ASN-PH-020919
ISSN: 2315-5388

International Journal of Basic, Applied and Innovative Research
IJBAIR, 2014, 3(1): 29 - 34
www.arpjournals.com; www.antrescentpub.com

RESEARCH PAPER

EFFECTS OF DIETARY *XYLOPIA AETHIOPICA* ON HEMATOLOGICAL PARAMETERS AND PLASMA LIPIDS IN MALE WISTAR RATS

[*1]Onyebuagu, P.C., [1]Pughikumo D.T. and [2]Aloamaka C.P.

Department of [1]Human Physiology, Niger Delta University, Bayelsa, Nigeria. [2]Human Physiology, Delta State University, Abraka, Nigeria.

***Corresponding author:** peteronyebuagu@gmail.com

Received: 20th January, 2014 **Accepted: 29th March, 2014** **Published: 31th March, 2014**

ABSTRACT

The effects of dietary intake of whole fruits of *Xylopia aethiopica* on blood parameters were studied. The PVC, Hb, MCV, MCHC, RBC, WBC, platelets, ESR, PT, APTT, cholesterol and triglyceride were estimated using standard methods. The fruits demonstrated significant increases ($p<0.05$) in Hb concentration, PCV, MCV and RBC counts, and significant decreases ($p<0.05$) in ESR and total cholesterol in the male Wistar rats. It is concluded that the whole fruits of *Xylopia aethiopica* exhibited positive effects on blood cell indices and properties, probably by virtue of its rich iron content, and that its reduction in serum total cholesterol is a welcome development as an anti-hyperlipidemia against the cardiovascular risk factors associated with elevated blood cholesterol levels.

Key words: *Xylopia aethiopica,* hematological parameters, Lipid profile, Wistar rats.

INTRODUCTION

The dried fruits of *Xylopia aethiopica* are used as spice, due to their strong aromatic quality, in the preparation of special local soups named “isi ewu” and “obe nta” in south-eastern parts of Nigeria. They are used in traditional medicine as a carminative, cough remedy, postpartum tonic in alleviating after-birth wounds, and as lactation aid (Murray, 1995). They are also employed in several traditional medicine remedies in most parts of Nigeria for the treatment of stomachaches, bronchitis, biliousness, dysentery (to mention a few), and externally applied as a poultice for headache and neuralgia. In combination with leaves of *Newbouldia laevis* (Bignoniaceae), or chieftaincy leaf, or ”ogilisi” in Igbo language, fruits of *X. aethiopica* is used for increasing menstrual blood flow, and is administered in combination with the root of *Blighia sapida* (Sapindaceae), or Isin in Yoruba, to terminate unwanted pregnancy - and thus, it is believed to have abortifacient properties (Muanya, 2008). In Southern Africa, traditional herbalists grind a mixture of seven dried fruits of *Xylopia aethiopica* and 21 leaves of *Rouwulfia vomitaria,* and administer the mixture, orally, to pregnant women at term to induce labour and achieve delivery (Gbile, 1989).

Most of the early works on *Xylopia aethiopica* had centered on its biologic activities, such as, antimicrobial (Iwu, 1993), antiparasitic (Tairu et al., 1999), insecticidal (Ojimelukwe and Okonkwo, 1999), antifungal (Awuah, 1986), antioxidant (Odukoya et al. (2005), diuretic and hypotensive (Semova *at al.*, 2001), antimalarial (Eftkins, 1997) and membrane stabilization (Ezekwesili *et al*., 2010). Nwafor (2013) demonstrated actions of *Xylopia aethiopica* against sperm production in laboratory animals, while its protective action on the liver and kidneys of Wistar rats was reported by Onyebuagu (2012). Nwafor *et al.* (2009) reported that methanolic extract of Xylopia *aethiopica in vivo* caused slight increases in some and decreases in other hematological parameters. However, the effect of dietary whole fruits of *Xylopia aethiopica* (the form in which the spice is consumed by humans), on blood have not been fully assessed.

Blood is the fluid of life and the medium in which most physiological and biochemical processes of the body are mediated. Any significant alterations to the homeostasis of this all-important medium might compromise many vital body functions and engender morbidity; hence, the use of blood as diagnostic aid in clinical medicine. This study therefore, aims to investigate the effect of dietary *Xylopia aethiopica* on hematological markers.

MATERIAL AND METHODS

Preparation of Fruits of Xylopia aethiopica: The whole dried fruits of *Xylopia aethiopica* were purchased from a local market in Ekpoma, Edo State, Nigeria. They were washed in clean water and air-dried for 10 hours, prior to drying in the oven at 40ºC for 12 hours. The dried fruits were then ground into powdered form. This was achieved by first pounding the whole dried fruits into small pieces using wooden mortar and pestle, and then grinding the pieces into powdered form using mechanical grinding machine. The desired amount of the powdered *Xylopia aethiopica* was measured using a sensitive digital weighing balance (Zohaus, Model CS 200, N.J. USA). The eventual prepared diet dose also contained cooked cassava starch as binder, and produced into dried crumbs, since Wistar rats prefer to hold their food with both hands to eat, while crouching on their hind limbs.

Preparation of Laboratory Animals: Twenty four (24) male Wistar rats were used in this study. They were young male Wistar rats, weighing 120-150g body weight. The rats were bought from the Animal House Unit of Department of Pharmacology, Niger Delta University, Bayelsa State, Nigeria, and housed in the research laboratory of the Department of Physiology, Niger Delta University.

Animal grouping: The rats were randomly divided into four groups of six rats per group and housed in standard rat cages for two weeks to acclimatize, prior to commencement of treatment.

Two categories of treatment diets were made available for use in this study: the control diet that contained normal rat chow and edible cassava starch only, and three treatment diets that contained the powdered *Xylopia aethiopica* at levels of 1.5% w/w, 2.5% w/w and 5% w/w of feed, with cassava starch as binder (and part of the feed). The rats were fed with the respective diet doses and tap water *ad libitum,* for six weeks.

Samples collection: The Wistar rats were sacrificed at the end of the treatment period by anesthesia in a chloroform chamber, and blood was collected via cardiac puncture. Aliquots of the blood from each animal were analyzed as follows: 0.2ml of blood was put into sample tube containing 0.1ml of Na_2 EDTA anticoagulant for analyses of Hb, red blood cell count, white blood cell count, white blood cell differential, platelet, ESR and PCV estimation. 2.5ml of blood from each animal was put into sample tube containing 0.3ml of 3.2% trisodium citrate for the determination of platelet poor plasma (PPP). 1.0ml of blood from the animals was put into another sample tube for the estimation of serum cholesterol and triglyceride concentrations.

Estimation of Blood Samples: The blood sample for PCV estimation was drawn into the capillary tube, one end of which was then sealed with laboratory clay. The tube was centrifuged at 11000 rpm for 5 minutes in a microhematocrit centrifuge (Model BL-135 D). The PCV was read using the microhematocrit reader (Hawskley Reader).

The Hb concentration, RBC count, WBC count, platelet count, mean corpuscular volume (MCV), and mean cell hemoglobin concentration (MCHC) were estimated using a semi-automatic hematological analyzer (SWELAB IED Model). The automatic counter utilized 2ml of blood in 16ml of commercially prepared diluents; and the blood cells were counted based on the principle of electronic impedance.

Estimation of Prothrombin Time (PT): An aliquot (0.1ml) of platelet poor plasma (PPP) sample from each animal was dropped into test tubes numbered 1-30. Thereafter, 0.1ml of commercially prepared control sample was dropped into tube no. 31. All the tubes were warmed at 37^0C for 3 minutes using water bath (but tube no. 31 was for 5mins). Then 0.2ml of prothrombin thromboplastin reagent was added to each test tube and the clotting time was recorded for each tube.

Estimation of Activated Partial Thromboplastin (APTT) Time: An aliquot (0.1ml) of APTT reagent (Darkez Ltd) was added to a pair of test tubes: one containing test sample, and the other serving as control. The two tubes were incubated at 37ºC for 5mins, before adding 0.1ml of freshly prepared $CaCl_2$ solution. The clotting time was recorded using electronic time piece.

Estimation of Erythrocyte Sedimentation Rate: To 0.4ml of 2.8% sodium citrate in a syringe was drawn the anti-coagulated blood sample to the 2ml mark. The mixture was then drawn by suction up the calibrated Westergreen tube to above the zero mark. The top of the tube was quickly closed with the index finger, and then carefully rotated till the upper level of blood was exactly at the zero mark. Then the clock was started and allowed to run for 1 hour, and the temperature also recorded. The height of the clear fluid above the upper limit of the red cells column after 1 hour was recorded in mm/hr.

Estimation of Serum Total Cholesterol and Triglyceride Concentration: These enzymatic methods was performed using the reagent kit chemo-analyzer (Hemolyzer 2000) – the principle of which is based on enzymatic hydrolysis and oxidation of cholesterol, to yield a reddish quinoneimine. The color intensity produced is proportional to the total serum cholesterol.

Statistical Analysis: All values were expressed in mean ± SEM. Data were statistically analyzed using Student's t-test. A P- value of 0.05 was considered to be significant.

RESULTS

The dietary treatment of male Wistar rats with varying doses of *Xylopia aethiopica* caused changes in blood cell parameters, hematological indices and plasma lipids. The results on the hematological indices (Table 1) showed significant ($p<0.05$) increases in mean serum concentrations of Hb, PCV and MCV, especially at treatment doses $\geq$ 2.5% w/w, compared to the control. The mean values of the MCV and MCHC were not significantly altered by the dietary treatment doses administered compared to the control.

The results of the mean values of the blood cell parameters (Table 2) showed significant increases in red blood cell count, but significant decreases in ESR in the rats which received the treatment doses of 2.5% and above, compared to the control. The slight increases in the mean values of WBC count and platelet count in the treated rats were however, not significant ($p>0.05$).

The result of the coagulation factors (Table 3) showed dose-dependent decreases in the prothrombin time (PT), and activated partial thromboplastin time (APTT), compared to the control values. The results on plasma lipid analyses (Table 4) showed significant dose-dependent decrease ($p<0.05$), especially at treatment doses of 2.5% w/w and 5% w/w, in serum total cholesterol concentration and slight decrease in the triglyceride concentration in the treated groups.

Table 1: Mean values of hematological indices in male Wistar rats fed varying doses of *Xylopia aethiopica*

Doses of *Xylopia aethiopica*	Mean values of Hematological Indices				
	Hb(g/dl)	PCV(%)	MCV (fl)	MCH (pg)	MCHC (g/dl)
Group I (Control)	11.81±0.21	44.32±0.36	69.68±0.77	18.24±0.11	28.56±0.23
Group II (1.5% w/w)	12.66±0.24	45.27±0.34	61.86±0.84	18.41±0.28	29.62±0.36
Group III (2.5% w/w)	13.48±0.12*	46.89±0.62*	60.94±0.21*	17.92±0.16	29.87±0.34
Group IV (5% w/w)	13.72±0.23*	47.79±0.22*	60.26±0.84*	18.06±0.74	30.62±0.88

n=6; Data presented as Means ± SD; *Significantly different, compared to control.

Table 2: Mean values of blood cells in male Wistar rats fed varying doses of *Xylopia aethiopica*

Doses of *Xylopia* aethiopica	Mean values of Blood Cells			
	ESR	RBC($x10^6$/µl)	WBC($x10^3$/ µl)	Platelet($x10^3$/ µl)
Group I (Control)	3.50±0.62	4.04±0.18	5.42±6.61	270.68±6.21
Group II (1.5% w/w)	3.00±0.71	4.84±0.78	6.37±3.47	277.28±8.77
Group III (2.5% w/w)	2.48±0.65*	5.37±0.62*	6.84±5.87	280.82±10.55
Group IV (5% w/w)	2.12±0.62*	5.87±0.39*	7.69±6.47	310.71±15.61

n=6; Data presented as Means ± SD; *significantly different, compared to control.

Table 3: Mean values of coagulation factors in male Wistar rats fed varying doses of *Xylopia aethiopica*

Doses of *Xylopia aethiopica*	Mean values of Coagulation Factors	
	PT	APTT
Group I (Control)	6.31± 0.48	17.67±1.87
Group II (1.5% w/w	5.78±0.69	17.38±1.28
Group III (2.5% w/w)	5.40 ±0.18	16.84± 0.72
Group IV (5% w/w)	5.10±0.61	16.24±1.37

n=6; Data presented as Means ± SD; *significantly different, compared to control.

Table 4: Mean values of serum lipids in male wistar rats fed varying doses of *Xylopia aethiopica*

Doses of *Xylopia aethiopica*	Mean Values of Serum Lipids	
	Total Cholesterol (mmol/l)	Triglyceride (mmol/l)
Group I (Control)	4.68±0.32	1.64±0.18
Group II (1.5% w/w	3.42±0.18	1.22 ±0.41
Group III (2.5% w/w)	1.88±0.21*	1.12±0.33
Group IV (5% w/w)	1.05±0.33*	1.04±0.47

n=6; Data presented as Means ± SD; *Significantly different, compared to control.

DISCUSSION

The effects of dietary *Xylopia aethiopica* on the affected hematological parameters were dose-dependent. The increase in Hb concentration in the animals that received the 2.5% w/w and above may be a reflection of similar increase in the RBC count, since Hb concentration is a function of the total RBC. These results strongly suggest the possible role *Xylopia aethiopica* may have in treatment-induced erythropoietic activity. This suggestion in supported by the study of Nworah *et al*. (2012) who reported the high content of iron in the fruits of *Xylopia aethiopica,* which make up 53.8% of the phyto-mineral composition of the fruits. Iron is required for the synthesis of heme – the main component in the synthesis of Hb. The lack of iron in the body is known to be the commonest cause of anemia, as a result of iron deficiency-induced reduction in erythropoietic activity (Ezeilo, 2009). It is tempting to suggest that the treatment-induced promotion of Hb synthesis, which, in turn, enhanced erythropoiesis, is responsible for the increases in the RBC indices such as Hb, PCV, MCV, MCH and MCHC observed in this study (Table 1). This assertion is supported by the study of Nwafor *et al*. (2009) who reported that the extract of the fruits of *Xylopia aethiopica* caused increases in the concentrations of Hb, PCV, and RBC count in albino rats.

The dose-dependent significant decrease in ESR may represent an outcome of the observed significant increase in the PCV in this study, since the ESR is a function of the rate of rouleux formation by the red blood cells. This effect of dietary *Xylopia aethiopica* on blood cells in this study is similar to that reported by Nwafor, (2013), who observed that extract of the fruits of *Xylopia aethiopica* caused increases in the RBC, WBC and platelets in albino rats. Ezeilo (2009) observed that increase in PCV and enriched plasma proteins (and increase in blood viscosity) engender reduction in ESR – which represent a well nourished state, while low PCV and low plasma proteins (serum albumin) causes elevation of ESR, as in the malnourished subject. On the other hand, the slight reductions in the coagulation factors (PT and APTT) by the ingestion of Dietary *Xylopia aethiopica* appear to be related to the equally slight increases in the platelet count observed in this study, considering the fact that a reduction in platelet number is associated with disorders of the hemostatic mechanisms (Fox, 1999). The findings on the effect of dietary *Xylopia aethiopica* on coagulation time observed in this study is similar to the report by Nwafor (2013), who observed slight decreases in the PT and APTT in albino rats treated with methanolic extract of fruits of *Xylopia aethiopica*.

The decreases in plasma lipids observed in this study is believed to be mediated via the documented *Xylopia aethiopica*-induced inhibition of dietary lipid absorption in the gastrointestinal tract, which is thought to be achieved via the reduction in the bile salts which are required for cholesterol absorption in the small intestine (Ostlund *et al.*, 2003; Onyebuagu *et al.,* 2013). The observed effects of dietary *Xylopia aethiopica* on plasma lipids concentrations in this study are similar to those reported by Nwafor (2013), who observed that extracts of *Xylopia aethiopica* demonstrated a dose-dependent reduction in serum total cholesterol (TC), triglyceride (TG), and low density lipoprotein cholesterol (LDL-C) levels, but caused increases in levels of high density lipoprotein cholesterol (HDL-C).

Conclusively, whole dry fruits of *Xylopia aethiopica* may promote blood parameters, probably, by virtue of its rich iron content, and may relegate total cholesterol and as such serve a beneficial role as an anti-hyperlipidemia against the risk factors associated with elevated blood cholesterol levels.

ACKNOWLEDGEMENT

The authors are very grateful to the following for their technical assistance in this study: Dr A. S. Oboma, and Mr. Charles Olotu.

REFERENCES

Awuah, R.T. (1980). Fungitoxic effects of extracts of some West African Plants. *Biol.; 115 (3):4551-453*

Eftkins, N.L. (1997). Antimalarial plants used in Northern Nigeria. *Trop. Doctor; 27(1):12-16*

Ezekwesili, C.N., Nwodo, O.F.C., Eneh, F.U. and Ogbunugafor, H.A. (2010). Investigation of the chemical composition and biological activity of *Xylopia aethiopica* Dunal (Annonaceae) *Afr. J. Biotechnology; 9 (43):7352-56.*

Ezeilo, G.C. (2009). Textbook of Physiology; 2ND Edition, Oxfrd University Press, New Delhi, India.

Fox, S.I. (1999). Human Physiology. Sixth Edition. WCB/McGraw-Hill, New York, USA.

Gbile, Z.O. (1989). Ethnobotany, Taxonomy and Conservation of Medicinal Plants. Forestry Research Institute of Nigeria, Ibadan, pp14-17.

Iwu, M. (1993). Handbook of African Medicinal Plants. CRS Press, Boca Roton. FL

Muanya, C. (2008). How plants can induce labour, heal after-birth wounds. The Guardian Newspaper. 17th Jan. 2008.

Murray, M. (1995). The Healing Powers of Herbs. Primes Publishing, Rocklin, CA.

Nwafor, A. (2013). Life Under Assault: Nowhere to Hide. *Inaugural Lecture Series, no. 102nd 14th March, 2013.*

Nwafor, A., Adienbo, M.O. and Egwurugwu, J.N. (2009). *In vivo* Effects of *Xylopia aethiopica* on Hemorheological Parameters in Guinea Pigs. *Afr. J. Appl. Zool. Environ. Biol.; 11: 79-81.*

Nworah, D.C., Nwafor, A. and Adienbo, M.O. (2012). HPLTC-Isolation and Characterization of Hydro-methanolic Chloroform Fraction of *Xylopia aethiopica* (In Press).

Odukoya, A.O., Ilori, O., Sofidiya, M.O., Animoh, A.O., Lawalm M.M. and Tade, I.O. (2005). Antioxidant activity of Nigerian Dietary Spices. *EJEAF; che-4 (6):1086-1093.*

Ojimelukwe, P.C. and Okonkwo, S. (1999). Effects of Preservation with *Xylopia aethiopica* on the Shelf Life of Cowpea Seeds. *J. Food Sci. Technol.;* 36(2):170-172.

Onyebuagu, P.C. (2012) Assessment of the Effect of *Xylopia aethiopica* on Reproductive Performance in Wistar Rats. PhD Thesis, Delta State University, Abraka, Nigeria.

Onyebuagu, P.C., Aloamaka, C.P. and Igweh, J.C. (2013). *Xylopia aethiopica* Lowers Plasma Lipid Precursors of Reproductive Hormones in Wistar Rats. *Int. J. Herbs and Pharm. Res.; 2(4): 48-53.*

Ostlund, R.E., Racette, B. and Stenson, W.F. (2003). Inhibition of Cholesterol Absorption by Phytosterol-replete wheat germ compared with Phytosterol-depleted wheat germ. *Am. J. Clin. Nutr.; 77(6): 1385-1389.*

Somova, L.I., Shodeh, F.O., Moodleya, K. and Govendera, Y. (2001) Cardiovascular and Diuretic Activity of Kaurene derivatives of *Xylopia aethiopica* and *Alipedea amatymbica. J Ethnopharmacology. 27(2-3):165-174.*

Tairu, A.D., Hoffmann, T. and Schieberle, P. (1999). Characteristics of key aroma compounds of dried fruits of *Xylopia aethiopica* (Dunal) A.Rich (Annonaceae) Using Aroma Extract Dilution Analysis. *J. Agric. Food Chem.; 47 (8) 3285-67.*

AUTHORS CONTRIBUTIONS

All the authors participated fully in this study. Onyebuagu P.C. was in charge of experimental design, protocols and discussion, Pughikumo D.T. was responsible for the interpretation and analysis of data and results, while Aloamaka C.P. was the overall supervisor of the project.