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Original Research Article

Anti -Anaemic Potentials of Tigernut Extract Administered on Rat Exposed to Phenylhydrazine Induced Toxicity

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Abstract

Anaemia is a public health problem that affect both the rich and poor, and it possess a serious challenge to the health care profession, consequently this research is therefore aim at investigating the anti - anaemic potentials of Tigernut (C. esculentus) extract administered to albino wistar rats exposed to Phenylhydrazine induced toxicity. Forty (40) male albino Wistar rats weighing between 180- 250g were used for this study. They were randomly divided into four (4) groups of ten (10) rats each. Control group received normal feed and drinking water. Extract group received 600mg/kg bw of aqueous extract of Tigernut orally, PHZ group received PHZ induction and PHZ + Extract group received PHZ induction + 600mg/kg bw of aqueous extract of Tigernut. The feeding regimens lasted for 4 weeks, after which blood samples were collected via cardiac puncture for estimation of different parameters. Results showed that ingestion of aqueous extract of *C. esculentus* was able to reverse the significant decrease in RBC ($p<0.01$), HB ($p<0.001$), PCV ($p<0.001$) and Fe^{+} ($p<0.01$) values occasion by PHZ induction, back to appreciable level. In conclusion ingestion of *C. esculentus* extract is capable of reversing the derogatory effect imposed on hemopoietic processes following PHZ induction. Since C. esculentus is cheap and readily available it can therefore be recommended for the management of anaemic condition pending the availability of a viable health facility.

Keywords: Anaemia, Red Blood Cells, Packed Cell Volume, Hemoglobin, Tigernut extract.

INTRODUCTION

Anaemia is a public health problem that affect both the rich and poor, and it possess a serious challenge to the health care profession, it is a blood disorder characterised by reduction in RBC count, Hb content and PCV [43]. In most cases it is life threatening and therefore require urgent attention and treatment. Where treatment is delayed life may be lost, not because there were no health facilities, but because they were not readily available for treatment, taking cognisance of the prevailing economic situation and accessibility to health facilities in some parts of Nigeria. To this end there is need to explore readily available nutrient sources that can serve therapeutic values taking cognisance of our vast wealth of vegetation. One of such sources are readily available plants that are scattered everywhere within are localities called Tigernut (*Cyperus esculentus*). *C. esulentus*, is a grass-like plant commonly found in seasonally flooded wetlands [9]. Despite its name, tigernut is not a real nut, it is actually a tuber [42]. It has other names such as earth nut, yellow nut sedge, chufa (Spanish), rush nut and edible galingale [34]. In Nigeria, it is called ofio, Aya and imumu [36]**.** *C. esculentus* had been reported to be a health food with rich nutritional composition and disease preventing properties [42]. It serves as daily ingredient and is also consumed as a snack due to its rich milky taste and rich sugar content [4, 14, 38]. It is rich in vitamins (B1, C & E), minerals such as calcium, magnesium, phosphorus, potassium, iron [3, 14] and some bioactive ingredients like salicyclic acid, alkaloids, terpenoids, saponins, steroids [37]. *C. esulentus* is considered to have adequate properties to fight respiratory infections and some stomach illnesses. It is considered as an effective remedy for diarrhoea and is a preventive measure for cyst, prostate, hernia and rectum deformation. It also prevents endometriosis or

fibrosis as well as blockage of the tip of the fallopian tube [9]. Aqueous extract of *C. esculentus* could be used as a possible fertility booster and to attenuate sperm toxicity and as a powerful aphrodisiac [14]. It is used in the management of diabetes mellitus [11] debility indigestion, dyspepsia and colitis [38, 2]. Finally consumption of aqueous extract of *C. esculentus* possess anti-atherogenic, antioxidant and hepato-protective effects [30, 37].

Many people consume this plant extract as normal beverage while others chew it claiming it to be medicinal. This research is therefore aim at investigating the anti- anaemic potentials of Tigernut (*C. esculentus*) extract administered to albino wistar rats exposed to Phenylhydrazine induced toxicity

MATERIALS AND METHODS

Experimental animals

Fourty (40) male albino Wistar rats weighing between 180- 250g were used for this study. These animals were obtained from the animal house of the Department of Physiology, University of Calabar, Nigeria. The animals were handled in accordance with standard procedures. Prior to the commencement of the experiments, the animals were acclimatized for one week and given free access to rodent chow (Vital Feeds Nigeria, Limited) and water *ad libitum*. Ethical approval was obtained from the Faculty of Basic Medical Sciences Ethics Committee. The research was carried out in compliance with the National Institute of Health Guide for care and use of Laboratory animals (NIH Publication No 823 revised 1978).

Preparation of *C. esculentus* extracts

Fresh tuber of Tiger nut (*C. esculentus*) was obtain from Gbogobiri market in Calabar, Cross River State, and was identified by the Chief Hebarium, Department of Botany University of Calabar. Voucher specimen deposited in the Departments herbarium number 2639 was kept for future reference. The tiger nut was washed to remove debris including other physical contaminants dried at room temperature. The dried samples were pulverized to powder form using grinding machine. 1400g of the powdered sample was soaked in 7000 ml of distilled water for 24 h. The mixture was then filtered with a white cotton (satin) material, followed with filter paper (Whatmann No.1) into beakers and placed in an oven. The filtrate was evaporated to dryness using a rotary evaporator at 40ºC. The extract was then collected into a sample bottle and stored in a refrigerator.

Toxicity Study on Tiger nut

Acute toxicity test was done according to standard procedure by Lorke [26]. Thirty six mice were used for the study. They were randomly selected and assigned to six batches containing six animals each. They were allowed a week for adaptation. Each batch received doses of extract intraperitoneally (1.64-104.48 mg/kg). Only the control group received normal saline intraperitoneally. They were all returned to their home cages and allowed free access to food and drinking water. The mortality in each group was assessed 24 hours after administration of the extract. The percentage mortalities were converted to probits and plotted against the log10 of the dose of the extract [26].

Experimental design

Fourty (40) male albino wistar rat weighing between 180-250g were randomly divided into four (4) groups of 10 rats each. The animals in group 3 and 4 were induced with 40mg/kg body weight phenylhydrazine intraperitoneally, prior to the commencement of the experiment.

Control group, received normal feed and drinking water

Extract group, received 600mg/kg bw of aqueous extract of Tigernut orally

PHZ group, received PHZ induction

Extract + PHZ group, received PHZ induction + 600mg/kg bw of aqueous extract of Tigernut.

The dose of 600mg/kg bw was arrived at following the estimation of Lethal toxicity (LD_{50}) value for the extract (LD_{50} =1872.22mg/kg). The administration was done orally and the experiment lasted for a period of 4 (four) weeks.

Collection of blood samples and analysis of different parameters

At the end of the administration, the animals were anaesthetized using chloroform and blood samples were collected via cardiac puncture with the help of 5ml syringe into EDTA and plain sample bottles. The blood in the plain sample bottles were allowed to clot for 2hrs. It was then centrifuged at 3000rpm for 10 minutes using centrifuge. The serum was collected into clean test tubes and then used for the estimation of various parameters.

Measurement of haematological parameters

The full blood analysis was done using automated hematology analyzer Sysmex model: kx-21N, Serial Number: A6695, as used by Archibong *et al*., [5] and Ofem *et al.*, [35].

Measurement of Electrolytes

Serum Na^+ and K^+ concentrations were determined using a flame photometer (Model 410C, Petracourt Ltd, England). Serum Cl^- concentration was determined using the end point calorimetric titration method [52]. Serum bicarbonate (HCO) concentration was measured using the modified method [51] as used by Archibong *et al*., [6].

Statistical Analysis

Results obtained were presented as mean± standard error of mean. Data obtained were analyzed by one way analysis of variance (ANOVA) followed by

post hoc student's Newman-keuls test using the SPSS computer program and p value less than 0.05 was considered statistically significant. The results were all presented in tabular form.

RESULTS

Red blood cell (RBC) Count

Table 1 shows comparison of the RBC count in the different experimental groups as stated thus, Control (6.45±0.04), Extract (6.87±0.73), PHZ group (4.24±0.04) and Extract + PHZ (6.55±0.17). The RBC count in the control group was significantly higher (P < 0.05) when compared with that of the PHZ group, but showed no significant difference when compared with that of the Extract treated groups respectively. It was also observed that the RBC count in the PHZ group was significantly lower (P<0.001) when compared with that of the extract treated groups respectively**.**

Hemoglobin (Hb) Concentration

Table 1 shows, comparison of Hb concentration in the different experimental groups as stated thus, Control (22.5±0.09), Extract (17.36±0.29), PHZ group (15.7±0.36) and Extract + PHZ (16.36±1.58). The Hb concentration in the control group was significantly higher (P < 0.01 and P < 0.001) when compared with that of the extract treated groups and PHZ group. It was also observed that the Hb concentration in the PHZ group was significantly lower (P<0.01) when compared with that of the extract treated groups respectively.

Packed Cell Volume (PCV)

Table 1 shows, comparison of PCV in the different experimental groups as stated thus, Control (39.7±0.16), Extract (37.3±3.63), PHZ group (25.2±0.13) and Extract + PHZ (37.4±0.46). The PCV in the control group was significantly higher (P < 0.001) when compared with that of the PHZ group, but showed no significant difference when compared with that of the Extract treated groups respectively. It was also observed that the PCV in the PHZ group was significantly lower (P<0.01) when compared with that of the extract treated groups respectively.

Table 1: Comparison of RBC, Hb and PCV in the control and treated groups

	RBC	HB	PCV
Control	6.45±0.04	22.5±0.09	39.7±0.16
Extract	6.87±0.73^{a}	17.36±0.29^{**,b}	37.3±3.63^{c}
PHZ	4.24±0.04**	15.7±0.36***	25.2±0.13***
Extract + PHZ	6.55±0.17^{a}	16.36±1.58^{***,b}	37.4±0.46^{c}

Values are represented as Mean ± SEM. *p<0.05, **p<0.01& ***p<0.001 vs control. a, b & c = p<0.01vs PHZ

Mean Corpuscular Volume (MCV)

Table 2 shows, comparison of MCV in the different experimental groups as stated thus, Control (54.6±0.68), Extract (57.8±1.96), PHZ group (61.6±0.24) and Extract + PHZ (57.0±0.89). The MCV in the control group was significantly lower (P < 0.01) when compared with that of the PHZ group, but showed no significant difference when compared with that of the Extract treated groups respectively. It was also observed that the MCV in the PHZ group was significantly higher (P<0.01) when compared with that of the extract treated groups respectively.

Mean Corpuscular Hemoglobin (MCH)

Table 2 shows, comparison of MCH in the different experimental groups as stated thus, Control (24.4±0.4), Extract (23.9±0.25), PHZ group (53.1±0.28) and Extract + PHZ (26.6±0.29). The MCH in the control group was significantly lower (P < 0.001) when compared with that of the PHZ and Extract + PHZ group respectively. It was also observed that the MCH in the PHZ group was significantly higher (P<0.01) when compared with that of the extract treated groups respectively.

Mean Corpuscular Hemoglobin Concentration (MCHC)

Table 2 shows, comparison of MCHC in the different experimental groups as stated thus, Control (39.7±0.74), Extract (43.9±0.07), PHZ group (91.0±1.76) and Extract + PHZ (46.5±0.16). The MCHC in the control group was significantly lower (P < 0.001 and P < 0.05) when compared with that of the Extract treated groups and PHZ group respectively. It was also observed that the MCHC in the PHZ group was significantly higher (P<0.01) when compared with that of the extract treated groups respectively.

Red Cell Distribution Width (RDW)

Table 2 shows, comparison of RDW concentration in the different experimental groups, as stated thus, Control (21.3±0.07), Extract (26.5±0.81), PHZ group (28.1±1.8) and Extract + PHZ (23.6±0.11). The RDW concentration in the control group was significantly lower (P < 0.001 & P < 0.05) when compared with that of the PHZ group and extract treated group. It was also observed that the RDW in the PHZ group was significantly higher (P<0.01) when compared with that of the extract treated groups respectively.

Table 2: Comparison of RBC indices in the control and treated groups

	MCV	MCH	MCHC	RDW
Control	54.6±0.68	24.4±0.4	39.7±0.74	21.3±0.07
Extract	57.8±1.96[a]	23.9±0.25[b]	43.9±0.07[*,c]	26.5±0.81[**,d]
PHZ	61.6±0.24[**]	53.1±0.28[***]	91.0±1.76[***]	28.1±1.8[***]
Extract + PHZ	57.0±0.89[a]	26.6±0.29[***,b]	46.5±0.16[***,c]	23.6±0.11[d]

Values are represented as Mean ± SEM. *p<0.05, **p<0.01& ***p<0.001 vs control. a, b, c & d = p<0.01 vs PHZ

White Blood Cell (WBC)

Table 3 shows, comparison of WBC count in the different experimental groups as stated thus, Control (27.18±0.28), Extract (28.23±2.51), PHZ group (7.91±0.01) and Extract + PHZ (10.49±0.61). The WBC count in the control group was significantly higher (P < 0.001) when compared with that of PHZ group but showed no significant difference when compared with that of the extract treated group. It was also observed that the WBC count in the PHZ group was significantly lower (P<0.01) when compared with that of the extract treated groups respectively.

Lymphocyte Count

Table 3 shows, comparison of Lymphocyte count in the different experimental groups as stated thus, Control (62.5±4.52), Extract (61.1±1.03) and PHZ group (60.05±1.28) and Extract + PHZ (44.6±4.34). The Lymphocyte count showed no significant difference when compared across the different experimental groups.

Monocyte Count

Table 3 shows, comparison of Monocyte count in the different experimental groups as stated thus, Control (22.4±1.92), Extract (14.3±0.76), PHZ group (10.7±0.09) and Extract + PHZ (11.76±2.7). The monocyte count in the control group was significantly higher (P < 0.001 and P < 0.05) when compared with that of the extract treated groups and PHZ group respectively. It was also observed that the monocyte count in the PHZ group was significantly lower (P<0.01) when compared with that of the extract treated groups respectively.

Granulocytes

Table 3 shows, comparison of granulocyte count in the different experimental groups as stated thus, Control (24.7±7.07), Extract (28.2±1.12), PHZ group (17.55±3.19) and Extract + PHZ (41.1±5.09) . The granulocyte count showed no significant difference when compared across the different experimental groups.

Table 3: Comparison of WBC and Differential count control and treated groups

	WBC	Lymphocyte	Monocyte	Granulocyte
Control	27.18±0.28	62.5±4.52	22.4±1.92	24.7±7.07
Extract	28.23±2.51[a]	61.1±1.03	14.3±0.76[*,b]	28.2±1.12
PHZ	7.91±0.01[***]	60.05±1.28	10.7±0.09[**]	17.55±3.19
Extract + PHZ	10.49±0.61[***,a]	44.6±4.34[**]	11.76±2.79[**,b]	41.1±5.09

Values are represented as Mean ± SEM. *p<0.05, **p<0.01& ***p<0.001 vs control. a & b = p<0.01 vs PHZ

Platelet Count

Table 4 shows, comparison of Platelet count in the different experimental groups as stated thus, Control (843±55.45), Extract (954.6±149.6), PHZ group (695.8±77.14) Extract + PHZ (744±6.71). The Platelet count was higher than that of the PHZ group but showed no significant difference when compared with that of extract treated group. It was also observed that the platelet count in the PHZ group was lower when compared with that of the extract treated groups respectively.

Mean Platelet Volume (MPV)

Table 4 shows, comparison of MPV in the different experimental groups as stated thus, Control (7.26±0.47), Extract (7.6±0.8), PHZ group (9.06±0.11) and Extract + PHZ (7.76±0.07). The MPV in the PHZ group was higher when compared across the different experimental groups

Platelet Distribution Width (PDW)

Table 4 shows, comparison of PDW in the different experimental groups as stated thus, Control (32.64±2.82), Extract (37.8±0.04), PHZ group (41.8±0.72) and Extract + PHZ (37.94±0.25). The PDW in the control group was significantly lower (P < 0.01) when compared with that of the PHZ group but showed no significant difference when compared with that of the extract treated groups. It was also observed that the PDW in the PHZ group was significantly higher (P<0.01) when compared with that of the extract treated groups respectively.

Platelet Large Cell Ratio (P-LCR)

Table 4 shows, comparison of PLCR in the different experimental groups as stated thus, Control (16.16±3.06), Extract (16.37±0.54), PHZ group (28.04±1.53) and Extract + PHZ (20.34±0.29). The PLCR in the control group was significantly lower (P < 0.01) when compared with that of the PHZ group but showed no significant difference when compared with

that of the extract treated groups. It was also observed that the PLCR in the PHZ group was significantly higher (P<0.01) when compared with that of the extract treated groups respectively.

Table 4: Comparison of Platelet count and Platelet indices in the control and treated groups

	PLT	MPV	PDW	P-LCR
Control	843±55.45	7.26±0.47	32.64±2.82	16.16±3.06
Extract	954.6±149.6	7.6±0.8	37.8±0.04^{a}	16.37±0.54^{b}
PHZ	695.8±77.14	9.06±0.11	41.8±0.72**	28.04±1.53***
Extract + PHZ	744±6.71	7.76±0.07	37.94±0.25^{a}	20.34±0.29^{b}

Values are represented as Mean ± SEM. **p<0.01& ***p<0.001 vs control. a & b = p<0.01 vs PHZ

Electrolyte and Mineral Concentration

Table 5 shows, comparison of Fe^{2+} concentration in the different experimental groups as stated thus, Control (45.75±0.23), Extract (55.25±3.58), PHZ group (38.5±0.97) and Extract + PHZ (48.28±1.38). The Fe^{2+} concentration in the control group was significantly lower (P < 0.01) when compared with that of the extract treated group, but was higher than that of the PHZ group. It was also observed that the Fe^{2+} concentration in the PHZ group was significantly lower (P<0.01) when compared with that of the extract treated groups respectively.

Table 5 shows, comparison of K^{+} concentration in the different experimental groups as stated thus, Control (17.45±0.69), Extract (19.2±0.00), PHZ group (16.1±0.04) and Extract + PHZ (16.6±0.18). The K^{+} concentration in the control group was significantly lower (P < 0.01) when compared with that of the extract treated groups, but was higher than that of the PHZ group. It was also observed that the K^{+} concentration in the PHZ group was significantly lower (P<0.01) when compared with that of the extract treated groups respectively

Table 5 shows, comparison of Na^{+} concentration in the different experimental groups as stated thus, Control (163.1±1.35), Extract (156.3±1.88), PHZ group (147.2±0.36) and Extract + PHZ (156.3±56). The Na^{+} concentration in the control group was significantly higher (P < 0.01 and P < 0.001) when compared with that of the extract treated groups and PHZ group respectively. It was also observed that the Na^{+} concentration in the PHZ group was significantly lower (P<0.01) when compared with that of the extract treated groups respectively

Table 5 shows, comparison of Cl^{-} concentration in the different experimental groups as stated thus, Control (117±1.12), Extract (114.5±0.89), PHZ group (93.16±0.16) and Extract + PHZ (114.5±0.00). The Cl^{-} concentration in the control group was significantly higher (P < 0.001) when compared with that of the PHZ group but showed no significant difference when compared with that of the extract treated groups respectively. It was also observed that the Cl^{-} concentration in the PHZ group was significantly lower (P<0.01) when compared with that of the extract treated groups respectively.

Table 5 shows, comparison of HCo^{-} concentration in the different experimental groups as stated thus, Control (21.6 ± 0.24), Extract (26.4 ± 0.25), PHZ group (21.0 ± 0.45) and Extract + PHZ (24.4 ± 0.25). The HCo^{-}concentration in the control group was significantly lower (P < 0.001) when compared with that of the extract treated groups respectively but showed no significant difference when compared with that of the PHZ group. It was also observed that the HCo^{-} concentration in the PHZ group was significantly lower (P<0.01) when compared with that of the extract treated groups respectively

Table 5: Comparison of Fe, K, Na and Cl in the control and treated groups

	Fe	K	Na	Cl^{-}	HCo^{-}
Control	45.75±0.23	17.45±0.69	163.1±1.35	117±1.12	21.6 ± 0.24
Extract	55.25±3.58^{*,a}	19.2±0.00^{*,b}	156.3±1.88^{**,c}	114.5±0.89^{d}	26.4 ± 0.25^{***,e}
PHZ	38.5±0.97*	16.1±0.04	147.2±0.36***	93.16±0.16***	21.0 ± 0.45
Extract + PHZ	48.28±1.38^{a}	16.6±0.18^{b}	156.3±56^{**,c}	114.5±0.00^{d}	24.4 ± 0.25***

Values are represented as Mean ± SEM. *p<0.05, **p<0.01& ***p<0.001 vs control. a, b, c, d & e = p<0.01 vs PHZ

DISCUSSION AND CONCLUSION

DISCUSSION

The result as obtained from this investigation is quite amazing and shows to a large extent the potential health benefits of *C. esculentus* extract ingestion.

The haematological analysis revealed that ingestion of *C. esculentus* extract was able to significantly reverse the decrease in RBC count, PCV and Hb concentration occasioned by PHZ induction. This result is further corroborated by earlier researches published by Augustine & Emmanuel [7] and Hanaa [20] which shows that ingestion of *C. esculentus* extract was able to boost the level of RBC, PCV and Hb.

This result is of significance because PHZ induction is known to cause haematotoxicity [29] which leads to the haemolytic anemia [12, 50]. PHZ induction increases the iron absorption in spleen, liver and duodenum thereby altering iron metabolism [41], as a result local demand and supply of Fe would increase erythropoeitic activity of the spleen so the size of spleen would be increased resulting in splenomegaly, these will summarily affect the process of Hb synthesis and erythropoiesis [50]. Finally PHZ induced anemia activate immune response which triggers phagocytosis in the spleen and liver, apart from this, administration of PHZ interferes with the binding of erythropoietin (EPO) with erythropoietin receptors (EPOR) so that JAK-STAT pathway would be affected. PHZ also showed genotoxic effect by creating single strand DNA damage [41], but these were all reversed following *C. esculentus* ingestion

The ability of the *C. esculentus* extract treatment to reverse decrease in level of RBC, PCV and Hb occasioned by PHZ induction, back to appreciable level maybe unconnected with the fact that extract of *C. esculentus* is rich in essential nutrients which include vitamins (B_1, B_9 C & E) and minerals such as calcium, magnesium and iron [1, 13, 14]. Increase availability of Fe (as shown by the result in table 5) is an important tool for Hb synthesis which is a necessary component for erythrocyte synthesis via erythropoiesis. Bioactive phytochemicals and nutrients in tigernut also include salicyclic acid, alkaloids, terpenoids, saponins, steroids, phosphorus and potassium that have a wide range of health promoting properties [37]**.**

The red cell indices result shows that ingestion of *C. esculentus* extract was able to significantly reverse the increase in the Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC) and Red cell distribution width (RDW-SD) occasioned by PHZ induction. This result agrees with findings by Criswell [12], Unami *et al.*, [49], and Shukla *et al.*, [45], which shows that PHZ increases MCV, MCH and MCHC, but this was reversed following the ingestion of aqueous extract of *C. esculentus*. The MCV which is the average volume of a single RBC size was shown to increase significantly following PHZ induction. It is important to note that MCV is known to increase in anaemic condition [43] therefore this is an indication that PHZ induction is capable of causing anaemia. In patience with anaemia, it is the MCV measurement that allows classification as microcytic (MCV below normal range), normocytic (MCV within normal range) or macrocytic (MCV above normal range) [47]. It is very likely therefore that the effects of the extracts on the RBC were normocytic since its values were not different from that of the control group. It is important to note that Increase MCH and MCHC as shown by the result occurs in Hyperchromia (a condition where the Hb is abnormally concentrated inside the Red cell such as in burn patient) and macrocytic anemia (a condition when the blood cells are too big, resulting from Vit B_{12} or Folic acid deficiency), it is an indication of vitamin deficiency, auto immune disorder, hemolytic anemia and liver disease. That value of these parameters were reversed following the ingestion of *C. esulentus*, is an indication of its potent value. RDW is a numerical measure of the variability in size (anisocytosis) of circulating erythrocytes [40]. This parameter is used in narrowing the differential diagnosis of anemia [28]. Increase RDW is an indicator of anisocytosis, that ingestion of *C. esculentus* extract could reverse the RDW value therefore suggests that the extract could cause the production of RBC that are less variable in size. Therefore, an anaemic condition is very unlikely in animals treated with *C. esculentus*.

WBC count, granulocytes and lymphocyte counts in the PHZ group was significantly lower when compared with that of control group and extract treated groups as revealed by the differential count study. The decrease in WBC count in PHZ group as presented by this study is important because it agrees with previous research published by Kale *et al.*, [22] and Pandey [39]. This to an extent is an indication that PHZ induction is capable of suppressing the defence mechanism and immune system at large, but this effect was reversed following extract treatment, in agreement with findings carried out by Augustine & Emmanuel [7] and Hanna [20]. WBC, granulocytes and lymphocyte counts are used in providing useful information for diagnosis in routine clinical evaluation of state of health of patience. That these parameters were reversed following ingestion of Tigernut extract back to appreciable level demonstrates its immune-stimulatory effect thereby enhancing the defence mechanism and boosting the immune system. Tigernut are known to contain important vitamins (Vit B9, B6, C & E) [10] which serve as antioxidants, they also play a major role in leucopoiesis and even formation of antibodies. This is an indication that the extract is capable of stimulating the hemopoietic system and can also alleviate severe leucopenic conditions.

The results showed that ingestion of *C. esculentus* was able to bring about a desirable reverse in platelet count and platelet indices (MPV, PDW, P-LCR) occasioned by PHZ induction. A decrease in platelet count could lead to excessive bleeding tendencies, purpura and leukemia which if severe may lead to death [19]. This tendency was reversed following the ingestion of *C. esculentus* extract. *C. esculentus* has been shown to bring about thrombocytosis [7] this means the extract may contain thrombopoietin-like agents which are capable of stimulating the release of thrombopoietin [16] pointing to the ability of the extract to boost platelet count even in deleterious situations like PHZ induction. Thereby ameliorating bleeding tendencies associated with thrombocytopenia, especially when triggered by blood loose

The ingestion of C. esculentus was not only shown to amiliorate this ugly trend occasioned by PHZ induction it was also able to produce platelets of normal volume, shapes and sizes as indicated by the platelet indices result. MPV is known to be an indicator and determinant of platelet function [53], including aggregation, release of thromboxane A2, platelet factor 4, beta-thromboglobulin [44] and expression of glycogen 1b and glycogen IIb/IIIa receptors [18, 48]. It is a newly emerging risk factor for athero-thrombosis. Increase in MPV has been documented in patients with metabolic syndrome, stroke and Diabetes Mellitus (DM) [33, 46]. Many studies have shown that increased MPV is one of the risk factors for myocardial infarction, cerebral ischemia and transient ischemic attacks [24, 25, 27, 31] and chronic vascular disease [15]. The MPV and other determinants of platelets function (PDW and PLC-R) are found to vary inversely with the platelet count in normal subjects [8, 25, 27, 31] and in chronic vascular disease [15], increase in these platelet indices value following PHZ induction is an indication of platelets destruction. Increase in MPV and PLCR are implicated in the etiology of cardiovascular diseases, also PDW has been found to be of some use in distinguishing essential thrombocythaemia (PDW increase) from reactive thrombocytosis (PDW normal) [8]. hence the reduction in their values back to appreciable level following extract ingestion as shown in this study is a good indication, this to an extent strongly supports the increase in platelet count also observed earlier which is an indication of the ameliorative potentials of *C. esculentus* ingestion.

The serum electrolyte result has revealed that there was a reduction of sodium ion concentration following the administration of *C. esculentus* extract, this may be due to the low concentration of sodium in the extract, or possibly due to the ability of the extract to potentiate excretion of sodium ions from the body. This was followed by a decrease in chloride concentration since sodium and chloride ions are always transported alongside [17]. This result is also very important because elevated Na^+ concentration predisposes one to high blood pressure [19], it therefore means that consumption of *C. esculentus* extract may be important in preventing high blood pressure. Potassium ion is the major cation inside the cell. *C. esculentus* extract brought about increased potassium ion concentration, this may be brought about by the decrease in serum sodium ions occasion by its excretion and reabsorption of potassium ions, since sodium and potassium ions are always exchanged in alternate manner by the Na^+/K^+ pump along the cell membrane [23] or probably the extracts are rich in potassium ion [37]. There was an increase in HCO_3 concentration in plasma of extract treated group. It is well known that bicarbonate is produced by the pancreas and it is essential in neutralizing the acidic pH produce by the acid in the gastrointestinal tract [19]. It maintains the acid-base buffering system of the blood. The serum electrolyte results is of great importance because it shows that *C. esculentus* ingestion is capable of preventing one from being predispose to atherogenesis or dyslipidaemic conditions.

CONCLUSION

Ingestion of *C. esculentus* extract is capable of reversing the derogatory effect imposed on hemopoietic processes following PHZ induction. Since *C. esculentus* is cheap and readily available it can therefore be recommended for the management of anaemic condition pending the availability of a viable health facility.

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AUTHORS CONTRIBUTION

This work was carried out in collaboration between all authors. Authors. O.S. E and N,A.N wrote the first draft of the manuscript, managed the literature and performed the statistical analysis, author M,C C and A,A.N designed the study, wrote the protocol and edited the manuscript, while authors O, E. A and O,D.O contributed in carrying out the feeding regimens and analysis of blood samples. All authors read and approved the final manuscript

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