India; approved the experimental design. Albino (Wistar) male rats of 150-200g (weight) were used for the study. Animals were housed in well ventilated room (temperature 23±2^{o}C, humidity 65-70% and 12h light/dark cycle) at Central Animal House, Department of Experimental Medicine, Rajah Muthiah Medical College and Hospital, Annamalai University. Animals were fed with standard pellet diet and water ad libitum. All studies were conducted in accordance with Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) norms and the National Institute of Health guidelines "Guide for the Care and use of Laboratory Animals".

Drugs and Chemicals

Silymarin, paracetamol were purchased from Micro labs Ltd., Bangalore, India, Carboxy Methyl Cellulose (CMC) was purchased from S.D. Fine Chemicals Ltd., Mumbai, India ethanol was purchased from CHANGSHU YANGYUAN CHEMICAL,CHINA, other solvents/reagents were analytical grade.

Paracetamol-Induced Liver Damage in Rats (Acute Model)

Five groups (I - V) each comprising of six albino (Wistar) male rats of 150-200g were selected. Group I served as control and received orally 0.5% Sodium CMC (1 ml each) for seven days. Groups III & IV rats received oral dose 100 and 200 mg/kg SRE respectively for 7 days. Group V Rats received oral dose of Silymarin (25mg/Kg body wt) for seven days. Paracetamol at a dose of 2gm/KG body wt p.o were administered on the 8th day to all animals in groups of II, III, and IV & V.

After 48 hrs administration of paracetamol dosing the rats were sacrificed cervical decapitation under Xylazine + Ketamine (16 + 100 mg/kg i.m.), blood samples were collected via abdominal aorta puncture the serum separated were used for the determination of diagnostic marker enzymes such as AST, ALT, ALP, bilirubin, albumin and total protein levels were analyzed in Secomam semi auto analyzer. [10-13].The results were expressed as mean ± SEM; differences in mean were estimated by means of ANOVA followed by "Dunnet's post hoc" test.

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Table: 1. Effect of SRE on biological parameters.

Groups	AST (U/L)	ALT (U/L)	ALP (U/L)	Total Bilirubin (mg/100ml)	Albumin (g %)	Total Protein (mg/dL)
Group I (Normal control)	55.30± 0.315	24.21± 0.496	96.10± 0.194	0.88± 0.008	3.69± 0.14	5.14± 0.035
Group II (Paracetamol control)	147.02 ±1.278***	61.11± 2.287***	392.13± 4.211***	2.16± 0.025***	1.47± 0.25***	3.13± 0.068***
Group III (SRE group)	62.43± 1.304***	28.36± 0.045***	141.26± 6.353***	0.94± 0.04**	2.92± 0.08**	4.01± 0.023***
Group IV (SRE group)	57.6± 1.352***	25.81± 0.344***	104.45± 5.697***	0.90± 0.008***	3.57± 0.04***	5.02± 0.026***
Group V (Standard Drug)	51.14± 1.254***	22.73± 0.547***	93.42± 4.354***	0.82± 0.37***	3.84± 0.12***	5.10± 0.045***

Values are mean ± SEM; n=6 in each group. Percentage inhibition/elevation compared to control. Group III, IV and V were compared with Group II; Group II was compared with Group I. Values are statistically significant at ** P< 0.01;*** P< 0.001.

DISCUSSION

Paracetamol is a known antipyretic, analgesic drug which produces hepatic necrosis at high doses and normally eliminated as sulfate and glucuronide conjugate. Administration of toxic doses of paracetamol the sulfation and glucuronidation routes become saturated and hence, higher percentages of paracetamol molecules are oxidized to highly reactive N-acetyl-p-benzoquinemine by cytochrome-450 enzymes. The Semiquinone radicals, obtained by one electron reduction of N-acetyl-p-benzoquineimine, can covalently binds to macromolecules of cellular membrane which increases the lipid peroxidation resulting in the tissue damage. Higher doses of paracetamol and N-acetyl-p-benzoquineimine can alkylate, oxidise intracellular GSH, results in the depletion of liver GSH pool subsequently leads to increased lipid peroxidation there by causes liver damage [14].

In the assessment of liver damage by paracetamol the determination of enzyme levels such as AST, ALT, ALP, bilirubin, albumin and total protein were largely used. Liver necrosis or membrane damage releases the enzyme into circulation which can be measured in serum. A high level of AST indicates liver damage, as well as cardiac infarction and muscle injury. ALT catalyses the conversion of alanine to pyruate and glutamate and is released in a similar manner. Therefore, ALT is more specific to the liver, and is thus a better parameter for detecting liver injury. Elevated levels of serum enzymes were indicative of cellular leakage and loss of functional integrity of cell membrane in liver. Serum ALP and bilirubin level on other hand are related to the function of hepatic cell. Increase in serum level of ALP is due to increased synthesis, in presence of increasing biliary pressure.

This present study evaluated the hepatoprotective effects of ethanolic extract of *Sida rhombifolia* (SRE) in paracetamol induced liver toxicity. Acute administration of paracetamol produced significant elevation in serum - AST, ALT, ALP & total bilirubin levels; and significant decrease in serum total protein and serum albumin level were found in toxic control group, when compared with the normal control group.

Treatment with SRE decreased the elevated serum levels of AST, ALT, ALP and total bilirubin levels towards the respective normal value that is an indication of stabilization of plasma membrane as well as repair of hepatic tissue damage caused by paracetamol. The serum total protein, serum albumin was almost approaching normal values and comparable with the results observed in standard group. The above changes can be considered as an expression of the functional improvement of hepatocytes, which may be caused by an accelerated regeneration of parenchyma cells.

Silymarin is a known hepatoprotective compound obtained from Silybum marianum is reported to have a protective effect on plasma membrane of hepatocytes and possess multiple mechanisms of actions against different hepatotoxic agents. The antioxidant property and cell regenerating functions as a result of increased protein synthesis were considered as most important actions [17]. The above results suggest that ethanolic extract *Sida rhombifolia* treated rats (100 and 200mg/kg) has gained normalcy against the hepatocellular injury caused by paracetamol during the 7 day treatment period and both dose levels were found almost equipotent. The result of these investigations was comparable and matches the previously reported protective effects of other plants. [15, 16]

From the results it was concluded that the ethanolic extract of *Sida rhombifolia* has significant action on paracetamol induced hepato-toxicity. Literature review shows that the *Sida rhombifolia* contains phenolic compound and flavanoids which may possess the possibility of hepatoprotective activity.

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