



## **Consumption of Tiger Nut (*Cyperus esculentus* L.) Improves Haematopoiesis in Wistar Rats**

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### **Authors' contributions**

*This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.*

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### **ABSTRACT**

**Background:** The consumption of tiger nut has often been relegated only to the poor due to its affordability.

**Aim:** This study is therefore aimed at assessing its effect on haematological parameters in Wistar rats.

**Materials and Methods:** Twenty-four adult Wistar rats were divided into four groups of six each. Animals in group A were administered normal saline solution while those in groups B, C and D were administered 1, 2 and 4 mL/kg body weight of undiluted tiger nut milk respectively for twenty-eight days. The administration was done 12 hourly via the oral route. At the end of the administration, the rats were sacrificed after an overnight fast under diethyl ether as anesthesia. Blood samples were collected via cardiac puncture. Haematological parameters were determined using standard methods.

**Results:** A dose-dependent increase was observed in packed cell volume (PCV), haemoglobin (Hb), red blood cell (RBC) and mean corpuscular haemoglobin concentration (MCHC) of animals treated with tiger nut milk when compared with those in animals in the control group. The increase was significant ( $p < 0.05$ ) at all instances except in the PCV of animals treated with 1 mL/kg body weight of tiger nut milk that were not significantly different from those in control animals. However,

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tiger nut milk had no significant ( $p < 0.05$ ) effect on mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) when compared with those in control animals. Tiger nut milk administration was observed to increase white blood cell (WBC), lymphocyte, neutrophil and platelet in a dose-dependent manner when experimental animals were compared with those in the control group. The increase was significant ( $p < 0.05$ ) at all instances except in the lymphocyte and neutrophil of animals treated with 1 mL/kg body weight of tiger nut milk that were not significantly ( $p < 0.05$ ) different from those in control animals.

**Conclusion:** Results of this present study indicate that tiger nut possesses haematopoietic properties and can be used to boost blood levels especially in anaemia patients. Furthermore, the plant possessed the ability to boost the immune system and thus defend the body against xenobiotics.

**Keywords:** Haematopoiesis; immune system; tiger nut; stress-resistance;

## 1. INTRODUCTION

Haematopoiesis is the production of all types of blood cells including formation, development, and differentiation of blood cells [1]. Prenatally, hematopoiesis occurs in the yolk sack, then in the liver, and lastly in the bone marrow. In the normal situation, hematopoiesis in adults occurs in the bone marrow and lymphatic tissues [2]. All types of blood cells are derived from primitive cells (stem cells) that are pluripotent (they have the potential to develop into all types of blood cells). In a healthy adult person, approximately  $10^{11}$ – $10^{12}$  new blood cells are produced daily in order to maintain steady-state levels in the peripheral circulation [1]. Anaemia increases in prevalence and severity as renal function decreases, it becomes much more common at reduced glomerular filtration rate. Depending on the severity, some of the symptoms of anaemia may include: pale skin, fatigue, weakness, loss of appetite, low haematocrit and hemoglobin in a RBC etc. Factors likely to contribute to anaemia in chronic kidney diseases include blood loss, shortened red cell life span, vitamin deficiencies, the “uremic milieu,” erythropoietin (EPO) deficiency, iron deficiency and inflammation [3]. However, the typical “anaemia of chronic renal insufficiency” is a result of a decreased production of red blood cells by the bone marrow. This defect in red blood cell production is largely explained by the inability of the failing kidneys to secrete hormone erythropoietin. This hormone is a necessary stimulus for the normal bone marrow to produce red blood cells [4].

*Cyperus esculentus* L. (also called tiger nut, chufa, atadwe, yellow nutsedge, and earth almond) is a crop of the sedge family widespread across much of the world. It is found in most of the Eastern Hemisphere, including Southern Europe, Africa, as well as the Middle East and the Indian subcontinent [5]. In Nigeria, tiger nut is

called Ofio by the “Yorubas”, “Akiausa” by the Igbos and “Aya” by the Hausas. It is cultivated for its edible tubers, called earth almonds or tiger nuts, as a snack food and for the preparation of *horchata de chufa*, a sweet, milk-like beverage. Tiger nut can be found wild, as a weed, or as a crop. It is an invasive species outside its native range, and is readily transported accidentally to become invasive. In many countries, tiger nut is considered a weed [6]. It is often found in wet soils such as rice paddies and peanut farms as well as well-irrigated lawns and golf courses during warm weather. The Scientific classification of *C. esculentus* is:

Kingdom	Plantae
Clade:	Tracheophytes
Clade:	Angiosperms
Clade:	Monocots
Clade:	Commelinids
Order:	Poales
Family:	Cyperaceae
Genus:	<i>Cyperus</i>
Species:	<i>C. esculentus</i>



**Fig. 1. Tiger nut [7]**

Despite its name, tiger nut is a tuber. However, its chemical composition shares characteristics with tubers and with nuts. This tuber is rich in

energy content (starch, fat, sugar, and protein), and dietary minerals (mainly phosphorus and potassium). The oil of the tuber was found to contain 18% saturated (palmitic acid and stearic acid) and 82% unsaturated (oleic acid and linoleic acid) fatty acids [8].

Tiger nut is a good source of calcium, iron, magnesium, phosphorus, ascorbic acid (Vitamin C), tocopherol (Vitamin E), dietary fibre as well as fats like oleic acid [8]. Tiger nut can be used to make a drink called kunnu aya by the Hausas. Tiger nut can be eaten raw, roasted, dried, baked or be made into a refreshing beverage called tiger nut drink [8]. A good way of incorporating the nut into your diet for those asking how to eat tiger nut is by eating them or by blending it and draining to make Kunnu Aya (tiger nut milk) which can also be sweetened with honey or date. Ogbuagu and Airaodion [7] recently reported that tiger nut milk boosts male fertility. This study is therefore aimed at assessing the effect of consumption of tiger nut on haematological parameters of Wistar rats.

## **2. MATERIALS AND METHODS**

### **2.1 Extraction of Milk**

Fresh tiger nut was purchased from a local market in Orita-Challenge Area of Ibadan, Nigeria. They were thoroughly washed in running tap water to remove contamination. The milk was extracted daily using electric juice extractor, and was preserved in the refrigerator at 4 °C before usage.

### **2.2 Experimental Design and Animal Treatment**

Twenty-four adult male Wistar rats with body weight between 150 and 180 g were used for this study. They were acclimatized for 7 days during which they were fed ad libitum with standard feed and drinking water and were housed in clean cages placed in well-ventilated housing conditions (under humid tropical conditions) throughout the experiment. All the animals received humane care according to the criteria outlined in the 'Guide for the Care and Use of Laboratory Animals' prepared by the National Academy of Science and published by the National Institute of Health. They were randomly divided into four groups of six rats each. Animals in group A were administered normal saline solution while those in groups B, C and D were

administered 1, 2 and 4 mL/kg body weight of undiluted tiger nut milk respectively for twenty-eight days. The administration was done 12 hourly via the oral route. At the end of the administration, the rats were sacrificed after an overnight fast under diethyl ether as anesthesia. Blood samples were collected by cardiac puncture into heparinized bottles. The blood samples were centrifuge for 10 minutes using a bench-top centrifuge (Centromix) and the supernatant plasma was then used for the determinations of the biochemical parameters.

### **2.3 Determination of Haematological Parameters**

The red blood cells (RBC) and white blood cells (WBC) counts were determined by the improved Neubauer haemocytometer method [9]. The haemoglobin (Hb) concentration was determined according to Jain [10], using the cyanomethaemoglobin method. The packed cell volume (PCV) was determined by the microhaematocrit method according to Dacie and Lewis [11]. Schilling method of differential leukocyte count was used to determine the distribution of the various white blood cells [12]. Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were computed according to Jain [10].

### **2.4 Statistical Analysis**

Data were subjected to analysis of variance using Graph Pad Prism. Results were presented as Mean  $\pm$  standard deviation. One way analysis of variance (ANOVA) was used for comparison of the means followed by Tukey's (HSD) multiple comparison test. Differences between means were considered to be significant at  $p < 0.05$ .

## **3. RESULTS**

A dose-dependent increase was observed in packed cell volume (PCV), haemoglobin (Hb), red blood cell (RBC) and mean corpuscular haemoglobin concentration (MCHC) of animals treated with tiger nut milk when compared with those in animals in the control group. The increase was significant ( $p < 0.05$ ) at all instances except in the PCV of animals treated with 1 mL/kg body weight of tiger nut milk that were not significantly different from those in control animals. However, tiger nut milk had no

significant ( $p < 0.05$ ) effect on mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) when compared with those in control animals (Table 1).

Tiger nut milk administration was observed to increase white blood cell (WBC), lymphocyte, neutrophil and platelet in a dose-dependent manner when experimental animals were compared with those in the control group (Table 2). The increase was significant ( $p < 0.05$ ) at all instances except in the lymphocyte and neutrophil of animals treated with 1 mL/kg body weight of tiger nut milk that were not significantly different from those in control animals at  $p < 0.05$ .

#### 4. DISCUSSION

In this study, a dose-dependent increase was observed in packed cell volume (PCV), haemoglobin (Hb), red blood cell (RBC) and mean corpuscular haemoglobin concentration (MCHC) of animals treated with tiger nut milk when compared with those in animals in the control group. The increase was significant ( $p < 0.05$ ) at all instances except in the PCV of animals treated with 1 mL/kg body weight of tiger nut milk that were not significantly different from those in control animals. However, tiger nut milk that were not significantly different from those in control animals. However, tiger nut milk had no significant ( $p < 0.05$ ) effect on mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) when compared with those in control animals as presented in Table 1. This might be an indication that there may be increased production of red blood cells; thus, suggesting the non-toxic nature of the plant to

red blood cells at this period of administration. This effect might be due to the high phytochemical content of tiger nut reported by Oguwike et al. [13].

The increase in the blood levels of erythrocyte parameters observed in this study might be suggestive that tiger nut possesses possible potentials to enhance erythropoietin release from the kidneys, which is the humoral regulator of RBC production and also affect the oxygen-carrying capacity of the blood and the amount of oxygen delivered to the tissues since red blood cells and haemoglobin (Hb) are very important in transferring respiratory gases [14,15]. This may be due to the high content of iron and proteins in the plant as reported by Bamishaiye et al. [16]. It is therefore possible that consumption of tiger nut by humans can help prevent anaemia especially in menstruating and pregnant women.

It has also been reported that values of RBC and associated parameters lower than normal ranges are indicative of anemic conditions while higher values are suggestive of polycythemia [17,18], thus, the 28-day treatment of animals with tiger nut milk may not have the potential to induce anemia nor polycythemia. This is in agreement with the findings of Bamishaiye et al. [16] who investigated the haematological parameters of albino rats fed on tiger nuts tuber oil meal-based diet. It is also consistent with the observation of Hassan [19] who studied the potential effect of tiger nut oil on some haemato-biochemical blood indices in male albino rats. Since the increase in the erythrocyte parameters observed in this study is dose-dependent, it therefore implies that a relatively high dose will yield better haematopoietic effect.

**Table 1. Effect of tiger nut milk on Erythrocyte parameters in Wistar rats after 28 days of administration**

Parameters	Control	1 mL/kg body weight of tiger nut milk	2 mL/kg body weight of tiger nut milk	4 mL/kg body weight of Tiger nut milk
PCV (%)	37.08±3.45 <sup>a</sup>	40.23±3.17 <sup>ab</sup>	46.03±2.94 <sup>b</sup>	59.25±6.11 <sup>c</sup>
Hb (g/dL)	9.41±0.47 <sup>a</sup>	12.44±1.05 <sup>b</sup>	14.52±0.93 <sup>bc</sup>	19.52±2.01 <sup>c</sup>
RBC (X10 <sup>12</sup> /L)	6.79±0.67 <sup>a</sup>	8.23±0.64 <sup>b</sup>	12.82±0.63 <sup>c</sup>	16.04±0.94 <sup>d</sup>
MCV (fL)	54.65±6.80 <sup>a</sup>	52.53±3.55 <sup>a</sup>	53.05±4.07 <sup>a</sup>	58.09±1.99 <sup>a</sup>
MCH (pg)	14.87±1.19 <sup>a</sup>	12.22±1.11 <sup>a</sup>	14.00±1.32 <sup>a</sup>	11.03±0.73 <sup>a</sup>
MCHC (g/dL)	25.38±2.74 <sup>a</sup>	31.00±1.68 <sup>b</sup>	33.88±4.04 <sup>bc</sup>	38.99±3.77 <sup>c</sup>

Values are presented as Mean±standard deviation with  $n = 6$ . Values with different superscript along the same row are significantly different at  $p < 0.05$ .

**LEGEND:** PCV = Packed Cell Volume; Hb = Haemoglobin; RBC = Red Blood Cell; MCV = Mean Corpuscular Volume; MCH = Mean Corpuscular Haemoglobin; MCHC = Mean Corpuscular Haemoglobin Concentration

**Table 2. Effects of tiger nut milk on White Blood Cells parameters and Platelets in Wistar rats after 28 days of administration**

Parameters	Control	1 mL/kg body weight of tiger nut milk	2 mL/kg body weight of tiger nut milk	4 mL/kg body weight of tiger nut milk
WBC (X10 <sup>9</sup> /L)	13.02±1.96 <sup>a</sup>	15.33±1.31 <sup>b</sup>	18.03±2.11 <sup>c</sup>	20.99±1.06 <sup>d</sup>
Lymphocyte (%)	38.08±6.79 <sup>a</sup>	41.46±5.01 <sup>ab</sup>	48.87±3.82 <sup>b</sup>	55.62±2.93 <sup>c</sup>
Neutrophil (%)	61.98±6.79 <sup>a</sup>	64.00±2.76 <sup>ab</sup>	69.72±3.83 <sup>bc</sup>	72.72±4.84 <sup>c</sup>
Platelet (X10 <sup>9</sup> /L)	418.02±1.96 <sup>a</sup>	423.22±7.83 <sup>b</sup>	428.24±9.63 <sup>c</sup>	431.63±7.32 <sup>d</sup>

Values are presented as Mean±standard deviation with n = 6. Values with different superscript along the same row are significantly different at p<0.05. WBC = White Blood Cell

Packed cell volume is the volume by percentage of red cells in whole blood. The increase in the content of haematological parameters of the blood of rats administered tiger nut milk for 28 days is an indication that the oxygen carrying capacity of the animals' blood is increased. The major function of the red blood cells is to transport haemoglobin, which in turn carries oxygen from the lungs to the tissues [20]. The Hb concentrations and haematocrit are values revealing the degree of anemia while the MCHC is a useful index of the average Hb concentration of the red cells [21]. Generally, low readings for RBC, Hb and hematocrit can indicate anaemia. According to Robins [22], in a toxic environment, RBC count significantly decreased but such was not observed in this study. The haemoglobin and packed cell volume of experimental rats were increased suggesting adequate iron status. This could be associated with the iron content of tiger nut which is a good source of non-heme iron [16,22]. Non-heme iron from plant sources has been reported not to be as readily available to the body as heme iron from animal sources which are well absorbed [23].

Tiger nut milk administration was observed to increase white blood cell (WBC), lymphocyte, neutrophil and platelet in a dose-dependent manner when experimental animals were compared with those in the control group (Table 2). The increase was significant (p<0.05) at all instances except in the lymphocyte and neutrophil of animals treated with 1 mL/kg body weight of tiger nut milk that were not significantly different from those in control animals at p<0.05. White blood cells, platelet, neutrophil, and lymphocytes are used to provide useful information for diagnosis in the routine clinical evaluation of the state of health of a patient [24]. Changes in the haematological system have a higher predictive value for human toxicity [25].

It has been emphasized that the high percentage of WBC especially lymphocytes are associated

with the ability of the animals to perform well under very stressful conditions [9]. This increase in the WBC and percentage lymphocyte counts suggests that the phytochemical compounds present in tiger nut elicited stress responses. The effect of this plant on the total WBC count could be due to the presence of flavonoid. This compound has an anti-inflammatory property and so has vital effect on inflammatory processes of some pathological states such as bacterial infection, malaria and liver diseases [9,26]. This might also imply that tiger nut milk may strengthen the immune system through many cytokines regulation. Tiger nut has also been reported to contain a good quantity of vitamin B<sub>1</sub> [13]. This might assist in balancing the central nervous system and helps the body to adapt to stress.

The significant increase observed in the neutrophil of experimental animals might be suggestive that consumption of tiger nut enhanced the body's ability to attack and destroy invading bacteria, viruses and other injurious agents (Phagocytosis). The significant increase in platelet count at p<0.05 observed in animals treated with tiger nut milk may be an indication that the plant has the propensity to stimulate the platelet-activating factor (PAF) and thus the blood clotting potentials. It could also be an indication that it has the potential to stimulate thrombopoietin production [27,28]. Moreover, the obtained positive change in haematological parameters in rats treated with tiger nut may be attributed to its high contents of unsaturated fatty acids which have been reported to improve certain haematological parameters [19].

## 5. CONCLUSION

The results of this present study indicate that tiger nut have haematopoietic properties and can be used to boost blood level especially in anaemia patients. Furthermore, the plant possessed the ability to boost the immune

system and thus defend the body against xenobiotics.

## CONSENT

It is not applicable.

## ETHICAL APPROVAL

Animal ethic Committee approval has been collected and preserved by the author.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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