Original Research Article

Assessment of the Impact of aqueous extract of *Telfairia occidentalis* on the haematological profile and blood film of wistar rats

ABSTRACT

Aims: This study investigated the impact of the aqueous extract of *Telfairia occidentalis* (TO) on the haematological profile and blood film of wistar rat.

Methodology: A total of 20 wistar rats randomly distributed into four groups A to D were acclimatized for two weeks; group A served as control, while groups B C and D were gavaged 100 mg/kg b/w (UGU 1), 150 mg/kg b/w (UGU 2), 250 mg/kg b/w (UGU 3) of aqueous extract of TO respectively, once every 48 hours for 60 consecutive days. After the exposure period, the surviving rats were examined and sacrificed. Blood samples were collected for full blood count and blood film preparation.

Results: The result of the study showed that TO casued an white blood cell count (25.48±1.28× 10^9 cells/L) when compared to the Control (17.50±0.10× 10^9 cells/L). There was equally an increase in the platelet count (810.95±43.05× 10^9 cells/L) when compared to the Control (420.50±56.50× 10^9 cells/L). However, there was a reduction in the red blood count of rats given dosage of the extract i.e. (6.75±0.42× 10^9 cells/L), (6.92±0.34× 10^9 cells/L) and (6.28±0.24× 10^9 cells/L) when compared with the Control (7.76.±0.06× 10^9 cells/L). It was also observed that rats administered 0.13g/ml has the lowest red blood cell count. Blood film analysis indicated that the presence of healthy leucocyte and erythrocyte cells.

Conclusion: In conclusion, this present study have shown that the potentials of aqueous extract of *T. occidentalis* contains potent antioxidant such as Flavonoids, Alkaloids, Tannins and Saponins that helps to regulate the haematological parameters of wistar rats.These antioxidants equally exerts a multitude of beneficial effect on cellular functions of both innate and adaptive immune system.

Keywords: blood film, haematology, full blood count, wistar rats, Telfairia occidentalis.

1. INTRODUCTION

Since the birth of medicine, people have relied on natural goods, particularly those derived from plants, to maintain their health. Tropical vine of *T. occidentalis* is produced in West Africa for its leaf vegetable and edible seeds. Among the plants other names are fluted gourd and fluted pumpkin. It is perennial, dioecious, and resistant to drought. It is low-lying shrub with broad, lobed leaves and long, twisting tendrils that spread over the ground (Horsfall and Spiff, 2005). It grows well in areas with high humidity and well-drained soils, gardens and small family farms. It belongs to Curcubitaceae family and has a simple, dark green veined leaf that may measure up to 18 cm broad and 35 cm long. The Curcubitaceae have been linked to human, according to one research (Esquinas-Alcazar *et al*., 1983). Watermelon & melon are all members of the Cucurbitaceae family (Oboh, 2005). Nutrients including protein, carbs, vitamins, and fiber may be found in *T. occidentalis (*Fasuyi, 2006). Oxalates, saponins, glycosides, are also found in this plant (Tindall, 1968).

An estimated 30 to 35 million indigenous Nigerians, including the Efik, Ibibio, and Urhobo, customarily utilize *T. occidentalis* (Akoroda, 1990). However, the Igbo ethnic group continues to produce the gourd as a food source and a medicine (Okoli *et al.,* 1983; Nwanna *et al*., 2008). Fluted gourds are often mentioned in Igbo mythology as having therapeutic abilities and were used as a blood tonic for those who were weak or ailing. The relevance of plant active components in agriculture and medicine has sparked a great deal of scientific interest in their biological activities. Majority of the functions of most plants in the natural world is still limited, despite the many research that has been conducted, it is necessary to do a thorough examination into the biological activities such as Ugu (*T. occidentalis*) and their impact in the haematological and blood profile of wistar rats.

2. MATERIAL AND METHODS

2.1 Collection, identification and laboratory analysis of edible plant

Fresh leaves of *Telfairia occidentalis* (fluted pumpkin) were purchased from Effurun Market in Effurun, Delta state in January, 2021; the taxonomic identity of the plant was confirmed at the Department of Plant Biology and Biotechnology, Faculty of Life Science, University of Benin, Benin City, Edo State, Nigeria.

2.2 Preparation of plant extracts

The purchased leaves were air-dried to crispiness in the laboratory (prevailing room temperature of 30 ± 2°C) for two weeks. The dried materials were reduced to coarse form using a pestle and mortar and further pulverized to very fine particles using Viking Exclusive Joncod pulverizing machine (Model: YLH2M2 - 4). The crude aqueous extract was prepared by decoction where 50g of the leaf powder extracted with 500mls of distilled water (via maceration) for 48hrs. The mixture was decanted and filtered using sterile whatman paper No 1. The filtrate was evaporated to dryness using a freeze dryer and reconstituted in distilled water to appropriate concentrations.

2.3 Experimental setup

Male wistar rats (6-7 weeks old) weighing within the range of 100g to 150g were obtained from the Anatomy Department, University of Benin, Nigeria. The rats were distributed randomly into four groups of six animals each for group A to D and allowed to acclimatized for 2 weeks. During acclimatization, the animals were housed in wooden cages with wire mess covers and fed with standard rodent chow (Bendel Livestock Feeds Limited, Ewu, Edo state, Nigeria) and given distilled water ad labium. After acclimatization, the rats were given different treatment protocol: Group A which was the

Control (CTR) was given distilled water; Group B, C and D rats were gavaged 100 mg/kg b/w (UGU 1), 150 mg/kg b/w (UGU 2), 250 mg/kg b/w (UGU 3) of aqueous extract of TO for 60 consecutive days (once every 48 hour) respectively. The rats were maintained in laboratory conditions; and had access to drinking water and standard rodent chow (Bendel Livestock Feeds, Ewu, Edo state, Nigeria®) *ad libitum*.

At the end of exposure period, survivors were fasted overnight and sacrificed under slight Anesthesia; then blood samples were collected. Blood was collected from the inferior vena cava of the rats with plain 5ml sterilized syringe into a vial containing 0.5 m EDTA for haematolgical analysis under a light anaesthesia. The blood sample was gently homogenised to ensure proper mixing of the blood with the anticoagulant, before taking it to the laboratory.

2.4 Laboratory analysis

In the laboratory, hematological analysis was carried out using Sysmx KX-21N automated machine (Sysmx corporation kobe, Japan) following the manufacturer's instructions. Briefly the sample was mixed and placed in contact with the sample probe for aspiration, when the buzzer sounds twice "beep, beep" and when the LCD screen displays ANALYZING, the sample was removed. Following this, the unit executed automatic analysis, and the result was displayed on the LCD screen and printed out. A drop of blood previously store in an EDTA bottle was placed on a slide. The blood was spread using the cover slip and left to dry at room temperature. The dried film was stained using Geimsa stain and left for 30 minutes. The film was rinsed with water and dehydrated using ascending grades of alcohol (starting from 70%, 90%, 96% and absolute); and cleared in xylene for 5 minutes. The section was mounted using shandom's mount (Distrene Dibutyl Phathalate xylene), covered with a cover slip and allowed to dry. The slides were examined using Leica CME light microscope (Model – 1349522X).

2.5 Data Analysis

All statistical analyses were conducted with Statistical Package for Social Scientists (SPSS) and Microsoft Excel computer software. Data are presented as mean±SE (n=5). One-way ANOVA was used to determine the differences among various groups. When the corresponding F test for differences among the treated group means was significant pair wise, comparisons between treated groups and corresponding negative control were determined using multiple comparison procedure of the Dunnett post-hoc test and differences were considered significant at $p<0.05$, $p<0.01$ and $p<0.001$ levels of significance.

3. RESULTS AND DISCUSSION

Studies have shown that haematological parameters represent a useful process in the diagnosis of many diseases as well as investigation of the lenght of damage to the blood (Onyeyili *et al*., 1991). This is relevant since blood constituents' change in relation to the physiological conditions of the animals. Haematological studies are vital because blood is a critical transport system of the body, and calculation of the haematological profile usually presents important information on the body's response to injury of all kinds, including hazardous injury (Schalm *et al*., 1975, and Ihedioha *et al*., 2004). Haematological constituents depicts the physiological functionality of the animal to its internal and external environments which include feed and feeding (Esonu *et al*., 2001) as well as drugs (Iheukwumere *et al*., 2007).

The white blood cell and differential count is usually carried out in order to produce information on the amount of the different white cells present in circulating blood (Cheesbrough, 2002). In this study the aqueous extract of *T. occidentalis* induced lymphocytosis ($16.00\pm5.40\times 10^9$ cells/L) of rats administered 0.07g/ml of the extract when compared to the Control ($12.45\pm1.35\times 10^9$ cells/L). Similarly, the extract of *T. occidentalis* induced the granulocytosis ($5.10\pm0.79\times 10^9$ cells/L) of rats administered 0.13g/ml of the

extract when compared to the Control (1.67±0.83× 10^9 cells/L). The monocytosis of the rats given *T. occidentalis* were equally induced (5.10±0.79× 10^9 cells/L) of rats administered 0.13g/ml of the extract when compared to the Control (3.38±0.42× 10^9 cells/L). Similar findings have been observed by Salmon *et al*., 2008). According to them the extract of *T. occidentalis*, significantly increased white blood cell count (25.48±1.28× 10^9 cells/L) when compared to the Control (17.50±0.10× 10^9 cells/L). The rise in the haematological indices observed following the treatment of the leaf extract might not be unconnected with the chemical composition of the leaves. The chemical composition shown to include proteins, fat, vitamin A, thiamine, riboflavin, nicotinamine, vitamin C (Aletor *et al.,* 1995) and minerals such as zinc, iron, calcium and magnesium (Archibong, 2002). Of all the white blood cell population counted, the lymphocytes were the mostly proliferated cells, followed by the granulocyte and monocytes. It is also possible that the membranes of these lymphocytes were oxidized as the rats were subjected to aqueous extract of *T. occidentalis*; as very high concentration of lymphocyte in the blood is suggestive of high degree of infection after trauma arising to high antibodies production (Abbas and Lichtman, 2003). High granulocyte levels indicates a high cellular damage/inflammation and depressed immunity; while increased monocyte count might be a sign of a serious infection, an autoimmune disorder or a blood disorder (Shugaba *et al*., 2012).

Table 1: Hematological profile of male wistar rats administered crude aqueous extract of TO leaf

	CTR	UGU 1	UGU 2	UGU 3
WBC (x 10^3/µl)	17.50±0.10	18.40±5.80	12.85±2.65	25.48±1.28
LYM (x 10^3/µl)	12.45±1.35	16.00±5.40	9.15±0.95	15.86±2.66
MID (x 10^3/µl)	3.38±0.42	1.70±0.20	2.20±1.00	4.61±0.60
GRA (x 10^3/µl)	1.67±0.83	0.70±0.20	1.50±0.70	5.11±0.80
LYM (%)	73.03±9.13	86.20±2.20	72.85±7.55	62.65±7.95
MID (%)	18.65±3.25	9.95±2.05	16.10±4.40	17.66±3.64
GRA (%)	8.32±5.88	3.85±0.15	11.05±3.15	19.69±4.31
RBC (x 10^6/µl)	7.76±0.06	6.92±0.34	6.75±0.42	6.28±0.24
HGB (g/dl)	16.67±0.37	14.10±0.80	13.70±0.70	12.18±0.58
HCT (%)	41.47±0.27	36.65±1.75	35.30±1.40	33.55±1.25
MCV (µm^3)	53.48±0.78	52.95±0.05	52.40±1.20	53.40±0.11
MCH (ρg)	21.43±0.63	20.35±0.15	20.30±0.20	19.32±0.12
MCHC (g/dl)	40.25±0.65	38.45±0.35	38.75±0.45	36.20±0.30
RDW-CV (%)	16.20±0.30	17.40±0.70	17.55±0.95	18.65±0.46
RDW-SD (µm^3)	30.88±0.68	32.10±1.90	31.15±2.95	37.01±1.09
PLT (x 10^3/µl)	420.50±56.50	493.00±75.00	558.00±13.00	810.95±43.05
MPV (µm^3)	8.03±0.13	7.05±0.25	7.25±0.25	7.14±0.06
PCT (%)	0.33±0.05	0.35±0.04	0.40±0.00	0.58±0.03
PDW (%)	17.88±0.58	16.70±2.20	15.95±0.35	15.97±0.14
P-LCR (%)	11.27±0.73	6.90±0.70	8.20±0.60	7.01±0.39

***NB**: All values are expressed as Mean±SE.*

One of the major roles of lymphocyte is their response to antigen (foreign bodies) by forming antibodies that circulate in the blood or in the development of cellular immunity Frandson (2003). Granulocytes such as neutrophils are the first-responders to inflammation and cell damage, while eosinophils are primarily associated with parasitic infections and an increase in their number may indicate such (Alberts, 2005). Ingestion of aqueous extract of *T. occidentalis*, as a matter of fact, may have induced an increase in the metabolic rate, with the resultant increase in the generation of free radicals with the attendant cellular damage. The immune system responds to these damages by the production of

oxidants during strenuous conditions. During such responses, free radicals are produced by the body, the first-responders to inflammatory cells to remove damaged cells. Being highly mobile, neutrophils swiftly congregate at a focus of infection, attracted by cytokines expressed by activated endothelium, mast cells and macrophages (Ear and McDonald, 2008). Monocytes help the immune system fight infection. These white blood cells have the ability to turn into dendritic cells and macrophages when the immune system detects a foreign substance. Dendritic cells identify foreign substances and communicate with B and T cells to help the body build immunity to a particular substance. Macrophages destroy parasites, bacteria and other organisms (Ugochukwu, 2003).

The reduction in haemoglobin concentration (12.17±0.58× 10^9 cells/L) when compared to the Control (16.67±0.37× 10^9 cells/L) and red blood cell counts (6.75±0.42× 10^9 cells/L) when compared to the Control (7.76±0.-06× 10^9 cells/L) is indicative of altered peripheral blood composition which is a perceived as disrupted hematopoietic process and interference with different stages of red cell synthesis and mature red blood cells. Heavy metals are known to cause inhibition of aminolevulinic acid dehydratase activity, thereby tempering the heme synthesis pathway. The reduction in hemoglobin, red blood cell and hematocrit may lead to insufficient levels of oxygen in the body (hypoxia). Same findings have also been reported by Hounkpatin *et al*. (2013) as a result of toxicity of cadmium, mercury and their combination; the study revealed a significant reduction in red blood cells (RBC), haemoglobin concentration (HGB) in wistar rats. Mannem (2014) upon thorough investigation, the toxicity of lead acetate on several haematological parameters in male wistar rats; and reported a significant decrease in the mean RBC values. Again, Nikolic *et al*. (2013) has shown that Pb, Cd and Cu intoxication significantly reduced values of erythrocytes, hemoglobin and hematocrit in induced wistar rats. The main function of the erythrocytes is to transport oxygen bound to hemoglobin from the lungs to the tissue. Thereafter, hemoglobin in erythrocytes is an excellent acid-base buffer, thus erythrocytes are the most responsible for the buffer capacity of whole blood (Ersley and Gabuzda, 1985). Hematocrit is used to measure blood carrying capacity and is directly associated with the HB% declination in this study. Reduction in hematocrit may be due to suppressed bone marrow hematopoietic system, causing iron deficiency in synthesis of haem protein of hemoglobin (Klauder and Petering, 1977; guyton and Hall, 1996). The insufficient hematocrit would show anemia or oligoheamia (Wepener *et al*., 1992). The changes in the blood HCT value has often been shown to be good indicator of heavy metal toxicity.

Platelets are cytoplasmatic fragments of bone marrow megakaryocytes (Laki, 1972). They are dynamic blood particles whose major function, along with the coagulation factors, is haemostasis, or the stoppage of bleeding. Platelets interact with each other, as well as with leukocyte and endothelial cells, searching the vascular bed for sites of injury, where they become activated (Machlus *et al*., 2014). In addition to their vital role in haemostasis and thrombosis, accumulating evidence demonstrates that platelets contribute to the inflammatory process, microbial host defense, wound healing, angiogenesis, and remodeling (Jain, 1975). Increased platelet count and plateletcrit in rats administered aqueous extract of *Telfairia occidentalis* was similar to the findings of Hounkpatin *et. al*. (2013). According to them, the increase in platelet count was as a result of chronic doses of cadmium, mercury and combined cadmium and mercury in wistar rats. Again, Barman *et al*. (2014) reported that the levels of platelet count (PLT), plateletcrit (PCT) and mean platelet mass (MPM) were significantly low and platelet distribution width (PDW), platelet large cell ratio (P-LCR) and mean platelet volume were high in workers involved in a lead-acid battery manufacturing plant. The increase may be as a result of the presence of substance in the leaf extract which may have resulted in the increased production of thrombocytes. Too many platelets can lead to certain conditions, including stroke, heart attack, or a clot in the blood vessels. Thrombocytosis may be caused by anemia due to iron deficiency, inflammation or infection (Cleveland clinic, 2017).

Plate 1b – 1d shows blood film of male rats given leaf extract of TO at various concentration. The micrographs did not show any tissue damage when compared to the control rat (Plate 1), as white blood cell showed mild lymphocytosis while erythrocytes showed normocytic cell +++, normochromic cells +++,no blood parasite seen. Erythrocytes in rats exposed to 100 mg/kg b/w and 50 mg/kg b/w crude aqueous extract of TO were nucleated. Platelet appeared adequate in number and normal in size.

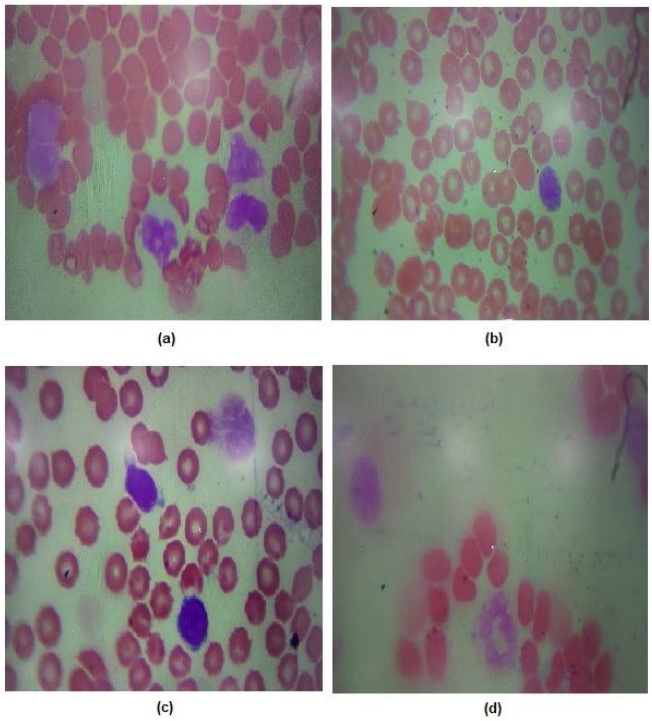

Plate 1: Blood film of (a) control rats (CTR) (b) rats exposed to 100 mg/kg b/w crude aqueous extract of TO (c) rats exposed to 150 mg/kg b/w crude aqueous extract of TO (d) rats exposed to 250 mg/kg b/w crude aqueous extract of TO (Eosin stain X 100)

4. CONCLUSION

In conclusion, this present study have shown that the potentials of aqueous extract of T. occidentalis contains potent antioxidants that helps to regulate the haematological parameters of wistar rats. These antioxidants equally exert a multitude of beneficial effect on cellular functions of both innate and adaptive immune system.

ETHICAL APPROVAL

This research design was reviewed and approved by the College of Science Ethical board, Federal University of Petroleum Resources, Effurun (CS/EMT/2021/006).

5 REFERENCES

1. Abbas, A.K. and Lichtman, A.H. (2003). *Cellular and Molecular Immunology*. 5th Edn., Saunders, Philadelphia.2146pp.
2. Ajayi, T. C., Omakaro, E. U. and Halin, N. K. D., 2000. Erythropoietic values of pumpkin leave extract Telfairia occidentalis in rabbits- a prelimary study. Nig J Physiol Sciences; (16): 1-2.
3. Akoroda., (1990). Ethnobotany of Telfairia occidentalis (Curcurbitaceae) among Igbos of Nigeria Econ. Bot., pp. 29-39.

4. Alada, A. R. A., 2000. The haematological effect of Telfaira occidentalis diet preparation. *African Journal of Biomedical Research. 3(20):* 185- 186.
5. Alberts, B. (2005). Leukocyte functions and percentage breakdown. *Mol. Biol. Cell*, 4: 41-43.
6. Aletor, V.A. and O.A. Adeogun, (1995). Nutrient and anti-nutrient components of some tropical leafy vegetables. Food Chem., 53: 375-379
7. Alimba, C.G. Bakare, A.A. and Latunji, C.A. (2006). Municipal landfill leachates induced chromosome aberrations in rat bone marrow cells. *African Journal of Biotechnology* 5(22): 2053-2057,
8. Badifu G.I., Akpapunan M.A., Mgbemere V .M., (1995). The fate of betacarotene in processed leaves of fluted pumpkin, a popular vegetable in Nigerian diet plant foods. Hum. Nutri; 48: 141-147.
9. Barman, T., Kalahasthi, R. and Rajmohan, H R (2014). Effects of lead exposure on the status of platelet indices in x. *Journal of Exposure Science and Environmental Epidemiology* 24:629-633
10. Cheesbrough, M. (2002). PCV and Red Cell Indices, Haematological Tests. **In**: Cheesbrough, M., editor. District Laboratory Practice in Tropical Countries, Part 2. 314pp.
11. Cleveland clinic (2017). *Thrombocytosis*. Available at: http://my.clevelandclinic.org/health/articles/thrombocytosis. Accessed: 12th February, 2017.
12. Ear, T. and McDonald, P.P. (2008). Cytokine generation, promoter activation and oxidant-independent NF-kappaB activation in a transfectable human neutrophilic cellular model. *BMC Immunol.,* 9: 14-14.
13. Ellman GL. 1959. Tissue sulfhydryl groups. Arch Biochem Biophys. 82: 70-77.
14. Ersley, A.H. and Gabuzda, T.G. (1985). *Basic pathophysiology of blood.* 3rd edition, Philadelphia, WB Saunders Co. 2954pp.
15. Eseyin O.A., Ebong P., Ekpo A., Igboasoiyi A., Oforah E. Hypoglycemic effect of the seed extract of Telfairia occidentalis in rat. *Pakistan J Biol Sci.* 2007;10(3):498–501.
16. Esonu, B.O., Emennalom, O.O., Udedibia, A.B.I., Herbert, U., Ekpor, C.F., Okoli, E.C. and Iheukwumere, F.C. (2001). Performance and Chemistry of Weaners Pigs fed raw mucuna bean (Velvet bean) meal. *Trop Anim Prod Invest.* 4:49–54.
17. Esquinas- Alcazar J.T., Gulick PJ, (1983). Genetic Resources of Cucurbitaeae Int Board for plant Genet, Resources, Rome.
18. Fasuyi, A.O., (2006). Nutritional potentials of some tropical vegetable leaf meals: chemical characterization and functional properties. Afr. J. *Biotech*., 5: 49-53.
19. Frandson, R.D. (2003). Anatomy and Physiology of Farms Animals. 6th Edition, Baltimore, MD: Lippincott Williams & Wilkins, 481pp.
20. Hamzah R. U, Jigam A. A, Makun H. A, Egwim E. C.(2013) Antioxidant properties of selected african vegetables, fruits and mushrooms: A review, mycotoxin and food safety in developing countries,
21. Horsfall, M Jnr. Spiff, A. I (2005). Equilibrium sorption study of Al^{3+} , Co^{2+} and Ag^{+} in aqueous solutions by fluted pumpkin (*Telfairia occidentalis Hook F*) waste biomass. Acta Chim Slov 52:174-181.
22. Hounkpatin, A.S.Y., Edorh, P.A., Guédénon, P., Alimba, C.G., Ogunkanmi, A., Dougnon, T.V., Boni, G., Aissi, K.A., Montcho, S., Loko, F., Ouazzani, N., Mandi, L., Boko, M. and Creppy, E.E. (2013). Haematological evaluation of Wistar rats exposed to chronic doses of cadmium, mercury and combined cadmium and mercury. *African Journal of Biotechnology* 12(23): 3731-3737..
23. Hounkpatin, A.S.Y., Edorh, P.A., Salifou, S., Gnandi, K., Koumolou, L., Agbandji, L., Aissi, K.A., Gouissi, M. and Boko, M. (2012). Assessment of exposure risk to lead and cadmium via fish consumption in the lacusrian village of Ganvié in Benin Republic. *J. Environ. Chem. Ecotoxicol.* 4(1):1-10.

24. Hutchins R .A., (1973).New method simplifies design of activated carbon systems. Chem Eng 80 : 133.
25. Ihedioha, J.T., Okafor, C. and Ihediola, T.E. (2004). The haematological profile of the Sprague Dawley out-bred albino rat in Nsukka. *Anim Res Intern.* 1:125–132.
26. Iheukwumere, F.C., Herbert, U., Iloeje, M.U. and Onyekwere, M. (2007). Physiological response of West African Dwarf does to progesterone injections: Haematology and serum biochemistry. *Proc. of 32nd annual conf. of NSAP.* 18–21.
27. Kayode O T., Kayode A A., Odetola (2009). Therapeutic effect of *Telfairia occidentalis* on Protein Energy Malnutrition – Induced Liver Damage 3 (3) 80-92
28. Klauder, D.S. and Petering, H.G. (1977). Anaemia of lead intoxication: A role of copper. *Journal of Nutrition* 7:1779-85
29. Machlus, K.R., Thon, J.N. and Italiano, J.E. (2014). Interpreting the developmental dance of the megakaryocyte: A review of the cellular and molecular processes mediating platelet formation. *British Journal of Haematology.* 165 (2): 227–36.
30. Mannem, P. (2014). Lead toxicity on hematological changes and amelioration with ginger (*Zingiber officinale*) extract in male albino rats*. International Journal of Advanced Research* 2(4): 23-28.
31. Mannem, P. (2014). Lead toxicity on hematological changes and amelioration with ginger (*Zingiber officinale*) extract in male albino rats*. International Journal of Advanced Research* 2(4): 23-28.
32. Misra HP, Fridovich I. The generation of superoxide radical auto-oxidation of hemoglobin. J. Biol. Chem .1972;247:6960- 6962.
33. Nikolic, R., Krstic, N., Jovanovic, J., Kocic, G., Cvetkovic, T.P. and Radosavljevic-Stevanovic, N. (2013). Monitoring the toxic effects of Pb, Cd and Cu on hematological parameters of Wistar rats and potential protective role of lipoic acid and glutathione. *Toxicol. Ind. Health.* 31(3): 239-246.
34. Nwanna E. E, Oboh G.(2007). Antioxidant and hepatoprotective properties of polyphenol extracts from *Telfairia occidentalis* (Fluted Pumpkin) leaves on acetaminophen induced liver damage. *Pakistan Journal of Biological Sciences*.
35. Nwanna, Esther Emem, et al. (2008) "Antioxidant and Hepatoprotective Properties of Telfairia occidentalis Leaf (Fluted Pumpkin)." Thesis and Dissertations (Biochemistry)