



Anti-Oxidant Protective Effects of *Cassia Alata* in Rats Exposed to Carbon Tetrachloride

^{1,*}WEGWU, M O; ¹AYALOGU, E O; ²SULE, O J

¹ Department of Biochemistry, University of Port Harcourt, P.M.B 5323, Port Harcourt E-mail: wewic2000@yahoo.com

² Departments of Medical Biochemistry, Niger Delta University Wilberforce Island, Bayelsa State

ABSTRACT: The protective activity of *Cassia alata* against carbon tetrachloride (CCl₄) – induced hepatotoxicity in rats was assessed. Crude extracts of petals of the plant in 0.5% ethanol were administered by intubation for 14 days prior to injection of 0.5ml Kg⁻¹ CCl₄ into the rats. Serum aspartate aminotransferase and alanine aminotransferase levels, 18 hours after CCl₄ administration, decreased significantly ($P \leq 0.05$) in rats treated with the extracts than in CCl₄ – treated rats only. These findings suggest that *Cassia alata* may be protective against hepatotoxin-induced liver damage in rats. @JASEM

A number of chemicals, including CCl₄ are known to cause necrosis of cell or tissue. The damage or death of tissue usually results in the leakage of the enzymes in the affected tissue(s) into the blood stream (Sieger *et al.*, 1985; Obi *et al.*, 2001). Serum or plasma enzyme levels have been used as markers for monitoring chemically induced tissue damages (Lin and Wang, 1986; Ngaha *et al.*, 1989). The enzymes L-Alanine aminotransferase (L-ALT) [E.C. 2.6.1.2] and L-Aspartate aminotransferase (L-AST) [E.C. 2.6.1.1] are important enzymes that are often employed in assessing liver injury (Jaeger *et al.*, 1975; Ngaha *et al.*, 1989; Obi *et al.*, 1998). The biotransformation of CCl₄ to metabolites is a cytochrome P450-mediated reaction that initiate lipid peroxidation and attendant tissue damage. Shenoy *et al* (2001) reported that the oxidative damage through free radical generation is among the various mechanisms involved in the hepatotoxic effects of CCl₄.

Some plant extracts have been shown to have protective antioxidant effects and are therefore hepatoprotective. Such plants would include *H. rosasinensis* (Obi *et al.*, 1998), *Celosia argentea* (Koji *et al.*, 1996), *Ginkgo biboba* (Shenoy *et al.*, 2001), among others. *Cassia alata*, a soft wooden plant, common in villages and clearings within the Delta zone of Nigeria has been reported by Elenwo (1997) to have some antimicrobial properties. The leaves and stem bark are taken orally after maceration and filtration for the treatment of heart burn, in some localities (Elenwo, 1997). The flowers and leaves are used for the treatment of ringworms and eczema.

This study was therefore designed to evaluate the anti-oxidant protective effects of *Cassia alata* in rats exposed to carbon tetrachloride (CCl₄).

MATERIALS AND METHODS

Animals: Twenty adult male Wistar albino rats (200-250g) used in this study were obtained from the Animal House, Department of Biochemistry, University of Port Harcourt, Port Harcourt, Nigeria. They were housed in standard cages (Griffin and George Modular Cage System) and left to acclimatize for 7-days to laboratory conditions before the commencement of the experiment. During the acclimatization, the animals were fed with pelleted rat chow and water *ad libitum*.

Chemicals: Absolute ethanol and formic acid were the products of BDH Chemical Company Ltd (Poole, England) while CCl₄ and chloroform were from May and Baker (Dagenham, England). Other materials include vegetable oil (Mazola produced for CPC, UK) and rat chow (Pfizer Nigeria Plc).

Preparation of extracts. The petals of *Cassia alata* were collected from Omuhuechi, Aluu in Rivers – State, Nigeria. Preparation of the extracts followed the method of Obi *et al.* (1998). 50g of petals of the plant were macerated in a mortar and introduced into a 250ml beaker. Initial extraction was achieved using 150ml of formic acid, ethanol, water (1:10:9 v/v/v) mixture. The extract was filtered after three hours through cheese cloth. This was repeated thrice and the filtrates were pooled. An equal volume of petroleum ether (bp 40-60°C) was employed in the ‘washing’ of the pooled filtrate in a separatory funnel. The separatory funnel and its content was shaken vigorously (manually at 5min.

intervals) for 2 hours and allowed to stand for 12 hours in order to eliminate carotenoids and chlorophyll from the extract. The washed extract was left at 4°C while the petroleum ether layer was discarded. The organic solvents were evaporated at 70°C using low temperature hot plate (B&T, Searle Company) and the residue filtered through a 0.45 micron filter paper using a vacuum pump (Compach). The concentrate was mixed with 5% (v/v) ethanol and left at 4°C in a refrigerator until used.

Experimental Procedure: The rats were divided into five experimental groups with each consisting of four rats. Rats in groups 1 and 2 received 2.5ml/kg body weight of 5% (v/v) aqueous ethanol by gavage. The rats in groups 3,4 and 5 were given 2.5ml/kg body weight of 1.0, 5.0 and 10.0% (v/v) solution of the crude extract concentrate in aqueous ethanol, respectively, by gavage. These treatments were for 14 days. CCl₄ dissolved in vegetable oil was administered intraperitoneally to rats in groups 2, 3, 4 and 5 at a dose of 0.5ml/kg body weight in 0.5ml of vegetable oil on the 14th day, after the administration of the extracts of *Cassia alata*. The rats in group 1 received 0.5ml of vegetable oil only intraperitoneally. Eighteen hours after the administration of CCl₄, the rats were anaesthetized in a chloroform saturated chamber. Blood samples were obtained by cardiac puncture from each rat by means of a 5ml hypodermic

syringe and needle and placed in an anticoagulant free bottle.

The serum was used for the assay of the hepatic enzymes activities (L-AST and L-ALT) using the Humazym M UV-test kits. The mean values of the control and test serum activities of a given enzyme were compared using the student's t-test (Zar, 1984). The significance level was set at P≤ 0.05.

RESULTS AND DISCUSSION

Serum L-AST and L-ALT induced by CCl₄ treatment and the effects of treatment with various concentrations of extracts of *Cassia alata* prior to treatment of rats with CCl₄ are shown in Tables 1 and 2, respectively. In the rats treated with CCl₄ only, there was a significant (P≤0.05) increase in the activities of the enzymes. A concentration-dependent decrease (P≤0.05) in the activities of both L-ALT and L-AST was observed in rats pretreated with extracts of *Cassia alata* prior to administration of CCl₄ compared with CCl₄ – treated rats alone. There was no significant difference (P≤0.05) in the activities of the enzymes in the control rats (Group 1) with those that received 10% extract with CCl₄ treatment (Group 5) for L-ALT. A similar trend was observed for L-AST in rats in Group 4 (5.0% extract) and Group 5 (10% extract).

Table 1. Effects of crude extracts of *Cassia alata* on serum L-ALT activity (U/l/mean mg protein) in CCl₄ – induced liver damage.

GROUP	CCl ₄ ml/kg	EXTRACT (%)	MEAN	S.E.M
1.	-	-	102.06 ^a	1.01
2.	0.5	-	213.75 ^b	1.32
3.	0.5	1.0	143.39 ^c	1.53
4.	0.5	5.0	119.29 ^d	1.34
5.	0.5	10.0	109.72 ^a	0.52

- Means with different superscripts are significantly different at the 0.05 level
 - Values are means for 4 replicates (n=4)
 - S.E.M = standard error of the mean.

One commonly used hepatotoxin in the experimental study of liver diseases is CCl₄ (Koji *et al.*, 1996; Obi *et al.*, 1998; Shenoy *et al.*, 2001). The toxicity of CCl₄ to the liver of mammals is largely as a result of the active metabolite, trichloromethyl radical (Johnston and Krueing, 1998). The trichloromethyl radical binds to tissue macromolecules and thus induce peroxidative degradation of membrane lipids of the endoplasmic reticulum which are rich in polyunsaturated fatty acids. Shenoy *et al.* (2001) postulated that such development would

ultimately lead to the formation of lipid peroxides that in turn yield other products, among which is malondialdehyde (MDA). MDA has been demonstrated to cause damage to the membrane. Hence, one of the principal causes of hepatotoxicity of CCl₄ is the lipid peroxidative degradation of biological membrane (Kaplowitz *et al.*, 1986; Cotram *et al.*, 1994).

Our preliminary investigations on the toxicity of CCL4 in male wistar albino rats indicate that administration of CCL4 concentrations above 0.6 mg/kg was lethal within 24-hours. Also CCL4 levels

below 0.4mg/kg was not lethal and liver damage was not obvious. These findings informed the application of 0.5mg/kg in this study to induce liver damage in rats. The CCL4 dose adopted in this work is in

agreement with that of Obi *et al.*(1998) who induced liver damage in rats by administering CCL4 levels of 0.5mg/kg

Table 2 Effect of crude extracts of *Cassia alata* on serum L-AST activity (U/l/mean mg protein) in CCL₄ – induced liver damage

GROUP	CCl ₄ ml/kg	EXTRACT (%)	MEAN	S.E.M
1.	-	-	107.26 ^a	1.04
2.	0.5	-	227.32 ^b	1.53
3.	0.5	1.0	140.38 ^c	1.30
4.	0.5	5.0	116.44 ^a	1.03
5.	0.5	10.0	110.75 ^a	2.08

- Means with different superscripts are significantly different at the 0.05 level
 - Values are means for 4 replicates (n=4)
 - S.E.M = standard error of the mean.

The results in this study indicate that CCL₄ caused an increase in the serum levels of marker enzymes: ALT and AST. Such elevation is indicative of liver injury, especially, the rise in L-ALT activity (Lin and Wang, 1986; Reinke *et al.*, 1988). The injection of 0.25ml of 1.0, 5.0 and 10.0% crude extracts / kg body weight of rats for 14 days prior to administration of 0.5ml/kg CCL₄ progressively caused a decline in hepatotoxicity than with CCL₄ alone. This is evidenced in the marked decrease in serum L-AST and L-ALT activity relative to the group treated with CCL₄ only. Shenoy *et al.* (2001) reported an elevation in the levels of MDA in liver of rats treated with CCL₄ only. They attributed the increase in MDA levels to enhanced lipid peroxidation, leading to tissue damage and failure of antioxidant defence mechanisms to prevent the formation of excessive free radicals. The results in this study suggest that pretreatment of rats for 14 days with *Cassia alata* prior to CCL₄ administration significantly ($P \leq 0.05$) reversed these changes. It would be deduced, therefore, that the antioxidant effects of crude extracts of *Cassia alata* could possibly be its mechanism of hepatoprotection.

In order to eliminate contamination with lipids, including lipid soluble materials, 50% aqueous ethanol slightly acidified with formic acid was employed in anthocyanin extraction present in the *Cassia alata* (Obi *et al.*, 1998). The extraction condition reported in this work ensured that the carotenes, tocopherols, chlorophyll, carotenoids, sterols, aglycones and catecols (Ibrahim and Baron, 1989; Van Sumere, 1989) were not present in the extract. Therefore, the anti-oxidant that is present in the extract is anthocyanin. This is in agreement with Obi *et al.* (1998) who reported the protection of *H. rosasinensis* anthocyanin against CCL₄ –induced liver damage in rats. The anthocyanin in the extract may have prevented liver damage by breaking the

sequence of events between the reductive dechlorination of CCL₄; abstraction of hydrogen ion from unsaturated fatty acids in the membrane; and peroxide formation. The anthocyanin may also have inhibited the activities of cytochrome P₄₅₀ Isoenzymes required for trichloromethyl radical production

Appreciation: The authors wish to express their profound gratitude to the University of Port Harcourt for the Senate Research Grant No UPH/DVC.AC/11/SD.022/COM.4 in partial support for this work.

REFERENCES

- Contram, R. S., Kumar, V. and Robbins, S. L. (1994). Cell injury and cellular death. In Robbin's Pathologic Basis of Diseases. 5th ed. Prism Book Pvt. Ltd. 379-430.
- Elenwo, E.N.(1997). Effectiveness of leaves of *C. alata* and *Vernonia myadalina* in the preservation of Okra fruits in the Niger Delta. Nig. Journ. Crop, Soil and Forestry. 3: 5-9.
- Ibrahim, R., Baron, D.(1989). Phenylpropanoids. *In:* Dey, P.M., Harborne, J.B.(E.ds),Methods in Plant Biochemistry.Vol.1, Plant Phenolics. Academic Press, London, pp. 75 – 111.
- Jaeger, R.J., Conolly, R.B., Murphy, S.D.(1975). Short term inhalation toxicity of halogenated hydrocarbon. Arch. Environ Health. 30:26-31.
- Johnston, D.E., Kroening, C.(1998). Mechanism of early CCL₄ toxicity in cultured rat hepatocytes. Pharmacol Toxicol. 83:231-39.

- Kaplowitz, N., Simon, F.R., Stolz A.(1986). Drug-induced hepatotoxicity. *Ann. Int. Med.* 104:826-39.
- Koji, H., Shigotohi, K., Purusotam, B., Tsuneo,N. (1996). Hepatoprotective effects of traditional medicines. Isolation of the active constituent from seeds of *C. argentea*. *Phytotherapy Research.* 10:387-392.
- Lin, J.K., Wang, C.J.(1986). Protection of crocin dyes in the acute hepatic damage induced by Aflatoxin B₁, and dimethylnitrosamine in rats. *Carcinogenesis* 7:595-599.
- Ngaha, E.O., Akanji, M.A., Madusolumuo, M.A. (1989). Studies on correlation between chloroquine induced tissue damage and serum enzyme changes in the rat. *Experientia* 45: 143-146.
- Obi, F.O., Usenu I.A., Osayande, J.O. (1998). Prevention of CCl₄-induced hepatotoxicity in the rat by *H. rosasinensis* anthocyanin extract administered in ethanol. *Toxicol* 131: 93-98.
- Obi F.O., Omogbai, L.A., Oriafo, O.S.J., Ovat, O. D. (2001). Effect of a short time post CCl₄ treatment interval on rat plasma enzyme levels and percentage mortality. *J. Appl. Sci. Environ. Mgt.* 6 (1): 5-8.
- Reinke, L.A., Lia, E.K. McCay, P.B. (1988). Ethanol feeding stimulates trichloromethyl radical formation from CCl₄ in liver. *Xenobiotica* 18:1311-1318.
- Van Sumere, C. F. (1989). Phenols and Phenolic Acids. *In: Dey, P.M., Harborne, J.B.(E.ds),Methods in Plant Biochemistry.Vol.1, Plant Phenolics.* Academic Press, London, pp. 29 – 73.
- Shenoy, K.A., Somayaji, S.N., Bairy, K.L.(2001). Hepatoprotective effects of *Ginkgo biloba* against CCl₄ – induced hepatic injury in rats *Indian J. Pharmacol.* 33: 260-266.
- Siegers, C.P., Horn, W., Younes, M.(1985). Effects of hypoxia on the metabolism and hepatotoxicity of CCl₄ and vinylidene chloride in rats. *Acta Pharmacol. Toxicol.* 56: 81-86.
- Zar, J.H. (1984). *Biostatistical Analysis.* Prentice Hall, Eaglewood Cliffs, N. J. USA. pp 21-34.