



Research Article

ISSN : 0975-7384  
CODEN(USA) : JCPRC5

## Evaluation of hepatoprotective activity of *Shorea tumbergaia* rox. against thioacetamide induced toxicity

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### ABSTRACT

Studies on the hepatoprotective activity of ethanol extract from the stem bark of *Shorea tumbergaia* and were carried out using thioacetamide-induced liver damage in albino rats. Levels of glutamic oxaloacetic transaminase, alkaline phosphatase, total bilirubin, total cholesterol, lactate dehydrogenase, and total protein in serum indicated hepatoprotective effect of the ethanol extract. Histopathological examination of liver sections confirmed that, pre-treatment with ethanol extract prevented hepatic damage induced by thioacetamide. The results were comparable with the standard hepatoprotective drug silymarin. The extract showed no signs of toxicity up to a dose level of 2000 mg/kg. It is suggested that, the presence of flavonoids in ethanol extract may be responsible for hepatoprotective properties. Results indicate hepatoprotective properties of ethanol extract of *Shorea tumbergaia*.

**Key words:** *Shorea tumbergaia*, Thioacetamide, Hepatoprotective, Ethanol extract, Silymarin.

### INTRODUCTION

*Shorea tumbergaia* (Dipterocarpaceae) is endemic to Seshachalam hills in Andhra Pradesh and is commonly known as thamba jalari. It is a large tree with ovate leaves having cordate base, entire margin and acute or acuminate apex. The stem and fruits have been used by various local tribes of Yanadi, Yerukula and Sugali and rural folk for a long time in Rayalaseema districts [1]. Ragini et al [2] reported antidiabetic and antioxidant activity of *Shorea tumbergaia*. Stem bark of *S tumbergaia* was found to contain anti-ulcer activity [3]. Ankanna and Savitramma revealed that the plant is source for different secondary metabolites like anthocyanins, emodins, triterpenoids, tannins, saponins, luecoanthocyanins, flavonoids, anthroquinones, phenols and glycosides [4]. Considering the role of flavonoids as anti oxidants in various liver disorders, an attempt was made to explore the hepatoprotective activity of stem bark of *S. tumbergaia*. The hepatoprotective activity of ethanol extract at various dose levels was assessed using thioacetamide as a hepatotoxin. Silymarin was used as positive control.

### EXPERIMENTAL SECTION

#### Plant material

The stems of *S. tumbergaia* were collected from foot hills of Tirumala and their entity was confirmed Dr Madhava Chetty, Botany department, SV University, Tirupathi, India. The voucher specimen (COG/TML/04/SVSK/2013) has been deposited in the Herbarium of the Institute.

#### Preparation of extracts

Powdered (200 g) bark of the plant was completely extracted with ethanol using soxhlet apparatus. The ethanol extract concentrated in vacuum yielded solid mass (15.6% w/w). Preliminary thin layer chromatographic studies of ethanol extract revealed the presence of flavonoids, phenolics, terpenoids and steroids. The ethanolic extract was tested for hepatoprotective activity at dose levels of 200 and 300 mg/kg po. The results were compared with a standard hepatoprotective drug silymarin (100 mg/kg). All the test substances were suspended in vehicle (5% acacia mucilage).

### Animals

Wistar albino rats of either sex weighing 175-225 g, maintained under standard husbandry conditions were used. Animals were allowed to take standard laboratory feed and water *ad libitum*. The experiments were performed after the experimental protocol was approved by the institutional animal ethics committee.

### Estimation of phenolic Content

The total phenolic content of the ethanol extract of *S. tumbuggaia* was estimated by the method of Folin ciocalteu [5]. Stock solution (0.5mg/ml) of the extract was prepared ethanol. From the stock solution 1ml of the extract was taken into a 25 ml volumetric flask. To this added 10 ml of water and 1.5 ml of Folin ciocalteu reagent. The mixture was kept aside for 5 min and then 4 ml of 20% sodium carbonate solution was added and volume was made up to 25 ml with double distilled water. The mixture was kept aside for 30 min and absorbance of blue colour developed was recorded at 765 nm. For the preparation of calibration curve the solutions of standard gallic acid was prepared in concentration range of 50 to 250 µg/ml.

### Estimation of total flavonoid content

The total flavonoid content of the ethanol extract of *S. tumbuggaia* was estimated by reported methods. The aluminium chloride colorimetric method was used for estimating Flavones, flavonols and isoflavones as reported by Chang et al [6]. Quercetin was used to make the calibration curve. From the stock solution of standard 0.1, 0.2, 0.3, 0.4 and 0.5 ml were taken which gave 10, 20, 30, 40 and 50 µg concentrations respectively. The diluted standard solutions (0.5 ml) were separately mixed with 1.5 ml of 95% ethanol, 0.1 ml of 10% aluminium chloride, 0.1 ml of 1M potassium acetate and 2.8 ml of distilled water. The absorbance of the reaction mixture was measured at 415 nm, after incubation at room temperature for 30 min. The amount of 10% aluminium chloride was substituted by the same amount of distilled water in blank. Similarly 0.5 ml of methanol extract of *S. tumbuggaia* (0.5 mg/ml) was reacted with aluminium chloride for determination of total flavonoid content.

### Toxicity studies

Acute toxicity studies were performed for ethanolic extract according to the acute toxic classic method [7] described by OECD. Female albino rats were used for acute toxicity study. The animals were kept fasting for overnight providing only water. The rats were divided into two groups of 3 animals each. The groups of rats were administered orally with ethanolic extract of *S. tumbuggaia* at a dose of 300 mg/kg. The animals were observed continuously after dosing during first 30 min, periodically for first 24 hr with special attention given during first 4 hr and daily thereafter, for a total of 14 days. As there was no mortality seen at this dose level, the procedure was repeated with further dose (2000 mg/kg) using fresh animals.

### Hepatotoxin and test substances

Test substances including silymarin were suspended in vehicle i.e. 5 % acacia mucilage for administration. Thioacetamide at a dose level of 100 mg/kg in water for injection was administered intra peritoneally.

### Thioacetamide-induced hepatotoxicity [8]

Rats were divided into 5 groups of 6 each, control, thioacetamide, silymarin and test groups. The rats of control group received a single daily dose of 5% acacia mucilage (1 ml/kg, p.o.). The rats of toxicant group received a single daily dose of vehicle (1 ml/kg, p.o.) for three days and a single intraperitoneal injection of thioacetamide (100 mg/kg) in water for injection, 30 min after the administration of the vehicle on the second day of treatment. The rats of silymarin group received silymarin (100 mg/kg p.o.) three times at 24 h intervals. Thioacetamide was administered 30 min after the second dose of silymarin while test groups were given orally a single daily dose of extracts in vehicle for three days and a single dose of thioacetamide (100 mg/kg i.p.) on the second day 30 min after the administration of respective test suspensions. After 48 h of thioacetamide administration i.e 4<sup>th</sup> day of the experiment the blood was collected and serum was used for determination of biochemical parameters.

### Assessment of liver function

Glutamic oxaloacetic transaminase (GOT), was estimated by a UV-Kinetic method based on the reference method of International Federation of Clinical Chemistry [9]. Alkaline phosphatase (ALKP) was estimated method by PNPP method [10], while total bilirubin (TBL) by Jendrassik and Grof method [11], total cholesterol (CHL) by CHOD-PAP method (Richmond, 1973 12), lactate dehydrogenase (LDH) by pyruvate method [13] and total protein (TPTN) by colour complexation with copper ions in an alkali solution [14]. All the estimations were carried out using standard kits on auto analyzer of Agappe make (Mispa Excel).

### Histopathological studies

Animals were sacrificed to remove the liver. The liver was fixed in Bouin's solution for 12 h, and then embedded in paraffin using conventional methods [15], cut into 5 µm thick sections and stained using haematoxylin-eosin dye. The sections were then observed for histopathological changes.

### Statistical Analysis

The mean values  $\pm$  SEM were calculated for each parameter. Percentage restoration against the hepatotoxin by the test samples was calculated by considering the difference between the hepatotoxin treated group and the control group as 100% restoration. For the determination of significant inter group difference each parameter was analyzed separately and one way analysis of variance (ANOVA) [16] was carried out. After that individual comparisons of group mean values were done using Dunnet's test [17].

### RESULTS

The phenolic content of ethanol extract of *S.tumbuggaia* was found to be 27% w/w representing the presence of various phenolic compounds like poly phenols, flavonoids, phenolic acids etc. From the calibration curve of the quercetin, the concentration of the flavonoids in the methanol extract of *S.tumbuggaia* was found to be 8.4% w/w. The ethanolic extract did not cause any mortality up to 2000 mg/kg and were considered as safe. Administration of thioacetamide (100 mg/kg i.p.) induced a marked increase in the serum levels of GOT, ALKP, TBL, LDH and CHL; and decrease in the levels of TPTN, indicating parenchymal cell necrosis. Significant decrease ( $p < 0.05$ ) in all the elevated levels of biochemical parameters except ALKP and significant ( $p < 0.05$ ) increase in depleted TPTN levels was observed with the groups of rats which received ethanolic extract at dose levels of 300 mg/kg as observed in case of silymarin treated group. Results of thioacetamide-induced hepatotoxicity are shown in Table 1.

Normal cellular architecture with distinct hepatic cells, sinusoidal spaces, and central vein (Figure 1a) was observed with histological examination of liver sections of control group. The examination of liver sections of rats administered with TAA showed hepatic cells with severe toxicity characterised by centrilobular necrosis along with various gradation of fatty changes comprising of tiny to large sized vacuoles, disarrangement of hepatic cells with blood pooling in sinusoidal spaces (Figure 1b). The liver sections of the rats administered with 300 mg/kg p.o. of ethanolic extract (Figure 1e) followed by TAA intoxications, showed lesser degree of visible changes similar to that observed in case of silymarin treated rat liver sections (Figure 1c) there by suggesting the protective effect of the extracts.

**Table 1: Effect of ethanolic extract of *S. tumbuggaia* on thioacetamide-induced hepatotoxicity in rats**

GROUP	SGOT	ALKP	TBL	LDH	CHL	TPTN
Control	115.60 $\pm$ 2.74	184.60 $\pm$ 4.89	0.09 $\pm$ 0.01	451.9 $\pm$ 1.92	55.67 $\pm$ 3.69	5.22 $\pm$ 0.40
Toxicant	453.01 $\pm$ 7.81	551.4 $\pm$ 13.47	0.56 $\pm$ 0.18	1085.2 $\pm$ 9.67	201.43 $\pm$ 8.58	2.15 $\pm$ 0.63
Silymarin	127.46 $\pm$ 5.97*	193.1 $\pm$ 3.17	0.14 $\pm$ 0.02*	557.0 $\pm$ 5.76*	67.24 $\pm$ 7.47*	5.71 $\pm$ 0.27**
EE 200	326.87 $\pm$ 15.28	260.3 $\pm$ 6.42	0.21 $\pm$ 0.04**+	830.8 $\pm$ 7.33**+	82.70 $\pm$ 7.70**+	7.39 $\pm$ 0.29**+
EE 300	137.51 $\pm$ 5.87**+	174.17 $\pm$ 42.2**+	0.11 $\pm$ 0.04**+	502.6 $\pm$ 17.2**+	71.93 $\pm$ 5.14**+	5.96 $\pm$ 0.36**+

Data represents the mean  $\pm$  SEM of six animals.

EE: Ethanol extract

\* Significant reduction compared to Thioacetamide ( $p < 0.05$ ). \*\* Significant increase compared to Thioacetamide ( $p < 0.05$ ).

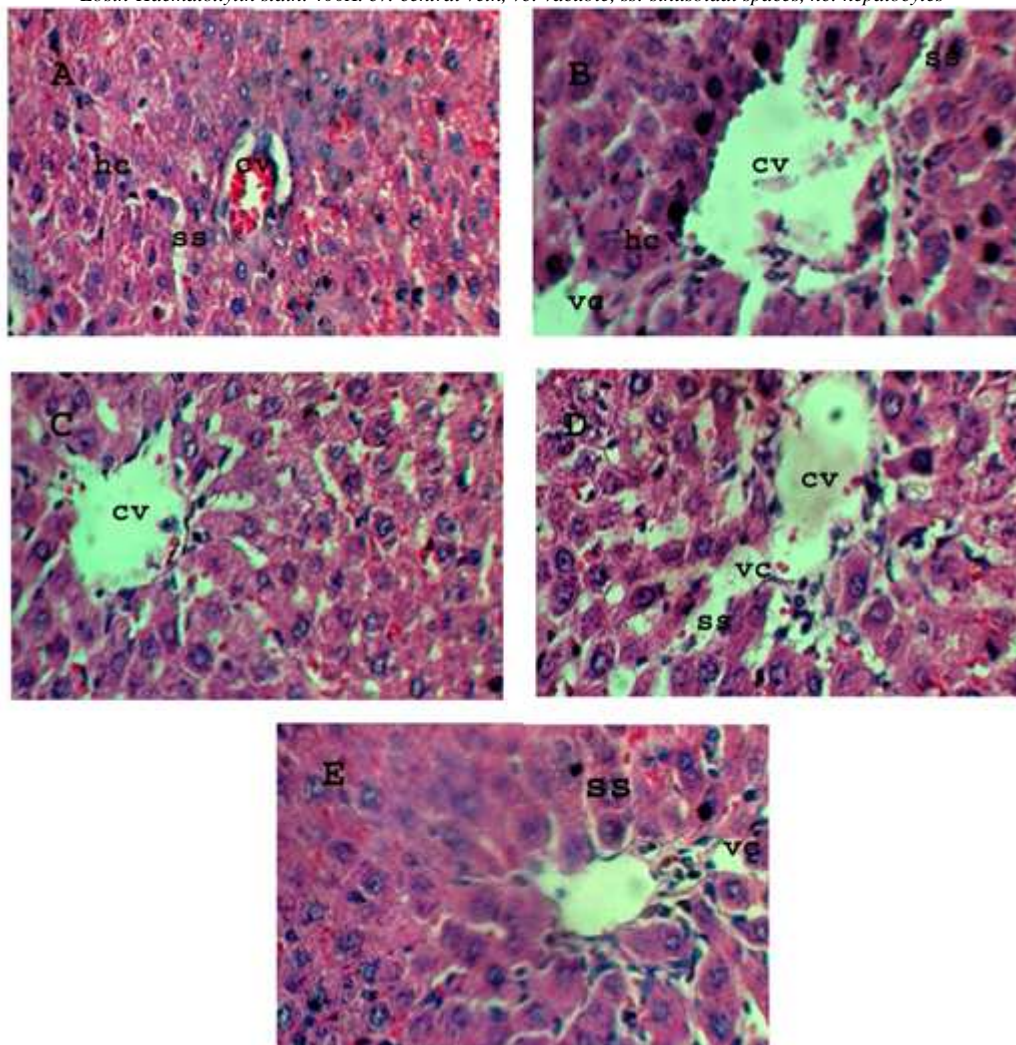
+ Non significant compared to Silymarin

### DISCUSSION

Administration of a single dose of thioacetamide in rats produces centrilobular hepatic necrosis. It gets metabolised to a toxic metabolite thioacetamide-S-oxide, a direct hepatotoxin, which is further metabolised, at least in part, by cytochrome P<sub>450</sub> monooxygenases. The subsequent product formed exerts hepatotoxicity by binding to hepatocyte macromolecules and causes centrilobular necrosis by generation of reactive oxygen species. The chronic thioacetamide exposure leads to cirrhosis in rats. It induces toxicity by altering semi permeable character of the cell membrane of hepatocytes resulting in an increased influx of calcium ions and leading to necrosis and finally death [18]. Therefore extracts under study antagonise the effect of thioacetamide by inhibiting cytochrome P<sub>450</sub> or by acting either as membrane stabiliser, thereby preventing the distortion of the cellular ionic environment associated with thioacetamide intoxication, or by preventing interaction of thioacetamide with the transcriptional machinery of the cells resulting in regeneration of hepatic cells.

Thus the hepatoprotective activity of these extracts may be due to their ability to affect the cytochrome P<sub>450</sub> mediated functions or stabilisation of endoplasmic reticulum resulting in hepatic regeneration. Many plants containing flavonoids are found to have hepatoprotective activity because of their anti-oxidant activity, hence it may be hypothesized that phenolics and flavonoids with their anti-oxidant properties, which are present in ethanolic extract of *S. tumbuggaia* are responsible for the hepatoprotective activity.

**Figure 1: Photomicrographs representing effect of ethanolic extract against thioacetamide -induced hepatotoxicity in rats**  
*a:Normal rat liver section; b: Liver section of the rat intoxicated with thioacetamide; c: Liver section of the rat treated with silymarin and intoxicated with Thioacetamide; d: Liver section of the rat treated with EE 200 mg/kg and intoxicated with Thioacetamide; e: Liver sections of the rat treated with EE 300 mg/kg and intoxicated with Thioacetamide*  
 Eosin-Haematoxylin stain. 400X. cv: central vein, vc: vacuole, ss: sinusoidal spaces, hc: hepatocytes



#### ACKNOWLEDGEMENTS

Authors are thankful to the management and principal of Creative Educational Society's college of pharmacy for providing the necessary facilities.

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