

Aphrodisiac Effect of Aqueous and Ethylacetate Leaf Extracts of *Alchornea cordifolia* on Male Spermatogenesis

¹L.N. Ebenyi, ²M.E. Ogbanshi, ²E.I. Akubugwo, ²K.N. Agbafor and ²O.R. Inya-Agha

¹Department of Biotechnology, Ebonyi State University, Abakaliki, Nigeria

²Department of Biochemistry, Ebonyi State University, Abakaliki, Nigeria

Abstract: This research investigated the effect of leaf extracts of *Alchornea cordifolia* on male spermatogenesis in albino rats. The dried leaf extracts were prepared with deionised water and ethylacetate. Chemical analyses of the extracts were performed using standard methods. The sperm cell count, motility and morphology were done using microscopic method. The effects of the extracts on male reproductive organ were investigated in 40 rats placed in groups (1 – 5). Group 1 acted as normal control while group 2 acted as positive control for the male groups. Groups 3, 4 and 5 received 200, 400 and 800 mg/kg body weight of aqueous and ethylacetate extracts for a period of 21 days. The effects of the extracts on sperm analysis showed that the percentage of the normal cells were significantly higher ($p < 0.05$) than that of the abnormal cells. The histological analysis showed that the extracts increased spermatogenesis at different doses but that of ethylacetate extract had the highest effect as compared with the positive controls. From the results of this study, it can be concluded that aqueous and ethylacetate extracts of *A. cordifolia* have potentials to improve infertility associated with low spermatogenesis through its chemical contents, antioxidant and antimicrobial activities.

Key words: *Alchornea cordifolia* • Libido • Aphrodisiac and Male Spermatogenesis

INTRODUCTION

Libido refers to an individual's desire for sexual activity. This can also be called sex drive. Factors that affect libido include psychological factors, biological factors and social factors. Personality and the level of stress an individual is exposed to are internal psychological factors that can affect sex drive [1]. Sex hormones (like testosterone and dopamine) and neurotransmitters associated with the nucleus accumbens regulate sexual drive in men. An individual's occupation and family are two social factors that can affect his sex drive. Medications, medical conditions, relationship issues, age and lifestyle can also affect sex drive. In both men and women, sexual desire is an important factor in the formation and maintenance of relationships and marriages [2]. A loss or lack of sexual function can adversely affect relationships. Throughout history, many drugs, foods, drinks and behaviours have been used to treat male infertility and at the same time increase sexual pleasure. Such substances can be often seen as aphrodisiac substances [3]. Wikipedia, the free encyclopedia defines

aphrodisiac as a substance that, when consumed, increases sexual desire. Generally, for a substance to be considered an aphrodisiac, it should be taken orally, greatly increase libido or sexual drive and should exert its action within some minutes or hours.

Alchornea cordifolia belongs to the family of Euphorbiaceae. The common names are Christmas bush and Dovewood. In Nigeria, it is called "ububo", in Igbo, "ipa esinyin", in Yoruba and "banbani" in Hausa. The plant is a strangling shrub or small evergreen plant that can grow up to 32 feet tall in swampy locations. It is propagated through seed or stem cuttings and grows well in very moist soil. The leaves and stems are used traditionally as a therapeutic agent in many countries in Africa as remedies for various conditions which includes enhancing libido and male infertility [4]. The stem bark is tinctured with local gin for its aphrodisiac effect [5]. It is used as an antidote for poison, as a sedative and antispasmodic.

The aim of this work was to verify the use of this medicinal plant locally as an aphrodisiac substance and to enhance male libido.

MATERIALS AND METHODS

Collection of Plant Materials: *Alchornea cordifolia* leaves were collected from Ndiagu Ogba in Ohaukwu Local Government Area of Ebonyi State. The leave was identified and authenticated by a plant Taxonomist at Enugu state University of Science and Technology, Enugu (ESUT).

Animals Used: Albino rats used in this study were wistar strain with average weight of 120-160 g obtained from the Animal House of the Faculty of Biological Sciences, University of Nigeria, Nsukka (UNN) and acclimatized for seven days in the Animal House of the Department of Biochemistry, Ebonyi State University, Abakaliki.

Methods

Extraction of Leaves of *Alchornea cordifolia*: The fresh leaves were washed and dried under ambient temperature before they were ground into fine powder using manual grinder and stored in an air tight container.

Preparation of Deionized Water Extract: The homogenized sample (250 g) was soaked in 500 ml of deionised water for 48 hours. The solution was filtered using a muslin cloth. The aqueous filtrate was evaporated to dryness using a rotary evaporator.

Preparation of Ethylacetate Extract: The homogenized sample (250g) was soaked in 500 ml of ethylacetate for 48 hours. The solution was filtered using a muslin cloth. The ethylacetate filtrate was evaporated to dryness using a rotary evaporator and stored in air-tight container.

Effect of Extracts on Reproduction: A total of 45 albino rats were used for the study and were divided into 9 groups each having 5 rats per cage. Group 1 received deionized water and acted as normal control. Group 2 received 50 mg/kg of clomifene citrate as standard drug for female fertility treatment while Group 3 received 25 mg/kg of proviron as standard drug for male fertility treatment respectively. Groups 4, 5 and 6 containing female rats only received 200, 400 and 800 mg/kg body weight of aqueous and ethylacetate leaf extracts respectively while Groups 7, 8 and 9 containing male rats received 200, 400 and 800 mg/kg each of aqueous and ethylacetate leaf extracts. At the end of the 21days treatment period, the animals were sacrificed under light anesthesia. The blood was drawn from the animals by puncturing the ocular median-cantus vein of the rats with

the aid of cappillary tubes, into non-heparinized tubes. After centrifugation at 2500 rpm for 10 minutes the serum was then separated and used for hormonal analysis of follicle stimulating hormone, luteinizing hormone, progesterone and testosterone.

For the male rats, sperm were collected by cutting the cauda epididymis and perfusing the cauda with 37 °C (0.9 %) normal saline. The epididymis perfusate was centrifuged at 2250 rpm for 10 mins. The pellet was suspended in 1.0 ml of normal saline. An aliquot of sperm suspension was used for the examination of sperm count, sperm motility and sperm morphology.

Evaluation of Sperm Count: Sperm count was evaluated using the method of [6] and the WHO manual for semen analysis (1999). Sperm counts were visually determined in a counting chamber by microscopy. 1 in 20 dilutions were made using bicarbonate formalin diluting fluid to mix properly. The improved Neubauer counting chamber was loaded with well mixed semen and allowed to stand for 3-5 minutes for spermatozoa to settle. The sperm count was evaluated by counting the four corners square and the central large square of the Neubauer counting chamber (improved) and the result was expressed as 10 per sperm dilution.

Calculation of Sperm Count:

Total number of sperm cells counted in 5 small squares = n

Multiplication factors 50,000 dilution factors = 20

i.e. sperm count per ml = n x 20 x 3

Where 3 is the number of ml of normal saline used in meshing the semen

Normal range of total sperm count = 20 million.

Evaluation of Sperm Motility: A drop of well mixed semen was placed on a slide and covered with a cover glass. The determination of sperm motility was done by counting both motile and non-motile sperms in at least 10 separate and randomly selected fields using x 10 objectives lens microscope to ensure uniform distribution of spermatozoa. The results obtained were written as percentage motility. The counting was repeated thrice to minimize standard error for each sample.

$$\text{Percentage of mobile spermatozoa} = \frac{\text{number of mobile spermatozoa}}{\text{Total number of counted spermatozoa}} \times 100 \%$$

Sperm Morphology: The sperm suspension was dropped and smeared onto a slide to obtain a uniform smear. After smearing, they were dried under air and stained with Liu's stain (Handsel Technologies Inc, Taipei, Taiwan) the slides were washed in water. While it was still wet, it was fixed with 95 % v/v ethanol for 5-10 minutes and allowed to air dry. It was then washed with sodium bicarbonate formalin solution to remove mucus. The smear was then rinsed with several changes of water and covered with dilute (1 in 20) carbon fuschin and allowed to stain for 3 minutes.

The stain was washed off using water and counter stain with 1 in 20 loffler's methylene blue for 2 minutes. The stain was then washed off in water and allowed to air dry. And then observed under a microscope for changes

in sperm morphology according to [7]. The criteria chosen for head abnormality were no hook, excessive hook, amorphous, pin, short head, big head and banana head. Coiled flagellum, bent flagellum tip were recorded as abnormal tailed sperm while those that have oval head with long tail were regarded as normal sperm. The results were reported in percentage i.e. the overall abnormal form.

RESULTS

Effect of Aqueous and Ethylacetate Extracts on Reproductive Organ: The results of the sperm analysis are illustrated with Figure 3 and 4. The results of the sperm analysis on rats treated with aqueous and

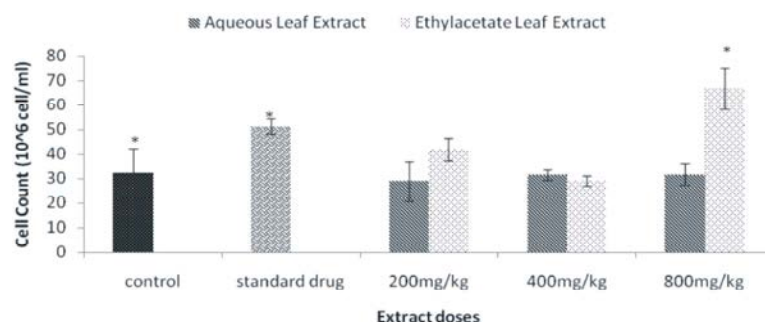


Fig. 1: Results of the Effect of Aqueous and Ethylacetate Extracts on Male Sperm Cell Count in Albino Rats.

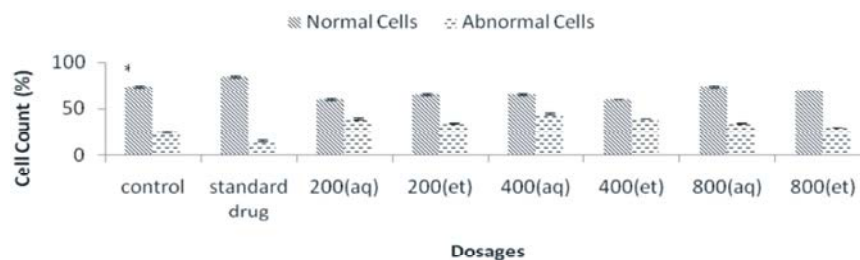


Fig. 2: Results of the effect of Aqueous and Ethylacetate Extracts showing the % difference between Normal Sperm Cells and Abnormal Sperm Cells in Albino Rats.

* Implies Significant Difference ($P < 0.05$) between Normal and Abnormal Sperm Cells. Plotted values are mean of three determination \pm SD.

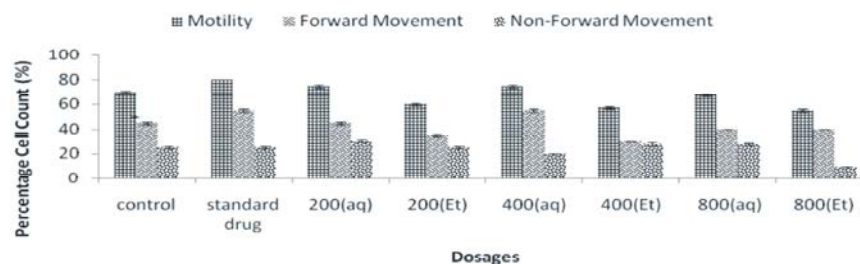


Fig. 3: Results of the Effect of Aqueous and Ethylacetate Extracts on Percentage Motility of Sperm Cells in Albino Rats.

* Implies Significant Difference ($P < 0.05$) between % Forward Movement and % Non-forward Movement as Compared with the Control. Plotted values are mean of three Determination \pm SD.

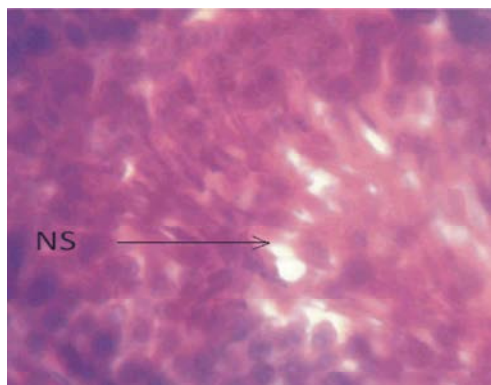


Plate 1: Photomicrograph of Control Testes of Albino rat Showing Normal Spermatogenesis (NS) Developing to Spermatozoa Through the Intermediate Stages from the Spermatogonium (H and E; x 600).

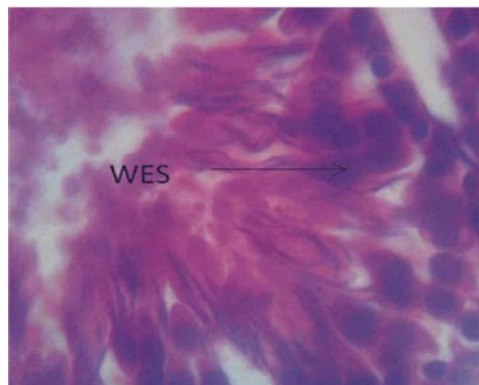


Plate 4: Photomicrograph of Rat Testis Treated with 800 mg/kg of Ethylacetate Extract Showing Well Enhanced Spermatogenesis (WES) as evidence of many Spermatozoa. This is Comparable to the Standard Drug or even better and is more enhanced than the Control.

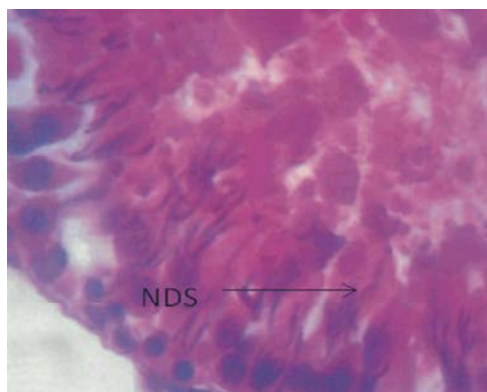


Plate 2: Photomicrograph of the Testis of Albino Rats treated with the Standard Drug Showing Normal Development of Spermatozoa (NDS) with Intermediate Stages Noted. (H and E x600).

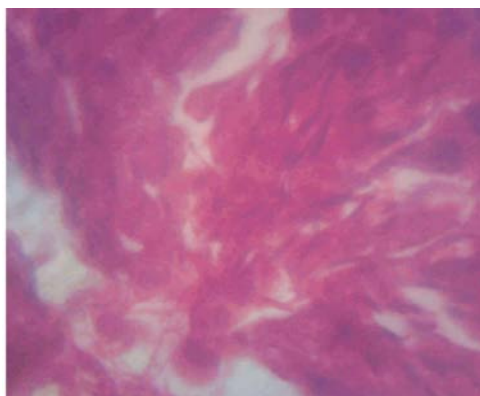


Plate 3: Photomicrograph of Albino Rat Testis treated with Aqueous Extract 800 mg/kg Showing Normal Spermatogenesis(S) but not Enhanced. (H and E x600).

ethylacetate extracts showed that in the sperm cell count, there was a significant increase ($P < 0.05$) in the cell counts of those administered ethylacetate extract highest dose as compared to the control group and standard drug. The results in Figure 5 also show a significant difference ($P < 0.05$) between the percentages (%) of normal sperm cells to that of abnormal sperm cells.

The results of the effect of the extracts on percentage (%) motility of the sperm showed that there was a significant difference ($P < 0.05$) between the % forward movement and none forward movement.

DISCUSSION

The medicinal plant *Alchornea cordifolia* studied in this work is a rich source of biologically important elements and may play a part in enhancing fertility. The result of the analysis showed that the extracts contained appreciable amount of important nutrient within the acceptable WHO limits [8]. These vitamins and minerals are important because they are required in the generation of DNA and RNA genetic materials, not just for the ovum but also for the spermatozoa preventing spina bifida in the new born [9].

Interestingly, several studies by [10], have also shown that reduction of zinc in the diet for men can also reduce their sperm count. Zinc is also an important constituent of the genetic makeup and a zinc deficiency is capable of causing chromosome changes which can lead to decreased fertility and a high risk of miscarriages. Men undergoing invitro fertilization treatment with their

spouses have been prescribed vitamin E supplements and pregnancy rates have, as a result, increased from 19 to 29 percentage.

In male rats, the number of sperm in the caudal epidemis was significantly increased $P < 0.05$ in rats treated with both extracts at different doses as compared to both normal and positive controls. Progressively, sperm motility increased significantly ($p < 0.05$) in the administered groups as compared with the control. Sperm morphological examination in the study revealed significant increase in the number of normal sperm. Saradha and Mathur, (2006), [11], indicated that an increased density of epididymal sperm could be correlated with testicular spermatogenic enhancement. This result is in accordance with [12], which opined that it is possible for an extract to mediate its effect by maintaining a proper balance in the serum levels of fertility hormones. This shows that the extracts at high dose act as an aphrodisiac substances that can increase male libido [13]. A corresponding increase was seen in the histology analysis. The control at magnification x600 showed normal spermatogenesis developing from the spermatogonium to spermatozoa through the intermediate stages with normal seminiferous tubules. The photomicrograph of the testis given proviron a standard drug for treatment of male hormonal imbalance also showed normal spermatozoa with increased spermatogenesis.

There was an increased number of seminiferous tubules in the aqueous leaf extract at 800 mg/kg at magnification X600 with spermatogenesis ongoing but not enhanced as compared to ethylacetate extract at 800mg/kg at magnification X600 with enhanced spermatogenesis. This is comparable to the standard drug (positive control) or even better and is more enhanced than normal control.

REFERENCES

1. Sleator, R.D., 2012. "Prediction of Protein Functions". *Methods in Molecular Biology*. *Methods in Molecular Biology*, 815: 15-24.
2. DeLamater, J.D. and M. Sill, 2005. "Sexual Desire in Later Life". *The Journal of Sex Research*, 42(2): 138-149.
3. Harding, S.M. and J.P. Velotta, 2011. "Comparing the Relative amount of Testosterone required to restore Sexual Arousal, Motivation and Performance in Male rats". *Journal of Hormonal Behaviour*, 59(5): 666-73.
4. Glenville, M., 2012. How to Improve your Fertility. http://www.marilynglenville.com/the_Foundation_of_Health.
5. Agarwal, A. and R.A. Saleh, 2002. Role of Oxidative Stress in Male Infertility: Rational, Significance and Treatment. *International Journal on Clinical Urology*, 29(4): 817-827.
6. Saradha, B. and P.P. Mathur, 2006. Induction of Oxidative Stress by Lindane in Epididymis of Adult Male Rats. *Journal of Environmental Toxicology and Pharmacology*, 22(8): 90-96.
7. Herson, P.S., I.P. Koerner and P.D. Hurn, 2009. Sex, Sex Steroids and Brain Injury. *Journal of Reproductive Medicine*, 27(3): 229-39.
8. Bassil, N., S. Alkaade and J.E. Morley, 2009. "The Benefits and Risks of Testosterone Replacement Therapy: A Review". *Journal of Therapeutic Clinical Risk Management*, 5(3): 427-448.
9. Basaria, S., A.D. Coviello, T.G. Travison, T.W. Storer, W.R. Farwell, A.M. Jette, R. Eder, S. Tennstedt, J. Ullor, A. Zhang, K. Choong, K.M. Lakshman, N.A. Mazer and R. Miciek, 2010. Adverse Events Associated with Testosterone Administration. *New England Journal of Medicine*, 363(2): 109-22.
10. Fishelson, L., O. Gon, V. Holdengreber and Y. Delarea, 2007. "Comparative Spermatogenesis, Spermatocytogenesis and Spermatozeugmata Formation in Males of Viviparous Species of Clinid Fishes (Teleostei: Clinidae, Blennioidei)". *The Anatomical Record: Advances in Integrative Anatomy and Evolutionary Biology*, 290(3): 311-23.
11. James, P.J., J.G. Nyby and G.A. Saviolakis, 2006. "Sexually Stimulated Testosterone Release in Male Mice (*Mus musculus*): Roles of Genotype and Sexual Arousal". *Journal of Hormonal Behaviour*, 50(3): 424-31.
12. Jones, T.H. and F. Saad, 2009. "The Effects of Testosterone on Risk Factors for and the Mediators of the Atherosclerotic Process". *Atherosclerosis*, 207(2): 318-27.
13. Neelesh, M., J. Sanjay, B.G. Vipin and V. Savita, 2011. Recent Studies on Aphrodisiac Herbs for the Management of Male Sexual Dysfunction. *Journal of Drug Research*, 68(1): 3-8.