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Acute toxicity studies of *Croton membranaceus* root extract

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ABSTRACT

Aim of the study: Croton membranaceus root and leaf extracts are used in the Bahamas to aromatize tobacco, in Nigeria to improve digestion, and in Ghana, for benign prostate hyperplasia. Despite claims of success there is paucity of information on its toxicity. The aim of this study was to determine if *Croton membranaceus* has acute toxicity properties.

Materials and methods: Roots were air-dried in a solar dryer for one week before milling. The powder was extracted with 96% ethanol, freeze-dried and re-extracted with distilled water and freeze-dried. 15 male Sprague–Dawley rats (180–200 g) were divided equally into 2 treatment groups [low dose (LD) and high dose (HD)], plus a control group (C). LD and HD received 1500 and 3000 mg/kg b.wt. *Croton membranaceus* aqueous extract, respectively, one time and observed for 14 days. Haematological [Full Blood Count and haemoglobin (Hb)], biochemical [bilirubin, alanine aminotransferase (ALA), aspartate aminotransferase (AST), total protein, albumin, globulin, alkaline phosphatise (ALP), γ-glutamyltranspetidase (GGT), urea, creatinine, creatinine kinase – Muscle and Brain (CK-MB), creatinine kinase – Total (CK-R)] examinations were performed.

Results: Control group's CK-MB (5444 ± 534 U/L) and LD group CK-MB (4014 ± 1016 U/L) were significantly different ($p < 0.05$). Control and the HD group CK-MB (3955 ± 1135 U/L) were significantly different ($p < 0.05$). Both LD and HD CK-R levels (697 ± 197 U/L and 732 ± 203 U/L, respectively), were lower than the control (1139 ± 220 U/L) at 48 h and 14 days ($p < 0.05$, $p < 0.05$, respectively). γ-GT levels of the HD group was 4.8 ± 0.4 U/L compared to the Control group value of 0.9 ± 0.2 U/L ($p < 0.05$).

Conclusions: Taking all factors into consideration, *Croton membranaceus* ingestion does not produce general acute toxicity. However, its creatinine kinase lowering ability could be explored.

1. Introduction

Croton comprises about 1200 species and occurs throughout the warmer regions of the world. It is best represented in America with about 65 species and also found in continental Africa with about 125 species in Madagascar. It was introduced into Nigeria in the 19th century and was described in 1864 in Ghana and Côte d' Ivoire [Flora 47:534 (1864)]. *Croton membranaceus* Müll.Arg. belongs to the Euphorbiaceae family. It is a monoecious herb or under shrub of up to 1–2 m tall. *Croton membranaceus* occurs in moist bush vegetation and savanna, at low altitudes and has a limited area of distribution. It is apparently uncommon (Abbiw et al., 2002) but has been cultivated in Ghana's Aburi Botanic Gardens.

In the Bahamas, the leaves are used to aromatize tobacco and in Nigeria they are used as a tonic and aromatic bitter, which improves digestion. The essential oil of the bark is used in aromatherapy to treat cough, fever, flatulence, diarrhea and nausea. These claims are anecdotal and very little has been documented. The antimi-

Abbreviations: ALT, alanine amino transferase; AST, aspartate amino transferase; AIN-93G, American Institute of Nutrition; ALB, albumin; ALP, alkaline phosphatise; ANOVA, analysis of variance; C, control; cDNA, complementary deoxyribonucleic acid; CK-MB, creatine kinase-muscle/brain; CK-R, creatine kinase-total; CSRPM, Center for Scientific Research in Plant Medicine; EDTA-2K, ethylenediamine-N,N,N′,N′-tetraacetic acid, dipotassium; GAFCO, Ghana Agriculture Food Company; γ-GT, γ-glutamyltranspetidase; HCT, haematocrit; HD, high dose; HGB, haemoglobin; LD, low dose; LD_{50}, lethal dose; LDH, lactate dehydrogenase; LYM %, lymphocytes percentage; LYM, lymphocyte count; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; MCV, mean corpuscular volume; MPV, mean platelet volume; PDW, platelet distribution width; P-LCR, platelet larger cell ratio; PLT, platelet; RBC, red blood cells; RDW-CV, coefficient of variation in red cell distribution width; RDW-SD, standard deviation in red cell distribution width; S–D, Sprague–Dawley; TP, total protein; WBC, white blood cells.

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crobial activity exhibited by the *Croton membranaceus* root extract supports its usefulness in treating secondary bacterial infection in measles as recently reported (Bayor et al., 2009). Furthermore, the root extract has been reported to exhibit markedly high cytotoxic activities particularly against human cancer cell lines (Ayim et al., 2007; Bayor et al., 2007). Finally, the root extract is used in formulations for the treatment and management of prostate and its related cancer in Ghana (Mshana et al., 2000).

Six compounds from the active ethyl acetate fraction of this extract, include a new furano-clerodane diterpenoid, crotomembranafuran, in addition to the known glutarimide alkaloid, (julocrotine, sitosterol, sitosterol-3-D-glucoside) labdane diterpenoid, gomojoside H and DL-threitol. The root bark contains scopoletin and julocrotine, as glutarimide alkaloid. It also contains calcium oxalate crystals.

Some preliminary tests on the activity of *Croton membranaceus* root extract have been undertaken, but more chemical and pharmacological research has to be done to evaluate its potential (Atakora, 2004). The isolation spectral data of julocrotin a glutarimide alkaloid from *Croton membranaceus* has also been reported (Aboagye et al., 2002).

In this study, the acute toxicity of *Croton membranaceus* root extract was investigated.

2. Materials and methods

At the commencement of the study, the protocol was reviewed and approved by the Institutional Animal Care and Use committee of the Noguchi Memorial Institute for Medical Research (NMIMR), University of Ghana, according to the Guidelines for Animal Studies.

2.1. Plant material

Croton membranaceus roots were collected from the Gyekiti Forest Reserve area in the Eastern region of Ghana. The plant was identified in its vernacular names by the farmers and authenticated by taxonomists from the Center for Scientific Research into Plant Medicine (CSRPM) herbaria, where voucher specimens of the plants have been kept for reference purposes.

2.2. Collection and extract preparation

After collection, the roots were air-dried in a solar dryer for one week before milling. About 1 kg of milled *Croton membranaceus* material was extracted with 96% ethanol for 24 h on a shaker at room temperature. Each extract was filtered and re-extracted with the same solvent for another 24 h. The pooled extracts were concentrated *in vacuo* at 50–55 °C before being transferred onto a freeze dryer to remove traces of the solvent and water. The marc from the ethanolic extractions was air-dried over-night, re-extracted with distilled water and the aqueous extract freeze-dried. The sample was stored in a cool dry area until use.

2.3. Subjects and experimental design

Fifteen (15) male Sprague–Dawley (S–D) rats (weighing about 150 g) were purchased from NMIMR and housed at the CSRPM, Mampong in the Eastern region of Ghana. During the acclimatization period, clinical observations of the animals were conducted as well as body weight measurement and the animals were found healthy. The animals used for the study were assigned into groups including a control group by the stratified random method according to their body weight. S–D rats fed *ad libitum* a standard chow diet (AIN-93G Formulation obtained from GAFCO – Ghana).

2.4. Housing conditions

Rats were housed in plastic cages with stainless steel tops in the animal care facility of the Center, where room temperature, humidity and ventilation were controlled. Rats were maintained at a 12-h light-cycle and were studied for 14 days. Prior to sacrifice, rats were euthanized by exsanguinations under ether anesthesia. Blood was sampled by cardiac puncture and all the visible organs and tissues were macroscopically examined and harvested.

2.5. Route of administration

The administration route was oral (gavage) in accordance with the main route of intake of *Croton membranaceus* by humans for medicinal purposes.

2.6. Acute toxicity test

Five (5) S–D rats constituted a group. Thus, three groups including the control group (C) were established. A single oral Low Dose (LD) of 1500 mg/kg b.wt and a single oral High Dose (HD) of 3000 mg/kg b.wt *Croton membranaceus* were reconstituted as an aqueous homogenous suspension containing 0.4% Tween 80. The highest dose 3000 mg/kg b.wt was selected based on previous studies (unpublished data). The administration volume was set at 1333 μl/kg b.wt. Group 1 the control group (C group) fed the normal chow diet and gavaged 400 μl 0.4% Tween 80 (once). Group 2, Low Dose group (LD group) and group 3, High Dose group (HD group) were gavaged with the extract as a once-off administration with the doses indicated above.

2.7. Clinical observations

The observation period was 14 days post administration. Clinical signs of toxidromes (such as rising fur, sluggish movement, draping, tremors, excitability, miosis, mydriasis, twitching, salivation, food intake, morbidity, etc.) and mortality were observed while dosing and after 0.5, 1, 3, and 6 h of administration. Thereafter, twice daily observations were made, up until the 14th day. Body weights were measured before dosing on the day of administration and every morning thereafter.

2.8. Laboratory examinations

All animals were housed individually in metabolic cages in order to obtain freshly voided urine. Urinalysis was performed after 48 h and on the 14th day. Urine was collected and examined for pH, protein, glucose, ketone bodies, bilirubin, occult blood and urobilinogen.

Haematological examinations were done 48 h post extract administration, and, on the 15th day at necropsy. Blood samples from the tail of the rats (48 h) and by cardiac puncture (15th day) were collected into EDTA-2K tubes for immediate analysis using the SYSMEX hematology autoanalyzer (Kobe, Japan). Reagents for the hematology autoanalyzer were obtained from STROMATOLYZER, (WH-USA). Leukocyte count, erythrocyte count, haemoglobin concentration, hematocrit, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), reticulocyte ratio, platelet count and differential leukocyte counts were determined.

Biochemical examinations were performed using blood collected into plain tubes. Blood samples were centrifuged for 5 min at 3000 rpm. The following biochemical assays were performed using SELECTRA JUNIOR VERSION 04 autoanalyzer for biochemical assays (VITAL SCIENTIFIC BV, NETHERLANDS). Total bilirubin, conjugated