

# Evaluation of Hepatoprotective activity of Stem Bark of *Homalium zeylanicum* in Rats

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## **Abstract:**

**Objective:** To evaluate the hepatoprotective activity of *Homalium zeylanicum* stem bark extracts against carbon tetrachloride (CCl<sub>4</sub>) induced hepatotoxicity.

**Materials and methods:** Hepatotoxicity was induced in male Wistar rats by intraperitoneal injection of CCl<sub>4</sub> (0.1 ml/kg/day for 14 days). Methanol extract of *Homalium zeylanicum* stem bark were administered to the experimental rats (250mg and 500mg/kg, p.o. for 14 days). The hepatoprotective effect of these extracts was evaluated by the assay of liver function biochemical parameters (total bilirubin, serum protein, alanine aminotransaminase, aspartate aminotransaminase, and alkaline phosphatase activities) and histopathological studies of the liver.

**Results:** In Methanol extract-treated animals, the toxic effect of CCl<sub>4</sub> was controlled significantly by restoration of the levels of serum bilirubin, protein and enzymes as compared to the normal and the standard drug Silymarin-treated groups. Histology of the liver sections of the animals treated with the extracts showed the presence of normal hepatic cords, absence of necrosis and fatty infiltration, which further evidenced the hepatoprotective activity.

**Conclusion:** Methanol extract of the stem bark of *Homalium zeylanicum* possesses significant hepatoprotective activity.

**Key words:** Hepatoprotective activity, *Homalium zeylanicum*, Carbon tetrachloride (CCl<sub>4</sub>).

## **INTRODUCTION:**

Liver is one of the largest organs in the human body and chief site for intense metabolism and excretion. Liver diseases are one of the major health problems in the world. These are caused by toxic chemicals, autoimmune disorders, infections and excess consumption of alcohol. The hepatotoxic chemicals can induce lipid peroxidation and oxidative damages. It is involved in almost all the biochemical pathways to growth, fight against the disease, nutrient supply, energy provision and reproduction<sup>[1,2]</sup>. Modern medicines have little to offer for alleviation of hepatic diseases and it is chiefly the plant based preparations which are employed for their treatment of liver disorders. But there are not much drug available for the treatment of liver disorders<sup>[3]</sup>.

Therefore, many folk remedies from plant origin are tested for its potential antioxidant and hepatoprotective liver damage in experimental animal model. Carbon tetrachloride (CCl<sub>4</sub>) induced hepatotoxicity model is widely used for the study of hepatoprotective effects of drugs and plant extracts<sup>[4]</sup>.

*Homalium zeylanicum* (Flacourtiaceae) is commonly known as "Liyan or Mukki". The bark is traditionally used in diabetes, rheumatism. The present study evaluates the hepatoprotective activity of *H. zeylanicum*.

## **MATERIALS AND METHODS:**

### **Collection of plant material and preparation of extract:**

Plant material used in this study consisted of bark of *Homalium zeylanicum*, collected in and around Tirumala hills, Andhra Pradesh. The plant was authenticated by Dr. Madhava chetti, Dept. Of Botany, S.V University, Tirupathi. The bark was coarsely powdered and subjected to methanol extract for 48 h by Soxhlet apparatus. The extracts were pooled together and concentrated in vacuum using rotary flash evaporator to obtain crude extract.

### **Experimental animals:**

Male wistar rats of (150-250g) were procured from Mahaveer enterprises, Hyderabad. Animals were housed in an environmentally control room and maintained at uniform laboratory conditions and provided with food and water *ad libitum*.

### **Evaluation of Hepatoprotective activity of *Homalium zeylanicum*:**

Rats were divided into five groups (n=5). Group I was served as control and received 2% acacia solution for 14 days. Group II to V were daily injected a mixture of Carbon tetrachloride at dose of 0.1ml/kg/day for 14 days. Group III served as standard group and administered Silymarin (100mg/kg/day p.o). Group IV and V treated with methanol extract of *Homalium zeylanicum* in the dose of 250mg and 500 mg/kg/day, p.o. (as per acute toxicity studies) for 14 days, respectively.

### **Assessment of Hepatoprotective activity:**

All the animals were killed on day 14 under light ether anesthesia. The blood samples were collected separately into sterilized dry centrifuge tubes and allowed to coagulate for 30 min at 37°C. The clear serum was separated at 2500 rpm for 10 min and biochemical investigations were carried out to assess liver function viz., total bilirubin, total protein, serum transaminases and serum alkaline phosphatase<sup>[5,6,7,8]</sup>.

### **Data analysis:**

The data was expressed as MEAN ± SEM for each group. Statistical analysis was performed by using student's "t" test. Values P<0.05 were considered statistically significant.

### **Histopathology:**

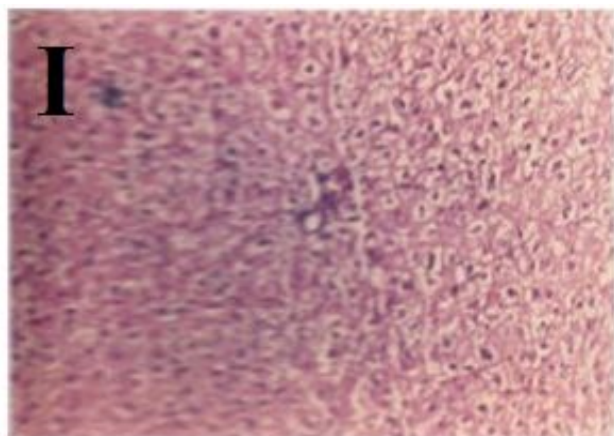
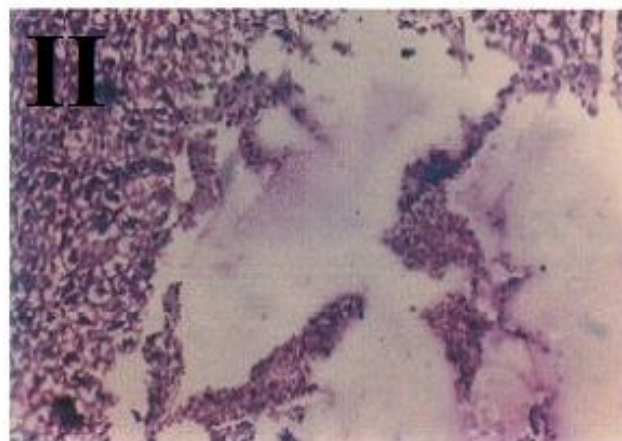
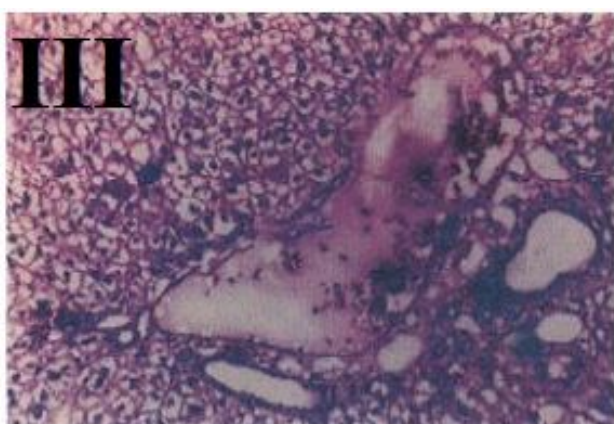
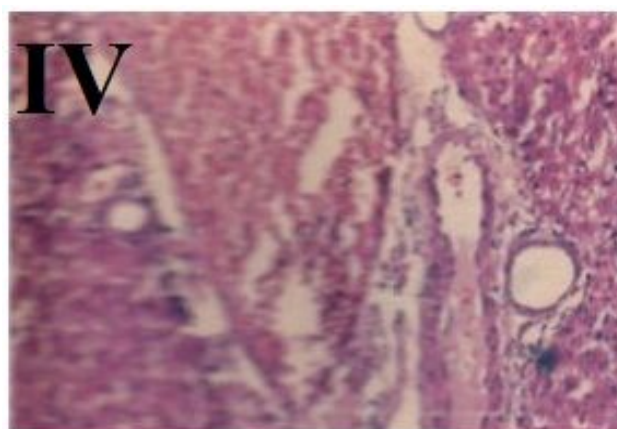
Liver was examined, weighed and stored in formalin 10% for 48 hrs and then with bovine solution for 6h and were processed for paraffin embedding using standard microtechnique<sup>[7]</sup>. A section of liver (5µm) stained with Alumhemotoxylin and Eosin was observed microscopically for histopathological studies.

## **RESULTS:**

The administration of CCl<sub>4</sub> to the animals resulted in a marked increase in total Bilirubin, Serum amino transaminases (AST and ALT) and Serum alkaline phosphatase activities. However, the Serum total protein level was decreased. The toxic effect of CCl<sub>4</sub> was controlled in the animals treated with the methanol extracts by way of restoration of the levels of the liver function biochemistry similar to that of the standard drug Silymarin. Among the different treated groups, significant Hepatoprotective activity was observed in those treated with methanol extract. The effects of marker enzymes were shown in table-1.

**Table-1: Effect of homalium zeylanicum extracts on ALP,AST,ALP,TP and Bilirubin**

Gr no:	Treatment mg/kg	ALT (U/ml)	AST(U/ml)	ALP (U/ml)	TP (g/ml)	Bilirubin(mg/dl)
I	Normal control	55.84±4.5	43.22±3.11	36.22±4.57	7.46±0.36	0.72±0.3
II	CCl <sub>4</sub> control	108.22±3.57	103.22±4.01	87.54±3.03	2.18±0.25	4.26±1.50
III	Ccl <sub>4</sub> +Silymarin(100)	74.53±4.21	68.28±2.55	44.25±2.02	6.86±4.8	1.06±0.46
IV	Ccl <sub>4</sub> +HZ extract(250)	69.23±2.54	62.14±2.52	51.00±5.46	3.72±0.52	2.15±0.86
V	Ccl <sub>4</sub> +HZ extract(500)	63.01±2.57	48.35±2.88	40.51±4.51	6.12±0.22	1.02±0.97

**OBSERVATION OF HISTOPATHOLOGY CHANGES IN THE LIVER:****Fig-1: Microview of liver tissue of normal Rat****Fig-2:Microview of liver tissue if ccl<sub>4</sub> induced Rat****Fig-3: Microview of methanolic extract of 250mg****Fig-4: Microview of methanol extract of 500mg**

**DISCUSSION:**

Carbon tetrachloride is the one of the most commonly used hepatotoxins in the experimental study of liver diseases<sup>[9]</sup>. It induces liver cell necrosis and apoptosis and can be used to induce Hepatic fibrosis or Cirrhosis by repetitive administration<sup>[10]</sup>. The hepatotoxic effect of Carbon tetrachloride is mainly due to its active metabolite, Trichloromethyl radical<sup>[11]</sup>. This activated radical bind covalently to the macromolecules and induce lipid peroxidation and forms lipid peroxides which produce damage to the membrane<sup>[12]</sup>. The increase in the levels of Serum bilirubin reflected the depth of jaundice and the increase in transaminases and Alkaline phosphatase which are cytoplasmic in location and released into circulation after cellular damages was the clear indication for the loss of functional integrity of the cell membrane<sup>[13,14]</sup>.

Amino transferases are present in high concentration in liver, an important class of enzymes linking Carbohydrate and Amino acid metabolism. Alanine amino transferase and Aspartate amino transferase are well known diagnostic indicators of liver disease.

In cases of liver damage with hepatocellular lesions and parenchymal cell necrosis, these marker enzymes are released from the damaged tissues into the blood stream<sup>[15]</sup>. In the present study, the activities of enzymes were found to increase in the hepatotoxic animals, and were significantly reduced in groups of methanolic extract of *Homalium zeylanicum*

administered rats as compared to that of toxicant rats. This confirms the protective effect of methanolic extract of *Homalium zeylanicum* against Carbon tetrachloride induced hepatic damage. The effect was more pronounced with 500mg/kg extract. A possible mechanism of the *Homalium zeylanicum* extract as Hepatoprotective may be due to its anti-oxidant effect or inhibition of Cytochrome P450. This might be due to the higher contents of flavonoids present in the extract which could have reduced the accumulation of toxic CCl<sub>3</sub> derived metabolites.

Histopathological examination of the liver section of the rats treated with toxicant showed intense centrilobular necrosis and vacuolization. The rats treated with Silymarin and extract along with toxicant showed sign of protection against these toxicants to considerable extent as evident from formation of normal hepatic cords and absence of necrosis and vacuoles.

**CONCLUSION:**

Overall, the results of the present study indicate that *Homalium zeylanicum* has Hepatoprotective activity against CCl<sub>4</sub> induced hepatotoxicity in rats.

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