

Effect of ethanol extract from *Shorea robusta* (Dipterocarpaceae) bark in paracetamol induced liver damage in rats

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Abstract

The Hepato protective Activity of the ethanol extract of *Shorea robusta* was investigated against paracetamol induced hepatic damage. Paracetamol at the dose of 3gm/ kg produced liver damages in rats manifested by the significant ($p < 0.0005$) rise in the level of SGOT, SGPT, ALP, (159.3 ± 3.637 ; 143.1 ± 1.215 ; 347.6 ± 15.42) compare to with respective control values (51.85 ± 1.527 ; 26.65 ± 1.095 ; 84 ± 9.824) respectively. Pre-treatment of rats with the plant extracts 200mg /kg, 400mg/kg and standard (Silymarin 50 mg/ kg) lowered significantly ($p < 0.0005$) respective serum (SGOT to 92.06 ± 2.473 & $73.97 \pm$; SGPT to 64.12 ± 2.27 & 45.22 ± 0 ; ALP to 7814; 195.8 ± 13.22 & 168.00 ± 10.16) respectively. It also shows in the reduction of cholesterol and total bilirubin respect to the paracetamol toxicity. In case of total protein paracetamol treated group decrease the total protein content, pre treatment with the plant extracts and silymarine there is an elevation of total protein contents. Histopathology of the liver cell shows less damages in the hepatic cell compare to the paracetamol treated group. On the basis of the investigation we may partially conclude that *S. robusta* can use to damage hepatic cell injury.

Keywords: Shorea robusta, Hepatoprotective, Paracetamol, Silymarin.

Introduction

The indigenous medicinal plants in the North East India are useful folk medicines. *Shorea robusta* is widely distributed in North Eastern state Tripura and throughout India [1]. *S. robusta* is a large such deciduous tree seldom quite leaflets found extensively among North East and Central India. Bark is reddish brown grey in colour and smooth and longitudinally a fissured. The plant is using for dysentery and for the plaster of fumigation. It is commonly given for weak digestion, gonorrhoea, and as an aphrodisiac [2, 3]. This plant is reported for various activity like, analgesic, [4] anti nociceptive & anti inflammatory [5], oxidative stress [6], Wound healing [7], anti ulcer [8]. The liver is the chemical factory which regulates to synthesizes, store and secretes important macromolecules in the body. As a result of that the healthy functioning of the liver determines the health status of an individual [9]. Liver regulates in synthesizing, store and secretes important macromolecules in the body. It has a strategic anatomical location and large capacity for metabolic transformation of the drug and other toxin entering from the gastro intestinal tract. As a result of that the healthy functioning of the liver determines the health status of an individual. Liver diseases are a global problem and the synthetic drugs are available for the treatments of liver disorders are believed to serious adverse effect on biological system [10]. Numerous medicinal plants and their formulation are used for liver disorder in ethno medicinal practice as well as traditional system of medicine in India [11]. Our aim is to determine the hepato

protective activity of Ethanol extract from *Shorea robusta* in paracetamol induced hepatic rats.

Materials and Method

Plant materials

Plant materials were collected from the forest of the Gomuti Dist. of Tripura and identified by Forest dept. of Tripura in the month of June 2012. Plant materials were identified and authenticated by Prof. P. Jayaraman, M.Sc., Ph.D; Director Plant anatomy research Centre, Tambaram, Chennai. Voucher specimens (No. PARC/2012/ 1276) was deposited for further reference. Plant materials (bark) were dried under shade, and make coarse powder by pulverized in our college laboratory.[12]

Preparation of extracts & Phyto chemical studies

The powder was defatted first in soxhlet apparatus with Pet. Ether for 18h and marc was subjected to extract with chloroform & 70% ethanol [13] respectively for 18 h. The extract was dried at 55°C in a vacuum distillation till condensed and the dried in hot air oven at 45°C till the solid mass obtained and kept in desiccators to get free from moisture. These were stored in bellow 10°C . Various phyto constituents present in the various extract was detected by respected chemical test [14, 15.]

Experimental animals



Wister albino rats either sex were used throughout the experiments. After randomization into various groups and before initiation of experiment the rats were acclimatized for a period of 7 days. Standard environmental condition such as temperature ($26 \pm 2^\circ\text{C}$) relative humidity (45-55%) and 12 hr dark/ light cycle were maintained in the quarantine. All the animals were fed with synthetic diet and water was allowed *ad-libitum* under strict hygienic condition. The entire animals were utilized for this study used according to the protocol approved by (No. IAE / SKIPS / 2012/ MAY08/ 1/ 12/ RAT-96/ MICE-36) Institutional Animal Ethics Committee.

Induction of hepatic injury

Certain drugs are also responsible for chronic hepatic disease such as chronic hepatitis fatty liver cirrhosis, and several vascular lesions of the liver [16]. The mechanism of acute hepatic injury depends upon the chemical compound and the species of animals used. We have studies hepato protective activity against paracetamol induced hepato toxicity. Paracetamol is one of the most powerful hepato toxins in terms of severity injury. It causes hepatic injury leading to biochemical changes having clinical features similar to those of acute viral hepatitis [17].

Experimental Design

Five groups of rats with six rats in each group are used for the study. The experimental groups were as follows:

Group I (Control): Were receive 2% gum acacia dissolved in distilled water at 1, 24 & 48 hrs P.O

Group II (Hepato toxic): Were administered with paracetamol (3 gm/ kg) ²¹ as a single dose on day one and received distilled water at 1, 24 & 48 hr P O following paracetamol administration.

Group III (Test group): Were administered paracetamol (3 gm/ kg) as a single dose on day one and received ethanol extracts (70%) of *S. robusta* (200mg/kg) at 1, 24 & 48 hr P.O following paracetamol administration.

Group IV (Test group): Were administered with paracetamol (3 gm/ kg) as a single dose on day one and received ethanol extracts of *S.robusta* (400mg/kg) at1, 24 & 48 hr P.O following paracetamol administration.

Group V (Standard drug): Were administered with paracetamol (3 gm/ kg) as a single dose on day one and received Silymarin (50mg/kg) at 1, 24 & 48 hr P O following paracetamol administration.

Screening of Anti diabetic Potency of Ethanol Extract of *S. robusta*

Haematological & biochemical analysis

For the haematological and biochemical analysis blood sample was collected by retro orbital puncture after 72 hr form overnight

fasted rats. Blood sample was centrifuge at 4000 rpm by Remi research centrifuge for 15 minutes. The plasma collected & plasma used for estimations of SGOT, SGPT, ALP, Cholesterol, Total protein & Total bilirubin by spectrophotometer assay by using Mispa semi auto analyser according to the Agappe diagnostic kits instruction manual [22,23].

Histopathological studies

Liver sample was immediately collected and fixed in 10% buffered formaldehyde solutions for a period at least 24 hr before for histopathological study. Samples were then embedded in paraffin wax with automatic tissue processors and five micron sections were prepared by using rotary microtome. This thin section was stains with hematoxylin & eosin and mounted on glass slides with canada balsam [24]. Degrees of liver damages were estimated at light microscope (Magnus lab photo microscope) in 10 X. The grades in liver damage to different group were assigned by numerical scores. The liver tissue was collected fixed in 10% formalin and stained with hematoxylin and eosin for photo microscopic observations.

Statistical analysis

All values are expressed as a Mean \pm SEM (n=6) One Way Analysis of Variance (ANOVA) followed by multiple comparison Tukey test. The minimum value of $p < 0.05$ was considered as significant. The results of diabetic control group was significant at $***p < 0.001$ compared to normal group and results from test groups was significant at $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ compared to hepatic control group.

Results

Hepatoprotective activity

Haematological & biochemical analysis

Administration of paracetamol causes a significant ($P < 0.05$) elevation of enzyme levels such as SGOT (Figure1A), SGPT (Figure1B), ALP (Figure 1C), Total Bilirubin (Figure 1D), serum cholesterol (Fig1E) level and decrease in total protein (Figure 1F) when compared to that of the control. There was a significant ($P < 0.05$) restoration of these enzyme levels on administration of the bark extract in a dose dependent manner and also by silymarin at a dose of 50 mg/kg. The reversal of increased serum enzymes in paracetamol induced liver damage by the extracts from *S.robusta* may be due to the prevention of the leakage of intracellular enzymes by its membrane stabilizing activity. Effective control of ALP, bilirubin and total protein levels points towards an early improvement in the secretory mechanism of the hepatic cells. The result is shown in the table: 1



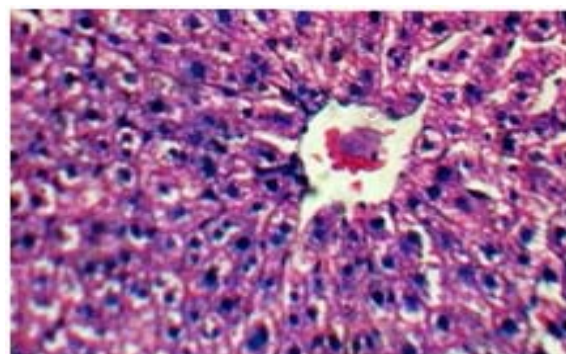
Table I: Effect of ethanolic extract of *Shorea robusta* on SGOT, SGPT, ALP, Cholesterol Total Protein, and Total Bilirubin.

GROUP	ALP	SGOT	SGPT	Cholesterol	Total protein	Total bilirubin
Control	184.900± 9.824	51.850± 1.527	26.65± 1.098	136.6± 0.7148	7.808± 0.4823	0.2953± 0.0176
Toxic	347.01± 15.42	159.3± 3.637	143.1± 1.215	209.5± 2.805	4.080± 0.124	2.465± 0.1340
Standard	130.9± 17.34*	66.18± 2.160***	32.49± 1.732***	162.9± 1.985***	6.188± 0.4194**	0.5633± 0.06946**
Dose I	195.80± 13.22***	92.06± 2.473***	64.12± 2.270***	187.4± 3.114***	4.442± 0.1905*	1.627± 0.06946**
Dose 2	168.0± 10.16***	73.97± 2.54***	45.22± 0.7814***	170.0± 1.497*	6.025± 0.1686**	0.5455± 9.07688**

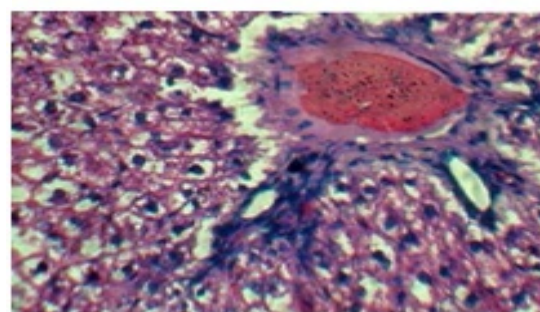
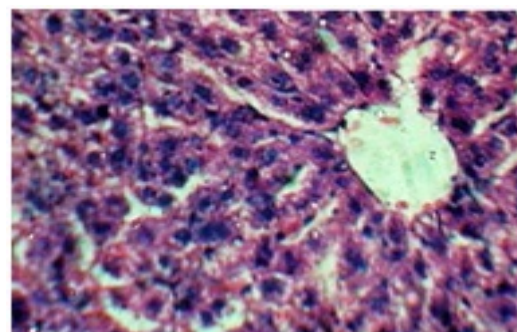
Values expressed as Mean ± SEM (n=6). *P< 0.05VsParacetamol treated group. ns = : significant; compared to paracetamol group. One way ANOVA followed by Dunne multiple comparison tests.

Histological parameter

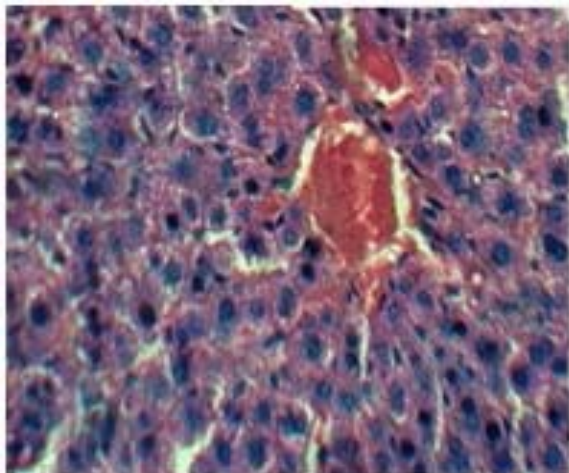
Histological profile of control animals showed normal hepatocytes (Figure 2A). The section of the liver of the toxic control group of animals exhibited severe intense congestion, hydropic degeneration, pyknosis and occasional necrosis (Figure.2B). The liver section of the silymarin treated animals showed normal hepatic architecture of few fatty globules (Figure.2C). The liver section of the animals treated with ethanol extracts from *S.robusta* (200mg/kg & 400mg/kg body weight) showed normal hepatic cords and absence of severe congestion, pyknosis and occasional necrosis (Figure 2D, & Figure 2E) indicating pronounced protection of hepatocytes by paracetamol induced hepatic damage.



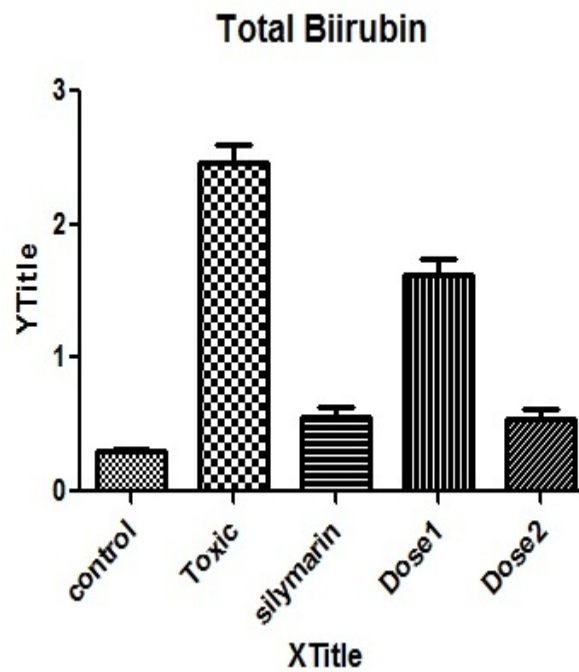
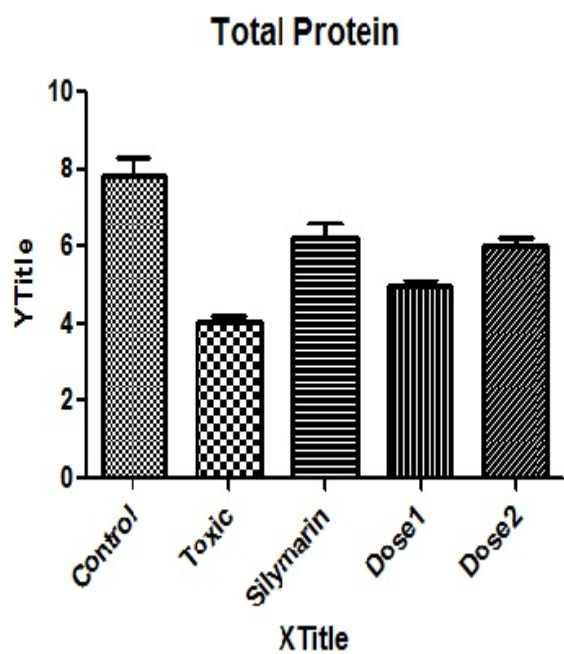
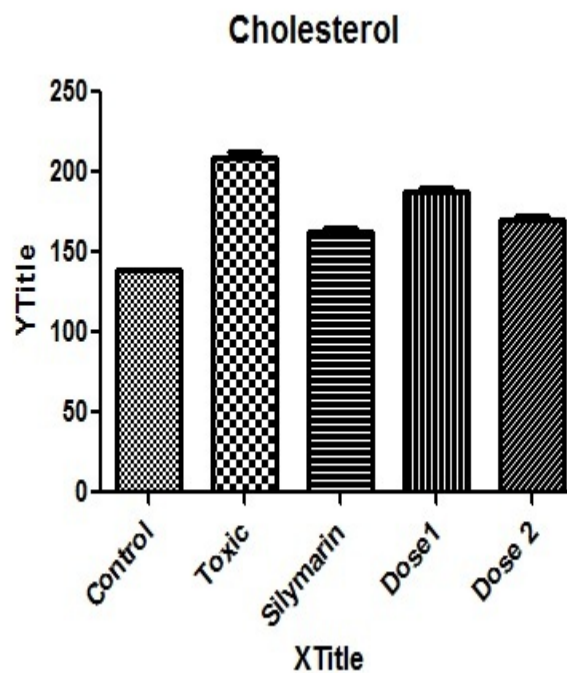
Dose I (200mg/kg)

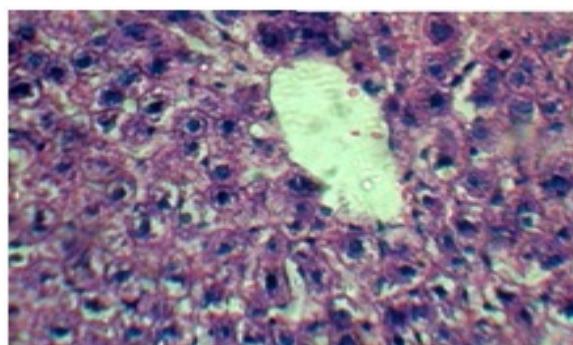
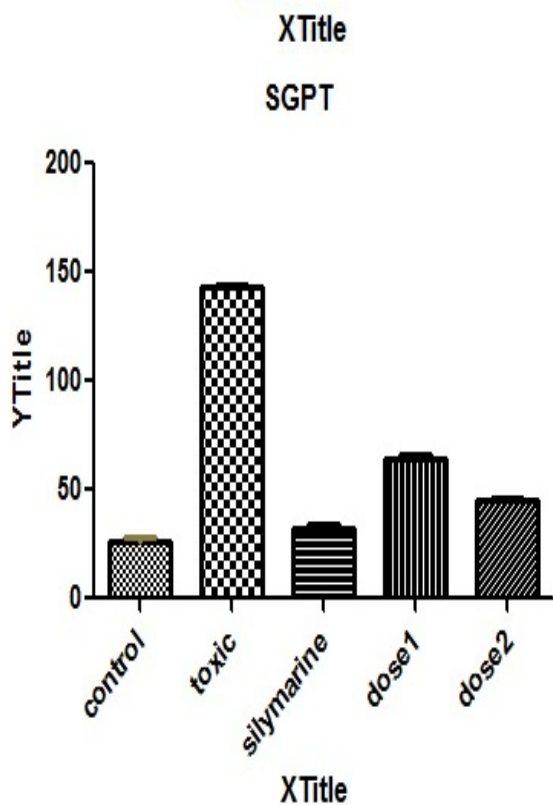
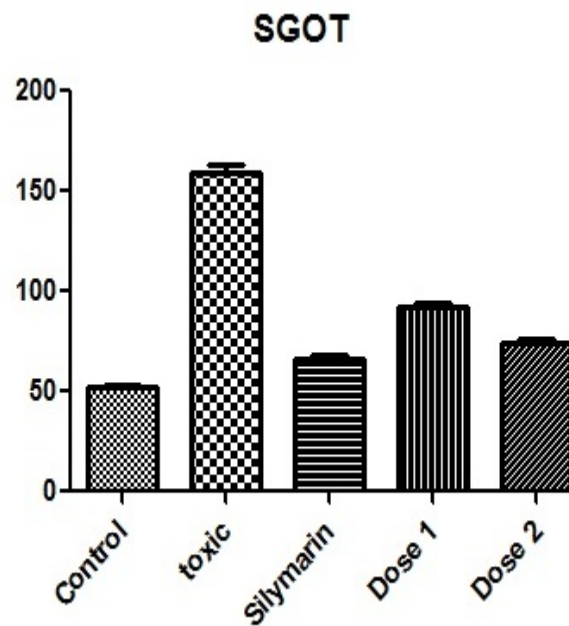
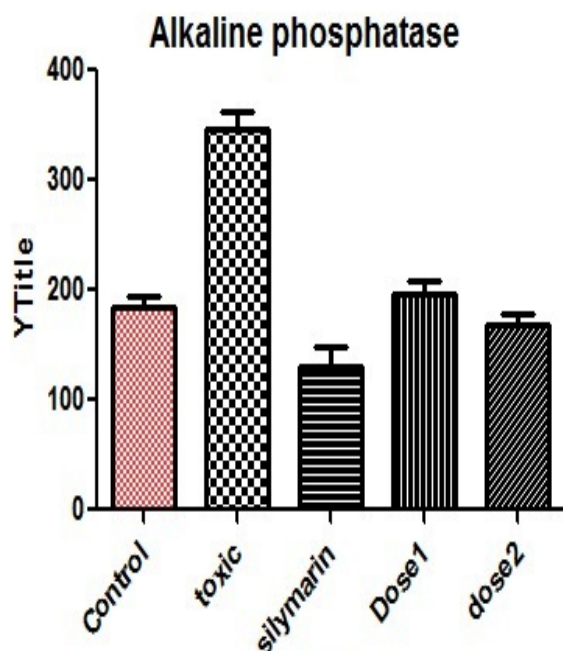


Toxic (paracetamol 3g/kg)



Normal Group





Dose II (400mg/kg)

Discussion

Indigenous herbal drugs and folk medicine is the treasure house of materials worthy of exploration. Many remedial measures claimed by the traditional system of medicine is mainly on empirical bases, on assumptions and on social beliefs. [26] Paracetamol, a well-known compound for producing chemical hepatic injury in rat has been used as an experimental model to test the potential hepatoprotective activity by several investigator [27]. It is mainly metabolized in the liver to glucuronide and sulphate conjugates that are subsequently excreted [28]. The hepatotoxicity of paracetamol has been attributed to the formation a highly reactive metabolite N-acetyl-P-benzoquinoneimine (NAPQI) by the hepatic cytochrome P-450 [29]. The elevated levels of serum enzymes are indicative of cellular leakages and loss of functional integrity of cell membrane in liver. It is established that serum enzymes such as ALP and AST levels were elevated in paracetamol-induced hepatotoxicity. Serum ALP and bilirubin levels on the other hand



are related to the function of hepatic cells [30]. Increase in serum level of these hepatic markers signifies structural and functional catastrophes of the hepatic systems, in our current study we are focusing on the magnitude of recovery of the paracetamol injured hepatic unit. Injury to the hepatocytes alters their transport function and membrane permeability, leading to leakage of enzymes from the cells [31]. The results were compared with the standard drug Silymarin and these effects were confirmed by our results. *S.robusta* at dose 200 mg/kg & 400 mg/kg significantly restored the altered ALP and total bilirubin levels, which is quantitatively comparable with the efficacy shown by Silymarin and also directly indicated the gross effectiveness of the drug extract on functional status of the liver. Paracetamol intoxication significantly lowered total protein levels and *S. robusta* at dose 200mg/ kg & 400mg / kg significantly increased protein levels more or less up to the normal benchmark. Histopathology of the liver samples revealed that the necrosis was reduced to few inflammatory cells in the rat treated with *S. robusta*. Hepato protective activity of *S. robusta* may be due to its phyto constituents. The efficacy of any hepato protective

drugs is dependent on its capacity of either reducing the harmful effect or restoring the normal hepatic physiology that has been distributed by hepatotoxins. Both silymarin and the plant extracts decreased paracetamol induced elevated enzyme levels in tested groups, indicating the protection for structural integrity of hepatocytic cell membrane or regeneration of damaged liver cells. In general, to use this plant as safe prophylactic agent, more studies should be carried out to know all the active components and their mechanism of actions whether synergistic or antagonist using different doses from this plant and another types of experimental animals for a long period in order to judgment if this plant could be used as safe agents or not in human therapy.

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