Sub-Acute Toxicity Studies of *Alchornea cordifolia* Leaf Extract in Swiss Albino Rats

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Abstract

The ethanolic leaf extract of *Alchornea cordifolia* (Schum. and Thonn.) Müll. Arg (Euphorbiaceae), a widely used traditional medicinal plant was assessed for possible sub-acute toxicity in Swiss albino rats. The rats were randomly distributed into five groups of four animals each. The groups were respectively administered 125, 250, 500 and 750 mg/kg body weight ethanolic leaf extract of *Alchornea cordifolia* intraperitoneally daily for (two weeks) 14 days. Normal saline was administered to the control group according to their body weights. The experimental animals were observed for another 14 days before the termination of the experiment. The weight of the animals was recorded daily throughout the duration of the study. The number of deaths in any group was recorded. All the surviving animals were sacrificed after 28 days. Blood samples were collected for biochemical and haematological analysis. Selected organs of the animals i.e., liver and kidney of both the dead and sacrificed animals were removed and stored in 10% formal saline ready for histopathological analysis. Administration of *Alchornea cordifolia* (0.125-0.75 g/kg, po daily) for two weeks (14 days) did not affect significantly the relative organ weights, blood chemistry and renal function. Histology of liver and kidney at dose levels up to 0.5 g/kg was normal and similar to vehicle-treated controls. However, liver sections of mice treated with 0.75 g/kg *Alchornea cordifolia* ethanolic leaf extract showed cloudy swelling of hepatocytes with vascular degeneration. These results suggest that *Alchornea cordifolia* is relatively non-toxic but has the propensity to induce hepatic injury at high doses.

Keywords: Sub-acute toxicity study, Ethanol extract; *Alchornea cordifolia*

Introduction

*Alchornea cordifolia* (Schum and Thonn.), is a plant of the family Euphorbiaceae. It is also known as Agyama in Ghana, Susubolonta in Sierra Leone, Casamancebugong in Senegal, Tshiya in Togo, Bondji Euphorbiaceae. It is also known as Agyama in Ghana, Susubolonta in

Experimental animals

Male albino rats of about 8 weeks old, weighing (84-100 g) were used for the experiment. The animals were housed in gauzed cages in the animal house behind the Biochemistry Department, Federal University of Technology, Owerri. Water and feed were made freely available for them and they were allowed to acclimatize for two weeks prior to commencement of experiment.

The male albino rats were divided into five groups, each consisting of four animals and fasted overnight prior to treatment. Group A, the control group received 2 ml/kg of normal saline daily intraperitoneally.

Materials and Methods

Plant extraction processes

The apparently healthy parts of the plant (leaves, root and stem bark) were harvested in large quantities and air-dried for about 3 weeks to a constant weight under shade in the laboratory. The dried samples were ground into powdered form using an electric grinder (Saisho 200 W) and stored separately. Using maceration method, 100 g of each powdered sample was soaked separately in 600 ml each of distilled water and ethanol of analytical grade respectively, for 72 hour. Each sample solution was filtered using Whatman No.1 filter paper. The aqueous and ethanol filtrates were separately concentrated using water-bath at 45°C. All the extracts were weighed and then stored in well stoppered containers and kept in a refrigerator at 4°C until used.

**Plant identification**

The Plant *Alchornea cordifolia* used for this research was collected from the Botanical Garden of School of Agricultural Technology, Federal University of Technology Owerri (FUTO) Nigeria. The plant was identified by Mr. Francis Iwunze of the Department of Forestry and Wild life, School of Agricultural Technology, FUTO. The plant was authenticated by a plant taxonomist, Dr EN Mbagnu of Imo State University Owerri, Nigeria. The plant was prepared and kept at the University herbarium with voucher number IMSU H524.

**References**

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Groups B, C, D and E were treated intraperitoneally with daily doses of ethanolic leaf extract, (i.e., 0.125 g/kg, 0.25 g/kg, 0.5 g/kg and 0.75 g/kg respectively), for 14 consecutive days. The ethanolic leaf extract chosen for this study showed the highest antimalarial activity in a previous study [8]. All the animals were monitored for another 14 days. The animals were monitored closely for clinical signs of toxicity; i.e., mortality, changes in behaviour patterns, convulsion, loss of sight, loss of appetite, etc. Body weight changes were also recorded daily through-out the 28 days of the study.

Sample collection and processing

At the end of the 28 days of the experiment, the animals were sacrificed by mild ether anesthesia. Blood samples were collected from the animals by cardiac puncture and dispensed into ethylene diaminotetraacetic acid (EDTA) tubes for haematological studies and plain tubes for serum used for evaluation of biochemical parameters. The animal organs (i.e., liver, kidneys and heart) were collected, weighed and stored in 10% formal saline for histopathological analysis.

Assessment of haematological parameters

The haematological parameters were analysed with the aid of an automated analyser (Sysmex XE-2100, Canada).

Biochemical Analysis

Assay of serum aspartate aminotransferase (AST) activity

Determination of AST activity was based on the method of Reitman and Frankel [9] using commercially available test kit (Randox Laboratories Ltd, UK). Briefly, 0.01 ml each of serum and distilled H2O were delivered into test tubes labeled Sample and Blank respectively. To both tubes, 0.5 ml of AST reagent I was added, appropriately mixed and incubated at 37°C for 30 min. Then, 0.5 ml of AST reagent II was added to all test tubes, mixed and incubated at 25°C for 20 min. Finally, 5.0 ml NaOH was added to the test tubes and mixed. Absorbance was taken at 510 nm against reagent blank after 5 min.

Assay of serum alanine aminotransferase (ALT) activity

This was based on the method of Reitman and Franke [9], using commercially available test kit (Randox Laboratories Ltd, UK). Briefly, 0.1 ml of serum and distilled water (dH2O) was delivered into test tubes labeled Sample and Blank respectively. Then, 0.5 ml of ALT reagent I was added into both tubes, mixed and incubated at 37°C for 30 min. Afterwards 0.5 ml of ALT reagent II was added to all test tubes, mixed and allowed to stand for 20 min at 25°C. Finally, 5.0 ml NaOH were added to the tubes, mixed and absorbance read against the reagent blank at 510 nm after 5 min and the activity calculated.

Assay of serum alkaline phosphatase (ALP) activity

ALP activity was assayed with the aid of a commercial test kit (Randox Laboratories Ltd, UK) according to the method described by Englehardt [10]. Briefly, 0.01 ml sample was added into labelled test tube. Then, 0.50 ml ALP reagent (containing diethanolamine buffer, magnesium chloride and p-nitrophenylphosphate) was added to the tube and mixed thoroughly. Absorbance was read against water blank at 405 nm and activity (U/l) determined.

Determination of serum total bilirubin concentration

The method that exploits the use of diazotized sulphanilic acid as described by Pearlman and Lee [11] and Zoppini et al. [12] was used with the aid of commercially available standard kit (Human Laboratories, Germany).

Determination of total protein concentration

The Biuret method as described by Gornall et al. [13] was employed for the determination of protein concentration. Test tubes were prepared and labelled as blank, standard and sample. To these, 0.02 ml of distilled H2O, standard protein and serum were added respectively. Then, to all test tubes 1.0 ml of total protein Reagent I was added, mixed and incubated at 25°C for 30 min. Absorbance of sample and standard were read against reagent blank at 500 nm and protein concentration calculated.

Determination of serum albumin concentration

This was determined as described by Doumas et al. [14]. Briefly, test tubes were prepared and labelled blank, standard and sample. To these, 10 μl of distilled H2O, standard albumin and serum were respectively added. Then, 1.0 ml Reagent A (containing Bromocresol reagent) was added to all tubes, mixed and incubated at 25°C for 10 min. Absorbance of sample and standard were read against blank at 630 nm and albumin concentration calculated.

Determination of urea and creatinine concentrations

Serum urea and creatinine concentrations were determined by Jaffe’s reaction and urease enzymatic method respectively using standard kits (Human Laboratories, Germany) [15].

Histopathological analysis of the liver and kidney

A histological study was carried out with the liver and kidney samples from different groups of the experimental animals. This was carried out to crosscheck the results that were obtained from biochemical assay. The method used was that described by Okoro [16].

Statistical Analysis

Data obtained were expressed as mean ± standard deviation. Statistical analysis was carried out using one way analysis of variance (ANOVA) and significance taken at P<0.05.

Results

Figure 1 shows the effect of ethanol extract of A. cordifolia on the body weight of the albino rats. It shows that there were dose-dependent significant (p<0.05) decrease in percentage body weight of the animals administered 125, 250 and 500 g/kg body weight of the ethanol extract. However, the animals administered the highest extract dose of 750 g/kg bw showed significant (p<0.05) increase in the body weight when compared with the control group that was administered the extract.

Generally, the biochemical parameters of the extract treated animals did not differ significantly (p>0.05) from the control (Table 1), except for ALT activity which was observed to be generally low in the treated groups but significantly (p<0.05) decreased only in the 250 g/kg and 750 g/kg extract groups, when compared with control.

Results of the study indicate that the administration of A. cordifolia leaf extract generally caused no variation in the haematological parameters of the rats. However, there was a significant (p<0.05) dose-dependent increase in the total white blood cell count of animals treated with the extract, when compared to the control (Table 2). On the other hand, there was a significant (p<0.05) dose-dependent decrease in the monocyte count of the treated animals.

The relative organ weights of the animals showed no significant (p>0.05) differences between those of the treated groups and the control.
Discussion

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 the treatment had no effect on the general condition and functional behaviour of the animals. The haematopoetic system is an important index of physiological and pathological status in man and group indicating that A. cordifolia at the doses used did not produce any damage in the form of organ swelling, atrophy or hypertrophy in the treated animals (Table 3).

There were no lesions observed in the liver tissues of rats treated with the ethanol leaf extract of A. cordifolia at 125 mg/kg, 250 mg/kg and 500 mg/kg body weight compared to the control (Plates 1-4). However, in the group treated with 750 mg/kg extract, a slight cloudy swelling of hepatocytes with vascular degeneration was observed (Plate 5). On the other hand, no lesions were observed in the kidney tissue sections of both the treated and control animals (Plates 6-10).

Table 1: Effect of ethanolic extract of Alchornea cordifolia on the serum biochemical parameters of rats treated with different doses of the extract.

| Parameters          | Control   | 125       | 250       | 500       | 750       |
|---------------------|-----------|-----------|-----------|-----------|-----------|
| AST (µL)            | 46.40 ± 2.87<sup>a</sup> | 47.75 ± 2.14<sup>a</sup> | 42.80 ± 2.67<sup>a</sup> | 45.00 ± 4.24<sup>a</sup> | 42.35 ± 2.25<sup>a</sup> |
| ALT (µL)            | 48.50 ± 2.83<sup>a</sup> | 44.25 ± 2.21<sup>a</sup> | 41.40 ± 2.50<sup>a</sup> | 44.25 ± 1.11<sup>a</sup> | 41.00 ± 2.48<sup>a</sup> |
| ALP (µL)            | 115.40 ± 3.69<sup>a</sup> | 115.40 ± 3.69<sup>a</sup> | 109.56 ± 2.75<sup>a</sup> | 114.00 ± 4.25<sup>a</sup> | 108.01 ± 5.82<sup>a</sup> |
| Total bilirubin (µmol/L) | 27.04 ± 6.48<sup>a</sup> | 26.51 ± 5.94<sup>a</sup> | 31.47 ± 5.46<sup>a</sup> | 32.49 ± 10.59<sup>a</sup> | 29.23 ± 3.59<sup>a</sup> |
| Conjugated bilirubin (µmol/L) | 10.29 ± 2.65<sup>a</sup> | 12.68 ± 4.58<sup>a</sup> | 11.29 ± 2.20<sup>a</sup> | 14.54 ± 4.76<sup>a</sup> | 13.25 ± 4.15<sup>a</sup> |
| Total protein (g/L) | 81.60 ± 3.17<sup>abc</sup> | 76.50 ± 5.49<sup>abc</sup> | 74.20 ± 4.91<sup>bc</sup> | 84.25 ± 2.10<sup>a</sup> | 84.25 ± 2.06<sup>a</sup> |
| Albumin (g/L)       | 47.00 ± 4.63<sup>a</sup> | 48.50 ± 4.53<sup>a</sup> | 45.30 ± 3.39<sup>a</sup> | 45.00 ± 2.83<sup>a</sup> | 49.97 ± 3.31<sup>a</sup> |
| Creatinine (µmol/L) | 99.02 ± 6.49<sup>a</sup> | 99.45 ± 9.10<sup>a</sup> | 99.50 ± 6.85<sup>a</sup> | 97.20 ± 5.40<sup>a</sup> | 98.62 ± 6.26<sup>a</sup> |
| Urea (mmol/L)       | 3.46 ± 0.60<sup>a</sup> | 4.73 ± 1.15<sup>a</sup> | 3.56 ± 0.40<sup>a</sup> | 4.30 ± 0.28<sup>a</sup> | 4.60 ± 0.39<sup>a</sup> |

Values are mean ± standard deviation. Values with different superscripts horizontally are statistically significant (p<0.05). AST, Aspartate aminotransferase; ALT, Alanine aminotransferase; ALP, Alkaline phosphatase.

Table 2: Effect of ethanolic extract of Alchornea cordifolia on haematological indices in rats treated for two weeks.

| Parameters          | Control   | 125       | 250       | 500       | 750       |
|---------------------|-----------|-----------|-----------|-----------|-----------|
| WBC (K/µL)          | 5.10 ± 0.63<sup>a</sup> | 6.83 ± 0.70<sup>a</sup> | 6.80 ± 1.07<sup>a</sup> | 7.15 ± 0.16<sup>a</sup> | 7.31 ± 0.21<sup>a</sup> |
| LYM (%)             | 79.10 ± 2.80<sup>a</sup> | 78.35 ± 3.06<sup>a</sup> | 79.60 ± 0.87<sup>a</sup> | 80.68 ± 1.15<sup>a</sup> | 80.52 ± 1.69<sup>a</sup> |
| NEU (%)             | 5.68 ± 1.40<sup>a</sup> | 5.98 ± 2.09<sup>a</sup> | 5.98 ± 1.03<sup>a</sup> | 6.20 ± 0.66<sup>a</sup> | 7.86 ± 2.59<sup>a</sup> |
| RBC (M/µL)          | 5.98 ± 0.21<sup>a</sup> | 5.55 ± 0.51<sup>a</sup> | 5.77 ± 0.14<sup>a</sup> | 5.68 ± 1.40<sup>a</sup> | 5.97 ± 0.31<sup>a</sup> |
| HGB (g/dl)          | 13.48 ± 0.51<sup>abc</sup> | 13.83 ± 0.47<sup>abc</sup> | 13.02 ± 0.51<sup>abc</sup> | 13.20 ± 0.40<sup>abc</sup> | 12.62 ± 0.26<sup>abc</sup> |
| Monocyte(%)         | 6.76 ± 0.03<sup>a</sup> | 6.50 ± 0.15<sup>bc</sup> | 6.55 ± 0.08<sup>abc</sup> | 6.41 ± 0.11<sup>abc</sup> | 6.30 ± 0.10<sup>abc</sup> |
| Eosinophil(%)       | 4.10 ± 1.47<sup>a</sup> | 4.35 ± 0.98<sup>a</sup> | 4.60 ± 0.99<sup>a</sup> | 5.68 ± 0.80<sup>a</sup> | 5.54 ± 0.66<sup>a</sup> |
| Basophil (%)        | 1.20 ± 1.42<sup>a</sup> | 1.60 ± 1.01<sup>a</sup> | 1.63 ± 0.88<sup>a</sup> | 1.67 ± 0.73<sup>a</sup> | 1.72 ± 0.86<sup>a</sup> |
| PLT (K/µL)          | 276.40 ± 95.49<sup>a</sup> | 302.20 ± 60.01<sup>a</sup> | 301.70 ± 65.66<sup>a</sup> | 348.50 ± 69.70<sup>a</sup> | 386.20 ± 99.82<sup>a</sup> |

Values are mean ± standard deviation. Values with different superscripts horizontally are statistically significant (p<0.05). WBC, white cell count; LYM, lymphocyte count; NEU, neutrophil; RBC, red blood cell; HGB, haemoglobin; PLT, platelet.
animals [17] and a sensitive target for toxic compounds [18] since it is initially exposed to a high concentration of toxic agents. This has a predictive value for toxicity in humans and animals and therefore analysis of blood is relevant to risk evaluation [19].

Results of the hematological analysis showed that *Alchornea cordifolia* has little or no effect on white blood cell (WBC), lymphocytes

| Organ   | Control   | 125  | 250  | 500  | 750  |
|---------|-----------|------|------|------|------|
| Heart   | 0.47 ± 0.04 | 0.49 ± 0.06 | 0.47 ± 0.02 | 0.40 ± 0.04 | 0.38 ± 0.03 |
| Liver   | 4.46 ± 0.59 | 5.08 ± 0.67 | 5.71 ± 0.63 | 4.67 ± 0.82 | 4.20 ± 0.48 |
| Kidney  | 1.20 ± 0.01 | 1.21 ± 0.09 | 1.23 ± 0.09 | 1.09 ± 0.13 | 1.07 ± 0.06 |
| Spleen  | 0.64 ± 0.13 | 0.74 ± 0.11 | 0.97 ± 0.26 | 0.73 ± 0.21 | 0.74 ± 0.09 |
| Stomach | 1.12 ± 0.07 | 1.17 ± 0.10 | 0.92 ± 0.31 | 1.25 ± 0.12 | 1.44 ± 0.03 |

Table 3: Effect of different doses of leaf ethanolic extract of *A. cordifolia* on relative organ weights of rats.
(LYM), neutrophils (NEU), red blood cells (RBC), haemoglobin concentration (HB), haematocrit (HCT), eosinophils, basophils 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 possible that the increase in neutrophils in the present study is related to the slight liver cell injury observed in the histopathological studies. The markers of renal function, urea and creatinine, were assessed based on reported kidney toxicity associated with the use of medicinal plants. 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 [20]. *Alchornea cordifolia* has also been reported to contain tannins and tannins are known to offer protection against nephrotoxicity [21].

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 [22]. 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 a high dose (750 mg/kg) of the extract. At a higher dose of the extract, it was observed that there were 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 125-500 mg/kg was reported to protect rats against paracetamol induced liver damage [23]. *Alchornea cordifolia* evoked mild liver damage at rather high dose (700 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 slight 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 [23] suggest that humans could take a maximum of 200 mg/kg of the extract daily. Though this appears to be much lower than the dosages used in the present study, observation of potential liver damage in mice observed previously 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 ethanolic leaf 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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