The Bioscientist Vol. 4(1): 41-50, 2016 Available online at http://www.bioscientistjournal.com

Full Length Research Paper

EFFECTS OF *CYPERUS ESCULENTUS* (TIGERNUT) MILK ON SERUM PROTEIN, HAEMATOLOGICAL INDICES AND ANTIOXIDANT ENZYMES IN WISTAR RATS

Ezekwesili, Chinwe.N[1] and Iyeke, Ephraim O[1]
[1]Department of Applied Biochemistry, Nnamdi Azikiwe University, Awka
* Corresponding Author: Email- cnezekwesili@yahoo.com Phone- 08033934218

ABSTRACT

The effects of *Cyperus esculentus* milk on the hematological parameters and antioxidant enzymes of albino rats were studied. *Cyperus esculentus* milk was extracted from the fresh tuber. Twenty-one rats were divided into three groups of seven animals each. Test groups (1 and 2) were orally administered 0.5 and 1.0 ml/100g body weight of *C. esculentus* milk respectively for six (6) weeks, while the rats in the control group (3) were given only standard rat chow. Hematological parameters and antioxidant enzymes activities were estimated using standard methods. Results showed no significant decrease in total protein ($p>0.05$) compared to the control, but significant and dose-dependent decrease ($p<0.05$) in the globulin fraction. In hemoglobin (HGB), Red blood cells (RBC), Packed cell volume (PCV), white blood cells (WBC) and platelets there was no significant difference ($p>0.05$) between the test and control. No significant changes in the activities of glutathione peroxidase and superoxide dismutase were observed whereas a non-significant decrease ($p > 0.05$) in catalase activity was recorded. Since elevated levels of white blood cells are indicative of immunologic and allergic responses, results therefore may indicate the absence of allergic and undesirable effects in the use of *C. esculentus* milk, even in its raw form, at the administered concentrations and for the feeding duration. The findings are also of health, nutritional and industrial importance as *Cyperus esculentus* milk may serve as a good source of plant milk.

Keywords: Tigernut, *Cyperus esculentus*, Hematology, Plant milk, Antioxidants

INTRODUCTION

Cyperus esculentus is commonly known as earth almond, tigernut, chufa, yellow Nutsedge and zulu nuts. In Nigeria, it is known as *Aya* in Hausa, *Ofio* in Yoruba and *Akihausa* in Ibo where three varieties (black, brown and yellow) are cultivated (Umerie *et al*., 1997). Among these, only brown and yellow varieties are readily available in the market. The yellow variety is preferred to all other varieties because of its bigger size, attractive colour and fleshier body (Belewu and Abodurin, 2008). Tigernut can be eaten raw, roasted, dried,

baked or made into a refreshing beverage called tigernut milk (Oladele and Aina, 2007). It can also be used as a flavoring agent for ice cream and biscuit (Cantalejo, 1997). *C. esculenta* tuber is rich in minerals such as phosphorous, potassium, calcium, magnesium and iron necessary for bones, tissue repair, muscles, and the blood stream. It contains proteins of high biological value with higher content of essential amino acids than the FAO/WHO proposed standards and therefore satisfies the amino acid needs of adults (Bosch *et al.,* 2005). *C. esculenta* is also rich in dietary fibre for effective treatment and prevention of many diseases including colon cancer, coronary heart diseases, obesity, diabetes and gastrointestinal diseases (Anderson *et al.*, 1994). The oil reduces blood triglycerides and low density lipoprotein-cholesterol (LDL-C), and increases high density lipoprotein-cholesterol (HDL-C) thereby preventing arteriosclerosis (Belewu *et al.*, 2006). It is also rich in antioxidant vitamins such as vitamins E and C essential for healing, and prevention of certain diseases whose pathogenesis are traceable to adverse reactions by free radicals (Mason, 2005). The consumption of antioxidant-containing foods may protect the immune system and as such delay the progression of HIV infection to AIDS (Elbim *et al.,* 1999). Tiger nut prevents heart disease and thrombosis, and activates blood circulation (Chukwuma *et al.,* 2010). Tiger nut contains a good quantity of vitamin B1, which assists in balancing the central nervous system and helps to encourage the body to adapt to stress (David, 2005). The effect of Tiger nut milk on blood protein levels, heamatological indices and endogenous antioxidant status has not been investigated scientifically in rat model.

This study was therefore designed to investigate the effects of prolonged intake of Tiger nut milk on serum proteins, blood leucocytes and the endogenous antioxidant enzymes which are the body's defense mechanisms against invading agents and free radicals.

MATERIALS AND METHODS

Collection of Tigernuts and Extraction of Tigernut milk

Fresh nuts of *Cyperus esculentus* was purchased from Hausa quarters at Regina Caeli road, Awka South L.G.A, Anambra state. The nuts were carefully handpicked and the viable ones selected. Ten kilogramme (10.0 kg) quantity was washed and the milk extracted with the aid of Binatone Juice Extractor, model JE-570. The fresh milk sample obtained was then stored in the refrigerator.

Experimental Design

The animals were divided into three groups of seven (7) rats per group (Control group, Treatment group (0.5 ml/100g) and Treatment group (1.0 ml/100g) respectively. The milk was administered to them orally with the aid of a stomach tube for a period of six (6) weeks and they were allowed access to the growers' marsh pellets *ad libitum*.

At the end of six (6) weeks, after overnight fast, the rats were anaesthetized with the aid of chloroform swabs and blood samples carefully collected via cardiac puncture. The blood was transferred into well labeled EDTA container and a clean, dry plain glass tube respectively. The blood in the glass tube was then allowed to clot and the serum was gently collected using a Pasteur pipette into a labeled plain tube. This was done for all the animals used.

Assay of Haematological Parameters

Hematological parameters such as hemoglobin concentration (HGB), packed cell volume (PCV), red blood cells (RBC), white blood cells (WBC) and platelets counts were estimated using Mindray Hematology Autoanalyser (BC-2800). This analyzer adopts the Coulter Principle to count WBC, RBC and PLT cells and to draw their corresponding histograms. The Hemoglobin concentration (HGB) was obtained by the colorimetric method and the results of the rest of the parameters were derived from those.

WBC (10^9/ L) is the number of leukocytes measured directly by counting the white blood cells passing through the aperture of the analyzer. Because the nucleated red blood cells (NRBC) can be mistaken by the analyzer for white cells, the system-generated result is usually corrected using the following formula:

$$WBC' = WBC \times \frac{100}{100 + NRBC}$$

Where WBC represents the system generated white cell number, NRBC the number of NRBCs counted in 100 white cells and WBC′ the corrected white cell number.

PLT (10^9/L) is measured directly by counting the platelets passing through the aperture.

Determination of Total Protein

Biuret method was used to determine the serum total protein content. Distilled water (0.2 ml), standard reagent (0.2 ml), and serum (0.2 ml) were added to the blank, standard, and test sample tubes respectively. Biuret reagent (1.0 ml) was then added to each tube. The test tubes were shaken and incubated for 30minutes at 25ºC. The absorbance of the sample (A_{sample}) and standard ($A_{standard}$) were read against the blank at a wavelength of 546nm.

The total protein concentration was then calculated using the formula:

$$\text{Total Protein Concentration} = \frac{(A_{sample})}{(A_{standard})} \times \text{standard conc. (g/dl)}$$

Determination of Serum Albumin

Bromocresol Green method was used to determine the concentration of serum albumin (Doumas *et al.,* 1971). Distilled water (0.01 ml), standard reagent (0.01ml), and serum (0.01 ml) were added to the blank, standard, and sample tubes respectively. Three millilitres (3.0 ml) of bromocresol concentrate was then added to each tube. The test tubes were shaken and incubated for 5minutes at 25ºC. The absorbance of the sample (A_{sample}) and standard ($A_{standard}$) were read against the blank at a wavelength of 640nm.

The serum albumin concentration was calculated using the formula:

$$\text{Albumin Concentration} = \frac{(A_{sample})}{(A_{standard})} \times \text{standard conc. (g/dl)}$$

Determination of Serum Globulin

This was calculated using differential method with the knowledge that total serum proteins consist of albumin and globulin

Antioxidant Enzyme Assays

Determination of Glutathione Peroxidase Activity

Serum glutathione peroxidase activity was estimated by the method of Beutler and Kelly as reported by Ogbunugafor *et al.,* 2013. The reaction

mixture contained 1.0 ml of 0.3M phosphate buffer (pH 7.4), 0.3ml of 10mM glutathione, 0.3ml of 15mM freshly prepared H_2O_2, and 1.37 ml of distilled water. The serum (0.1ml) was added to the mixture in the cuvette, shaken and absorbance read at 340nm. Extinction coefficient of 1.622 x $10^{-3}M^{-1}cm^{-1}$ was used to calculate enzyme activity and expressed in unit mg $protein^{-1}$.

Determination of Catalase Activity

Determination of serum catalase activity was carried out according to the method of Beers and Seizer as outlined by Ogbunugafor *et al*., (2013).The reaction mixture (2.2 ml) contained 0.l ml of serum, 0.1ml H_20_2 and 2.0 ml of phosphate buffer (50 mM, pH 7.0). The absorbance of the test sample was read against blank devoid of serum. Decrease in absorbance due to decomposition of H_20_2 was measured in UV spectrophotometer at 30 seconds interval for 3minutes. The extinction coefficient for H_20_2 used at 240nm for the enzyme activity calculation was 40.0 $m^{-1}cm^{-1}$ and the result expressed in unit mg $protein^{-1}$.

Determination of Superoxide Dismutase Activity

The method of Sun and Zigma as described by Ogbunugafor *et al*., (2013) was adopted. The reaction mixture (3.0 ml) contained 2.95ml of sodium carbonate buffer (0.05mM and pH 10.2) and 0.02 ml of serum sample. Epinephrine (3 M, 0.03 ml) in 0.005N HCl was used to initiate the reaction. The reference cuvette contained 2.95 ml buffer, 0.02 ml of water and 0.03ml of epinephrine. Enzyme activity was estimated by measuring the change in absorbance at 480nm for 3-5minutes. An extinction coefficient of 4020 $m^{-1}cm^{-1}$ was used to calculate enzyme activity and expressed in unit of mg $protein^{-1}$.

The formula used in calculating the concentration of enzyme is given as

$$\text{Enzyme activity} = \frac{\text{OD / min} \times V}{\sum \times v}$$

v = volume of the sample.

V = Total volume of the mixture.

$\sum$ = Molar extinction coefficient.

OD/min = average absorbance per min.

Statistical Analysis

All the data was expressed as Mean ± S.D. and statistical significance was evaluated by one way analysis of variance (ANOVA) using SPSS17 at 95% confidence interval.

RESULTS AND DISCUSSION

The results of the haematological analyses showed that the white blood cell count of the control group, 0.5 ml/100g group and 1.0 ml/100g group were 14.33 × 10^9 /L, 12.48 × 10^9 /L and 13.82 × 10^9 /L respectively. There was no significant change ($p<0.05$) in the White Blood Cell count of the test groups (0.5 ml/100g and 1.0 ml/100g) when compared to the control (Fig.1).The red blood cell count of the control group, 0.5 ml/100g group and 1.0 ml/100g group were 6.93 ± 0.51 × 10^{12} /L, 6.09 ± 0.80× 10^{12} /L and 6.60 ± 0.64× 10^{12} /L respectively.

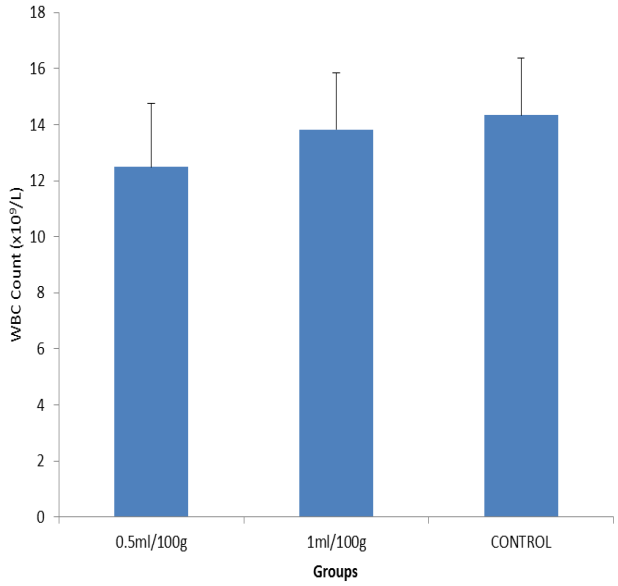

Fig. 1: Effect of *C. esculentus* milk extract on rat white blood cell count after 6 weeks of intake. n=7. No significant change was observed (p>0.05)

The values for the test groups were not significantly different (p>0.05) from that of the control (Fig.2). Likewise, the hemoglobin concentration of the test animals treated with 0.5 ml/100g and 1.0 ml/100g of the milk (11.58 g/dl and 12.37 g/dl respectively) did not vary significantly (p>0.05) when compared to that of the control animals (13.42 g/dl) (Fig.3).

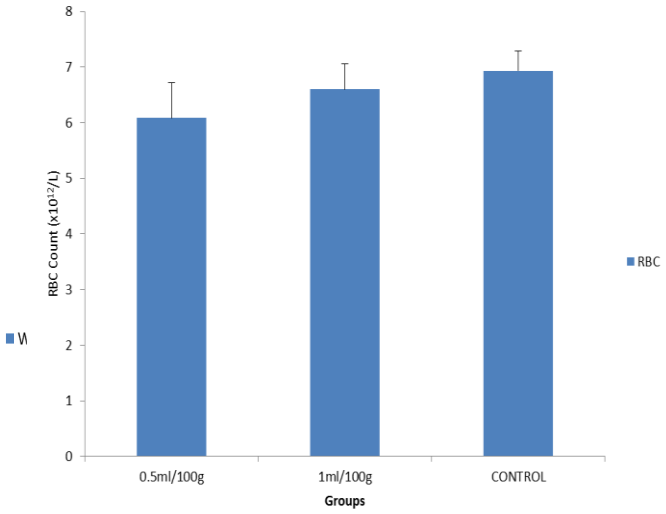

Fig.2: Effect of *C. esculentus milk* on Red Blood Cell Count of rats after 6 weeks of intake (n=7). Insignificant reduction in RBC Count was observed with the milk (p>0.05)

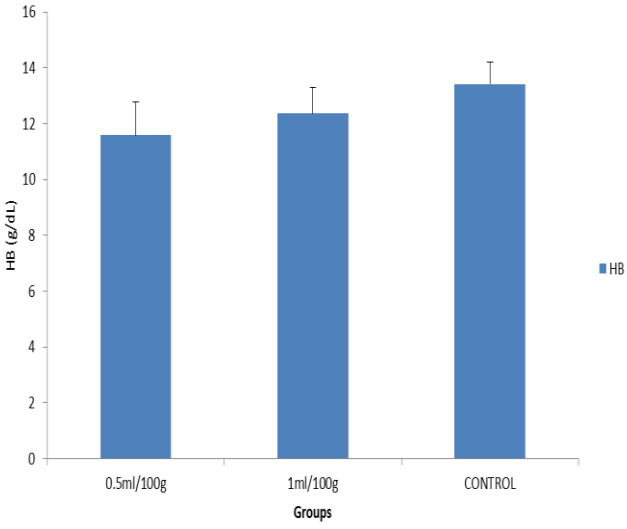

Fig.3: Effect of *C. esculentus* milk on rat haemoglobin concentration after 6 weeks of intake (n=7). No significant reduction was observed (p>0.05).

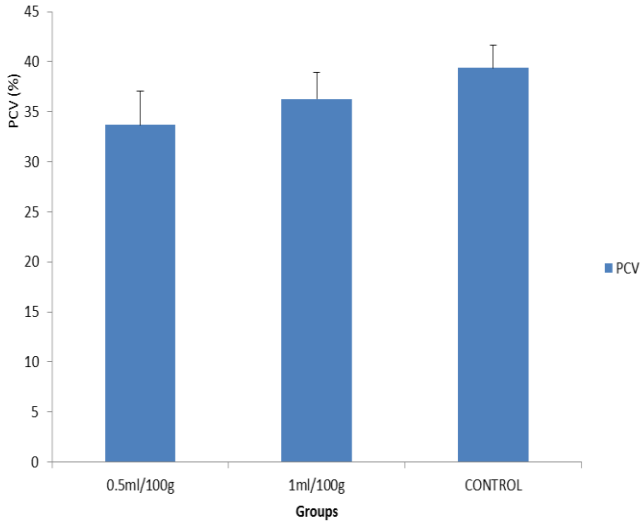

Fig.4: Effect of *C.esculentus* milk on rat packed cell volume after 6 weeks of intake (n=7). No significant decrease was observed (p>0.05).

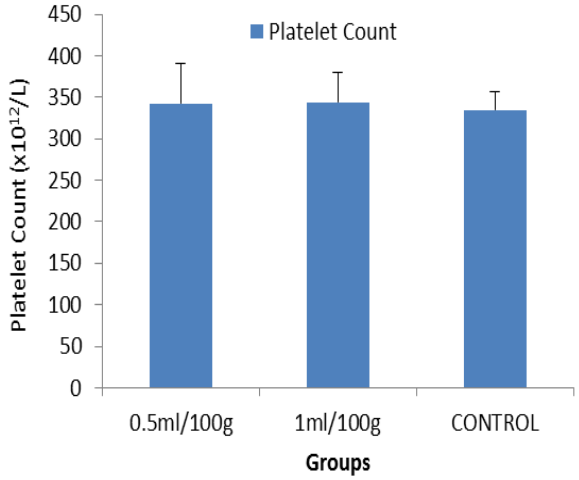

Fig.5: Effect of *C. esculentus* milk on rat blood platelet count after 6 weeks of intake (n=7). No significant change was noted.

The result presented in Figure 4 showed that the pack cell volume for the control group, 0.5 ml/100g group, and 1.0 ml/100g group were 39.37%, 33.68% and 36.28% respectively. No significant alteration ($p<0.05$) in the pack cell volumes of the test groups (0.5 ml/100g and 1.0 ml/100g) were observed when compared to the control. In fig. 5, no significant change in blood platelet count was detected following the intake of the milk. Administration of the higher dose of the milk (1.0 ml/100g) increased the values of all the haematological parameters (although non-significantly, $p>0.05$) without causing any adverse reaction in the animals. These results therefore may suggest the absence of toxic or undesirable effects in the use of *Cyperus esculentus* milk even in its raw form. Agbabiaka *et al*., 2013, reported decreased haemoglobin concentration, red blood cell count and packed cell volume in birds fed *C. esculentus* based diet. These observations support findings by Bamishaiye *et al*., 2009. In their report, no significant difference in all the haematological parameters (Hb, RBC Count, PCV, Mean cell haemoglobin concentration (MCHC), WBC Count and Platelet Count) in albino rats fed *C. esculentus* oil based diet was observed.

Our findings also revealed that the total protein content of the control group was 7.3 g/dl, that of the 0.5 ml/100g group was 6.12 g/dl and for the 1.0 ml/100g group it was 6.05g/dl. This shows that the intake of *C. esculenta* milk (0.5ml/100g and 1ml/100g) decreased the total protein concentration of the test rats when compared to the control. This decrease was not significant ($p<0.05$), but a significant decrease in the globulin fraction was recorded (Figures 6 and 7). The effects of the milk were not dose-dependent. The decrease in globulin could be responsible for the observed decreases in total protein and the haemetological

parameters due to reduced synthesis of haemoglobin in the erythrocytes.

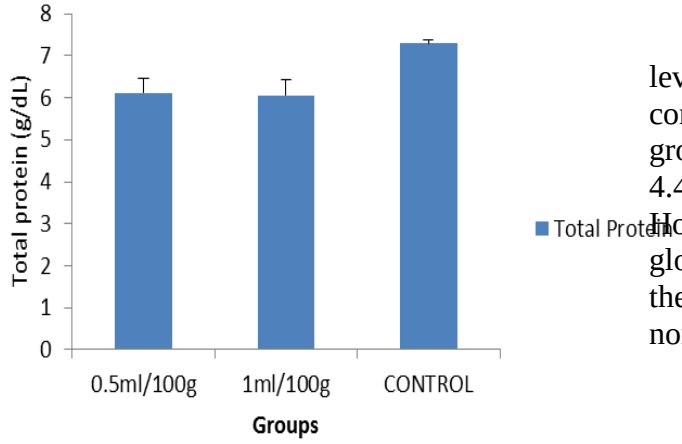

Fig.6: Effect of *C. esculentus* milk on total protein concentration of rat. There was an Non-significant decrease ($p>0.05$) in the total protein concentration of the test groups (0.5 ml/100g and1.0 ml/100g) when compared to the control (n=7).

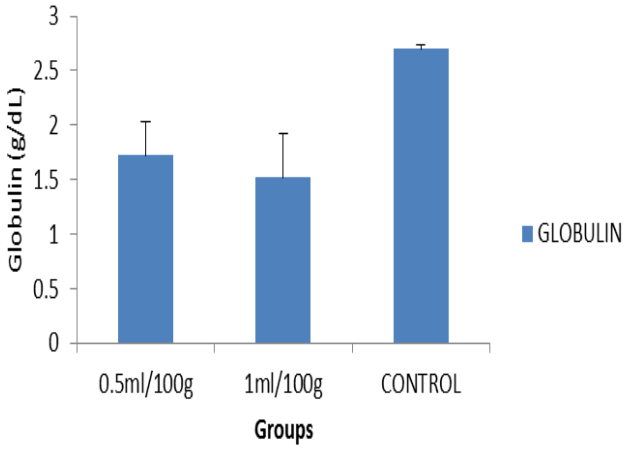

Fig.7: Effect of *C. esculentus* milk on the globulin concentrations of rat. There was a significant decrease ($p<0.05$) in the globulin concentration of the test groups (0.5 ml/100g and 1.0 ml/100g) when compared to the control.

No observable change in the albumin level was noted (Fig.8).The albumin contents of the control group, 0.5 ml/100g group and 1.0 ml/100g group were 4.60 g/dl, 4.40 g/dl and 4.52 g/dl respectively. However, these decreases in protein and globulin may be of no biological value since the observed values are still within the normal ranges.

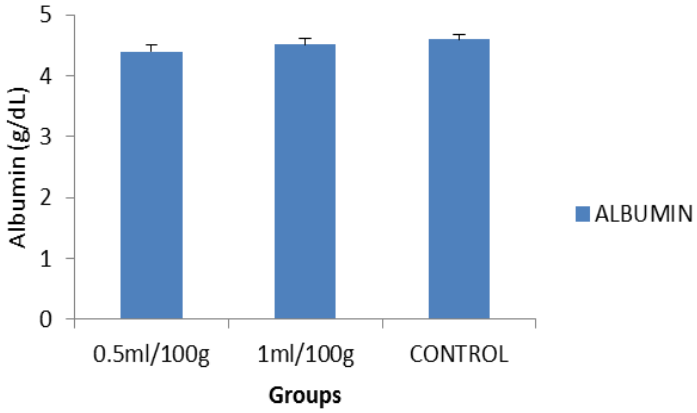

Fig.8: Effect of *C. esculentus* milk on blood albumin level. There was no significant change ($p>0.05$) in the albumin concentration of the test groups (0.5 ml/100g and 1.0 ml/100g) when compared to the control.

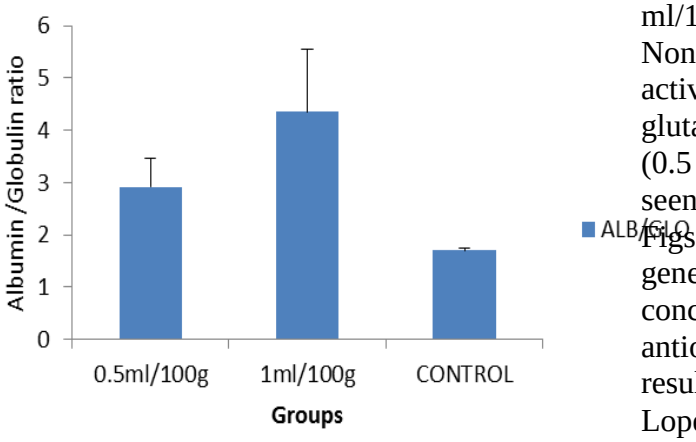

Fig.9: Effect of *C. esculentus* milk on the albumin – globulin ratio. Significant increase ($p<0.05$) in A/G ratio of the test groups (0.5 ml/100g and 1.0 ml/100g) were observed.

The value of albumin – globulin ratio (A/G) is an index of the health status with regard to nutrition. The A/G ratios observed in the tigernut fed rats (Fig.9) were an improvement of what was observed in the control group of rats indicating a better nutritional status as a result of the intake of the milk. Slight and insignificant changes in serum total protein and albumin after four weeks of oral administration of the ethanol extract of tiger nut were also reported by Akpojotor *et al*., 2015.

In vitro antioxidant effects of nuts demonstrated by their ability to inhibit lipid-peroxidation and oxidative DNA damage have been documented (Lopez-Uriarte *et al*., 2009). The results of the investigation of effects of tiger nut milk intake on endogenous antioxidant enzymes showed that the catalase activity of the control group, 0.5 ml/100g group and 1.0 ml/100g group were 0.319 units/mg protein, 0.145 units/mg protein and 0.016 units/mg protein respectively. There was no significant difference ($p<0.05$) in the catalase activity of the test groups (0.5 ml/100g and 1.0 ml/100g) when compared to the control. Non-significant differences ($p<0.05$) in the activities of superoxide dismutase and glutathione peroxidase of the test groups (0.5 ml/100g and 1.0 ml/100g) were also seen when compared to the controls (see Figs: 10, 11, and 12). This implies that the generation of free radicals, which concomitantly stimulates the activities of the antioxidant enzymes, was minimized. The results therefore support the reports by Lopez-Uriarte *et al*., 2009.

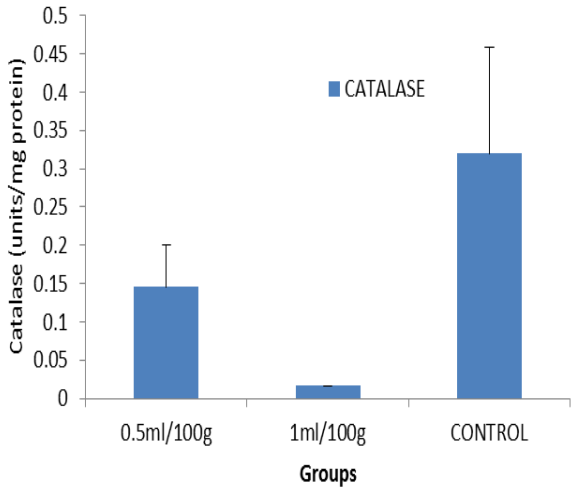

Fig.10: Effect of *C. esculentus* milk on serum catalase activity of rat. No significant difference ($p>0.05$) between the test groups (0.5 ml/100g and 1.0ml/100g) and the control was observed.

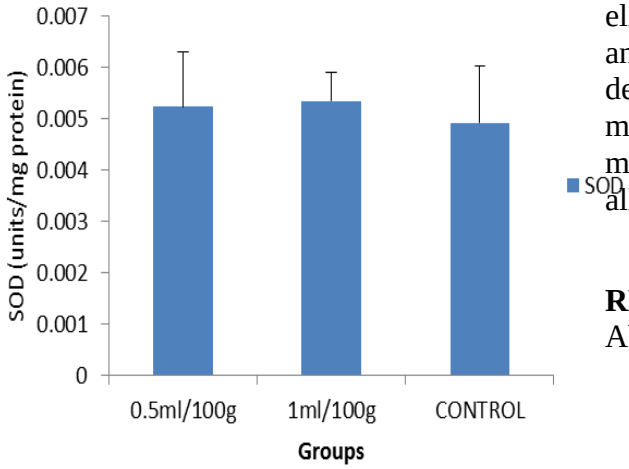

Fig.11: Effect of C. esculentus milk on serum superoxide dismutase activity of rat. No significant change ($p>0.05$ in activity was observed.

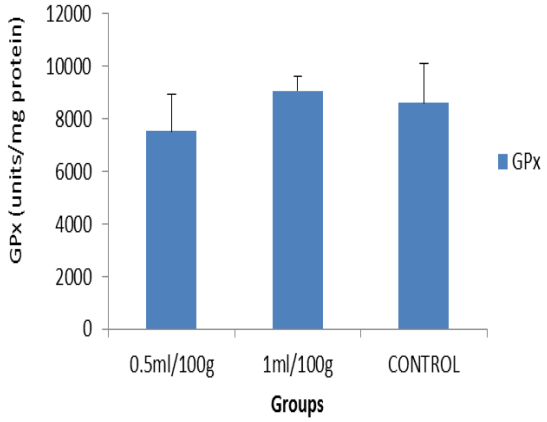

Fig.12: Effect of *C. esculentus* milk on serum glutathione peroxidase activity. No significant change ($p>0.05$) was observed.

CONCLUSION

In conclusion, the research findings show that *Cyperus esculentus* milk is adequate for maintaining a healthy nutrition, it does not trigger increase in white blood cell number and by implication may not elicit immunological or allergic challenges and does not encourage the accumulation of destructive free radicals. *Cyperus esculentus* milk could serve as a good source of plant milk especially for those individuals who are allergic to dairy milk and its products.

REFERENCES

Abaejoh, R., Djomdi I., Ndjouenkeu, (2006). Characteristics of tigernut (*Cyperus esculentus*) tubers and their performance in the production of a milky drink. *J food process. Preserv.*,30: 145-163.

Agbabiaka, L.A., Madubuike, F.N., Ekenyem, B.U., Esonu, B.O. (2013). Effect of tiger nut based diets on haematological and serum biochemistry of broiler finisher. *Agric Biol J North America*, 4(3): 186 – 191.

Akpojotor, P., Njoku, B., Kyrian, U.N. (2015). Effects of ethanolic extract of *Cyperus esculentus* (Tiger nut) on some liver function parameters using albino wistar rats. *European Journal of Pharmaceutical and Medical Research*, 2(5): 1705 – 1715.

Anderson, J.W., Smith B.M. and Gustafson, N.J. (1994). Health benefits and practical aspects of high fibre diets. *Am. J. Clin. Nutr*., 59: 1242S-1247S.

Bamishaiye, O., Muhammad, N., Bamishaiye, O. (2009). Haematological parameters of albino rats fed on tiger nuts (*Cyperus esculentus*) tuber oil meal – based diet. The International Journal of Nutrition and Wellness, 10(1).

Belewu, M.A., Abodunrin A.O. *(*2008). Preparation of kunun from an

unexploited rich food source: tiger nut (*Cyperus esculentus*). *Pak J Nutr* 7:109–11.

Belewu, M.A. (2006). A functional approach to dairy science and technology. Adlex Publisher, Ilorin, Nigeria.

Bosch, L., Alegria, A. and Farri, R. (2005). RP-HPLC determination of tigernut and orgeat amino acid contents. *Food Sci. Technol. Inter.*, 11: 33-40.

Cantalejo, M.J. (1997). Analysis of volatile components derived from raw and roasted earth almond (*Cyperus esculentus* L.). J. Agric. Food Chem.45: 1853-1860.

Chukwuma, E.R., Obiama, N. and Christopher, O.I. (2010). The phytochemical composition and some Biochemical effect of Nigerian Tigernut (*Cyperus esculentus*. L) tuber. *Pakistan Journal of Nutrition*, 9(7): 709-715.

David, A.B. (2005). "Tiger nut". A Dictionary of Food and Nutrition.Encyclopedia.com: http://www.encyclopedia.com/

Devries, F. and Feuke, T. (1999). Chufa (Cyperusesculentus) A weedy cultivar or cultivated weed? Econ. Bot., 45: 27- 37.

Doumas, B.T., Watson, W.A. and Biggs, H.G. (1971). Albumin standards and the measurement of serum albumin with Bromocresol green. Clin Chim Acta.

Elbim ,C., Pillet, S. and Prevost, M.H. (1999). Redox and activation status of monocytes from human immunodeficiency virus-infected patients. *Journal of Virology*, 73 (6): 4561-4566.

Lopez – Uriarte, P., Bullo, M., Casas – Augustench, P., Babio, N., Salas – Salvadó, J. (2009). Nuts and oxidation, a systematic review. Nutr Rev., 67: 497 – 508.

Mason,D.(2005).TigernutsIn:http://www.nvsuk.org.uk/growing_show_vegetables_1/tiger_nut.pp . Accessed December, 2009.

Ogbunugafor, H.A., Igwo – Ezikpe, M.N., Igwilo, I.O., Salisu, T., Ezekwesili, C.N. (2013). A *Cajanus cajan*: Potentials as functional food. *The Bioscientist*, 1(2): 119 – 126.

Oladele, A.K. and Aina, J.O. (2007). Chemical composition and functional properties of flour produced from two varieties of tiger nut (*Cyperus esculentus*). *Afr. J. Biotechnol*., 6: 2473-2476.

Sun, M. and Zigma, S. (1978). An Improved spectrophotometric assay of superoxide dismutase based on epinephrine auto oxidation. *Analytical Biochemistry*.90:81-89.

Umerie, S.C., Okafor, E.P., Uka, A.S. (1997). Evaluation of the tubers and oil of *Cyperus esculentus. Bioresour. Technol*., 61: 171-173.