

Effect of Methanolic Extract of *Vernonia Amygdalina* on Reproductive Parameters in Male Albino Rats

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Abstract: *Vernonia amygdalina* is a shrub that grows throughout the tropical Africa. Several studies have reported the nutritional, anti-malaria and anti-helminthic effects of its extracts but there is scanty information on its effect on reproduction. This study was designed to investigate the effect of its methanolic extract on reproductive parameters in male Albino rats. Methanolic extract of *Vernonia amygdalina* is designated as MEVA. MEVA was prepared using cold maceration. The extract was administered for 30 days for andrological studies. Distilled water (0.5 mL) served as the control. Plasma testosterone levels were assayed using Enzyme-Link Immunosorbent Assay (ELISA) and semen analysis was done microscopically, histology of the testes was also done. Data were analyzed using ANOVA at $p < 0.05$. Treatment of rats with all the doses of MEVA caused significant decrease in testosterone levels, sperm motility and sperm count relative to control; while, there was no significant change in sperm viability. MEVA also caused severe germinal erosion and necrosis in the seminiferous tubules in the testes.

Key words: *Vernonia amygdalina*, sperm count, Albino rats, testosterone, sperm motility

INTRODUCTION

Vernonia amygdalina belongs to the family of Asteraceae. It is commonly called bitter leaf in English language, Shuwaka in Hausa language, Onugbu in Igbo language and Ewuro in Yoruba language.

It is a highly appreciated vegetable in West and Central Africa and can be consumed in various dishes (Bosni *et al.*, 1995). Medicinally, the leaves are widely used for fevers and are known as quinine substitute (Challand and Willcox, 2009). It is used to prepare cough medicine in Ghana (Akinpelu, 1999) and the root infusion is taken in Nigeria as an antihelminthic as well as for enteritis and rheumatism (Ainslie, 1937).

Pharmacological studies have shown that the leaf extract of *V. amygdalina* has both hypoglycemic and hypolipidemic properties in experimental animals and so could be used in the management of diabetes, hypertension, etc (Akah and Okafor, 1992). The extracts of *V. amygdalina* have been reported to have analgesic and antipyretic effects (Tekoba *et al.*, 2002). The aqueous extract of *V. amygdalina* has also been reported to have anti-oxidant property (Nwanjo and Nwokoro, 2004).

However, due to paucity of information from literature on the effect of *V. amygdalina* on reproductive

parameters in male Albino rats, this study therefore aim at investigating the effect of methanolic extract of *V. amygdalina* on these reproductive parameters.

MATERIALS AND METHODS

Experimental animals: Adult male Albino rats weighing between 160 and 180 g bred in the Animal House of Physiology Department, LAUTECH, Ogbomoso were used. They were housed under standard laboratory conditions with a 12 h daylight cycle and had free access to feed and water; they were acclimatized to laboratory conditions for 2 weeks before the commencement of the experiments. All experiments were carried out in compliance with the recommendations of Helsinki's declaration on guiding principles on care and use of animals.

Plant materials: Fresh specimens of *V. amygdalina* harvested from a local farm in Ogbomoso, Nigeria were authenticated in the taxonomy unit of the Department of Pure and Applied Biology, LAUTECH, Ogbomoso.

Preparation of Methanolic Extract of *Vernonia Amygdalina* (MEVA): Large quantities (1.56 kg) of the

fresh specimens of *V. amygdalina* were washed free of soil and debris and the roots were separated from the leaves and stems. The leaves and stems were air-dried for 4 weeks and the dried specimens were pulverized using laboratory mortar and pestle.

Weighed portion (556 g) of the pulverized specimens were macerated with 70% methanol (1:2 wt./vol.) for 72 h at room temperature (26-28°C). The resulting solution was then filtered using a wire-gauze and a sieve with tiny pores (0.25 mm). The 70% methanol was later evaporated using steam bath to give a percentage yield of 10.24% of the starting material.

About 10 g of the Methanolic Extract of *V. Amygdalina* (MEVA) was dissolved in 100 mL of distilled water to give a concentration of 0.1 g mL⁻¹. The dosages of the extract administered in this study were in accordance with those reported by Challand and Willcox (2009).

Experimental design: Here, 24 animals were randomly divided into four groups with each group consisting of 6 rats. The four groups of rats were subjected to the following oral treatments once a day for 30 days:

- Group I rats received 50 mg kg⁻¹ body weight of MEVA
- Group II rats received 100 mg kg⁻¹ body weight of MEVA
- Group III rats receive 150 mg kg⁻¹ body weight of MEVA
- Group IV rats received 1.0 mL of distilled water as the control group

About 24 h (day 31) after the last dosing of the four groups, blood samples were collected and the animals were then euthenised by cervical dislocation for semen analysis. Histological preparation of the testes was also carried out.

Body weight: Body weight of each rat was monitored on weekly basis.

Collection of blood sample: Blood samples were collected through the medial cantus into EDTA bottles for hormonal assay.

Hormonal assay: Plasma samples were assayed for testosterone using the Enzyme-Linked Immunosorbent Assay (ELISA) technique using the Randox kit.

Semen collection: The testes were removed along with the epididymides. The caudal epididymides were separated from the testes, blotted with filter papers and lacerated to collect the semen.

Semen analysis

Progressive sperm motility: This was done immediately after the semen collection. Semen was squeezed from the caudal epididymis onto a pre-warmed microscope slide (27°C) and 2 drops of warm 2.9% sodium citrate was added, the slide was then covered with a warm cover slip and examined under the microscope using x400 magnification. And 10 fields of the microscope were randomly selected and the sperm motility of 10 sperms was assessed on each field. Therefore, the motility of 100 sperms was assessed randomly. Sperms were labelled as motile, sluggish or immotile. The percentage of motile sperms was defined as the number of motile sperms divided by the total number of counted sperms (i.e., 100) (Mohammad-Reza *et al.*, 2005).

Sperm viability (Life/dead ratio): This was done by adding 2 drops of warm Eosin/Nigrosin stain to the semen on a pre-warmed slide, a uniform smear was then made and dried with air; the stained slide was immediately examined under the microscope using x400 magnification. The live sperm cells were unstained while the dead sperm cells absorbed the stain. The stained and unstained sperm were counted and the percentage was calculated (Laing, 1979).

Sperm morphology: This was done by adding 2 drops of warm Walls and Ewas stain (Eosin/Nigrosin stain can also be used) to the semen on a prewarmed slide, a uniform smear was then made and air-dried; the stained slide was immediately examined under the microscope using x400 magnification (Laing, 1979). And 5 fields of the microscope were randomly selected and the types and number of abnormal spermatozoa were evaluated from the total number of spermatozoa in the 5 fields; the number of abnormal spermatozoa were expressed as a percentage of the total number of spermatozoa.

Sperm count: This was done by removing the caudal epididymis from the right testes and blotted with filter paper. The caudal epididymis was immersed in 5 mL formol-saline in a graduated test-tube and the volume of fluid displaced was taken as the volume of the epididymis. The caudal epididymis and the 5 mL formol-saline were then poured into a mortar and homogenized into a suspension from which the sperm count was carried out using the improved Neubauer haemocytometer under the microscope.

Testicular histology: After weighing the testes, they were immediately fixed in Bouin's fluid for 12 h and the Bouin's fixative was washed from the samples with 70% alcohol. The tissues were then cut in slabs of about 0.5 cm transversely and the tissues were dehydrated by passing through different grades of alcohol: 70% alcohol for 2 h,

95% alcohol for 2 h, 100% alcohol for 2 h, 100% alcohol for 2 h and finally 100% alcohol for 2 h. The tissues were then cleared to remove the alcohol, the clearing was done for 6 h using xylene. The tissues were then infiltrated in molten Paraffin wax for 2 h in an oven at 57°C; thereafter, the tissues were embedded. Serial sections were cut using rotary microtome at 5 µm. The satisfactory ribbons were picked up from a water bath (50-55°C) with microscope slides that had been coated on one side with egg albumin as an adhesive and the slides were dried in an oven. Each section was deparaffinized in xylene for 1 min before immersed in absolute alcohol for 1 min and later in descending grades of alcohol for about 30 sec each to hydrate it. The slides were then rinsed in water and immersed in alcoholic solution of hematoxylin for about 18 min. The slides were rinsed in water then differentiated in 1% acid alcohol and then put inside a running tap water to blue and then counterstained in alcoholic eosin for 30 sec and rinsed in water for a few seconds, before being immersed in 70, 90% and twice in absolute alcohol for 30 sec each to dehydrate the preparations. The preparations were cleared of alcohol by dipping them in xylene for 1 min. Each slide was then cleaned, blotted and mounted with DPX and cover slip and examined under the microscope. Photomicrographs were taken at 40, 100 and x400 magnifications.

Statistical analysis: The mean and Standard Error of Mean (SEM) were calculated for all values. Comparison between the control and experimental groups was done using one-way Analysis of Variance (ANOVA) with Duncan's multiple range test. Differences were considered statistically significant at $p < 0.05$.

RESULTS

Effect on body weight: The administration of all treatment dose of MEVA to the rats for 30 days caused non-significant ($p > 0.05$) changes in body weight relative to their control (Fig. 1).

Effect on hormonal levels: Treatment of rats for 30 days with all the treatment doses of MEVA caused significant ($p < 0.05$) decrease in testosterone levels relative to the control (Fig. 2).

Effect on sperm characteristics: Treatment of rats for 30 days with all the various doses of MEVA (except 100 mg kg⁻¹) caused significant ($p < 0.05$) decrease in sperm motility relative to the control. All the treatment doses of MEVA caused non-significant ($p > 0.05$) decrease in sperm viability (life/dead) (Fig. 3). MEVA (except

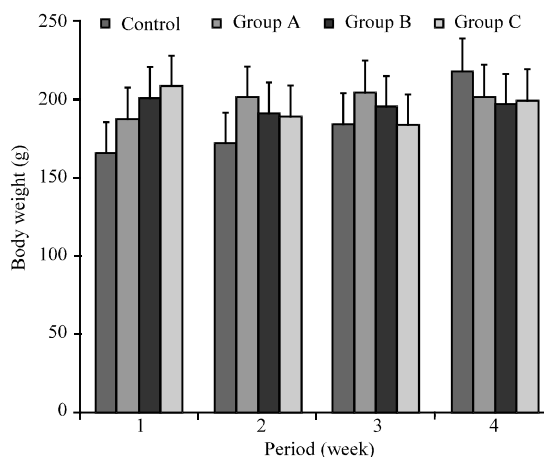


Fig. 1: Body weight changes in control rats and rats treated with MEVA for 30 days

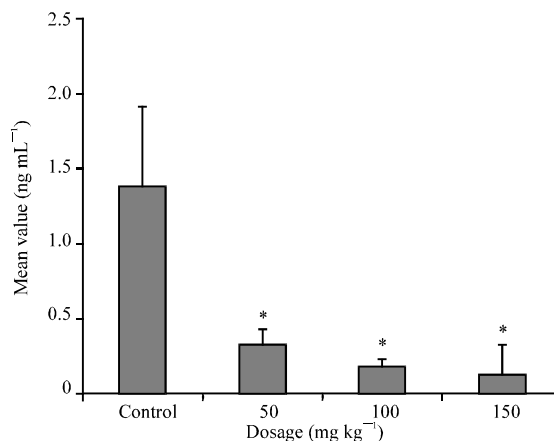


Fig. 2: Effect of treatment of rats for 30 days with MEVA on plasma testosterone levels

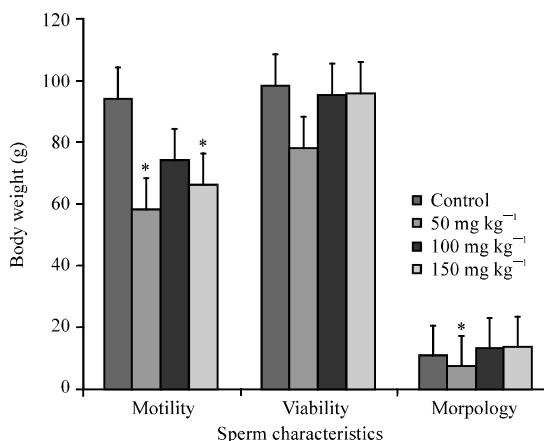


Fig. 3: Spermogram showing the effect of MEVA on sperm characteristics after treatment of rats for 30 days (n = 6; * $p < 0.05$)

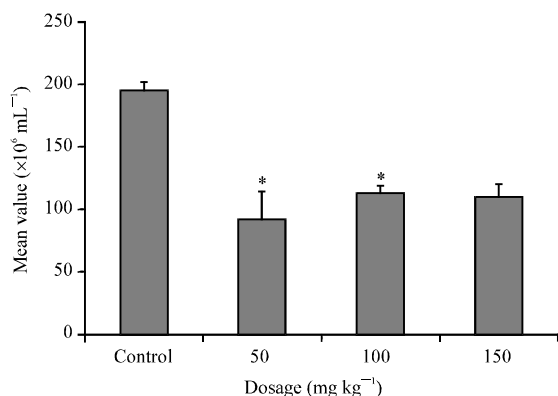


Fig. 4: Spermogram showing the effect of MEVA on sperm count after the treatment of rats for 30 days (n = 6; *p<0.05)

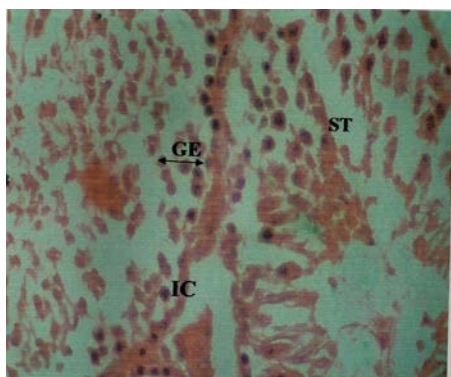


Fig. 5: Effect of 0.5 mL of distilled water (control) on the testis after treatment of rats for 30 days at x400; photomicrograph showing the Seminiferous Tubules (ST) with normal Germinal Epithelium (GE)

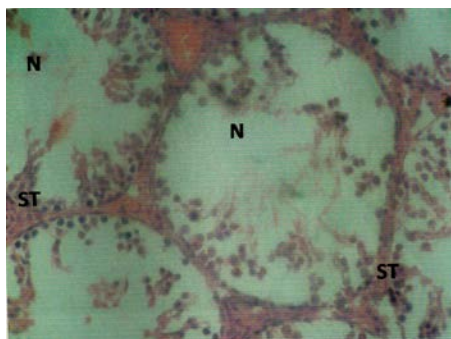


Fig. 6: Effect of 100 mg kg⁻¹ body weight of MEVA on the testes at x400; photomicrograph showing severe germinal erosion and Necrosis (N) in most of the seminiferous tubules

50 mg kg⁻¹ body weight) caused non-significant (p>0.05) increase in the percentage of abnormal sperm cells; while,

all the treatment doses of MEVA (except 150 mg kg⁻¹ body weight) caused significant (p<0.05) decrease in sperm count (Fig. 4).

Histopathological findings: Treatment of rats for 30 days with various doses of MEVA caused severe germinal erosion and necrosis in the seminiferous tubules; while, the control rats presented with normal germinal epithelium in the seminiferous tubules (Fig. 5 and 6).

DISCUSSION

The extract caused non-significant changes in body weight of rats after treatment for 30 day, this suggests the extract was not toxic as well as non-androgenic in nature, since androgens are known to possess anabolic activities. Similar report was given by Gonzales *et al.* (2006) in rats treated with *Lepidium meyenii* extracts.

The extract caused significant decrease in testosterone levels. Similar report was given by Das *et al.* (2009) in rats treated with *Aegle marmelos* extract. This decrease in testosterone levels could indicate that the extract inhibits the mechanism intervening in the process of hormone synthesis in the Leydig cells.

The andrological results show that treatment of rats for 30 days with the extract caused significant decrease in sperm motility. Similar report was given by Venma *et al.* (2002) in rats treated with *Sarcotemma acidum* extract. This suggests that the extract was able to permeate the blood-testis barrier with a resultant alteration in the microenvironment of the seminiferous tubules, since it has been reported that the decrease in sperm motility caused by chemical agents was due to their ability to permeate the blood-testis barrier (Baldessarini, 1980) and thus, creating a different microenvironment in the inner part of the wall of the seminiferous tubules from that in the outer part (Bloom and Fawcett, 1975).

There was a statistically non-significant decrease in sperm viability as well as a non-significant increase in the percentage of morphologically abnormal sperm cells induced after treatment of rats with the extract. This could be due to the ability of the extract to either interfere with the spermatogenic processes in the seminiferous tubules, epididymal functions or activities of testosterone on hypothalamic release factor and anterior pituitary secretion of gonadotropins which may result in alteration of spermatogenesis (William, 2000; Bowman and Rand, 1985).

Sperm count is considered to be an important parameter with which to assess the effects of chemicals on spermatogenesis (Reddy *et al.*, 1997). Spermatogenesis is influenced by the hypothalamic-adenohypophyseal, Leydig Cell System relating gonadotrophin releasing hormone, leutinizing hormone and androgen. This implies

that the decrease in sperm count caused by extract in the treated rats might be as a result of decrease in plasma level of testosterone because this hormone has been reported to be important in the initiation and maintenance of spermatogenesis (Christensen, 1975). Similar report was given by Krishnamoorthy *et al.* (2007) in *Terminalia chebula* extract treated rats. Treatment of rats with the extract caused severe germinal erosion and necrosis. This could be due to insufficient amount of testosterone, since it has been reported that testosterone is essential for the growth and division of the germinal cells of the seminiferous tubules (Burger and de Kretsner, 1989). Similar result was obtained in rats treated with *Colebrookia oppositifolia* (Gupta *et al.*, 2001).

CONCLUSION

It can be concluded that *Vernonia amygdalina* has deleterious effect on the reproductive functions in male Albino rats.

REFERENCES

- Ainslie, J.R., 1937. The List of Plants used in Native Medicine in Nigeria. Imperial Forestry Institute, Oxford, UK.
- Akah, P.A. and C.L. Okafor, 1992. Blood sugar lowering effect of *Vernonia amygdalina* Del, in an experimental rabbit model. *Phytother. Res.*, 6: 171-173.
- Akinpelu, D.A., 1999. Antimicrobial activity of *Vernonia amygdalina* leaves. *Fitoterapia J. Study Med. Plants*, 70: 432-434.
- Baldessarini, R.J., 1980. Drugs and the Treatment of Psychiatric Disorders. In: The Pharmacological Basis of Therapeutics, Goodman, L.S. and A. Gilman (Eds.). Macmillan Publishing Co., New York, pp: 301-417.
- Bloom, W. and D.W. Fawcett, 1975. Male Reproductive System. In: The Textbook of Histology, Bloom, W. and D.W. Fawcett (Eds.). Saunders Co., Philadelphia.
- Bosni, M.L.K., P.O. Osuji, A.K. Tuah and N.N. Umunna, 1995. *Vernonia amygdalina* as a supplement to teff straw (*Eragrostis tef*) fed to Ethiopian Menz sheep. *Agroforest. Syst.*, 31: 229-241.
- Bowman, W.C. and M.J. Rand, 1985. The Reproductive System and Drugs Affecting the Reproductive Systems. In: Textbook of Pharmacology, Bowman, W.C. and M.J. Rand (Eds.). 2nd Edn., Blackwell Scientific Publications, Oxford, pp: 1-8.
- Burger, H. and D. de Kretsner, 1989. The Testis. Raven Press, New York.
- Challand, S. and M. Willcox, 2009. A clinical trial of the traditional medicine *Vernonia amygdalina* in the treatment of uncomplicated malaria. *J. Altern. Complement Med.*, 15: 1231-1237.
- Christensen, A.C., 1975. Leydig Cell. In: Handbook of Physiology, Greep, P.O. and E.B. Astwoods (Eds.). American Physiological Society, Washington, DC.
- Das, U.K., D. De, K. Chatterjee, C. Mallick, T.K. Bera and D. Ghosh, 2009. Antigonadal effect induced by hydro-methanolic extract of leaf of *Aelgle mermelos* in male rat: Effect of hCG co-administration. *J. Med. Plants Res.*, 3: 728-735.
- Gonzales, C., J. Rubio, M. Gasco, J. Nieto, Y. Sandra and G.F. Gonzales, 2006. Effect of short-term and long-term treatments with three ecotypes of *Lepidium meyenii* (MACA) on spermatogenesis in rats. *J. Ethnopharmacol.*, 103: 448-454.
- Gupta, R.S., R.K. Yadav, V.P. Dixit and M.P. Dobhal, 2001. Antifertility studies of *Colebrookia oppositifolia* leaf extract in male rats with special reference to testicular cell population dynamics. *Fitoterapia*, 72: 236-245.
- Krishnamoorthy, P., S. Vaithinathan, A. Vimal-Rani and A. Bhuvanewari, 2007. Effect of *Terminalia chebula* fruit extract on lipid peroxidation and antioxidative system of testis of albino rats. *Afr. J. Biotechnol.*, 6: 1888-1891.
- Laing, J.A., 1979. Fertility and Infertility in Domestic Animals. 3rd Edn., Bailliere Tindall, London.
- Mohammad-Reza, P., D. Farzaneh, T.K. Taherch and P.P. Zoherb, 2005. The effects of hydroalcoholic extract of *Actinidia chinensis* on sperm count and motility and blood levels of estradiol and testosterone in male rats. *Ach. Iranina Med.*, 8: 211-216.
- Nwanjo, H.U. and E.A. Nwokoro, 2004. Antidiabetic and biochemical effects of aqueous extract of *Vernonia amygdalina* leaf in normoglycaemic and diabetic rats. *J. Innov. Life Sci.*, 7: 6-10.
- Reddy, C.M., D.R.K. Murthy and S.B. Patil, 1997. Antispermatic and androgenic activities of various extracts of *Hibiscus rosa sinensis* in albino mice. *Indian J. Exp. Biol.*, 35: 1170-1174.
- Tekoba, A.M., A.O. Onabanjo, O.O. Amole and P.M. Emeka, 2002. Analgesic and antipyretic effects of the aqueous extract of *Vernonia amygdalina*. *West Afr. J. Pharm.*, 16: 68-74.
- Venma, P.K., A. Sharma, A. Mathur, P. Sharma, R.S. Gupta, S.C. Joshi and V.P. Dixit, 2002. Effect of *Sarcostemma acidum* stem extract on spermatogenesis in male albino rats. *Asian J. Androl.*, 4: 43-47.
- William, K.W., 2000. Hormones and Hormone Antagonists. In: Remington: The Science and Practice of Pharmacy, Gennaro, A.R. (Ed.). Vol. 11, 20th Edn., Lippincott Williams and Wilkins, Baltimore, Md., pp: 1390-1391.