ORIGINAL ARTICLE

Discovery Phytomedicine 2019, Volume 7, Number 3: 118-127

Acute and sub-acute oral toxicity and phytochemical profile of *Croton menyharthii* plant from Tana River County Kenya

Emma Wanjiku Kagunya,[1,2*] Catherine Kaluwa Kaingu,[3] James Mucunu Mbaria[1]

ABSTRACT

Croton menyharthii root bark is used to manage female reproductive ailments in Tana River County, Kenya. The plant treats dysmenorrhea, prevents abortion, stops post-partum hemorrhage and is also a contraceptive. Toxicological and phytochemical profile of the plant is still unknown. Preliminary phytochemical screening of Dichloromethane-Methanol and aqueous *Croton menyharthii* root bark extracts was carried out as per method used by Kisianan et al., 2019. Acute oral toxicity study was conducted using female rats by using OECD 423 guidelines whereas the sub-acute toxicity study was carried out using OECD 407 guidelines. General behavior, adverse effects and mortality were keenly observed throughout the experimental period. Food intake, water intake, body weight, organ weight, hematological and biochemical parameters were evaluated. Alkaloids, saponins, phenols, cardiac glycosides and tannins were present in both organic and aqueous extract. Both extracts had acute oral toxicity greater than 2000 mg/kg. In the sub-acute toxicity study, there was a significant dose-dependent decrease in the levels of total protein in rats treated with 200 ($P<0.006$), 400 ($P<0.00$) and 800 mg/kg ($P< 0.00$) aqueous extract relative to the control. None of the extracts caused a significant effect on haematological parameters. Long term administration of *Croton menyharthii* root bark extract is associated with significant alterations in renal physiology. Given the finding, we recommend the judicious use of the root bark extracts of *Croton menyharthii* particularly when long term use is being considered.

*Correspondence to:
Emma Wanjiku Kagunya, Department of Public Health, Pharmacology & Toxicology, Faculty of Veterinary Medicine, University of Nairobi, Nairobi, Kenya. Nairobi, Kenya. Department of Pharmacy, Thika Level 5 Hospital, P.O Box 227-01000, Thika, Kenya.
kiggzemma13@gmail.com

Cite This Article: Kagunya, E.W., Kaingu, C.K., Mbaria, J.M. 2020. Acute and sub-acute oral toxicity and phytochemical profile of *Croton menyharthii* plant from Tana River County Kenya. *Discovery Phytomedicine* 7(3): 118-127. DOI: 10.15562/phytomedicine.2020.135

INTRODUCTION

Croton menyharthii is a small tree/shrub that may grow up to a height of 4m.[1] The plant is distributed worldwide and occurs on rocky outcrops, dry woodlands and in dense marshy thickets in countries such as Zambia, Angola, Namibia, South Africa, Swaziland, Botswana, Kenya, Malawi, Ethiopia, Tanzania, Somalia, Mozambique, and Zimbabwe in Africa.[1]

Croton menyharthii has a variety of medicinal uses. In Somalia, fresh/dried roots are used for the management of dysmenorrhea, tapeworms and hepatitis. The root bark is used for the management of intestinal obstruction. Moreover, antimalarial and anti-influenza potential of the plant has also been reported.[2] In Tana River, it is locally known as "Muyama or Mualikaji". The plant concoction is used for the management of prolonged and/or irregular menses, post-partum hemorrhage, infertility, spontaneous abortions and for fertility regulation. Some women inhale smoke from the plant burnt leaves to ease menstrual pains.[2] The plant disrupts the estrous cycle of female *Wistar* rats and causes a significant dose-dependent reduction in fertility and implantation index.[3] Other studies reports the presence of three flavonols and an indole alkaloid that caused marked antimicrobial and enzyme inhibition activities.[4] However, no data is available on the toxicology profile of the plant. The present study aims to determine the acute, sub-acute toxicity and phytochemical compounds of the root bark extracts of *Croton menyharthii*.

MATERIALS AND METHODS

Study area

Croton menyharthii root bark samples were collected from Tana River County in Kenya. The plant is extensively used as a fertility regulator particularly in Garsen, Idsowe and Ngao villages.[5]

Sample collection and identification

Fresh *Croton menyharthii* root barks were collected from Tana River County and transported to the School of Biological Sciences of the University of Nairobi where botanical identification was done. A voucher specimen was preserved (CK021). Plant material preparation

Tap water was used to wash off adhering particles/debris from the plant root bark. The roots were dried and chopped into small pieces using a knife. The pieces were ground into a fine powder, packed in air tight containers and stored in dry, dark and well-ventilated storage cabinets.

[1]Department of Public Health, Pharmacology & Toxicology, Faculty of Veterinary Medicine, University of Nairobi, P.O Box 30197-00100, Nairobi, Kenya. P.O. Box 29053-00625, Nairobi, Kenya.
[2]Department of Pharmacy, Thika Level 5 Hospital, P.O Box 227-01000, Thika, Kenya.
[3]Department of Veterinary Anatomy and Physiology, University of Nairobi, P.O Box 30197-00100, Nairobi, Kenya.

Aqueous extract preparation

Kaingu *et al.,* 2017 protocol was used.[6] Briefly, about 1300 grams of the root bark powder of *Croton menyharthii* was weighed, mixed with distilled water in a volumetric flask at a ratio of 1:6 (w/v), and macerated for 48 hours at room temperature with continuous shaking. The resulting mixture was then filtered, and the filtrate lyophilized. The percentage yield of the extract was then calculated.

Dichloromethane-methanol extract preparation

Kisianan *et al.,* 2019 protocol was used.[7] Approximately 1800 grams of *Croton menyharthii* root bark powder was weighed and divided into six portions (each 300 grams in weight). The portions were mixed with 800 ml of 1:1 ratio of dichloromethane and methanol mixture (v/v). The mixture was macerated for 72 hours while continuously being shaken. The filtrate was collected in a round-bottomed flask. Filtrate volume was reduced *in vacuo* using a rotary evaporator. The residual solvent was removed by the use of sand bath operating at 50°C for 5 days. The product was weighed and percentage yield calculated.

Qualitative phytochemical screening

Croton menyharthii root bark extract phytochemical components were identified using qualitative methods.[8,9] Tannins, flavonoids, anthraquinones, alkaloids, terpenoids, saponins, phenols and glycosides were evaluated.

Experimental animals and ethics clearence

Ethical clearance from the Faculty of Veterinary Medicine Biosafety, Animal use and ethics committee was sought before study commencement (REF: FVM BAUEC/2019/185).

Sixty four female *Wistar* rats were bought from the Department of Public Health, Pharmacology and Toxicology (PHPT), University of Nairobi. The rats were aged between 6-12 weeks old. They were nulliparous and non-pregnant. They weighed between 180 to 210 grams. They were housed in polypropylene cages measuring 31×41×18cm and lined with wood shavings (a maximum of 5 rats per cage). All rats acclimatized for 7 days to standard animal house conditions (temperature: 25 ± 3°C; relative humidity 56–60%; 12 hours of light and 12 hours of darkness). The animals were fed on standard rat pellets from a commercial supplier (Unga Group Plc, Kenya) and water provided *ad libitum*.

Acute toxicity testing

The acute oral toxicity study was carried out according to the Organization for Economic Cooperation and Development (OECD) test guideline 423.[10]

Croton menyharthii aqueous extract

Twelve rats were randomly assigned to four experimental groups; (Group 1-4). All animals were labeled using picric acid to facilitate their identification. Food was withheld overnight before dosing but water was provided *ad libitum*. The weight of the animals was taken on day 0 (fasting) and just before the administration of *Croton menyharthii* extract. Test substances were administered via oral gavage. 300 mg/kg was selected as the starting dose as per the OECD test guideline 423.[10]

1ml of distilled water was administered to the control rats (group 1). Test groups received 300 (group 2) and 2000 mg/kg (group 3) respectively. The fourth group received 5000 mg/kg as a limit dose if no mortality was recorded at 2000 mg/kg for both extracts.

Croton menyharthii Dichloromethane-methanol extract

Extra virgin oil was administered to the control (group 1), test groups received 300 (group 2) and 2000 mg/kg (group 3) respectively.[10] In all groups, food was withheld for a further four hours after extract administration. The animals were intensely observed for signs of toxicity for the first thirty minutes and every half hour up to four hours.[10] Changes in skin and fur color, mucous and eye membranes, respiratory system, circulatory system, autonomic and central nervous system and somatomotor activity were noted.[10] Other effects including tremors, convulsions, salivations, diarrhoea, coma and death were also recorded.[10] The animals were thereafter observed after 24 hours and daily thereafter for fourteen days.[10] Individual body weights of the animals were taken on day 7 and day 14.[10] All the animals were then humanely euthanized and macroscopy of all major organs undertaken.

Sub-acute toxicity

The OECD test guidelines 407 was used to carry out the sub-acute toxicity study.[11] A total of 40 animals were used. The animals were randomly assigned to 8 groups with 5 rats each. All animals in a group were labelled as either 1-5 using picric acid for ease of identification.

Group 1 (control) received 1ml of distilled water. Groups 2, 3 and 4 were the test groups. These rats received 200, 400 and 800 mg/kg *Croton menyharthii a*queous extract respectively once daily for 28 days.[11]

Croton menyharthii dichloromethane extract

Group 5 (control) received 1ml of extra virgin oil. Groups 6, 7 and 8 were used as test groups. They

received 200, 400 and 800 mg/kg dichloromethane-methanol extract respectively.[11]

All rats were individually monitored for signs of toxicity or mortality as an end point throughout the entire course of the study. The animals were weighed on the first day and weekly (7 days) thereafter. Feed and water consumption were evaluated weekly.

Blood sample ccollection and analysis

After 28 days, all rats were anesthetized and approximately 5 ml of blood was collected via cardiac puncture into Labex® tubes containing Ethylene diamine tetra acetic acid (EDTA) and kept at -20°C. This blood was used for hematological (blood) parameter analysis including total red blood cell count (RBC), hematocrit, mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), mean cell volume (MCV), total white blood cell count (WBC) and thrombocyte count. Approximately 5 ml of blood was collected into clot activator tubes and the blood allowed to stand for 30 minutes before being centrifuged at 3000 revolutions per minute to obtain serum which was transferred to 2ml eppendorf tubes and stored at -20°C. This blood was used for biochemical parameter analysis including aspartate aminotransferase (AST), alanine aminotransferase (ALT), total protein (TP), blood urea nitrogen (BUN) and creatinine.

Histopathological studies

Once blood was collected, all animals were humanely euthanized and post-mortem carried out. Internal organs were examined for gross pathological changes. Additionally, the liver and kidney of both control and treatment group animals were excised, washed with physiological saline and their wet weights determined. The relative organ-body weight ratio of all the animals was calculated using the formula below.

$$\text{Relative organ weight} = \frac{\text{Absolute organ weight}}{\text{Weight of the animal}} \times 100$$

Statistical analysis

The weight of the animals, biochemical and hematological parameters were expressed as mean ± standard deviation of the mean. One-way analysis of variance (ANOVA) was used to analyze the means using IBM SPSS Statistics software version 21. To find out if there was a significant difference among the groups and the controls, a Tukey post hoc test was performed. Differences were considered statistically significant at P *0.05*

RESULTS

Extract yield

Qualitative phytochemical profile of the extracts

Acute oral toxicity study

Observation of treated and control animal for toxicity signs

Effects of aqueous and organic extracts on the weekly mean body weights

5000 mg/kg *Croton menyharthii* aqueous extract caused a significant *(P<0.013)* change in the mean weight gain of treated rats relative to the control over a

Table 1 **Description, yield and solubility of *C. menyharthii* root bark extracts**

Extract	Description	Yield	Solubility
Aqueous Croton menyharthii	Dark brown powder	6.25% w/w	Highly soluble in water
Dichloromethane-methanol Croton menyharthii	Dark brown tar-like substance	3.03% w/w	Sparingly soluble in water Soluble in extra virgin oil

Table 2 **Phytochemical compounds present in *Croton menyharthii* root bark extracts**

Compound	Aqueous extract	DCM-Methanol extract
Tannins	Present	Present
Flavonoids	Absent	Absent
Anthraquinones	Absent	Absent
Alkaloids	Present	Present
Terpenoids	Absent	Absent
Saponins	Present	Present
Phenols	Present	Present
Cardiac glycosides	Present	Present

14-day period (Figure 2). 300 and 2000 mg/kg *Croton menyharthii* aqueous extract produced a non-significant increase (*P<0.178* and *P< 0.536*) respectively in the mean weight of treated rats relative to the control over a 14-day period (Figure 2). 300, 2000 and 5000 mg/kg *Croton menyharthii* organic extract caused a non-significant increase *(P<0.848, P<0.933,* and P< *0.604* respectively) in the mean weight of treated rats compared to the control over a 14-day period.

Gross necropsy

No changes in the color, size or macroscopic pathology of major organs including liver, kidneys, spleen, heart, lungs, stomach, small and large intestines were observed in rats administered with both the aqueous and organic extracts.

Sub-acute toxicity studies

Physical and clinical effects of the extracts

No major clinical or physical effects were observed in all test rats after the 28-day treatment regime for both *Croton menyharthii* aqueous and dichloromethane-methanol extracts even at the highest dose (800 mg/kg body weight). However, all *Croton menyharthii* dichloromethane-methanol extract treated rats scrambled for water a few minutes after extract administration. All rats displayed excessive grooming particularly around their mouths. They were also rubbing their mouths against the cage walls and the floor. There was no mortality in any of the test groups.

Effects of *Croton menyharthii* extract on feed consumption

Tables 4 and 5 show the average weekly feed consumption of rats that received both extracts over 28-day period. 400 mg/kg body weight *Croton menyharthii* aqueous extract caused a significant difference *(P<0.016)* in feed consumption rate relative to the control (Table 4). However, there was a non-significant difference *(P>0.05)* in feed consumption at 200 and 800 mg/kg body weight relative to the control (Table 4).

200 mg/kg *Croton menyharthii* dichloromethane-methanol extract caused a significant difference *(P<0.033)* in average feed consumption rate relative to the control (Table 5). However, there was a non-significant difference (*P>0.05*) in feed consumption rate in animals that received 400 and 800 mg/kg body weight *Croton menyharthii* dichloromethane-methanol extract relative to the control (Table 5).

Effects of Croton menyharthii extracts on water consumption

Croton menyharthii aqueous extract caused a significant difference in the water consumption in rats that received 200 and 400 mg/kg (P*<0.040; P<0.03)* respectively relative to the control (Table 6). However, at 800 mg/kg there was no significant difference (*P<0.05)* in the mean water consumption relative to the control (Table 6). *Croton menyharthii* dichloromethane-methanol extract caused non-significant changes in water consumption over the 28 day treatment period relative to the control (Table 7).

Effects of Croton menyharthii extracts on the weight of animals

None of *Croton menyharthii* aqueous extract doses caused a significant change in the mean weight of animals over the study period (Table 8).

At all treatment doses, *Croton menyharthii* dichloromethane-methanol extract caused a non-significant change in the mean weekly weight of treatment animals relative to the control (Table 9).

Effect of *Croton menyharthii* aqueous extract *on hematological parameters*

At all treatment doses, *Croton menyharthii* aqueous extract caused a non-significant change in the mean values of the hematological parameters relative to the controls (Table 10).

Effect of Croton menyharthii dichloromethane extract *on hematological parameters*

At all treatment doses, *Croton menyharthii* dichloromethane-methanol extract caused a non significant change in the mean values of the hematological parameters relative to the controls.

Effect of Croton menyharthii aqueous extracts on biochemical parameters

Croton menyharthii 200, 400 and 800 mg/kg aqueous extract caused a significant decline in total protein levels in the treatment animals relative to the control (Table 12).

Effect of Croton menyharthii dichloromethane-methanol extract on biochemical parameters

200, 400 and 800 mg/kg *Croton menyharthii* dichloromethane-methanol extract had a non significant effect on the mean values of the biochemical parameters relative to the control.

Gross macroscopy of major organs

On macroscopic examinations, there was no pathological difference on major organs of rats in all the *C. menyharthii* aqueous and dichloromethane-methanol root barks extract treatment groups when compared to the control. There was no change in texture, size, colour of the heart, lungs, liver, stomach, small and large intestines, spleen and kidneys.

Table 3 **Effects of *C. menyharthii* root bark extracts on wistar rat physical and behavioral parameters**

	Control (AQCRM)	AQCRM 300 mg/kg	AQCRM 2000 mg/kg	AQCRM 5000 mg/kg	Control (DMCRM)	DMCRM 300 mg/kg	DMCRM 2000 mg/kg	DMCRM 5000 mg/kg
Respiration	_	+	+	+	_	+	+	+
Circulation	_	_	_	_	_	_	_	_
Skin and fur	_	_	_	_	_	_	_	_
Eye color	_	_	_	_	_	_	_	_
Grip	+	+	+	+	+	+	+	+
Sound response	+	+	+	+	+	+	+	+
Response to touch	+	+	+	+	+	+	+	+
Locomotion	+	+	+	+	+	+	+	+
Urination	+	+	+	+	+	+	+	+
Defecation	+	+	+	+	+	+	+	+
Diarrhoea	_	_	_	_	_	_	_	_
Writhing reflex	+	+	+	+	+	+	+	+
Lethargy	_	+	+	+	_	+	+	+
Sedation	_	+	+	+	_	+	+	+
Tremors	_	_	_	_	_	_	_	+
Convulsions	_	_	_	_	_	_	_	_
Mortality	_	_	_	_	_	_	_	_

Table 4 **The effect of *Croton menyharthii* aqueous root bark extract on the average weekly feed consumption in test and control animals**

Treatment	Week 1	Week 2	Week 3	Week 4	*p-value*
Control	109.73±16.15	101.67±6.53	101.44±11.56	96.86±22.46	-
200	112.70±19.79	105.33±21.95	112.69±11.01	112.67±12.29	*0.282*
400	111.32±23.69	128.11±8.30	122.55±12.40	112.12±23.73	*0.016*
800	96.76±40.67	110.10±4.46	108.50±10.93	112.65±16.59	*0.737*

Table 5 **The effect of *Croton menyharthii* dichloromethane-methanol root bark extract on average weekly feed consumption in test and control animals**

Treatment	Week 1	Week 2	Week 3	Week 4	*p-value*
Control	84.87±24.47	76.97±5.83	75.91±9.15	73.43±6.09	-
200	98.63±13.84	93.06±6.91	93.15±7.97	94.03±17.58	0.033
400	90.98±15.79	82.57±3.80	73.65±12.45	75.14±22.56	0.950
800	97.70±16.12	75.94±3.59	75.65±11.07	74.12±15.10	0.936

Table 6 **Effect of the aqueous root bark extracts of *Croton menyharthii* on the average weekly water consumption in treatment and control group animals**

Treatment	Week 1	Week 2	Week 3	Week 4	*P-value*
Control	139.28±26.37	129.29±11.34	148.57±13.76	141.43±55.67	-
200	161.43±21.74	160.71±24.23	153.57±39.02	168.57±48.71	*0.040*
400	155.71±38.13	187.86±18.68	168.57±38.37	175.71±48.60	*0.003*
800	143.57±50.23	156.43±22.86	160.00±40.52	167.86±38.28	*0.111*

Table 7 **Effect of *Croton menyharthii* dichloromethane-methanol extract on average weekly water consumption in treatment animals relative to the control**

Treatment	Week 1	Week 2	Week 3	Week 4	*P-value*
Control	129.29±66.61	137.14±31.60	129.29±7.32	125.71±38.88	-
200	147.14±18.90	158.57±11.80	164.29±22.81	147.14±43.96	*0.100*
400	142.14±17.04	135.71±10.58	125.00±21.41	117.14±46.45	*1.000*
800	140.71±22.44	123.57±11.80	142.86±12.86	131.43±49.05	*0.895*

Table 8 **Effect of graded doses of *Croton menyharthii* aqueous extract on the mean weekly weight of treatment animals relative to the control**

Treatment	Day 0	Day 7	Day 14	Day 21	Day 28	*P-value*
Control	188.90 ± 14.28	221.38 ± 22.20	223.30 ± 26.60	225.19± 25.48	230.11 ± 26.63	-
200	176.23 ± 12.17	203.95 ± 10.29	198.85 ± 8.17	208.67 ± 10.56	227.76 ± 14.90	*0.620*
400	169.23 ± 12.81	198.91 ± 11.66	214.08 ± 8.71	217.72 ± 8.44	230.74 ± 9.56	*0.767*
800	169.41 ± 12.22	189.81 ±14.18	196.84 ± 13.84	198.73 ± 16.98	214.26 ± 16.31	*0.229*

Table 9 **Effect of graded doses of *Croton menyharthii* dichloromethane-methanol extract on the mean weekly weight changes**

Dose	Day 0	Day 7	Day 14	Day 21	Day 28	*P-value*
control	183.47 ± 15.76	199.42 ± 21.24	209.13 ± 24.64	215.71 ± 27.21	220.68 ± 30.44	-
200	178.90 ± 13.14	199.70 ± 16.60	207.70 ± 18.62	211.23 ± 18.26	220.31 ± 18.04	*0.997*
400	178.29 ± 14.28	205.97 ± 19.14	215.31 ± 17.26	220.67 ± 16.26	228.02 ± 18.96	*0.984*
800	164.16 ± 8.38	188.28 ± 11.51	200.42 ± 13.84	206.46 ± 14.83	216.96 ± 14.21	*0.797*

Table 10 **Effect of *Croton menyharthii* aqueous extract graded doses on hematological parameters**

Parameter	Control	200 mg/kg	*P Value*	400 mg/kg	*p value*	800 mg/kg	*p value*
RBC (x10^6/µL)	7.47±1.03	7.76±1.04	***0.098***	7.37±1.03	***0.988***	7.12±1.56	***0.979***
WBC (x10^3/µL)	9.50±1.05	11.71±5.70	***0.829***	12.84±1.92	***0.587***	14.17±3.98	***0.318***
Platelets (x10^3/µL)	682.00±180.53	875.00±188.49	***0.315***	769.00±128.78	***0.617***	714.00±78.84	***0.617***
Hb (gm/dL)	14.10±2.57	14.00±1.55	***1.00***	14.00±1.27	***1.00***	13.60±3.09	***0.992***
PCV (%)	42.3±7.89	41.1±4.87	***0.993***	41.5± 4.36	***0.998***	40.4±9.32	***0.978***
MCV (fL)	56.60±2.14	53.10±3.68	***0.263***	56.5±2.65	***1.00***	56.60±0.96	***1.00***
MCH (pg)	18.80±0.50	18.00±0.75	***0.498***	19.10±1.00	***0.942***	19.10±0.70	***0.955***
MCHC (g/dL)	33.20±0.53	34.00±1.51	***0.690***	33.80±0.66	***0.842***	33.70±0.94	***0.888***

RBC: Red blood cells, WBC: White blood cells, Hb: Haemoglobin, PCV: Packed cell volume, MCV: Mean corpuscular volume, MCH: Mean corpuscular haemoglobin, MCHC: Mean corpuscular haemoglobin concentration. Values expressed as mean ± SD (n=5).

Effect of *Croton menyharthii* aqueous extract on the relative organ-body weight ratio

200, 400 and 800 mg/kg *Croton menyharthii* aqueous extract caused a non significant difference on the relative organ-body weight ratios of treated animals relative to the control (Table 14).

Effect of *Croton menyharthii* dichloromethane-methanol extract on the relative organ-body weight ratio

200 mg/kg bodyweight *C. menyharthii* dichloromethane-methanol extract caused a significant difference (P<0.045) in the relative organ-body weight ratios of the liver (Table 15).

DISCUSSION

Croton comprises of a multitude of species (about 1200) and occurs throughout the world.[12] Therefore, it is not a surprise that there has been a lot of interest in the toxicology of these species.[12–19] The toxicology of *Croton zambesicus, Croton membraneceous, Croton lobatus, Croton stellatopilosus, Croton macrostachyus,* and *Croton penduliforus* has been studied in human cancer cell lines,[19] brine shrimp,[17]

Table 11 **Effects of graded doses of *C. menyharthii* dichloromethane-methanol extract on hematological parameters in treatment animals relative to the control**

Parameter	Control	200 mg/kg	*p value*	400 mg/kg	*p value*	800 mg/kg	*p value*
RBC (x 10^6/μL)	7.38±0.77	6.89±1.64	***0.883***	8.18±0.48	***0.638***	8.25±0.24	***0.576***
WBC (x10^3/μL)	11.73±1.86	11.65±2.28	***1.00***	14.48±1.92	***0.175***	9.38±1.89	***0.281***
Platelets (x10^3/μL)	513.00±342.43	696.00±76.64	***0.574***	582.25±116.99	***0.959***	769.00±138.48	***0.306***
Hb (gm/dL)	14.30±1.79	13.20±3.12	***0.851***	15.20±0.65	***0.898***	15.00±0.95	***0.936***
PCV	41.8±5.07	39.30±8.56	***0.896***	44.3±1.33	***0.899***	43.90±1.51	***0.939***
MCV (fL)	56.60±3.04	57.40±1.64	***0.962***	54.20±1.81	***0.451***	53.20±2.12	***0.183***
MCH (pg)	19.30±1.01	19.10±0.53	***0.990***	18.60±0.44	***0.578***	18.20±1.07	***0.273***
MCHC (g/dL)	34.10±0.62	33.40±1.07	***0.698***	34.20±0.49	***0.995***	34.30±1.21	***0.992***

RBC: Red blood cells, WBC: White blood cells, Hb: Haemoglobin, PCV: Packed cell volume, MCV: Mean corpuscular volume, MCH: Mean corpuscular haemoglobin, MCHC: Mean corpuscular haemoglobin concentration. Values expressed as mean ± SD (n=5).

Table 12 **Effect of *Croton menyharthii* aqueous extract on biochemical parameters in treated animals relative to the control**

Parameter	Control	200 mg/kg	*p value*	400 mg/kg	*Pvalue*	800 mg/kg	*p value*
ALT mg/l	118.00±13.89	119.00±30.23	***1.000***	136.00±23.34	***0.681***	109.00±21.50	***0.955***
AST ml/l	219.00±46.41	202.00±27.80	***0.976***	241.00±103.57	***0.950***	153.00±18.91	***0.428***
T.P g/l	87.00±6.86	75.00±2.55	***0.006***	69.00±3.42	***0.000***	69.50±3.54	***0.000***
BUN	8.00±1.00	9.00±0.25	***0.980***	8.00±0.45	***0.898***	7. 00±1.72	***0.445***
Creatininemg/dl	47.00±2.24	52.00±5.88	***0.971***	63.00±29.47	***0.526***	47.00±5.73	***1.000***

ALT: Alanine aminotransferase, AST: Aspartate amino transferase, TP: Total protein, BUN: Blood urea nitrogen. Values are expressed as mean ± SD of five animals.

Table 13 **Effect of graded doses of *Croton menyharthii* dichloromethane-methanol extracts on biochemical parameters in treatment animals relative to the controls**

Parameter	Control	200 mg/kg	*p value*	400 mg/kg	*p value*	800 mg/kg	*p value*
ALT mg/l	111.00±31.05	107.00±22.05	***0.990***	133.00±28.95	***0.583***	108.00±11.63	***0.999***
AST ml/l	191.00±66.42	162.00±8.50	***0.851***	185.00±34.32	***0.997***	164.00±42.32	***0.820***
T.P g/l	75.00±2.62	75.00±4.59	***0.961***	75.00±2.16	***0.997***	70.00±4.21	***0.304***
BUN	8.00±1.00	9.00±0.25	***0.980***	8.00±0.45	***0.898***	7.00±1.72	***0.445***
Creatininemg/dl	46.00±3.78	43.00±2.32	***0.993***	45.00±4.38	***0.987***	47.00±11.68	***0.998***

ALT: Alanine aminotransferase, AST: Aspartate amino transferase, TP: Total protein, BUN: Blood urea nitrogen. Values are expressed as mean ± SD; n=5.

Table 14 **Effect of *Croton menyharthii* aqueous extract on the relative organ-body weight ratio of treated animals relative to the control**

Treatment	Liver	*P value*	Left kidney	*p value*	Right kidney	*P value*
Control	0.03700 ± 0.00399	_	0.003746 ± 0.000388	_	0.003845 ± 0.000466	_
200 mg/kg	0.03802 ± 0.00380	0.969	0.00373 ± 0.000250	1.000	0.003935 ± 0.000278	0.957
400 mg/kg	0.03919 ± 0.00174	0.770	0.003628 ± 0.000269	0.897	0.003794 ± 0.000172	0.992
800 mg/kg	0.03797 ± 0.00469	0.211	0.003696 ± 0.000196	0.997	0.003874 ± 0.000224	0.558

Table 15 **Effect of *Croton menyharthii* dichloromethane-methanol extract on the relative organ-body weight ratio of treat animals relative to the control**

Treatment	Liver	*P value*	Left kidney	*P value*	Right kidney	*P value*
Control	0.03825 ± 0.002301	_	0.003817 ±0.000462	_	0.004084 ± 0.000372	_
200 mg/kg	0.03156 ± 0.002749	*0.045*	0.003885 ± 0.000442	*0.991*	0.003643 ± 0.000232	*0.174*
400 mg/kg	0.03523 ± 0.005741	*0.563*	0.003546 ± 0.000270	*0.668*	0.003505 ± 0.000351	*0.051*
800 mg/kg	0.03361 ± 0.002586	*0.220*	0.003977 ± 0.000282	*0.906*	0.003773 ± 0.000311	*0.443*

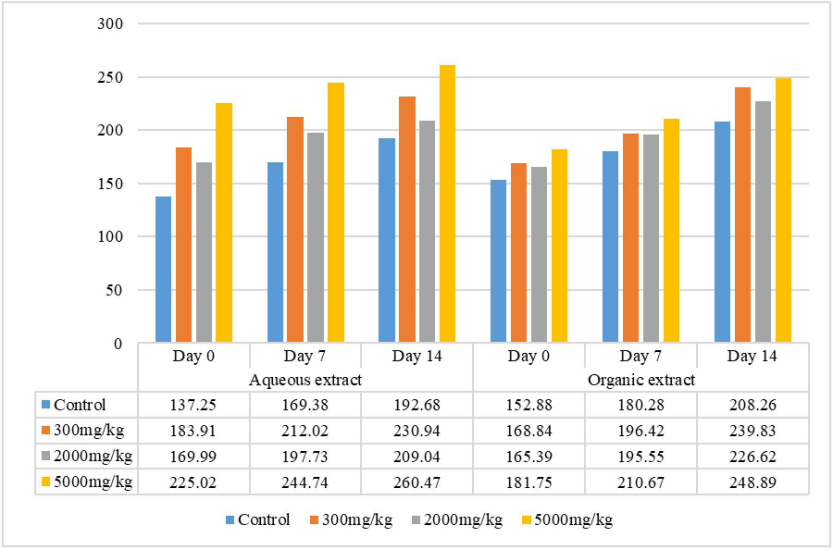

Figure 2 Changes in the mean weight of animals treated with root bark extracts of *C. menyharthii* in the acute toxicity protocol

and in rats and mice.[12–16,18] Methanol,[13,19] water,[14,15] ethanol,[12,16] a mixture of water and methylene chloride,[17] and petroleum ether were used[18] to prepare leaf,[12,13,15,16] root,[12,14] stem bark,[17] and seed oil[18] extracts. In this study, it was established that root bark extracts of *Croton menyharthii* prepared using water and a mixture of dichloromethane and methanol had LD_{50} values of > 2000 mg/kg body weight. This finding was similar to that reported by Lagnika *et al.,* 2016 using *Croton lobatus*.[15] Drowsiness and lethargy seen in rats within 30 minutes of receiving test extracts might be due to the presence of alkaloids. According to Njeru *et al.,* 2013, alkaloids have a CNS depressant effect.[20]

The 28 day oral dose using both *C. menyharthii* extracts did not cause any adverse physical effects on the treatment animals. This seems to be in agreement with the results shown by studies using *Croton membranous* and *Croton zambesicus*.[13,14] However, the fact that animals that received *C. menyharthii* dichloromethane-methanol extract rubbed their mouths on the cage walls and floor may be a sign of irritation. The presence of tannins and alkaloids in medicinal plants may be a cause of irritation in animal models due to their bitterness.[20] There were no significant changes in the hematological parameters in animals treated with both *C. menyharthii* extracts suggesting that both extracts may not have an effect on the bone marrow and spleen. Probably both extracts might not cause peripheral blood cells destruction or stimulate antibodies against blood forming precursors. These findings corroborate those of Sharif *et al*., 2015 who reported similar findings while studying the methanol extract of *Euphorbia pulcherrima*.[21]

The observation of a non-significant dose dependent increase in RBCs and Hb and decline in the levels of MCV and MCH may imply that the extract might induce microcytic anemia.[22] This finding is similar to that reported by Okonkwo *et al*., 2019.[23] The *C. menyharthii* aqueous extract produced a significant dose dependent decrease in protein levels similar to the effect of *Croton macrostachys* in goats Gadir *et al.,* 2003.[24] Djimeli *et al.,* 2017 also reported a reduction in the levels of serum protein in mice brought about by *Alchornea cordifolia*.[25] This might be due to the presence of tannins which have been reported to cause protein malabsorption.[26] This finding is similar to that reported by Usman *et al.,* 2014 on a study in rats using *Euphorbia lateriflora*.[27] The low concentration of urea in animals treated with both extracts may be related to similar reduced levels of protein observed. Evidence suggests that urea is a product of protein metabolism and therefore a low level of protein will likely result in a low level of urea.[28] Probably the results observed may actually have more to do with malabsorption than the effect of the extract on the kidneys. Macroscopic examination of major organs revealed non-significant changes. The tested extracts did not produce any visible effects on the organs. Similar outcomes have been reported in other Croton species.[15,16]

The decrease in the mean relative-organ weight ratio of the liver in animals treated with 200 mg/kg *C. menyharthii* organic extract may be due to inter-group and/or intra-animal physiological variations. However, these findings are in contrast to Chaotham *et al*., 2013 who reported a dose dependent significant increase in the relative organ weight of the liver and kidneys of rats treated with *Croton stellatopilosus* extract.[16]

The non-toxic effect of the dichloromethane-methanol extract of *C. menyharthii* on the liver contrasts other studies on *Croton zambesicus* and *Croton cajucara*.[29,30] Akinloye *et al.,* 2015[29] reported liver injury and necrosis in rats treated with *Croton zambesicus* extract and Rodriguez *et al.,* 2004[30] reported nuclear alterations, microvacuolar degeneration and turbid tumefaction in rat livers treated with the extract of *Croton cajucara* extract.

In this study, the *C. menyharthii* organic extract produced inflammation of the renal tubules, that resulted in lymphocytic infiltration and multifocal hemorrhage of the renal parenchyma. Probably the extract might be toxic to the kidney on long term use. Other researchers have reported similar results on *Acalypha wilkesiana*.[31] In their report, Makoshi *et al.,* 2016 established that a 14-day oral administration of the aqueous leaf extract of *Acalypha wilkesiana* produced severe necrosis

and degeneration of the renal tubules and glomeruli but also caused hepatotoxicity in rats.[31] Biochemical parameters however do not correlate with the kidney histopathological findings. There was a non-significant difference compared to the controls. A study by Gounden and Jialal argues that by the time a rise in serum creatinine levels is detected, kidney function would have reduced by up to 50%.[32] Furthermore, the authors suggest that serum creatinine might be a more precise means of evaluating renal function than urea.[32] Therefore, in this study our findings on the histopathological changes resulting from the administration of the organic extract of *C. menyharthii* could be representative of early signs of kidney damage. The aqueous extract however did not cause any histopathological changes in treated animals. This is probably because, oil-based vehicles (extra virgin oil) tends to increase the rate and extent of absorption of both fat-soluble and sparingly water-soluble substances from the intestine. This may result in the substances being delivered faster and in larger quantities causing an increased likelihood of toxicity than aqueous-based vehicle.[33]

CONCLUSIONS

Long term administration of the root bark extracts of *Croton menyharthii* is associated with significant alterations in the renal physiology of rats. Given this finding, we recommend the judicious use of the root bark extracts of *Croton menyharthii* particularly when long term use is being considered.

REFERENCES

1. Flora of Zimbabwe: Species information: Croton menyharthii.
2. Schmelzer GH, Achigan-Dako EG, Bosch CH. Medicinal plants of tropical Africa. Conclusions Recomm based PROTA. 2010;11:108.
3. Kaingu CK, Odumaa JA. Potential of Croton menyharthii and Uvariodendron kirkii aqueous extracts as female Novel contraceptives. Discov Phytomedicine. 2019;6:112–8.
4. Aderogba MA, Ndhlala AR, Rengasamy KRR, Van Staden J. Antimicrobial and selected in vitro enzyme inhibitory effects of leaf extracts, flavonols and indole alkaloids isolated from Croton menyharthii. Molecules. 2013;18:12633–44.
5. Kaingu CK, Oduma JA, Mbaria JM, Kiama SG. Medicinal plants traditionally used for the management of female reproductive health dysfunction in Tana River County, Kenya. TANG [HUMANITAS Med. Association of Genuine Traditional Korean Medicine; 2013;3:17.1-17.10.
6. Kaingu CK, Oduma JA, Mbaria JM, Kiama SG. Effects of Croton menyharthii and Uvariodendron kirkii extracts on ovarian corpora lutea and reproductive hormones. Discov Phytomedicine. DiscoverSys, Inc.; 2017;4:21.
7. Kisianan Kenana J, Mbaria JM, Kaluwa Kaingu C, Onyango Okumu P, Mbaria JM, Kaingu CK. Ph ton The Journal of Ethnobiology and Traditional Medicine Ph ton Toxicological and Phytochemical Evaluation of Uvariodendron kirkii [Internet]. J. Ethnobiol. Tradit. Med. 2019. Available from: https://sites.google.com/site/photonfoundationorganization/home/the-journal-of-ethnobiology-and-traditional-medicine
8. Harborne AJ. Phytochemical methods a guide to modern techniques of plant analysis. springer science & business media; 1998.
9. Evans WC. Trease and Evans' Pharmacognosy E-Book. Elsevier Health Sciences; 2009.
10. Guideline O, Testing FOR, Chemicals OF. Acute Oral Toxicity – Acute Toxic Class Method. 2001;1–14.
11. Guidelines O. Repeated dose 28-day oral toxicity study in rodents. Oecd Guidel Test Chem. 2008;1–13.
12. 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.
13. C OJ, A OD. Oral acute toxicity study of methanol leaf extracts of Croton Zambesicus in mice. Merit Res J Pharm Pharm Sci. 2016;2:1–5.
14. Maroyi A. Review of Croton membranaceus Mûll. Arg.: phytochemical, pharmacological and toxicological perspective. J Complement Med Res. ScopeMed International Medical Journal Management and Indexing System; 2018;9:24.
15. Rats LIN, Lagnika L, Tchachedre M, Laleye A, Sanni A. Research Article TOXICOLOGICAL EFFECT OF AQUEOUS EXTRACTS OF Croton. 2016;2:1–8.
16. Chaotham C, Chivapat S, Chaikitwattana A, De-Eknamkul W. Acute and chronic oral toxicity of a partially purified plaunotol extract from Croton stellatopilosus Ohba. Biomed Res Int. 2013;2013.
17. Mbiantcha M, Tb N, Bl N, Tane P, Kamanyi A. Preliminary Assessment of Toxicity of Croton Macrostachyus Stem Bark (Euphorbiaceae) Extracts. 2013;3:113–32.
18. Abiola OS, Selina OO, Olubunmi DA, Abiola MO. Acute toxicity effect of Croton penduliflorus (Euphorbiaceae) Seed Oil. Nig Q J Hosp Med. Lagos University Medical Society; 2015;25:80–5.
19. Ayim JA, Bayor MT, Phillips RM, Shnyder SD, Wright CW. The evaluation of selected ghanaian medicinal plants for cytotoxic activites. J Sci Technol. 2008;27:16–22.
20. Njeru SN, Matasyoh J, Mwaniki CG, Mwendia CM, Kobia KG. A Review of some phytochemicals commonly found in medicinal plants. Int J Med Plant. 2013;105:135–40.
21. Sharif HB, Mukhtar MD, Mustapha Y, Baba G, Lawal AO. Acute and subchronic toxicity profile of Euphorbia pulcherrima methanol extract on Wistar albino rats. Adv Pharm. Hindawi; 2015;2015.
22. Urrechaga E, Hoffmann JJML, Izquierdo S, Escanero JF. Differential diagnosis of microcytic anemia: the role of microcytic and hypochromic erythrocytes. Int J Lab Hematol. 2015;37:334–40.
23. Okonkwo CO, Ohaeri OC, Atangwho IJ. Haematological changes in rats exposed to insecticidal oils from the leaves of Cassia occidentalis and Euphorbia milii. Heliyon. Elsevier; 2019;5:e01746.
24. Gadir WSA, Onsa TO, Ali WEM, El Badwi SMA, Adam SEI. Comparative toxicity of Croton macrostachys, Jatropha curcas and Piper abyssinica seeds in Nubian goats. Small Rumin Res. Elsevier; 2003;48:61–7.
25. Djimeli MN, Fodouop SPC, Njateng GSS, Fokunang C, Tala DS, Kengni F, et al. Antibacterial activities and toxicological study of the aqueous extract from leaves of Alchornea cordifolia (Euphorbiaceae). BMC Complement Altern Med. BioMed Central Ltd.; 2017;17.
26. Barszcz M, Taciak M, Skomiał J. A dose-response effects of tannic acid and protein on growth performance, caecal fermentation, colon morphology, and β-glucuronidase activity of rats. J. Anim. Feed Sci. 2011.

27. Usman M., Sule M., Gwarzo M. Toxicological studies of aqueous root extract of Euphorbia lateriflora (Schum and Thonn) in rats. J Med Plants Stud. 2014;2:58–62.
28. Higgins C. Urea and the clinical value of measuring blood urea concentration. 2016.
29. Akinloye OA, Balogun EA, Idowu ; O M O, Njoku ; H, Ugbaja RN. Biochemical, Toxicological, and Histological Changes associated with the use of Croton zambesicus as Anti-Malarial Decoction [Internet]. Pacific J. Sci. Technol. 2015. Available from: http://www.akamaiuniversity.us/PJST.htm
30. RodrÍguez JA, Hiruma-Lima CA, Souza Brito ARM. Antiulcer activity and subacute toxicity of trans-dehydrocrotonin from Croton cajucara. Hum Exp Toxicol. Sage Publications Sage CA: Thousand Oaks, CA; 2004;23:455–61.
31. Makoshi MS, Oladipo OO, Gotep JG, Forcados GE, Shu ML, Chinyere CN, et al. Safety evaluation of Acalypha wilkesiana in albino rats and BHK-21 cell line. Comp Clin Path. Springer; 2016;25:543–8.
32. Gounden V, Jialal I. Renal Function Tests. StatPearls. StatPearls Publishing; 2019.
33. Marty MS, Domoradzki JY, Hansen SC, Timchalk C, Bartels MJ, Mattsson JL. The effect of route, vehicle, and divided doses on the pharmacokinetics of chlorpyrifos and its metabolite trichloropyridinol in neonatal sprague-dawley rats. Toxicol Sci. 2007;100:360–73.