

Full Length Research Paper

Acute oral toxicity study of *Mystroxylon aethiopicum* root bark aqueous extract in albino mice

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Acute oral toxicity of *Mystroxylon aethiopicum* root bark aqueous was evaluated in albino mice of either sex. In this study, five groups of mice were orally treated with doses of 1000, 2000, 3000, 4000 and 5000 mg/kg body weight of crude extract. The mortality, signs of toxicity and body weights were observed individually for two weeks. At the end of the two weeks study, all animals were sacrificed and the hematological and biochemical parameters as well as organ weights relative to body weight of each animal were determined. No mortality, signs of toxicity and abnormalities in vital organs were observed in the entire period of study for both treated and control groups of mice. Additionally, there were no significant changes ($p > 0.05$) in the blood hematology and biochemical analysis. However, the body weights of all mice increased significantly. The *M. aethiopicum* root bark aqueous extract were found to have a high safe margin when administered orally. Hence, the extract can be utilized for pharmaceutical formulations.

Key words: *Mystroxylon aethiopicum*, acute oral toxicity, albino mice.

INTRODUCTION

The use of medicinal plants has received great attention in the world as an alternative to conventional drugs and the demand for these remedies has recently increased (Phani and Kumar, 2014). Plant based medicines have been used by traditional healthcare in most parts of the world for thousands of years (Newman et al., 2000). According to the World Health Organization, 80% of the populations in developing countries rely on traditional medicines for their healthcare (WHO, 2007). Some of these traditional medicines involve the use of crude plant extracts in the form of infusion, decoction or tincture which may contain an extensive diversity of molecules,

often with indefinite biological effects (Olowa and Nuñez, 2013).

The *Mystroxylon aethiopicum* (Celastraceae) is a small to medium sized evergreen tree that grow up to 12 m high (Pope, 1995). The plant grows in a wide range of habitats including the forest margins, evergreen forests, open woodland, riverine fringes and also on termite mounds and rocky ridges (Burrows and Willis, 2005). In Africa, the plant is widely distributed in Ethiopia, Sudan, South Africa, Namibia, Angola, Cameroon, Madagascar, Seychelles and Comoro (Curtis and Mannheimer, 2005). In Tanzania, the species is found in highlands of Arusha

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and Kilimanjaro regions, where it locally known as “*Oldonyanangu*” in Maasai language (Kokwaro, 1993). Traditionally, the plant is consumed by many ethnic groups in Africa for the management of hemorrhagic diarrhea, stomach, respiratory tract infections, coughs and anemia (Boera et al., 2005; Iwu, 2014). In Kenya, root bark extract of this plant is reportedly to be used in making a kind of tea which is drunk as a stomach medicine, particularly by children (Kokwaro, 1993). Despite the wide use of *M. aethiopicum* in the management of different diseases, there is lack of scientific studies regarding the prevalence of the toxicological profile of this plant. This study hypothesize that extracts of *M. aethiopicum* is safe for usage in traditional medicine since it has been used by many communities in Africa as an alternative to conventional drugs. The aim of this paper therefore was to determine the safety profile of the aqueous root bark extracts of *M. aethiopicum* in albino mice.

MATERIALS AND METHODS

Plant materials and preparation of extracts

The plant materials were collected from Imbibya village in Arusha rural district, Tanzania. Plant species were identified by Mr. Gabriel Laizer, a botanist from the Tropical Pesticide Research Institute (TPRI) and voucher specimen coded MA-001 was kept at Nelson Mandela African Institution of Science and Technology (NM-AIST). Root barks were harvested without affecting the plant, air dried under the shade and pulverized into fine particles using electric blender. Pulverized materials (250 g) were added to a 1 L of distilled water maintained at 30°C for 4 h and allowed to cool to room temperature. The extracts were sieved and centrifuged at 5000 rpm for 10 min. The supernatant was collected and filtered using Whatman No. 1 filter paper and dried by freezing to eliminate water by sublimation. The extracts were stored in a deep freezer at -20°C for further activities.

Experimental animals

Albino mice of both sexes, weighing between 19 and 20 g and aged 3 to 4 weeks were randomly obtained from the Plant Protection Division of the Tropical Pesticides Research Institute (TPRI) Arusha, Tanzania. The animals were allowed to stay in cages with sawdust litters in a controlled temperature environment of about 23°C. Lighting was controlled to supply 12 h of light and 12 h of darkness for each 24 h period.

Ethical consideration

Prior to the experimental work, an ethical clearance with notification number NIMR/HQ/R.8a/Vol. IX/2145 was given by the National Health Research Ethics Sub-Committee (NatHREC) of the National Institute for Medical Research (NIMR) in Tanzania.

Experimental design

Acute oral toxicity test was done according to OECD guideline number 425 of 2001 (OECD, 2001). The mice were acclimatized for

7 days before experimentation. Before dosing with extract, the mice were starved for 4 h with access to adequate drinking water only. The mice were divided into a control group and five experimental groups with six mice each (3 males and 3 females). Body weights of the mice were determined and the dose was calculated in accordance with their body weights. The control group received 1% Tween 80 in normal saline only by the oral route (5 ml/kg body weight), whereas the animals in the treatment group were administered oral doses of 1000, 2000, 3000, 4000 and 5000 mg/kg body weight, respectively, of crude extract that was dissolved in 1% Tween 80 in normal saline. Food was withheld for 1 h after administration of the extract but not water. The mice were observed regularly for mortality and any sign of toxicity such as change in skin and fur, eyes, respiratory effects, mucus membrane, diarrhea and sleep.

By the 14th day, all mice were weighed and blood samples were collected by cardiac puncture into two vacutainer tubes for each animal. The first vacutainer tube contained anti-coagulant substance (EDTA) and the second vacutainer tube was plain. Hematological parameters including red blood cell (RBC), mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), white blood cell (WBC), lymphocyte and hemoglobin concentration (Hb) were determined using the blood samples contained in the EDTA tubes. The blood samples contained in plain vacutainer tubes were centrifuged at 4000 rpm for 10 min and the serum obtained were subjected to biochemical parameters which included cholesterol, protein, bilirubin and alkaline phosphate (ALP). After blood collection, all mice were sacrificed and dissected for macroscopic organ analysis. The internal organs such as liver, heart, lungs, spleen and kidneys were carefully removed and weighed.

Statistical analysis

The student's t-test was employed to compare mean body weight between day zero and day fourteen for both control and treated groups of mice while one way analysis of variance (ANOVA) was used in multiple comparisons of the means for organ weight, hematological and biochemical data between the control and treated groups of animals. All statistical analysis was performed using STATISTICA software version 8 (StatSoft, 2007) with the level of significance set at $p < 0.05$

RESULTS

Findings from this study indicate that there were no signs of toxicity in mice of both control and treated groups up to a dose level of 5000 mg/kg body weight. All animals were normal throughout the study period and they survived until the end of the 14th day of experimentation.

Body weight changes

The body weights of the control and treated mice with aqueous extract of root barks of the plant are shown in Table 1. Results from this study revealed that there were a gradual increase in body weights of both control and treated groups of mice.

Hematological parameters

The results of hematological parameters of control and

Table 1. Body weight (g) values of control and mice treated with *M. aethiopicum* root bark aqueous extract measured during the acute toxicity study.

Dose (mg/kg BW)	Sex	Mean at day 0	Mean at day 14	Mean difference	p-value
Control	M	20.355 ± 0.078	24.677 ± 0.400	4.322	0.000448
	F	19.316 ± 0.065	23.416 ± 0.219	4.100	0.000057
1000	M	20.321 ± 0.047	24.217 ± 0.062	3.896	0.000001
	F	19.248 ± 0.044	23.310 ± 0.047	4.062	0.000000
2000	M	20.220 ± 0.060	24.309 ± 0.089	4.089	0.000003
	F	19.311 ± 0.145	23.288 ± 0.079	3.977	0.000018
3000	M	20.292 ± 0.115	23.738 ± 0.357	3.446	0.000782
	F	19.149 ± 0.050	23.260 ± 0.119	4.111	0.000006
4000	M	20.393 ± 0.072	24.147 ± 0.260	3.754	0.000154
	F	19.437 ± 0.100	23.502 ± 0.248	4.065	0.000108
5000	M	20.223 ± 0.052	23.643 ± 0.106	3.42	0.000008
	F	19.141 ± 0.064	23.202 ± 0.137	4.061	0.000011

Values are expressed as mean ± SEM, M = male, F = female, BW = body weight.

treated mice are shown in Table 2. These results show that there were no significant changes ($p > 0.05$) in hematological parameters, both in the control and treated groups of mice after 14 days of treatment with *M. aethiopicum* root bark aqueous extract. All values of hematological parameters such as red blood cell, mean cell volume, mean cell hemoglobin, mean cell hemoglobin concentration, white blood cell, lymphocyte and hemoglobin concentration remained within normal limits throughout the experimental period.

Biochemical parameters

In this study, no significant changes ($p > 0.05$) were observed at all doses in alkaline phosphate (ALP) and cholesterol levels between the control and treated group of mice as shown in Table 3. Furthermore, the values of protein and bilirubin did not differ significantly ($p > 0.05$) in the treated mice as compared to the control group.

Macroscopic examination

The macroscopic examination of the internal organs of animals revealed no difference between the control and treated mice groups after administration even with higher dose of 5000 mg/kg body weight.

Organ weight

The organ weights relative to body weights of the animals were determined and results are summarized in Table 4. Findings from this study indicated that there were no significant differences ($p > 0.05$) in weight changes of each organ between the control and treated mice at all doses.

DISCUSSION

Medicinal plants have been used worldwide for thousands of years in the form of crude drugs such as tinctures, teas, poultices, powders and other herbal formulations (Gurib, 2006). *M. aethiopicum* is among the medicinal plant which is known for many traditional applications in humans (Schmidt et al., 2002). The root bark extract has been commonly used by many populations in Africa for medicinal purposes (Burkil, 2004). Despite the usage of this plant as traditional medicine, there are few studies on the safety evaluations. Therefore, experimental screening methods using animal models are essential to ascertain the safety of this plant.

In this study, the *M. aethiopicum* root bark aqueous extract did not affect the body weight of the treatment mice as compared to the control mice. The gradual body weight gain shown by animals against the extract, provide circumstance evidence that the administration of the crude extract has negligibly level of toxicity on the growth of the animals. According to Raza et al. (2002) and Teo et al. (2002), the reduction in gain body weight is a sensitive indicator of toxicity after exposing the animals to the toxic substances and it is usually significant if such losses are more than 10% of the initial weight. Findings from this study are in agreement with previous study conducted by Ndukui et al. (2014) who reported weight gain in albino rats treated with aqueous leaf extract of *M. aethiopicum* in Uganda.

Blood parameters analysis is relevant to risk evaluation as the hematological system has a higher predictive value for toxicity in humans when assay involves animals (Olson et al., 2000). Blood is an important index of physiological and pathological status in both animals and humans and the parameters usually measured are red

Table 2. Hematological values of control and mice treated with *M. aethiopicum* root bark aqueous extract measured during the acute toxicity study.

Parameter	Sex	Dose (mg/kg BW)						p-value
		Control	1000	2000	3000	4000	5000	
RBC (m/mm ³)	M	8.099 ± 0.265	8.058 ± 0.145	7.948 ± 0.071	7.978 ± 0.234	8.311 ± 0.114	8.308 ± 0.091	0.525153
	F	7.323 ± 0.064	7.283 ± 0.015	7.250 ± 0.021	7.287 ± 0.038	7.373 ± 0.056	7.377 ± 0.187	0.864938
MCV (fl)	M	47.167 ± 0.186	46.867 ± 0.203	47.057 ± 0.030	46.860 ± 0.146	46.910 ± 0.124	47.070 ± 0.032	0.542368
	F	49.130 ± 0.214	49.083 ± 0.020	49.313 ± 0.041	48.993 ± 0.219	49.017 ± 0.284	48.700 ± 0.062	0.308154
MCH (pg)	M	18.800 ± 0.321	19.043 ± 0.159	18.233 ± 0.120	18.333 ± 0.088	18.733 ± 0.285	19.033 ± 0.176	0.073604
	F	19.000 ± 0.190	18.790 ± 0.114	19.327 ± 0.059	19.217 ± 0.164	18.893 ± 0.131	19.233 ± 0.103	0.085373
MCHC (g/dl)	M	34.600 ± 0.404	34.190 ± 0.107	34.700 ± 0.379	34.467 ± 0.186	34.303 ± 0.170	35.087 ± 0.297	0.325877
	F	33.157 ± 0.230	32.930 ± 0.268	33.293 ± 0.091	32.850 ± 0.189	33.370 ± 0.151	33.360 ± 0.064	0.249899
WBC (m/mm ³)	M	9.433 ± 0.376	8.890 ± 0.137	9.273 ± 0.057	9.303 ± 0.126	9.933 ± 0.234	9.323 ± 0.026	0.060657
	F	8.177 ± 0.127	7.943 ± 0.217	7.953 ± 0.159	8.327 ± 0.039	8.070 ± 0.192	8.253 ± 0.052	0.383882
LYM (%)	M	74.533 ± 0.338	74.300 ± 0.058	74.200 ± 0.115	75.133 ± 0.120	74.520 ± 0.321	74.907 ± 0.254	0.094817
	F	73.693 ± 0.059	73.670 ± 0.176	73.640 ± 0.052	73.653 ± 0.035	73.583 ± 0.043	73.373 ± 0.028	0.144279
Hb (g/dl)	M	14.967 ± 0.233	14.965 ± 0.120	15.167 ± 0.088	15.100 ± 0.208	14.967 ± 0.233	15.010 ± 0.116	0.942121
	F	14.390 ± 0.021	14.383 ± 0.032	14.363 ± 0.028	14.417 ± 0.034	14.413 ± 0.054	14.393 ± 0.048	0.919693

Values are expressed as mean ± SEM, M = Male, F = Female, BW = Body weight, RBC = Red blood cell, MCV = Mean cell volume, MCH = Mean cell hemoglobin, MCHC = Mean cell hemoglobin concentration, WBC = White blood cell, LYM = Lymphocyte, Hb = Hemoglobin concentration

Table 3. Clinical biochemical values of control and mice treated with *M. aethiopicum* root bark aqueous extract measured during the acute toxicity study.

Parameter	Sex	Dose (mg/kg BW)						p-value
		Control	1000	2000	3000	4000	5000	
Cholesterol (mg/dl)	M	86.975 ± 0.187	86.993 ± 0.136	87.079 ± 0.269	86.996 ± 0.092	87.019 ± 0.164	87.105 ± 0.192	0.993597
	F	88.068 ± 0.145	88.246 ± 0.309	87.742 ± 0.387	88.048 ± 0.100	88.150 ± 0.108	87.896 ± 0.995	0.974879
Total protein (g/dl)	M	5.073 ± 0.136	5.184 ± 0.052	4.883 ± 0.150	5.228 ± 0.042	4.840 ± 0.192	5.197 ± 0.278	0.428318
	F	6.191 ± 0.238	5.993 ± 0.239	6.180 ± 0.138	5.841 ± 0.157	6.412 ± 0.111	6.546 ± 0.174	0.147521
Direct bilirubin (mg/dl)	M	0.602 ± 0.036	0.588 ± 0.026	0.629 ± 0.008	0.614 ± 0.010	0.592 ± 0.019	0.622 ± 0.008	0.670220
	F	0.705 ± 0.017	0.678 ± 0.038	0.728 ± 0.015	0.682 ± 0.024	0.695 ± 0.050	0.736 ± 0.016	0.660283
ALP (U/L)	M	71.449 ± 0.410	71.777 ± 0.190	72.048 ± 0.201	71.613 ± 0.163	71.680 ± 0.154	70.855 ± 0.189	0.058343
	F	71.865 ± 0.321	71.593 ± 0.332	72.190 ± 0.053	72.155 ± 0.085	71.976 ± 0.226	72.255 ± 0.093	0.322070

Values are expressed as mean ± SEM, M = Male, F = Female, BW = Body weight, ALP = Alkaline phosphate

study has shown that acute oral ingestion of *M. aethiopicum* root bark aqueous extract did not

lymphocyte and hemoglobin concentration (Vaghasiya et al., 2011). The normal range of

these parameters can be altered by the intake of some toxic plants (Ajagbonna et al., 1999). This

Table 4. Organ-body weight values of control and mice treated with *M. aethiopicum* root bark aqueous extract measured during the acute toxicity study.

Organ	Sex	Organ-body weight index (%)						p-value
		Dose (mg/kg BW)						
		Control	1000	2000	3000	4000	5000	
Liver	M	4.658 ± 0.181	4.630 ± 0.087	4.502 ± 0.116	4.547 ± 0.219	4.534 ± 0.185	4.601 ± 0.057	0.973370
	F	4.441 ± 0.008	4.432 ± 0.005	4.413 ± 0.001	4.419 ± 0.007	4.348 ± 0.043	4.405 ± 0.015	0.055842
Heart	M	0.533 ± 0.110	0.446 ± 0.050	0.444 ± 0.062	0.411 ± 0.067	0.319 ± 0.149	0.468 ± 0.083	0.720114
	F	0.348 ± 0.002	0.341 ± 0.006	0.345 ± 0.004	0.334 ± 0.006	0.342 ± 0.005	0.343 ± 0.005	0.427091
Lungs	M	0.696 ± 0.033	0.661 ± 0.048	0.611 ± 0.011	0.598 ± 0.026	0.611 ± 0.018	0.678 ± 0.074	0.446295
	F	0.535 ± 0.004	0.535 ± 0.002	0.534 ± 0.003	0.533 ± 0.006	0.528 ± 0.003	0.535 ± 0.001	0.699071
Spleen	M	0.638 ± 0.013	0.626 ± 0.015	0.612 ± 0.026	0.630 ± 0.007	0.574 ± 0.028	0.608 ± 0.019	0.289455
	F	0.438 ± 0.008	0.434 ± 0.004	0.434 ± 0.001	0.431 ± 0.003	0.432 ± 0.004	0.421 ± 0.005	0.293800
Kidney (R)	M	0.634 ± 0.012	0.606 ± 0.006	0.625 ± 0.012	0.614 ± 0.008	0.618 ± 0.009	0.599 ± 0.007	0.166221
	F	0.543 ± 0.002	0.534 ± 0.003	0.538 ± 0.004	0.542 ± 0.003	0.537 ± 0.002	0.538 ± 0.003	0.352114
Kidney (L)	M	0.620 ± 0.013	0.559 ± 0.023	0.590 ± 0.022	0.583 ± 0.026	0.602 ± 0.026	0.570 ± 0.023	0.484740
	F	0.543 ± 0.001	0.541 ± 0.001	0.542 ± 0.001	0.542 ± 0.001	0.541 ± 0.001	0.542 ± 0.000	0.665622

Values are expressed as mean ± SEM, M = male, F = female, BW = body weight, R = right, L = left.

study has shown that acute oral ingestion of *M. aethiopicum* root bark aqueous extract did not cause any change in hematological parameters for both control and treated mice verifying the nontoxic nature of the extract. Findings from this study validate the safety nature of the extract through oral administration. This explains why there is no reported case of intoxication due to the use of this plant among the Maasai communities in Tanzania (Kokwaro, 1993).

Disease or response to toxic substances is indicated by alterations in the biochemical parameters which are the sensitive indicators of organ function or metabolic defects (Reddy et al., 2013). Liver plays a major role in the metabolism and detoxification of compounds that reach the liver and hence it serves as a prime target organ for drugs and toxic substances (Reddy et al., 2013). A liver function test such as alkaline phosphate (ALP) is therefore useful in determining the extent of damage (Shah et al., 2011). In the

same perspective, liver is the major site for cholesterol synthesis or disposal in mammals (Gautam and Goel, 2014). In this study, there were no significant changes in ALP and cholesterol levels for both control and treated mice, suggesting that *M. aethiopicum* root bark aqueous extract had no effects on the liver function and cholesterol metabolism of the mice and therefore strengthen the safety utilization of the plant in traditional medicine. Safety nature of the root bark extract of the plant is also indicated by other biochemical parameters such as protein and bilirubin which did not differ significantly as compared to control group of mice. These results support the reported use of *M. aethiopicum* by traditional healers in Uganda for treating various diseases with no severe adverse effect (Ndukupi et al., 2014).

Macroscopic examination of internal organs of the experimental animals in this study did not reveal any abnormalities, presence of lesions or

changes in the color for both control and treated group and therefore suggest that *M. aethiopicum* root bark aqueous extract is potentially safe for human consumption. In toxicological studies, internal organs such as liver, hearts, lungs, spleen and kidneys are primarily affected by metabolic reactions caused by the toxicants (Dybing et al., 2002).

Organ weight is an important index to diagnose whether the organ was exposed to the injury or not (Jothy et al., 2011). In this study, the weights of internal organs were not statistically significantly in both control and treated group of mice, indicating that the extract is virtually nontoxic. The non-toxicity shown by *M. aethiopicum* root bark aqueous extract towards albino mice, ratify the safety profile of the aqueous root bark extract of the plant. Results from this study collaborate with the previous cytotoxicity investigation study of the same plant growing in Uganda, which did not show significant changes

in organ weight of albino rats (Ndukui et al., 2014).

Conclusion

This study presents evidence of nontoxic effects of *M. aethiopicum* root bark aqueous extract in animal models. No mortality or any sign of toxicity was observed in mice treated with the extract and therefore establishing its safety in use. The hematological and biochemical analysis showed no adverse effects between control and treated groups of mice. Furthermore, the plant extract did not induce any damage to the vital body organs and therefore considered as relatively safe for utilization especially in rural communities where conventional drugs are unaffordable due to their high costs. However, a detailed experimental analysis of sub-acute toxicity remains unveiled to complete the safety profile of this plant.

Conflict of Interests

The authors have not declared any conflict of interests.

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