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Assessment of the reproductive impact of Ethiopian pepper (*Xylopia aethiopica*) on rabbit bucks

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Additional keywords

Testis.
Semen.
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SUMMARY

The study was conducted to assess the effect of *Xylopia aethiopica* on the reproductive functions of rabbit bucks. To achieve this objective, rabbit bucks aged 46-48 weeks and an average weight of 2.98 kg were randomized into three treatment groups of six animals per treatment group designated as T1 (control), T2 and T3. The inclusion levels of *Xylopia aethiopica* in the diets for the designated groups were 0 %, 0.5 % and 1.0 % for T1 (control), T2 and T3 respectively. The rabbits were fed for 56 days before start of semen collection which lasted for four weeks at a semen collection regime of twice a week followed by histopathological study of the testes. The results of this study showed that groups fed diet containing 0.5 % *Xylopia aethiopica* had significantly ($p<0.05$) higher sperm motility (73.77 %), concentration (145.93x106/mm^3), total ejaculate (104.58x109/mm^3) and viability (79.03 %) than those fed 0 % and 1.0 %. There were no significant ($p>0.05$) differences in the semen volume, libido and average weight gain between the treatments. The testicular photomicrograph showed degenerative changes at a higher inclusion rate (1.0 %) of *Xylopia aethiopica* in the diet. The present findings suggest that excessive and uncontrolled dietary inclusion of *Xylopia aethiopica* may cause damage to the testes, thus impairing testicular functions and possibly compromise male fertility. On the other hand, *Xylopia aethiopica* may be useful in enhancing spermatogenic processes in so far as its consumption is regulated.

Evaluacion del impacto reproductivo de la pimienta etiópica (*Xylopia aethiopica)* en conejos

Palabras clave adicionales

Testículos.
Semen.
Xylopia aethiopica.

RESUMEN

El estudio pretende evaluar el efecto de *Xylopia aethiopica* sobre las funciones reproductivas del conejo macho. Se emplearon conejos macho de 46-48 semanas con un peso medio de 2,98 kg, agrupados aleatoriamente en tres tratamientos en grupos de 6. Los niveles de inclusión de *Xylopia aethiopica* en las dietas fueron: 0 % (T1, control), 0.5 % (T2) y 1.0 % (T3). Los conejos fueron alimentados durante 56 dias antes de empezar la recolección de semen, que se prolongó durante cuatro semanas, a base de dos obtenciones semanales, seguidas de un estudio histopatológico de los testículod. Los resultados de este estudio mostraron que que los animales que consumieron el tratamiento T2, tuvieron mayor ($p<0,05$) movilidad espermática (73,77%), concentración (145,93x106/mm^3), volumen total de eyaculado (104,58x109/mm^3) y viabilidad (79,03 %) que los que recibieron los tratamientos T1 y T2. No hubo diferencias significativas ($p>0,05$) en volumen seminal, libido y ganancia media de peso entre tratamientos. Las microfotografías, mostraron cambios degenerativos en el testículo con la mayor proporción de *Xylopia aethiopica* (1%) en la dieta. Estos hallazgos sugieren que una inclusión dietética, excesiva e incontrolada de *Xylopia aethiopica* puede causar daños en los testículos y, posiblemente, comprometer la fertilidad del macho. Por otro lado, *Xylopia aethiopica*, puede ser útil para mejorar los procesos de la espermatogénesis, cuando su nivel de consumo es controlado.

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INTRODUCTION

There has been an increasing demand for the use of plant products in enhancing male fertility and management of some physiological disorders. This is due to low cost, easy availability and lesser side effects of organic medicinal products compared to their synthetic counterpart. Hence, plant material are continuously scrutinized and explored for their beneficial effects. A larger number of these tropical plants and their extract have shown beneficial therapeutic effects including fertility enhancing and contraceptive compounds, anti-

oxidant, anti-inflammatory, anti-cancer, anti-microbial and aphrodisiac (Raji *et al*., 2006). In view of the concerns on male animal infertility in our livestock industry, the scope of the biologic actions of this commonly used botanical remedy needs to be assessed. Among the promising medicinal plants, *Xylopia aethiopica*, commonly called Ethiopia pepper or Negro pepper is an angiosperm of the Annonaceae family. It is a tropical evergreen tree growing up to 20 meters tall and bearing aromatic seeds locally known as "uda" by the southern eastern part of Nigeria. Traditionally, the fruits are used as spices; and aqueous decoction is used especially after child birth probably for its antiseptic properties and to arrest bleeding (Nnodim *et al*., 2011). This fruit of *X. aethiopica* has been reported to act as antioxidant, hypolipidemic and hypoglycaemic agent hence, confirming to its use as an anti-diabetic agent used in traditional medicine for treating diabetes. This plant has a wide spectrum of biological activities and has played a crucial role in traditional medicines because of their valuable physiological and pharmacological properties (Ogbonnia *et al*., 2008). The seeds contain bitter principles, alkaloids, glycosides, saponnis, tannins, sterols, carbohydrate, protein and free fatty acid, mucilage's and acidic compounds (Burkhills, 1985) some of which might be responsible for the reported uses. There have been a number of contradictory reports on the effect of *X. aethiopica* on male fertility. Therefore, this study was intended to bridge the gap in our continued efforts to validate the effects of *X. aethiopica* on male reproductive function.

MATERIALS AND METHODS

Experimental location: The experiment was carried out at the Rabbit Unit of the Teaching and Research Farm of the College of Animal Science and Animal Production, Michael Okpara University of Agriculture, Umudike, Abia state, Nigeria.

Experimental animals and management: Eighteen healthy rabbit bucks aged 46-48 weeks with an average weight of 2.98 kg were used for this study. The animals were quarantined for two weeks during which they were treated against ecto and endo-parasite. The animals were provided commercial grower feed supplemented with *Panicum maximum, Calopogonium mucunoides* and *Centrosema pubescens* during the quarantine period in order for the animals to adapt to their new environment before the introduction of experimental diets. They were also provided *ad-libitum* access to clean drinking water throughout the experimental period.

Table I. Composition of the experimental diets (Composición de las dietas experimentales).

Ingredients (kg)	T_1	T_2	T_3
Maize offal	44.33	41.83	41.33
Palm kennel cake	44.32	43.82	43.32
Soybean meal	8.35	8.35	8.35
Xylopia aethopica	-	0.5	1.0
Bone meal	2.50	2.50	2.50
Premix	0.25	0.25	0.25
Salt	0.25	0.25	0.25
Calculated nutrient composition. Crude protein	16.16	16.06	16.08
Metabolisable energy (Kcal kg^{-1})	2699.31	2698.31	2697.32

Experimental procedures and design: The experiment was a completely randomized design (CRD). The 18 rabbits were randomly assigned to 3 treatment groups of 6 rabbits each designated as T_1 (control), T_2 and T_3. The treatments T1, T2 and T3 consisted of 0 %, 0.5 % and 1.0 % inclusion levels of dried ground *X. aethiopica*, respectively, incorporated in the compounded diets. The diets of the experimental animals and the composition of *X. aethiopica* are presented in **table I** and **II**, respectively. The rabbits were fed for 56 days before start of semen collection.

Data collection and evaluation

Semen collection: Two weeks prior to semen collection, the rabbit bucks were trained to serve an artificial vagina (AV) using a teaser rabbit doe. On the 57th day following the administration of the experimental diets, the 18 bucks used in this study were placed on a semen collection schedule of twice per week. One ejaculate was collected from each rabbit buck once between 08:00 to 13:00 (local time) on Mondays and Thursdays for 4 consecutive weeks. The rabbit doe was taken to the buck's cage and the doe was held in position for service. When the male attempted to mount, the AV was strategically placed below the belly of the doe in such a way that the penis of the male was introduced into the AV. The temperature of the inner liner rubber sleeve of the AV was adjusted to 40-42°C at the time of semen collection. Lubrication of the inner sleeve was performed using glycerine.

Estimation of semen traits: Semen evaluation involved the estimation of both microscopic and macroscopic indices. Ejaculate volume was read-off directly in millimeters from a calibrated glass collection tube attached to the AV. Sperm motility percentage score was subjectively assessed in a drop of fresh semen on a warm glass slide covered with a warm cover slip and examined using a microscope at x40 magnification. Sperm cell concentration ($\times 10^6$ /mm^3) was determined using a haemocytometer at a dilution of 1 in 200 in a solution of 45 mL normal saline and 5 mL formalin. Total sperm ($\times 10^9$ per ejaculate) was determined by multiplying the semen ejaculate volume by the sperm cell concentration. Morphological examination of the semen was done by performing different counts of the morphologically normal and abnormal sperm cell types on eosin/nigrosin stained preparations. Libido was estimated by observing the reaction time (seconds) which elapsed between exposure of a buck to a doe and the first copulation (serving the AV).

Histological studies

Tissue processing: At the end of semen collection period, the experimental rabbits were sacrificed using *captive bolt* followed by immediate exsanguinations. Thereafter, the testes of the rabbits were harvested, transversely cut and fixed in 10% formal saline for

Table II. Composition of Ethiopian pepper (*X. aethiopica*) (Composición of la pimienta etiópica (*X. aethiopica*)).

Proximate composition	value
Dry matter (%)	91.7
Crude protein (%)	7.44
Ash (%)	3.30
Ether extract (%)	2.40
Crude fibre (%)	2.30
Mineral and vitamin composition	
Magnesium (%)	0.44
Calcium (%)	0.09
Sodium (ppm)	10.5
Potassium (ppm)	5.23
Iron (mg/100 g)	1.82
Phosphorous (mg/ 100g)	7.73
Vitamin C (IU)	8.80
Vitamin E (IU)	6.70

48 hours, and processed for paraffin wax embedding with a Shandon Duplex automatic tissue processor by dehydrating through 70%, 90% and two changes of absolute alcohol for 120 minutes each. Clearing was achieved through two changes of xylene for two hours each; and impregnation with two changes of paraffin wax for 2 hours each. The impregnated tissues were then embedded in molten paraffin wax and allowed to cool to get solidified. Then sections were cut at 5-7 µ thickness with a rotatory microtome. The cut sections were stained by hematoxylin and eosin (H&E) method. All sections were examined under light microscope using × 400 magnification. Photographs of tissues were taken with Olympus photomicroscope for observation and documentation of histopathology.

Statistical Analysis: The data generated were analysed using ANOVA. Significant means were separated using Duncan multiple range test at 5 % level of significance.

RESULTS AND DISCUSSION

The results of semen evaluation, libido and weight of rabbit bucks treated with varying levels of *X. aethiopica* are presented in **table III**. The result of this study showed that the fruits of *X. aethiopica* caused marked variations ($p<0.05$) in mass sperm motility, ejaculate concentration, total sperm, and sperm viability. Other measured parameters such as volume, libido, average total weight and average weight gain were not significantly affected ($p>0.05$) by the treatment. The mean semen volume agrees favourably with the findings of Herbert and Acha (1995) and Brackett (2004) who reported that rabbit semen volume varied between 0.4-0.71 ml. Although the semen volume recorded in this study showed no significant ($p>0.05$) differences among the treatment groups, the slight numerical increase in the 0.5 % treated group is indicative of an enhanced semen production at this inclusion rate. The observed trend of ejaculate indices in response to the treatment doses suggested that a higher inclusion rate (1.0 %) of *X. aethiopica* produced suppressive effect on semen parameters. Sperm motility is an important index in reproductive examination because it demonstrates the vigour with which sperm cells are propelled during the process of fertilization. The result of sperm motility in this study showed significant increase ($p<0.05$) at the 0.5 % rate compared with the control and 1.0 % treatment groups which recorded a mean value of 53.33 % and 32.00 % respectively. Nwangwa (2012) observed an anti-fertility effect of *X. aethiopica* and reported a significant ($p<0.05$) and dose dependent decrease in sperm motility of Wistar rats treated with ethanolic extract of *X. aethiopica* at 0.5 and 1.0 ml. Contrastingly, the findings of Oguike and Archibong (2011) showed a significantly higher mean motility values (56.67, 63.54 and 72.55 % for control, 1.0 %, and 2.0 % respectively) in rabbit bucks fed dietary supplement of Ethiopian pepper relative to those in the control group. The mean value of 73.77 % sperm motility obtained for rabbit bucks in 0.5 % group in this study is in agreement with 70 % and above reported by Kobal and Kosec (1996) and Brackett (2004) for good quality fresh sperm. Although the mean motility values in the control and 1.0 % group were significantly ($p<0.05$) lower and below the value described by Kobal and Kosec (1996) and Brackett (2004) for quality semen, a low score of 30 % has been reported by Pineda (2003) as minimally acceptable spermatozoal motility levels for fresh ejaculates advocated by some breeding organizations. However, in order to achieve fertilization, such low motility values should be compensated with a higher sperm concentration index.

The administration of *X. aethiopica* in this study is also shown to significantly decrease sperm concentration at a higher dose of 1.0 % ($44.33 \times 10^6/mm^3$) compared with 0.5 % ($145.93 \times 10^6/mm^3$) and control group ($97.33 \times 10^6/mm^3$). Except for the lower value of $44.33 \times 10^6/mm^3$ in 1.0 % group, observations made on semen concentration in this study are consistent with the range of 50 to 350 $\times 10^6/mm^3$ reported by Brackett (2004) and also similar to what was obtained by Hafez (1970) and Herbert and Adejumo (1993) for composite rabbit bucks. The 1.0 % treated animals also exhibited a significant decrease ($p<0.05$) in sperm viability compared with 0.5 % and the control groups. In an earlier study, Burkhills (1985) had documented that *X. aethiopica* alongside other plants can cause adverse effects on the reproductive system of males through affecting the sperm count, motility and viability. Decrease in sperm motility and viability suggests alteration of sperm maturation in the epididymides in the rabbits fed higher level (1.0 %) of *X. aethiopica.* A similar observation in this study was reported by Nwangwa (2012) who found a significant ($p<0.05$) decrease in sperm concentration, sperm viability and a non significant decrease in the percentage of sperm with normal morphology of Wistar rats treated with ethanolic extract of *X. aethiopica*. More recently, Onyebuagu *et al*. (2015) reported that *X. aethiopica* caused significant decreases in ratio of testicular weight to body weight, sperm motility, sperm count, and alterations in the histology of the testes.

Table III. Mean values of semen characteristics, libido and weight of rabbit bucks treated with *Xylopia aethiopica* (Valores medios para las características del semen, libido y el peso de los conejos tratados con *Xylopia aethiopica*).

Parameters	T_1	T_2	T_3	SEM
Volume (ml)	0.52	0.70	0.49	0.10
Mass motility (%)	53.33[b]	73.77[a]	32.00[c]	3.32
Ejaculate Conc. ($\times 10^6$/mm^3)	97.33[b]	145.93[a]	44.33[c]	8.05
Total Sperm ($\times 10^9$/mm^3)	47.87[b]	104.58[a]	21.89[b]	14.52
Sperm viability (%)	64.67[ab]	79.03[a]	54.67[b]	4.79
Libido (seconds)	12.90	17.30	15.50	2.23
Average total weight (kg)	3.18	3.20	3.20	0.01
Average weight gain (kg)	0.04	0.04	0.04	0.01

[a, b, c]Means on the same row bearing different superscripts are significantly different ($p<0.05$).

On the other hand, Woode *et al*. (2011) reported increase in serum testosterone and luteinizing hormone levels as well as a significant increase in sperm count in Sprague Dawley rats administered alcoholic extract of *X. aethiopica*. Also, Oguike and Archibong (2011) found a dose dependent significant ($p<0.05$) increase in sperm concentration and live sperm cells in rabbits administered *X. aethiopica* at an inclusion level of 1 an 2 % in feed. The disparity in the contrasting potentials of *X. aethiopica* with other authors who reported increased semen parameters at a higher level may be ascribed to specie differences in *X. aethiopica* or choice of solvent for phytonutrient extraction which expectedly may have influenced the composition of phytoconstituents and consequent results in reported studies. However, the report of histopathological section of the testis in this study clearly revealed the interference of *X. aethiopica* on spermatogenesis which consequently influenced the outcome of the semen parameters observed in **table II**. There are a number of probable mechanisms for the anti-gonadal activities of *X. aethiopica* at a higher level. It is possible that at 0.5 % level of dietary supplemention, the Ethiopian pepper enhanced some *in vivo* antioxidant status and

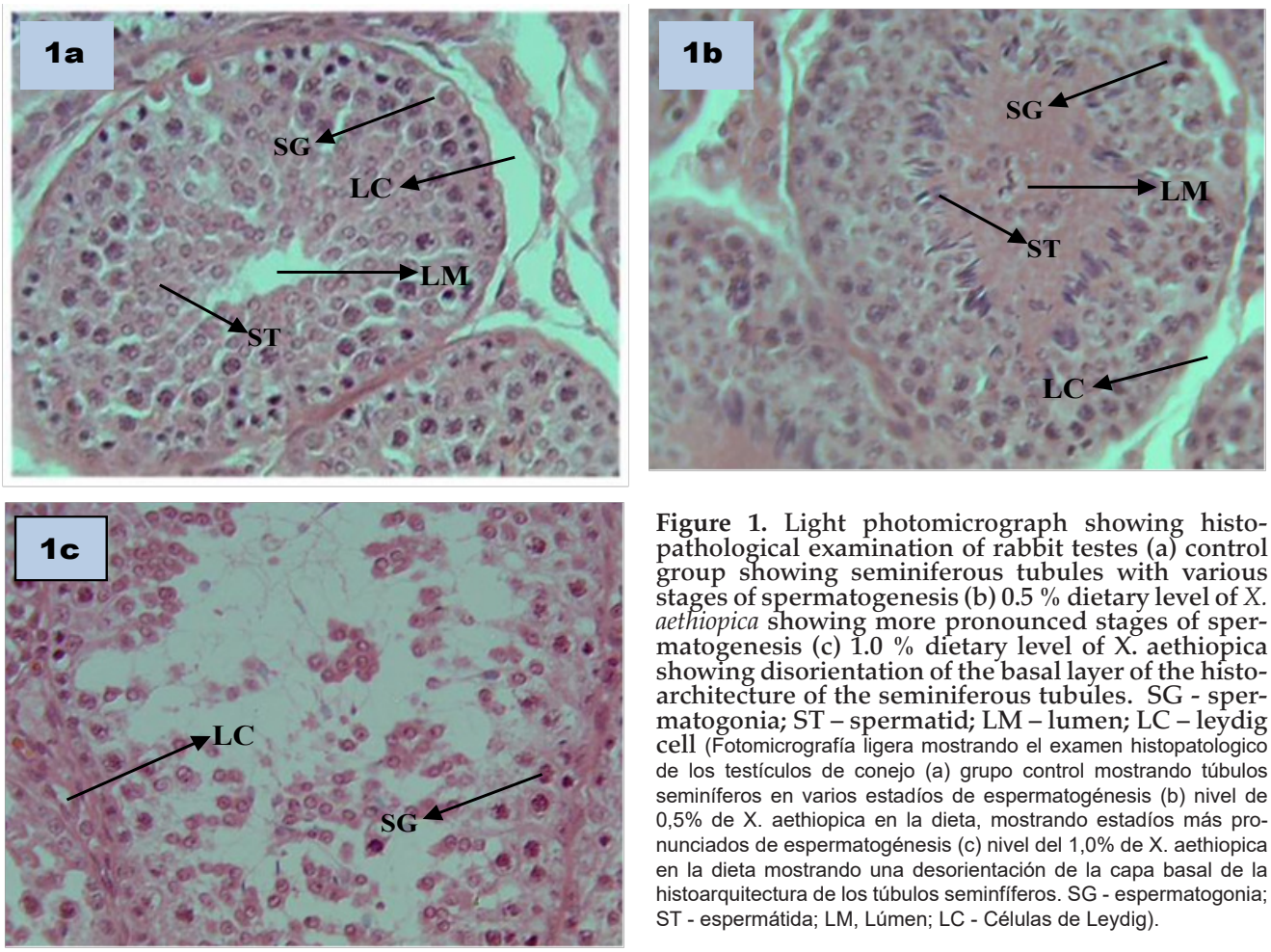

Figure 1. Light photomicrograph showing histopathological examination of rabbit testes (a) control group showing seminiferous tubules with various stages of spermatogenesis (b) 0.5 % dietary level of *X. aethiopica* showing more pronounced stages of spermatogenesis (c) 1.0 % dietary level of X. aethiopica showing disorientation of the basal layer of the histoarchitecture of the seminiferous tubules. SG - spermatogonia; ST – spermatid; LM – lumen; LC – leydig cell (Fotomicrografía ligera mostrando el examen histopatologico de los testículos de conejo (a) grupo control mostrando túbulos seminíferos en varios estadíos de espermatogénesis (b) nivel de 0,5% de X. aethiopica en la dieta, mostrando estadíos más pronunciados de espermatogénesis (c) nivel del 1,0% de X. aethiopica en la dieta mostrando una desorientación de la capa basal de la histoarquitectura de los túbulos seminfíferos. SG - espermatogonia; ST - espermátida; LM, Lúmen; LC - Células de Leydig).

maintains testicular membrane integrity for optimum spematogenesis. This could be attributed to the phenolic contents and the *in vitro* antioxidant properties of Ethiopian pepper (Pandey and Rizvi, 2009; George and Osioma, 2011; Adefegha and Oboh, 2012). Levels above 0.5 % may have exerted a direct inhibitory action on the testis, or have affected the pituitary causing changes in gonadotrophin concentrations and thus subsequent spermatogenic impairment. Studies have shown that *X. aethiopica* exhibits cytotoxicity and antiproliferative activity in human cervical cancer cells (Adaramoye *et al*., 2011). The reduction in semen parameters and the dose dependent degeneration of the seminiferous tubules in the testis of rabbits treated with *X. aethiopica* in the present study as shown in Fig 1 is thus in conformity with the earlier studies indicating the cytotoxicity of *X. aethiopica* (Asekun and Adeniyi, 2004; Adaramoye *et al*., 2011; Onyebuagu *et al*., 2015).

The light photomicrograph showing histopathological examination of rabbits testes used in this study is shown on **figure 1**. Transverse sections of testes of rabbit bucks in the control and 0.5 % group as seen in **figure 1a** and **b** showed normal histoarchitecture of the seminiferous tubules. Sertoli and Leydig cells of normal size were present. The number of seminiferous tubules was normal with bundles of normal spermatozoa showing all the various stages of spermatogenesis. Administration of a higher dose (1.0 %) of *X. aethiopica* caused visible cytotoxic activity by clearing all matured spermatozoa, germ cells and shows disorientation of the basal layer of the histoarchitecture of the seminiferous tubules **(figure 1c)**. The 1.0 % treated group showed a destroyed tunica albuginea and depleted germ cell layers, disorganization of the normal regular layering of the various stages of spermatogonia and spermatocytes. Nwangwa (2012) in his histopathological study on the effect of ethanolic extract of *X. aethiopica* on male reproductive organ observed disorientation of the basal layer of the histoarchitecture of the seminiferous tubules in the testes of Wistar rats that received higher dose of 1 ml for 28 days. Another study by Onyebuagu *et al*. (2015) shows that 5% w/w dietary dosage of dried whole fruits of *X. aethiopica*, fed to male albino rats for 21 days resulted to decreased spermatogenesis, reduction in the relative number of sertoli cells, and enlargement of the lumen of the seminiferous tubules bordering the apical surfaces of the sertoli cells.

CONCLUSION

The present findings suggest that excessive and uncontrolled dietary inclusion of *X. aethiopica* may cause damage to the testes, thus impairing testicular functions and possibly compromise male fertility. On the other hand, *X. aethiopica* may be useful in enhancing spermatogenic processes in so far as its consumption is regulated.

BIBLIOGRAPHY

Adaramoye, O.A.; Sarkar, J.; Singh, N.; Meena, S.; Changkija, B.; Yadav, P.P.; Kanojiya, S. and Sinha, S. 2011. Antiproliferative action of *Xylopia aethiopica* fruit extract on human cervical cancer cells. *Phytother Res,* 25: 1558-1563.

Adefegha, S.A. and Oboh, G. 2012. Effect of diets supplemented with Ethiopian pepper [*Xylopia aethiopica* (Dun.) A. Rich (Annonaceae)] and Ashanti pepper [*Piper guineense* Schumach. et Thonn (Piperaceae)] on some biochemical parameters in normal rats. *Asian Pacific J Trop Biomed,* 2: 559-567.

Asekun, O.T. and Adeniyi, B.A. 2004. Antimicrobial and cytotoxic activities of the fruit essential oil of *Xylopia aethiopica* from Nigeria. *Fitoterapia. 75*: 368-370.

Brackett, B.G. 2004. Male Reproduction in Mammals. In Duke's Physiology of domestic animals 12th ed. Edited by Reece, W.O. Cornel University Press. Ithaca. Pp. 670-688.

Burkhills, H.M. 1985. Useful plants of west tropical Africa. 2nd ed. Royal Botanical Garden. Kew. UK.

George, B.O. and Osioma, E. 2011. Phenolic content and total antioxidant capacity of local spices in Nigeria Afr *J Food Sci*, 5: 741-746.

Hafez, E.S.E. 1970. Rabbits. In: Hafez, E.S.E. (Ed). Reproduction and breeding techniques for laboratories animals. Lea and Febiger. Philadelphia. pp. 273-298

Herbert, U. and Acha, C.O. 1995. Preliminary observations on the seminal characteristics of rabbits raised in two locations in South Eastern Nigeria. *Nigerian J Anim Prod,* 22: 108-112.

Herbert, U. and Adejumo, D.O. 1993. An artificial vagina for collecting rabbits semen in the tropics. 18th Annual conference for Nigerian Society for Animal Production. Federal University of Technology. Owerri. Nigeria. March, 21-25, 1993.

Kobal, S. and Kosec, M. 1996. Macroscopic and microscopic semen properties in white New Zealand rabbits. *J Physiol Pharmacol* 47(2 suppl. 1): 153.

Nnodim, J.K.; Emejulu, A.; Amaechi, A. and NwosuNjoku, E. C. 2011. Influence of *Xylopia aethiopica* fruits on some haematological and biochemical profile. *Almeeen J Med Sci,* 4: 191-196.

Nwangwa, E.K. 2012. Antifertility effects of ethanolic extract of *Xylopia aethiopica* on male reproductive organ of wistar rats. *Amer J Med Med Sci*, 2: 12-15.

Ogbonnia, S.; Adekunle, A.A.; Bosa, M.K. and Enwuru, V.N. 2008. Evaluation of acute and sub-acute toxicity of *Alstonia congensis* Engler bark and *Xylopia aethiopca* (Dunal) A. Rich (Annonaceae) fruits mixtures used in treatments of diabetes. *African J Biotechnol*. 7: 701-705.

Oguike, M.A. and Archibong, C.B. 2011. Effect of Ethiopian pepper (*Xylopia aethiopica*) on the testis and semen quality of rabbit bucks. *J Adv Dev Res,* 2: 294-298.

Onyebuagu, P.C.; Kiridi, K. and Aloamaka, C.P. 2015. Dietary *Xylopia aethiopica* reduces fertility capacity of male wistar rats. *J Natur Sci Res, 5*: 47-52.

Pandey, K.B. and Rizvi, S.I. 2009. Plant polyphenols as dietary antioxidants in human health and disease. *Oxid Med Cell Longev*, 2: 270-278.

Pineda, M. H. 2003. Male Reproductive system. In: Pineda, M.H. (Ed.). McDonald's Veterinary endocrinology and reproduction. 5th ed. Lowa State Press. Lowa. pp. 239-271.

Raji, Y.; Fadare, O.O.; Adisa, R.A. and Salami, S.A. 2006. Comprehensive assessment of the effect of *Sphenocentrum jollyanum* root extract on male reproductive activity in albino rats. *Reprod Med Biol*, 5: 283-292.

Woode, E.; Alhassan, A.; Abaidoo, C.S. 2011. Effect of ethanolic fruit extract of *Xylopia aethiopica* on reproductive function of male rats. *Int J Pharm Biomed Res*, 2: 161-165.