

Research Article

Subacute Oral Toxicity Assessment of *Alchornea cordifolia* (Schumach and Thonn) Müll Arg (Euphorbiaceae) Extract in Rats

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Abstract

Purpose: To assess *Alchornea cordifolia*, a medicinal plant with numerous biological actions and uses in traditional medicine, for possible toxicity in rats.

Methods: The probable effect of the ethanol extract of *Alchornea cordifolia* (250 - 2000 mg/kg, p.o.) by gavage was evaluated on blood cellular elements and chemistry, as well as on the weight and histology of vital organs of male adult Sprague-Dawley rats.

Results: Daily administration of the extract for two weeks did not cause significant changes in most haematological indices and blood chemistry. However, a dose-dependent increase ($p < 0.01$) in neutrophils was observed. Relative organ weights were comparable in control and treated groups. Histopathological assessment of liver sections of treated-rats showed normal architecture at doses < 1000 mg/kg. However, in animals treated with 1000 and 2000 mg/kg, cloudy swelling of hepatocytes with vacuolar and hydropic degeneration were evident. Kidney architecture at all dose levels was normal.

Conclusion: The results of the study show that administration of the ethanol extract of *Alchornea cordifolia* to male adult rats by gavage evoked histopathologic changes in the liver at doses > 1000 mg/kg. These findings call for caution in the use of *Alchornea cordifolia* especially in high doses.

Keywords: *Alchornea cordifolia*, Rats, Subacute oral toxicity, Neutrophils, Hepatocytes, Hydropic denegeration

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INTRODUCTION

The World Health Organization (WHO) estimates that nearly 70 % of the world population depend on traditional medicine, especially medicinal plants, for their primary health care needs [1]. Concerns have, however, been raised by researchers regarding the safety of such botanical products. *Alchornea cordifolia* (Schumacher and Thonn) Müll. Arg. (Euphorbiaceae) is a shrub found along the coastal regions of West Africa. Traditionally, pulverized leaves of *Alchornea cordifolia* are used to treat wounds, sores and cuts [2]. Phytochemical analysis of the leaves has identified the presence of several compounds including flavonoids [3]. Reports on the biological activity of *Alchornea* suggest that it is antibacterial [4] spasmolytic [3], anti-inflammatory [5], hepato-protective [6], anti-diarrhoeal [7], antioxidant [8] and antimicrobial [9]. We reported previously on the propensity of *Alchornea* alcoholic extract to provoke hepatic damage in mice [10]. In view of possible interspecies variation in response to toxic agents, we were prompted to investigate the effect of *Alchornea* extract in rats with the objective of expanding the knowledge on the safety profile of this popular medicinal plant.

EXPERIMENTAL

Collection and identification of plant material

The fresh leaves of the plant *Alchornea cordifolia* were obtained from the Kwame Nkrumah University of Science and Technology botanical gardens and authenticated by Dr. Kofi Annan of the Department of Pharmacognosy, Faculty of Pharmacy and Pharmaceutical Sciences, College of Health Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana. A voucher specimen (No. FPPS-DCOL. 123) was kept at the herbarium of the Department of Pharmacognosy, Faculty of Pharmacy and Pharmaceutical

Sciences, College of Health Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.

Preparation of *Alchornea cordifolia* extract

The leaves of the plant were sun-dried for one week and then powdered in a hammer mill. The powder (800 g) was extracted by cold maceration with 70 % alcohol (5 L). The alcohol was evaporated in a rotary evaporator attached to a thermochiller (Buchi 700, Recirculation chiller) at a temperature of 20 °C. The residue was freeze-dried to obtain a brown sample of the crude extract (yield: 10.80 %w/w), subsequently referred to as the extract in this study.

Experimental animals

Male adult Sprague-Dawley rats (130 - 195 g) were purchased from Noguchi Memorial Institute for Medical Research, University of Ghana, Legon, Accra, Ghana and maintained in the animal house of the Department of Pharmacology, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana. They were housed in groups of six in stainless steel cages (34 x 47 x 18 cm) with soft wood shavings as bedding, fed with normal commercial pellet diet (GAFCO, Tema, Ghana) and given water *ad libitum*. The animals were humanely handled throughout the experiment in accordance with ethical guidelines for the care of laboratory animals (EEC directive of 1986:86/609 EEC) [11]. The protocols were approved (Ref. No. D/COL/ECA-8-2010) by the Department's Ethics Committee.

Subacute treatment

Male Sprague-Dawley rats (130 - 195 g) were obtained and divided into five groups (n = 6). The groups received 250, 500, 1000, 2000 mg/kg extract in 2 % tragacanth (p.o.) daily for 2 weeks. The control group received tragacanth 2 % only throughout the 2-week period. The animals were monitored closely

for signs of toxicity, and at the end of the two-week period, the rats were euthanized by cervical dislocation and blood was collected into tubes for analysis.

Blood analysis

Haematological analyses were performed on whole blood collected into tubes with ethylenediaminetetraacetic acid (EDTA). White blood cell (WBC), lymphocytes (LYM), mid cells (MID), neutrophils (NEU), red blood cells (RBC), haemoglobin concentration (HB), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), red cells distribution weight (RDW) and platelets (PLT) were determined by an automatic analyzer (CELL DYN 1700, Abbott Diagnostics Division, Abbott Laboratories, Abbott Park, IL, USA).

Biochemical analysis

Biochemical analyses were performed on serum obtained after centrifugation of whole blood (without anticoagulant) at 3000 rpm for 5 min. Determination of total proteins; total bilirubin, indirect bilirubin, albumin, globulin and A/G ratio were performed using an automatic analyzer (Random Access Chemistry System ATAC 8000, élan diagnostic laboratories, Brea, CA, USA). Levels of the liver enzymes alanine aminotransferase (ALT), alkaline phosphatase (ALP) and γ -glutamyl aminotransferase (GGT) were also determined. Analysis for blood urea nitrogen (BUN), creatinine, sodium, potassium and chloride were performed as well.

Organ weight determination

The following organs were quickly removed and individually weighed: liver, spleen, kidneys, heart, stomach, testes and lungs. Macroscopic appearances of the organs were observed and the relative weight of each organ was calculated. The liver and kidney were fixed in 10 % formalin for histopathological studies.

Histopathological examination

For microscopic examination, whole liver and kidney were fixed in 10 % neutral buffered formalin. Sections (2.0 mm) were placed in tissue preparation cassettes and processed with an auto processor (Microm STP120, Spain) for embedding in paraffin. Tissue sections of 7.0 μ m were cut and stained with hematoxylin and eosin (H and E).

Statistical analysis

Results were expressed as mean \pm standard error of mean (SEM). Statistical analysis was performed by one-way ANOVA using Graph Pad Prism for Windows version 4.02 (Graph Pad software, San Diego, CA, USA), followed by Neuman Keuls test to evaluate significant differences between the groups. Differences were considered significant at $p < 0.05$

RESULTS

Behavior of the treated animals

When the animals were observed closely over the period for signs of toxicity, no abnormal behavior, motor or neurological disorder was noticed. The treatment did not affect the gastrointestinal or respiratory systems. There was no mortality or changes in locomotor activity. Food and water intake were normal and there were no significant differences between the body weight of the treated and control groups over the 14-day period (Figure 1).

Blood analysis

For haematological evaluation, there was no significant change in most of the parameters analyzed (Table 1). However, there was a significant dose-dependent increase in the neutrophil count of animals treated with the extract compared to the control. The level of lymphocyte decreased in rats treated with 2000 mg/kg compared to the control (Table 1).

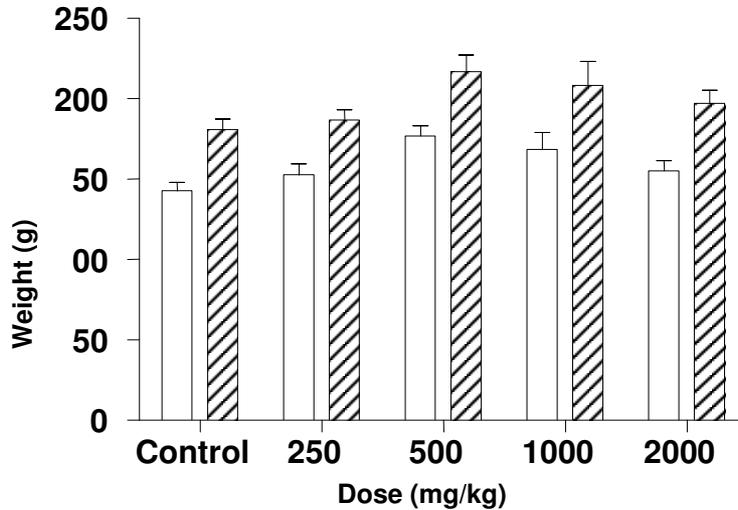


Fig 1: Weight of rats before (plain bars) and after (striped bars) 14-day treatment with *Alchornea cordifolia* extract.

Table 1: Effect of *Alchornea cordifolia* on haematological indices in rats treated for two weeks.

Parameter	Dose (mg/kg)				
	Control	250	500	1000	2000
WBC (K/ μ L)	10.34 \pm 1.77	8.70 \pm 1.01	9.58 \pm 0.38	8.88 \pm 0.98	8.08 \pm 1.20
LYM (%)	83.63 \pm 3.07	81.94 \pm 1.11	76.70 \pm 1.69	76.52 \pm 2.59	71.64 \pm 2.35*
MID (%)	13.03 \pm 1.87	13.32 \pm 0.35	16.10 \pm 0.93	15.62 \pm 2.02	17.34 \pm 2.00
NEU (%)	3.35 \pm 1.42	4.74 \pm 1.01	7.20 \pm 0.88*	7.86 \pm 0.73*	11.02 \pm 0.86**
RBC (M/ μ L)	6.01 \pm 0.20	5.84 \pm 0.07	6.00 \pm 0.32	5.98 \pm 0.21	5.77 \pm 0.14
HB (g/dL)	13.48 \pm 0.51	13.83 \pm 0.47	13.02 \pm 0.51	13.20 \pm 0.40	12.62 \pm 0.26
HCT (%)	35.80 \pm 1.43	35.20 \pm 0.91	34.75 \pm 1.80	34.15 \pm 1.09	32.78 \pm 0.47
MCV (fl)	59.62 \pm 1.47	59.97 \pm 0.96	57.95 \pm 0.99	57.12 \pm 0.80	56.84 \pm 0.66
MCH (pg)	22.44 \pm 0.60	22.98 \pm 0.29	21.80 \pm 0.54	22.08 \pm 0.35	22.16 \pm 0.24
MCHC (g/dL)	37.66 \pm 0.57	38.14 \pm 0.49	37.58 \pm 0.68	38.65 \pm 0.10	39.02 \pm 0.24
RDW (%)	15.86 \pm 0.66	16.02 \pm 0.45	15.30 \pm 0.32	14.67 \pm 0.29	16.12 \pm 0.34
PLT (K/ μ L)	276.40 \pm 95.49	302.20 \pm 60.01	401.70 \pm 65.66	348.50 \pm 69.70	446.20 \pm 99.82

Values are expressed as means \pm SEM (n = 6); (*) indicates significant (p < 0.05) and ** (p < 0.01) compared to control (Newman Keuls test)

Serum biochemical analysis

Biochemical parameters determined did not differ from control (Table 2). Similarly, renal function parameters investigated did show any differences as compared to the control groups.

Relative organ weights

We observed no significant differences between the treated groups and the control group.

Table 2: Effect of *Alchornea cordifolia* extract on serum biochemistry of rats treated for two weeks.

Parameter	Doses (mg/kg)				
	Control	250	500	1000	2000
Total bilirubin (µmol/L)	24.97±7.72	47.03±9.21	48.56±20.31	33.52±13.60	63.61±18.00
Direct bilirubin µmol/L	7.52±1.92	13.11±2.45	13.34±5.76	9.58±3.65	16.76±4.73
Total protein (g/L)	87.00±2.30	93.00±4.57	87.20±4.63	86.80±5.54	90.80±1.93
Albumin (g/L)	38.80±1.59	41.17±1.11	41.60±0.40	38.00±2.55	39.80±1.02
Globulin (g/L)	4.82±0.27	5.18±0.39	4.56±0.43	4.88±0.37	5.10±0.13
Albumin/globulin ratio	0.82±0.09	0.82±0.05	0.94±0.08	0.80±0.06	0.78±0.02
AST (U/L)	206.80±27.32	219.80±15.41	252.80±14.95	212.80±16.93	247.00±22.07
ALT (U/L)	88.20±17.07	99.50±14.23	116.20±6.67	101.00±5.30	112.40±11.63
ALP (U/L)	389.80±26.04	282.20±56.42	295.00±9.64	351.00±50.00	282.00±0.00
GGT (U/L)	9.60±0.51	19.17±5.44	9.67±3.18	10.75±2.10	14.50±3.50

Values are expressed as means ± SEM (n = 6), no significant differences between control and treated groups (p > 0.05) (Neuman Kuels test)

Histopathological features

There were no changes in the liver of rats treated with 250 mg/kg and 500 mg/kg extract compared to the control (Figure 2). However, in the groups treated with 1000 mg/kg and 2000 mg/kg extract, we observed cloudy swelling of hepatocytes with vacuolar and hydropic degeneration respectively. These changes were diffuse throughout but less severe around the portal tracts as depicted in Figure 2 which is a representative photomicrograph of six animals

As Fig 3 indicates, examination of the kidneys did not show any differences between the extract-treated group and control (at all dose levels).

DISCUSSION

The therapeutic potential of natural products has increased considerably in recent years with renewed awareness that natural products have the potential to cause toxicity since they contain active constituents [12].

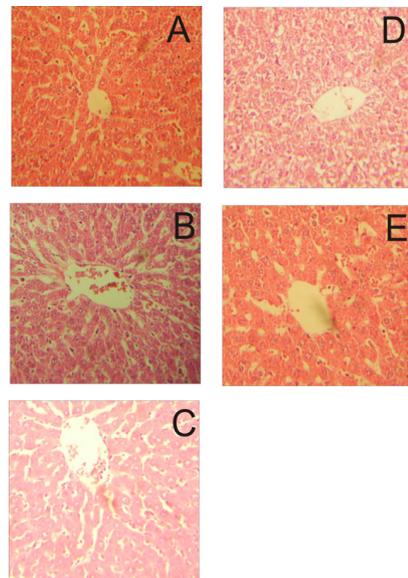


Figure 2: Photomicrograph (x 400) of liver sections of rats treated with *Alchornea cordifolia* extract for 14 days. Tissues fixed in 10 % neutral buffered formalin were processed and stained with hematoxylin and eosin. Panel A shows the liver section for vehicle-treated control animals, panel B (250 mg/kg), panel C (500 mg/kg), panel D (1000 mg/kg) and panel E (2000 mg/kg) of extract. In panels D and E, cloudy swelling of hepatocytes with vacuolar and hydropic degeneration are seen. The changes are diffused throughout but less severe around the portal tracts

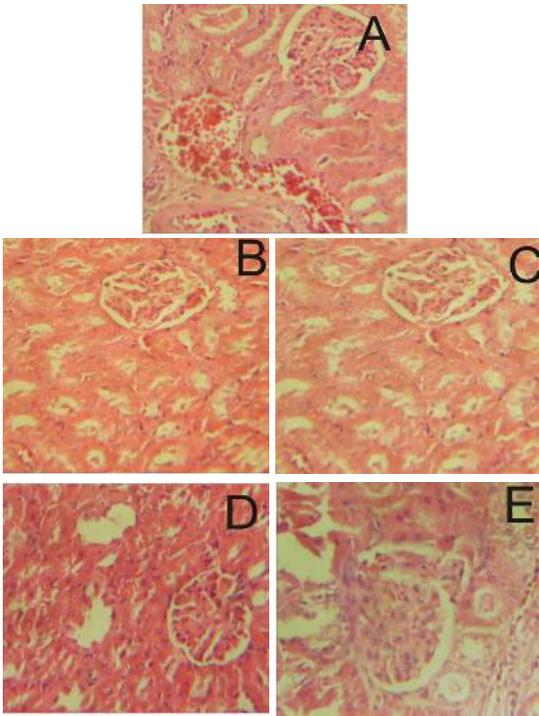


Figure 3: Photomicrograph (x 400) of kidney sections of rats treated with *Alchornea cordifolia* extract for 14 days. Tissues fixed in 10% neutral buffered formalin were processed and stained with hematoxylin and eosin. Panel A shows the kidney section for vehicle-treated control animals, panel B (250 mg/kg), panel C (500 mg/kg), panel D (1000 mg/kg) and panel E (2000 mg/kg) of extract. No changes were observed in the treatment groups compared to the vehicle-treated control.

There has therefore been a growing interest in the safety assessment of herbal medicines over the past few years.

In the present study, we investigated the possible toxicity of *Alchornea cordifolia* extract, a potential medicinal agent in rats. Following a 14-day treatment with the extract, the animals were closely monitored for signs of toxicity. Normally, loss of more than 10 % of initial body weight is an indicator of adverse effects [13]. In our study, we did not find significant changes in the body weight or the behavior of the animals. The slight

increase in body weight after the 14-day treatment can be ascribed to normal growth of the animals over the period. Food and water intake were normal and no deaths occurred. The relative weights of vital organs of animals did not show significant differences compared to the vehicle-treated control with no evidence of organ swelling, atrophy or hypertrophy in the animals. These observations indicate that our treatment had no effect on the general condition and functional behavior of the animals.

The hematological system has a predictive value for toxicity in humans and therefore analysis of blood is relevant to risk evaluation [14]. Results of the hematological analysis showed that *Alchornea cordifolia* has little or no effect on white blood cell (WBC), lymphocytes (LYM), mid cells (MID), neutrophils (NEU), red blood cells (RBC), haemoglobin concentration (HB), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), red cells distribution weight (RDW) and platelets (PLT). However, a dose dependent increase in neutrophils (NEU) was observed. Neutrophils interact with foreign compounds and microorganisms and destroy them by the process of phagocytosis. They are also known to be involved in the pathology of various inflammatory conditions. It is plausible that the increase in neutrophils in the present study is related to the liver cell injury observed in the histopathological studies. Lymphocytes are also known to circulate in blood and migrate to injured tissues. This may account for the decrease in lymphocyte numbers at the highest dose of treatment.

The markers of renal function, urea and creatinine, were assessed based on reported kidney toxicity associated with the use of medicinal plants [15]. These parameters were not affected in treated animals. *Alchornea cordifolia* has been shown to contain flavonoids [3]. Some of these flavonoids have been demonstrated to inhibit nephrotoxicity

because of its strong antioxidant activity [16]. *Alchornea cordifolia* has also been reported to contain tannins and tannins are known to offer protection against nephrotoxicity [17]. It is possible that these constituents offered protection to the treated animals in the present study.

Aspartate transaminase (AST) and Alanine transaminase (ALT) are considered as indicators of liver function. High levels of AST, ALT and ALP in serum are usually indicative of disease and necrosis in the liver of animals. Alkaline phosphatase (ALP), Gamma glutamyl transpeptidase (GGT), albumin, globulin, bilirubin and total protein were the other biochemical parameters analyzed. Analysis of these parameters is important since there are several reports of liver toxicity related to the use of medicinal plant products [18]. These parameters did not differ in the treated and the vehicle-treated groups, indicating that *Alchornea cordifolia* caused no adverse effect on the hepatic and renal systems.

Histopathological observations in the liver however did not correlate with the biochemical findings especially at high doses (1000-2000 mg/kg). At high doses of *Alchornea* treatment, we observed degenerations in the liver suggesting early signs of liver cell injury. This type of liver injury is however known to be reversible upon withdrawal of the toxicant allowing the liver cells to regenerate. This early stage of liver injury may not necessarily reflect in elevation of liver enzymes.

In contrast to the present findings, *Alchornea cordifolia* at doses of 200-500 mg/kg was reported to protect rats against paracetamol-induced liver damage [6]. *Alchornea* evoked mild liver damage at rather high doses (1000 - 2000 mg/kg) in the present work. This may be intriguing but the extract probably contains several components with different pharmacological actions. The proportion of specific toxicants may be increased at high doses. Though the liver damage detected in

the histopathology studies was not associated with corresponding elevation of liver transaminases, the potential toxicity of the extract especially at high doses cannot be ignored.

Estimates based on recommended dosage of dried *Alchornea cordifolia* leaves (maximum 50 g per litre of water; 4 cups daily) in traditional medicine [19] suggest that humans could take a maximum of 200 mg/kg of our extract daily. Though this appears to be much lower than the dosages used in the present study, observation of potential liver damage in mice previously [10] and the fact that dosages in herbal remedies are never precise due to non-standardization, call for caution in the routine use of the plant.

CONCLUSION

The results of the study indicate that though the alcohol extract of *Alchornea cordifolia* is relatively non-toxic to the haematological and renal systems, it is potentially hepatotoxic in rats if administered at high doses. Though these findings cannot be directly extrapolated to man in view of possible species differences and possible differences in metabolic activation, the present results suggest that caution should be taken in the use of the plant product especially at high doses.

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