Anti-atherogenic and anti-ischemic potentials of *Croton membranaceus* observed during sub-chronic toxicity studies

Dan K. Afriyie, George A. Asare[1], Kwasi Bugyei, Isaac Asiedu-Gyekye, Ben A. Gyan[2], Samuel Adjei[3], Phyllis Addo[3], Archibald Sittie[4], Alexander K. Nyarko[5]

Department of Pharmacology, University of Ghana Medical School, [1]Department of Medical Laboratory Sciences, School of Allied Health Sciences, [2]Department of Immunology, Noguchi Memorial Institute for Medical Research, University of Ghana, [3]Animal Experimentation Unit, NMIMR, [4]Center for Scientific Research into Plant Medicine, Mampong, Akuapim, Ghana, [5]Clinical Pathology, NMIMR

Submitted: 19-02-2012 Revised: 28-03-2012 Published: 08-01-2013

ABSTRACT

Background: *Croton membranaceus* (*CM*) is used for benign prostate hyperplasia treatment. **Objective**: Sub-chronic toxicity studies are non-existent and provided the basis for this study. **Materials and Methods**: 90 days oral administration of a low dose (LD) (30 mg/kg b. wt.), medium dose (MD) (150 mg/kg b. wt.), and high dose (HD) (300 mg/kg b. wt.) *CM* aqueous root extract to 3 groups ($n = 6$ each) of male Sprague-Dawley rats, alongside a control group, was undertaken. Urinalysis, hepato-renal function tests, lipid profile, cardiac enzymes, and routine hematology tests were performed. **Results**: Triglyceride levels (C = 1.05 ± 0.19, LD = 0.64 ± 0.08, MD = 0.55 ± 0.04, HD = 0.50 ± 0.02 mmol/L) were significantly reduced ($P < 0.05$). Very low density lipoprotein (C = 0.48 ± 0.09, LD = 0.29 ± 0.04, MD = 0.25 ± 0.02, HD = 0.23 ± 0.01 mmol/L) decreased significantly ($P < 0.05$). Cardiac enzymes-creatinine kinase (C = 568 ± 172, LD = 315 ± 79, MD = 441 ± 209, HD = 286 ± 81 IU/L) decreased markedly ($P < 0.05$) alongside lactate dehydrogenase (C = 2675 ± 875, LD = 1667 ± 1229, MD = 1186 ± 442, HD = 855 ± 239 IU/L) ($P < 0.05$). **Conclusion**: *C. membranaceus* aqueous root extract is non-toxic but demonstrates anti-atherogenic and anti-ischemic potentials.

Key words: Anti-atherogenic, anti-ischemic, *Croton membranaceus*, sub-chronic, toxicity

Access this article online

Website: www.phcogres.com

DOI: 10.4103/0974-8490.105640

Quick Response Code:

INTRODUCTION

Croton membranaceus, a plant of the Genus Euphorbiaceae, is an emerging plant of interest from the medicinal point of view. Its aqueous root extract is currently employed, in varying dosage forms, for the treatment and management of prostatic cancers and other related tumors.[1-2] The anti- microbial activities of the methanol root extracts have also been confirmed,[3] likewise, its usefulness in treating secondary bacterial infections in a clinical condition such as measles by some natives in Ghana.[2] Phytochemical investigations into the ethyl acetate fraction of the root extract led to the isolation of a new furano-clerodane diterpeniod, crotomembranafuran in addition to known glutarimide alkaloid, julocrotine; beta-sitosterol; beta- sitosterol-3-D-glucoside; labdane diterpioid, gomojoside H, and DL-thrietol.[4] Furthermore, marked cytotoxic activity of crotomembranafuran, beta-sitosterol-3-D-glucoside, and DL-thrietol against human prostate (PC-3) cells has been reported.[5]

The promising anti-tumor potential of many medicinal plants including *C. membranaceus* has also led to concerns over safety and efficacy despite their long history of use, and the perception that such "natural" products offer a safer alternative to conventional medicines.[6] On the contrary, the toxicity of medicinal plants has been known to man since their therapeutic effects became known. Although cytotoxic evaluations of some selected Ghanaian medicinal plants including *C. membranaceus* lend credence to their use in traditional medicines for the treatment of cancer,[4] extensive toxicological studies have not been documented on *C. membranaceus* to confirm its safety, neither has its anti-atherogenic potential being reported. Furthermore, toxicological data on extracts of medicinal plants are currently required as part of the evaluation

Address for correspondence:
Dr George A. Asare, Chemical Pathology Unit, Department of Medical Laboratory Sciences, School of Allied Health Sciences, College of Health Sciences, University of Ghana, P.O. Box KB 143, Korle-bu, Accra, Ghana. E-mail: gasare@chs.edu.gh

process of these plants in many countries to ensure safety before marketing.[7-8] Additionally, toxicity studies on medicinal plants provide insight into specific toxic effects on target organs, the mode of toxic action, and also dosing range guidance for other toxicity studies.[9-10] However, the virtual non-existence of extensive toxicological and other therapeutic data on *C. membranaceus,* despite claims of success in its use for prostatic tumors and other related cancers, limits its widespread acceptance as a potential anti-tumor, anti-atherogenic, and anti-ischemic agent. Indeed, numerous drugs used in cancer chemotherapy exhibit cell toxicity with the potential to induce genotoxic, carcinogenic, and teratogenic effects on non-tumor cells.[11]

The study, therefore, revealed anti-atherogenic and anti-ischemic properties of *Croton membranaceus*, although the aim was the determination of the possible sub-chronic toxic effects of the aqueous root extract using male Sprague-Dawley (SD) rats.

MATERIALS AND METHODS

Plant Material

C. membranaceus roots were collected from the Gyekiti Forest Reserve area in the Eastern region of Ghana by Dr. Archibald Sittie of the Center for Scientific Research into Plant Medicine (CSRPM). The plant was identified by its vernacular names by the farmers and authenticated by taxonomists from the CSRPM herbaria where voucher specimens (CSRPM 2110) of the plants have been kept for reference purposes. The roots were carefully washed with water, sun-dried for two weeks, pulverized, packaged in sample bottles, labeled appropriately, and stored at room temperature (25°C-27°C).

Preparation of Aqueous Extract

One thousand grams (1000 g) of dry powdered *C. membranaceus* root was macerated for 24 hours with 4000 mL of distilled water and heated for 1 hour. The extract was filtered through medical gauze to separate it from the residue. Another 3000 mL of distilled water was added to the residue, macerated for a further 24 hours, and the above procedure repeated to obtain a second extract. The extracts were pooled and freeze-dried using Freeze Dryer Gamma 1-16/2-16 LSC (2004 version). The dry yield of the extracts was weighed (20.6 g) and stored in a sealed container in a refrigerator between 2°-8°C until use.

Experimental Animals

Twenty-four healthy, young male Sprague-Dawley (S-D) rats (6-8 weeks) weighing 100-150 g were purchased from the Animal Experimentation Department of the Noguchi Memorial Institute for Medical Research (NMIMR). The rats were housed in the University of Ghana Medical School Animal Experimentation and Care Unit, and were treated humanely. During the acclimatization period, clinical observations on the animals were conducted as well as body weight determinations, and the rats were found healthy. Rats were assigned into groups including a control group. S-D rats were housed in metal cages with stainless steel tops in the animal care facility where room temperature, humidity, and ventilation were controlled. S-D rats fed *ad libitum* a standard chow diet (AIN-93G formulation, obtained from GAFCO-Ghana). Rats were maintained at a 12-h light-cycle and prepared for various experiments. Prior to sacrifice, rats were anesthetized and later euthanized. All visible organs and tissues were macroscopically examined and harvested after blood sampling by cardiac puncture.

Experimental Design

Sub-chronic Toxicity Test

The protocol adopted followed the OECD (1998) protocol[9] using a total of 24 healthy S-D male rats. Rats were randomly divided into 4 groups of 6 each. Three of the groups were given single doses of 30, 150, and 300 mg/ kg b. wt. of the aqueous extract by oral gavage, respectively, for 90 days, while the control group received distilled water equivalent to the volume of extract given. Rats in the different groups were weighed weekly from day 0 to the 90th day. All animals were observed for apparent clinical signs of toxicity, morphological, and behavioral alterations during the experimental period. The weights of animals, food, and water consumption were also monitored during this period. Urine and blood samples were collected from individual rats on the 91st day of the study for urinalysis, hematological and biochemical assays. Blood was collected by cardiac puncture, and major organs such as the liver, heart, and kidneys harvested from each rat for macroscopic and histological studies, before sacrifice.

Urine Analysis

Urine samples were collected from individual animals on the 91st day and analyzed for glucose, bilirubin, ketone, nitrite, pH, blood, protein, urobilinogen, leukocytes employing standard procedures with the aid of URIPATH test kit (Plasmatec Laboratory Products-UK-Lot 100114).

Hematological Assessment

Blood was collected into EDTA (ethylenediaminetetracetate)-2K tubes and analyzed for red blood cell counts (RBC), hemoglobin levels (HGB), hematocrit (HCT), mean cell volume (MCV), mean cell hemoglobin concentration (MCHC), packed cell volume (PCV), white blood cell counts (WBC) (total and differential), and platelets count (PLT) using a Sysmex-KX-2IN hematology auto analyzer.

Reagents for hematological analyzes were obtained from Stromatolyser (WH-USA).

Biochemical Assessment

Blood collected into gel separator tubes was processed for serum by allowing it to clot and centrifuging for 5 minutes at 3000 rpm. Serum samples were stored at –20°C until use. The serum was analyzed for levels of sodium ions, potassium ions, chloride ions, total protein, albumin, total bilirubin, alkaline phosphatase (ALP), alanine aminotransferase (ALA), aspartate aminotransferases (AST), total cholesterol, low density lipoprotein (LDL), high density lipoprotein (HDL), very low density lipoprotein (VLDL), urea, creatinine, lactate dehydrogenase (LDH), creatine kinase MB (CK-MB) (Muscle and Brain), and creatine kinase NAC (Total CK) using SELECTRA JUNIOR AUTOANALYZER (VITAL SCIENTIFIC BV, VERSION 04, NETHERLANDS).

Pathology

Harvested heart, liver, and kidney tissues were freed of fat and connective tissues, blotted with clean wipes, examined macroscopically, and weighed to obtain the organ-to-body weight ratios.[12] 3 μm sections of the heart, kidney, and liver were dried at 61°C in an oven and dewaxed in two changes of xylene for 5 minutes each. They were rehydrated by passing through two changes of absolute ethanol, 5 minutes each before passing through tap water. The sections were stained in Mayer's hematoxylin for 5 minutes, blued in tap water before staining in alcoholic eosin for 45 seconds. The stained sections were dehydrated in increasing concentrations of ethanol, cleared in xylene and mounted in-dibutyl phthalate in xylene (DPX).

Statistical Analyzes

Statistical analyzes of the data was done using SPSS (Statistical Package for Social Sciences) version 16. Results were expressed as mean±standard error of mean (SEM), n=6. Significant differences between dose groups and controls were evaluated by performing a one-way ANOVA (followed by Newman-Keuls multiple comparison test). If ANOVA showed significant differences, *post hoc* analysis was performed with Tukey test. P values less than 0.05 were considered significant.

RESULTS

Organ Weight and Relative Organ Weight of Male S-D Rats

No toxidromes or mortality was observed. Administration of aqueous extract of *C. membranaceus* at all dose levels did not result in any significant differences in mean organ weights of the heart, liver, or kidney of the treated groups compared to the control. The highest mean organ weights (in grams) obtained for the heart (C=0.98±0.02, LD=0.95±0.04, MD=0.93±0.02, HD=1.02±0.03), liver (C=7.43±0.37, LD=7.87±0.40, MD=7.88±0.26, HD=8.38±0.54), and kidney (C=1.63±0.08, LD=1.60±0.08, MD=1.62±0.06, HD=1.77±0.08) were observed in the HD group. Furthermore, a dose-dependent increase in organ weights was observed in the liver. However, the body to organ ratios did not show significant differences.

Urinalysis

Results obtained from the urinalysis data did not reveal any noticeable differences in urobilinogen, glucose, bilirubin, specific gravity, blood (RBC), nitrite, and leukocytes of control and treated groups. Traces of ketones and proteins were observed in both control and treated groups, and urine pH appeared to be slightly acidic with increasing dose in the medium and high dose groups.

Hematological Indices

From Table 1, the total WBC declined slightly comparing all the test groups to the control group. Lymphocyte count showed a similar decline that was not dose-dependent and was not statistically significant. However, the 15% decline in the % lymphocytes was significant between the C group and the HD group. HGB increased moderately comparing the test groups to the control group. However, this was not in a dose-dependent manner and was not statistically significant. RBC showed a progressive increase from the C group to the HD group. RBC differences were, however, not significant. Similarly, the rest of the hematological parameters did not show significant differences.

BIOCHEMICAL ASSAYS

From Table 2, the renal function profile (urea, creatinine, sodium, potassium) did not show significant changes. Chloride remained virtually unchanged. Similarly, hepatic enzymes did not show significant differences. However, AST and ALT values declined in a dose-dependent manner. The total protein, albumin, and globulin did not show significant changes [Table 3].

From Table 4, values of CK-NAC for the test groups were lower than the control group. CK-NAC reductions ranged from 47%-54% in the test groups. Similarly, CK- MB values were lower in the test groups compared to the control group by 45%-50%, from the LD to the HD. Significant CK-NAC differences were observed between the control group and the MD dose group (P=0.013) as well as in the control and HD group (P=0.009). Similarly, significant CK-MB differences occurred between the control and MD group (P=0.038) as well as between the control and the HD group (P=0.012). From the

afore-mentioned, it is generalized that about 50% serum CK reduction takes place as a result of *C. membranaceus* administration.

LDH values declined in a dose-dependent manner by 48% (LD), 56% (MD), and 68% (HD). Significant differences were observed between the control and MD groups (P=0.018) as well as between the control and HD groups (P=0.011) [Table 4].

Some lipid profile parameters demonstrated remarkable changes. Total cholesterol and HDL increased slightly in the test groups compared to the control groups. Increases, however, were not significant. Similarly, LDL values showed slight changes that were not significant. TG levels, however, decreased with dose increase by 39% (LD), 48% (MD), and

Table 1: Hematological indices of the control group, Low Dose group (LD=30 mg/kg b.wt.), medium dose group (MD=150 mg/kg b. wt.), and high dose group (HD=300 mg/kg b.wt.) on day 91 after the administration of *C. membranaceous aqueous* root extract on male Sprague-Dawley rats

Hematological parameters	Groups of S-D rats given *C. membranaceus*			*P* values
	Control	Group 1 (LD) (30 mg/kg b. wt.)	Group 2 (LD) (150 mg/kg b. wt.)	Group 3 (HD) (300 mg/kg b. wt.)
WBC × 10^3/μL	7.78±0.77	5.63±0.74	6.27±0.88	6.55±1.41 NS
RBC × 10^6/μL	7.58±0.25	7.97±0.07	8.09±0.14	8.15±0.16 NS
HGB (g/dL)	13.53±0.42	14.25±0.21	14.47±0.15	14.23±0.27 NS
HCT (%)	45.2±1.53	47.52±0.53	48.15±0.63	47.00±0.79 NS
MCV (fL)	59.65±0.51	59.67±1.03	59.62±0.92	57.92±0.64 NS
MCH (pg)	17.83±0.41	17.9±0.30	17.9±0.28	17.42±0.22 NS
MCHC (g/dL)	29.98±0.57	29.98±0.34	30.05±0.18	30.14±0.20 NS
PLT × 10^3/μL	782±45.04	767±22.95	739±25.27	712±28.16 NS
LYM (%)	83.57±0.78	80.78±1.21	81.9±1.25	68.2±6.36* 0.0145
LYM# × 10^3/μL	6.33±0.72	4.57±0.63	5.133±0.74	4.05±0.60 NS

Data are expressed as mean±SEM. Test values carrying superscripts are statistically different from the control parameters. *P<0.05. n=6 , WBC=White Blood Cells; RBC=red Blood Cells; HGB=Hemoglobin; HCT=hematocrit; MCV=Mean Corpuscular Volume; MCH=Mean Corpuscular Hemoglobin; MCHC=Mean Corpuscular Hemoglobin Concentration; PLT=Platelet; LYM %=Lymphocytes percentage; LYM=lymphocyte count

Table 2: Renal function indices of the control group, low dose group (LD=30 mg/kg b.wt.), medium dose group (MD=150 mg/kg b. wt.), and high dose group (HD=300 mg/kg b.wt.) on day 91 after the administration of *C. membranaceous* aqueous root extract on male Sprague-Dawley rats

Renal function tests	Groups of S-D rats given *C. membranaceus*				*P* values
	Control	Group 1 (LD) (30 mg/kg b. wt)	Group 2 (MD) (150 mg/kg b.wt.)	Group 3 (HD) (300 mg/kg b.wt.)	
URE (mmol/L)	6.59±0.46	6.12±0.34	6.57±0.17	6.16±0.19	NS
CR (mmol/L)	57.22±4.65	59.58±1.51	59.52±4.03	56.92±1.52	NS
K (mmol/L)	5.19±0.20	5.00±0.26	4.81±0.15	5.20±0.41	NS
Na (mmol/L)	139.2±0.48	140.2±0.48	139.8±0.48	140.5±0.56	NS
Cl (mmol/L)	99.55±0.67	99.58±0.63	99.75±0.52	99.52±0.74	NS

Data are expressed as mean±SEM. Not significant when compared to the Control (P>0.05). n=6 , URE=urea; CR=creatinine; Na=sodium; K=potassium; Cl=Chloride

Table 3: Liver function tests of the control group, low dose group (LD=30 mg/kg b.wt.), medium dose group (MD=150 mg/kg b. wt.), and high dose group (HD=300 mg/kg b.wt.) on day 91 after the administration of *C. membranaceous* aqueous root extract on male Sprague-Dawley rats

Liver function tests	Groups of S-D rats given *C. membranaceus*				*P* values
	Control	Group 1 (LD) (30 mg/kg b. wt.)	Group 2 (MD) (150 mg/kg b. wt)	Group 3 (HD) (300 mg/kg b. wt.)	
TBIL umol/L)	0.71±0.21	0.89±0.21	0.91±0.23	0.90±0.22	NS
ALT (U/L)	103.8±19.06	67.3±24.74	51.68±7.36	47.93±7.77	NS
AST (U/L)	320.7±66.29	167.8±74.77	137.27±74.06	103.20±19.37	NS
ALP (U/L)	261.1±24.5	257.2±11.02	251±6.12	276.53±3.13	NS
TP (g/L)	56.42±1.12	61.3±1.97	61.52±1.99	60.88±1.16	NS
ALB (g/L)	30.73±0.54	32.25±0.70	31.97±0.38	32.35±0.42	NS
GB (U/L)	25.68±0.96	29.08±1.47	29.5±1.67	29.48±1.06	NS

Data are expressed as mean±SEM. Not significant when compared to the Control (P>0.05). n=6 , TBIL=total bilirubin; ALT=alanine aminotransferase; AST=Aspartate aminotransferase; ALP=alkaline phosphatase; TP=total protein; ALB=albumin; GB=globulin

Table 4: Arteriogenic and ischemic biomarkers of the control group, Low Dose group (LD=30 mg/kg b.wt.), medium dose group (MD=150 mg/kg b. wt.), and high dose group (HD=300 mg/kg b.wt.) on day 91 after the administration of *C. membranaceous* aqueous root extract on male Sprague-Dawley rats

Lipid / Cardiac enzymes profiles	Groups of S-D rats given *C. membranaceus*			*P* values	
	Control	Group 1 (LD) (30 mg/kg b.wt.)	Group 2 (MD) (150 mg/kg b.wt.)	Group 3 (HD) (300 mg/kg b.wt.)	
TCHOL (mmol/L)	1.29±0.09	1.44±0.08	1.35±0.06	1.43±0.08	NS
TG (mmol/L)	1.05±0.19	0.64±0.08	0.55±0.04**	0.50±0.02**	0.005/0.0068**
HDL (mmol/L)	0.76±0.04	0.85±0.04	0.78±0.02	0.86±0.02	NS
LDL (mmol/L)	0.32±0.04	0.30±0.04	0.32±0.05	0.38±0.05	NS
VLDL (mmol/L)	0.48±0.09	0.29±0.04	0.25±0.02**	0.23±0.01**	0.007/0.0076**
CK-NAC (IU/L)	657±268	434±106	626±247*	303±57**	0.009**/0.013*
CK-MB (IU/L)	568±172	315±79†	441±209*†	286±81*	0.012*/0.038†
LDH (IU/L)	2675±875	1667±1229	1186±442†	855±239*	0.011*/0.018†

Data are expressed as mean±SEM. Test values carrying superscripts are statistically different from the Control parameters. *P<0.05, **P<0.01, †P<0.05. n=6 , TCHOL=Total Cholesterol; TG=Triglyceride; HDL=High Density Lipoprotein; LDL=Low Density Lipoprotein; VLDL=Very Low Density Lipoprotein; CK-NAC=Total Creatine Kinase; CK-MB=Creatine Kinase-Muscle Brain

52% (HD). Differences were statistically significant between the control and MD groups (P=0.0068) as well as between the control and HD groups (P=0.005). In the same manner, VLDL levels declined in a dose-dependent manner from 40% (LD)-48% (HD). Decreases were very significant, P=0.0076-control verses MD group, and P=0.007-control verses HD group [Table 4]. A 40%-50% reduction in anti-atherogenic markers was generally observed.

Pathological examination

Macroscopic examination of the heart, liver, and kidney of the treated groups compared to the control group did not reveal any sign of abnormality or damage, which could be attributed to the extract. Furthermore, histological assessment of sections of these organs did not show any differences or abnormality, which could be attributed to the extract. The photomicrographs of the histological sections of the heart, kidney, and liver of the control and treated groups are shown in Figures 1-3.

DISCUSSION

Toxicity studies using animal models are often employed to determine the health risk associated with a potential medicinal plant. Clinical signs of toxidromes are the first signs of toxicity followed by mortality. In the absence of obvious toxidromes and mortality, elevations of analytes and organ changes (both macroscopic and microscopic) after plant extract administration are good markers of possible toxicity. In certain cases, however, toxicity studies may reveal a depression of certain biomarkers. Such depressions may be suggestive of the presence of some therapeutic agents in the medicinal plant. The need, therefore, to screen all medicinal plants in use for toxicity and side effects cannot be over-emphasized.

Hematological analyzes are also appropriate for risk assessment as such changes in blood cells provide useful information of toxicity in humans. In this study, the absence of deleterious effects on the WBC, RBC, HGB, HCT, MCV, MCH, HCHC, and PLT largely suggests that the hematological parameters were generally unaffected, apart from the percentage lymphocytes that showed significant decreases with the high-dose group. In an acute toxicity study of *C. membranaceus*, similar decreases were observed but were not statistically significant.[13] Lymphocytes are produced in the bone marrow and are involved with the immune system. Several factors may account for low lymphocyte levels. Some of which include infection, aplastic anemia, and some nerve disorders. Okokon *et al.* reported of reduced lymphocyte percentage when the ethanol root extract of *C. zambesicus* was administered to rats at 27, 54, and 81 mg/kg b. wt.[14] However, decreases were not statistically significant. The reduced levels of lymphocytes by the high dose of the extract in this study may have important immunomodulatory effects on the effector cells of the immune system and phagocytic activity as similarly observed in other medicinal plants.[15-16] Furthermore, since there were no reductions in HCT, MCHC, and MCH (except for the high dose group), normocytic and hypochromic anemia in the treated groups can be excluded.[17]

Renal function indices such as urea, creatinine, and electrolytes examine the excretory capacity of the nephrons as well as the electrolyte regulatory function of the distal convoluted tubules. The profile of the renal function tests and histology [Figure 2] suggest the absence of nephrotoxicity. Marginal changes in the urea concentration were observed. However, this did not follow any dose-specific pattern. Similarly, renal handling of electrolytes was not affected by *C. membranaceus*. Although Okokon *et al.* observed slight increases in creatinine in a dose-dependent fashion, after the administration of the ethanol

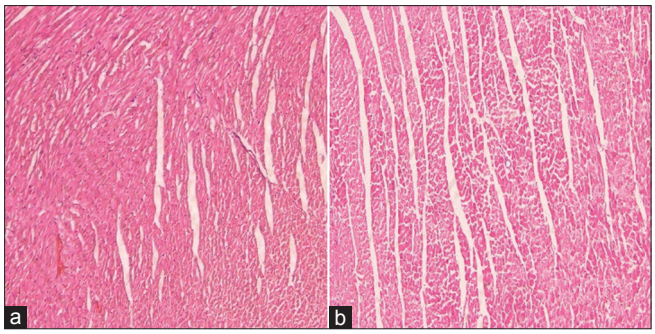

Figure 1: Photomicrographs of transverse sections of the heart of the control group (a) and the *C. membranaceus* treated group (300 mg/ kg b. wt) after sub chronic administration of the extract, (b) (H and E, ×20)

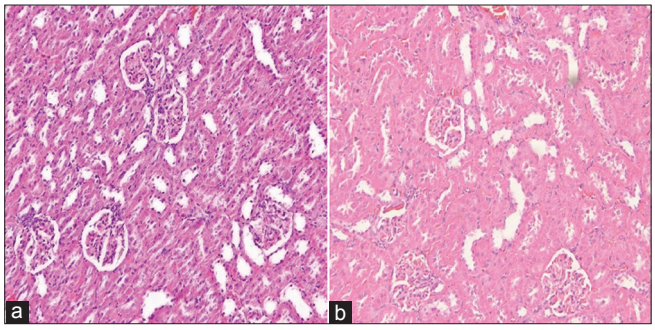

Figure 2: Photomicrographs of transverse sections of the kidney of the control group (a) and the *C. membranaceus* treated group (300 mg/kg b. wt) after sub chronic administration of the aqueous root extract (b) (H and E, ×20)

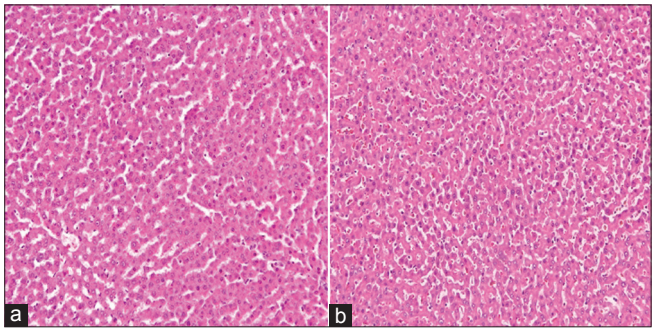

Figure 3: Photomicrographs of transverse sections of the liver of the control group (a) and the *C. membranaceus* treated group (300 mg/kg b. wt) after sub chronic administration of the aqueous root extract (b) (H and E, ×20)

root extract of *C. zambesicus*, changes were not significant.[14] Furthermore, in that study, sodium was slightly elevated in all the test groups and was significant, demonstrating nephrotoxicity as cited in other studies.[18-19] Urinalysis of renal metabolites and filtrates is often used as an indirect or corroborative measure of nephrotoxicity or kidney damage. Results from the urinalysis performed showed that there were no significant changes in urobilinogen, glucose, bilirubin, specific gravity, blood (RBC), nitrite, leukocytes, ketones, proteins, and urine pH in the control and treated groups as similarly observed in the acute toxicity study of the extract.[13] These results suggest that the extract was not nephrotoxic and neither did it induce acidosis, alkalosis, ketonuria, and hyperbilirubinemia.

Liver enzymes are particularly important in determining the hepatocellular integrity of the liver. It is only during tissue damage that these enzymes leak out of the tissue and become elevated in the serum. Elevations in ALT and AST are, therefore, an indication of hepatocellular damage. Previous studies of *C. membranaceus* at higher doses of 3000 mg/kg b. wt. did not show statistical differences between the test groups and the control group.[13] However, in this study, both ALT and AST levels were reduced by more than 50% after daily administration of *C. membranaceus* for 90 days. On the contrary, a similar sub-chronic toxicity study on the ethanol crude root extract of *C. zambesicus* resulted in significant increases in ALT, AST, and ALP, suggesting hepatotoxic effects of the extract.[14] The findings from this study, therefore, indicate that the extract does not cause hepatocellular injury nor does it disturb hepatocytes membrane integrity as observed in Figure 3.[20-23]

A significant elevation of serum ALP levels is usually a characteristic finding in obstructive jaundice or in cholestatic liver disease.[24] With respect to serum levels of ALP in this study, insignificant reduced levels were observed in the low and medium dose groups compared to the control group, suggesting that the extract does not cause obstructive jaundice or intrahepatic cholestasis. The liver's ability to promote protein synthesis after *C. membranaceus* administration is reflected by the absence of reduced levels in the protein fractions. Increase in the total protein, albumin, and globulin, therefore, indicate promotion of protein synthesis as equally observed by Okokon *et al.* with *C. zambesicus*. In the absence of adverse findings, these results suggest some level of hepato-protection offered by *C. membranaceus*.[14]

Changes in the levels of atherogenic analytes provide relevant information on the predisposition of the heart to atherosclerosis and its associated coronary heart disease. From the lipid profile results, it is possible that *C. membranaceus* in the bloodstream reduced the amount of volatile fatty acids (VFA) or the non-esterified fatty acids or prevented demobilization of lipids from the adipose tissue. With reduced lipids entering the hepatic component, acetate or aceyl CoA production reduces, thereby reducing the amount of cholesterol entering the microsomal pool and hence a reduction in the microsomal transfer protein that is available to the smooth endoplasmic reticulum for VLDL assembly through the golgi apparatus. Furthermore, a reduced production of acetyl CoA affects TG production

in the microsomal pool, which eventually reduces microsomal transfer protein production and subsequently VLDL production. The exact mechanism of action of *C. membranaceus* is uncertain at this stage. However, the data demonstrates a reduction in TG and VLDL. A change in lipoprotein metabolism can result from a change in synthesis, processing, or catabolism of plasma lipoprotein particles. A change in hepatic synthesis of VLDL can thus lead to hypo- or hyper-synthesis of TG-rich lipoproteins. Cardiovascular diseases are associated with high levels of VLDL.[25] A low production of VLDL as seen in this study implies a lower risk for atherosclerotic cardiovascular disease development. This possible cardio-protection of *C. membranaceus,* perhaps due to enhanced catabolism of TG, could be due to increased activity of lipoprotein lipase, a key enzyme in the removal and degradation of TG from circulation. Furthermore, the anti-ischemic potentials of *C. membranaceus* are seen from the results of the cardiac enzyme profile. From this study, LDH, total CK, as well as CK-MB reduced significantly, especially in the HD group. In conclusion, these results agree with previous studies suggesting no toxicity but anti-atherogenic and anti-ischemic effects of *C. membranaceus* aqueous root extract;[13] a suggestion that will require further investigations.

REFERENCES

1. Aboagye FA, Sam GH, Massiot G, Lavaud C. Julocrotine, a glutarimide alkaloid from Croton membranaceus. Fitoterapia 2000;71:461-2.
2. Mshana NR, Abbiw DK, Addae-Mensah I, Adjanouhoun E, Ahyi MR, Ekpere JA *et al*. Traditional Medicine and Pharmacopoeia, Contribution to the revision of ethnobotanical and Floristic Studies in Ghana, OAU/STRC Technical Report; 2000. p. 67.
3. Bayor MT, Gbedema SY, Annan K. The antimicrobial activity of Croton membranaceus, a species used in formulations of measles in Ghana. J Pharmacognosy and Phytother 2009;1:047- 051.
4. Ayim JA, Bayor MT, Philips RM, Shnyder, SD, Wright CW. The evaluation of selected Ghanaian medicinal plants for cytotoxic activities. J Sci Tech (Ghana) 2007;27:16-22.
5. Bayor MI. The anticancer and other bioactivity investigations on the extract and some compounds of Croton membranaceus (Euphobiaceae). PhD Thesis. Department of Pharmaceutical Chemistry, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana: KNUST Press, Kumasi, 2008.
6. Adewunmi CO, Ojewole JA. Safety of traditional medicines, complementary and alternative medicines in Africa. Afr J Trad Comp Alt Med 2004;1:1-3.
7. Morgan DG, Kelvin AS, Kinter LB, Fish CJ, Kerns WD, Rhodes G. The application of toxicokinetic data to dosage selection in toxicology studies. Toxicol Pathol 1994;22:112-23.
8. Gericke N. The regulation and control of traditional herbal medicines. An international overview with recommendations for the development of a South African Approach. Traditional Medicines Programme at the University of Cape Town. Working draft document, University of Cape Town, Cape Town: 1995. (Unpublished) http://apps.who.int/medicinedocs/pdf/whozip57e/whozip57e.pdf (Accessed - December 2011).
9. OECD. Test no. 408: Repeated dose 90-day oral toxicity study in rodents. OECD Guidelines for the Testing of Chemicals, Section 4: Health Effects. pp 1-10, Paris, France, OECD Publishing; 1998.
10. OECD. Organization of European Cooperation and Development Guidelines for testing Chemical. Acute Oral Toxicity-Fixed Dose Procedure-420. Research Triangle Park, NC, USA: NIH Publication No. 02-4501; 2001. p. O-4 - O-5. Available from: http://www.iccvam.niehs.nih.gov/docs/acutetox_docs/udpProc/udpfin01/vol_2.pdf. [Last accessed 2011 Nov].
11. Chung KT, Wong TY, Wei CI, Huang YW, Lin Y. Tannins and human health: A review. Crit Rev Food Sci Nutr 1998;38:421-64.
12. Adeyemi O, Akindele J, Nwumeh K. Acute and Subchronic toxicological assessment of Byrsocarpus coccineus Schum. and Thonn (Connaraceace) aqueous leaf extract. Int J App Res Nat Prod 2010;3(2). Available at: <http://www.ijarnp.org/index.php/ijarnp/article/view/25>. Date accessed: 16 Dec. 2011.
13. Asare GA, Sittie A, Bugyei K, Gyan BA, Adjei S, Addo P, *et al*. Acute toxicity studies of Croton membranaceus root extract. J Ethnopharmacol 2011;134:938-43.
14. Okokon JE, Nwafor PA, Ekpo MD. Subchronic toxicity studies of the ethanolic root extract of Croton zambesicus. Pak J Pharm Sci 2010;23:160-9.
15. Oyedemi SO, Yakubu MT, Afolayan AJ. Effect of aqueous extract of Leonotis leonurus (L.) R. Br. leaves in male Wistar rats. Hum Exp Toxicol 2010;29:377-84.
16. Nayak S, Mengi S. Immunostimulant activity of noni (Morinda citrifolia) on T and B lymphocytes. Pharm Biol 2010;48:724-31.
17. Keele CA, Neil E, Joels N. The internal environment: The red blood corpuscles. In: Wright S, editor. Wrights Applied Physiology. oxford: Oxford University Press; 1983. p 32-5.
18. Adebayo HA, Guang-Zhi Z, Jun-Ting F, Chang-Jiu JI, We-Jun H, Jun-Ju X, *et al.* Biochemical, heamatological and histopatholaogical studies of extract of Ageratum conyzoides L in Sragues-Dawley rats. J Med Plants Res 2010;4:2264-72.
19. Sireeratawong S, Lertprasertsuke N, Srisawat U, Thuppia A, Ngamjariyawat A, Suwanlikhid N, *et al.* Acute and Sub chronic toxicity study of the extract from Tiliacora triandra (Colebr.) Diels in rats. Songklanakarin J Sci Tech 2008;30:611-9.
20. Aliyu R, Adebayo AH, Gatsing D, Garba IH. The effects of ethanolic leaf extract of Commiphora africana (Burseraceae) on rat liver and kidney functions. J Pharmacol. Toxicol 2007;2:373-9.
21. Dobbs NA, Twelves CJ, Gregory W, Cruickshanka C, Richards MA, Rubens RD. Epirubicin in patients with liver dysfunction: Development and evaluation of a novel dose modification scheme. Eur J Cancer 2003;39:580-6.
22. Kew MC. Serum aminotransferase concentration as evidence of hepatocellular damage. Lancet 2000;355:591-2.
23. Plaa GL, Hewitt WR, du Souich P, Caillé G, Lock S. Isopropanol and acetone potentiation of carbon tetrachloride-induced hepatotoxicity: Single versus repetitive pretreatments in rats. J Toxicol Environ Health 1982;9:235-50.
24. Davern TL, Scharschmidt BF. Biochemical Liver Function Tests. In: Friedman FMLC, Sleisenger MH, editors. Sleisenger and Fordtrans' Gastrointestinal and Liver Disease Pathophysiology. USA: Diagnosis and Management, Elsevier Science; 2002.
25. Alaupovic P, Blankenhorn DH. [Determination of potentially atherogenic triglyceride-rich lipoprotein particles]. Klin Wochenschr 1990;68 Suppl 22:38-42.

Cite this article as: Afriyie DK, Asare GA, Bugyei K, Asiedu-Gyekye I, Gyan BA, Adjei S, *et al*. Anti-atherogenic and anti-ischemic potentials of *Croton membranaceus* observed during sub-chronic toxicity studies. Phcog Res 2013;5:10-6.

Source of Support: From the College of Health Sciences, University of Ghana, Legon, Ghana, **Conflict of Interest:** None declared.