

MODULATORY EFFECT OF *DRACAENA MANNI* LEAF EXTRACT ON SOME BIOCHEMICAL PARAMETERS OF WISTAR RATS TREATED WITH CARBON TETRACHLORIDE (CCl₄)

C. A. ANOSIKE, K. I. ANYANWU AND P. E. JOSHUA

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ABSTRACT

The modulatory/protective effect of the ethanol extract of *Dracaena mannii* leaves on some biochemical parameters of male Wistar rats treated with Carbon tetrachloride (CCl₄) was studied. Sixteen male Wistar rats of 100 - 170g body weight (bw) were divided into four groups of four rats each designated - group I (control), groups II, III, and IV. Groups II, III, and IV were injected intraperitoneally with 5ml/kg body weight (bw) of CCl₄; control was injected with 5ml/kg corn oil. Groups III and IV were administered with 200mg/kg bw and 400mg/kg bw of the extract respectively, 48hrs after CCl₄ injection, and the rats sacrificed after 5 days. Result from the study showed that administration of 200mg/kg bw of the extract significantly lowered (P < 0.05) CCl₄ induced elevations in serum alanine aminotransferase (ALT), serum aspartate aminotransferase (AST), total and conjugated bilirubin. Administration of 400mg/kg bw of the extract also reduced CCl₄ induced elevations in these parameters, but not as significantly as that of 200mg/kg dose. This suggests that the protective effect of the *Dracaena mannii* leaf extract occurs at low doses, higher doses may be toxic.

KEYWORDS: *Dracaena mannii*, ethanol extract, rats, biochemical parameters.

INTRODUCTION

Medicinal plants contain a variety of phytochemical substances which have appreciable physiological and pharmacological effects on man and animals. Traditional herbalists use most plants with pharmaceutical importance to treat one disease or the other (Iwu, 1993). The plant *Dracaena* (Dracaenaceae) which has been reported to have high biological activity (Okunji *et al.*, 1996) is used traditionally by herbal medicine practitioners in the treatment of a variety of illnesses. *Dracaena mannii* plant locally referred to as "Akuku" in Hausa, "Odo" in Igbo, "Ope - kannakanna" in Yoruba and "Kalala Kwabwe" in Tshilub grows as a shrub in most tropical countries including Nigeria (Jackson, 1990). There are some 120 species of the plant genus distributed in Central Africa, South Africa and Southeast Asia. The majority of the species are native to Africa. South Africa has three recognised species; *D. aletiformis*, *D. mannii*, *D. transvaalensis*, each of which occurs in a different habitat (Jackson, 1990).

Spirocorazole, an antifungal saponin isolated from two species of *Dracaena* - *D. mannii* and *D. cirborea* has been shown to have strong molluscicidal activity (Okunji *et al.*, 1991; Okunji *et al.*, 1996). The red sap (dragon's blood) secreted by some species of *Dracaenaceae* is used medicinally as a wash to stop bleeding and promote healing, to treat chest pains, internal trauma and post partum bleeding. It is also used as a colourant on furniture and violins (Venter 1996).

This work was aimed at evaluating the protective/modulatory effect of *Dracaena mannii* leaf extract on CCl₄ induced toxicity in rats.

MATERIALS AND METHODS

Animals

Male Wistar rats of weight 100-170g were used for the experiment. They were obtained from the animal house of the Faculty of Biological sciences, University of Nigeria, Nsukka. The rats were maintained *ad libitum* on water and growers mash bought from Nsukka market.

Plant material

Dracaena mannii leaves were collected from the environs of Nsukka, Nigeria and identified by Mr. Alfred Ozioko of the department of Botany, University of Nigeria, Nsukka. Voucher specimens were deposited in the herbarium unit of the department.

Chemicals and Reagents

CCl₄ was purchased from Aldrich Chemical Co. All other chemicals used in this study were of analytical grade and products of May and Baker, England; BDH, England and Merck, Darmstadt, Germany. Reagents used for all the assays were commercial kits and products of Randox, USA; QCA, Spain; Teco (TC), USA; Biosystem Reagents and Instruments, Spain.

Extraction

The leaves of *Dracaena mannii* were dried under room temperature (25°C ± 15°C) for two weeks, pulverized into coarse form with a milling machine and macerated in absolute ethanol for 24hrs before filtering with a white filter cloth. The resulting ethanol extract was evaporated to dryness using a rotary evaporator at an optimum temperature of 40°C ± 5°C. The weight of the dry extract was determined (35.05g). This was made into 1 litre aqueous solution and stored in the fridge until used.

Phytochemical analysis

Preliminary phytochemical tests were carried out on the ethanol extract of the leaves using the methods of Harbone, (1973) and Trease and Evans (1996). Tests for the presence or absence of phytochemical compounds using the above methods involve the addition of an appropriate chemical agent to the extract of the plant in a test tube. The mixture is shaken vigorously or gently as the case may be. The presence or absence of phytochemicals such as saponins, flavonoids, alkaloids, tannins, terpenoids, steroids etc. is observed.

Acute toxicity tests

The acute toxicity tests were carried out by the method of Lorke (1983) to define the range of lethal dose and

safe range for the extract. The LD₅₀ of the crude ethanol extract was calculated to be 800mg/kg. All doses used in this study were therefore carefully chosen to exclude the lethal range.

Experimental Design

Sixteen adult male albino rats were equilibrated for seven days, randomly divided into four groups of four rats each and housed in separate cages. They were fasted for 12hrs prior to the experiment. CCl₄ was dissolved in corn oil in the ratio of 3:1 and administered to the rats. Rats in group I (control) were intraperitoneally injected (i.p.) with corn oil. Rats in groups II, III and IV were injected (i.p.) with 5mg/kg CCl₄ (single dose). After 48hrs, 200mg/kg and 400mg/kg bw of *Dracaena mannii* leaf extract was administered to groups III and IV respectively. The rats were sacrificed after 5 days.

Preparation of serum

Blood was obtained from the rats by heart puncture technique into centrifuge tubes. Serum was prepared by centrifugation for 10mins at 3000rev/hr in a bench centrifuge. The clear supernatant was used for the biochemical tests carried out.

Biochemical analysis

Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and serum alkaline phosphatase were estimated colorimetrically using Randox reagent enzyme kits based on the methods of Reitman and Frankel (1957), and King and Kind (1954) respectively. Total protein based on Lowry (1951) and Bilirubin based on Jendrassik rxn (1938) were also determined using reagent kits.

Statistical analysis

Data were analysed as mean \pm SD. Difference between means was assessed by a two-tailed Student's T- test. $P < 0.05$ was considered statistically significant.

RESULT AND DISCUSSION

The result of the phytochemical tests revealed the presence of saponins, resin, carbohydrates, alkaloids and glycosides in the ethanol extract of *Dracaena mannii* leaves. Flavonoids, tannins and steroids were however absent. Okunji *et al.*, (1996) isolated two saponins from the fruit pulp of *Dracaena mannii*. Acute toxicity test of the extract gave an LD₅₀ of 800mg/kg. Doses were chosen to exclude the lethal range. Result from table 1 show that CCl₄ treated rats had significant increases ($P < 0.05$) in serum alanine aminotransferase, total and conjugated bilirubin, and non-significant increases ($P > 0.05$) in serum aspartate aminotransferase and total protein as compared to control. Treatment of the rats with 200mg/kg bw of the extract significantly reduced ($P < 0.05$) the CCl₄ induced elevation in both ALT and AST. At the administration of 400mg/kg bw of the extract, there was also reduction in the enzyme levels, but it was not as marked as that observed for 200mg/kg b/w. There was no significant difference ($P > 0.05$) between the alkaline phosphatase (ALP) of the control and CCl₄ treated rats. Treatment with the extract also elicited no significant change in ALP values. There was a significant and non-significant reduction in total protein at the administration of 200mg/kg bw and 400mg/kg bw of the extract respectively as compared to CCl₄ treated rats. Total and conjugated bilirubin of CCl₄ treated groups were significantly increased (P

< 0.05) as compared to control. A significant reduction ($P < 0.05$) in total and conjugated bilirubin was observed at the treatment with 200mg/kg bw and 400mg/kg bw of the extract. The effect was however not dose dependent. CCl₄, as an industrial solvent is known to elicit hepatocarcinogenic changes in laboratory animals (Amdur *et al.*, 1991; Guven *et al.*, 2003) due to the ability of the animals to metabolically break down CCl₄ in vivo, into intermediates that cause damage to the liver (Schinella and Becker, 1975). CCl₄ - induced lipid peroxidation as reported by Sipes *et al.* (1977) is highly dependent on its bioactivation by the cytochrome p-450 system of the liver to the trichloromethyl radical (CCl₃) and in the presence of oxygen, to trichloromethyl peroxy radical (CCl₃O₂) which is more reactive. The first metabolite; trichloromethyl free radical is believed to initiate the biochemical events that ultimately culminate in liver cell necrosis and damage (Pohl *et al.*, 1984). Exposure to CCl₄ leads to free radical generation in other tissues such as heart, lung, testis, brain and blood and also induce acute renal injuries (Ahmad *et al.*, 1987; Perez *et al.*, 1987; Szymonik-Lesiuk *et al.*, 2003). The liver damage caused by acute exposure of CCl₄ shows clinical symptoms such as jaundice, swollen and tender liver and elevated levels of liver enzymes in the blood (Tirkey *et al.*, 2005). Increased levels of ALT and AST of groups treated with CCl₄ in this study showed that CCl₄ induced liver damage thereby causing the liver enzymes to leak out into the bloodstream. These serum enzymes found within organ and tissues are released into the blood following cellular necrosis, increases in cell membrane permeability or proliferative changes and are used as diagnostic measure of liver damage (Sanjiv, 2002). Tirkey *et al.*, (2005) reported a marked rise in serum levels of ALT, AST and bilirubin at the administration of CCl₄. His study also reported CCl₄ - induced hepatic oxidative stress, as demonstrated by CCl₄ induced generation of peroxy radical which lead to a significant reduction of the antioxidant enzymes - catalase and superoxide dismutase in the liver. Bilirubin, a major breakdown product of haemoglobin rises when there is liver damage or injury (Sanjiv, 2002). At elevated levels, it causes the discolouration of the skin known as jaundice. Elevation of total bilirubin as a result of decreased uptake and conjugation of bilirubin by the liver is caused by liver cell dysfunction, while increased levels of direct or conjugated bilirubin is due to decreased secretion from the liver or obstruction of the bile ducts (Sanjiv, 2002). In this present study, CCl₄ induced hepatic damage, as represented by elevated levels of ALT, AST and bilirubin. Reduction of the serum aminotransferases, total and conjugated bilirubin by the *Dracaena mannii* leaf extract suggest a modulatory effect of the extract on CCl₄ induced liver cell damage. Protective effect of various natural products on CCl₄ induced hepatotoxicity has been reported (Hsiao *et al.*, 2003; Tirkey *et al.*, 2005). Also studies done with Ginseng (Joeng *et al.*, 1996) showed that the antioxidant property of ginsenosides contributes to its protection against CCl₄ induced hepatotoxicity. The hepatoprotective effect of the *Dracaena mannii* extract against CCl₄ induced toxicity observed in this study was mainly at the administration of the lower concentration (200mg/kg bw of the extract, suggesting that higher doses of *Dracaena mannii* extract maybe toxic to the animal. In conclusion, this hepatoprotective effect of *Dracaena mannii* against CCl₄ toxicity suggest that the plant may also possess some antioxidant properties which helped to combat the CCl₄ induced oxidative stress in the liver.

Table I: Effect of *Dracaena manni* leaf extract on some biochemical parameters in rats after CCl₄ administration.

PARAMETERS	GROUPS			
	Control	CCl ₄ 5ml/kg b.w	CCl ₄ + 200mg/kg D.manni Extract	CCl ₄ + 400mg/kg D. manni Extract
Alanine aminotransferase (U/L)	32.25 ± 13.89 ^a	55.50 ± 25.40 ^b	24.75 ± 3.30 ^a	32.75 ± 4.5 ^a
Aspartate aminotransferase (U/L)	106.0 ± 16.00 ^a	127.25 ± 15.92 ^a	72.25 ± 17.56 ^b	112.75 ± 16.24 ^a
Alkaline phosphatase (U/L)	83.25 ± 5.50	88.50 ± 3.51	92.25 ± 7.27	85.75 ± 6.65
Total protein (g/dl)	63.50 ± 6.60 ^a	73.25 ± 9.36 ^a	54.25 ± 9.91 ^b	62.25 ± 6.85 ^a
Total bilirubin (mg/dl)	0.33 ± 0.32 ^a	1.25 ± 0.85 ^b	0.21 ± 0.09 ^a	0.38 ± 0.06 ^a
Conjugated bilirubin (mg/dl)	0.28 ± 0.16 ^a	0.68 ± 0.45 ^b	0.14 ± 0.01 ^a	0.25 ± 0.07 ^a

Values are mean ± SD

Results with different superscript (^{a, b}) on the same row are statistically significant (P < 0.05).

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