

Hepatoprotective Effect of *Clerodendrum inerme* Linn. Ethanolic Extract.

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Twenty four rats were divided into four equal groups. The control group received distilled water intraperitoneally. The second group received carbon tetrachloride 0.5 ml/kg for seven days while the third group was injected with carbon tetrachloride 0.5 ml/kg and simultaneously administered with *Clerodendrum inerme* extract 200 mg/kg orally for seven days. The fourth group received carbon tetrachloride 0.5 ml/kg intraperitoneally together with silymarin 200 mg/kg orally for seven days. At the end of treatment blood samples collected by direct cardiac puncture and serum were used for the marker enzymes aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, as well as cholesterol and triglyceride content. Rats treated with the ethanolic extract of *C. inerme* showed a significant decrease in the levels of the markers. The results obtained indicate that *C. inerme* Linn. protects the liver against carbon tetrachloride induced hepatotoxicity.

Key words: Hepatoprotective, Glutathione, *Clerodendrum inerme*, carbon tetrachloride, aspartate amino transferase, alanine amino transferase, alkaline phosphatase.

INTRODUCTION

Renewed interest in the biological activities of medicinal plants emerged in the early 1980s when the Council for Scientific and Industrial Research, India published data on the screening of biological activities of many medicinal plants using experimental models [1]. Recently the use of herbal preparation in the management of various conditions has been rapidly increasing in India. The users believe that herbal preparations are safe although the ingredients may not have been structurally characterized [2]. *Clerodendrum inerme* Linn. (Verbenaceae) is used as a febrifuge, antipyretic and as a substitute for quinine in the treatment of malarial fevers [2]. In India, traditionally, the powdered leaves were mixed with camphor, garlic or pepper and used for edema, muscular pain as well as rheumatic pain while the roots were used for venereal diseases [2]. There is a growing interest in correlating phytochemical constituents of plants with their pharmacological activities [3].

In the present study, the effect of the extract of *Clerodendrum inerme* Linn. on serum enzymes

in rats treated with the hepatotoxic agent carbon tetrachloride was studied. Carbon tetrachloride destroys hepatocytes and interferes with cellular metabolism and transportation thereby elevating the level of serum enzymes such as alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST) as well as serum triglycerides and cholesterol.

MATERIALS AND METHODS**Collection of plant material**

Fresh plants were collected from Thinnelvely district in South India. The botanical identity was established with the help of a plant taxonomist at the Tamil Nadu Agricultural University, Coimbatore, A voucher specimen, number TAU/2005-11/11235, was deposited at the same institution.

Preparation of the extract

The air dried leaves of *C. inerme* were ground. The resulting powder was stored in airtight containers at room temperature, protected from sunlight. About 500 g of the powder was soaked

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in 1.5 l of 50 % aqueous ethanol for 2 weeks at room temperature with occasional shaking. The extract was filtered and the solvent evaporated under reduced pressure to yield 150 g of a greenish brown semisolid residue which was used for the study.

Experimental protocol

Twenty-four male albino rats of weight 150-220 g were selected for the study. The animals were provided with rat chow and water *ad libitum*. They were divided into four groups consisting of six animals each. The groups were administered with the following substances for 7 days:

Group I (control): Distilled water intraperitoneally (i.p.).

Group II: CCl₄ 0.5 ml/kg i.p. for seven days.

Group III: CCl₄ 0.5 ml/kg i.p. and *Clerodendrum inerme* extract 200 mg/kg orally simultaneously for seven days [2].

Group IV: CCl₄ 0.5 ml/kg i.p. and silymarin 200mg/kg orally simultaneously for seven days [3].

At the end of experimental period blood samples were collected by direct cardiac puncture and the serum was tested for the marker enzymes AST, ALT and ALP as well as cholesterol and triglycerides levels.

The rats were sacrificed and the livers removed immediately. The liver homogenates were prepared in 50 % 0.05 M sodium phosphate buffer pH 7.0 prepared according to the Indian Pharmacopoeia and centrifuged at 700 rpm for 10 min at 4 °C. The supernatant was used for the estimation of glutathione content. The glutathione content was estimated in the liver homogenate using DTNB [4]. The absorbance was measured at 412 nm and the results expressed as reduced glutathione (GSH) mg/g of wet tissue.

The statistical analysis was carried out using

ANOVA. The level of significance was set at $p < 0.001$.

RESULTS

Carbon tetrachloride is an hepatotoxicant which causes elevated levels of the serum enzymes AST, ALT and ALP as well as cholesterol and triglycerides levels. This was observed during the experiment as shown in Table 1. The ethanolic extract of *C. inerme* produced a significant decrease in the levels of the serum enzymes ALT, AST, ALP as well as triglycerides and cholesterol as compared to CCl₄ treated groups.

Table 1. Effect of *C. inerme* extract on serum enzymes ALT, AST and ALP in rats treated with CCl₄.

Groups	Serum Enzymes (I.U./litre)		
	ALT	AST	ALP
I	41.4±1.7	57.8±2.9	36.8±2.4
II	285±11.7	98.9±4.0	312.2±18.3
III	46.4±1.9	62.1±2.5	35.8±3.4
IV	54.2±2.2	74.2±9.0	38.2±4.4

Values are expressed as mean ±SD, $p < 0.001$

There was a rise in hepatic reduced glutathione content after treatment with *C. inerme* as shown in Table 2. All the effects of *Clerodendrum inerme* were comparable with those of silymarin, a standard hepatoprotective substance.

Table 2: Effect of *C. inerme* extract on the content of triglycerides, cholesterol and glutathione in rats treated with CCl₄

Groups	Triglycerides (mM/litre)	Cholesterol (mM/litre)	Glutathione (mg/g)
I	56.8±4.0	40.0±3.2	7.9±0.1
II	97.9±11.8	95.1±10.4	5.3±0.2
III	59.2±2.5	45.2±4.1	7.1±0.1
IV	60.0±5.0	48.3±5.7	7.5±0.2

Values are expressed as mean ±SD, $p < 0.001$.

DISCUSSION

The effect of the ethanolic extract of *Clerodendrum inerme* on the serum enzymes in rats treated with the hepatotoxic agent CCl₄ was studied. Carbon tetrachloride destroys the hepatocytes and interferes with cellular metabolism and transport [5] which is manifested in the serum enzyme levels. The metabolism of CCl₄ leads to release of CCl₄ free radicals which initiate peroxidation and degradation of fatty acids in the membranes.

Carbon tetrachloride also causes accumulation of fat in the liver by interfering with the transfer of triglycerides from the liver into plasma [6]. Due to destruction of hepatocytes, the serum enzyme levels increase. Aspartate aminotransferase (AST) is a mitochondrial enzyme while ALT is a cytosolic enzyme present in the liver. Serum estimation of ALT is of great value in assessing liver cell injury. Aspartate aminotransferase levels may rise in acute liver necrosis. Rise in total serum cholesterol, which is normally excreted in the

bile is also observed.

The activated CCl₄ radical binds to macromolecules and induces peroxidative degradation of membrane lipids of the endoplasmic reticulum which is rich in polyunsaturated fatty acids. This leads to the formation of lipid peroxides which in turn give rise to other products like malondialdehyde that cause damage to the membranes [7]. This lipid peroxidation of the biomembrane is one of the principal causes of hepatotoxicity by CCl₄ [8].

The results obtained in this study show that *C. inerme* extract significantly reduced serum enzymes, triglycerides and cholesterol and significantly increased the glutathione level. These findings are indicative of hepatoprotective activity. This shows that simultaneous treatment with *C. inerme* protects the liver against CCl₄ induced hepatotoxicity. Further biochemical parameters like lipid peroxidation, total protein, albumin and histopathological studies are needed to elucidate the mechanism of hepatoprotective action of *C. inerme* Linn.

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