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The Effect of Aqueous Stem Bark Extract of *Sclerocarya birrea* (Hoechst) on Alcohol Carbon Tetrachloride Induced Liver Damage in Rats

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Abstract: *Sclerocarya birrea* (Hochst) is widely used in Nigeria and some African countries as medicine for the treatment of various ailments. In the present study the effect of the aqueous extract of *Sclerocarya birrea* was investigated against alcohol-carbon tetrachloride induced hepatocellular injury in rats over a period of 21 days. The aqueous stem bark extract was administered orally by gavage to the rats at a dose of 2, 5 and 8 mg kg^{-1} body weight, respectively from days 15 to 21, while a single dose of carbon tetrachloride (CCl_4; 0.1 mL kg^{-1} body weight in pure corn oil) was administered subcutaneously on day 20 to induce hepatotoxicity. At the of end the experimental period, blood was collected for the assessment of serum levels of Alanine aminotransferase (ALAT), Aspartate aminotransferase (ASAT), Alkaline phosphatase (ALP), bilirubin, albumin and protein levels. The liver tissue obtained was used for histopathological assessment of liver damage. The levels of ASAT, ALP and Albumin were significantly ($p<0.05$) increased in the rats administered 2 mg kg^{-1} but was more ($p<0.001$) in the 5 mg kg^{-1} groups. Histopathological studies show vacuolar cytoplasmic degeneration, multiple foci of hepatocyte cloudy swelling and focal areas of hepatocyte necrosis with macrophage infiltration providing supportive evidence for the biochemical analysis with greater toxicity in the groups administered 2 and 5 mg kg^{-1} of extract. This study demonstrates that the aqueous extract of the stem bark of *Sclerocarya birrea* extract possess possible hepatotoxic and antihepatotoxic activity at low and high doses, respectively.

Key words: *Sclerocarya birrea*, alcohol-carbontetrachloride, hepatoprotective, induced hepatotoxicity

INTRODUCTION

Sclerocarya birrea (Hoechst) family anacardiacea is a tree found widespread in the savannah regions of Africa from Ethiopia in the North to Southern Africa (Hutchinson and Dalziel, 1957). Pharmacological studies indicate that the stembark of this plant possess antidiarrhoel (Galvez *et al*., 1993), antibacterial, antinflamatory, antimalarial (Venter and Venter, 1996) and anthelminthic (Sparg *et al*., 2000) properties.

In view of its many uses and the fact that it is administered without studying its adverse effects especially in patients with conditions that compromise the function of the liver, the present study was undertaken to examine the possible effect of the aqueous extract of this plant on alcohol -CCl_4 induced hepatotoxicity in rats.

MATERIALS AND METHODS

Plant material: The stem bark extract of *Sclerocarya birrea* was collected from Bauchi State in the Savannah region of Nigeria and identified by a plant taxonomist of the Department of Biological Sciences University of Maiduguri, Nigeria.

It was sun dried, pulverized to coarse powder and stored at room temperature of 32±4°C and <30% relative humidity.

Chemicals: Absolute ethanol (Glissons), Carbon tetrachloride (May and Baker) and pure corn oil (Sigma).

Animals: A total of 25 adult wistar albino rats (150-280 g) of both sexes were obtained from the animal facility centre of the National Veterinary Research Institute Vom, Plateau State, Nigeria.

The animals were kept in plastic cages at room temperature of 32 ±4°C and <30% relative humidity with a 12 h light/dark cycle. They had access to drinking water and standard laboratory diet (Sanders SEEPC feed PLC, Jos, Nigeria) *ad libitum*. Identification was by colour tattoo.

Extract preparation: Water extraction was performed according to the method described by the World Health Organization (WHO, 1992). A total of one hundred grams (100 g) of the stem bark powder of *Sclerocarya birrea* were subjected to exhaustive soxhlet extraction in distilled water (500 mL) for 42 h. The extract was concentrated in a

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water bath until a constant dark sticky residue was obtained. This was further oven dried and maintained in a desiccator until a constant weight was obtained. The dried stem bark extract obtained was stored in a tightly stoppered container at -4°C until required. Stock solution of the extract was prepared by dissolving 2 g weight of the powdered root bark in 20 mL of normal saline and the concentration used was 0.1 g mL^{+}.

Experimental design: The animals were randomly divided into five groups of five rats each and their initial body weights taken and recorded.

Control groups: Group I served as the negative control and no drug or extract was administered for the period of the experiment.

Group II was the treatment control and received 40% alcohol (20 mL kg^{-1} body weight) for twenty-one days. On day 20 of the study period, carbon tetrachloride (1:1 in pure corn oil, 0.1 mL kg^{-1} body weight) was administered. No extract was given.

Treatment groups: Groups III - V received 40% alcohol (20 mL kg^{-1} body weight) for 21 days. From day 15 to 21, group III received 2 mg kg^{-1}, group IV received 5 mg kg^{-1} and group V received 8 mg kg^{-1} body weight of the aqueous extract. On day 20, carbon tetrachloride (1:1 in corn oil, 0.1 mL kg^{-1} body weight) was administered to all the treatment groups.

Route of administration: Alcohol (40%) and extract were given intragastrically via oral incubations while carbon tetrachloride was administered subcutaneously.

Collection of blood samples: Animals were weighed and sacrificed by transection of the jugular vein. Blood collection was at the site of transection and plasma serum was obtained by centrifuging the blood samples at 12,000 rpm for 5 min.

Biochemical analysis: Serum levels of ALAT, ASAT, ALP, bilirubin, albumin and protein levels were assayed by standard methods (Reitman and Frankel, 1957; Doumas *et al*., 1982).

Histological analysis: The liver tissues removed, were carefully dissected out, fixed in Bouins fluid and processed for light paraffin sections and stained with Haematoxylin and Eosin.

Statistical analysis: Data obtained were analysed using the Statistical Package for Social Scientist (SPSS 11.0) and results were expressed as the mean value±standard error of mean. Differences among means of various groups were determined by student's t-test. A probability level of less than 5% ($p<0.05$) was considered significant.

RESULTS

Effects of alcohol, CCl_4 and extract on mean body weight: Mean body weight was reduced in all but the negative control group though the reduction was not statistically significant ($p>0.05$) (Table 1).

Effects of alcohol, CCl_4 and extract on biochemical parameters: A significant ($p<0.05$) increase in the serum level of ALP was observed when the negative control was

Table 1: Effects of the administration of alcohol, carbontetrachloride and extract on mean body weights of rats

	Dose (kg^{-1})						
Groups	Alcohol (mL)	CCl_4 (mL)	Extract (mg)	Initial body weight (g)	Final body weight (g)	Body weight difference (g)	% Weight change (g)
I	0	0	0	282.0±18.44	302.8±19.61	22.72	7.5
II	20	0.1	0	154.85±6.62	151.74±13.37	3.14	2.0
III	20	0.1	2	210.10±11.67	204.06±13.37	6.04	2.9
IV	20	0.1	5	232.62±13.24	223.16±12.46	9.46	4.1
V	20	0.1	6	186.84±13.21	171.98±14.30	14.86	8.0

Results are presented as Means±SEM, N = 5

Table 2: Effects of the administration of alcohol, carbontetrachloride and extract on biochemical parameters of the rats liver

	Dose (kg^{-1})									
Groups	Alcohol (mL)	CCl_4 (mL)	Extract (mg)	ASAT (IU L^{-1})	ALAT (IU L^{-1})	ALP (IU L^{-1})	T/P (g L^{-1})	ALB (g L^{-1})	C/B (mmol L^{-1})	T/B (mmol L^{-1})
I	0	0	0	53.4±5.72	23.0±0.17	139.8±5.00	61.4±2.32	33.2±1.77	2.4±0.25	6.2±0.37
II	20	0.1	0	60.0±4.30	23.4±1.94	180.1±2.11	61.4±0.87	35.8±0.97	3.0±0.55	5.8±0.37
III	20	0.1	2	80.8±2.22*	35.0±1.82	267.4±12.5*	59.6±0.40	29.2±1.28	2.8±0.37*	7.0±0.32
IV	20	0.1	5	195.2±4.4*	115.0±6.0*	266.0±5.14*	59.0±0.32	26.4±0.25	3.2±0.38*	8.6±0.40*
V	20	0.1	8	91.4±4.20*	33.4±1.21	204.2±2.27	59.2±1.28	28.2±0.37	2.4±0.4*	6.0±0.32

Significance relative to treatment control (Group II)* = $p<0.001$, N = 5 Results are presented as Means±SEM. ASAT, Aspartate Aminotransferases. ALAT, Alanine Aminotransferases. ALP, Alkaline Phosphatase. T/P, Total Protein. ALB, Albumin. C/B, Conjugated Bilirubin. T/B, Total Bilirubin

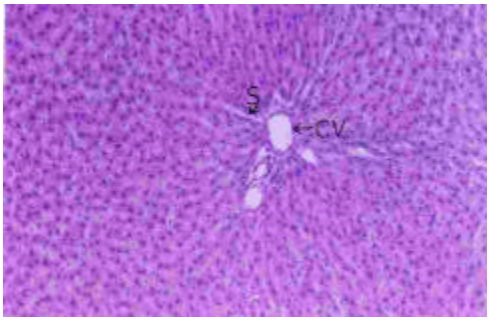

Fig. 1: Liver section of control group showing normal hepatic parenchyma with a Central Vein (CV) and Sinusoids (S), H and E stain. X 200

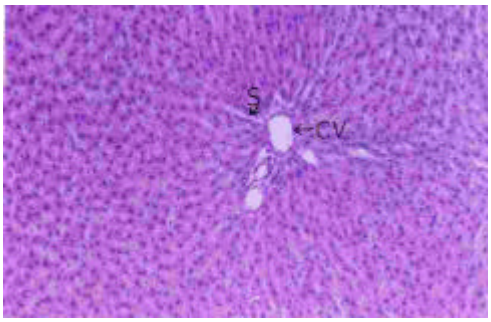

Fig. 4: Liver section of rat treated with 5 mg kg^{-1} of extract showing Cloudy swelling (X). H and E stain X 200

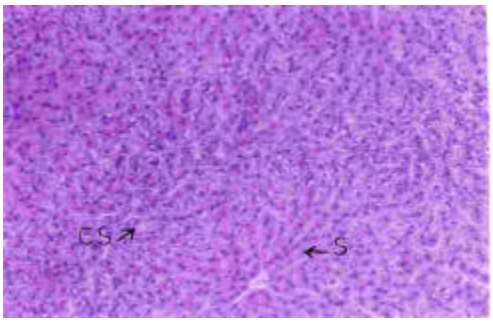

Fig. 2: Liver section of rat treated with alcohol (20 mL kg^{-1}) Showing Central Vein (CV), sinusoids (S) and Cloudy Swelling (CS) H and E stain. X 200

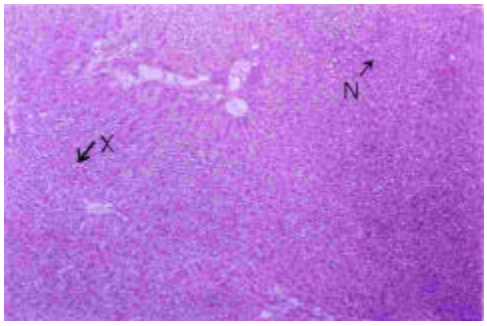

Fig. 5: Liver section of rat treated with 8 mg kg^{-1} of extract showing Cloudy swelling (x) and necrotic areas (N). H and E stain X 100

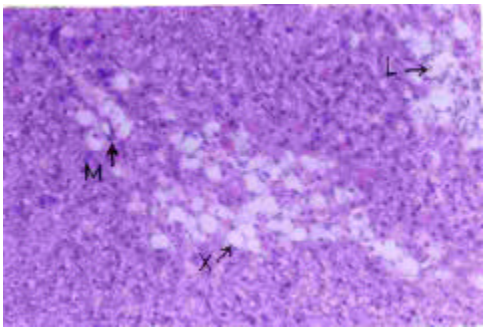

Fig. 3: Liver section of rat treated with 2 mg kg^{-1} of extract showing Lymphocyte (L), macrophages (M), cloudy swelling (x). H and E stain X 200

compared with the positive control. There was also a significant ($p<0.05$) increase in the level of this enzyme when the negative controls were compared with the positive controls and those in Groups III and IV which were administered 2 and 5 mg kg^{-1} body weight of the aqueous extract, respectively. The levels of ASAT were significantly ($p<0.05$) higher in all the groups that were administered the extract when compared to the negative control (Table 2).

Histopathologic findings: No histological or macroscopic alterations were found in the liver tissues of the negative control group (Fig. 1), however there was widespread vacuolar cytoplasmic degeneration in the positive controls (Fig. 2).

In the groups administered the extract, histological alterations varied with respect to the dose administered; the group administered 2 mg kg^{-1} body weight of the aqueous extract showed multiple foci of hepatocyte cloudy swelling, focal areas of hepatocyte necrosis with infiltration of macrophages (Fig. 3). The degenerative changes observed were more pronounced in the group of animals administered 5 mg kg^{-1} body weight of the aqueous extract (Fig. 4) but occurred to a lesser extent in

the group administered 8 mg kg^{-1} body weight of the aqueous extract (Fig. 5).

DISCUSSION

The loss in mean body weight observed in all treatment groups in this study (Table 1), though not statistically significant ($p>0.05$) could be attributed to the loss of appetite observed during the experimental periods which might have lead to decrease in food intake or may have been caused by lesions in the intestine and this might have affected the digestion and/or absorption of nutrients (Rabo, 1998). It may also have been caused by a probable central nervous effect of the plant extract on the satiety centre via the ventromedial nuclei of the hypothalamus (Guyton and Hall, 1996).

A plethora of findings from previous studies have shown that alcohol and CCl_4 cause elevations in serum levels of ALAT and ASAT by causing damage to liver cells (Peralta *et al*., 1992). We tried to investigate the effects of *Sclerocarya birrea* extract in the development of functionally compromised liver tissue. The positive control group showed increases in the levels of these enzymes, but only increases in ALP levels were significant ($p<0.05$). However, the extract in the dosage range used, caused significant ($p<0.05$) increase in the levels of ALAT, ASAT, ALP, Conjugated and Total Bilirubin. This increase was greater at lower doses of 2 $mgkg^{-1}$ and most striking at 5 mg kg^{-1}body weight of the extract, respectively. A notable observation is a decrease ($p>0.05$) in the levels of these parameters observed with administration of higher doses of extract (8 mg kg^{-1} body weight of the aqueous extract).

Phytochemical analysis showed that *Sclerocarya birrea* contains flavonoids like catechin-3-Galloy ester (Peralta *et al*., 1992) and procyanidin (Galvez *et al*., 1993) as well as alkaloids (Hussain and Deeni, 1991) and tannins (Galvez *et al*., 1993). Alkaloids have the quality of being cytotoxic (Zimmerman, 1978) while flavonoids exhibit antioxidant qualities, thus are effective scavengers of super oxide anions and are therefore hepatoprotective (Ramanathan *et al*., 1989). Carbon tetrachloride toxicity on the other hand is attributed to the free radical generation (CCl_3^-), which may cause lipid peroxidation, thus altering the permeability of the liver cell membranes, resulting to leakage of enzymes from cells (Singh *et al*., 2005).

Alkaline phosphatase levels increase remarkably in diseases that impair bile formation and to a lesser extent in hepatocellular diseases (Kumar and Clarke, 2002). In this study, serum total bilirubin was significantly increased ($p<0.05$) in the groups administered 2 and 5 mg kg^{-1} body weight of extract, respectively indicating impaired bile formation.

Histopathologic findings reveal liver damage of varying degrees and confirm earlier findings which showed that alcohol- CCl_4 induced liver damage in rats exhibit features such as centrilobular vacuolation, swelling and necrosis of hepatic cells (Peralta *et al*., 1992). This effect was prominent in the low (2 mg kg^{-1}) extract group and quite mild in the high (8 mg kg^{-1}) extract group.

This study demonstrates that the aqueous extract of the stem bark of *Sclerocarya birrea* possess possible hepatotoxic activity on alcohol carbon tetrachloride induced liver damage at low dose and hepatoprotective activity at higher dose. A further study to validate the effect of the plant extract at higher doses is recommended.

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