

Hepatoprotective activity of leaves of *Zanthoxylum armatum* DC in CCl₄ induced hepatotoxicity in rats

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Zanthoxylum armatum DC (Rutaceae) is extensively used in indigenous system of medicine as a tonic, carminative, stomachic and anthelmintic. In the present study, the hepatoprotective activity of the leaves ethanolic extract of *Z. armatum* (EEZA) was evaluated in CCl₄-induced hepatotoxicity in rats. The extract at a dose of 500 mg/kg registered a significant decrease in the levels of serum glutamyl oxalacetic acid transaminase (SGOT), serum glutamyl pyruvate transaminase (SGPT), alkaline phosphatase (ALKP), and serum bilirubin (SBLN) and liver inflammation, which was supported by histopathological studies on liver, thus exhibited a significant hepatoprotective activity. The phytochemical screening of defatted ethanolic extract showed the presence of sterols, alkaloids, flavonoids, and reducing sugars.

Keywords: *Zanthoxylum armatum*, Hepatoprotective activity.

The liver is the prime organ concerned with various states of metabolic and physiologic homeostasis of the organism¹. In modern medicine, there is no specific cure for such disease such as infectious hepatitis and liver cirrhosis. Treatment of many liver diseases is symptomatic and often disappointing, since much is still obscure about their etiology. There is, however, a plethora of drugs in the indigenous system of medicine said to be useful in these disease².

Zanthoxylum armatum DC (Rutaceae) commonly known as “darmar” or “Nepali Dhaniya” in Hindi is an armed scandent, erect shrub, 6 m tall or more with dense foliage, found in the hot valleys of the Himalayas from Jammu to Bhutan at altitudes of 1,000-2100 m and in Eastern Ghats in Orissa and Andhra Pradesh at 1,200 m. The bark, fruits and seeds are extensively used in indigenous system of medicine as a tonic, carminative, stomachic and anthelmintic. The stem has exhibited hypoglycemic activity in the

preliminary trials. The fruits and seeds are employed as an aromatic tonic in fever and dyspepsia^{3,4}.

The *in vitro* antioxidant activity of the *Z. armatum* has been reported in various models by us⁵. A number of alkaloids viz. berberine, dictamnine, magnoflorine, xanthoplanine, sikimmianine and γ -fagarine have been reported from its stem-bark, wood and roots⁴. The stem bark and leaves also contain an essential oil and resins⁶. Experimental evidences show that free radicals are reported to be involved in the pathogenesis of liver injury⁷. Also, it is established that plants having antioxidant property also exert hepatoprotective action⁸. As *Z. armatum* has shown significant antioxidant activity and also contain flavonoids⁴, therefore, we have investigated the hepatoprotective activity of ethanolic extract of leaves in the rats.

Materials and Methods

All chemicals used were of analytical grade. Silymarin was obtained from Sigma Chemical Co., USA and other chemicals were obtained from SISCO Research Laboratories Pvt. Ltd, Mumbai, India.

Plant material

The *Zanthoxylum armatum* were procured from the Plant Physiology Division, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Krishi Nagar, Jabalpur, M.P. (India) and authenticated by the taxonomic division, National Herbarium of Cultivated Plants, National Bureau of Plant Genetic and Resources, New Delhi. A voucher specimen (vide accession no. NHCP/NBPGR/2007/100/2225 dated 22/08/2007) was retained in our laboratory. The plant material was dried under shade at room temperature, reduced to moderately coarse powder and extracted successively with petroleum ether (60-80°C) and 95% ethanol using soxhlet apparatus. The ethanolic extract was dried under vacuum (yield, 6.67%). The defatted ethanolic extract of *Zanthoxylum armatum* (EEZA) was used for the preliminary phytochemical screening and hepatoprotective studies.

Preliminary phytochemical screening

A preliminary phytochemical screening was carried out for the extracts employing the standard procedure revealed the presence of various phytoconstituents viz. alkaloids, steroids, terpenes, flavonoids, saponins, tannins, glycosides, carbohydrates and proteins⁹.

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Animals and acute toxicity studies

Wistar albino rats of both sexes (weighing 130-170 g) were used in the present study. They were housed in clean polypropylene cages (38 × 23 × 10 cm) with not more than three animals per cage and maintained under standard laboratory condition (temperature 25 ± 2°C) with dark and light cycle (12/12 h) and provided standard pellet diet (Hindustan Lever, Kolkata, India) and water *ad libidum*. The study was approved by Institutional Animal Ethics Committee.

Acute toxicity study using Wister albino rats was performed for the extract according to the acute toxic classic methods as per OECD guidelines¹⁰. The animals were kept fasting for overnight providing only water, and after which the extract was administered orally 500 mg/kg body wt⁵ and observed for 14 days. The animals were observed continuously for 3 h and then observed each h during 24 h administering the extract for any change in general behavior or other physiological activities as per OECD guidelines. If mortality was observed in 2 out of 3 animals, then the dose administered was assigned as toxic and if mortality was observed in 1 animal, the dose administered was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for further higher dose i.e. 2000 mg/kg.

Assessment of hepatoprotective activity

The rats were divided in to four groups of six rats each. The animals of group I and II served as control and carbon tetrachloride (CCl₄) control received vehicle (0.1% tween 80, 10 ml/kg b. w.). Group III served as standard and received silymarin (100 mg/kg b. w. in 0.1% tween 80) and group IV was given EEZA (500 mg/kg b. w. in 0.1% tween 80). All administration of doses was made by gastric intubations once daily for 7 days.

On the 8th day 1 h after the administration of last dose, the animals of groups II, III and IV were given an intraperitoneal injection of CCl₄ with an equal quantity of liquid paraffin (0.5 ml/kg b. w.). All the animals were then fasted for 24 h, anaesthetized and the blood was collected by cardiac puncture. The liver was quickly dissected, washed with ice-cold saline and stored in freezer. The blood samples were allowed to coagulate at room temperature for 1 h. Serum was separated by centrifugation at 12,000 rpm at 4°C for 5 min¹¹.

Biochemical estimation

Serum was analyzed for various biochemical parameters i.e., serum glutamyl oxalacetic acid

transaminases (SGOT, AST) serum glutamyl pyruvate transaminases (SGPT, ALT)¹² and alkaline phosphatase (ALKP)¹³ and serum bilirubin (SBLN)¹⁴.

Histopathological studies

The hepatoprotective activity was confirmed through histopathological studies on liver of rats. Slices of liver were cut and washed in Ringer's solution soaked with filter paper for 1.5 min. Then liver slices were fixed in Carnay's fluid I (ethanol: chloroform: glacial acetic acid 6:3:1) and processed for paraffin embedding following the standard microtechniques. Sections of liver, stained with aqueous haematoxylin and alcoholic eosin were observed microscopically for histopathological changes¹⁵.

Statistical analysis

The data represent Mean ± S.E.M. Results were analyzed statistically by one-way ANOVA, followed by Students 't' test. The minimum level of significance was set at $P < 0.001$ compared to control. The entire statistics were estimated by using Sigma Stat 3.5™, statistical software.

Results and Discussion

Phytochemical screening for the ethanolic extract of *Z. armatum* revealed the presence of phytoconstituents like sterols, alkaloids, phenolics, flavonoids and reducing sugars. The extract did not cause any mortality up to 2000 mg/kg and considered as safe.

CCl₄-induced hepatotoxicity

The results of CCl₄-induced hepatotoxicity are represented in Table 1. CCl₄ intoxication in normal rats significantly elevated the levels of SGOT, SGPT, ALKP, SBLN and liver inflammation, indicating acute hepatocellular damage and biliary obstruction. The rats that received 500 mg/kg of EEZA showed a significant ($P < 0.001$) decrease in the SGOT, SGPT, ALKP, and SBLN levels and liver inflammation, compared to induced control group.

Normal histology of rat liver showed sinusoidal degeneration (Fig. 1a). The liver sections of the rats treated with CCl₄ showed cellular degeneration hydropic changes which were more around the central vein and fatty changes with wide spread hepatocellular necrosis and centrolobular necrosis (Fig. 1b). The liver section of EEZA-treated showed micro fatty changes with dense collection of lymphoid cells, suggesting evidence of very little necrosis or degeneration. There was no hepatocellular damage, except small arrears of focal degeneration and sinusoidal dilation in treated rat livers (Fig. 1c and d).

Table 1—Effect of defatted ethanolic extract of *Zanthoxylum armatum* (EEZA) on CCl₄-induced hepatotoxicity in rats. (n = 6).

[Values expressed as mean ± SEM]

	SGPT (IU/L)	SGOT (IU/L)	SALP (IU/L)	Bilirubin (mg/dl)		Liver weight (g)
				Total	Direct	
Group I (Control)	53.25 ± 3.83	382.50 ± 21.32	196.68 ± 1.09	0.93 ± 0.03	0.17 ± 0.08	3.21 ± 0.22
Group II (Induction control)	268.32 ± 23.04***	702.45 ± 6.6***	489.23 ± 5.7***	3.14 ± 0.24***	1.29 ± 0.25***	3.38 ± 0.24**
Group III (Standard) 100 mg/kg b.w.)	78.6 ± .33	408.29 ± 4.68	213.55 ± 4.27	1.64 ± 0.82	0.19 ± 0.03	3.22 ± 0.01
Group IV (EEZA) (500 mg/kg b.w.)	104 ± 9.75*	438.00 ± 7.0**	249.59 ± 6.07*	1.82 ± 0.38**	0.24 ± 0.88*	3.32 ± 0.10**

*p<0.05 compared to control; **p<0.02 compared to control; ***p<0.001 compared to control.

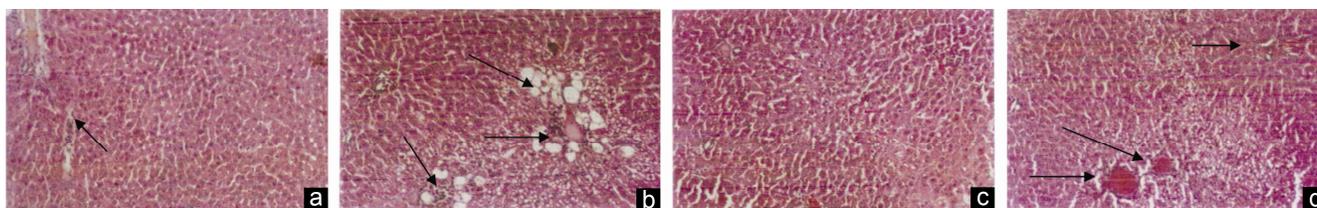


Fig. 1—Histopathology of liver tissues at X 400 magnification [(a): Group I (control) - section showing central vein surrounded by hepatic cord of cells (normal architecture); (b): Group II (CCl₄ treated) - section showing patches of liver cell necroses, inflammatory collections and accumulation of fatty lobules around central vein; (c): Group III (Standard silymarin treated) - almost near normal; and (d): Group IV (treated with ethanolic extract of *Z. armatum*)-minimal inflammatory cellular infiltration. Almost near normal liver architecture. Regeneration of hepatocytes around central vein]

CCl₄ is biotransformed into cytochrome P450 in the liver endoplasmic reticulum to the highly reactive trichloromethyl free radical which in turn reacts with oxygen to form a trichloromethyl peroxy radical, which may attack lipids on the membrane of endoplasmic reticulum more readily than trichloromethyl free radical. The trichloromethylperoxy radical leads to elicit lipid peroxidation, the disruption of Ca²⁺ homeostasis, elevation of hepatic enzymes and finally results in cell death¹⁶.

The results obtained from the present study indicated that the EEZA exhibited hepatoprotective effect against CCl₄-induced liver damage by normalizing the elevated levels of the hepatic enzymes. This suggested the possibility that EEZA is able to condition the hepatocytes, so as to cause accelerated regeneration of parenchyma cells, thus providing protection against membrane fragility and decreases of leakage of the marker enzymes into the circulation as compared to silymarin. It is also reported to have protective effect on the plasma membrane of hepatocytes¹⁷. The results support the use of this plant for the treatment of hepatitis as oriental traditional medicine.

Flavonoids have been reported as active substances for the treatment of hepatitis induced by chemicals¹⁸

and virus¹⁹ *in vitro* and *in vivo*. Ethanolic extract of *Z. armatum* showed positive results for the presence of phenolics and flavonoids during preliminary phytochemical screening. The possible mechanism may be that the antioxidant potentiality of flavonoids can scavenge free radicals and protect the cell membrane from destruction. Hence, the transaminases (ALT/AST) may not leak into blood from the necrotic hepatocytes.

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