Research Article

Effects of crude methanol extract of *Cucumis metuliferus* fruits (Cucurbitaceae) on White Blood Cells in Cockerels

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Abstract

Background: *Cucumis metuliferus*, a fruit used by poultry farmers in some parts of Nigeria to combat some poultry diseases such as Fowl Typhoid, Gumboro and Newcastle disease. The people of Plateau State in Nigeria eat the fruit believing that it cures HIV/AIDS. White blood cells are known to fight against diseases, hence the need to study the effect of this fruit on white blood cells. **Hypothesis/Purpose:** To find out the effect of the prolonged administration of the extract on total white blood cell (WBC) count, differential leucocyte count (DLC) and absolute differential leucocyte count (ADLC) was calculated. This will establish the plant use in the treatment of diseases. **Material and methods:** Twenty seven weeks 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 crude methanol extract (CME), (200, 400 and 600 mg/kg respectively). **Results:** There was a significant ($p<0.05$) increase in the value of total WBC from day 14 in all the treated groups while those that were given 600 mg/kg had a significant ($p<0.05$) increase from day 7. The result of the DLC showed that there was a significant ($p<0.05$) increase on lymphocytes percent in all the treated groups from day 7 when compared to their day 0, while the percentage of monocytes, neutrophils, eosinophils and basophils showed a significantly ($p<0.05$) decrease in all the treated groups. The result of absolute DLC showed a significant ($p<0.05$) increase in the values of lymphocytes, monocytes, neutrophils and eosinophils, while that of basophils showed no significant difference throughout the periods of experiment in all the treated groups. **Conclusion:** The results of this study showed that the plant *Cucumis metuliferus* increased the level of white blood cells and may probably stimulate the immune system.

***Keywords*:** *Cucumis metuliferus*, white blood cell, cockerels

Introduction

The use of plant resources mainly for herbal medicine, food, forage etc in Nigeria represents a long history of human interaction with the environment. These herbs have many potential clinical and therapeutic applications in the modern medical setting, as numerous studies have revealed that they contain bioactive components (Usman et al., 2014a; Gotep, 2011; Jimam et al., 2011), which have resulted in a better understanding of their physiological, therapeutic and clinical actions (Merken et al., 2001; Zheng and Wang, 2001). Plant extracts possess antioxidant (Bellucio et al., 2008), antibacterial (Usman et al., 2014b) and perhaps immunoregulatory (Feizi and Dadian 2012; Kumari et al., 2012) effects. The plant *C. metuliferus* contains beta carotene and vitamin E, which are of health benefit and are of importance by helping to strengthen the immune system

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(Usman et al., 2015).

White blood cells (also called leucocytes or immune cells) are cells which form a component of the blood. They help to defend the body against infectious disease and foreign materials. White blood cells are found in large numbers in the lymphatic system, the spleen and in other body tissues (Anon, 2015). Immunity is divided into two parts, the innate immunity and the adaptive one, based on the speed and specificity of the immune reaction. The innate immune system encompasses the elements of the immune system, such as, neutrophils, monocytes, complement, cytokines, and acute phase proteins, which provide immediate host defense (Parkin and Cohen, 2001). Its' responses typically involve the participation of many different cell types, including neutrophils, macrophages, monocytes, natural killer cells, and dendritic cells (Dempsey et al., 2003). The innate response is rapid but lacks specificity. Karami et al. (2013) showed that Japanese quails fed dietary plant extracts significantly increased the counts of lymphocytes, basophils and eosinophil. Similarly, Lee et al. (2007) demonstrated that methanol extracts of traditional medicinal plants commonly used in Korea are capable of affecting various *in vitro* parameters of chicken innate immunity. Barret (2003) showed, by *in vitro* experiments that *Echinacea purpurea* increases activity of natural killer cells. Also, Feizi and Dadian (2012) demonstrated that the extract of *Echinacea purpurea* increased the immunity level of broilers. The adaptive response is more precise and has memory, so subsequent exposure leads to a more vigorous and rapid response, but the development of the adaptive response takes several days or weeks (Dempsey et al., 2003). The adaptive response includes two stages. First, the antigen is recognized by antigen-presenting cells and presented to the antigen specific T or B cells leading to cell priming, activation and differentiation, which usually occurs within the specialized environment of lymphoid tissue. Second, the activated T cells leave the lymphoid tissue to the disease site and the antibodies are released from activated B cells into blood and tissue fluids, and then to the infective place. In this immune system, three important components: antigen-presenting cells, T lymphocytes, and B lymphocytes, drive the targeted effector responses (Parkin and Cohen, 2001). Kumari et al. (2012) showed that herbal feed additives augment humoral and cell mediated immune responses in broilers.

This study was designed to investigate the effect of *Cucumis metuliferus* on white blood cells (total, differential and absolute), to see whether it will increase or decrease their levels 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 extracted by maceration using methanol (analar grade) according to the method of Sofowora (2008).

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), 2012.

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 methanol fruit extract, that is CME, (200, 400 and 600 $mgkg^{-1}$ 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 white blood cell count (total and differential). The absolute differential leucocytes count was calculated.

Total White Blood Cell (WBC) Count (mm^3)

This was done using a haemocytometer as described by Schalm et al. (1975). The total leucocytes count was calculated using the formular:

WBC per mm^3 = cells counted x 20 (1.2 dil) x 10 (0.1 mm depth) mm

Differential Leucocyte Count (DLC) (%) or Relative DLC

DLC was determined according to Schalm et al. (1975). Each white cell was reported in percentage as:

$$\text{Percentage cell type (\%)} = \frac{No.of\ that\ cell\ type}{Total\ WBC\ count}$$

Absolute Differential Leucocyte Count (ADLC)

The value of absolute DLC was calculated using the formular below:

Absolute DLC = % DLC × Total WBC count

Statistical analysis

Data were analysed using the Computer Statistical Software Package, GraphPad Instat (2000) using one way analysis of variance (ANOVA) and results expressed as mean ± standard deviation (S.D) where $p < 0.05$ was considered significant (Armitage, 1980).

Results

Effect of methanol extract on total White Blood Cell (WBC) Count

There was a significant ($p<0.05$) increase in the value of total WBC from day 14 in all the treated groups while those that were given 600 mg/kg had a significant ($p<0.05$) increase from day 7. The control group also had a significant ($p<0.05$) increase on day 21 and 7 days after treatment withdrawal when compared to the day before dosing (day 0) (Table 1). However, values obtained 7 days post-treatment when compared with those of day 21 after treatment, in all the treated groups were significantly ($p<0.05$) decreased as shown in table 1.

Table 1. The effect of the methanolic extract of *Cucumis metuliferus* on the total WBC ($x10^3/mm^3$) count

Treatment (mg/kg)	**Days of Treatment** (Mean±S.D) mm^3; n = 5				**Days of Treatment withdrawal**
Dose of Extract	0	7	14	21	7
Control (distilled water)	11.82 ± 0.72	12.28 ± 1.12	12.86 ± 0.64	13.60 ± 1.34*	13.62 ± 1.28*
200	11.68 ± 0.62	13.48 ± 0.23	15.52 ± 0.26*	24.96 ± 1.11*	20.14 ± 0.66*a
400	12.00 ± 0.53	13.74 ± 0.09	16.24 ± 0.59*	25.56 ± 0.87*	21.36 ± 1.16*a
600	11.88 ± 0.76	13.94 ± 0.21*	17.18 ± 0.61*	26.20 ± 0.55*	22.48 ± 0.71*a

Table 2. The effects of methanol extract of *Cucumis metuliferus* on differential leucocyte count (DLC) %

Parameters	**Treatment (mg/kg) Dose of Extract**	**Days of Extract Treatment** (Mean DLC ± S.D.) % ; n = 5				**Days of Treatment Withdrawal**
		0	7	14	21	7
Lymphocytes	Control (distilled water)	61.00 ± 0.71	60.60 ± 0.55	60.60 ± 0.55	61.80 ± 0.84	61.60 ± 0.89
	200	59.60 ± 0.55	62.60 ± 0.55*	64.80 ± 0.84*	66.80 ± 1.64*	64.60 ± 0.55*a
	400	60.40 ± 0.55	63.60 ± 0.55*	66.00 ± 1.00*	71.20 ± 0.84*	65.40 ± 0.89*a
	600	59.60 ± 0.89	63.60 ± 1.14*	66.20 ± 1.30*	72.60 ± 0.55*	66.20 ± 0.84*a
Monocytes	Control (distilled water)	7.00 ± 0.45	7.00 ± 0.00	7.00 ± 0.00	6.60 ± 0.55	6.40 ± 0.55
	200	6.80 ± 0.45	4.80 ± 1.10*	5.00 ± 0.71*	4.20 ± 0.45*	5.60 ± 0.55a
	400	6.60 ± 0.55	3.80 ± 0.45*	4.80 ± 0.45*	3.80 ± 0.45*	5.40 ± 0.55a
	600	7.00 ± 0.00	3.60 ± 0.55*	4.60 ± 0.55*	3.60 ± 0.55*	5.00 ± 0.71*a
Heterophils (Neutrophils)	Control (distilled water)	27.00 ± 0.71	27.60 ± 0.55	27.60 ± 0.55	27.00 ± 1.00	27.60 ± 0.55
	200	28.60 ± 0.89	29.60 ± 0.55	25.60 ± 1.14*	25.20 ± 1.92*	25.00 ± 0.71*
	400	27.40 ± 0.55	29.40 ± 0.55	24.60 ± 1.82*	21.20 ± 1.10*	24.40 ± 0.89*a
	600	28.00 ± 0.71	29.00 ± 1.73	24.20 ± 1.79*	20.20 ± 0.45*	24.20 ± 0.45*a
Eosinophils	Control (distilled water)	4.40 ± 0.55	4.80 ± 0.45	4.80 ± 0.45	4.20 ± 0.45	4.20 ± 0.45
	200	4.20 ± 0.45	3.00 ± 0.00	4.00 ± 0.71	3.60 ± 0.89	4.40 ± 0.55
	400	4.60 ± 0.55	3.20 ± 0.45*	3.60 ± 0.55	3.20 ± 0.45*	4.40 ± 0.55
	600	4.40 ± 0.55	3.60 ± 0.55	4.20 ± 0.45	3.20 ± 0.45	4.00 ± 0.71
Basophils	Control (distilled water)	0.60 ± 0.55	0.00 ± 0.00	0.00 ± 0.00	0.40 ± 0.55	0.20 ± 0.45
	200	0.40 ± 0.55	0.00 ± 0.00	0.60 ± 0.55	0.40 ± 0.55	0.40 ± 0.55
	400	1.0 ± 0.00	0.0 ± 0.0x	1.0 ± 0.0	0.60 ± 0.55	0.40 ± 0.55
	600	1.0 ± 0.00	0.2 ± 0.45	1.0 ± 0.0	0.40 ± 0.55	0.60 ± 0.55

*($p<0.05$) significant when compared to the day before dosing; a ($p<0.05$) significant when compared to values of day 21 of treatment

Table 3. The effect of the methanol extract of *Cucumis metuliferus* on absolute differential leucocyte count (ADLC)

Parameters	Treatment (mg/kg) Dose of Extract	Days of Treatment				Days of Treatment Withdrawal
		0	7	14	21	7
Lymphocytes	Control (Distilled Water)	7183.20 ± 349.83	7440.80 ± 669.99	7792.40±375.64	8410.00 ± 904.90*	8398.20 ± 905.68
	200	6961.00 ± 368.80	8438.40 ± 156.02*	10057.40±236.50*	16618.80±744.71*	13008.40 ± 354.02*a
	400	7247.80 ± 323.30	8738.40 ± 60.75*	10721.40±502.97*	18196.60 ± 572.10*	13964.80 ± 671.95*a
	600	7077.80 ± 407.76	8867.40 ± 277.88*	11345.40±646.88*	19021.40±436.35*	14881.20 ± 487.83*a
Monocytes	Control (Distilled Water)	824.60 ± 46.27	859.60±78.32	900.20±44.99	902.00±151.56	872.20±117.00
	200	793.00 ± 47.47	647.20±148.93	775.20±102.46	1046.60±97.26	1129.00±129.48*
	400	792.20 ± 77.25	522.40±63.98	779.20±74.70	970.20±109.59	1150.60±94.79*
	600	671.60 ± 332.67	501.80±76.44	791.80±112.24	944.20±152.52	1126.00±180.22*
Heterophils (Neutrophils)	Control (Distilled Water)	3183.20±244.56	3388.40±303.01	3549.40±191.99	3662.00±274.26	3753.60±279.13
	200	3387.00±195.67	3990.00±96.48	3973.00±186.89	6298.40±666.64*	5036.00±243.55*a
	400	3287.80±154.9	4039.40±68.88*	3990.60±255.83*	5425.40±456.29*	5216.80±430.67*
	600	3329.00±274.29	4040.60±207.47*	4150.20±200.55*	5293.40±199.52*	5440.20±198.95*
Eosinophils	Control (Distilled Water)	516.40 ± 48.63	591.20 ± 90.76	618.00 ± 72.68	568.00 ± 43.82	569.80 ± 54.40
	200	492.00 ± 79.03	404.40 ± 6.84	620.20 ± 105.45	893.60 ± 196.61*	887.40 ± 126.60*
	400	552.20 ± 72.14	439.80 ± 62.77	586.40 ± 103.41	816.20 ± 98.45x	942.00 ± 146.59*
	600	522.80 ± 74.08	501.80 ± 76.44	720.80 ± 70.51	837.40 ± 107.50*	897.20 ± 147.66*
Basophils	Control (Distilled Water)	72.60 ± 66.46	0.00 ± 0.00	0.00 ± 0.00	56.00 ± 77.01	26.20 ± 58.58
	200	47.00 ± 64.52	0.00 ± 0.00	94.20 ± 86.00	102.60 ± 140.73	79.20 ± 108.47
	400	120.00 ± 5.34	0.00 ± 0.00	162.40 ± 0.86	151.60 ± 138.49	85.80 ± 117.72
	600	118.80 ± 7.60	28.40 ± 3.50	171.80 ± 6.14	103.60 ± 141.88	135.40 ± 123.65

*($p<0.05$) significant, when compared to the day before dosing; a($p<0.05$) significant, when compared to values of day 21 of treatment; n = 5 cockerels

Effect of methanol extract on Differential Leucocyte Count (DLC) (%)

There was a significant ($p<0.05$) increase on lymphocytes percent in all the treated groups from day 7 when compared to their day 0 (Table 2). The result of the 7 days post treatment of lymphocytes as shown in Table 2 showed a significant ($p<0.05$) decrease when compared to their day 21. The percentage of monocytes, neutrophils, eosinophils and basophils showed a significant ($p<0.05$) decrease in all the treated groups.

The result of 7 days post treatment when compared to day 21 showed that only monocytes and neutrophils had a significant ($p<0.05$) increase (Table 2).

Effect of methanol extract of *Cucumis metuliferus* on Absolute Differential Leucocyte Count

The result of the absolute DLC of lymphocytes as shown in Table 3 showed that all the treated groups had a significant ($p<0.05$) increase from day 7 to 7 days post treatment, however, when the values of 7 days post treatment was compared to its day 21 all the treated groups had a significant ($p<0.05$) decrease.

The result of absolute DLC of monocytes as shown in Table 3 showed a significant ($p<0.05$) increase in all the treated groups only after 7 days treatment withdrawal.

The result of absolute DLC of heterophils (neutrophils) as shown in Table 3 showed that those treated with 400 and 600 mg/kg extract had a significant ($p<0.05$) increase from day 7 while those given 200 mg/kg had a significant ($p<0.05$) increase from day 21.

There was a significant ($p<0.05$) increase in the level of eosinophils of all the treated groups from day 21 (Table 3). The result of absolute DLC of basophils showed no significant difference throughout the periods of experiment in all the treated groups (Table 3).

Discussion

WBC plays a prominent role in disease resistance through the process of phagocytosis and generation of antibodies. The result in this study shows that the methanol extract of *C. metuliferus* significantly ($p<0.05$) increased WBC count in the cockerels. This tends to show that the plant might help in boosting the immune system. This result is different from that of Wannang et al. (2007) who reported a decrease in the value of total WBC at 500 and 1000 mg/kg of the aqueous extract of *C. metuliferus*. Schalm et al. (1975) reported that leucocytes exercise their function as free macrophages in the tissue in defense against microbial agents and that monocyctes in particular, have a great ability for phagocytosis or pinocytosis.

The results of differential leucocyte count in this study showed a significant ($p<0.05$) increase in the values of lymphocytes and a significant ($p<0.001$) decrease of monocytes. Lymphocytosis is a common feature in viral infection such as mumps and measles and very high counts

are seen in whooping cough. Lymphopenia (a decrease in the lymphocyte count), is often found in patients undergoing radiotherapy and chemotherapy (Baker et al., 1998). However, lymphopenia can also occur due to increased demand on the system for lymphocytes in both immune and inflammatory responses (Mbaya et al., 2008). The increase in the number of lymphocytes indicates that medicinal plants such as *Cucumis metuliferus* improve the phagocytic capacity of the immune system, and would predispose the animals to enhance immunological responses to infections. Monocytes exhibit phagocytic activity and migrate into tissues to become macrophages. Monocytes and macrophages possess biologically active chemicals involved in inflammation mediation and the destruction of invading organisms. Monocytes also have an important immunological role in antigen processing (Campbell and Ellis, 2007). This result shows that lymphocytosis is primarily responsible for the increases in WBC count and the potential stimulating effect of *C. metuliferus* on the immune system as reported by Schalm et al. (1975). From the result of this work, it may probably mean that the fruit of *C. metuliferus* increases antibody activity (or level).

The amount of granulocytes (heterophils, eosinophils and basophils), were significantly ($p<0.05$) lower when compared to the control group. Avian neutrophils are devoid of myeloperoxidase, but despite this, they are still capable of phagocytosis. In the early phase of bacterial infections, neutrophils are mobilized within a few hours; neutrophils also participate in viral and parasitic infections. Avian neutrophils phagocytize and destroy microorganisms using oxygen-dependent and oxygen-independent mechanisms (Campbell and Ellis, 2007). Absolute neutrophil number increases during stressful conditions as a response to endogenous corticosteroid release. The exact function of the avian eosinophils is unknown; however, avian eosinophils may serve as modulators of inflammation in delayed hypersensitivity response (Campbell and Ellis, 2007). Eosinophils are seen to increase in some stress factors, for example, Maxwell (1987) reported increased levels of eosinophils in ducks, up to 8-12 % following injection of horse blood serum. Also, Kontecka et al. (1999) found elevated proportions of eosinophils in ducks exposed to stress caused by limited access to drinking water. In broiler chickens, a similar effect was observed three hours after ochratoxin administration which caused a 3.7 % points increase in the levels of eosinophils (Moura et al., 2004). On the other hand, Nowaczewski et al. (2006) found no significant differences in agranulocytes and granulocytes level between pheasants fed complete diet with or without vitamin C supplementation (anti-stress agent). Avian basophils are frequently found in the peripheral blood, in contrast to mammalian basophils which are rarely found in blood film of normal animals. Avian basophils participate in acute, inflammatory and type IV hypersensitivity reactions (Campbell and Ellis, 2007). The function of avian basophils is not fully known. Avian basophils produce, store and release histamine, so they may function in immediate hypersensitivity reactions. Conversely, the avian basophil plays an important role in type I hypersensitivity reactions, which is a function that is different from the basophil of the mammals. A stress-related basophilia has been demonstrated to occur in chickens subjected to food restriction, but the response may be age or duration-dependent (Campbell and Ellis, 2007). Although, the basophils level in this study is within the normal range, there was a significant ($p<0.05$) decrease on day 7 from 1.0 ± 0.00 % to 0.0 ± 0.0 % of those treated with 400 mg/kg extract. This may probably mean that the birds were not under any stress nor exposed to allergic agents.

The absolute differential leucocyte count showed a significant ($p<0.05$) increase in the levels of agranulocytes (lymphocytes and monocytes) and granuloctes (heterophils and eosinophils) during treatment and 7 days post-treatment when compared to the levels obtained at day 0. There was no significant difference in the values of basophils during and after treatment. The absolute differential leucocyte count was calculated because the absolute value is much more important than the relative value, which is expressed as a percentage.

The results of this study showed that the plant *C. metuliferus* may probably stimulate the immune system.

Conclusion

Immunity can be boosted by choosing the right product(s) that can increase the effectiveness of white blood cells. The results of this study showed that the plant *Cucumis metuliferus* increased the level of white blood cells and may probably stimulate the immune system. In plateau State of Nigeria, people infected with HIV/AIDS believe in taking the fruits, because they claim it boosts their immune system (personal/oral communication, 2012). Perhaps that may be true.

Conflict of Interest

Authors wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

Disclosure Statement

The authors have nothing to disclose.

Authors' Contributions

Joy Gararawa Usman, Olufunke Adebola Sodipo and Umar

Kyari Sandabe set the practical and performed the statistical analysis. Joy Gararawa Usman and Bitrus Wampana collected blood sample and performed the laboratory analysis. Joy Gararawa Usman, Ayi Vandi Kwaghe and Nendir John Haruna Umaru helped with the literature review. All authors read and approved the final manuscript.

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Lists of Abbreviations

ADLC:	Absolute Differential Leucocyte Count
ANOVA:	One Way Analysis of Variance
CIOMS:	Council for International Organizations for Medical Science
CME:	Crude Methanol Extract
DLC:	Differential Leucocyte Count
HIV/AIDS:	Human Immunodeficiency Virus/ Acquired immune deficiency Syndrome
ICLAS:	International Council for Laboratory Animal Science (ICLAS),
WBC:	White Blood Cell