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Modulating Role of Bitter Leaf on Spermatogenic and Steroidogenesis Functions of the Rat Testis

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ABSTRACT

The characteristic bitter taste of *Vernonia amygdalina* has been attributed to its anti-nutritional contents such as alkaloids, saponins, tannins and glycosides. Recently the spermigraphic effect of the plant in male rats has been reported. The present study examined the effect of bitter leaf extract on 30 days oral administration of 50, 100, 200 mg kg⁻¹ and 60 days oral administration of 200 mg kg⁻¹ b.wt. of the extract on some fertility indices in male wistar rats. At 30 days administration, the extract produced a significant and dose dependent increase (p<0.05) in sperm concentration, percentage motility, morphology and percentage live sperm. There was a decrease in the blood level of Follicle Stimulating Hormone (FSH) but no significant increase in the levels of Leutinizing Hormone (LH) and Testosterone. Administrations of bitter leaf extract at higher dosage (200 mg kg⁻¹ b.wt.) and duration provoked varying degrees of testicular degeneration ranging from a significant reduction in sperm concentration, motility, percentage normal morphology, percentage number of live sperm to a significant increase in the number of percentage abnormal sperm. Evidences suggest that higher dosage of bitter leaf (*Vernonia amygdalina*) extract administered for a longer duration could be deleterious to the testes.

Key words: Bitter leaf, *Vernonia amygdalina*, infertility, testosterone, leutinizing hormone, spermatozoa

INTRODUCTION

The fertility enhancing capacity of plant extract has been reported in numerous studies. In recent time, paramount attention is been shifted from synthetic drugs to natural plant products (Akunna *et al.*, 2013). Various plants that were once considered of little or no importance are now studied and subsequently developed into drugs, with minimal side effects (Izevbigie, 2003). Some wild herbs and spices have been shown to be most effective, relatively non-toxic and have substantial scientific documentation to attest to their efficacy in infertility management (Izevbigie *et al.*, 2004). Bitter leaf (*Vernonia amygdalina*) is an important plant in eastern part of Nigeria where it is known as "Olugbu" and eaten as a vegetable. The plant tree is about 1-3 m in height with a green leaf of about 6 mm diameter (Igile *et al.*, 1994, 1995a). Its antioxidative components have been documented (Jisaka *et al.*, 1992). Several reports on its anti-malarial (Masaba, 2000; De Madureira *et al.*, 2002; Tona *et al.*, 2004), antiviral (Vlietinck *et al.*, 1995; Ogbulie *et al.*, 2007), laxating (Ho, 1994), antitumor (Jisaka *et al.*, 1993a, b; Izevbigie *et al.*, 2005),

antihelmintics (Alawa *et al.*, 2000, 2002; Osinubi, 2007), antidiabetic (Adaramoye *et al.*, 2009), hypolipidemic (Adaramoye *et al.*, 2007, 2008a), activities of *V. amygdalina* has been documented.

In this study, the pro-fertility properties of *V. amygdalina* extract in male Wistar rats through seminal fluid analysis were determined.

MATERIALS AND METHODS

Plant materials and the aqueous extraction procedure: Samples of leaves were collected from a farm in Ojo lagos, Nigeria in the month of September, 2012. They were transported to the laboratory of Anatomy department of Lagos State University College of Medicine, Ikeja Lagos where it was authenticated by a staff in the herbarium of the Department of Botany, University of Lagos, Lagos, Lagos state, Nigeria and a voucher specimen no. DSN 71 was deposited for reference. The leaves were thoroughly washed in sterile water and was washed and air-dried to a constant weight in the laboratory. The dried material was pulverized into a dry powder using a mortar and pestle. About 250 g of the powder was extracted with 3.75 L of distilled water. The filtrate was concentrated in an electric Oven at 50°C until a semisolid residue was obtained. The percentage yield of the extract was calculated.

Animals: Sprague-Dawley rats were obtained from a breeding stock maintained in the Animal House of the College of Health Sciences, Lagos state university college of Medicine and were authenticated. The animals were housed in well ventilated wire wooden cages in the Animal Facility of the Department of Anatomy, Lagos State University College of Medicine (LASUCOM), Ikeja, Lagos. An approval was sought and obtained from the Departmental Ethical Committee on Animal Use. The rats were maintained under standard natural photoperiodic condition of twelve hours of light alternating with twelve hours of darkness (i.e., L: D; 12:12h photoperiod) at room temperature (25-26°C) and humidity of 65±5%. They were allowed unrestricted access to water and rat chow (Feedwell Livestock Feeds Ltd, Ikorodu, Lagos, Nigeria). They were allowed to acclimatize for 15 days before the commencement of the experiments. The weights of the animals were estimated at procurement, during acclimatization, at commencement of the experiments and twice within a week throughout the duration of the experiment, using an electronic analytical and precision balance (BA210S, d = 0.0001 g) (Satorius GA, Goettingen, Germany). Experimental procedures involving the animals and their care were conducted in conformity with International, National and institutional guidelines for the care of laboratory animals in Biomedical Research and Use of Laboratory Animals in Biomedical Research as promulgated by the Canadian Council of Animal Care (CCAC, 1985). Further the animal experimental models used conformed to the guiding principles for research involving animals as recommended by the Declaration of Helsinki and the Guiding Principles in the Care and Use of Animals (WMA/APS, 2002).

Animal grouping and experimental design: Fifty male adult (12-14 weeks old) Sprague Dawley rats weighing 190-215 g were used. The rats were randomly divided into five groups of ten rats each such that the average weight difference between and within groups did not exceed±20% of the average weight of the sample population. The first group (A) animals were each given distilled water 2.5 mL kg⁻¹ b.wt./daily/orally for 30 days. These animals served as control. The group B, C and D rats were treated with 50, 100 and 200 mg kg⁻¹ b.wt./daily/orally, respectively for 30 days. Group E rats were treated with 200 mg kg⁻¹ b.wt./daily/orally for an extended period of 60 days. The extract were administered once daily by 12 noon for six days (Monday to Saturday) within a week. All the animals were sacrificed 24 h after the last dosing.

Animal sacrifice and sample collection: The rats were at the time of sacrifice first weighed and then anaesthetised by placing them in a closed jar containing cotton wool sucked with chloroform anaesthesia. The abdominal cavity was opened up through a midline abdominal incision to expose the reproductive organs. Then, the testes were excised and trimmed of all fat. Blood samples collected through cardiac puncture for hormonal analysis. The hormone analysis (Testosterone, Follicle stimulating hormone and Leutenizing hormone) was carried out using Immunoassay (ELISA) method (Yakubu *et al.*, 2007). The testes and epididymis from the rats were carefully dissected out and weighed independently. The testes from each rat were carefully exposed and removed. They were trimmed free of the epididymides and adjoining tissues.

Evaluation of epididymal sperm concentration: As described by Saalu *et al.* (2011), spermatozoa in the right epididymis were counted by a modified method of Yokoi and Mayi (2004). The epididymis was minced with anatomic scizzors in 5 mL physiologic saline, placed in a rocker for 10 min and allowed to incubate at room temperature for 2 min. After incubation, the supernatant fluid was diluted 1:100 with solution containing 5 g sodium bicarbonate and 1 mL formalin (35%). Sperm number was determined with the new improved Neuber's counting chamber (haemocytometer). Approximately 10 VL of the diluted sperm suspension was transferred to each counting chamber of the haemocytometer and was allowed to stand for 5 min. This chamber was then placed under a binocular light microscope using an adjustable light source. The ruled part of the chamber was then focused and the number of spermatozoa counted in five 16-celled squares. The sperm concentration was then calculated multiplied by 5 and expressed as $[X] 10^6 \text{ mL}^{-1}$, where $[X]$ is the number of spermatozoa in a 16-celled square.

Sperm progressive motility: This was evaluated by an earlier method by Sonmez *et al.* (2005). The fluid obtained from the left cauda epididymis with a pipette was diluted to 0.5 mL with Tris buffer solution. A slide was placed on light microscope with heater table, an aliquot of this solution was on the slide, and percentage motility was evaluated visually at a magnification of $\times 400$. Motility estimates were performed from three different fields in each sample. The mean of the three estimations was used as the final motility score. Samples for motility evaluation were stored at 35°C.

Sperm morphology: The sperm morphology was evaluated with the aid of light microscope at $\times 400$ magnification. Caudal sperm were taken from the original dilution for motility and diluted 1:20 with 10% neutral buffered formalin (Sigma-Aldrich, Oakville, ON, Canada). Five hundred sperm from the sample were scored for morphological abnormalities. Briefly, in wet preparations using phase contrast optics, spermatozoa were categorized. In this study a spermatozoon was considered abnormal morphologically if it has a rudimentary tail, round head or detached head and was expressed as a percentage of morphologically normal sperm.

Statistical analysis: The obtained data were expressed as Mean \pm SD of number of experiments ($n = 10$). A homogenic level among the groups was tested using Analysis of Variance (ANOVA) (55). Where heterogeneity occurred, the groups were separated using Duncan Multiple Range Test (DMRT). A value of $p < 0.05$ and $p < 0.005$ was considered to indicate a significant difference between groups.

RESULTS

Sperm count and sperm motility: As shown in Table 1, the mean sperm count and motility for the control group of rats treated 2.5 mL kg⁻¹/day/oral of distilled water were 129.5±5.2 and 70.3±3.3%, respectively. The group of rat that were treated with 50 mg kg⁻¹/day/oral of the extract (Group B) demonstrated a non significant increase in their sperm count and motility when compared to the control values. The group C rats had a significant (p<0.05) increase their sperm count and motility when also compared to the control group. The group that had 150 mg kg⁻¹ b. wt. of extract showed a significant (p<0.05) decrease in sperm concentration and sperm motility. The group of rat treated with 150 mg kg⁻¹ b.wt. of bitter leaf extract for longer duration (60 days) had a significant decrease in sperm concentration and motility (p<0.005 and p<0.05, respectively).

Sperm progressivity and sperm morphology: There was no significant difference in the sperm progressivity between the control and Group B and C rats. However, groups D and E rats showed sluggish linear progression when compared to the control group of rat. Although, the group B rats had a high percentage number of normal sperm similar to the control, there was a significant (p<0.05) reduction in percentage number of abnormal sperm. The Group C rats had a significant (p<0.05) increase in percentage number of normal sperm and a significant (p<0.005) decrease in percentage number of abnormal sperm. Group D and E rats both had a significant (p<0.05) decrease in the percentage number of normal sperm and a significant (p<0.005 and p<0.05) elevation in percentage number of abnormal sperm (Table 1).

Livability (Live/dead ratio): The group B rats had non-significant increase in percentage number of live/dead sperm (80.1±9.3%) when compared to the value (75.4±7.3%) of control group of rats. There was a significant (p<0.05) increase in percentage number of live/dead sperm of group C rats (92.5±1.2%) when compared to those of the control. The group of animals in D and E had a significant (p<0.05) reduction in percentage number of live/dead sperm (73.01±0.3% and 60.2±6.3%, respectively) when compared to the control group.

Serum testosterone levels, follicle stimulating and luteinizing hormone: Although, there was a non-significant increase in the serum testosterone level in both group B and C, there was a decrease in the serum testosterone level of group C and D rats (0.19±0.05, 0.20±0.01, respectively) with that of the group E being significantly (p<0.05) lower (0.14±0.07). The extract produced a

Table 1: Effect of bitter leaf extract on different hormones after 30 and 60 days treatment period

Treatment groups (mg kg ⁻¹)	Sperm count (×10 ⁶ mL ⁻¹)	Sperm motility (%)	Progressivity	Normal morphology (%)	Abnormal morphology (%)	Live/dead ratio livability (%)
A	129.5±5.2	70.3±3.36	a ₁	78.4±0.5	26.6±8.1	75.4±7.3
B	136±7.1	75.3±9.3	a ₁	80.8±3.1	24.2±5.1*	80.1±9.3
C	148±1.2*	80.5±4.3*	a ₁	90.7±7.0*	9.3±1.2**	92.5±1.2*
D	120.1±0.3*	70.0±2.4*	b ₁	72.3±2.3*	38.1±6.7*	73.01±0.3*
E	100.4±0.6**	65.3±0.5*	b ₁	69.0±6.7*	40.3±2.4**	60.2±6.3

*, **Represent significant decreases and increases at p<0.05 and p<0.005, respectively when compared to the control values, values are Means±S.E.M, n = 10 in each group, a₁: Rapid linear progressive motility, b₁: Show sluggish linear or non-linear motility, Group A: 2.5 mL kg⁻¹ b.wt. of distilled water (Control), Group B: 50 mg kg⁻¹ b.wt. of bitter leaf extract for 30 days, Group C: 100 mg kg⁻¹ b.wt. of bitter leaf extract for 30 days, Group D: 200 mg kg⁻¹ b.wt. of bitter leaf extract for 30 days, Group E: 200 mg kg⁻¹ b.wt. of bitter leaf extract for 60 days

Table 2: Effect of bitter leaf extract on serum level of testosterone, follicle stimulating and luteinizing hormone after 30 and 60 days treatment period

Treatment group (mg kg ⁻¹)	Testosterone (ng mL ⁻¹)	FSH (miu mL ⁻¹)	LH (miu mL ⁻¹)
A	0.18±0.02	0.14±0.2	0.09±0.01
B	0.19±0.05	0.13±0.5	0.10±0.03
C	0.20±0.01	0.10±1.0	0.11±0.01
D	0.16±0.03	0.10±8.1	0.07±0.03
E	0.14±0.07*	0.11±0.1	0.06±4.3

*,**Represent significant decreases and increases at p<0.05 and p<0.005, respectively when compared to the control values, values are Means±S.E.M. n = 10 in each group, FSH: Follicle stimulating hormone, LH: Leutinizing hormone, Miu: Milli international unit, ng: Nanogramme, Group A: 2.5 mL kg⁻¹ b.wt. of distilled water (Control), Group B: 50 mg kg⁻¹ b.wt. of bitter leaf extract for 30 days, Group C: 100 mg kg⁻¹ b.wt. of bitter leaf extract for 30 days, Group D: 200 mg kg⁻¹ b.wt. of bitter leaf extract for 30 days, Group E: 200 mg kg⁻¹ b.wt. of bitter leaf extract for 60 days

non-significant decrease in the blood level of Follicle Stimulating Hormone (FSH) of all the treated groups (0.13±0.5, 0.13±0.5, 0.10±1.0, 0.10±8.1, 0.11±0.1, respectively) when compared to the value of the control group (0.14±0.2). Also, there was a non-significant increase in the level of luteinizing hormone in the treated groups (0.10±0.03, 0.11±0.01, 0.07±0.03, 0.06±4.3, respectively) when compared to the control (0.09±0.01) (Table 2).

A spermatozoon was considered abnormal morphologically if it had one or more of the following features: Rudimentary tail, round head and detached head.

DISCUSSION

Bitter leaf has a unique nutritional and phytochemical property which has numerous physiological, biochemical and morphological benefits. It is known that consumption of vegetables is essential for a healthy life due to their antioxidative properties (Saalu *et al.*, 2011; Akunna *et al.*, 2013). The results from our study demonstrated the modulating and the testiculotoxic role of bitter leaf extract in a dose dependent manner.

Evidenced in our study, was an improved sperm concentration, motility, percentage normal morphology and percentage number of live sperm of the groups of animal treated with 50 and 100 mg kg⁻¹ bitter leaf extract for a duration of 30 days when compared to the control group of rats. These finding suggests that administration of extract from Bitter leaf (*Vernonia amygdalina*) successfully increases the sperm qualities which are in accordance with the report of Oyeyemi *et al.* (2008b) and Longe *et al.* (1983) in which there was a significant improvement observed in all the sperm parameters of rats treated with Bitter leaf rats. The administration of bitter leaf (*Vernonia amygdalina*) could increase glucose metabolism leading to the production of pyruvate which is known to be the preferred substrate essential for the activity and survival of sperm cells (Egbinike *et al.*, 1986; Dua and Vaidya, 1996).

As antioxidants, the flavonoids and vitamins in bitter leaf extract could maintain sperm morphology, sperm survival and sperm function and therefore be regarded as a steady supply of additional nutrients to the treated rats over the control groups. The improved sperm characteristics in the treatment group suggested that bitter leaf extract could produce a stimulatory effect on the hypothalamus. Although, our result a showed decrease in the blood level of follicle stimulating hormone, the increase in the level of serum testosterone and luteinizing hormone of group B and C rats indicates the modulating potentials of bitter leaf extract in rats.

Studies show that treatment with antioxidants improves steroidogenesis by enhancing the primary effect of on leydig cell endocrine function along with increased circulatory testosterone production and stimulation of spermatogenesis (Prasad and Rajalakshmi, 1989).

Previously in our laboratory, we have shown that higher dosage of antioxidants could become pro-oxidants thereby damaging cellular structures (Iwalewa *et al.*, 2005; Saalu *et al.*, 2010).

Reported in the present study is the fact that administrations of bitter leaf extract at higher doses (200 mg kg⁻¹ b.wt.) and duration provoked varying degrees of testicular degeneration ranging from a significant reduction in sperm concentration, motility, percentage normal morphology, percentage number of live sperm to a significant increase in the number of percentage abnormal sperm. Although, the mechanism of action of this effect is still unclear, alkaloids one of the active components of bitter leaf have been implicated (Igile *et al.*, 1995b; Adaramoye *et al.*, 2008b). It is postulated that these alkaloids could be bioactivated thereby releasing metabolites, which bind to cell molecules and cross-link DNA causing cytotoxicity (Saalu *et al.*, 2010; Oyeyemi *et al.*, 2008a).

CONCLUSION

The spermiogram and hormonal evaluation obtained from this study provide evidence suggesting that higher dosage of bitter leaf (*Vernonia amygdalina*) extract administered for a longer duration could be deleterious to the testes. It is even more significant when one considers reports that lower mammal possess more efficient xenobiotic biotransformation system.

REFERENCES

- Adaramoye, O.A., J. Achem, O.O. Akintayo and M.A. Fafunso, 2007. Hypolipidemic effect of *Vernonia amygdalina*, a tropical vegetable in rats fed on high cholesterol diet. *J. Clin. Lipidol.*, 1: 467-467.
- Adaramoye, O., B. Ogungbenro, O. Anyaegbu and M. Fafunso, 2008a. Protective effects of extracts of *Vernonia amygdalina*, *Hibiscus sabdariffa* and vitamin C against radiation-induced liver damage in rats. *J. Radiat. Res.*, 49: 123-131.
- Adaramoye, O.A., O. Akintayo, J. Achem and M.A. Fafunso, 2008b. Lipid-lowering effects of methanolic extract of *Vernonia amygdalina* leaves in rats fed on high cholesterol diet. *Vasc. Health Risk Manage.*, 4: 235-241.
- Adaramoye, O., O. Akintayo, J. Achem and M.A. Fafunso, 2009. Lipid lowering effects of methanolic extract of *Vernonia amygdalina* leaves in rats fed on high cholesterol diet. *Atheroscler. Suppl.*, 10: 1119-1119.
- Akunna, G.G., L.C. Saalu, B. Ogunlade, A.O. Ojewale and L.A. Enye, 2013. Consumption of bay leaf (a food spice) may be a safe and effective treatment for male infertility resulting from partial ligation of the left renal vein in wistar rat: Study suggest. *Am. J. Res. Commun.*, 1: 123-142.
- Alawa, C.B.I., J.O. Gefu, N.P. Chiezey, P.A. Abdu, S.O. Magaji, L.O. Eduvie and I.A. Adeyinka, 2000. Screening of *Vernonia amygdalina* for Anthelmintic Properties. Proceedings of the International Workshop on Ethno-Veterinary Practices, August 4-18, 2000, Kaduna, Nigeria, pp: 49-55.
- Alawa, J.P., G.E. Jokthan and K. Akut, 2002. Ethnoveterinary medical practice for ruminants in the subhumid zone of northern Nigeria. *Prev. Vet. Med.*, 54: 79-90.
- CCAC, 1985. Guide to the Handling and use of Experimental Animals. Vol. 23, NIH Publications, Ottawa, USA., pp: 45-47.

- De Madureira, M.D.C., A.P. Martins, M. Gomes, J. Paiva, AP. da Cunha and V. do Rosario, 2002. Antimalarial activity of medicinal plants used in traditional medicine in S. Tome and Principe Islands. *J. Ethnopharmacol.*, 81: 23-29.
- Dua, A.A. and S.R. Vaidya, 1996. Sperm motility and morphology as changing parameters linked to sperm count variations. *J. Postgraduate Med.*, 42: 93-96.
- Egbunike, G.N., W. Branscheid, J. Pfisterer and W. Holtz, 1986. Changes in porcine sperm lactate dehydrogenase isoenzymes during sperm maturation. *Andrologia*, 18: 108-113.
- Ho, C.T., 1994. *Food Phytochemicals and Cancer Prevention*. American Chemical Assoc., Washington, DC.
- Igile, G.O., W. Oleszek, M. Jurzysta, S. Burda, M. Fanfunso and A.A. Fasanmada, 1994. Flavonoids from *vernonia amygdalina* and their antioxidant properties. *J. Agric. Food Chem.*, 42: 2445-2448.
- Igile, G.O., W. Olesezk, S. Burda and M. Jurzysta, 1995a. Nutritional assessment of *Vernonia amygdalina* leaves in growing mice. *J. Agric. Food Chem.*, 43: 2162-2166.
- Igile, G., W. Oleszek, M. Jurzysta, R. Aquino, N. Tommas and C. Pizza, 1995b. Vernoniosides D and E, Two novel saponins from *Vernonia amygdalina* Del. *J. Natural Prod.*, 58: 1438-1443.
- Iwalewa, E.O., C.O. Adewunmi, N.O. Omisore, O.A. Adebajji and C.K. Azike *et al.*, 2005. Pro- and antioxidant effects and cytoprotective potentials of nine edible vegetables in Southwest Nigeria. *J. Med. Food*, 8: 539-544.
- Izevbigie, E.B., 2003. Discovery of water-soluble anticancer agents (edotides) from a Vegetable found in Benin City, Nigeria. *Exp. Biol. Med.*, 228: 293-298.
- Izevbigie, E.B., J.L. Bryant and A. Walker, 2004. A novel natural inhibitor of extra cellular signal-regulated kinases and human breast cancer cell growth. *Exp. Biol. Med.*, 229: 163-169.
- Izevbigie, E.B., M. Opata and J.L. Bryant, 2005. Deleterious side effects of tamoxifen may be ameliorated by aqueous *Vernonia amygdalina* leaf extracts. *J. Nutr.*, 135: 3037-3060.
- Jisaka, M., H. Ohigashi, T. Takagaki, H. Nozaki and T. Tada *et al.*, 1992. Bitter steroid glucosides, vernoniosides A1, A2, A3 and related B1 from a possible medicinal plant: *Vernonia amygdalina* used by wild chimpanzees. *Tetrahedron*, 48: 625-632.
- Jisaka, M., H. Ohigashi, K. Takegawa, M. Hirota, R. Irie, M.A. Huffman and K. Koshimizu, 1993a. Steroid glucosides from *Vernonia amygdalina*, a possible chimpanzee medicinal plant. *Phytochemistry*, 34: 409-413.
- Jisaka, M., H. Ohigashi, K. Takegawa, M.A. Huffman and K. Koshimizu, 1993b. Antitumoral and antimicrobial activities of bitter sesquiterpene lactones of *Vernonia amygdalina*, a possible medical used by wild chimpanzees. *Biosci. Biotechnol. Biochem.*, 57: 833-834.
- Longe, O.G., G.O. Farinu and B.L. Fetuga, 1983. Nutritional value of the fluted pumpkin (*Telfairia occidentalis*). *J. Agric. Food Chem.*, 31: 989-992.
- Masaba, S.C., 2000. The antimalarial activity of *Vernonia amygdalina* Del. (Composital). *Trans. R. Soc. Trop. Med. Hyg.*, 94: 694-695.
- Ogbulie, J.N., C.C. Ogueke and F.C. Nwanebu, 2007. Antibacterial properties of *Uvaria chamae*, *Congronema latifolium*, *Garcinia kola*, *Vernonia amygdalina* and *Aframomium melegueta*. *Afr. J. Biotechnol.*, 6: 1549-1553.
- Osinubi, A.A., 2007. Effects of *Vernonia amygdalina* and chlorpropamide on blood glucose. *Med. J. Islamic World Acad. Sci.*, 16: 115-119.
- Oyeyemi, M.O., O. Oluwatoyitr, O.O. Ajala Leigh and T.F. Adesiji, 2008a. The spermogram of male wistar rats treated with aqueous leaf extract of *Vernonia amygdalina*. *Foliav Eterinaria*, 2: 98-101.

- Oyeyemi, M.O., O.O. Leigh, O.O. Ajala, A.O. Badejo and B.O. Emikpe, 2008b. The effects of Ugu *Telfairia occidentalis* leaves on the testis and spermatozoa characteristics in male Albino rat. *Folia Vet.*, 52: 102-105.
- Prasad, M.R.N. and M. Rajalakshmi, 1989. Spermatogenesis and Accessory Gland Secretions. In: Textbook of Biochemistry and Human Biology, Talwar, G.P., L.M. Srivastava and L.M. Moudgil (Eds.). 2nd Edn. Prentice-Hall of India Pvt. Ltd., New Delhi, India, pp: 883.
- Saalu, L.C., A.A. Osinubi, A.A. Akinbami, O.E. Yama, A.O. Oyewopo and B.U. Enaibe, 2011. *Moringa oleifera* Lamarck (drumstick) leaf extract modulates the evidences of hydroxyurea-induced testicular derangement. *Int. J. Applied Res. Nat. Prod.*, 4: 32-45.
- Saalu, L.C., T. Kpela, A.S. Benebo, A.O. Oyewopo, E.O. Anifowo and J.A. Oguntola, 2010. The dose-dependent testiculoprotective and testiculotoxic potentials of *Telfairia occidentalis* Hook f. leaves extract in rat. *Int. J. Applied Res. Nat. Prod.*, 3: 27-38.
- Sonmez, M., G. Turk and A. Yuces, 2005. The effect of ascorbic acid supplementation on sperm quality, lipid peroxidation and testosterone levels of male Wistar rats. *Theriogenology*, 63: 2063-2072.
- Tona, L., R.K. Cimanga, K. Mesia, C.T. Musamba and T.de Bruyne *et al.*, 2004. *In vitro* antiparasitic activity of extracts and fractions from seven medicinal plants used in the democratic republic of Congo. *J. Ethnopharm.*, 93: 27-32.
- Vlietinck, J., L. van Hoof, J. Totte, A. Lasure, D.V. Berghe, P.C. Rwangabo and J. Mvukiyeumwami, 1995. Screening of hundred Rwandese medicinal plants for antimicrobial and antiviral properties. *J. Ethnopharmacol.*, 46: 31-47.
- WMA/APS, 2002. Guiding principles for research involving animals and human beings. *Am. J. Physiol. Regul. Integr. Comp. Physiol.*, 283: R281-R283.
- Yakubu, M.T., M.A. Akanji and A.T. Oladiji, 2007. Effect of oral administration of aqueous extract of *Fadogia agestis* (Schweinf. Ex.Hiern) stem on some testicular function indices of male rats. *J. Ethnopharmacol.*, 115: 288-292.
- Yokoi, K. and Z.K. Mayi, 2004. Organ apoptosis with cytotoxic drugs. *Toxicology*, 290: 78-85.