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Acute toxicity studies of *Myrsine africana* aqueous seed extract in male Wistar rats on some hematological and biochemical parameters

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Abstract

Background: Natural medicinal products have gained recognition worldwide in the treatment and control of diseases. One of the major concerns as they are used is the lack of adequate pharmacological and toxicological data to support their use. *Myrsine africana* is traditionally used as human and veterinary anthelmintic.

Methods: Two groups of male Wistar rats were orally given as a single dose 1000 and 5000 mg/kg body weight of the *M. africana* extract respectively. Hematological and biochemical assays of each animal blood were done after 48 h and at day 14.

Results: No animal died in the entire study period. The median lethal dose (LD_{50}) of the seed extract was estimated to be above 5000 mg/kg body weight in Wistar rats. Red blood cells, packed cell volume and creatinine in rats fed with 5000 mg/kg body weight were found to be significantly elevated from the control at 48 h. At day 14, thrombocytes and aspartate aminotransferase were significantly elevated in the high dose group while urea level had decreased significantly in the treatment groups.

Conclusions: *M. africana* extract was found to have a high safe margin validating its wide use. However, caution should be exercised when using this extract as was indicated by the altered parameters. It is therefore recommended that lower doses than 1000 mg/kg body weight should be used for treatment.

Keywords: Biochemical parameters; Extract; *Myrsine africana*; Rats; Toxicity

Background

Since antiquity, man has used plants to treat common diseases even long before mankind discovered the existence of microbes. An 80 % of the population in the non-developed countries depends on traditional medicine for their primary health care [1]. According to International Development Research Centre (IDRC), 85 % of Africans use herbal remedies in their routine health care in Sub-Sahara Africa [2]. Natural products remedies are esteemed safer and less damaging to the human body than the synthetic drugs [3]. The safety of herbal medicine has continually been questioned due to reported illness and fatality of the test animals under study [4]. Toxicological studies are important in hazard identification stage of safety assessment of drugs. Regulatory safety assessment for natural products relies on both the assessment of cases of adverse reactions and the review of published toxicity information effects [5]. Earliest report of the toxicity of herbs originated from Galen, a Greek pharmacist and physician. He showed that herbs do not contain only therapeutic constituents, but that they may also contain harmful substances [6]. Acute toxicity is a term used to describe the adverse effects that are caused by a single exposure of a toxic substance or brief multiple exposures over a very short span of time –usually less than 24 h. The described acute toxicity should occur within 14 days of administration of the substance [7].

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Acute toxicity studies are usually done to establish the median lethal dose (LD_{50}) of a substance [8].

Myrsine africana also called Cape Myrtle or African boxwood is a *Myrinaceae* and is an evergreen shrub growing to 2 m at a slow rate. The plant native to Africa and Asia and usually grows well in dry parts. *Myrsine africana* typically has dense, dark-green to red foliage and produces tiny bright purple berries which are edible. The seeds and roots of *M. africana* are widely used for livestock and human as an anthelmintic, especially in the treatment of tapeworms [9, 10]. This plant is also used for the treatment of diarrhea, rheumatism, toothache pulmonary tuberculosis and relieving hemorrhage [11]. *M. africana* is traditionally used as a fragrance in tea, carminative, spice, appetizer and flavoring agent [12]. There are no published conventional safety studies on *Myrsine africana* despite widespread use as an anthelmintic [12–14]. This study was therefore projected to evaluate this plant acute toxicity effect on the hematological and biochemical parameters in order to validate its use.

Methods

M. africana seeds were collected from the Samburu area of Kenya. Identification and authentication was done at the herbarium, School of Biological Sciences, University of Nairobi.

Extraction of seeds materials

The *M. africana* seeds were cleaned in tap water and rinsed in distilled water. After air-drying at room temperature (22-26 °C) to a constant weight, the seeds were ground to a uniform powder using an electric mill. The powder (100 g) was soaked in 1 L distilled water for 48 h. The mixtures was filtered through cotton wool and then with filter paper (125 mm) after which the filtrate was frozen at -20 °C for 24 h followed by freeze drying. The powdered extract was packed and sealed in air tight polythene bags, stored in the refrigerator at 4 °C and used within five days.

Laboratory animals

A total of 15 male Wistar albino rats (195 – 225 g) were obtained from the animal house of the department of Biochemistry, University of Nairobi. The animals were housed in the research room of this department and the temperatures were maintained at 27– 30 °C. The room was well ventilated and maintained on light for 12 hours and 12 h darkness. The rats were provided with the standard rat pellets and clean water *ad libitum*. The animal studies were in compliance with the ethical procedure for the care and use of laboratory animals approved by the "Animal care and use committee (ACUC)" of the Faculty of Veterinary Medicine University of Nairobi.

Experimental design for acute toxicity

The animals were randomly assigned into three groups of five rats each. They were kept overnight fasting prior to extract administration. Group 1 served as the control and the rats were administered with 2 ml distilled water once. Two concentrations of the aqueous extract, 1000 and 5000 mg/kg body weight were constituted each in 2 ml distilled water respectively. These extracts were orally given as a single dose and only once to groups 2 and 3 respectively using a gavage. Food was withheld for further 3 h post extract administration.

Animal bleeding, hematological and biochemical assays

Blood was collected at from each rat at 48 h and the 14^{th} day post extract administration via the tail lateral vein using a 2 ml hypodermic syringe and needle when the animal was restrained. A blood aliquot (1.3 ml) was put into the EDTA tubes and thoroughly mixed for hematological analysis. The remaining 0.5 ml of the blood was put in the plain tube for biochemical analysis. Hemoglobin concentration (HB), packed cell volume (PCV), red blood cells (RBC), white blood cells (WBC), mean corpuscular hemoglobin (MCH), mean cell volume (MCV), mean corpuscular hemoglobin concentration (MCHC) and thrombocytes were analysed within six hours using "*Melet Schoesing* MS4" hematological analyzer. The blood in the plain tubes was immediately centrifuged at 3000 revolutions per minute for 10 min to extract serum which was stored at −20 °C and used within 12 h for biochemical assays. Aspartate aminotransferase (AST), alanine aminotransferase (ALAT), total proteins, creatinine, urea were assayed for each rat using commercial kits according to the manufacturer's protocol using "UVmini-1240 *UV–vis*" spectrophotometer manufactured by "Shimadzu".

Physical observation

Clinical observations were made after every 30 min post extract administration for the first four hours and latter once a day up to the 14^{th} day. Mortality, moribund, ill health or reaction to treatment, such as changes in skin and fur, eyes and mucus membranes, behavior pattern, tremors, salivation, diarrhea, sleep and coma were observed. Weight recording was done before extract administration, at 48 h, day 7 and day 14.

Statistical analysis

The analysis was done using SPSS 17.0 and the results were expressed as mean ± standard deviation of the mean (SD). One-way analysis of variance (ANOVA) was employed for between and within group comparison. 95 % level of significance ($p \leq 0.05$) was used for the statistical analysis.

Results and discussion

In this study, the rats in the control and treated groups were monitored daily until day fourteen and they showed no toxic signs. This study suggested that *M. africana* aqueous extract has an $LD_{50} > 5000$ mg/kg body weight in albino Wistar rats since none of the experimental animals died within the 14 days study period. According to [15] substances with LD_{50} values less than 5000 mg/kg body weight are classified as substances with low toxicity. The weight change of the treated animals was not significantly different from the control, Table 1, validating the low toxicity of this extract. It was suggested that this extract did not cause any inflammation in the animals. This was reflected by the non-significant increase in the white blood cells, Tables 2 and 3. White blood cells are usully increased when the immune system is triggered thus offering protection to the body from infection by foreign organisms [16]. Red blood cells (RBC) and packed cells volume (PCV) at 48 h in 5000 mg treatment group were significantly elevated and both normalized at day 14, Tables 2 and 3, suggesting that there may have beeen a correlation between these responses and dosage [17]. An increased RBC and unaltered WBC suggestes a strong immuno-modulatory activity of *Myrsine africana* extracts [18]. Hemoglobin concentration (HB), Mean cell volume MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) at 1000 and 5000 mg/kg body weight did not have significant differences with the control suggesting that *Myrsine africana* at these doses did not induce anaemia in the rats. This is a mark of high safety margin of the extract through oral route justifying its widespread use by traditional healers in Kenya [19]. Thrombocytes (platelets) in the treated groups increased, Tables 2 and 3 both at 48 h and at day 14 strongly suggest that there could be some bioactive compounds in *Myrsine africana* seeds that can enhance thrombopoiesis [20].

Acute toxicity is usually defined as the adverse change(s) occurring immediately or a short time following a single or short period of exposure to a substance or substances [21]. The assessment of hematological parameters could be used to reveal the deleterious effect of foreign compounds including herbal extracts on the blood constituents of animals. They are also used to determine possible alterations in the levels of biomolecules such as enzymes, metabolic products, and the normal functioning of the organs [22].

The liver plays a major role in the metabolism and detoxification of compounds that reach the liver and hence the prime target organ for drugs and toxic substances [23]. The significantly elevated AST at the 14th days in both treatment groups, and not at 48 h is an indication of cellular damage [24]. ALAT is more specific to the liver and thus a better parameter for detecting liver injury [25]. In this study there was no significant difference of ALAT of the treated groups with the control group

Table 1 Weight profile for the male Wistar rats in *M.africana* acute toxicity testing

Time	Control	1000 mg	5000 mg
Initial weight	217.4 ± 9.4	222.4 ± 5.8	224.9 ± 5.6
At 48 h	219.2 ± 8.9	219.1 ± 8.0	222.7 ± 7.0
7 days	226.9 ± 8.7	226.9 ± 7.6	232.9 ± 6.6
14 days	239.2 ± 8.6	243.2 ± 10.4	246.6 ± 10.5
Total gain	22.0 ± 1.5	20.8 ± 5.4	21.7 ± 7.5
% weight gain	8.5	7.9	8.2

Table 2 Effect of *M.africana* on hematological and biochemical measurements in male Wistar rats at 48 h

Parameter	Control	1000 mg/	5000 mg
WBC (10^3/μL)	10.1 ± 2.7	10.9 ± 5.3	8.1 ± 1.5
RBC (10^6xμL)	5.6 ± .22	6.1 ± 1.3	***7.6 ± 9.8**
PCV (%)	35.1 ± 1.5	34.3 ± 8.1	***44.1 ± 5.3**
Hb (g/dL)	14.0 ± .81	13.9 ± 2.5	14.5 ± .32
MCV(fL)	63.9 ± .60	57 ± 2.7	59.7 ± 1.2
MCH(pg)	24.0 ± 1.2	24.3 ± 1.2	22.3 ± 3.0
MCHC (g/dL)	38.5 ± .91	43.5 ± 3.2	32.4 ± 5.3
THROMB (10^3/μL)	336.2 ± 59.8	441 ± 99	419 ± 8.9
AST (U/L)	45.6 ± 15.1	41. 6 ± 7.3	38.1 ± 12.0
ALAT(U/L)	46.4 ± 2.4	42.1 ± 4.9	44.6 ± 4.2
Total proteins (g/dL)	7.7 ± 5.2	7.5 ± .86	8.2 ± .9
Creatinine (mg/dL)	1.7 ± .36	4.2 ± 4.5	***15.6 ± 2.1**
Urea (mg/dL)	42.9 ± 3.7	46.8 ± 3.8	46.8 ± 8.0

*Significantly different

Table 3 Effect of *M.africana* on hematological and biochemical measurements in male Wistar rats at day14

Parameter	Control	1000 mg/kg/bwt	5000 mg/kg bwt
WBC (10^3/μL)	9.1 ± .34	12.27 ± 1.4	18 ± 3.1
RBC (106xμL)	5.7 ± .57	6.37 ± .31	6.3 ±. 62
PCV (%)	35.2 ± 3.1	38.9 ± 1.7	37.3 ± 2.6
Hb(g/dL)	12.8 ± 1.5	15.2 ± .94	15.2 ±. 71
MCV(fL)	63 ± 1.4	59.9 ± .78	60.1 ± 4.0
MCH(pg.)	23.8±. 82	23.2 ± .61	22.4 ± 4.7
MCHC (g/dL)	38 ± 1.1	39.4 ± 1.2	39 ± 1.4
Thromb. (10^3/μL)	186 ± 20	288 ± 44.7	***564 ± 71**
AST (U/L)	34.46 ± 7.5	***57.2 ± 5.9**	***55.4 ± 4.1**
ALAT(U/L)	44.7 ± 6.4	45 ± 4.6	48.1 ± 5.9
Total proteins(g/dL)	8.7 ± 2.2	6.5 ± .76	8.7 ± 1.8
Creatinine (mg/dL	1.8 ± 1.3	1.9 ± .78	1.9 ± .22
Urea (mg/dL)	42.8 ± 6.2	*21.8 ± 5.0	***27.8 ± 4.7**

*Significantly different

suggesting that *M.africana* seed extract may not obviously cause liver toxicity. Creatinine level was significantly elevated at a dose of 5000 mg/kg body weight at 48 h but not at day14, an indication that the extract was effectively eliminated by the kidneys [26]. A biologically active components capable of reducing urea content in the animals could have been present in the extract as it was indicated by the low urea values in day 14. Inefficient absorption of proteins, tubular necrosis and liver diseases are among the factors that can cause reduced serum urea [27].

Conclusions

Caution should be exercised when using this natural product extract as was indicated by the altered biochemical parameters; AST, urea and creatinine. It was recommended that lower doses than the studied ones should be used for treatment. Further studies to evaluate sub-acute and chronic effect of this extract are recommended.

Competing interests

The authors declare no conflict of interest. There is no financial or non-financial competition.

Authors' contributions

JM provided the plant material and helped in designing and structuring the project. PM contributed in the animal handling and the extract administration while the major work was done by ZK. All authors read and approved the final manuscript.

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