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Haematopoietic Properties of Ethanolic Fruit Extract of *Musa Acuminata* on Albino Rats

***Okon, J. E.; Esenowo, G. J. Afaha, I. P. and Umoh, N. S.**
Department of Botany and Ecological Studies, University of Uyo, P. M. B. 1017, Uyo. Akwa Ibom state-Nigeria
*Corresponding Author: joesplendid@yahoo.com

ABSTRACT
The haematopoietic properties of ethanolic fruit extract of Musa acuminata was investigated on Albino rats. A phytochemical content of the extract was examined. Median lethal dose (LD_{50}) of the ethanolic fruit extract was evaluated through intraperitoneal (i.p.) route. The 10%, 20% and 30% of the LD_{50} were used as the working doses. Twenty five (25) Albino rats were randomly divided into five groups of four rats each. Group I, II and III were receive 500 mg/kg, 1000 mg/kg and 1500 mg/kg of Musa acuminata ethanolic fruit extract respectively. Group III served as negative control and receive normal saline and feed, group V served as positive control and receive 10 ml/kg of folic acid (Standard drug). The oral administration lasted for 21–days. The results of the phytochemical screening reveal the presence of alkaloids, saponnins, tannins, terpenes, flavonoids, anthraquinones and cardiac glycosides. The LD_{50} studies showed that the mice treated intraperitoneally tolerated a considerably high dose of 5000 mg/kg without any manifestations. The extract at the doses administered was found to increase in dose-dependent fashion on PCV and Hb ($P<0.05$ for 500 mg/kg and $P<0.05$ for 1000 mg/kg and 1500 mg/kg), RBC ($P<0.05$ for 1000 mg/kg and 1500 mg/kg) and marginal increases that were non significant ($P<0.05$ for 500 mg/kg); MCH and MCV ($P<0.05$ and $P<0.01$ for 1000 mg/kg and 1500 mg/kg respectively), 500 mg/kg was not significant. MCHC had no significant change. WBC recorded marginal increases that were not significant ($P<0.05$). Similarly, the differential white blood cell recorded marginal increases that were not significant. The results of this study thus supports the haematopoietic potentials of the extract as blood booster.
Keywords: Haematopoietic, *Musa acuminata*, ethanolic, phytochemical screening.

INTRODUCTION

Musa acuminata commonly known as blood bananas or bluggoe plantain banana. It is a perennial herbs belonging to the family Musaceae. The trunk (known as the pseudostem) is made up of tightly packed layers of leaf sheaths emerging from completely or partially buried corms [13].

The inflorescence of *Musa acuminata* grows horizontally or obliquely from the trunk. The individual flowers are white to yellowish-white in colour and are negatively geotropic (that is, growing upwards and away from the ground). Randy [13] reported that both male and female flowers are present in a single inflorescence. Female flowers located near the base (and develop into fruit), and the male flowers located at the tip-most top-shaped bud in between leathery bracts [7].

The rather slender fruits are berries, the size of each depends on the number of seeds they contain. Each fruit can have 15 to 62 seeds. Each fruit bunch can have an average of 161.76 ± 60.62 fingers with each finger around 2.4 cm by 9 cm in size. The seeds of *Musa acuminata* are around 5 to 6mm in diameter. They are subglobose or angular in shape and very hard. The tiny embryo is located at the end of the micropyle. Each seed of *Musa acuminata* typically produce around four times its size in edible starchy pulp (the parenchyma, the portion of the bananas we eat), around 0.23 cm^3 [13].

Anaemia is a medical condition in which the red blood cell count or haemoglobin is less than normal. The normal level of haemoglobin is generally different in males and females. For men, anaemia is typically defined as haemoglobin level of less than 13.5 gram/100ml and in women as haemoglobin of less than 12.0 gram/100ml [8]. The red blood cells carry oxygen around the body, using a particular protein called haemoglobin. The bone marrow needs enough dietary iron and some vitamins to manufacture haemoglobin. If the body do not have enough iron in the diet, the body will draw on the small reserves of iron stored in your liver. Once this reservoir is depleted, the red blood cells will not be able to carry oxygen around the body effectively [8].

Traditional medicine practitioners in Akwa Ibom State are using *Musa acuminata* fruits to treat anaemia, but this information is not properly documented. This research is carried out to ascertain the efficacy of the plants use in traditional medicine practice for anaemia treatments. The objective of this

study is determine the effects of ethanolic extract of *Musa acuminata* on haemopoietic parameters on the experimental rats

MATERIALS AND METHODS

Plant Collection and Authentication

The fresh fruits of *Musa acuminata* used in this research were obtained from Obio Offot in Uyo Local Government Area in Akwa Ibom State, Nigeria in June 9th, 2012. The plant samples were identified and authenticated by Dr. (Mrs.) M. E. Bassey, a plant taxonomist in the Department of Botany and Ecological Studies, University of Uyo, Uyo, Nigeria. The voucher specimen were prepared and deposited in the herbarium (*Musa acuminata*: Afaha, UUH 3019 Uyo) of the Department.

Preparation of Plant Extracts

Musa acuminata fruits sample were shed-dried and pulverised by graded using a manual blender. Five hundred grammes (500g) of the coarse powdered sample of *Musa acuminata* fruits was macerated with 1000 ml of 70% ethanol and allowed to stand for 72 hours hours. The solution was filtered using glass funnel packed with cotton wool. The filtrate was evaporated to dryness by heating in a water bath at 40^0C which give a yield of 73.7 g of semi-dry extract with black colour. This was reconstituted in distilled water to an appropriate concentration for administration and phytochemical screening.

Collection and Maintenance of Animals

Healthy male and female sex albino rats weighing 150 – 250 g were obtained from the animal house of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Uyo, Uyo and maintained according to the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals.

Phytochemical Screening

The experiment was carried out in the Department of Pharmacognosy and Natural Medicine, University of Uyo, Uyo. The phytochemical screening involves the simple chemical test to detect the presence of secondary metabolites. The methods of Trease and Evans [14] was used for Phytochemical screening.

Determination of Median Lethal Dose (LD_{50})

Swiss albino mice weighing 25-32 g were dose by the intraperitoneal (i.p.) route using the method of Lorke [9]. The animals were administered with 5000 mg/kg, 4000 mg/kg, 3000 mg/kg, 2000 mg/kg, 1000 mg/kg and 500 mg/kg of *Musa accuminata* extract in 5 groups of 5 mice each. The animals were observed for manifestation of physical signs of toxicity and the number of death within 24 hours was recorded. The LD_{50} was calculated as the geometric mean of the maximum dose producing 0% mortality and the minimum dose producing 100% mortality. Food was withdrawn for 18 hrs before the onset of the experiment according to methods of Amresh *et al.* [3].

$$LD_{50} = \sqrt{D_0 \, X \, D_{100}}$$

Where: D_0 = Maximum dose producing 0% mortality

D_{100} = Minimum dose producing 100% mortality

Experimental Design

Twenty five (25) Albino rats weighing 150 – 250 g were randomly assigned into 5 sets: 5 animals per group for haematological and haemopoietic activities. 10%, 20% and 30% of LD_{50} were used as working doses (low, middle and high dose respectively).

Groups:

Group I receive 10 ml/kg of normal saline.

Group II receive 10 mg/kg of haematinic (folic acid).

Group III receive 500 mg/kg of *Musa acuminata* extract.

Group IV receive 1000 mg/kg of *Musa acuminata* extract.

Group V receive 1500 mg/kg of *Musa acuminata* extract.

The daily administration (orally) was for fourteen days (21-days) and during these period animals were allowed for free access to feed and water *ad libitum*.

Blood Samples Collection

After twenty one days of oral administration of the soluble fraction of the two extracts, forty eight hours after the last dose were administered to each of the groups, the animals were anesthetised with chloroform, dissected to exposed the cardiac cavity of the heart, blood was obtained using a sterile syringe (5ml) by cardiac puncture and carefully discharged into ethylene diamine tetraacetic acid (EDTA) bottle by running it down the side of the bottle, covered and rolled gently to mix with EDTA so as to avoid clotting. The sample bottles were labelled accordingly for all the 5 groups.

Haematological Analysis

The methods of Alada [2], Lewis *et al.* [8] and Choudhari and Deshmukh [4] was adopted to determine the Packed cell volume (PCV), haemoglobin concentration (Hb conc.), red blood cell count (RBC count), white blood cell count (WBC count), platelet count (PLT count), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular volume (MCV), absolute count of lymphocytes (Lym.#), absolute count of neutrophils (Neut.#), platelet distribution width (PDW), mean platelet volume (MPV), reticulocyte count (Retics. count) and mean corpuscular haemoglobin (MCH). All the haematological parameters was analysed using Mindary 5 Path Differential BC 5300 Autoanalyser at University of Uyo Teaching Hospital (UUTH), Uyo.

Statistical Analysis

Data were expressed as mean ± S.E.M (standard error of the mean) of three replicates and were subjected to statistical analysis using the student's t-test by comparing the control with the treated groups. Probability limit was set at ninety five percent (95%) level of significance ($P<0.05$) as described by Ubom [15].

RESULTS

The results of phytochemical screening investigated from the fruit extract of *Musa acuminata* revealed the presence of alkaloids, saponins, tannins, terpenes, flavonoids, anthraquinones and cardiac glycosides (Table I). The mice treated through intraperitoneal (i.p) route with a single dose of 500-5000 mg/kg of fruit extract (water soluble fraction) showed decrease in writing, respiration distress, decreased limb and no death was recorded with 24 hours post administration of the extract, since the extract is non-toxic, the LD_{50} was taken to be 5000 mg/kg.

The results of the effects of oral administration of *Musa acuminata* fruit extracts for 21-days on haematological indices. Haemoglobin (Hb), Packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelet (PLT), white blood cell (WBC), platelet distribution width (PDW) and mean platelet volume (MPV) are shown in Tables 1 and 2. Administration of the different percentages of *Musa acuminata* fruits by rats caused significant increases in the PCV, Hb conc. RBC count, PLT count, and MCV (Table 1 and 2). White blood cell count and its differentials were non-significant in the extracts. There was a progressive increase in the haematological indices as the concentrations of *Musa acuminata* extracts increased i.e. 10%, 20% and 30%. The high dose in group III shows the most significant responses in the extracts.

Table 1: Results of phytochemical screening of *Musa acuminata* fruit extract

Test	Inference
Alkaloids	
Dragendorff's reagent	++
Mayer's reagent	++
Picric acid	++
Saponins	
Frothing test	++
Anthraquinones	
Borntrager's test	+++
Cardiac glycosides	
Salkowski test	+++
Terpenes	+++
Flavonoids	+++
Shinoda test	

Key:

+++ - Abundance

++ - Moderate +

\- Trace

Table 2: Haematological Indices of Rat Treated with Ethanolic Extract of *Musa acuminata* fruit.

Treatment/ Groups	RBC	HB	HCT/PCV	MCV	MCH	MCHC	MPV
I: Low dose (500 mg/kg)	6.11+0.21	11.0+0.51	34.2+1.08*	55.97+0.91*	18.00+0.25	22.23+17.45*	6.50+0.00
II: Middle dose (1000 mg/kg)	6.54+0.18*	12.07+0.60	37.63+2.27*	58.0+1.37*	18.63+0.67	33.77+4.02*	6.33+0.15
III: High dose (1500 mg/kg)	6.82+0.06*	12.63+0.21*	39.43+1.33*	57.8+2.15*	18.53+0.46	32.07+0.67*	6.57+0.06
IV: + control (10 ml/dl)	5.8+0.8	14.7+0.6	46.6+1.4	84.6+0.4	28.5+0.2	33.6+0.12	7.4+0.3
V:-ve Control (10 ml/dl)	6.44+0.16	11.77+0.15	35.4+0.43	56.4+1.81	17.83+0.46	33.47+1.11	6.33+0.15

Values represent mean ± standard deviation of 3 replicates
*Significant at P< 0.05.

Table 3: Platelet and White Blood Cell and its Differential Count of Wistar Albino rats treated with Ethanolic Extract of *Musa acuminata* fruit.

Treatment/Groups	PLT	PDW	WBC	LYM#	NEUT#
I: Low dose (500 mg/kg)	729+62.69*	7.50+0.00*	8.64+6.09	85.37+5.37	11.97+4.53
II: Middle dose (1000 mg/kg)	869+149.08*	7.07+0.21*	17.6+4.11	89.10+6.20	8.73+5.20
III: High dose (1500 mg/kg)	889.10+6.20*	7.57+0.38*	17.2+3.13	89.00+4.83	9.13+4.29
IV: + Control (10 ml/dl)	736+52.6	14.6+2.1	12.6+4.7	86.6+1.8	8.8+1.7
V: Control (10 ml/dl)	89.00+4.83	7.27+0.06	12.8+2.0	86.9+1.00	11.00+0.82

Values represent mean ± standard deviation of 3 replicates.
*Significant at P< 0.05.

DISCUSSION

The results of this work showed that *Musa acuminata* fruits are abundant in cardiac glycosides. Similar results were obtained by Farine et al. [6] and Trease and Evans [14] who worked on *Morinde citrotolia* and *Digitalis pupurea* respectively. They reported the presence of cardiac glycosides in these plants. They showed that cardiac glycosides can be used in the treatment of diseases associated with the heart and they are currently used by herbalist to treat tumour in Akwa Ibom state [12]. The cardiac glycosides found in *Musa acuminata* fruit could be used for treatment of heart disease problems.

In *Musa acuminata* fruit saponins are present in moderate amount. This result is in line with the study of Okoko [10] who reported that the presence of saponins in *Mucuna pruriens*. The work indicated that saponins have the properties of precipitating proteins, cholesterol-binding and haemolysis. Flavonoids were present in *Musa acuminata* fruit in abundant, Trease and Evans [14] stated that some of the biological functions of flavonoids include protection against allergies, free radicals, platelet aggregation microorganisms, ulcers, hepatotoxins and tumors [11]. Saponins was moderately present, saponins, on the other hand, was reported to have the properties of precipitation of proteins, cholesterol-binding and haemolysis.

Other phytocomponents such as alkaloids and glycosides found in these plants also do not have properties relating to increased haematopoiesis. Thus, saponins present in *Musa acuminata* fruit can help the body fight against infections and microbial invasions.

The results of haematological tests showed a significant (P<0.05) increase in packed cell volume (PCV), haemoglogin concentration (Hb.), red blood cell count (RBC), and platelet (PLT). Similar results were obtained by Esenowo *et al.* [5] who suggested that the leaves of *Peristrophe bicalculata* (RETZ) Nees are capable of increasing the packed cell volume, haemoglogin concentration, and red blood cells. Their studies confirmed the use of *Peristrophe bicalculata* leaves to restore lost blood during excessive bleeding. *Musa acuminata* can be used to restore lost blood during excessive bleeding.

The mean values of packed cell volume (PCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular volume (MCV), mean platelet volume (MPV), and platelet distribution width (PDW) were significantly ($P<0.05$) increased while the total white blood cell count and its differentials were reduced when compared with the control groups in *Musa acuminata* extracts. Similar observations were made by Ahumibe and Braide [1] who suggested that increase in red blood cells stimulates cytokine erythropoietin. It is likely that increase in red blood cells in *Musa acuminata* extracts may stimulate cytokine erythropoietin. This study confirmed the use of *Musa acuminata* in diets.

CONCLUSIONS AND RECOMMENDATION

Musa acuminata fruit extracts contains bioactive components and are good potentials for clinical applications as they significantly increased blood parameters with good hamatopoietic properties. Therefore, it has haemopoietic, stimulating, enhancing and protective properties. Its use as antianaemia is therefore supported. Based on the findings from this research work, further investigations are also needed to exploit its relevant therapeutic effect to substantiate its ethnomedicinal usage of *Musa acuminata*.

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