ANTIHEPATOTOXIC ACTIVITY OF AQUEOUS EXTRACT OF *CASSIA ALATA* (LINN) LEAVES AGAINST CARBON TETRACHLORIDE INDUCED LIVER DAMAGE IN RATS

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ABSTRACT

The effect of oral administration of aqueous extract of leaves of *Cassia alata* in various doses (2.5 - 20.0 $mgkg^{-1}$) for 7 days, on hepatic damage induced by administration of 45% EtOH (20 $mlkg^{-1}$) and CCl_4 (0.1 ml kg^{-1}) in rats has been investigated. Biochemical parameters including levels of serum tranaminases (GOT and GPT), serum bilirubin and plasma prothrombin time have been determined to assess liver cell damage and liver function. Significant increases in the levels of serum tranaminases 88:14 $\pm$ 29.89 U/L, ($P<0.01$) GOT and 76.00 $\pm$ 31.19 U/L ($P < 0.05$) GPT were reduced by 22.7% and 32.9% respectively. In addition, prothrombin time, 19.97 $\pm$2.02 sec. ($P<0.005$) was reduced by 48.75% and bilirubin contents (0.31 $\pm$ 0.10 mg/dl, direct and 1.38 $\pm$ 0.98 mg/dl total) were decreased by 44.2% and 58.1% respectively. The results showed that the levels of serum transaminases (GOT and GPT), serum bilirubin and plasma prothrombin time raised by the EtOH/CCl_4 treatment were dose-dependently reduced by the oral administration of the extract. The observed hepato-protective activity of the extract confirms this aspect of the use of *Cassia alata* in traditional medicine for the treatment of cirrhosis and hepatitis.

INTRODUCTION

Cassia alata (Caesalpinacease) popularly known as 'ringworm cassia' is a medicinal plant used in many parts of the world. It is well known in West Africa, where it goes by various names. For example, the Fanti people of Southern Ghana call it 'Nsenpi' whilst in Sierra Leone it is called 'Nje-pai' by the Mendes. The Yorubas of Southern Nigeria call it 'Asuwon'. The various parts of the plant are used for several medicinal purposes. Thus decoctions of the leaves are used as laxative and in the treatment of skin diseases and liver problems especially hepatic jaundice (Irvine, 1961; Hutchinson and Dalziel, 1954). The bark and seeds may be used in pest control (Grainage *et al.*, 1986). In Ghana its use in the treatment of skin diseases and in purgation are well known. Furthermore, the use of the leaves in the treatment of liver problems has been adopted by traditional medicine practitioners in the coastal areas (especially the central region of Ghana) for a very long time (Personal communication).

Cassia alata flourishes in the forest areas of West Africa. It is a shrub which grows all year round and flowers during November to January. Although the plant is well investigated (Anonymous, 1992; Ayim, 1986), its use in the treatment of hepatic jaundice in folk medicine is not well documented. This investigation was undertaken to study the possible hepatoprotective effect of aqueous extract of the leaves of the plant.

MATERIALS AND METHODS

Plant Material

Cassia alata leaves were collected from the main campus of the University of Cape Coast, Cape Coast, Ghana. The herbarium specimens were identified in the Botany Department of the Faculty of Science of the same University. The leaves were dried at 40°C for a period of one week, ground and sieved with a sieve. The powdered leaves were kept in cellophane bags and stored at 4°C.

Animals

Male albino rats of the Wistar strain weighing 150 -200g were obtained from the National Veterinary Research Institute, Vom, Plateau State, Nigeria. They

were housed under a standard lighting regimen and fed standard diet (Nutrifeeds, Nigeria Ltd., Kano) and water *ad libitum*.

Drugs and other Chemicals

Carbon tetrachloride (CCl_4) May and Baker Ltd., England; Ethanol (EtOH), Prolabo, Paris, France); GOT, GPT, prothrombin time and bilirubin estimation kits (Randox Laboratories Ltd. Ardmore, U.K.). All other chemicals were of analytical grade.

Preparation of Aqueous Extract

The extraction procedure was carried out according to the method of Mittal *et al.* (1981) and Fernando *et al.* (1991). A 200g portion of the powdered leaves was mixed with a litre of distilled water in a two-litre beaker and boiled for 1½hr. It was allowed to cool to 40°C and then sieved through a cheese cloth. The liquid part was filtered using Whatman No. 1 paper. The filtrate was then evaporated until the final volume was 400 ml (1 ml of the extract represent 0.5g of the dry powder). The extract was then stored in the refrigerator until use.

Phytochemical Analysis

The phytochemical screening of the extract was performed using the methods of Sims (1968), Trease and Evans (1978) and Odebiyi and Sofowora (1978). Tests for tannins, alkaloid, saponins, phlobatannins, simple sugars, flavonoids and cardiac glycosides were carried out according to standard procedures..

Induction of Hepatotoxicity

The experimental animals were divided into six groups of six rats each. Group I (control) was administered with normal saline (1.0 ml kg^{-1}) for 21 days and on the 22nd day, blood samples were taken by cardiac puncture and diagnostic estimations performed. Group II - VI served as the model for EtOH/CCl_4-induced hepatotoxicity. Prior to the administration of the extract, rats in groups II - VI were given 45% EtOH (v/v, 20 ml kg^{-1} body weight orally) for 21 days. Rats in groups III - VI also received various doses (2.5, 5.0, 10.0 and 20.0 ml kg^{-1}) of the extract from the 15th to 21st day of study. On the 20th day, they were injected with CCl_4 (1:1 in groundnut oil and 0.1 ml kg^{-1} body weight s.c.). They were sacrificed 48 hrs later (Tripathi *et al.*, 1991). Blood was collected and plasma and serum samples prepared and stored at -20°C for the following estimations to assess the liver function. The plasma was used to determine prothrombin time according to the method of Curtains and Marc (1974). The serum collected was used to assay glutamate pyruvate transaminase (GPT), glutamate oxaloacetate transaminase (GOT) levels by the method of Retiman and Frankel, (1957) and bilirubin (total and direct), by the Malloy and Evelyn (1973) method.

RESULTS

Phytochemical Studies

The results of the preliminary phytochemical screening showed the presence of tannins, phlobatannins, simple sugars, flavonoids, alkaloids and anthraquinone glycosides.

Effect of Extract on EtOH/CCl_4-induced Hepatotoxicity

As shown in Table 1, there was a high significant increase in the levels of all the biochemical parameters (GPT, GOT, serum bilirubin and plasma prothrombin time) estimated as a result of EtOH/CCl_4 treatment of the experimental animals. When compared, the prothrombin time in the EtOH/CCl_4 group was 19.97 ± 3.62 sec, while the control value was 3.41 ± 0.52 sec ($p < 0.005$). The control value of the GPT was 22.31 ± 2.60 U/L while the EtOH/CCl_4-treated group was 76.00 ± 31.19 U/L ($p < 0.05$). The GOT in the EtOH/CCl_4-treated group was 88.14 ± 29.89 U/L and the corresponding control value obtained was 13.75 ± 4.34 U/L, thus showing a significant ($p < 0.01$) increase. The direct bilirubin value was 0.31 ± 0.10 mg/dl for the control and 0.69 ± 0.15 mg/dl for the EtOH/CCl_4-treated group, whilst the total bilirubin was 0.45 ± 0.30 mg/dl for the control and 1.37 ± 0.99 mg/dl for the EtOH/CCl_4-treated groups.

Pre-treatment of the experimental animals with the extract for 7 days (15th day to 21st day of study) brought about significant reductions in the levels of GPT, GOT, bilirubin and plasm prothrombin time (Table 1). Increasing the dose from 2.5 ml kg^{-1} body weight, brought about decreases in the prothrombin time and GOT. The direct bilirubin content changed from 56.3 12% and the total bilirubin content changed from 63.8 to 5.7% compared to the EtOH/CCl_4-treated group of rats. In all cases, the extract in the doses indicated was effective in reducing the pre-treatment in the EtOH/CCl_4 group.

DISCUSSION

In this study, hepatotoxicity was induced by prolonged administration of alcohol and then short administration of carbon tetrachloride. Chronic alcohol

consumption is known to be associated with the appearance of excess fat in the parenchymal cells, causing a complex and acute change in which there is liver cell death, appearance of an intracellular hyaline and an acute inflammation progressing into a degenerative/regenerative fibrotic disease, cirrhosis. Cirrhosis and hepatitis may occur because of enhanced lipid peroxidation produced during the microsomal metabolism of ethanol. Alcohol pretreatment stimulates the toxicity of carbon tetrachloride due to increased production of toxic reactive metabolites of the compound, such as the trichloromethyl metabolite by the microsomal mixed function oxidase system (Lieber *et al.*, 1974; Bowman and Rand, 1988). This activated radical binds covalently to the macromolecules and induces peroxidative degradation of membrane lipids of endoplasmic reticulum rich in polyunsaturated fatty acids. This lipid peroxidative degradation of bio-membranes is one of the principal causes of hepatotoxicity (Dhawan *et al.* 1991).

In the present study, the hepatotoxicity of the EtOH/CCl_4 (in toxic doses) has been manifested by the increase in the values of the biochemical parameters (prothrombin time, GPT, GOT and bilirubin) of plasma and serum from treated rats as compared with the untreated animals (control) (Table 1). This is in agreement with the findings of Tripathi *et al.* (1991).

The extract in various doses (2.5, 5.0, 10.0 and 20.0 ml kg^{-1}) decreased the level of the biochemical parameters assayed. Increasing the dose from 2.5 ml kg^{-1} to 20.0 ml kg^{-1} led to a decrease in the prothrombin time (71.70 to 22.98%), GPT (69.7 to 36.7%) and GOT (45.8 to 23.2%). The direct bilirubin content changed from 56.3 to 12.1% and the total bilirubin decreased from 63.8 to 5.7%. In all cases, the extract showed a dose-related effectiveness when compared with the EtOH/CCl_4-treated group. These findings indicated that the extract of *Cassia alata* leaves, possess potential hepatoprotective activity. The chemical nature of the active ingredients responsible for the hepatoprotective activity is unknown. However, it has been reported that flavonoids, as well as vitamins A and C have antioxidant properties (Anonymous, 1992).

The phytochemical screening revealed the presence of flavonoids and these might be implicated in the hepatoprotective activity of the extract. The result of this study, therefore, provide an experimental basis for the use of decoctions of *Cassia alata* as a hepatoprotective drug for the management of cirrhosis and hepatitis in folk medicine.

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Table 1: Effect of aqueous leaf extract of *cassia alata* on some biochemical Parameters in rats with etoh/ccl_4 induced hepatotoxicity

Treatment	Prothrombin Time (sec.)	GPT (U/L)	GOT (U/L)	Bilirubin (mg/dl)	
				Direct	Total
I. Control	3.41 ± 0.52	22.32 ± 2.60	13.75 ± 4.34	0.31 ± 0.10	0.45 ± 0.30
II. EtOH/CCl_4	19.97 ± 3.62^{a}	76.00 ± 31.19^{c}	88.14 ± 29.89^{b}	0.69 ± 0.15^{NS}	1.38 ± 0.98^{NS}
III Dose of Extract 2.5 (ml kg^{-1})	14.32 ± 2.94^{b}	53.02 ± 9.81^{NS}	40.44 ± 15.31^{b}	0.39 ± 0.18^{b}	0.89 ± 0.20
IV. " " 5.0	9.50 ± 1.86^{b}	42.11 ± 7.94^{NS}	33.09 ± 10.01^{b}	0.20 ± 0.54^{NS}	0.37 ± 0.36^{NS}
V. " " 10.0	7.82 ± 2.83^{b}	35.42 ± 12.56^{NS}	28.48 ± 14.37^{b}	0.10 ± 0.13	0.14 ± 0.21^{NS}
VI. " " 20.0	4.59 ± 3.24^{b}	27.92 ± 13.01^{b}	20.48 ± 13.01^{ns}	0.08 ± 0.15^{b}	0.08 ± 0.11^{b}

All values are mean of six separate determinations $\pm$ S.D., Significant difference (I versus II; III - IV versus II) between groups. a = $P<0.005$, b = $P<0.01$, C = $P<0.05$

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