



# Anti-anaemic and Anti-leukopenic Effects of Hydromethanolic Extracts of Tiger Nut and Ginger on Alloxan-induced Diabetic Rats

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## Authors' contributions

This work was carried out in collaboration among all authors. All the authors contributed to the success of the work and its publication. Author SCI designed and supervised the work and edited the initial drafts.

Authors KAU and FON participated in carrying out the entire work and they wrote the first draft. Author FON also contributed in editing the drafts. Authors WCO and SAO participated in collecting the materials used and in the statistical analysis. All authors read and approved the final manuscript.

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## ABSTRACT

**Aims:** This study investigated the anti-anaemic and anti-leukopenic effects of hydromethanolic (1:4) extracts of *Cyperus esculentus* (Tiger nut) and *Zingiber officinale* (Ginger) on the alloxan-induced diabetic albino rats.

**Place:** The *in vivo* study took place in the Department of Biochemistry, Micheal Okpara University of Agriculture Umudike, Abia state.

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**Methods:** Fresh *Cyperus esculentus* and *Zingiber officinale* was air dried at room temperature, grounded into fine powder and extracted with hydromethanol in ratio of 20:80 using Soxhlet extraction method. The albino rats (total number of 36) were randomly divided into 6 groups of 6 rats each: Group 1 (Normal control); Group 2 (Diabetic); Group 3 (Glibenclamide); Group 4 (500mg/dl *Cyperus esculentus*); Group 5 (500mg/dl *Zingiber officinale*) and Group 6 (250mg/dl *Cyperus esculentus* + 250 mg/dl *Zingiber officinale*, 50:50) were administered orally. Group 2 to 6 were administered with 160mg/kg alloxan monohydrate intraperitoneally to induce a diabetic state. The haematological parameters determined were PCV, HB, RBC, TWBC, MCV, MCH and MCHC.

**Results:** The diabetic group treated with the Glibenclamide and 500 mg/kg *Zingiber officinale* extract had significant recovery (compared with the normal control) from the diabetic- induced depletion of Hb, PCV and RBC than the diabetic group treated with 250 mg/kg *Cyperus esculentus* + 250 mg/kg *Zingiber officinale* extracts. 500 mg/kg *Cyperus esculentus* extract showed the least recovery from diabetic induced anaemia.

**Conclusion:** Ginger extract showed value in the recovery from anaemia and leucopenia associated with diabetes having significantly improved the values of RBC, PCV, haemoglobin and TWBC in the diabetic rats more than the effects of Tigernut and combined (ginger and tigernut) extracts. Ginger extract treatment might also increase the defense mechanism of the body against infections in diabetes.

**Keywords:** Antidiabetic; Anti-anaemic; Anti-leucopaenic; *Z. officinale*; *C. esculentus*.

## ABBREVIATIONS

PCV : Packed Cell Volume,  
HB : Haemoglobin,  
RBC : Red Blood Cell,  
TWBC : Total white Blood Cell Count,  
MCV : Mean Cell Volume,  
MCH : Mean Cell Haemoglobin  
MCHC : Mean Cell Haemoglobin Concentration.

## 1. INTRODUCTION

Haematological parameters are measurements that are related to the blood and blood-forming organs. There is need to monitor haematological parameters in diabetic subjects [1]. Several hematological changes affecting the red blood cells (RBCs), white blood cells (WBCs), and the coagulation factors are shown to be directly associated with Diabetes mellitus [2] other hematological abnormalities reported in Diabetes mellitus patients include RBCs, WBCs, and platelet dysfunction [3,4]. Anaemia in diabetes mellitus may be due to the oxidative destruction of circulating red blood cells by the administered alloxan monohydrate [5] had reported that in diabetics, hypochromic anaemia may be due to falling in the body's iron content caused by increasing oxidation.

*Cyperus esculentus* (Tiger nut.) also called 'chufa sedge', is a tuber known under various names such as nut grass, earth or ground almond, yellow nut and edible galingale. It is also called "ayaya" in Hausa, "ofio" in Yoruba [6,7] and "Aki-Hausa" in Igbo. It is commonly used as a healthy food for humans and animals in some

parts of the world like Africa, Europe and America [8]. *Cyperus esculentus* has been shown to contain alkaloid, saponin, flavonoid, glycoside, tannin, carbohydrates, reducing sugar, and phenols [9]. Flavonoids are phenolic substances known to be synthesized by plants which possess some biological properties such as anti-inflammation, anti-diabetic, and cell proliferation activities. Alkaloids, saponins, and tannins are known to have antimicrobial activity, as well as other physiological activities [10,11]. *Cyperus esculentus* contains some minerals such as P, K, Ca, Fe, Zn, Mg, traces of Cu and Mn as well as some vitamins like E and C. In addition, high content of soluble glucose and unsaturated fatty acids (oleic acid and linoleic acid) [12]. The medicinal properties of *Cyperus esculentus* are rarely discussed, although its usage in orthodox activities is well known [13].

*Zingiber officinale*, commonly known as Ginger, is an ancient spice consumed worldwide for culinary and medicinal purposes. It is also called "Jinja" in Igbo, "Cithar" in Hausa and "Atale" in Yoruba. The plant has a number of chemicals responsible for its medicinal properties, such as anti-arthritis, anti-inflammatory, anti-oxidant, anti-diabetic, antibacterial, antifungal, anticancer, etc. [14,15,16,17]. The phytochemical composition of the *Z. officinale* has been extensively studied in the past studies. *Z. officinale* is reported to possess essential oils, phenolic compounds, flavonoids, carbohydrates, proteins, alkaloids, glycosides, saponins, steroids, terpenoids and tannin as the major phytochemical groups. These

phytochemicals play an important role in the medicinal property of this plant [18,19].

This study focused on determining and comparing the anti-anaemic and anti-leucopaenic potentials of *Cyperus esculentus* (Tiger nut) and *Zingiber officinale* (Ginger) extracts in alloxan-induced diabetic Wistar rats.

## 2. MATERIALS AND METHODS

### 2.1 Plants

Fresh *Cyperus esculentus* and *Zingiber officinale* were obtained from Ekeonunwa, Owerri municipal in Imo state, Nigeria, and identified at the Botany Department, Micheal Okpara University of Agriculture Umudike.

### 2.2 Chemicals

Chemicals used in this study included Methanol (extra pure) Manufactured by Loba Chemie Pvt. Ltd. 107 Wodehouse road, Mumbai 400005, India, Alloxan monohydrate Manufactured by Qualikems fine Chem Pvt. Ltd. Plot no. 68/69, G.I.D.C. Industrial estate, Nandesari, Vadodara, Gujarat. Glibenclamide Manufactured by Nigeria German Chemicals Plc. Km 38 Abeokuta express way, Otta, Ogun state, Nigeria.

All chemicals were purchased from Body Scientific Chemicals Aba Nigeria.

### 2.3 Equipment

The equipment used was obtained from the Department of Biochemistry, Micheal Okpara University of Agriculture Umudike, Abia state.

They include a thermometer, micropipette (perfect, USA), refrigerator (Haier thermocool, England), spectrophotometer, syringe, water bath, weighing balance, petri dish, glassware, beakers, capillary tube, micro-haematocrit centrifuge, Soxhlet extractor, hemocytometer, test tubes, gavage, glucometer (Accucheck), glucose stripes.

### 2.4 Preparation of Extracts

Extracted liquid was obtained from the grounded air-dried *Cyperus esculentus* and *Zingiber officinale* using the Soxhlet extractor as adapted from [20]. They were extracted with 350ml of hydromethanol which consists of 20% water and 80% of methanol. After which was put in the oven for 40°C and allowed to evaporate to form a semi Solid.

### 2.5 Animals

The experimental animals used for the study were 36 Wistar albino rats (*Rattus norvegicus*)

with a weight of (90-117g). The rats were obtained from the Animal holding unit of the Department of Biochemistry, Micheal Okpara University of Agriculture Umudike. The animals were kept in well-ventilated and clean cages at an average room temperature of 30°C. The rats were fed with cubes produced by Livestock Feeds PLC and bought from the local shop and water ad libitum which was changed daily. The rats were allowed to acclimatize to the new environment for 7 days.

The toxicity study was also carried out with an additional 36 albino Wistar rats.

#### 2.5.1 Alloxan-Induced diabetic animal model

Albino Wistar rats weighed (90-117g) after fasting for 24hrs. Animals in the diabetic group were subjected to a single intraperitoneal injection of alloxan monohydrate at 160 mg/kg body weight [21], freshly dissolved in sterile distilled water. The rats were given a 5% glucose solution after 12 hours of alloxan injection to drink overnight to counter hypoglycemic shock. 4 days after the alloxan injection, fasting blood glucose (FBG) was determined using a glucometer with the model (Accu-chek active, Roche Diagnostic). Rats showing FBG above 200 mg/dl were considered diabetic, as described by [22].

#### 2.5.2 Administered amount of Alloxan in ml

1g of alloxan dissolved in 20 mls of distilled H<sub>2</sub>O  $\rightarrow$  1g/20ml = 0.05g/ml  $\times$  1000 = 50mg/ml

For every animal weighed (dose administered) = Alloxan dosage (mg) X body weight(g) / Concentration X 1000 (Eq 1)

Alloxan dosage for a 117g of rat = 160mg/Kg X 117g / 1000g = 18.2mg

Amount of alloxan per dose = 50mg is contained in 1ml

18.20mg = 1ml/50mg X 18.20mg = 0.37ml

Therefore, each rat was given 0.4ml of the Alloxan solution.

### 2.6 Acute Toxicity Study/ Determination of Median Lethal Dose (LD<sub>50</sub>)

This was done using the Lorke method [23]. A total of 36 Wistar rats (18 rats each for *Cyperus esculentus* and *Zingiber officinale*) were used for the study. The study was carried out in two phases for each extract (*Cyperus esculentus* and

*Zingiber officinale*). In the first phase, three groups; A-C of 3 rats each were orally gavaged with distilled water (10 ml/kg), 10 mg/kg, 100 mg/kg, and 1000mg/kg (lower doses) of the tiger nuts extract respectively. Rats in group A served as the normal control. The rats were observed for signs and symptoms of toxicity and mortality over a period of 24 hours. The same procedure was repeated with the ginger extract.

In the second phase, three groups; with 3 rats in each group, were given (orally) distilled water, 1600 mg/kg, 2900 mg/kg, and 5000mg/kg (higher doses) of the extracts respectively. Rats in Group 1 served as the normal control. The rats were observed for 24 hours, post-administration. The same procedure was followed with the ginger extract.

In each phase of the acute study, the rats were allowed for another 7 days to observe any delayed toxicity. The median lethal dose (LD<sub>50</sub>) of the two extracts was then calculated using the formula adopted by [24].

$$LD_{50} = \sqrt{\frac{\text{Least dose with mortality} \times \text{Highest dose without mortality}}{\text{Eq 2}}}$$

## 2.7 Experimental Design

The rats were divided into 6 groups of six rats each: Group 1: Normal control rats were administered with 1 ml of distilled water once daily for 14 days. Group 2: Diabetic control rats were administered with 1 ml of distilled water once daily for 14 days. Group 3: Diabetic rats were treated with *Cyperus esculentus* extract (500 mg/kg body weight/day) for 14 days. Group 4: Diabetic rats were treated with *Zingiber officinale* extract (500 mg/kg body weight/day) for 14 days. Group 5: Diabetic rats were treated with both *Cyperus esculentus* and *Zingiber officinale* extract (50:50) (500 + 500mg/kg) for 14 days. Group 6: Diabetic rats were treated with Glibenclamide (5 mg/kg body weight/day) for 14 days.

Extracts of tiger nut, ginger, and Glibenclamide were administered to the experimental albino rats based on design groups through the oropharyngeal cannula for 14 days. Their respective body weight sample was taken before and after treatment of oral administration of drugs [25].

## 2.8 Collection of Blood Samples

Blood samples were taken from each rat by terminal bleeding from the heart and transferred into a clean EDTA container (thoroughly mixed) ready for haematological investigations.

## 2.9 Evaluation of Haematological Parameters

The Haematological parameters evaluated following (25) were: haematocrit (Packed cell volume), Haemoglobin Concentration, Red Blood Cell Count, Total White Blood Cell Count, Mean cell volume, Mean cell haemoglobin, and Mean cell haemoglobin concentration.

## 2.10 Statistical Analysis

All the data were tested for normally using Mann-Whitney's normality test. Statistical comparisons were performed for haematology and glucose levels using one-way analysis of variance (ANOVA) followed by Duncan's multiple range tests to separate the mean. The results were presented in tables and expressed as mean ± SEM and p-value less than 0.05 (p<0.05) was considered statistically significant. The data were analyzed using SPSS version 23.0.

## 3. RESULTS

Table 1 represents the acute toxicity test of *Cyperus esculentus* and *Zingiber officinale* on albino Wistar rats at different doses. The acute toxicity test of *Cyperus esculentus* and *Zingiber officinale* extracts showed no death in both phases of the experiment at different doses.

**Table 1. Acute toxicity test of *Cyperus esculentus* (C.E) and *Zingiber officinale* (Z.O)**

Phase 1	Number of rats	Dosages mg/kg body weight	Mortality of C.E	Mortality of Z.O
Group A	3	10mg/kg	0/3	0/3
Group B	3	100mg/kg	0/3	0/3
Group C	3	1000mg/kg	0/3	0/3
Phase 2				
Group A	3	1600mg/kg	0/3	0/3
Group B	3	2900mg/kg	0/3	0/3
Group C	3	5000mg/kg	0/3	0/3

#### 4. DISCUSSION

Most of the natural plant extracts used in traditional medicine are believed to be safe, compared to synthetic drugs, even when there are no toxicological records or scientific evidence to this belief [26], hence the need to carry out acute toxicological studies on the two extracts (tiger nut and ginger) using rats.

As shown in Table 1, after 24 hours post administration of the tigernut and ginger extracts, at both high and low doses no death was recorded in all the extract groups and the distilled water group. Therefore, the result of the LD<sub>50</sub> of the two extracts were estimated to be ≥ 5000mg/kg body weight in mouse which is a likely indication that the two extracts are non-toxic. It is important to recall that the Organization of Economic Cooperation and Development (OECD), Paris, recommended LD<sub>50</sub> value < 50mg/kg as very toxic; > 50 ≤ 500 mg/kg as harmful; > 500 ≤ 2000 mg/kg as not harmful or toxic; and ≥ 5000 mg/kg body weight as very safe [27]. Also, according to [28,29] reported that substances with an LD<sub>50</sub>> 1000 mg/kg are of low toxicity or are relatively safe. Hence, the two extracts; tigernut and ginger with an LD<sub>50</sub> ≥ 5000 mg/kg can be said to have a very high safety margin.

The result presented in Fig. 1 showed that the Hb concentration, PCV, and RBC in the diabetic untreated rats were significantly (p<0.05) reduced from 14.72g/dL, 38.60%, 6.09 × 10<sup>6</sup>/mm<sup>3</sup> (normal control) to 7.84g/dL, 20.40%, 3.25 × 10<sup>6</sup>/mm<sup>3</sup>, respectively. Whereas the diabetic

group treated with 500mg/kg CE showed significant recovery by an average of 10.52g/dL (Hb), 27.60% (PCV), 4.42 × 10<sup>6</sup>/mm<sup>3</sup> (RBC), and Glibenclamide (standard drug) showed significant recovery by of 13.72g/dL (Hb), 34.00% (PCV), 5.45 × 10<sup>6</sup>/mm<sup>3</sup> (RBC), (compared with the normal control).

From the results on the effects of the extract on the haematological values of the diabetic rats presented in Fig. 1, it was observed that haematological parameters including RBC, PCV, and Hb were significantly low in the diabetic untreated rats when compared with the normal control group. This suggests that the diabetic agent used in this study potentially caused a significant level of anaemia, and the extent of recovery observed in the treated groups showed that 500 mg/kg tigernut extract behaved as potent anti-anaemic agents much like the standard antidiabetic (Glibenclamide) drug in diabetes.

Patients with diabetes suffer the consequences of impaired renal function earlier in the course of their disease than do their nondiabetic counterparts [30]. In diabetic nephropathy (DN), anaemia tends to be more severe than in non-diabetic renal disease and occurs at an earlier stage of the disease. Anaemia in diabetes mellitus may be due to the oxidative destruction of circulating red blood cells by the administered alloxan monohydrate [31] had reported that in diabetics, hypochromic anaemia may be due to a fall in the body's iron content due to increasing oxidation.

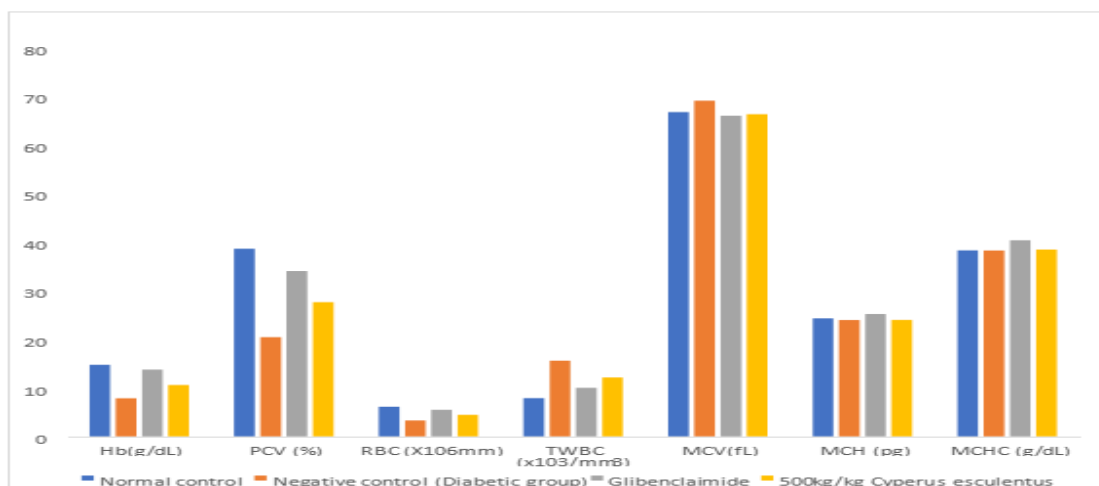


Fig. 1. Effect of *Cyperus esculentus* on the haematological parameters of alloxan induced diabetic rat

Fig. 1 is a chart that shows the effect of 500mg/kg of *Cyperus esculentus* extracts on the haematological parameter of alloxan induced diabetic rat.

The induction of diabetes mellitus did not significantly alter the mean values of MCH and MCHC, but caused significant ( $p < 0.05$ ) increase in the mean value (69.06fL) of MCV (Mean Corpuscular Volume) in the diabetic untreated group compared with an average value of 66.90fL (tiger nut extract) and 65.96fL (Standard drug) obtained in the treated groups, suggest progressive return from macrocytic cells (which is characteristics of haemolysis or excessive loss of blood, and corresponding effect via compensatory mechanism by the haemopoetic tissues) to normocytic cells by the treatment extracts. The mean value of MCV in Gilbenclamide treated group was statistically similar to the mean value obtained in the 500 mg/kg tigernut extract treated group. This effect might be due to the phytochemical content of tiger nut as reported by [32].

Results obtained for 500 mg/kg tiger nut in this study is consistent with the findings of [33], who reported that tiger nut extract significantly increased the values of RBC, PCV, and Hb in a dose-dependent manner in rats, although the values of MCV and MCHC were not significantly altered in that study. This positive effect of tiger nut on RBC values also corroborates with the findings of [34], who reported a significant increase in RBC, Hb, and PCV values in rats following treatment with tiger nut extract.

#### 4.1 Effect of 500 Mg/Kg Ginger on the Haematological Parameters of Alloxan-Induced Diabetic Rat

The results presented in Table 2 and Fig. 2 showed that the Hb concentration, percentage mean PCV, and the average number of RBC in the diabetic untreated rats were significantly ( $p < 0.05$ ) reduced from 14.72g/dL, 38.60%,  $6.09 \times 10^6/\text{mm}^3$  (normal control) to 7.84g/dL, 20.40%,  $3.25 \times 10^6/\text{mm}^3$  respectively. Whereas the diabetic group treated with the 500mg/dl Ginger showed significant recovery, 13.28g/dL, 32.40%,  $5.21 \times 10^6/\text{mm}^3$  respectively (compared with the diabetic, 500mg/dl tiger nut and combined extracts).

In this experiment, the increased RBC count of *Z. officinale*-treated rats, when compared to the negative control, could be due to the lowered

lipid peroxide level in the RBC membrane leading to a decreased susceptibility of RBCs to haemolysis. Because non-enzymatic glycosylations of membrane proteins correlate with hyperglycemia [35], it might be said that *Z. officinale* produced its effect by decreasing the elevated glucose concentration in *Z. officinale*-treated rats.

Total White blood cells (TWBC) are involved in the body defense against infection [36]. It has been suggested that the body's defense mechanism against infection is disturbed due to the disturbed WBC function in diabetes [37]. In this experiment, we demonstrated that *Z. officinale* increased the lowered WBC to control levels. This result indicates that *Z. officinale* treatment might also increase the defense mechanism of the body against infections in diabetic rats.

The induction with the diabetic agent did not significantly alter the mean values of MCH (Mean Corpuscular Haemoglobin), and MCHC (Mean Corpuscular Haemoglobin Concentration) implying normal Corpuscular Haemoglobin Concentration, but caused significant ( $p < 0.05$ ) increases in the mean value (69.06 fL) of MCV (Mean Corpuscular Volume) in the diabetic untreated group, compared with an average value of 65.96fL (standard drug) and 66.29fL (ginger extract) obtained in the treated groups, suggest progressive return from macrocytic cells (which is characteristics of haemolysis or excessive loss of blood, and corresponding effect via compensatory mechanism by the haemopoetic tissues) to normocytic cells by the treatment extracts. The mean value of MCV in Gilbenclamide treated group was statistically similar to the mean value obtained in the 500 mg/kg ginger extract-treated group.

The result of the TWBC showed a significant increase from the normal mean value of  $7.85 \times 10^3/\text{mm}^3$  to  $15.57 \times 10^6/\text{mm}^3$  in the diabetic untreated group compared with 500 mg/kg ginger extract treated groups,  $8.24 \times 10^3/\text{mm}^3$ , whose mean TWBC value was not significant ( $p > 0.05$ ) and almost the normal range. The mean value of TWBC in Gilbenclamide treated group was statistically the same ( $p > 0.05$ ) as the mean value obtained in the 500 mg/kg ginger extract-treated group.

This study is consistent with the findings of [38] who reported significant changes in RBC and WBC values in rats following treatment with Ginger extract.

#### 4.2 Effect of 500 Mg/Kg Tiger Nut and 500 Mg/Kg Ginger on the Haematological Parameters of Alloxan-Induced Diabetic Rat

The results showed that the Hb concentration, percentage mean PCV, and the average number of RBC in the diabetic untreated rats were significantly ( $p < 0.05$ ) reduced from 14.72g/dL, 38.60%,  $6.09 \times 10^6/\text{mm}^3$  (normal control) to 7.84g/dL, 20.40%,  $3.25 \times 10^6/\text{mm}^3$  respectively. Whereas, the diabetic group treated with

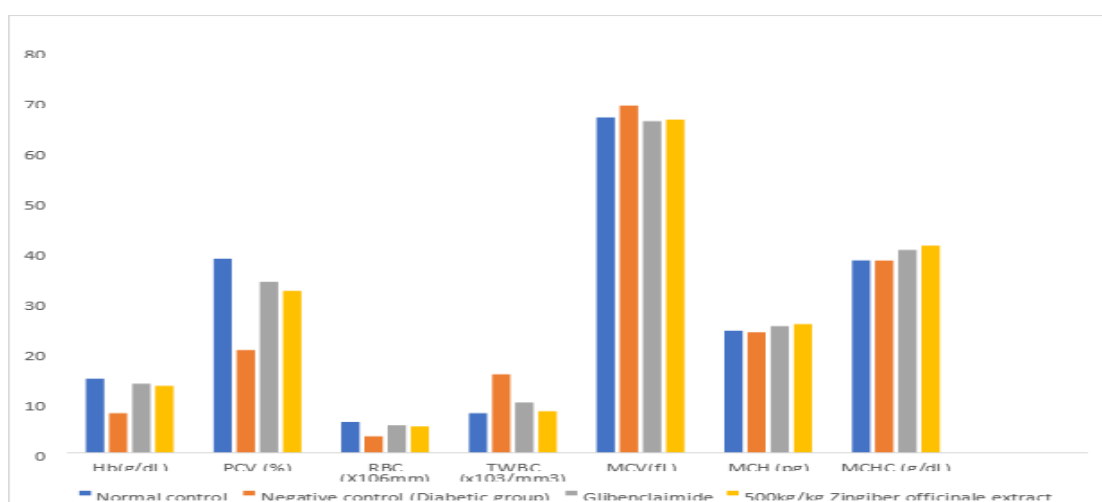
combined 500mg/kg extracts showed significant recovery to an average of 12.64g/dl(Hb), 32.20% (PCV), and  $5.09 \times 10^6/\text{mm}^3$  while Glibenclamide showed significant recovery (compared with the normal control). This showed that both extracts significantly improved haematological values in diabetic rats. This effect of the extract may be due to their iron content.

Fig. 2 is a chart that shows effect of 500mg/kg of *Zingiber officinale* on the haematological parameters of alloxan induced diabetic rat.

**Table 2. Haematological data obtained from the Control Groups and after 14-day treatments with *Cyperus esculentus* and *Zingiber officinale* extracts of alloxan-induced diabetic rats**

Animal Groups	Hb(g/dL)	PCV (%)	RBC ( $\times 10^6/\text{mm}^3$ )	TWBC ( $\times 10^3/\text{mm}^3$ )	MCV (fL)	MCH (pg)	MCHC (g/dL)
Normal control	14.72±0.26 <sup>a</sup>	38.60±1.50 <sup>a</sup>	6.09±0.26 <sup>a</sup>	7.85±0.85 <sup>c</sup>	66.71±0.49 <sup>b</sup>	24.26±0.65 <sup>a</sup>	38.26±0.84 <sup>b</sup>
Negative control	7.84± 0.62 <sup>c</sup>	20.40±0.81 <sup>c</sup>	3.25±0.14 <sup>c</sup>	15.57±0.88 <sup>a</sup>	69.06±0.60 <sup>a</sup>	23.94±0.93 <sup>b</sup>	38.24±1.62 <sup>b</sup>
Std. control	13.72±0.25 <sup>a</sup>	34.00±0.54 <sup>a</sup>	5.45±0.07 <sup>a</sup>	9.97±0.50 <sup>c</sup>	65.96±0.20 <sup>b</sup>	25.17±0.20 <sup>a</sup>	40.35±0.30 <sup>a</sup>
500mg/kg C.E	10.56±0.29 <sup>b</sup>	27.60±1.16 <sup>b</sup>	4.42±0.16 <sup>b</sup>	12.10±0.77 <sup>a</sup>	66.90±0.33 <sup>b</sup>	23.95±0.80 <sup>a</sup>	38.44±1.48 <sup>b</sup>
500mg/kg (Z.O)	13.28±0.88 <sup>a</sup>	32.40±2.61 <sup>a</sup>	5.21±0.41 <sup>a</sup>	8.24±0.65 <sup>c</sup>	66.29±0.51 <sup>b</sup>	25.57±0.58 <sup>a</sup>	41.19±0.96 <sup>a</sup>
500mg/kg (C.E+Z.O)	12.64±0.41 <sup>a</sup>	32.20±1.01 <sup>a</sup>	5.09±0.17 <sup>a</sup>	9.67±0.94 <sup>b</sup>	66.85±0.40 <sup>b</sup>	24.81±0.44 <sup>a</sup>	39.26±0.53 <sup>b</sup>

(NOTE: *Cyperus esculentus* (C.E) *Zingiber officinale* (Z.O). standard (std) control (Glibenclamide). Values are presented as mean ± Standard Error of Mean. Means compared along columns with different superscripts signifying significantly different at  $p < 0.05$ , superscript a, b, c, and d signifies mean differences, where values with the letter a means that it was significantly higher than the value with superscript b while b was significantly higher than superscript c and c is significantly higher than superscript d. RBC (Red Blood Cell); PCV (Packed Cell Volume); Hb (Haemoglobin); TWBC (Total White Blood Cell); MCV (Mean Corpuscular Volume); MCH (Mean Corpuscular Haemoglobin) and MCHC (Mean Corpuscular Haemoglobin Concentration)



**Fig. 2. Effect of *Zingiber officinale* on the haematological parameters of alloxan induced diabetic rat**

The role of iron in haemoglobin and red blood cells formation is well established [39]. Iron in green leafy vegetables is reportedly responsible for the haematinic effects of such plants [40]. The positive effects exerted by tiger nut and ginger on the haematological values of diabetics may also be due to their reported antioxidant effects [41, 42]. Antioxidant agents are known to be major actors in reversing the haematological anomalies associated with diabetes mellitus [43]. Another likely mechanism for the observed effects may be that the extracts enhanced the synthesis of erythropoietin, a hormone that stimulates the production of RBCs in the bone marrow initially disrupted by the administration of alloxan.

The result of the TWBC showed a significant increase following treatment with the extracts, suggesting that the extracts could enhance the responsiveness of the rat immune system indicating an immune involvement in the resultant anaemia. Furthermore, the rapid rise in TWBC mean values following the chemical induction of diabetes mellitus may be due to the activation of a secondary immune response via activation of B lymphocytes thereby inducing an amnestic response and secondly, induced membrane damage rendering erythrocytes susceptible to recognition and destruction by macrophages. This observed WBC anomaly was however ameliorated at the end of the treatment period with tiger nut and ginger extracts. Improvements in WBC count in rats treated with these extracts have been reported [25,29].

This study suggested that the diabetic agent (alloxan) used in this study potentially caused a significant level of anaemia, and the extent of recovery observed in the treated group (500 mg/kg tigernut + 500 mg/kg ginger extract) showed that the combined extract behaved as a potent antidiabetic agent much like the standard antidiabetic (Gilbenclamide) drug. The Group treated with the combined extract of 500 mg/kg tigernut extract and 500 mg/kg ginger extract gave better activity than 500 mg/kg tigernut extract, suggesting possible synergistic antidiabetic activity. This may be why extracts from tiger nut and ginger have over the years been used in traditional medicine [44].

## 5. CONCLUSION

*Cyperus esculentus* (tigernut) and *Zingiber officinale* (ginger) extracts could be safe when used orally in the management of anaemia and leucopenia associated with diabetes mellitus having significantly improved the values of RBC,

PCV, haemoglobin, and total white blood cells in the experimental diabetic rats. This study supports the use of these extracts in traditional medicine for the management of diabetes and its associated complications. However, further studies are required with the plant extracts. Such studies should include measurement of RBC fragility and serum folic acid, iron, cobalt, vitamin B12, calcium levels, and renal functions to demonstrate the exact mechanism of actions of these extracts on increased RBC count of diabetic rats. *Z. officinale* extract treatment might also increase the defense mechanism of the body against infections in diabetic rats.

## CONSENT

It's not applicable.

## ETHICAL APPROVAL

Ethical approval was obtained from the Ethical Committee of the School of Health Technology, Federal University of Technology Owerri, Nigeria.

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## COMPETING INTERESTS

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

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