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TOXICITY PROFILE OF AQUEOUS LEAF EXTRACTS OF *CITROPSIS ARTICULATA* AND *MYSTROXYLON AETHIOPICUM* IN MALE ALBINO RATS

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ABSTRACT

Aim: Many serious adverse effects of new xenobiotics are detected thorough toxicological studies. In this present study, the safety profile of *C.articulata* L. (Rutaceae) and *M.aethiopicum* (Celastraceae) leaf aqueous extracts was evaluated by acute and sub-chronic toxicity tests in male wistar rats. **Methods:** Acute oral toxicity was investigated in 8 weeks old Swiss mice (18-24g) that were divided in 4 groups (n=4mice) given extract doses ranging from 7,500-12,000 mg/kg and 15,000-22,500 mg/kg for *M.aethiopicum* and *C.articulata* respectively. In sub-chronic toxicity, male rats 8 weeks old weeks were divided into 8 groups (n=6). The two aqueous extracts were administered a single daily dose of 150, 300 and 450 mg/kg body weight orally for 21 consecutive days and at the 22th day, the organ weights, hematological, histological, serum biochemical parameters were determined. **Results:** The LD_{50} of *C.articulata* and *M. aethiopicum* in Swiss mice were 18,985 mg/kg and 9,708 mg/kg body weight, respectively. General signs of toxicity due to large oral exposure to *C.articulata* were; hyper urination, diarrhea and hypo activity, while for *M. aethiopicum* were; Paralysis of hind limbs, defecation, respiratory distress, loss of balance and circling movement. Convulsions were observed in both extracts at death point. In

the sub chronic toxicity test, no mortality was observed during the course of the entire study in rats treated with both extracts. Both extracts caused a dose dependent statistically significant reduction ($p<0.001$) in monocytes of the treatment groups. The dose of 150mg/kg of *M.aethiopicum* caused a significant increase ($p<0.001$) in Neutrophils, Eosinophils and Basophils. There was a statistically significant increase ($p<0.05$) in Haemoglobin, lymphocytes, Red blood cells, Hematocrit and Mean Packed Cell Volume at 450mg/kg dose of *M.aethiopicum*. In serum biochemical parameters both aqueous extracts caused a statistically significant reduction ($p<0.05$) in serum Alanine transferase enzyme. The aqueous extract of *C.articulata* caused a significant ($p<0.01$) dose dependent reduction on serum alkaline phosphatase enzyme and on plasma urea. *M.aethiopicum* caused a significant increase ($p<0.01$) in plasma Creatinine levels. There were no significant effects on Total bilirubin and Aspartate transferase with both extracts. There was a dose dependent increase in liver weight for both extracts with the highest of (32.83%, $p<0.001$) at dose of 450mg/kg of *C.articulata*. Histopathological examination revealed no significant patho- physiological changes in the kidney and intestines of the treatment -groups. However the higher dose (450mg/kg) of *M.aethiopicum* showed pneumonitis and edema of lungs, focal areas of hepatic and perivascular degenerations with lymphocytes infiltration of the liver tissues. **Conclusion:** This study indicates that the LD50 values are above 5000mg/kg which is said to be experimentally safe for use under OECD guidelines. However prolonged use of higher doses of both extracts could result to Heamatological, biochemical and Histopathological changes in the living system , this implies the extracts potential to cause chronic toxicity.

Keywords: Acute toxicity; Heamatological, Biochemical; Histopathological; *Citropsis articulata*; *Mystroxylon aethiopicum*.

INTRODUCTION

Many serious toxic reactions caused by new potential drugs may be detected by routine toxicological testing in animal models before use in human trials. Experimental studies have shown that predictable, “dose time- dependent” reactions are likely to be revealed in animal experiments. It is the details of these experimental studies that form the basis of experimental toxicology that is applied to new drug discovery and development. Most of the idiosyncratic adverse effects especially the unpredictable ones when not related to time or dose are considerably more difficult to identify in preclinical drug evaluation (Frank, 2008). Most xenobiotics are now subject to stringent government requirements for safety testing before they can be marketed. This is especially true for pharmaceuticals, food additives, pesticides,

herbal remedies and industrial chemicals. Exposure of the public to inadequately tested drugs or environmental agents has resulted in several notable disasters in the world at large (WHO, 2004).

Acute toxicity tests are generally the first tests conducted for newly developed xenobiotics. They provide data on the relative toxicity likely to arise from a single or brief exposure. It is an initial assessment of toxic manifestations on health hazards likely to arise from short-term exposure to drugs and is one of the initial screening experiments performed with all compounds (Shetty *et al*., 2007). Sub-chronic toxicity studies are conducted to determine what side effects will arise from repeated administration of a drug at lower dosages than those used in acute toxicity studies. This also helps in determining safe dosages to be used in the initial human clinical trials. The clinical signs, body weight, and food consumption are important parameters for monitoring when conducting sub-chronic testing of a substance. Complete hematology, serum chemistry and histopathology profiles are determined at least at the end of the administration period and in some cases at intervals during the period of administration. The descriptions of toxic effects elicited in the sub chronic and chronic studies allow clinicians conducting clinical trials to know which side effects to anticipate in order to protect the patient volunteers from the detrimental effects of a potential drug (Paget *et al*., 1964).

The use of herbal medicines have received greater attention in the world as an alternative to orthodox medicine and the demand for these remedies has currently increased (Daswani *et al*., 2006). According to world health organization 80% of the population in developing countries relies on herbal medicine (WHO, 2008). In this regard, experimental screening methods are important in order to ascertain the safety and efficacy of traditional and herbal products (Mythilpriya *et al*., 2007).Despite the wide use of the leaves of *C.articulata* and *M.aethiopicum* by communities in Uganda on management of erectile dysfunction, very few investigations have been published in the literature about their toxicological profile. Therefore, the purpose of this study was to determine the safety profile of the aqueous leaf extracts *C.articulata* and *M.aethiopicum* in Swiss mice and Wistar male rats.

MATERIALS AND METHODS

Study Design

This was an experimental study design conducted at the Division of Pharmacology and Toxicology, College of Veterinary Medicine, Animal Resources and Biosecurity (COVAB).

Collection, identification and extraction of the plant material

Fresh leaf samples were collected from western Uganda and transported in black polythene to Makerere Herbarium, Department of Botany for identification; authentication and voucher specimen numbers NJ2 and NJ5 were deposited. The leaves samples of C. *articulata* and M. *aethiopicum* were later washed in clean running tap water and spread on clean benches for air drying for 1 week to a constant weight. The dry leaf samples were pulverized to powder using an electrical grinder into a coarse powder. Two hundred and fifty grams of the powder were weighed (Mettler PJ300, Germany) and each separate plant powder was boiled in 2 liters of distilled water for 20 minutes. The mixture was allowed to cool and filtered using Whatman® filter paper No. 2 (Whatman® International Ltd, England). Thereafter, the filtrate was freeze dried at pressure 32pa, with original temperature set at -47^{o}C and then maintained at 0^{o}C for 36hrs. The solid extracts were used to determine the percent yield and stored at 4^{o}C for later use. Fresh aliquot portions of the extract were weighed and dissolved in distilled water (at room temperature) for use on each day of the experiment.

Experimental animals

Forty eight disease free Wistar Male albino rats (n =48, weighing 150- 200 g, 8 weeks old) and Swiss mice (n=24(1:1, Male: Female, weighing 16-22 g, 8 weeks old) were purchased from College of Veterinary Medicine, Animal resources and Biosecurity, Animal house. The animals were housed in a normal environmental condition (temperature 24±1°C; 12:12 day/night; relative humidity of 60±5%), fed with standard rodent pellet (Engaano Millers Limited-Kampala) and water given *ad libitum*. Animal care and handling conformed to international guidelines (OECD, 2008).

Acute toxicity procedure

The acute toxicity study was conducted as per the OECD guidelines 420 (OECD, 2001) with limit test dose of 2,000 and 5,000 mg/kg used (OECD, 2002; Miller and Tainter; 1944; Gosh, 1984). The 8 weeks old mice were divided into two groups of 2 animals each (males and females). The control group received normal saline. A stock concentration of 200 mg/ml extract was prepared. Following an overnight fasting period, body weight of the mice was determined and dosed per individual body weight. Signs of toxicity and mortality were observed after 1, 2, 4 and 8 hour and twice daily for the next 7days post exposure. For the second phase doses above 5000mg/kg were used with a constant multiplication factor. And

the percentage mortality was transformed into probit and presented as Log dose against Probits curve.

Sub acute-Toxicity Study

Healthy adult Male wistar rats weighing 150-200 gm were divided into 8 groups of 6 animals each and were housed under standard conditions (temperature 24±1°C; 12:12 day/night; relative humidity of 60±5%).The control animals (Group-8) received 10 ml/kg of normal saline and the other (Group1-6) received 150, 300 and 450 mg/kg body weight of *C.articulata* and *M.aethiopicum* respectively with Group 7 receiving 100µg/kg of Testosterone intraperitoneally for 21 days.

Body and organ weight

The body weights of the rats were determined on day 1, 14 and 21 of the study. At the end of the treatment period, 3 animals each from the various treatment groups were sacrificed by euthanasia 18 hours post-treatment. The relative organ weight (ROW) of the fresh liver, kidney and heart were immediately determined.

Hematological and Biochemical tests

On day 21, the animals were fasted for 18hrs after the last treatment, they were anaesthetized with diethyl ether and blood samples collected through cardiac puncture with 1ml whole blood collected in heparinised and non- heparinised vacuitainer respectively for hematological and biochemical parameters. The hematological parameters determined using Nihon Kohden Celtac F coulter machine were white blood cells, hemoglobin, red blood cells, mean corposular volume, mean corposular hemoglobin concentration, platelets, lymphocytes, neutrophils and basophils. The blood in non-heparinised vacuitainer was used for liver functional test (Alanine aminotransferase, alkaline phosphatase, Aspartate transferase and Total bilirubin) and renal function test (Creatinine, Uric acid and Urea) using COBAS INTEGRA 400 automated machine.

Histological tissue preparation

Organs such as liver, kidney, lung and intestine were freshly removed, washed and transferred to an ice cold saline solution. Portions of these organs were fixed in 10% buffered formalin for 24hours after which were trimmed and loaded in cassettes ready for processing. Samples were processed using an automatic tissue processer (Histokinette, TP1020 Vi.ii) through increasing concentrations of ethanol (70%, 80%, 90%, 96%, and 100%) and cleared

in 2 changes of paraffin wax and embedded. Followed by tissue blocking, sectioned by microtome (Leica RM2235, Germany) of 3-5µm thickness and the slides were stained with Hematoxylin- eosin.

Statistical analysis

The percentage mortality in the acute toxicity was transformed in Probits and graph of log dose against Probits plotted. The hematological, biochemical, bodyweight and organ weight results were expressed as mean value ± Standard Error of the Mean (SEM). Differences Within study group were performed by one way analysis of variance using ANOVA test. The significant difference between the groups was considered at $P<0.05$ level of significance with dunnet multiple comparison tests.

RESULTS

Acute toxicity

The calculated LD_{50} of aqueous extracts of *C. articulata* and *M. aethiopicum* were found to be 18,985 and 9,708 mg/kg respectively (Figure 1A and B). Aqueous extracts of *M.aethiopicum* showed a lower LD_{50} value which according to EPA and OECD (2008) classification brackets of toxic substance is claimed to be experimentally safe. The outstanding behavioral change observed in both extracts was convulsions at time of death, with other changes differing per respective extracts (Table 1 and 2).

Table 1: Doses used and mortality of mice in *C. articulata* treated groups

Group	Dose Mg/kg	Log Dose	Number used	No Dead	% Dead	Probit	Signs of toxicity observed
1	15,000	4.18	4	0/4	0	3.04	Hyper urination, diarrhea, hypo activity and convulsions at dead point
2	17,500	4.24	4	1/4	25	4.44	
3	20,000	4.30	4	2/4	50	5.10	
4	22,500	4.35	4	4/4	100	6.55	

n=4

1A **1B**

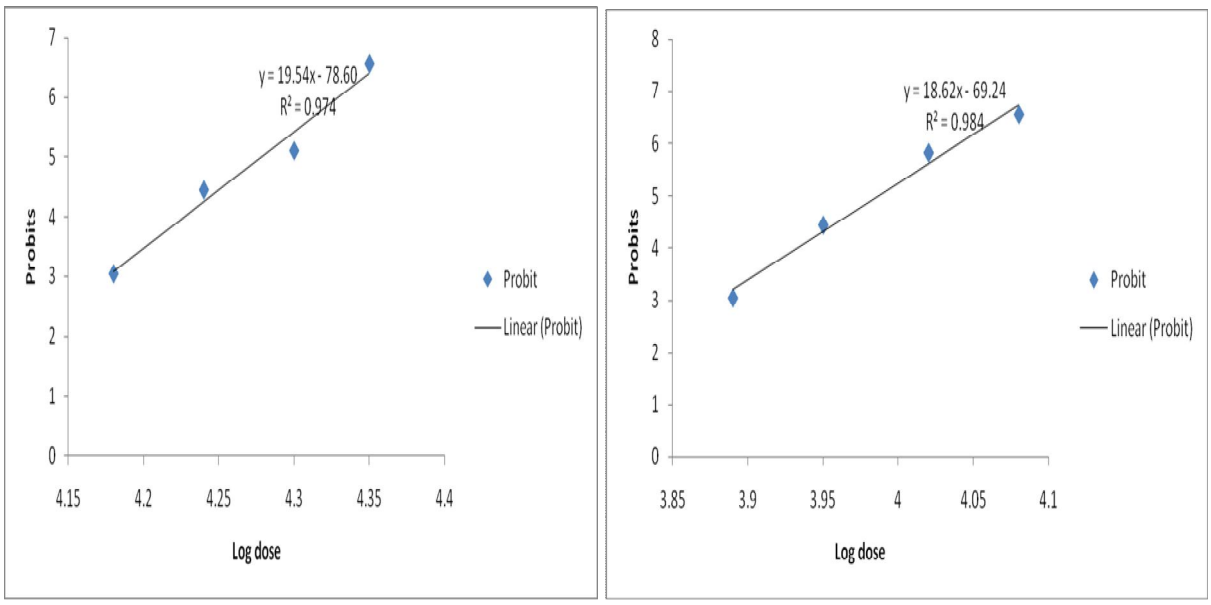


Figure1A and B: Log dose vs Probits curve for LD_{50} determination for *C. articulata* and *M. aethiopicum* in mice. Calculated LD_{50} value for *C. articulata* and *M.aethiopicum* were 18,985 mg/kg and 9,708 mg/kg respectively.

Table 2: Doses used and mortality of mice in *M. aethiopicum* treated groups

Group	Dose Mg/kg	Log Dose	Number used	No Dead	% Dead	Probit	Signs of toxicity observed
1	7,500	3.89	4	0/4	0	3.04	Paralysis of Behind limbs, defecation, constrained breathing, loss of balance. with circling movement and convulsions at death point
2	9,000	3.95	4	1/4	25	4.44	
3	10,500	4.02	4	3/4	75	5.82	
4	12,000	4.08	4	4/4	100	6.55	

n=4

Body weight changes

The aqueous dose of 450 mg/kg of *C. articulata* demonstrated a percentage increase in body weight as 53.49%,54.01%,65.55%, with that of *M. aethiopicum* also showing 41.91%, 50.05% and 58.70% with $p<0.001$ when compared with both negative and positive control on days 7, 14 and 21 respectively. The dose of 150mg/kg of *C. articulata* on day 7 had a 37.91% increase on bodyweight with $p<0.01$ when compared with both control groups, while that of 300 mg/kg had 34.75% and 23.84% on day 7 and 14 with $p<0.05$ when compared with positive control group only. The dose of 150 mg/kg of *M. aethiopicum* had a 47.13% ($P<0.05$ compared to both controls) and 53.39% ($p<0.05$, compared only to positive control) increase

on body weight on days 14 and 21 concurrently. On day 14 the dose of 300mg/kg of *M. aethiopicum* had an increase on bodyweight with 30.77% ($p<0.05$, when compared to positive control only) (Table 3).

Table 3: Effects of body weight on rats treated with aqueous extracts of *C.articulata* and *M.aethiopicum* for 21 days

Treatment Groups	Day 0	Day7	Day14	Day21
Normal control	89.30±6.87	97.67±6.69 (9.37%)	108.3±7.57 (21.27%)	123.7±7.01 (38.52%)
Positive control	90.79±4.13	105.0±3.69 (15.65%)	92.81±4.07 (2.22%)	113.3±2.84 (24.79%)
C. articulata 150mg/kg	95.85±2.61	131.5±5.12 (37.19%)**Aa	121.6±3.30 (26.86%)	122.2±3.19 (27.49%)
300mg/kg	92.54±7.50	124.7±7.37 (34.75%)*a	114.6±5.13 (23.84%)*a	112.4±5.04 (21.46%)
450mg/kg	94.41±3.34	144.9±6.17 (53.49%)***Aa	145.4±6.18 (54.01%)***Aa	156.3±6.86 (65.55%)***Aa
M.aethiopicum 150mg/kg	89.51±4.99	125.0±4.76 (39.65%)A	131.7±4.66 (47.13%)*Aa	137.3±4.39 (53.39%)*a
300mg/kg	91.23±9.48	115.4±5.75 (26.49%)	119.3±5.82 (30.77%)*a	123.6±5.19 (35.48%)
450mg/kg	104.5±7.17	148.3±8.14 (41.91%)***Aa	156.8±6.41 (50.05%)***Aa	158.7±6.07 (58.70%)***Aa

Values expressed as mean±SEM, n=3, $p<0.05$, *$p<0.05$, **$p<0.01$, ***$p<0.001$, A- Significant when compared with Negative control, a- significant when compared with Positive control groups and %- percentage.

Changes in organs weight

The effects of organs with aqueous extract treated group showed a dose- dependent increase in weight. There was a dose dependent percentage increase in liver weight in doses of 150 (16.87), 300 (17.46) and 450(20.48%) mg/kg of M.aethiopicum respectively. The dose of 450 mg/kg of *C. articulata* had a statistical significance increase in liver weight ($p<0.001$, 32.83%) when compared to both negative and positive control groups. There was no statistically significant increase in the weight of lungs and kidney in all extracts treatment groups when compared to both negative and positive control (Table 4).

Table 4: Effects on organ weights of male rats treated with aqueous extracts of *Citropsis articulata* and *Mystroxylon aethiopicum*.

Organs Weighed	Controls		*Citropsis articulata*			*Mystroxylon aethiopicum*		
	Negative	Positive	150mg/kg	300mg/kg	450mg/kg	150mg/kg	300mg/kg	450mg/kg
Liver	6.70±0.06	7.10±0.20 (5.97%)	6.76±0.32 (0.89%)	6.87±0.42 (2.54%)	8.90±0.20*** Aa (32.83)	7.83±0.09*Aa (16.87%)	7.87±0.12 **Aa (17.46%)	8.07±0.24***Aa (20.48%)
Kidney	1.03±0.09	1.07±0.09 (3.88%)	1.33±0.24 (29.13)	1.40±0.03 (35.92%)	1.47±0.07 (42.71%)	1.13±0.07 (9.71%)	1.03±0.09 (0%)	1.40±0.06 (35.92%)
Lungs	0.97±0.07	0.93±0.12 (4.12%)	1.30±0.06 (34.02)	1.13±0.17 (16.49)	1.13±0.09 (16.49%)	0.93±0.03 (-4.12%)	1.10±0.10 (13.40)	1.30±0.15 (34.02%)

Values expressed as mean±SEM, n=3, $p<0.05$, *$p<0.05$, **$p<0.01$, ***$p<0.001$, A-Significant when compared with Negative control, a-significant when compared with Positive control groups and %- percentage.

Hematological parameters

Generally there was no statistically significant (p>0.05) increase in Neutrophils; MCV; MCH; MCHC; RDW-CV and Platelets in all extracts treatment groups of *C. articulata* and *M. aethiopicum*. However, there was a statistically significant (P<0.001) increase in Monocytes in all aqueous extracts treatment groups of *C. articulata* and *M.aethiopicum* compared to both normal and positive control groups. The doses of 300 and 450mg/kg of *M.aethiopicum* showed a statistically significance (p<0.05) increase in lymphocytes and also significance increase of; HCT, RBC's and HGB in the dose of 450mg/kg respectively when compared with positive control group. There was statistically increase in levels of HCT at the dose of 450 mg/kg of *C. articulata*. However the doses of 300 and 450mg/kg of *M. aethiopicum* showed a significant reduction of WBC's when compared to the positive control only (Table 5).

Table 5: Hematological parameters values of rats treated with various doses of aqueous extracts of *C. articulata* and *M. aethiopicum* for 21 days.

parameters	Controls		*Citropsis articulata*			*Mystroxylon aethiopicum*		
	Negative	Positive	150mg/kg	300mg/kg	450mg/kg	150mg/kg	300mg/kg	450mg/kg
WBC (10^3UL)	11.53± 1.69	16.66± 1.77	16.77± 1.84	15.14± 0.78	14.06± 1.58	15.07± 1.23	10.27± 1.41*a	9.85± 1.30*a
NEUT (10^3UL)	8.40± 0.26	7.55± 0.25	8.03± 0.64	7.88± 0.35	7.37± 0.21	10.10± 0.56	8.12± 1.56	8.12± 0.96
LYMP (10^3UL)	75.82± 0.66	74.03± 1.33	79.32± 0.84	79.53± 0.83	78.21± 0.95	72.90± 2.45	81.22± 1.66**a	81.48± 1.83*Aa
MONO (10^3UL)	13.43± 0.79	8.58± 0.77**A	0.98± 0.14***Aa	0.91± 0.03***Aa	1.30± 0.20***Aa	7.45± 1.57***A	5.80± 0.77***A	5.17± 1.36***Aa
EO (10^3UL)	0.65± 0.13	0.40± 0.03	0.34± 0.01`	0.22± 0.01	0.24± 0.04	1.08± 0.34	1.07± 0.20	0.32± 0.08
BASO (10^3UL)	1.30± 0.17	1.28± 0.16	0.72± 0.14	0.61± 0.12	0.58± 0.09	3.92± 0.65***Aa	2.05± 0.32	1.28± 0.36
RBC (10^3UL)	7.86± 0.57	8.61± 0.41	8.21± 0.17	8.43± 0.34	8.76± 0.20	8.68± 0.18	8.28± 0.14	9.37± 0.47*A
HGB (g/dl)	13.93± 0.92	15.73± 0.66	14.85± 0.16	14.68± 0.56	15.82± 0.59	15.40± 0.26	14.87± 0.27	16.88± 1.08*A
HCT (%)	48.02± 2.62	53.35± 2.45	52.92± 1.08	50.98± 2.11	56.13± 1.96*A	54.83± 0.90	53.05± 1.03	58.23± 2.03**A
MCV (fL)	60.77± 1.24	61.28± 0.52	63.32± 1.24	60.28± 1.07	62.98± 1.22	62.29± 0.51	63.30± 1.20	61.53± 1.12
MCH (pg)	17.80± 0.17	17.83± 0.01	17.92± 0.18	17.52± 0.19	17.83± 0.35	17.38± 0.14	17.80± 0.17	18.22± 0.25
MCHC (g/dl)	29.20± 0.47	28.97± 0.23	28.15± 0.40	29.02± 0.30	28.20± 0.21	28.02± 0.06	27.85± 0.40	29.58± 0.77
RDW-CV (%)	22.82± 0.37	22.83± 0.52	22.22± 0.48	22.27± 0.49	22.53± 0.24	24.30± 0.36	22.52± 0.66	24.82± 0.81

PLT (10^3UL)	628.5± 58.74	618.0± 10.61	741.5± 47.47	662.7± 32.17	570.0± 60.01	736.0± 60.13	632.0± 26.46	625.0± 39.01
MPV ((fL)	8.87± 0.10	9.48± 0.27	9.67± 0.07	9.75± 0.35	9.55± 0.23	9.53± 0.32	8.82± 0.11	10.20± 0.34**A

Values expressed as mean±SEM, n=3, p<0.05, *p<0.05, **p<0.01, ***p<0.001, A-Significant when compared with Negative control, a- significant when compared with Positive control, Mean corpuscular volume (MCV), Neutrophil (Neut), Lymphocytes (LYMP), Monocytes (MONO), Eosinophils (EO), Basophils (Baso), Red blood cells(RBC), Hemoglobin(HGB), Hematocrit (HCT), Mean concentration hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC), Platelets (PLT), Mean packed cell volume (MPV).

There was a dose dependent statistically significant reduction (p<0.05) in the serum level of Alanine transferase (ALT) at all doses of *C. articulata* and *M. aethiopicum* respectively. The doses of 300 and 450mg/kg of C. articulata caused a statistically significant reduction (p<0.01) in the serum levels of Alkaline phosphatase (ALP2S) and urea levels. However the doses of 450mg/kg of *C. articulata* caused a statistically significance increase (p<0.01) in serum levels of Creatinine (table 6).

Table 6: Serum biochemical parameters of the rats treated with various doses of *C. articulata* and *M. aethiopicum* for 21 days

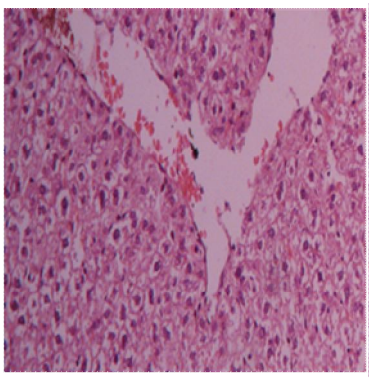
Parameters	**Controls**		***Citropsis articulata***			***Mystroxylon aethiopicum***		
	Negative	**Positive**	**150mg.kg**	**300mg/kg**	**450mg/kg**	**150mg/kg**	**300mg/kg**	**450mg/kg**
ALT(U/L)	533.2±74.27	440.3±243.6	107.1±3.617*Aa	173.3±48.03*Aa	163.7±16.00*Aa	181.2±17.05*Aa	123.9±12.83*Aa	112.0±13.41*Aa
AST (U/L)	500.8±62.37	580.3±323.8	173.9±11.74	315.4±64.66	199.3±23.11	246.7±39.96	204.0±7.823	159.7±7.11
ALP2S (U/L)	303.0±30.27	355.2±37.12***a	183.7±9.538**Aa	176.0±16.19**Aa	249.2±9.748	275.7±20.72	263.3±18.73	284.5±35.92
BILT2 (umol/l	6.040±0.12	5.67±2.211	2.97±0.96	3.03±0.89	2.02±0.19	1.97±0.23	2.53±0.68	1.32±0.08
CREJ2 (umol/l)	34.50±1.12	33.50±0.99	35.33±2.92	40.00±3.83	33.17±0.60	40.33±1.92	39.33±2.17	45.83±3.72**Aa
UREAL (mmol/l)	6.97±0.13	7.30±0.30	5.28±0.16**Aa	5.48±0.25**Aa	6.18±0.17	7.12±0.11	6.33±0.39	6.32±0.42

Values expressed as mean±SEM, n=6, p<0.05, *p<0.05, **p<0.01, ***p<0.001, A-Significant when compared with Negative control, a- significant when compared with Positive control.

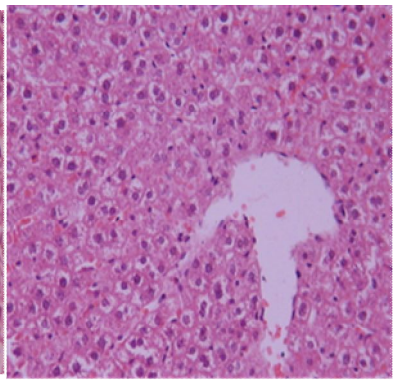
Histopathological findings

At the dose rate of 450mg/kg of *C. articulata* and *M. aethiopicum*, there was no significant lesions observed in the kidneys and intestines of all treatment groups (Figure 3 E, F, K and L). The dose of 450mg/kg of *C. articulata* and *M. aethiopicum* caused significant changes in the lungs of treated groups. Pneumonitis and edema of the lung was microscopically observed in the lung (Figure 3 H and I). The 450 mg/kg dose of *M. aethiopicum* showed focal areas of perivascular degeneration and lymphocytes infiltration of the liver (Figure 3 C).

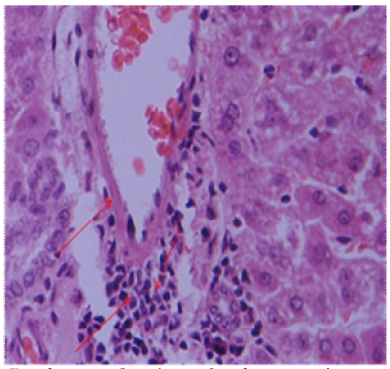
Normal liver A **_C. articulata_ (450mg/kg) B** **_M. aethiopicum_ (450mg/kg) C**



No observable changes (x200) , the scale bar is 100µm

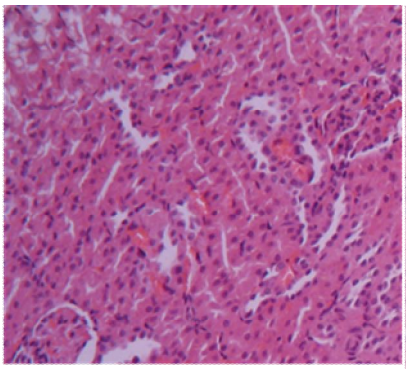


No significant changes (x200) , the scale bar is 100µm

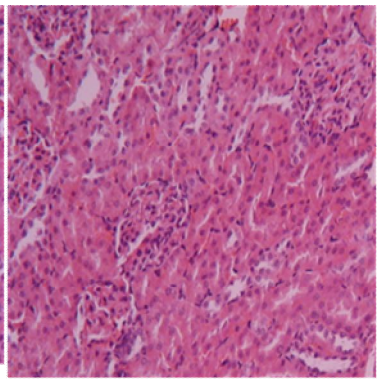


Focal areas of perivascular degeneration and Lymphocytes infiltration, (x200) , the scale bar is 100µm

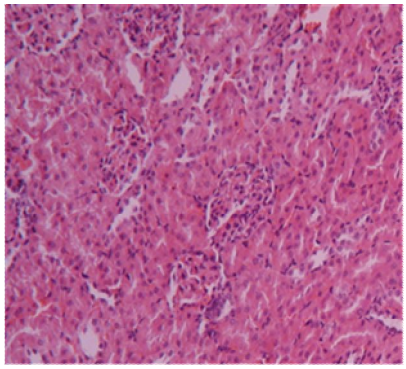
Normal kidney D **C.articulata (450mg/kg) E** **M.aethiopicum (450mg/kg) F**



No observable changes: x200, the scale bar is 100µm



No observable changes: x200, the scale bar is 100µm



No significant lesions observed: x200, the scale bar is 100µm

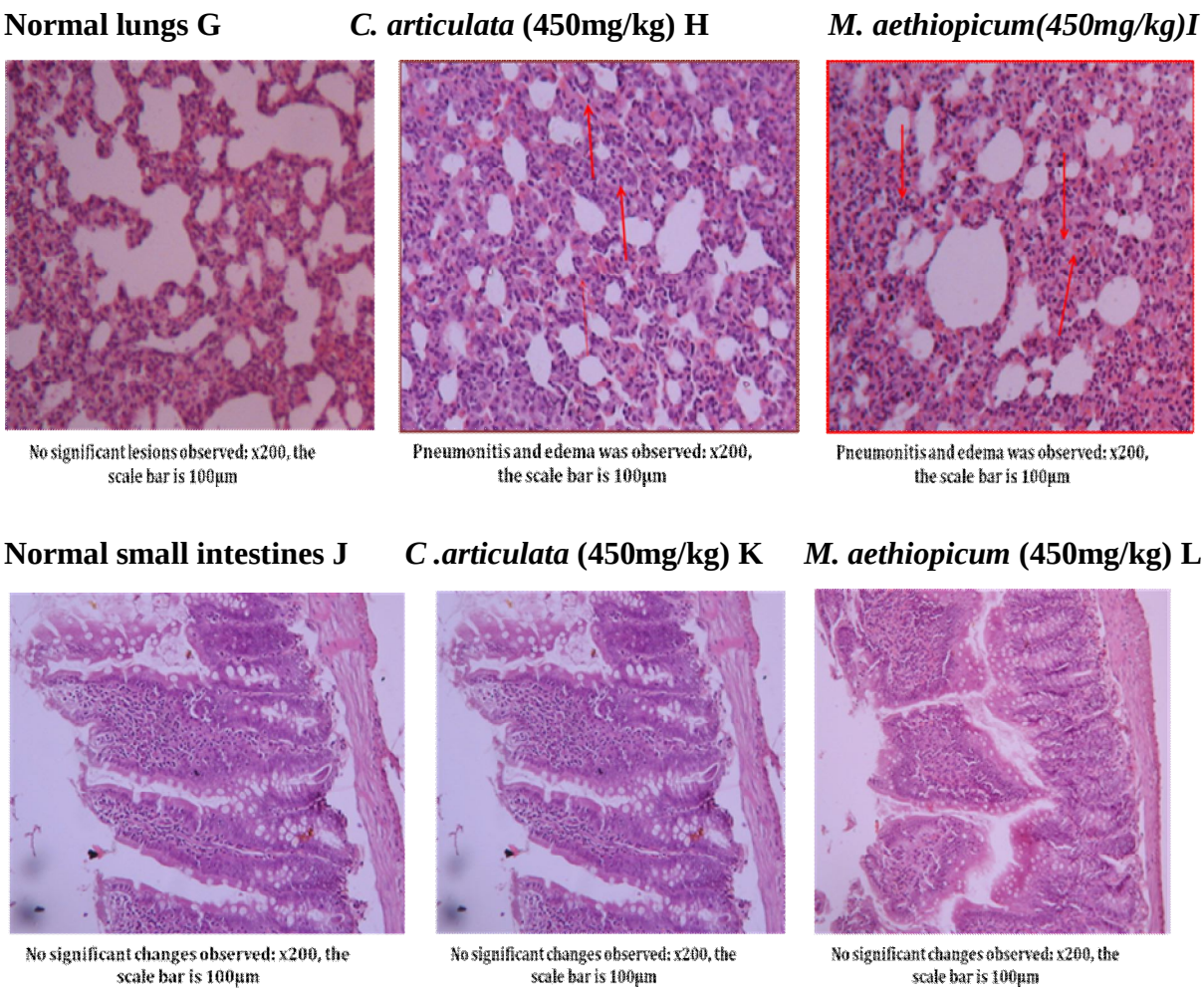


Figure 3: Histopathological findings of the liver (A-C), kidney (E-F), lung (G-I) and small intestinal (J-L) tissues of rats treated with aqueous extracts of *C. articulata* and *M. aethiopicum* for 21 days.

DISCUSSION

Most of medicinal agents have been derived from nature for thousands of years and an impressive number of modern drugs have been isolated from natural sources. The World Health Organization suggested that effective, cheap, safe and locally available plants, be used as substitutes for drugs development (WHO, 2004). Herbal medicine is still the mainstay of about 75-80% of the world population, mainly in the developing countries for primary health care (Godkar and Godkar, 2003). Herbal medicines have received greater attention as an alternative to clinical therapy and the demand for these remedies has currently increased. The increase in number of users as opposed to the scarcity of scientific evidence on the safety of the medicinal plants, have raisen regarding toxicity and detrimental effects of these remedies. This medicinal plants commonly contain various bioactive principles which have the

potential to cause beneficial and/or detrimental effects. Although there are many traditional herbal medicines available, only a few have been verified by clinical trials, their efficacy and safety are still questioned by consumers (Cheng *et al.*, 2009). Experimental screening methods are therefore important in order to ascertain the safety and efficacy of traditional, herbal products and also to establish the active component of the herbal products (Mythilpriya *et al.*, 2007).

In this study we evaluated the acute and sub-chronic toxicity profile of *C. articulata* and *M. aethiopicum* in male albino rats. Single oral dose of 5000mg/kg of *C. articulata* and *M. aethiopicum* did not cause any mortality while clinical signs of toxicity observed were hyper urination, diarrhea, hypo activity, paralysis of behind limbs, defecation, constrained breathing, loss of balance, circling movement and convulsions; this could be due to the effects of the extracts on the parasympathetic system of the autonomic nervous system. The LD_{50} of *C. articulata* and *M. aethiopicum* were found to be 18,985 and 9,708 mg/kg respectively. Therefore *M. aethiopicum* aqueous extract had a lower LD_{50} as compared to *C. articulata.* This finding is in agreement with Clarke and Clarke (1967), OECD (2008), who reported that any compound or drug with oral LD_{50} estimates greater than 1000 or 5000 mg/kg body weight could be considered to be of low toxicity and experimentally safe.

The percentage increase of aqueous extract of *C. articulata* and *M. aethiopicum* on body weight was remarkable on day 7, 14 and 21 with the dose of 450 mg/kg having a highly significant increase ($p<0.001$) in all treatment groups. This indicates that the plant extracts could not affect the food metabolism and utilization process in the body nor suppressing the satiety centre. Both extracts exhibited a dose-dependent increase in freshly weighed organ weight with *M. aethiopicum* showing a highly significant increase in liver weight in all treatment groups when compared with negative and positive control groups, this would be attributed to Phytoecdysteroids found in this plants (Dinan, 2001). One of the most interesting properties of ecdysteroids is their ability in animals to increase growth, protein content, and muscle mass, also known as anabolic activity (Hikino et al. 1972).

The assessment of hematological parameter is essential in determining the toxic effects of new xenobiotics system on erythropoiesis process of a living system. According to Orong *et al.*, 2010, hematopoietic system is one of the most sensitive targets for toxic chemicals and an important index of physiological and pathological status in humans and animals. Both extracts showed a statistically significant reduction ($p<0.05$) in levels of Monocytes with *M.*

aethiopicum causing a significant ($p<0.05$) dose dependent reduction in white blood cells, this could be associated with imbalance in the level of hematological parameter synthesis and catabolism. *M .aethiopicum* caused a dose –dependent significant ($p<0.05$) increase in the levels of lymphocytes, hematocrit, red blood cells and hemoglobin. This shows that *M. aethiopicum* might induce the process of erythropoiesis resulting in increased blood cells synthesis. *C. articulata* dose of 450mg/kg resulted into significant increase in the level of Hematocrit. Both aqueous extracts had no significant effects on Neutrophils, Basophils, mean corpuscular hemoglobin, RDW-CV and platelets.

The clinical monitoring of serum marker enzymes as biochemical parameters are associated with health indices and are of diagnostic significance in routine health evaluation. The assessment of liver and kidney function is very important in evaluating toxicity of modern and traditional medicines, since these organs play major roles in metabolism of xenobiotics in the body. Measurement and monitoring of Alanine amino transaminase (ALT) and Aspartate amino transaminase (AST) are largely used in the assessment of liver damage by drugs or any other hepatotoxin (Ramaiah, 2011). The liver and heart release ALT and AST and an elevation in their plasma concentrations are indicators of liver and heart damage (Wasan *et al.*, 2001; Mythilypriya *et al.*, 2007). However, ALT is more specific to the liver and is thus a better parameter for detecting liver injury (Ozer *et al.*, 2008). In this study serum biochemical parameters were studied to evaluate the possible alterations in hepatic and renal functions influenced by the aqueous extracts of *C. articulata* and *M. aethiopicum*. Serum biochemical parameters related to hepatic function namely; AST and Total Bilurubin contents exhibited no significant alterations as compared to the negative and positive controls. However, both extracts exhibited a significant ($p<0.05$) dose-dependent reduction in ALT. The aqueous extracts of *C. articulata* also cause a significant ($P<0.01$) reduction in the levels of ALP**.** The significant decrease observed in the level of ALT and AST is suggestive that both aqueous extracts may not possess hepatotoxic effect and equally could not have caused some toxic effects on the heart tissue (Crook, 2006). However the ratio of AST: ALT was above 1 in all the treatment groups. A mild or higher level of AST indicates liver injury or myocardial infarction (Cheesebrough., 1991; Feldman *et al.*, 2000) and the ratio of AST/ALT may be employed in disease diagnosis. An AST/ALT ratio greater than 1 suggests myocardial infarction, while a ratio less than 1 may be due to the release of ALT from the affected liver (Sacher and McPherson, 1991) this was outlined in the histopathological sections. It is also well known that almost all xenobiotics are eliminated through renal excretion hence it was

found necessary to estimate the effects of the aqueous extracts on kidney functions tests (Bhattacharya, 2012). Kidney functions related to Serum biochemical parameters were urea and Creatinine. The aqueous extract of *C. articulata* demonstrated a statistically significant ($p<0.01$) dose- dependent increase in serum urea level with no significant effect on Creatinine, while *M. aethiopicum* demonstrated significant increase ($p<0.01$) in Creatinine at dose of 450mg/kg with respect to positive and normal control group animals. Lack of effect of each extract individually on Creatinine and urea is suggestive of no kidney damage specifically by renal filtration mechanism (Crook, 2006) or probably indicates that both extracts did not interfere with the renal capacity to excrete these metabolites.

The histological sections of Kidneys and intestines of all extracts treated groups showed no observable significant lesions. However, both extracts at the higher doses caused pneumonitis and edema of the lungs, which could have been attributed to asphyxiation during dosing. The higher dose of *M.aethiopicum* caused significant focal area of perivascular degeneration on the liver this could be associated with the above 1 ratio of AST: ALT which suggests underlying liver damage.

CONCLUSION

In conclusion, the present findings have shown that aqueous extracts of *C. articulata* and *M. aethiopicum* when used in lower doses are most likely not to produce severe toxicological risk which is demonstrated by their high LD_{50} values. However, further investigations of the observations made in this study at the cellular level in a chronic study and their underlying mechanism of changes is highly recommended.

CONSENT

Consent was sought from School of biomedical Sciences research and ethical committee, College of Health Sciences and Uganda Council of Science and technology.

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CONFLICT OF INTEREST: The author's hereby declare no conflict of interest in this work.

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