

## Research Article

### Effects of Aqueous Extract of *Cyphostemma glaucophilla* Leaves on Some Specific and Non-specific Immune Responses in Albino Rats

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**Abstract:** *Cyphostemma glaucophilla* is used in the management of kwashiorkor, because impaired immune responses is associated with malnourishment the modulatory activity of aqueous leaves extract of *Cyphostemma glaucophilla* on primary and secondary humoral responses, *in vivo* leucocyte mobilization, Delayed Type Hypersensitive Reaction (DTHR), haemoglobin, packed cell volume and white blood cell count were evaluated. The extract at 250 and 500 mg/kg stimulated significant ( $p < 0.05$ ) dose dependent increase in primary and secondary sheep red blood cell specific antibody titre comparable to Levamisol. Extract at doses of 100 and 250 mg/kg induced leucocyte mobilization while 500 mg/kg inhibited leucocyte mobilization. The extract at 250 and 500 mg/kg produced significant ( $p < 0.05$ ) inhibition of DTHR in rats by 20.24 and 67.77%, respectively. The extract also induced significant ( $p < 0.05$ ) increases in packed cell volume by 3.0% at the lowest dose of 0.5 mg/kg and 11.2% at the highest dose of 2.0 mg/kg. However, the extract was insensitive to haemoglobin and total white blood cell count. Result has established cellular and humoral immunomodulatory activities of *Cyphostemma glaucophilla* aqueous leaves extract.

**Keywords:** *Cyphostemma glaucophilla*, humoral response, hypersensitivity, immunomodulatory, leucocyte mobilization

## INTRODUCTION

In countries where the diets of growing children, is deficient in proteins, immune deficiency is caused by such severe malnutrition. Cell mediated immunity and antibody responses are impaired, as a consequent of deficiency of helper T cells (Joos and Tamm, 2005).

Immunity from disease is actually conferred by two cooperate defense system; viz: non specific (innate) immunity and specific (adaptive) immunity (Guyton and Hall, 2006), which could be altered by substances to either enhance or suppress their ability to resist invasion by pathogen (Janeway, 2005).

Though it is believed that the cause of kwashiorkor is lack of proteins with more or less adequate energy, Heird (2008) have recorded that there is impaired immune responses and high risk of infections consequent on reduced synthesis of protein.

There has been a growing interest in identifying and characterizing natural compounds with immunomodulatory activities (Ganachari *et al.*, 2004). Compounds which appear to stimulate the human immune responses are being sought for the treatment of immune deficiency disease or for generalized immuno suppression following drug therapy; for combination

therapy with antibiotics or as adjuncts for vaccines (Nworu *et al.*, 2007).

*Cyphostemma glaucophilla* is a medicinal plant which belongs to the family of *Vitaceae*, *Cyphostemma* species are caudiform and use to belong to the genus *Cissus* (Eggli, 2002). The aqueous leaf extract is used successfully to treat kwashiorkor in many parts of Kogi and Kwara states of Nigeria. Result of acute toxicity (Ojogbane *et al.*, 2010) indicated that extract had no adverse effect at the limit per oral dose of 3000 mg/kg body weight. The leaf extract induces increases in liver total proteins and plasma proteins especially albumin (Omale and Okafor, 2008; Ojogbane *et al.*, 2007). In addition to its lipid lowering effect (Ojogbane *et al.*, 2007), the extract has anti-inflammatory activity (Ojogbane *et al.*, 2011).

Although, there is no data on the effect of *Cyphostemma glaucophilla* on the immune system, Herbalist administers the extract to healthy children with the hope/thought that it keeps/maintain their health. The present study was conducted to determine the effect of extract on the body defense mechanism. In this study, we are presenting our initial findings on the effect of extract on humoral and cell mediated immune responses.

## MATERIALS AND METHODS

### Collection and extraction of plant material:

*Cyphostemma glaucophilla* leaves were collected from the bank of River Niger along Idah-Ibaji road in Kogi State of Nigeria and characterized by A.O Ozioko of Botany Department, University of Nigeria, Nsukka. They were washed to remove dirt, dried at room temperature and pulverized with a milling machine into a coarse powder. A portion (100 g) of the pulverized leaves was macerated in five volume (w/v) water for eighteen hours with two changes of solvent. The filtrate was evaporated in a water bath to get the dry residue (yield = % starting material).

**Animals:** The Wistar albino rats used for this study were obtained in March, 2012 from the Faculty of Biological Sciences Animal House, University of Nigeria, Nsukka, Nigeria. The rats of either sex, aged between 7 and 9 weeks and weighing (110-150 g) were housed under standard conditions (25±2°C and 12 h light/dark cycle). They were fed with standard pellets (Top Feed Nigeria Ltd.) and had access to clean drinking water.

**Antigen:** Fresh sheep blood was obtained from the Animal Farm of the Faculty of Agriculture, Kogi State University, Anyigba, Nigeria. Sheep Red Blood Cells (SRBCs) were washed three times in copious volume of pyrogen-free sterile normal saline by centrifugation at 3000×g for 10 min on each occasion. The washed SRBCs were adjusted to a concentration of 10<sup>9</sup> cells/mL for immunization and challenge.

**Humoral Antibody (HA) synthesis:** Rats were immunized by an intraperitoneal injection (ip) of 0.1 mL of 10<sup>9</sup> SRBC/mL on day 0 and challenged by similar i.p injection of the same amount on day 5. Primary antibody titre was determined on day 5 (before the challenge) and secondary titre on day 10 by the haemagglutination technique (Nelson and Mildenhall, 1967). The extract (100, 250 and 500 mg/kg, respectively) body weight was administered 3 days prior to immunization and continued daily for 5 days after the challenge to three groups of rats B, C and D. Group A received normal saline (0.85% NaCl; 5 mL/kg) while group E was administered levamisole. Blood samples were obtained by retro-orbital puncture in test tubes and allowed to clot, centrifuged at 3000×g for 10 min to obtain the serum. For each sample, a 25 mL serum was obtained after centrifugation and serially diluted two fold in 96U-well micro titre plates using pyrogen free sterile normal saline. The last well on each roll contained sterile normal saline as control. The diluted sera was challenged with 25 mL of 1% (v/v) SRBC in the plate and then incubated at 37°C for 1 hour the highest dilution giving rise to visible haemagglutination was taken as antibody titre. The antibody titres were expressed in graded manner and the minimum dilution (1/2) being ranked as 1 (calculated as log of the dilution

factor). The mean ranks of different treatment groups were compared for statistical significance.

**In vivo leucocyte mobilisation:** Five groups of five animals each was used for this experiment. The effect of the extract on in vivo leucocyte migration induced by inflammatory stimulus was investigated by the method of Ribeiro *et al.* (1991).

One hour after oral administration of extract (100, 250 and 500 mg/kg, respectively) to groups B, C and D, each rat in the group (n = 5) received intra peritoneal injection of 0.5 mL of 3% (w/v) agar suspension in normal saline. The rats were sacrificed 4 h later and the peritoneum washed with 5 mL of 5% solution of EDTA in phosphate.

**Delayed Type Hypersensitive Reaction (DTHR):** Method of Naved *et al.* (2005): Five groups of five animals each were used for this experiment.

DTHR was induced in rats using SRBC as antigen. Animals were sensitized by subcutaneous injection of 0.02 mL of 10<sup>9</sup> cell/mL SRBC (day 0) in the plantar region of right hind paw and challenged on day 5 by subcutaneous injection of the same amount of antigen into the left hind pad. The oedema produced by antigenic challenge in the left hind paw was measured as the difference in the paw thickness before and 24 h after the challenge. The paw thickness was measured with a pocket size screw gauge. The extract, (100, 250 and 500 mg/kg, respectively) was administered orally to groups B, C and D three days prior to sensitization and continued till the challenge. The control group (A) received normal saline while group E was administered levamisole (immunostimulant).

**Determination of Packed Cell Volume (PCV), Haemoglobin (Hb) and White Blood Cell (WBC) estimation:** The haematocrit method of Alexander and Griffiths (1993) was used to estimate the PCV, Haemoglobin concentration was by the method of Dacie and Lewis (1991) while the white blood cell estimation was by the visual method of Dacie and Lewis (1984).

### Experimental design for assay of PCV, Hb and WBC:

Five groups A, B, C, D and E of five animals each (110-150 g) of either sex were given normal saline (0.85% NaCl; 5 mL/kg), 0.5, 1.0, 1.5 and 2.0 mg/kg body weight oral daily doses of extract using stomach tubes for 14 days respectively. Twenty 4 h after the last administration, animals were anaesthetized and blood samples were collected via cardiac puncture into heparinised tubes using sterile syringes and were used to determine the concentration of PCV, Hb and WBC.

**Statistical analysis:** Results were analyzed by one way analysis of variance using SPSS version 18, differences of the means were considered significant at (p<0.05).

Table 1: Extract induced stimulation in humoral antibody response  
Humoral antibody responses (mean titre±SEM)

Group	Treatment (mg/kg)	Primary	Secondary
A	Normal saline (5 mL/kg)	3.0±0.18 <sup>a</sup>	3.7±0.24 <sup>a</sup>
B	100	4.0±0.20 <sup>b</sup>	4.7±0.23 <sup>b</sup>
C	250	5.1±0.24 <sup>c</sup>	6.0±0.30 <sup>c</sup>
D	500	5.3±0.24 <sup>c</sup>	6.5±0.42 <sup>c</sup>
E	Levamisol 2.5	5.5±0.12 <sup>c</sup>	6.6±0.01 <sup>c</sup>

Values with different superscript (<sup>a</sup>, <sup>b</sup>, <sup>c</sup>) are statistically significant (p<0.05)

## RESULTS

**Effect of extract on primary and secondary humoral immune response in rats:** On Table 1, the extract stimulated a significant (p<0.05) dose dependent elevation of primary and secondary Sheep red blood cells specific antibody titre in the test groups B, C and D when compared with the control (A). However the humoral antibody stimulation caused by extracts at doses of (250 and 500 mg/kg, respectively) in groups C and D are comparable to that produced by levamisol.

**Effect of extract on in vivo leucocyte mobilization in rats:** On Table 2, even though the standard drug levamisol did not produce remarkable effect, the extract produced a biphasic effect; doses of 100 and 250 mg/kg induced increase in peritoneal leucocyte mobilization but at 500 mg/kg extract inhibited peritoneal leucocyte mobilization.

**Effect of extract on delayed type hypersensitivity reaction in rats:** On Table 3, the extract produced significant (p<0.05) dose dependent inhibition of delayed type hypersensitivity. The lowest dose (100

mg/kg) produced an inhibition of 9.23% in group B while higher doses 250 and 500 mg/kg (vide groups C and D respectively) produced inhibitions of 20.24 and 65.77% respectively. However, levamisol (an immunostimulatory drug) stimulated DTH by -4.17%.

**Effect of extract on Packed Cell Volume (PCV), Haemoglobin (Hb) and White Blood Cell count (WBC):** Table 4 shows that the extract induced a dose dependent significant (p<0.05) increase in the concentration of PCV when compared with the control group (A) that received normal saline, the extract produced increases in the PCV of rats (Group B-E) that received scalar amounts of the extract. While the PCV increased to 38.0±1.26% among animals that were treated with 0.5 mg/kg extract, those that received 1.0 and 2.0 mg/kg extract responded with 40.0±1.41% and 46.2±1.48% increase in PCV from the control value of 35.0±1.79%. These increases were statistically significant (p<0.05). This treatment had no significant (p>0.05) effect on the concentration of Hb and total WBC count.

## DISCUSSION

The most prevalent cause of immunodeficiency worldwide is severe malnutrition which affects as much as 50% of the population in some impoverished communities (Gerald *et al.*, 2008). Availability of complement component and Phagocyte function are compromised during malnutrition which will directly affect pathogen elimination. Significant low level of complement is a feature of malnutrition (Mayer, 2006).

On Table 1, extract stimulated a significant (p<0.05) dose dependent elevation in primary and secondary humoral immune responses to sheep red

Table 2: Biphasic effect of extract on *in vivo* leucocyte mobilisation in rats

Group	Treatment (mg/kg)	Total leucocyte count (mm <sup>-3</sup> )	Percentage leucocyte mobilization	Percentage neutrophil	Percentage lymphocytes
A	Normal saline (5 mL/kg)	765.0±59.2 <sup>a</sup>		56.40±1.30	43.60±0.12
B	100	787.0±55.0 <sup>b</sup>	32.68 <sup>a</sup>	72.00±0.51	28.00±1.20
C	250	834.0±55.0 <sup>c</sup>	39.29 <sup>b</sup>	65.30±0.52	34.70±1.35
D	500	565.0±51.2 <sup>d</sup>	-47.61 <sup>c</sup>	63.40±0.58	36.60±1.30
E	Levamisol 2.5	767.0±55.0 <sup>c</sup>	35.75 <sup>d</sup>	70.00±1.29	30.00±1.30

Values with different superscript (<sup>a</sup>, <sup>b</sup>, <sup>c</sup>, <sup>d</sup>, <sup>e</sup>) in a column are statistically significant (p<0.05)

Table 3: Extract induced inhibition of delayed type hypersensitive reaction in rats

Group	Treatment (mg/kg)	Paw volume (mm)	Percentage inhibition of DTHR
A	Normal saline (5 mL/kg)	0.336±0.000	
B	100	0.305±0.010	9.23 <sup>a</sup>
C	250	0.268±0.000	20.24 <sup>b</sup>
D	500	0.115±0.000	65.77 <sup>c</sup>
E	Levamisol 2.5	0.350±0.010	-4.17 <sup>d</sup>

Negative sign shows stimulation of delayed type hypersensitivity; Values with different superscript (<sup>a</sup>, <sup>b</sup>, <sup>c</sup>, <sup>d</sup>) in a column are statistically significant (p<0.05)

Table 4: Extract induced increase of PCV and relative insensitivity of HB and WBC concentration to extract treatment

Group	Treatment (mg/kg)	PCV (%)	Hb (g/dL)	WBC (N X 10 <sup>3</sup> /mm <sup>3</sup> )
A	Normal saline (5 mL/kg)	35.0±1.79 <sup>a</sup>	6.20±1.75 <sup>a</sup>	2.30±0.41 <sup>a</sup>
B	0.5	38.0±1.26 <sup>b</sup>	6.40±1.41 <sup>a</sup>	2.40±0.41 <sup>a</sup>
C	1.0	40.0±1.41 <sup>c</sup>	6.50±1.41 <sup>a</sup>	2.40±0.32 <sup>a</sup>
D	1.5	43.5±1.95 <sup>d</sup>	6.40±1.40 <sup>a</sup>	2.35±0.40 <sup>a</sup>
E	2.0	46.2±1.48 <sup>c</sup>	6.50±1.70 <sup>a</sup>	2.40±0.50 <sup>a</sup>

Values with different superscript (<sup>a</sup>, <sup>b</sup>, <sup>c</sup>, <sup>d</sup>, <sup>e</sup>) in a column are statistically significant (p<0.05)

cell antigen in rats. Antibody synthesis requires the cooperation of at least three major cell types; the microphage, the B-lymphocytes and T-lymphocytes (Janeway, 2005). The secondary titres are expectedly higher, since subsequent antigenic stimulations of priory-sensitized animals may result in high antibody production, as there is now an expanded clone of cells with memory of the original antigen available to proliferate into mature plasma cells (Agerberth and Gudmundsson, 2006). This property will enhance humoral immune protection of the animal which is mediated through opsonisation, direct neutralization of antigen, agglutination of antigen and activation of complement system to cause lyses and death of antigen cells (Zen and Parkos, 2003).

On Table 2, extract at lower doses (100 and 250 mg/kg, respectively) produced a significant ( $p < 0.05$ ) increase in Agar induced leucocyte mobilization. It has been observed that the chemotatic movement of neutrophils towards the foreign body is the first and the most important step in phagocytosis (Ganachari *et al.*, 2004). This activity may help to increase the general resistance of the body against microbial infections. The Polymorphous Neutrophils (PMNS), which engulf and eliminate invading micro organism, was the most mobilized

Delayed type hypersensitivity is know to be initiated by reactions between antigen specific T-cell and the antigen which results in the release of lymphokines that affect a variety of cell types especially microphages (Le *et al.*, 2004). From present result, the inhibition of delayed type hypersensitive reaction by extract is an indication of its ability to modulate cell mediated immune response. This mechanism may be related to the anti-inflammatory properties of the plant which has been reported in earlier studies (Elejo *et al.*, 2012). This inhibition can occur by immune deviation which entails steering T-cells towards an IL-4 producing TH2 or TC2 phenotypes (Goronzy and Weyand, 2007).

Pathological changes in kwashiorkor include a low level of Packed Cell Volume (PCV) and Haemoglobin (Hb) both of which consequently lead to anaemia (Wardlaw *et al.*, 2004). The result on Table 4 showed that the extract induced a significant ( $p < 0.05$ ) increase in the concentration of PCV. This effect will also reflect in an increase in the concentration of Red Blood Cell (RBC) because (Mayer, 2006) had reported that PCV is also a function of RBC concentration hence it is a representation of the percentage of RBC in blood. The possibilities that extract could have induced erythropoiesis in the bone marrow remain to be established. The observation showing non significant ( $p > 0.05$ ) effect of extract on the concentration of Haemoglobin indicated that the integrity of the RBC was maintained and that there was no haemolysis of the RBC. This is in agreement with previous studies

(Ojogbane and Nwodo, 2010) that the aqueous extract of *Cyphostemma glaucophilla* stabilizes the erythrocyte membrane.

Leucocytosis may occur in hepatic damage (Kawai and Akira, 2006) non significant effect of extract on the concentration of white blood cell count is an indication that the extract does not cause hepatic damage (Ojogbane *et al.*, 2012). It is observed from the result of this study that the aqueous extract of *Cyphostemma glaucophilla* leaf has immunomodulatory effect on both the cell mediated and humoral components of the immune system and has potential benefit in reversing anaemia in kwashiorkor. These activities partly explain why the claim by traditional medicine practitioners from Igala speaking areas of Kogi State Nigeria may not be wild.

## CONCLUSION

The result of this study justifies its inclusion in herbal tonics as immune-boosting agent and has established cellular and humoral immunomodulatory activities of *Cyphostemma glaucophilla* aqueous leaf extract.

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