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Research Paper

HAEMATOLOGICAL EFFECTS OF FLUTED PUMPKIN (*TELFAIRIA OCCIDENTALIS*) LEAVES IN RATS

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The effects of different concentrations of ethanol extract of fluted pumpkin (*Telfairia occidentalis*) leaves on some haematological parameters: Packed Cell Volume (PCV), Haemoglobin (Hb) concentrations, Total White Blood Cell (TWBC) count and Platelet count were evaluated using 16 Wister strain albino rats as subjects. In the experiment, the rats were divided into 4 groups of 4 rats each and the rats were served different concentrations of *T. occidentalis* ethanol extract for seven (7) days. The result showed that the group of rats which received 300 mg/kg and 200 mg/kg leaves extract had a significant increase ($p < 0.05$) in PCV and Hb concentration, those administered 100 mg/kg of the extract had significant increase ($p < 0.05$) in PCV only. There was no significant increase ($p < 0.05$) in the WBC and platelet counts of all the groups when compared with the control. It therefore follows that *T. occidentalis* has haematopoietic properties.

Keywords: *Telfairia occidentalis*, Packed Cell Volume (PCV), Haemoglobin (Hb) concentrations, Total White Blood Cell (TWBC) count and Platelet count

INTRODUCTION

Botanical Description of Fluted Pumpkin (*Telfairia occidentalis*) Leaves

Telfairia occidentalis is a tropical vine grown in West Africa as a leaf vegetable and for its edible seeds (Badifu *et al.*; 2001). Common name for the plant include fluted gourd, fluted pumpkin and

ugu in Igbo, gbaroko in Yoruba and umeke in Edo. This creeping vegetable spread low across the ground with lobed leaves and long twisting tendrils (Okoli *et al.*, 1999). The plant is dioecious, perennial and drought-tolerant. It thrives best in soils rich in organic matter and during the early part of the rainy season. The fruit of the plant is large and inedible reaching about 13 kg. The seed

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can be fermented for several days and eaten as slurry (Badifu *et al.*, 2001). The root and leaves have been shown to contain highly toxic alkaloids and saponin (Akube, 1990; Akindahunsi *et al.*, 2005).

Fluted pumpkin is a source of protein, oil, fat, minerals and vitamins (Onyenuga, 1998; Ifon, 1999; Okoli *et al.*, 1999; Aletor and Adeogun, 1995). The leaves of this vegetable are used in the preparation of several delicacies in southern Nigeria. One of which is Edikang Ikong soup (a popular delicacy of the Efik/Ibibios in Cross River and Akwa Ibom State in Nigeria). *T. occidentalis* can be grown in garden and farmed as a vegetable. It can survive for 3-4 years if there is moisture in the soil (Achinewu, 1997).

Fluted pumpkin plays important role in human and livestock nutritions. The poor state of economy in developing countries has made consumption of high protein food out of reach of more than 65-70% of the (Nworgu, 2004). One of the ways of solving this problem is to use unconventional sources of protein and leaf protein to supplement the diet of man farm animals. The herbal preparation of the plant has been employed in the treatment of sudden attack of convulsion, malaria and anaemia (Adedapo *et al.*, 2002).

Apart from the nutritional (Okoli *et al.*, 1993) agricultural and industrial importance (Akoroda, 1990), the plant is also medicinally useful. It possesses anti inflammatory (Oluwole, 2003), antibacterial (Odoemena, 1995), erythropoietic (Ajayi *et al.*, 2000), anti cholesterol (Esseyin *et al.*, 2005a) and anti diabetic (Eseyin *et al.*, 2000, Eseyin *et al.*, 2005b) activities.

SCIENTIFIC CLASSIFICATION OF FLUTED PUMPKIN

(*Telfairia occidentalis*)

Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Curbitales
Family	Curcubitaceae
Genus	<i>Telfairia</i>
Species	<i>Telfairia occidentalis</i>
Botanical name	<i>Telfairia occidentalis</i>

STATEMENT OF THE PROBLEM

The shortage of animal protein particularly in developing countries in Africa (Nigeria inclusive) has necessitated investigation of several novel sources of protein. This acute shortage of protein had been attributed to the phenomenal rise in the price of animal feeds which account for about 75-85% of the recurrent production cost in intensive monogastric animal production in Africa (Fetuga, 1977). This has escalated price of animal product thus making animal protein generally beyond the reach of the average citizen in the developing countries.

With the teaming population, there is an attendant rise in the demand for animal product in recent years but a careful survey of some relevant literatures and policy statement on the supply and consumption of animal protein reveals a growing concern with the widening gap between estimated protein requirement and actual protein consumption in many tropical developing countries including Nigeria (Fasuyi, 2007).

AIMS AND OBJECTIVES

The major objective of this research work was to study by evaluating the haematological effect of different concentrations of leaf meal prepared from *Telfairia occidentalis* on Wistar strain albino rats.

MATERIALS AND METHODS

Animal Model

Wistar strain albino rats obtained from the animal house, University of Nigeria, Nsukka, Nigeria were used for the study. The rats were housed in wire mesh cages under standard conditions (temperature 25-29°C, 12 h light and 12 h darkness cycles) and fed with standard rat pelleted diet and watered.

Plant Material

The fresh leaves of *Telfairia occidentalis* (fluted pumpkin) were purchased from a local market in Elele, Rivers State, Nigeria, authenticated by Chief Pharmacist, F N Osuala of the department of Pharmacognosy, Madonna University, Elele Campus. The leaves were washed thoroughly to remove contaminants from the farmland. The leaves were shade dried and reduced to a powdery form by grinding.

Food Sample/Diet Composition

The diet given to the rats was the normal rat chow obtained from Bendel feed and flour mills limited, Ewu. Below is the comprehensive composition of the feed.

Nutrients	Concentration
Crude protein	14.5%
Crude fat	4.8%
Crude fibre	7.2%

Crude ash	8.0%
Calcium	0.8%
Phosphorous	0.62%
Lysine	0.6%
Available phosphate	0.29%
Methionine + cysteine	0.5%
Vitamin A	8,000 IU
Vitamin D ₃	2,400 IU
Vitamin E	15 mg
Vitamin B ₂	4 mg
Vitamin C	50 mg
Manganese	30 mg
Zinc	30 mg
Sodium	0.15%
Metabolisable energy	2,300 Kcal/kg

(Source: Vital feed limited)

Experimental Design

16 albino rats were used as animal model. They were housed in four wire mesh cages containing four rats in each cage. The rats were weighed before the experiment. The rats were made to acclimatize for 1 week before the experiment began. They were allowed to feed and water freely throughout the period which the experiment lasted. Administration of the plant extract was by oral route.

Order of Placement

- GROUP 1: The rats in this group were administered feed, distilled water and 100 mg/kg of plant extract.
- GROUP 2: Administered feed, distilled water and 200 mg/kg of plant extract.

GROUP 3: Administered feed, distilled water and 300 mg/kg of plant extract.

GROUP 4: Served as control. Administered feed and distilled water only.

Reagents and Chemicals Used

Turks fluid was used for total white blood cell determination. Ammonium oxalate for platelet count, Drabkins fluid was used for haemoglobin concentration.

METHODOLOGY

Extraction

222 g of the powdered form of the leaves of *T. occidentalis* was soaked in 80% ethanol, stirred for 24 h after every 1 h interval. After 24 h, it was sieved with filter paper. The extract was evaporated to concentration by using the steam method and was dried further using the hot air oven. 1 g of extract was then dissolved in 10 ml of normal saline.

Collection of Blood Samples

Collection of blood samples was carried out at the end of seven days experiment. While under chloroform anaesthesia, blood was collected using sterile needles and syringes from each rat through heart puncture and transferred into sterilized bottles containing Ethylene Diamine Tetra Acetic acid (EDTA).

ANALYSIS OF BLOOD SAMPLES

Packed Cell Volume (PCV) Estimation

The packed cell volume was determined using the microhaematocrit centrifuge (Jouan A₁₃ model).

Principle of the Test

The packed cell volume is that proportion of whole blood occupied by red cells, expressed as a ratio (litre/litre). Anticoagulated blood in a glass capillary of specified length, bore size, and wall thickness is centrifuged in a microhaematocrit centrifuge at RCF 12,000-15,000 xg for 3-5 min to obtain constant packing of the red cells. A small amount of plasma remains trapped between the packed red cells. The PCV value is read from the scale of a haematocrit reader or calculated by dividing the height of the red cell column by the height of the total column of blood. (Cheesbrough, 2004)

MATERIALS

Microhaematocrit centrifuge, Capillary tubes, Sealant (plasticin), Microhaematocrit reader, EDTA anticoagulated blood.

Procedure

1. Plain capillary tubes were three-quarter filled with mixed EDTA anticoagulated blood.
2. Using a sealant, one of the unfilled ends were sealed.
3. The tubes were placed on the slots of the microhaematocrit rotors and balanced with the sealed ends against the rim gasket to avoid breakage.
4. The inner lid was positioned carefully to avoid dislodging of the tubes.
5. It was centrifuged for 5 min at 12,000 xg.
6. Immediately after centrifuging, the PCV was read using a haematocrit reader and recorded.

Haemoglobin (Hb) Concentration

The haemoglobin (Hb) concentration was measured spectrophotometrically by cyanomethaemoglobin method.

Principle of the Test

Whole blood is diluted 1 in 201 in a modified Drabkin's solution which contains potassium ferricyanide and potassium cyanide. The red cells are haemolyzed and the haemoglobin is oxidized by a ferricyanide to methaemoglobin. This is converted by a cyanide to stable haemoglobincyanide (HiCN). Absorbance of the HiCN solution is read in a spectrophotometer at wavelength 540 nm or in a filter colorimeter using a yellow green filter.

Materials

EDTA anticoagulated blood, spectrophotometer, cuvette, timer, Drabkin's neutral diluting fluid, micropipette, cotton wool.

Procedure

1. 0.02 ml of well mixed EDTA anticoagulated blood was carefully dispensed into 4 ml Drabkin's neutral diluting fluid.
2. The solution in the tube was gently mixed with the blood and left at room temperature, protected from sunlight for 4-5 min.
3. The wavelength of the spectrophotometer was set at 540 nm. The Drabkin's fluid was used to zero the spectrophotometer and the absorbance of the patient's sample was read.

Calculation

$$\text{Hb} = \frac{\text{OD of Test} \times \text{Conc of standard} \times \text{Dilution factor}}{\text{OD of Std.}}$$

$$\text{Dilution factor} = \frac{1}{200} \div \frac{1}{200} = \frac{1}{200} \times \frac{200}{1} = 1$$

Platelet Count

The platelet count was obtained using the haemocytometer.

Principle of Test

Blood is diluted 1 in 20 in a filtered solution of ammonium oxalate reagent which lyses the red cells. Platelets are counted microscopically using an improved neubauer ruled counting chamber and the number of platelets per litre of blood calculated.

Materials

Improved neubauer ruled counting chamber, Ammonium oxalate reagent, Coverslips, Pipettes, Hand counter, EDTA anticoagulated blood, clean tubes, cotton wool.

Procedures

1. 0.38 ml of ammonium oxalate was dispensed into each of the tubes.
2. 200 µl (0.02 ml) of well mixed anticoagulated and blood was added to each tubes and mixed properly.
3. Using a Pasteur pipette, one of the grids of the chamber was filled with the sample.
4. The chamber was left undisturbed for 20 min to allow the cells to settle.
5. Using the 10x objective, the rulings were focused and the central square of the chamber was brought into view. The 40x objective was then used to focus the small platelets which appeared as small bright fragments.
6. The platelets in the small squares were counted and the number of platelets reported per litre of blood.

Calculation

$$\text{Counts/litre} = \frac{\text{Number of cells counted} \times \text{Dilution factor} \times 10^6}{\text{Volume counted}}$$

$$\text{Dilution factor (DF)} = 20$$

Volume counted = total area of squares counted x depth.

$$\text{Depth} = 0.1 \text{ mm}^3$$

Let the number of cells counted = N

$$\text{Counts/ litre} = \frac{N \times 20^* \times 10^6}{0.2^+ \times 0.1^{\mu}}$$

where * = 1 in 20 dilution of blood, + = 0.2 mm² area counted, μ = 0.1 mm depth of chamber.

Total White Blood Cell (TWBC) Count

The total white blood cell count was estimated using the haemocytometer.

Principle of Test

White blood cell is diluted 1 in 20 in an acid reagent which haemolyses the red cells (not the nucleus of the nucleated red cells), leaving the white cells to be counted. White cells are counted microscopically using an improved Neubauer ruled counting chamber (haemocytometer) and the number of WBC's per litre of blood calculated. (Cheesbrough, 2004).

Materials

Improved Neubauer ruled counting chamber, EDTA anticoagulated blood, White blood cell diluting fluid, Hand counter, Pasteur pipettes, Counting chamber, Timer, Clean tubes.

Procedure

1. 0.38 ml of the diluting fluid was measured into each of the tubes.
2. 20 μ l of EDTA anticoagulated blood was added to it and well mixed.
3. The counting chamber was assembled making sure that the central grid areas of the

chamber and the special haemocytometer cover glass are completely clean and dry.

4. The diluted blood sample was remixed and using a Pasteur pipette, the grids of the chamber were filled with the sample.
5. The chamber was left undisturbed for 2 min to allow time for the white blood cells to settle.
6. Using the 10x objective with the condenser iris closed sufficiently to give full contrast, the rulings of the chamber and white cells were focused until they appeared as small black dots.
7. The cells in the four large corner squares of the chamber were counted.
8. The number of the white cells per litre of blood was reported by dividing the total number of cells counted by 2 then divide the number obtained by 10.

Calculation

$$\text{WBC Count (per litre)} = \frac{\text{Cells counted} \times 20^* \times 10^6}{4^+ \times 0.1^{\mu}}$$

where * = 1 in 20 dilution of blood, + = 4 mm² area counted, μ = 0.1 mm depth of chamber.

$$\text{Count/litre} = \text{Cells counted} \times 50 \times 10^6 / \text{L}$$

DATA ANALYSIS

Table 1 shows the comparison of mean \pm standard deviation of group 1 and control.

Table 2 shows the comparison of mean \pm standard deviation of group 2 and control.

Table 3 shows the comparison of mean \pm standard deviation of group 3 and control.

Table 1: Comparison of Mean \pm Standard Deviation of Group 1 and Control

Parameters	Test	Control	Pvalue
PCV (l/l)	0.36 \pm 2.36	0.30 \pm 2.16	0.002*
Hb (g/dl)	8.80 \pm 0.83	8.57 \pm 0.44	0.724
Platelets(x10 ⁹ /l)	93.5 \pm 17.5	113.5 \pm 19.1	0.329
TWBC (X10 ⁹ /l)	8.4 \pm 0.32	7.9 \pm 1.1	0.497

Note: * P < 0.05 significant.
Key: PCV = Packed Cell Volume, Hb = Haemoglobin Concentration, WBC = White Blood Cell

Table 2: Comparison of Mean \pm Standard Deviation of Group 2 and Control

Parameters	Test	Control	Pvalue
PCV (l/l)	0.36 \pm 1.29	0.30 \pm 2.16	0.030*
Hb (g/dl)	9.75 \pm 0.52	8.57 \pm 0.44	0.001*
Platelets(x10 ⁹ /l)	135.0 \pm 18.4	113.5 \pm 19.1	0.134
TWBC (X10 ⁹ /l)	9.1 \pm 2.8	7.9 \pm 1.1	0.497

Table 3: Comparison of Mean \pm Standard Deviation of Group 3 and Control

Parameters	Test	Control	Pvalue
PCV (l/l)	0.38 \pm 2.22	0.30 \pm 2.16	0.019*
Hb (g/dl)	13.1 \pm 1.71	8.57 \pm 0.44	0.007*
Platelets(x10 ⁹ /l)	126.3 \pm 11.8	113.5 \pm 19.1	0.065
TWBC (X10 ⁹ /l)	9.8 \pm 3.1	7.9 \pm 1.1	0.416

RESULTS

The results of the effects of different concentrations of ethanol extracts of *Telfairia occidentalis* leaves on Packed Cell Volume, Haemoglobin (Hb) concentration, Total White Cell (TWBC) count, and platelets counts is shown on Table 1. Oral administration of the extract for one week resulted in significant increases in the PCV and Hb on rats treated with 300 mg/kg and 200 mg/kg of the extract with $p < 0.05$. The group 1 rats that received 100 mg/kg had a significant increase ($p < 0.05$) on the PCV only. There are no

significant increases on the platelets and total white blood cell counts in groups 1, 2, and 3 when compared with the values in group 4 (control) ($p < 0.05$).

DISCUSSION

The present study has shown that the ethanol extract of fluted pumpkin (*Telfairia occidentalis*) leaves caused significant increase in PCV and Haemoglobin (Hb) concentration on rats fed with the extract for 7 days. From the result, there is significant increase ($p < 0.05$) on the PCV and Hb

of rats served 300 mg/kg and 200 mg/kg of the extract and PCV of rats served 100 mg/kg of the extract.

The increases in the haematological indices observed in this study is consistent with the observation made when rats fed with the diet preparation of the air-dried leaves of *T. occidentalis* for 4 weeks (Alada, 2000). The result of this study differs from the report of Salman, *et al.* (2008). In their result, the aqueous extract of *Telfairia occidentalis* also significantly increased ($p < 0.05$) the WBC count. The increases in the haematological indices observed following treatment with *T. occidentalis* leaves ethanol extract might not be unconnected with the chemical composition of its leaves. The chemical composition has shown to include proteins, fat, vitamin A, thiamine, riboflavin, nicotinamide, vitamin C (Aletor *et al.*, 1995) and minerals such as zinc, iron, calcium and magnesium (Archibong, 2002). The amino acid profile of *T. occidentalis* had also been shown to be very rich and includes alanine, aspartate, glycine, glutamine, histidine, lysine, methionine, tryptophan, cysteine, leucine, arginine, serine, threonine, phenylalanine, valine, tyrosine and isoleucine (Fasuyi, 2006). Some of these constituents are well established haematopoietic factors that have direct influence on the production of blood in the bone marrow.

Moreover, the amino acids derived from *T. occidentalis* could also contribute to the increase in Hb concentration. The significant increase observed in this study is however inconsistent with the insignificant change in haematological parameters observed when birds were fed with the dietary preparation of the sun-dried leaves of the plant (Fasuyi *et al.*, 2007). The significant

change observed with the denaturing of active ingredients especially proteins in the leaves during exposure to sunlight.

CONCLUSION

The fluted pumpkin leaves extract is a good source of protein, amino acid, vitamins and mineral supplement. The concentration at which it is ingested plays a very important role. 300 mg/kg and 200 mg/kg of fluted pumpkin leaves extract had effects on the PCV and Hb of rats which fed on them by increasing the values of PCV and Hb when compared with the control group of rats that fed on feed and distilled water only. These increases were observed after 7 days of administration of the extract. It therefore follows that *Telfairia occidentalis* has haematopoietic properties.

RECOMMENDATION

Fluted pumpkin leaves is a highly nutritious vegetable which is predominantly grown by small-scale farmers and commonly consumed by majority of people. It is therefore advised that this haematinics, which has also been proven to be an anti-inflammatory, anticholesterolemic and antidiabetic farm product should be planted in the gardens and is recommended to be taken as food in very large quantities to boost blood production and fight anaemia.

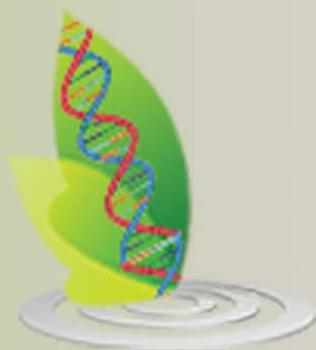
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