

# Hepatoprotective Activity of Methanolic Extract of *Rhyncosia Beddomei* Baker Against Ethanol Induced Hepatotoxicity

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## Abstract

In the present study, hepatoprotective activity of methanolic extract of *Rhyncosia beddomei* Baker leaves in ethanol induced hepatotoxicity was investigated. The activity was assessed against ethanol induced hepatotoxicity by measuring the levels of serum enzymes like SGOT, SGPT and ALP, total proteins, total bilirubin, and triglycerides. Further, hepatic tissues were also subjected to histopathological studies. The extract showed significant hepatoprotective activity at 200 mg and 400 mg/kg b.w by decreasing the levels of SGPT, SGOT, ALP, total bilirubin, triglycerides and increasing the level of total proteins when compared to toxicant group. The histopathological studies further supported the activity. The results of the present study proved the hepatoprotective potential of methanolic extract of *Rhyncosia beddomei* Baker

**Key words:** *Ecbolium viride* (Forssk.) Alston, Paracetamol, Hepatoprotective Activity, Serum Enzymes, Histopathological Studies.

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## 1. INTRODUCTION

Liver is one of the major organ responsible for maintenance of metabolic functions, secretion, and storage including regulation of various physiological processes. It also synthesizes useful principles and detoxicates toxic substances. Hepatotoxicants like alcohol, toxic chemicals, infections etc induce liver diseases mainly through lipid peroxidation. Thus, hepatic diseases considered as most health serious problems [1]. Its damage is always associated with increase in tissue lipid peroxidation, cellular necrosis, and depletion in the tissue GSH levels. In addition to the above, serum levels of many biochemical markers like SGPT, SGOT, ALP, triglycerides, cholesterol, bilirubin are elevated and total proteins depleted. Hepatic disorders have been recognized worldwide as an important cause of morbidity and mortality in man and animals all over the globe [2]. Herbs are known to play a major role in the treatment liver disorders and many traditional healers have claimed that numerous medicinal plants can be extensively used for the treatment of various liver disorders. *Rhyncosia beddomei* Baker commonly known as Adavikandi, in Telugu belongs to the family fabaceae. The leaves are reported to contain flavanoids, alkaloids, glycosides, triterpenoids and reported to be useful as abortifacient, antibacterial, antidiabetic and hepatoprotective. Leaves are also used for wounds, cuts, boils and rheumatic pains by adivasi tribes [3, 4]. Traditional uses and phytoconstituents reported of this plant prompt us to take up this study.

## 2. MATERIALS AND METHODS

### 2.1 Collection of plant material

The plant material was collected from vicinity of Tirumala hills, Tirupati, identified and authenticated by Dr. Madhava chetty, Asst. Professor, Botany Dept, Sri Venkateswara University, Tirupati ( Voucher specimen No : RB-222 ).

### 2.2 Preparation of plant extracts

The leaves of the plant were separated, washed and dried at room temperature. After complete drying, it was powdered in a multi mill grinder and passed through a 60 mesh sieve. Dried coarse powdered drug was subjected to successive solvent extraction using Soxhlet apparatus (petroleum ether, benzene, chloroform and methanol) and macerated with chloroform water.

### 2.3 Phytochemical Screening

Extracts obtained on successive solvent extraction and maceration was subjected to phytochemical screening for the detection of various phytoconstituents [5, 6].

### 2.4 Animal studies

#### 2.4.1 Experimental animals

The pharmacological studies were carried out on albino Wistar rats of either sex weighing 150-200 g. The animals were housed in the animal house, maintained in controlled temperature (27±2 °C) and light cycle (12 hr light and 12 hr dark). They were fed with rat feed and water *ad libitum*. The study protocol was approved by the institutional Animal Ethical Committee of MSRCP (IAEC certificate No: MSRCP/P-11 2010, dated 3/12/2010).

#### 2.4.2 Acute toxicity studies

Acute toxicity study was performed on methanol extract of *Rhyncosia beddomei* (MERB) following OECD-423 guidelines [7]. After fasting overnight, rats were administered with extract of MERB in a single dose up to the highest dose of 2000 mg/kg orally. The animals were observed continuously for 1 h and then hourly for 6 h and finally after every 24 h up to 15 days for any toxicological symptoms.

#### 2.4.3 Experimental design

Animals were divided into 5 groups containing six animals each

Group I was served as normal control and received normal saline. Group II was maintained as toxicant group received 2

ml of 15% v/v ethanol at a single dose per day (p.o) for 30 days. Group III was orally administered with standard Silymarin at 25 mg/kg once daily for 30 days and 2 ml of 15% v/v ethanol at a single dose per day (p.o) for 30 days. Group IV and V were orally administered once daily for 30 days with MERB extract at a dose of 200 & 400 mg/kg b.w respectively and 2 ml of 15% v/v ethanol at a single dose per day (p.o) for 30 days.. After the treatment period all the animals were anaesthetized for the collection of blood from retro-orbital sinus [8]. The blood was collected from retro-orbital sinus of the animals by anaesthizing under light ether anaesthesia using a heparinised capillary tube. Then it was allowed to clot and serum was separated from clotted blood by centrifugation at 8000 rpm for 10 min. The separated serum was used for the estimation of SGPT (ALT), SGOT (AST), alkaline phosphatase (ALP), total proteins, total bilirubin and triglycerides. Liver was carefully excised and washed in ice cold normal saline solution and pressed between filter paper pads and weighed. A portion of liver (one animal of each group) was preserved in 10% neutral formalin for histopathology studies.

### 3. EVALUATION STUDIES

#### 3.1 Biochemical estimation

Serum was used for the estimation of Serum glutamate pyruvate transaminase (SGPT), Serum glutamate oxaloacetate transaminase (SGOT) [9], Alkaline phosphatase (ALP) [10], triglycerides [11], total proteins [12] and total bilirubin [13]. All these estimations were performed following International Federation of Clinical chemistry and Laboratory medicine (IFCC) standard procedures. All the determinations were carried out using standard kits (Agappe diagnostics, Span Diagnostics) by using Semi-automatic B4B Diagnostic Division Chemistry Analyzer CA-2005 Ranbaxy diagnostic division.

#### 3.2 Histopathology studies

Paraffin sections were prepared from formalin fixed liver samples and stained with haematoxylin and eosin. Histological samples were categorized based on the extent of hepatic injury [14].

#### 3.3 Statistical analysis

All values are expressed as Mean $\pm$  SEM and tested with One Way Analysis of Variance (ANOVA) followed by Tukey-Kramer multiple comparison test.

## 4. RESULTS AND DISCUSSION

Phytochemical screening of different extracts reveal methanolic extract was a good source of flavonoids, tannins, alkaloids and other phenolic compounds. Alkaloids, flavonoids and saponins known to possess hepatoprotective activity [15] and hence, the methanolic extract was selected for the study. Acute toxicity studies of methanolic extract at the dose of 300mg/kg and 2000mg/kg showed no toxic symptoms or death in any of the animals up to one week and till the end of the study, hence 1/10 and 1/5<sup>th</sup> of highest dose were selected for the studies. Hepatotoxicity was induced by administering ethanol and the level of marker enzymes, total proteins, total bilirubin, and triglycerides is shown in Table 1. The serum levels of SGPT, SGOT, ALP, triglycerides and bilirubin were increased significantly, while the level of total proteins decreased in toxicant group. The treatment with the extract altered serum parameters significantly. SGPT levels

were enhanced by fivefold in toxicant group and it was significantly reduced in the MERB (p<0.001 for 200 mg/kg and 400 mg/kg) treated groups. Similarly, there was a threefold increase in SGOT levels in hepatotoxic group, this was significantly reduced in the extract treated groups (p<0.001 for 200 & 400 mg/kg). ALP levels were almost doubled with toxicant group and was significantly reduced in MERB (p<0.001) for 200 and 400 mg/kg) treated groups. Serum levels of total protein decreased substantially in the positive control group. MERB at both doses elevated the levels of total proteins (p<0.001). Total bilirubin level was increased by five folds in the positive control group and extract treated group significantly reduced the levels at 200 mg and 400mg/kg (p<0.001). Twofold increase in serum triglyceride levels was observed in toxicant group, and it was significantly reduced by extract treated groups at 200 mg/kg and 400 mg/kg (p<0.001). Standard Silymarin at 100 mg/kg, significantly reduced the liver weight, serum levels of SGPT, SGOT, ALP, total bilirubin, triglycerides and increased the total protein levels (p<0.001), which almost brought back to the normal levels. Alcohol consumption is known to cause fatty infiltration, hepatitis and cirrhosis of liver due to enhanced lipid peroxidative reaction during the microsomal metabolism of ethanol. Alcohol can induce *in vivo* changes in membrane phospholipid composition and fluidity, because of an increase in hepatic lipid peroxidation which may eventually affect cellular functions results in loss of membrane structure and integrity. Ethanol inhibits glutathione peroxidase, decrease the activity of catalase, superoxide dismutase, along with increase in levels of glutathione in liver due to the damaging effects of free radicals produced following ethanol exposure or alternatively could be due to a direct effect of acetaldehyde, an oxidative product of ethanol[16]. Elevated levels of serum enzymes, triglycerides, total bilirubin were significantly reduced and total proteins level was enhanced by methanolic extract at both dose levels.

Liver photomicrographs of histopathological studies are shown in fig 1. The liver architecture remains intact in normal control group with hepatocytes, sinusoids, central veins and portal tracts appear within normal limits. Ethanol treated group (positive control) showed partial loss of normal liver architecture. The central vein appears mildly dilated and perivenular hepatocytes show extensive ballooning degeneration bridging across the lobules. The sinusoids are within normal limits and there is a moderate lymphocytic infiltration in periportal region with spillover into the adjacent hepatocytes associated with hemorrhage. In Silymarin treated group, liver architecture was preserved. The perivenular hepatocytes showed ballooning degeneration with several hepatocytes showing evidence of regeneration. There is a mild lymphocytic infiltration in amongst the hepatocytes. Liver photomicrograph of MERB 200 mg/kg treated group showed no alteration of liver architecture with moderate lymphocytic infiltration, light regeneration of hepatocytes, ballooning degeneration and hemorrhage. MERB 400 mg/kg treated group showed normal liver architecture with minimal lymphocytic infiltration, regeneration of hepatocytes and ballooning degeneration.

## 5. CONCLUSION

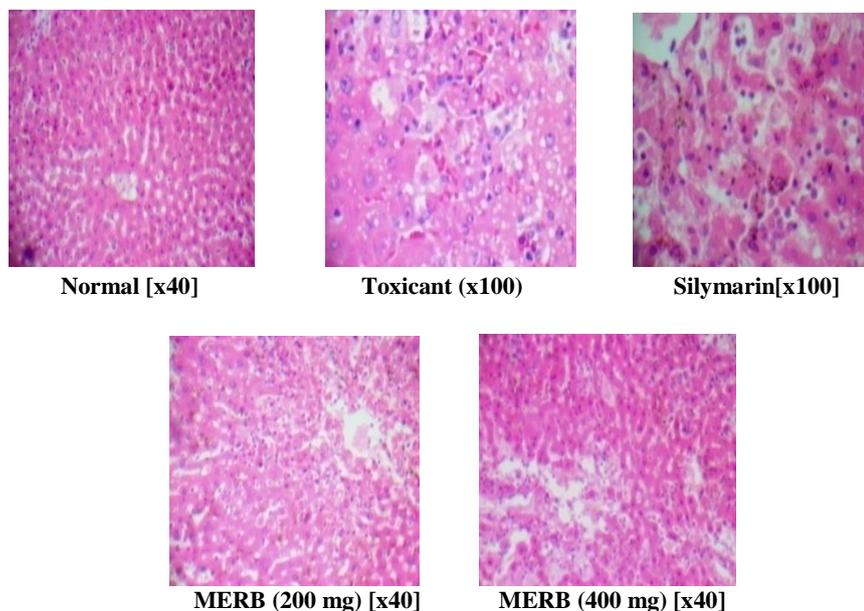
The results obtained in the present study indicated that the methanolic extract of *Rhynchosia beddomei* leaves possess significant hepatoprotective activity and histopathological studies further support the activity. The activity may be due

to the presence of phytoconstituents like flavonoids, alkaloids and phenolic compounds.

Groups	SGPT (U/I)	SGOT (U/I)	ALP (U/I)	Total proteins (g/dl)	Total bilirubin (mg/dl)	Triglycerides (mg/dl)
Normal control	37.4 ±3.44	83.07 ±7.71	148.59 ±7.46	8.422 ±0.404	0.485 ±0.113	80.5 ±4.79
Positive control	195.99 ±8.863	239.73 ±16.3	285.15 ±9.63	5.390 ±0.542	2.247 ±0.079	161.15 ±5.41
Silymarin (25 mg)	80.51 ±8.5***	113.25 ±7.81***	169.5 ±12***	7.830 ±0.2***	0.741 ±0.10***	86.65 ±4.34***
MERB (200mg)	135.29 ±5.6***	171.7 ±7.54***	214.24 ±8.9***	7.221 ±0.21***	1.24 ±0.06***	126.47 ±4.89***
MERB (400mg)	97.07 ±9.0***	131.71 ±8.62***	174.98 ±7.6***	7.632 ±0.19***	0.756 ±0.08***	89.05 ±8.35***

**Table 1 Effect of MERB on serum parameters in ethanol induced hepatotoxicity**

Values are expressed as Mean ± SEM. Data compared against positive control group. One way analysis of variance (ANOVA). \* p<0.05, \*\* p<0.01, \*\*\* p<0.001 Tukey-Kramer multiple comparison test



**Fig. 1 Effect of MERB on liver architecture in ethanol induced hepatotoxicity**

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