A Preliminary Assessment of *Cucurbita Maxima* Leaves from Cameroon on Haematological Parameters in Albino Rats

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**Abstract**

*Cucurbita maxima* (locally called pumpkin plant) are widely used in North West region of Cameroon as indigenous medicine and food for the management of anemia since time immemorial. The current study of this plant was undertaken to establish the effect of this plant on hematological parameters of albino rats and also to attempt to ascertain its safety for use. For this purpose, 25 g/day powdered plant in the ratio of 1:2 with the normal feed was given orally to the test mice. 1 tablet of Irofolate (200 mg of ferrous sulphate and 0.25 mg of folic acid) moistened in 25 g normal feed was administered orally/day to the positive control mice and the normal feed (25 g) moistened with clean water was administered orally/day to the negative control. All rats were observed daily for up to six days for clinical signs of physiological abnormalities. Twenty four hours following the last administration, all rats were killed and examined for gross pathological lesions and blood samples collected for hematological parameters. Hematological parameters showed a significant difference amongst the test group mice compared with the control group mice.

**Keywords:** *Cucurbita maxima*; Pumpkin; Albino rats; Hematological; HB; PCV; Cameroon

**Introduction**

Anemia is one of a wide spread public health problem in the world especially amongst the elderly and children. WHO estimates the number of anemia people worldwide to be a staggering 3.5 billion in the developing countries and that approximately 50% of all anemia can be attributed to iron deficiency. The global distribution of the disease burden of Iron deficiency anemia is heavily concentrated in Africa and WHO Regional Southeast Asia-D. These regions bear 71% of the global mortality burden and 65% of the disability-adjusted life years lost [1]. The most highly affected population groups in developing countries are pregnant women (56%), school age children (53%), non-pregnant women (44%), and preschool children (42%). However, another group that requires attention as well: older children, half of whom are anemic (51%). In industrialized countries, the most affected groups are pregnant women (18%) and preschool children (17%), followed by non pregnant women and older adults, both at 12%. A percentage of the population shows that such conditions as anemia, thrombocytopenia, leucopenia and low blood cell indices values are due to malnutrition. These conditions are most often seen in children especially those in rural areas [2].

Apart from children, anemia is a common disease condition in adults who are diagnosed with cancer or have undergone a surgical operation. The cost of treatment of post surgical infections in sub Saharan countries with some antibiotics as reported in Uganda, which is often expensive, can also lead to a plastic anemia [3].

Although there are various drugs used for the treatment of anemia in Cameroon, they are not affordable to many poor people, and this trend is similar in most developing countries [4-5]. In addition, the rural populations in various parts of the world do not have adequate access to high quality drugs for the treatment of anemia [6], so depend heavily on plants and herbal products for the treatment of diseases and anemia [7].

The plant kingdom has served as a source of shelter, nutrition, medicine, and completes the ecosystem of so many species of organisms. Plants have been reported to be widely used in most Cameroonians communities especially vegetables. Many plants have been shown to contain certain bioactive minerals and compounds that help to boast up systems in the body.

*Cucurbita maxima* commonly known as pumpkin, is one such plant that is frequently being used as food as well as traditional medicine for long days [8-9]. In 2001, Yongabi et al. reported the use of pumpkin seed extracts in the treatment of semen infection. *Cucurbita maxima* belongs to family cucurbitaceae. It is large climbing herb, annual or perennial. Its aerial part consists of flexible succulent stem with trifoliate leaves. It is widely cultivated throughout the India and other warm regions of world for use as vegetable as well as medicine.

According to Calli et al., it is used in most countries as anti-diabetic, antitumor, antihypertensive, anti-inflammatory, immunomodulatory and antibacterial agents [10]. This plant has also been shown to contain Vitamin A, beta-carotene, Thiamine (Vitamin B1), Riboflavin (VitaminB2), Niacin (Vitamin B3) Pantothenic acid (B5) Vitamin B6, Folate (Vitamin B9) Vitamin C, Vitamin E, Calcium, Iron, Magnesium, Phosphorus, Potassium, Sodium, and Zinc. Apart from just the ethnobotanical uses of pumpkin, herbal medicine in general is increasing popular and often the medicine of the common person [11-13].

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In this paper, the results of the evaluation of the effect of *Cucurbita maxima* leaves extract on the hematological indices of albino rats is presented.

**Materials and Methods**

**Research design**

An experimental design was used. Fresh leaves of *C. maxima* were collected from Kumbo (Bui Division, North West Region Cameroon) rinsed in clean water, dried and ground to powder form. It was then kept in an air tight container till use, kept away from sunlight.

Laboratory animals were fed with the test plant (*Cucurbita maxima*) with positive and negative controls set alongside for 6 days and bled for blood samples each. Samples were then analyzed for hematological values manually and with automation. Gross anatomy was done to verify for signs of organ toxicity. The entire study was done in the laboratory of Science for Life Foundation Bamenda, Cameroon, from April to May 2013.

**Methods**

A dose of 25 g/day/rat of *C. maxima* powdered leaf was considered to be the minimum dosage to be given per day. A total of 6 rats (of approximately the same age: 3.5 to 4 months and weight (300 g) grouped into 3 groups was identified as Test, Positive control and Negative control (i.e. 2 rats per group). To the negative control, only the feed (25 mg/group/day) was administered for the duration of feeding. The positive control mice received a mixture of 1 tablet of Irofolate (200 mg of ferrous sulphate and 0.25 mg of folic acid) and 25 g of normal feed/day. To the test group 1:2 preparations of 65 g of powdered plant were put into lithium heparin test tubes for hemato logical examination. All rats were examined carefully for gross physiological changes, with particular emphasis on the liver, kidney, lungs, heart and spleen.

**Hematological parameters**

All values were done using automatic analyzers and results confirmed by manual methods. The mean values for each parameter was established. All tests were done in duplicate to minimize error.

**Packed Cell Volume (PCV)**

The packed cell volume, also called haematocrit, was used to calculate the Mean Cell Volume (MCV) and Mean Cell Haemoglobin Concentration (MCHC). The packed cell volume is that proportion of whole blood occupied by red cells, expressed as a ratio. Anticoagulated blood in a glass capillary of specified length bore size, and wall thickness is centrifuged in a microhaematocrit centrifuge at 12000-15000 rpm for 3-5 minutes 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 microhaematocrit reader or calculated by dividing the height of the red cells column by the total column of blood.

**Methodology:**

To about three quarters, a plain capillary was filled with well mixed anticoagulated blood. Using a sealant material, the indicated end of the capillary tube was sealed. The filled capillary in one of the numbered slots of the microhaematocrit rotor was carefully located with the sealed end against the rim gasket (to prevent breakage). Centrifuge for 3-5 minutes (12000-15000 rpm), using the shorter time when the rpm is 15000 g. Immediately after centrifuging, the PCV was read off. Caution was exercised by first checking that there was no leakage of blood from the capillary or leakage. To read the PCV in a hand-held microhaematocrit reader, the base of the cell column (above the sealant) was aligned on the “O” line and the top of the plasma on line 100. The PCV read off from the scale. The reading point is the top of the red cell column, just below the buffy coat layer (WBC and platelets).

**Haemoglobin (Hb):**

Haemoglobin is the oxygen-carrying elements in the body. They are heterogeneous proteins produced in developing erythroblasts. Each human Haemoglobin (Hb) consists of a tetramer of 2α and 2β globin chains bound to a single heme moiety. Heme contains one ferrous iron (Fe²⁺) atom carried in a porphyrin ring.

**Methodology:** From the relation PCV = 3 × Hb, the Hb is directly calculated for each sample indicating that Hb = PCV/3.

\[
Hb (g/dl) = \frac{PCV(\%)}{3}
\]

**White Blood Cell (WBC):** In this case, the WBC count is used to investigate possible leucopenia or leukocytosis due to the presence of the feed, and not merely the investigation of infections.

**Principle:** Whole blood is diluted 1 in 20 in an acid reagent which haemolyses the red cells (not the nucleus of 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 per liter of blood calculated.

**Methodology:** A 0.35 ml of the diluting fluid into a term tube using the 1 ml graduated pipette was prepared. Using the 20 µl pipette 0.02 ml of a sample of capillary blood or EDTA venous blood was added. The blood was expelled 3 times in the diluting fluid by squeezing and releasing the rubber tubing. It was mixed by gently tapping the bottom of the tube a few times the dilution of the blood now was 1:20. The test tube was gently tapped to mix the diluted blood. The counting chamber was filled with the diluted blood using the Pasteur pipette. Air bubbles were avoided the counting chamber not over filled. The counting chamber was placed on the microscope stage to allow the cells to settle for 2 minutes. The counting Chamber was well focused. The cells in 4 large squares of the counting chamber were counted. The number of cells in 1 µl whole blood as follows was calculated as per the following equation:

\[
\text{WBC} / \mu l = \frac{\text{no of cells counted} \times \text{DF}}{\text{Area} \times \text{Depth of chamber}}
\]

**Platelets Count:**

**Principle:** The blood was diluted with a 1% ammonium oxalate solution which completely haemolysis the red cells living only the platelets to be counted in the counting chamber.

**Procedure:** A 0.38 ml of diluting fluid was pipette into a test tube, filled in the 20 µl pipette to the mark with blood and wiped off the outside of the pipette. The first drop of blood was wiped away. The content was expelled into the diluting fluid and washed out by drawing up the fluid and expelling it into the fluid a couple of times. The counting chamber was set up with its cover glass in position. Using
the Pasteur pipette, the counting chamber was filled with one large drop of the diluted blood. The counting chamber was placed on the microscope stage and using the 10 x objective locates the central square of the counting chamber. The platelets in 4 corner squares plus one middle square in the central roll area of the chamber were considered as per the following calculation:

**Calculation:**

\[
\text{Platelets} / \mu l = \frac{\text{No of Platelets counted} \times DF}{\text{Area} \times \text{Depth of chamber}}
\]

**Differential Count:** The examination of thin blood films in this case is important in the investigation of appearance and morphological changes in blood cells. The thin blood films were prepared, dried and fixed with 95 per cent methanol, and stained by Giemsa method. Thereafter, the slides were dried and viewed under the microscope using the oil immersion objective.

**Methodology:** The slide was placed on a staining rack and covered with methanol for 2-3 mins. The smear was covered with a 1 in 10 dilution of stock giemsa stain for 10 - 15 mins. It was then washed off the stain with clean water. The reverse side of the slide was cleaned and placed on a draining rack for the film to air-dry.

**MCV (Mean Cell Volume):** Information on red cell size was determined from the MCV, expressed in femtolitres (fl), was increased and when the cells were microcytic, the MCV was reduced.

**Haemoglobinization of red cells was with the terms:**

- **Normochromic:** Normal staining of red cells as seen when haemoglobinization was described. The cells usually contain a small area of central pallor (no more than one third of the cell’s diameter) due to the biconcavity of red cells.

- **Hypochromic:** Pale staining of red cells, as seen when haemoglobinization was evaluated. Hypochromic cells usually show an increased area of central pallor.

**MCH (Mean Cell Haemoglobin):** The amount of haemoglobin in picograms (pg) in an average red cell was described for both treatment and controls. It was taken that when red cells are hypochromic, the MCH is reduced and when the cells are macrocytic, the MCH is increased.

**MCHC (Mean Cell Haemoglobin Concentration):** The concentration of haemoglobin in g/l in 1 litre of packed red cells was evaluated for all the treatments. When red cells are hypochromic and microcytic, the MCHC is reduced.

**Calculations:**

- **MCV (Fl)**:
  \[
  \text{MCV (Fl)} = \frac{PCV (\%)}{RBC (in \text{ millions})} \times 10
  \]

- **MCH (Pg)**:
  \[
  \text{MCH (Pg)} = \frac{Hb \left(\frac{g}{dl}\right)}{RBC (in \text{ millions})} \times 10
  \]

- **MCHC (%)**:
  \[
  \text{MCHC} (%) = \frac{Hb \left(\frac{g}{dl}\right)}{PCV (\%)} \times 100
  \]

**Results**

After 6 days of administration, gross anatomical examination did not show any sign of toxicity. Some haematological parameters were not significantly different amongst tests and control groups Table 1 except for values of Hb, PCV and RBC count which were significantly different amongst the test and control groups. Figures 1 and 2.

**Discussion**

This study has revealed the medical importance of this plant
through its anti anemic activity by boasting the Hb, RBC, HCT and MCHC values in the test rats as shown in Table 1. Significant elevation in the levels of Hb, RBC, HCT and MCHC agrees with previous results reported by other authors on the rich phytochemistry of the plant. Analyses of the plants suggest that the plant contains Vitamin A, beta-carotene, Thiamine (Vitamin B1), Riboflavin (Vitamin B2), Niacin (Vitamin B3) Pantothenic acid (B5) Vitamin B6, Folate (Vitamin B9) Vitamin C, Vitamin E, Calcium, Iron, Magnesium, Phosphorus, Potassium, Sodium, and Zinc significantly Iron, Vitamin B6, B9 and other bioactive ingredients. These ingredients are vital for the formation of RBC and Hb [3].

From Table 1, the WBC count shows increase counts coupled with increase platelet counts probably due to the stress and trauma the mice went through during exsanguinations. The Giemsa stained blood smears for the test and control groups showed microcytic cells confirming the low MCV (Table 1) values for their samples even after manual calculations. The MCHC values for the two showed a normal range (Table 1). The reason behind the reduction in the MCV value is as a result of inadequate vitamin B12 supply in the test mice since the plant is reported to be deficient in Vitamin B12.

MCV and MCHC values in the negative control mice showed increase ranges (Table 1) confirming the macrocytic cells in the Giemsa stained smears. Gross anatomical examination of organs in situ after dissection revealed no significant change from the control mice indicative of the fact those C. maxima was is not toxic to the cells of the mice. The conclusion from this study is that C. maxima reduce anemia and is not toxic when consumed. It is therefore recommended for nutritional support for patients with acute anemia in Cameroon.

**Conclusion**

A number of conclusions can be drawn from this study: One is that C. maxima reduces anemia as shown in the elevated values of Hb, PCV and MCHC in the test rats. Another is that C. maximum is non toxic when consumed, as no sign of toxicity was observed in the test rats. This conclusion also lends credence to the ethno botanical evidence collected from the field, which suggest that the plant has been used as a vegetable in many soup recipes in most cultures in Cameroon since antiquity. The high cocktail of minerals such as potassium, Calcium and Iron may have served as a precursor to the synthesis of hemoglobin. It is, therefore, recommended for nutritional support for patients with acute anemia in Cameroon.

**References**