

EFFECT OF *CLAUSENA DENTATA* (WILLD.) M. ROEM. AGAINST PARACETAMOL INDUCED HEPATOTOXICITY IN RATS

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ABSTRACT

Ethanol extract of *Clausena dentata* (Willd.) M. Roem (Rutaceae) was evaluated for hepatoprotective activity in rats. The plant extract (250 mg/kg, p.o.) showed a remarkable hepatoprotective activity against acetaminophen-induced hepatotoxicity as judged from the serum markers for liver damage. Acetaminophen induced a significant rise in aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP), total bilirubin, gamma glutamate transpeptidase (GGTP) and decrease in total protein. Treatment of rats with ethanol extract (250 mg/kg) significantly ($P < 0.001$) altered serum marker enzymes levels to near normal against acetaminophen treated rats. The activity of the extract was comparable to the standard drug, silymarin (50 mg/kg, p.o.). Histopathological changes of liver sample were compared with respective control. Results indicate that *Clausena dentata* possesses hepatoprotective effect on acetaminophen-induced hepatotoxicity in rats.

Keywords: *Clausena dentata*, acetaminophen, biochemical parameters, histopathology.

INTRODUCTION

Herbal medicines have recently attracted much attention as alternative medicines useful for treating or preventing life style related disorders and relatively very little knowledge is available about their mode of action. There has been a growing interest in the analysis of plant products which has stimulated intense research on their potential health benefits. Liver, the key organ of metabolism and excretion has an immense task of detoxification of xenobiotics, environmental pollutants and chemotherapeutic agents. Hence, this organ is subjected to variety of diseases and disorders. Several hundred plants have been examined for use in a wide variety of liver disorders.

Clausena dentata (Willd.) Roem is a small tree plant, belonging to the family of Rutaceae and found in India, Sri Lanka and China (Agarwal, 1981). It is popularly known as Anai chedi in Tamil. *Clausena dentata* is used by local peoples of Yercaud and Boda Hills for its medicinal and nutritive value. The phytochemical studies of the plant have revealed the presence of volatile oils of four furanoid terpenic compounds, α -clausenan, rosefuran (γ -clausenan) and diclausenans A and B (Rao and Subramanian, 1934) and furanoterpenes (Subba Rao *et al.*, 1984). The root bark was found to contain coumarins (Govindachari *et al.*, 1968). The present study was undertaken to investigate the hepatoprotective activity of methanol extract of the stem bark of *Clausena dentata* against acetaminophen induced hepatotoxicity in rats.

MATERIALS AND METHODS

Plant materials and preparation of extract

The stem bark of *C. dentata* was collected from the Yercaud, Salem Dist, India. The stem bark were dried in the shade and pulverized to a coarse powder. The powder was then packed into soxhlet apparatus and subjected to hot continuous percolation using ethanol (95% v/v) as solvent. The extract was concentrated under vacuum and dried in a vacuum desiccator and then suspended in 5% gum acacia for hepatoprotective studies.

Animals

Male Wistar rats (100-125 g) were procured from Tamilnadu Veterinary College, Chennai, India. They were housed in microlon boxes with standard laboratory diet and water *ad libitum*. The study was conducted after obtaining Institutional animal ethical committee clearance.

Hepatoprotective activity

The animals were divided into 3 groups, each group comprising 6 animals. Group I served as control and received 2 ml/kg of saline daily for 7 d orally. Group II rats were similarly treated as group I. Group III rats were received silymarin 50 mg/kg p.o. for 7 days and Group IV were treated with alcohol extract of *C. dentata* at a dose of 250 mg/kg respectively for 7 d. On the seventh day acetaminophen (2 g/kg, p.o.) was administered, 30 min after the last dose to all rats except rats in group I (Raj Kapoor *et al.*, 2002). After 36 h, all the rats were

sacrificed under light ether anesthesia; blood was collected in sterile centrifuge tube and allowed to clot. Serum was separated by centrifuging at 2500 rpm for 15 min and used for the biochemical assays.

Assessment of liver function

Biochemical parameters i.e., aspartate amino transferase (AST), alanine amino transferase (ALT) (Reitman and Frankel, 1957), alkaline phosphatase (ALP) (Kind and King, 1954), γ -glutamyl transpeptidase (GGTP) (Szasz, 1969), total bilirubin (Mallay and Evelyn, 1937) and total protein (Lowry *et al.*, 1951) were analyzed according to the reported methods. The liver was removed and morphological changes were observed. A portion of liver was fixed in 10% formalin for histopathological studies.

Histopathological studies

Liver slices fixed for 12 hrs in Bouin's solution were processed for paraffin embedding following standard micro techniques (Galigher and Kozloff, 1971). 5 μ m sections of liver stained with alum haematoxylin and eosin, were observed microscopically for histopathological changes.

STATISTICAL ANALYSIS

The values were expressed as mean \pm SEM. Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Tukey multiple comparison tests. P values < 0.05 were considered as significant.

RESULTS AND DISCUSSION

The effect of *C. dentata* on serum marker enzymes are presented in table 1. The levels of serum AST, ALT, ALP, total bilirubin, GGTP were markedly elevated and that of protein decreased in acetaminophen treated animals, indicating liver damage. Administration of *C. dentata* extract at the dose of 250 mg/kg remarkably prevented acetaminophen-induced hepatotoxicity in rats (P<0.001). The effect of *C. dentata* was comparable with that of standard drug silymarin (table 1).

Histopathological studies, showed acetaminophen to produce extensive vascular degenerative changes and centrilobular necrosis in hepatocytes. Treatment with silymarin and *C. dentata* extract produced mild degenerative changes and absence of centrilobular necrosis when compared with control (fig. 1). All these results indicate a hepatoprotective potential of the extract.

In recent years, many studies have been undertaken with traditional medicines, in an attempt to develop new drugs for liver disorders (Liu, 1989). In the present study, we used acetaminophen for liver damage induction, to investigate whether the plant extract *C. dentata* could decrease efficiently the toxicity produced by these hepatotoxicant.

Acetaminophen (Paracetamol) is a widely used antipyretic-analgesic drug produces acute hepatic damage on accidental over dosage. It is established that, a fraction of acetaminophen is converted via the cytochrome P₄₅₀ pathway to a highly toxic metabolite; N-acetyl-p-benzoquinamine (NAPQI) (Dahlin *et al.*, 1984) which is normally conjugated with glutathione and excreted in urine. Overdose of acetaminophen depletes glutathione stores, leading to accumulation of NAPQI, mitochondrial dysfunction (Parmar and Kandakar, 1995) and the development of acute hepatic necrosis. Several P₄₅₀ enzymes are known to play an important role in acetaminophen bioactivation to NAPQI. P₄₅₀ 2E1 have been suggested to be primary enzyme for acetaminophen bioactivation in liver microsomes (Raucy *et al.*, 1989). Studies demonstrated that acetaminophen induced hepatotoxicity can be modulated by substances that influence P₄₅₀ activity (Mitchell *et al.*, 1973).

In the assessment of liver damage by acetaminophen the determination of enzyme levels such as AST, ALT is largely used. Necrosis or membrane damage releases the enzyme into circulation and hence it can be measured in the serum. High levels of AST indicates liver damage, such as that caused by viral hepatitis as well as cardiac infarction and muscle injury, ALT catalyses the conversion of alanine to pyruvate 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 are indicative of cellular leakage and loss of functional integrity of cell membrane in liver (Drotman and Lawhan, 1978). Serum ALP, bilirubin and total protein levels 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 (Muriel and Garcipiana, 1992).

Administration of acetaminophen caused a significant (P<0.001) elevation of enzyme levels such as AST, ALT, ALP, GGTP, total Bilirubin and decrease in total protein when compared to control. There was a significant (P<0.001) decrease of these enzyme levels on administration of the *C. dentata* at a dose of 250 mg/kg. The reversal of increased serum enzymes in acetaminophen-induced liver damage by the extract may be due to the prevention of the leakage of intracellular enzymes by its membrane stabilizing activity. This is in agreement with the commonly accepted view that serum levels of transaminases return to normal with the healing of hepatic parenchyma and the regeneration of hepatocytes (Thabrew and Joice, 1987). Effective control of ALP, bilirubin and total protein levels points towards an early improvement in the secretory mechanism of the hepatic cells.

Table 1: Effect of *Clausena dentata* on serum marker enzymes in acetaminophen induced hepatotoxicity in rats

Treatment	Dose (mg/kg)	AST U/L	ALT U/L	ALP U/L	Total protein mg%	Total bill mg%	GGTP U/L
Control	-	92.0±2.17	113.6±1.4	452±5.12	6.15±0.34	0.73±0.04	87.0± 1.2
Acetaminophen	2000	290.5±1.4 ^a	324.5±2.8 ^a	776±6.45 ^a	4.6±0.23 ^b	0.97±0.03 ^b	129.6±2.7 ^a
Silymarin	50	151.4 ±6.63 ^c	89.2±3.6 ^c	228.4 ±5.42 ^{b,c}	8.12± 0.560	0.72±0.03 ^c	35.3±1.78 ^c
<i>C. dentata</i> + Acetaminophen	250	152.0±1.9 ^{a,c}	215.3±3.2 ^{a,c}	480 ±3.90 ^{b,c}	7.1±0.20 ^c	0.86±0.05	91.0±1.02 ^c

N = 6; Values are expressed as mean ± SEM, ^aP< 0.001; ^bP< 0.01 Vs. Control, ^cP< 0.001 Vs. Acetaminophen Data were analyzed by using one-way ANOVA followed by Tukey multiple comparison test.

Histopathological Slides

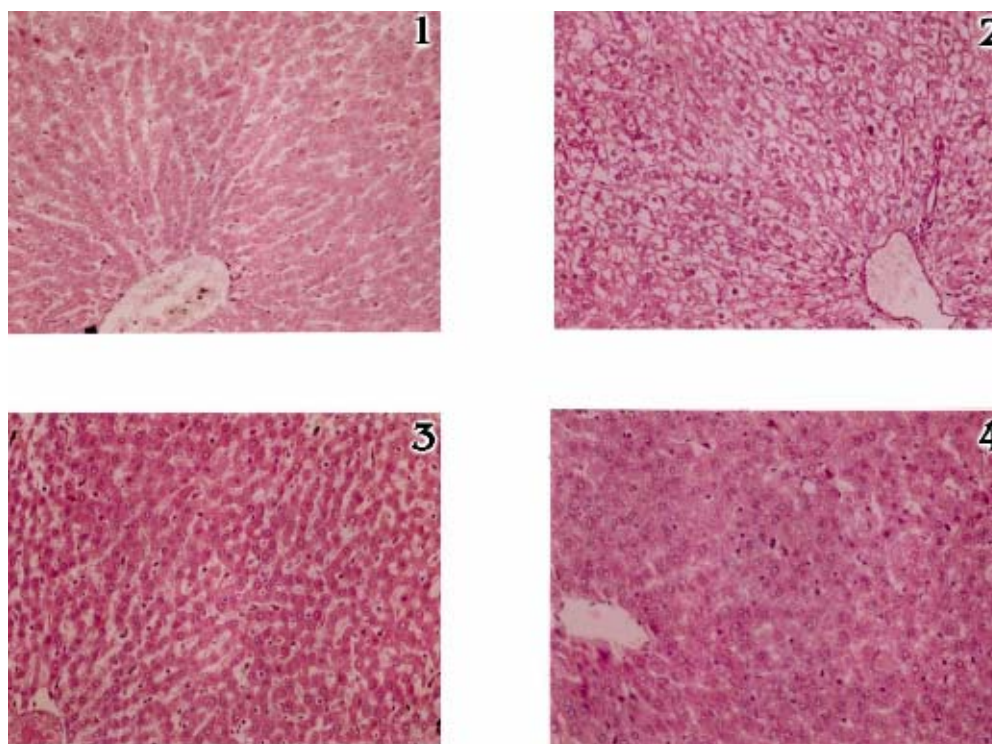


Fig. 1: Effect of *Clausena dentata* on acetaminophen induced liver damage in rat (X 10)

[1] liver from rat treated with saline shows normal cellular architecture with distinct hepatic cells, sinusoidal space and a central vein [2] liver from rat treated with acetaminophen exhibited severe hepatocyte degeneration and necrosis [3 & 4] liver treated with silymarin and *Clausena dentata* (250 mg/kg, p.o.) plus acetaminophen shows normal architecture with mild hepatocyte degeneration.

The efficacy of any hepatoprotective drug is dependent on its capacity of either reducing the harmful effect or restoring the normal hepatic physiology that has been disturbed by a hepatotoxin. The plant extract decreased acetaminophen induced elevated enzyme levels in test group, indicating the protection of structural integrity of hepatocytic cell membrane of damaged liver cells.

Extensive vascular degenerative changes and centrilobular necrosis in hepatocytes was produced by acetaminophen. Treatment with alcohol extract of *C. dentata* produced only mild degenerative changes and

absence of centrilobular necrosis, indicating its hepatoprotective efficiency.

Preliminary phytochemical studies reveal the presence of flavonoids in ethanolic extract of *C. dentata*. Flavonoids are hepatoprotectives (Seevola *et al.*, 1984; Wegner and Fintelmann, 1999). The observed hepatoprotective activity of *C. dentata* may be due to the presence of flavonoids. Further studies to characterize the active principles and to elucidate the mechanism are in progress.

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