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Joy Gararawa Usman

a) National Veterinary Research Institute
Vom, Plateau State, Nigeriab) Department of Veterinary Physiology,
Pharmacology and Biochemistry,
Faculty of Veterinary Medicine,
University of Maiduguri, Maiduguri,
Borno State, Nigeria

Olufunke Adebola Sodipo

Department of Clinical Pharmacology
and Therapeutics, College of Medical
Sciences, University of Maiduguri,
Maiduguri, Borno State, Nigeria

Ayi Vandi Kwaghe

Livestock Department and Pest Control
Services, Federal Ministry of
Agriculture and Rural Development,
Area 11, Garki, Abuja, Nigeria

Bitrus Wampana

Department of Veterinary Physiology,
Pharmacology and Biochemistry,
Faculty of Veterinary Medicine,
University of Maiduguri, Maiduguri,
Borno State, Nigeria

Nendir John Haruna Umaru

National Veterinary Research Institute
Vom, Plateau State, Nigeria

Umar Kyari Sandabe

Department of Veterinary Physiology,
Pharmacology and Biochemistry,
Faculty of Veterinary Medicine,
University of Maiduguri, Maiduguri,
Borno State, Nigeria

Correspondence:

Joy Gararawa Usman

a) National Veterinary Research Institute
Vom, Plateau State, Nigeriab) Department of Veterinary Physiology,
Pharmacology and Biochemistry,
Faculty of Veterinary Medicine,
University of Maiduguri, Maiduguri,
Borno State, Nigeria

Email: najocheri[at]yahoo.com

Effects of crude methanol extract of the fruit of *Cucumis metuliferus* (Cucurbitaceae) on some haematological parameters in Cockerels

Joy Gararawa Usman*, Olufunke Adebola Sodipo, Ayi Vandi Kwaghe, Bitrus Wampana, Nendir John Haruna Umaru, Umar Kyari Sandabe

ABSTRACT

The ripe fruits of *C. metuliferus* were collected in Vom village, Jos South Local Government Area, Plateau State, Nigeria. The fruits were washed and sliced using clean knives, and then air dried in the laboratory and pulverized. The dried powder was serially extracted using solvents of different polarities (n-hexane, chloroform, methanol and distilled water). The biochemical research involving animals was carried out according to the principles of Council for International Organizations for Medical Science (CIOMS) and the International Council for Laboratory Animal Science (ICLAS), 2012 and was approved by the Ethics Committee, Faculty of Veterinary Medicine, University of Maiduguri, Borno State, Nigeria. Twenty, 7-week old cockerels were used for this study. They were randomly divided into four groups of 5 chicken each (groups A, B, C and D). Cockerels in group D served as the untreated control and were given only feed and distilled water daily for a period of 28 days. The cockerels in groups A, B and C were treated daily orally with graded doses of the most active of the fruit extracts, that is crude methanol extract (CME), (200, 400 and 600 mg/kg⁻¹ respectively). Haematological effect of the prolonged administration of the extract was examined in cockerels. Birds treated with 200, 400 and 600 mg/kg of the extract exhibited significant (p<0.05) increase in PCV, Hb and RBC count on day 14 and 21 after treatment. There was a significant (p<0.05) decrease of MCV and MCH throughout the periods of experiment of the treated groups, while MCHC significantly (p<0.05) decrease after 7 days post-treatment.

Keywords: *Cucumis metuliferus*, Haematological effect, Cockerels.

INTRODUCTION

Plants have been used for various purposes since prehistoric times [1, 2] and medicinal herbs are being increasingly studied by pharmacological researchers [3]. Indian Ayurveda medicine used herbs as early as 1900 BC describing about 700 medicinal plants [4]. Herbal medicine was also important from early days in Europe. Dioscorides, who became popular with natural remedies about 60 AD, described over 600 plants and plant extracts [5]. It was not until recently that more attention was drawn to these practices. According to the World Health Organization (WHO) more than 80% of the world's populations rely on traditional medicine for their primary healthcare, majority of which use plants or their active principles [6]. Various plants of the family Cucurbitaceae have been shown to increase haematological parameters in man and animal [7, 8, 9, 10]. Increased attention on ethnoveterinary medicine (EVM) is justified because it is accessible, easy to prepare and administer at little or no cost at all [11]. These practices may be the only option in areas where conventional services are economically unavailable or cannot be effectively reached [12]. Many EVM practices do work and make sound veterinary sense [13].

The health benefits of the fruits of *Cucumis metuliferus* are in the nutrients it contains. These nutrients are made up of good levels of vitamin C, iron and potassium. It also has some amounts of phosphorus, magnesium, zinc, calcium, copper and sodium [14]. Two antioxidant compounds have been identified in the melon seeds; γ -tocopherol and α -tocopherol. Both are organic types of vitamin E with many health benefits to the body cells and organs, such as the red blood cells, skin, muscles, nerves and heart [14]. Studies on the acute toxicity levels of the fruits have been reported in cockerels [15] and in rats [16]. The aim of this study, therefore, is to investigate the effect (if any) of the fruit on some haematological indices in cockerels.

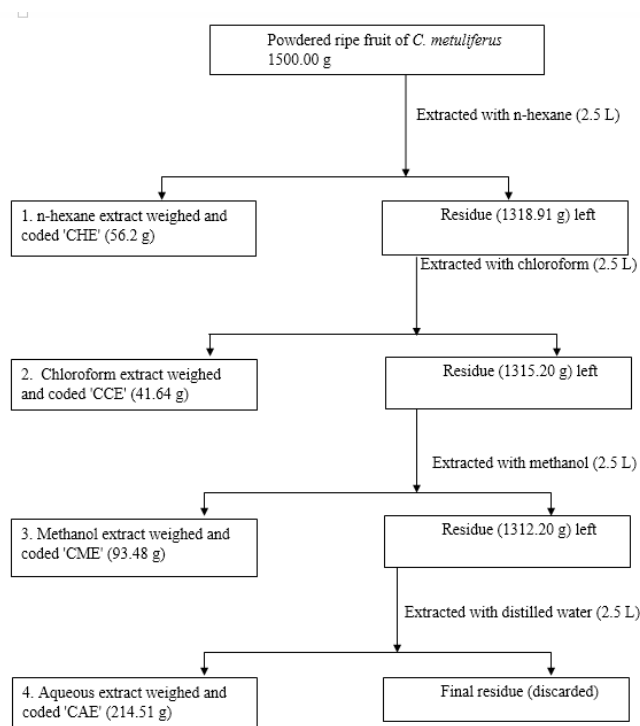
MATERIALS AND METHODS

Sample Collection and Identification

The fruits of *C. metuliferus* were collected in Vom village, Jos South Local Government Area, Plateau State, Nigeria in Nov. 2012. The plant was identified and authenticated by a plant Taxonomist in the Department of Biological Sciences, University of Maiduguri, Maiduguri. This was kept in an air-tight container until used.

Preparation of the Extracts

The ripe fruits from the plant were washed and sliced using clean knives, and then air dried in the laboratory and pulverized using a mortar and pestle. The powder (1.5 kg) was weighed and stored at room temperature in an air tight bottle, prior to use. The dried powder was serially extracted by maceration using solvents of different polarities (n-hexane, chloroform, methanol and distilled water) according to the method of Sofowora [17] as shown in scheme 1. Solvents were of analar grade.



Scheme 1: Cold extraction profile of the air-dried powdered fruit of *Cucumis metuliferus*

Experimental Animals

Day old chickens were purchased from Ghamba Consultancy and Enterprises, Wulari, Maiduguri and kept at the Veterinary Physiology,

Pharmacology and Biochemistry Laboratory, University of Maiduguri, for Intensive Management. Throughout these periods, food and water were provided *ad libitum*. The feed given was pelletised Vital Feed, (Grand Cereals Ltd.), Zawan Roundabout, Jos, Plateau State. The biochemical research involving animals was approved by the Ethics Committee, Faculty of Veterinary Medicine, University of Maiduguri, Borno State, Nigeria and was carried out according to the principles of Council for International Organizations for Medical Science (CIOMS) and the International Council for Laboratory Animal Science (ICLAS), [18].

Sub-Acute Toxicity Study

Twenty, 7-week old cockerels were used for this study. They were randomly divided into four groups of 5 chicken each (groups A, B, C and D). Cockerels in group D served as the untreated control and were given only feed and distilled water daily for a period of 28 days. The cockerels in groups A, B and C were treated daily orally with graded doses of the most active of the fruit extracts, that is CME, (200, 400 and 600 mg/kg respectively). Effect of the prolonged administration of the extract was examined. Blood was obtained from the wing vein on weekly basis and was used for the determination of haematological parameters such as red blood cell count (RBC), haemoglobin (Hb), and packed cell volume (PCV). Mean corpuscular volume (MCV) (fl) [19], mean corpuscular haemoglobin (MCH) (pg) [19] and mean corpuscular haemoglobin concentration (MCHC) (g/dl) [19] were calculated.

Statistical Analysis

Data were analysed using the Computer Statistical Software Package, GraphPad Instat [20] using one way analysis of variance (ANOVA) and results expressed as mean \pm standard deviation (S.D) where $p < 0.05$ was considered significant [21].

RESULTS

The Effect of the Methanol Extract of *Cucumis metuliferus* on Packed Cell Volume (PCV), Haemoglobin (Hb) and Red Blood Cell (RBC) Count in Cockerels

Birds treated with 200, 400 and 600 mg/kg of the extract exhibited significant ($p < 0.05$) increase in PCV, Hb and RBC count on day 14 and 21 after treatment. There is also a significant ($p < 0.05$) increase in PCV in cockerels treated with the extract at 600 mg/kg on day 7 of treatment (Table 1). Similarly, a significant ($p < 0.05$) increase in RBC count was observed in birds treated with the extract at 400 and 600 mg/kg after 7 days post treatment.

At the seventh day post treatment there was a significant ($p < 0.05$) decrease in the haemoglobin concentration in the treatment group with 400 mg/ml extract.

Table 1: The effect of the methanol extract of *Cucumis metuliferus* on packed cell volume (PCV), haemoglobin (Hb) and red blood cell (RBC) count of cockerels

Parameter	Treatment Dose of Extract (mg/kg)	Days of Treatment				Days post treatment (Withdrawal)
		0	7	14	21	7
Mean ± S.D; n = 5						
PCV (%)	Control (distilled water)	25.40 ± 0.55	25.00 ± 0.71	26.40 ± 0.55	26.60 ± 0.55	25.20 ± 0.84
	200	25.00 ± 0.71	25.60 ± 0.55	27.60 ± 0.55*	29.20 ± 0.84*	27.80 ± 0.84*
	400	24.60 ± 0.55	25.80 ± 0.45	28.80 ± 0.84*	29.60 ± .55*	27.60 ± 0.55 ^a
	600	25.00 ± 1.00	26.60 ± 0.55*	29.40 ± 0.55*	29.60 ± 0.55*	28.20 ± 0.84*
Hb (g/dl)	Control (distilled water)	9.40 ± 0.75	9.00 ± 0.14	9.16 ± 0.17	9.66 ± 0.13	9.72 ± 0.16
	200	9.04 ± 0.43	9.12 ± 0.30	10.28 ± 0.33*	10.80 ± 0.14*	8.64 ± 0.79 ^a
	400	9.12 ± 0.43	9.24 ± 0.26	10.56 ± 0.22*	10.92 ± 0.11*	8.88 ± 0.09 ^a
	600	9.08 ± 0.54	9.40 ± 0.32	10.76 ± 0.09*	10.92 ± 0.11*	9.74 ± 0.55 ^a
RBC (x10 ⁶ /mm ³)	Control (distilled water)	1.47 ± 0.06	1.63 ± 0.05	1.80 ± 0.14	1.95 ± 0.05*	2.00 ± 0.12*
	200	1.81 ± 0.06	1.90 ± 0.05	2.66 ± 0.45*	3.05 ± 0.34*	2.62 ± 0.36*
	400	1.39 ± 0.10	2.09 ± 0.12*	3.02 ± 0.31*	3.39 ± 0.14*	2.87 ± 0.17 ^a
	600	1.42 ± 0.09	2.17 ± 0.16*	3.22 ± 0.25*	3.35 ± 0.09*	2.99 ± 0.28*

* (p<0.05) significant when compared to the day before dosing

^a (p<0.05) significant when compared to values of day 21 of treatment

Effect of Methanol Extract on Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) of Cockerels

throughout the periods of experiment of the treated groups, while MCHC significantly (p<0.05) decrease after 7 days post-treatment. The control group also had a significant (p<0.05) decrease (Table 2).

There was a significant (p<0.05) decrease of MCV and MCH

Table 2: Effect of the methanolic extract of *Cucumis metuliferus* on mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) of cockerels

Parameter	Treatment (mg/kg) Dose of Extract	Days of Extract Treatment				Days of Treatment Withdrawal
		0	7	14	21	7
(Mean ± S.D.); n = 5						
MCV (fl)	Control	173.38 ± 3.93	153.18 ± 8.09	147.30 ± 10.32*	136.20 ± 4.23*	126.76 ± 0.73*
	200	138.06 ± 4.61	134.93 ± 3.83	106.35 ± 8.37*	96.51 ± 9.29*	107.86 ± 14.85*
	400	178.08 ± 11.05	123.57 ± 5.60*	96.21 ± 11.74*	87.48 ± 4.00*	96.37 ± 7.25*
	600	176.27 ± 5.39	123.07 ± 8.47*	91.81 ± 8.29*	88.45 ± 2.26*	95.18 ± 10.93*
MCH (pg)	Control	64.23 ± 6.15	55.12 ± 1.82*	51.13 ± 3.85*	49.48 ± 2.07*	48.81 ± 2.42*
	200	49.89 ± 1.65	48.09 ± 2.38	39.51 ± 6.06*	35.74 ± 3.95*	33.52 ± 5.40*
	400	66.09 ± 5.90	44.24 ± 1.81*	35.28 ± 4.17*	32.27 ± 1.37*	30.87 ± 2.93*
	600	64.04 ± 3.80	43.45 ± 2.36*	33.60 ± 2.88*	32.63 ± 0.78*	33.31 ± 3.55*
MCHC (g %)	Control	37.03 ± 3.24	36.02 ± 1.17	34.70 ± 0.84	36.33 ± 1.01	38.61 ± 1.66
	200	36.15 ± 1.06	35.63 ± 1.15	37.25 ± 1.05	35.03 ± 3.96	31.09 ± 2.81*
	400	37.10 ± 2.30	35.81 ± 0.48	36.69 ± 1.18	36.90 ± 0.31	32.22 ± 4.39*
	600	36.35 ± 2.22	35.33 ± 0.67	36.61 ± 0.62	36.90 ± 0.31	34.63 ± 2.71

* (p<0.05) significant when compared to the day before dosing

Control (administered distilled water)

DISCUSSION

PCV significantly (p<0.05) increased in all the treatment and remains so 7 days post treatment withdrawal. This result showed that the plant *C. metuliferus* increased the level of chicken PCV. This is in agreement with the work of Wannang *et al.* [22], that the aqueous extract of *C. metuliferus* significantly increased the level of PCV in

rats when given at 1000 mg/kg. An increase in the PCV value of rabbits treated with *C. metuliferus* against trypanosomiasis was also reported [23]. The normal PCV of chickens ranges between 23 and 55 % [24]. Therefore, a PCV of less than 23 % is suggestive of anaemia and a PCV greater than 55 % suggests dehydration or erythrocytosis (polycythemia) [24]. The result in this study showed a significant (p<0.05) increase in PCV value. Considering the importance of the

PCV as an indicator of blood dilution, it could be opined that the fruit of *C. metuliferus* may probably be used in the treatment of anaemia. This result therefore validates its use in the treatment of anaemia as reported by Burkill [25].

There was a significant ($p < 0.05$) increase in the levels of Hb. The level of Hb was shown to increase when *C. metuliferus* was given to rats at 500 and 1000 mg/kg (22). Haemoglobin, the primary component of erythrocytes, transports oxygen and carbondioxide, it is also considered to be an important measure of an animal to withstand some levels of respiratory stress.

There was a significant ($p < 0.05$) increase in the level of RBC in all the treated groups in this study. This is in contrast with that of Wannang *et al.* [22] who reported a significant ($p < 0.05$) decrease of RBC at 500 mg/kg and a significant ($p < 0.05$) increase at 1000 mg/kg of the aqueous extract of *C. metuliferus* in rats. Jimam *et al.* [26] reported that the tissues of spleen and pancreas of rats treated with 500 and 1000 mg/kg of the ethanol extract of *C. metuliferus* appeared normal. Spleen and stem cells in bone marrow give rise to RBC and various types of white blood cell (WBC). The increases in the blood indices could be related to the chemical composition of the fruit of *C. metuliferus*. It may therefore be inferred that some of the active components in this plant act on the bone marrow to stimulate the production and differentiation of haematopoietic stem cells. Karaye *et al.* [27] reported the various amino acids and volatile organic compounds found in *C. metuliferus*. Usman *et al.* [15] reported the various phytochemicals present in the fruit of *C. metuliferus*. Some of these constituents are well established haematopoietic factors that have direct influence on the production of blood in the bone marrow [28]. For instance, iron is a well-established haematopoietic factor and deficiency of it produces anaemia. The level of iron present in the fruit of *C. metuliferus* 2.6 mg/223 g [14] may help in the production of RBC. It has been shown in this study that there was an increase level of RBC in cockerels administered the fruit extract of *C. metuliferus*. The main function of the erythrocytes is to carry oxygen from the lungs to the tissues. Anaemia occurs when the haemoglobin level, haematocrit and /or erythrocyte count are below the normal range. This can be as a result of impaired erythrocyte production, increased erythrocyte destruction or blood loss. Ascites is related frequently to the phenomenon of hypoxemia resulting from reduced oxygen content of blood [29]. From the results obtained in this study on PCV, Hb and RBC, it may probably be deduced that the fruit extract of *C. metuliferus* increases the oxygen carrying capacity of RBCs and could probably be used in the treatment of hypoxemia and ascites.

The values of MCV, MCH and MCHC are used to classify anaemia. The values of MCV, MCH and MCHC in this present work were higher on the day before dosing (day 0). It may be deduced that the fruit extract may probably be used in the treatment of macrocytic anaemia. A significant change in the mean size of the erythrocytes will be reflected in the MCV. Erythrocytes subpopulation of large cells, that is MCV, more than normal most likely represent cells recently released from the haematopoietic tissue and is indicative of macrocytic anaemia. MCV values less than normal are indicative of microcytic anaemia [24]. MCH is the total haemoglobin in one RBC. MCH is decreased in all anaemia. From the result of this study, the value of MCH in this work ($30.87 \pm 2.93 - 66.09 \pm 5.90$ pg) was seen to be slightly below the normal range (33-45 pg) only after treatment withdrawal, but increased above the normal range during treatment. Thus, it may be deduced that *C. metuliferus* could be a good agent for managing anaemic animals. The level of iron present in the fruit [14]

can be of help in the production of RBC, since iron is a vital component of haeme. The value of MCHC can be used to determine the level of hypochromatic erythrocytes, a significant hypochromacia will be reflected as a decrease in the MCHC [24]. In most anaemia, the value of haemoglobin is decreased more and the haematocrit is decreased less, thus, it can be seen that in most anaemia the value of MCHC is decreased because of decrease in haemoglobin, except in sickle cell anaemia where the value of MCHC is increased due to dehydration [24]. From the result of this study the value of MCHC ($31.09 \pm 2.81 - 37.25 \pm 1.05$ g %) was seen to be within the normal range (26-35 g %) or slightly above normal. Thus it may be deduced that the plant *C. metuliferus* may be used to treat anaemia.

CONCLUSION

In conclusion, the result of this study showed that the plant *C. metuliferus* may be used as haematinics as well as in the treatment of malnutrition. The presence of iron in the fruit may have stimulated the production of RBC. This study has therefore confirmed the folkloric use of *C. metuliferus* as a haematinic agent.

Conflict of Interest

Authors have declared that they have no competing interests.

Disclosure Statement

The authors have nothing to disclose.

Authors' Contributions

JGU, OAS, and UKS set the practical and performed the statistical analysis. JGU and BW collected blood sample and performed the laboratory analysis. JGU, AVK and NJHU helped with the literature review. All authors read and approved the final manuscript.

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