American Journal of Medical and Biological Research, 2015, Vol. 3, No. 5, 124-127
Available online at http://pubs.sciepub.com/ajmbr/3/5/1
© Science and Education Publishing
DOI:10.12691/ajmbr-3-5-1

Cissus populnea (Guill & Perr): A Study of the Aqueous Extract as Potential Spermatogenic Enhancers in Male Wistar Rats

Anthony B. Ojekale[1,*], Oladipupo A. Lawal[2], Peter I. Jewo[3], Jamiu. A. Oguntola[1], Latifat O. Abdul[1]

[1]Department of Biochemistry, Lagos State University, PMB 0001 LASU Post Office, Ojo, Lagos, Nigeria
[2]Department of Chemistry, Lagos State University, PMB 0001 LASU Post Office, Ojo, Lagos, Nigeria
[3]Department of Anatomy, Lagos State University College of Medicine, P.M.B. 21266, Ikeja, Lagos, Nigeria
*Corresponding author: anthony.ojekale@lasu.edu.ng

Received April 29, 2015; Revised June 16, 2015; Accepted June 28, 2015

Abstract Background: There is a global observed decline in male fertility over the years consequent on various factors affecting spermatogenesis. Some Nigerian males across various geographic and socio-economic strata employ the use of herbals such as *Cissus populnea* extracts amongst others to manage this and also as herbal aphrodisiacs. This study assessed the spermatogenic effects of aqueous extract of *Cissus populnea* stem bark. **Methodology**: *Cissus populnea* stem bark was extracted with water, concentrated and lyophilized. Male wistar rats were orally administered with 150 mg/kg body weight of aqueous extract (*Cissus populnea*) over a 64 day period. Sperm of the experimental animals was collected, and the parameters (count, motility, morphology) analysed. Data were analysed with SPSS 19. **Principal findings**: The oral administration of *C. populnea* extract over a 64 day period to male wistar rats resulted in a four-fold increase in sperm count in the test rats (145±55) compared with the control group (44±17). Testicular histology shows better packed spermatozoa in group of rats treated with *Cissus populnea*. **Conclusion/significance**: The findings suggest that oral administration of *Cissus populnea* aqueous extract improves spermatogenesis in male wistar rats.

Keywords: *Cissus populnea, Vitaceae, aqueous extract, spermatogenesis, wistar albino rats, male fertility*

Cite This Article: Anthony B. Ojekale, Oladipupo A. Lawal, Peter I. Jewo, Jamiu. A. Oguntola, and Latifat O. Abdul, "*Cissus populnea* (Guill & Perr): A Study of the Aqueous Extract as Potential Spermatogenic Enhancers in Male Wistar Rats." *American Journal of Medical and Biological Research*, vol. 3, no. 5 (2015): 124-127. doi: 10.12691/ajmbr-3-5-1.

1. Introduction

Man since early times had always wanted to leave progenies behind as a means of continuity of his lineage and proof of his procreative ability[1]. Both the ability to engage in sexual trysts and consequently impregnating females culturally leaves a man with a feeling of well being and massages (boosts) his ego. The inability of a man to either have full penetrative sex or impregnate the

opposite gender due to erectile dysfunction or infertility is becoming quite a common feature, and has been on the increase for some time now [2-4]. There is growing evidence to support global declining fertility in males viz sperm concentration, motility and morphologically normal [5,6,7,8,9]. Various reasons including but not limited to .age[10], environment [11], exposure to/ use of GSM devices [12], disease conditions [13,14], and others are adduced for this decline. Tangential to decreasing male fertility is a concomitant drop in libido which is mostly but not all times age related [15]. Other reasons such as oxidative stress [16], weight, disease conditions, and lifestyle are some of the establisehd factors that affect libido in men.

There are quite a number of herbals presently available and commercially sold in the public domain in Nigeria which the manufacturers claim to enhance both libido and spermatogenesis.

Plants like Yohimbine [17,18], *Lepidium meyenii* [19], *Cochlospermum planchonii* [20] and Ginseng [21,22] have been reported to be of use as aphrodisiacs and spermatogenesis enhancers.Due to multifaceted reasons such as cost, scarcity, adulteration etc, quite a number of people now patronize and use herbal remedies as therapy for ailments in most parts of Nigeria [23] and indeed other parts of the world at large [24]. Depending on region, there exists a legion [25,26,27] of herbal remedies being marketed as spermatogenesis enhancers and aphrodisiacs. A popular herbal preparation among these aphrodisiacs in the South Western part of Nigeria is the aqueous extract of *Cissus populnea* stem bark [2,28].

The plant *Cissus populnea* Guill & Perr (Vitaceae) has been reported to possess numerous biological activities [2,29-34]. The plant is also used in the treatment of sore breast, indigestion, veneral diseases, intestinal parasites,

oedema and eye problems. Previous phytochemical studies on different parts of *C. populnea* reported the presence of saponins, tannins, anthraquinones, cyanogenic glycoside, flavonoids, carotenoids, triterpenoids, and ascorbic acid [2,29,35,36]. *C. populnea* aqueous stem bark extract have been reportedly used as aphrodisiac/fertility enhancer for males among the Yoruba in South Western part of Nigeria [2], and various possible scientific reasons adduced for these have also been reported [3,37,38]. In continuation of our studies on the aqueous extract of *C. populnea* stem bark [2,28], this present in vestigation is aimed at unraveling the spermatogenic potentials of *C. populnea* stem bark.

2. Materials and Methods

2.1. Sample Collection/extraction

The stem bark of *Cissus populnea* was purchased from a traditional herbal practitioner in Lagos state and authenticated at the herbarium of the University of Lagos. The extraction was carried out as earlier reported [2]. The extract was lyophilized and the resultant powder stored in an air tight container at 4°C until needed. The lyophilized extract was reconstituted with distilled water for oral administration to the experimental animals [2].

2.2. Experimental Animals

Adult male Wistar rats weighing between 120 - 160 g were obtained from the Department of Anatomy, Lagos State University College of Medicine, Ikeja, Lagos, Nigeria and used for this study. The animals were housed in well ventilated cages and maintained in the animal house of the Department of Biochemistry, Lagos State University, Ojo, Lagos for seven (7) days before the commencement of the drug treatment. The animals were fed with commercial rat feed (Nemeith livestock feeds Ltd. Nigeria) and water *ad libitum* under normal laboratory environmental conditions. The study protocol was approved by the Institutional Ethical Committee. The oral administration regime followed our earlier reported dosage [2].

2.3. Animals

The procured Wistar rats were housed in well ventilated cages, kept in the University animal house and allowed to acclimatize in their new environment for 7 days. The animals were fed daily with commercial rat feed and water *ad libitum*.

The animals were grouped thus:

Group I: Animals orally administered with *Cissus populnea* extracts daily for 64 days

Group II: Animals administered with distilled water daily for 64 days.

Group III: Animals administered with Addyzoa (Commercially available polyherbal formulation).

2.4. Blood and Organs Collection

The animals were sacrificed at the end of 64 days under ketamine anesthesia. Their testes and accessory sex organs were carefully dissected out, trimmed of fat and weighed. The epididymis was used for estimation of sperm parameters. The testis was fixed in 10% formol saline solution and processed for histology.

2.5. Determination of Epidemical Sperm Parameters

Sperm density was determined by modifying a method previously described [25]. Briefly, the caudal epididymis was dissected out, several incisions were made in it, and it was suspended in 1ml of buffered physiological saline. The preparation was allowed to stand for 10 minutes in an incubator at 37°C to allow sperm swim up. One drop from this preparation was placed on a heated slide for motility estimation. The slides were examined using a CETI (UK), phase contrast light microscope at magnifications from 100 to 400. The microscopic field was scanned systematically, and spermatozoa encountered were assessed as motile or non-motile. An estimate of the percentage of motile sperm was made [44]. Sperm count was determined using the improved Neubauer haemocytometer. A dilution ratio of 1:20 from each well-mixed sample was prepared by diluting 50µl of epididymal spermatozoa suspended in physiological saline with 950µl of diluents. The diluent was made by adding drops of 10 % formalin to the buffered saline to immobilize sperm. The counting chamber was charged from this suspension carefully to avoid sperm accumulating in its trough. Sperm morphology was assessed from slides prepared with one drop from the original sperm suspension. The slides were air-dried, stained with heamatoxylin for 2minutes, washed and dried again. They were examined in dark field microscopy for abnormalities in head, neck, mid-piece or tail regions.

2.6. Histology

This followed a procedure as described [45]. Briefly, the testes was cut into 0.5 cm thick slabs and fixed in Bouin's fluid for 72 hours. They were then passed through graded alcohol, cleared in xylene, embedded in molten paraffin and blocked out. Serial sections of 5µm thick were cut from these blocks and stained with haematoxylin and eosin stains. They were examined under light microscopes (CETI, UK) at magnifications ranging from 400 to 1000.

3. Results and Discussion

3.1. Sperm Parameters

The administration of aqueous extract of *C. populnea* caused elevation in all sperm parameters (sperm motility, morphology and total count) assessed in this study. The elevation in motility and proportion of normal sperm cells was not significant. The extract however caused a four-fold increase in total sperm count; (144.75 ± 55.35) for *C. populnea* treated group compared to (43.75 ± 17.09) and (45.7 ± 13.280 for water and Addyzoa treated groups, respectively (Table 1). There was also an elevation in absolute weight of the testis and prostate in both *C. populnea and* Addyzoa treated groups when compared to water-treated control group (Table 2).

Table 1. Sperm analysis

Sperm parameters	Control	*C. populnea*	Addyzoa
Motility (%)	8.75 ± 4.75	28.75 ± 5.82	13.75 ± 4.43
Morphology (%)	normal: 70 ± 7.5 abnormal: 30 ± 7.5	normal: 77.5 ± 4.663 abnormal: 22.5 ± 4.63	normal: 77.5 ± 4.66 abnormal: 22.5 ± 4.63
Total count ($x10^6$)	19.75 ± 12.70	144.75 ± 55.35	45.7 ± 13.28

Values expressed as mean ± SEM, n =5
*p<0.05 vs control.

Table 2. Organ weights

Organ weights	Control	*C. populnea*	Addyzoa
Testis	left:0.94 ± 0.17 right:0.98 ± 0.17	left:1.27 ± 0.10 right:1.21 ± 0.13	Left: 1.16 ± 0.03 Right: 1.14 ± 0.01
Epididymis	left:0.15 ± 0.05 right:0.21± 0.08	left: 0.22 ± 0.03 right: 0.21 ± 0.03	left: 0.23 ± 0.02 right: 0.22 ± 0.03
Prostate	0.25 ± 0.03	0.33 ± 0.11	0.32 ± 0.11

Values expressed as mean ± SEM, n =5
*p<0.05 vs control.

3.2. Histology

Testicular histology was well preserved in all animal groups in this study. Tubules were better packed with mature spermatozoa in group 2 (animals administered with *C. populnea*) than in the other two groups (Figure 1 and Figure 2). Only a small proportion of tubules showed germ cell loss especially in the ad-luminal areas. Interstitium was also normal in all experimental groups with normal Leydig cells visible in many areas (Figure 3).

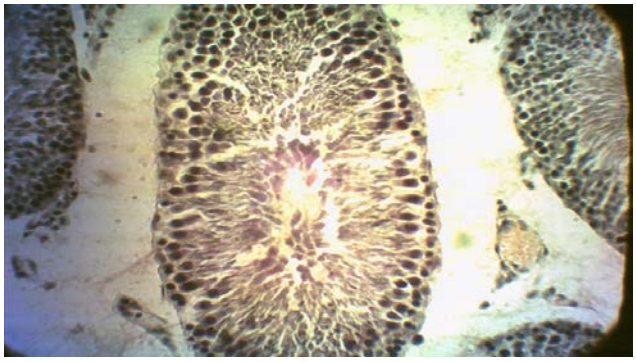

Figure 1. Photomicrograph of cross section of testis from control showing normal tubular outline and complement of germ cells. H&E ×400

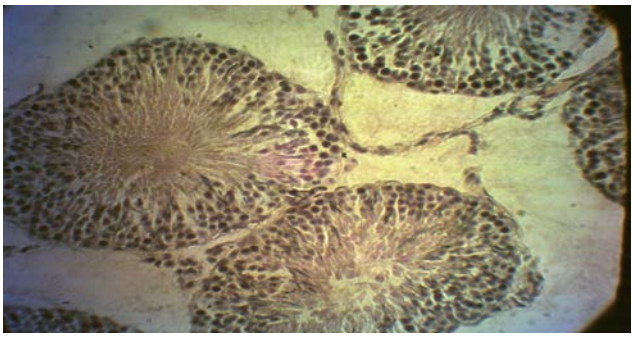

Figure 2. Photomicrograph of cross section of testis from *C. populnea* treated rat showing normal tubular outline and complement of germ cells with adluminal areas packed with mature spermatozoa. H & E x 400

In this study, we examined the effects of aqueous extract of *C. populnea*, an herbal preparation used widely in some parts of Africa as an aphrodisiac and pro-fertility agent on sperm parameters and histology of the testes in albino rats. The effects were also compared with *Addyzoa* (a commercially purchased fertility enhancing polyherbal formulation). The oral administration of *C. populnea* aqueous extract over a 64 day period caused a significant increase in all sperm parameters especially in sperm density, where there was an observed four-fold increase compared to control. This finding corroborates those from a number of previous investigators [29,37,39]. The plant extract reportedly ameliorated testicular damage induced by flutamide in pre-pubertal rats [40]. The plant has also been reported to increase cell proliferation in Sertoli cell lines *in vitro* [37]. Sertoli cells play critical roles in spermatogenesis, and this may well be a key pathway for the pro-fertility effects of the plant extracts. Much of the effects of FSH on sperm production are exerted through actions on Sertoli cells. Sertoli cells support all cells of the germ series as they pass through the various stages of development from primordial spermatogonia to fully differentiated free spermatozoa. They are the only cells that traverse the blood brain barrier in the seminiferous epithelium, and it is believed that their number determines to a large extent the rate of spermatogenesis [41,42]. Spermatogenesis is adversely affected by oxidative stress, consequent upon an excessive production of reactive Oxygen species (ROS), or impairment in the antioxidant defense mechanisms [43]. The spermatogenic effect of *C. populnea* extract observed in this study is likely due to the presence of secondary metabolites (phenolics) like glycosides and saponins present [2,35] that exert their pharmacological effects as antioxidants by mopping up ROS to minimize their damaging effect on sperm cells and associated tissues like testes [29]. The presence of high concentrations of vitamin C and Zn in *C. populnea* as reported [3] corroborates the enhanced sperm formation in this study, as both levels of Zn and Vitamin C are correlated in male fertility.

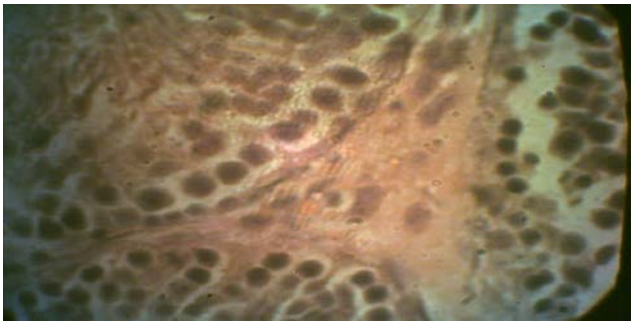

Figure 3. Photomicrograph of cross section of testis from *C. populnea* treated rat showing normal arrangement of germ cells. There is however slight sloughing of cells in the basal area of the tubule on the left of this field. Interstitium and visible Leydig cells are normal. H & E ×1000

The folkloric use of the aqueous stem bark extract of *C. populnea* by the indigenous males in some parts of Nigeria is justifiable with the increment in the observed sperm count of the experimental rats in this study.

References

[1] Ayala, F.J., A Synopsis. In Handbook of Evolution, Vol. 2: The Evolution of Living Systems (Including Hominids) ed. F.M.W.a.F.J. Ayala. Vol. 2. 2005, KGaA, Weinheim: Wiley-VCH Verlag GmbH & Co.

[2] Ojekale, A.B., et al., Phytochemisty and spermatogenic potentials of aqueous extract of Cissus populnea (Guill. and Per) stem bark. ScientificWorldJournal, 2006. 6: p. 2140-6.

[3] Ogunlesi, M., et al., Determination of the concentrations of zinc and vitamin C in oysters and some medicinal plants used to correct male factor infertility. Journal of Natural Products, 2009. 2: p. 89-97.

[4] Report., J., Report of optimal evaluation of the infertile man. Fertil Steril., 2004. 82(1): p.:123-130.

[5] Auger, J., et al., Decline in semen quality among fertile men in Paris during the past 20 years. New England Journal of Medicine, 1995. 332(5): p. 281-285.

[6] Bostofte, E., J. Serup, and H. Rebbe, Has the fertility of Danish men declined through the years in terms of semen quality? A comparison of semen qualities between 1952 and 1972. International journal of fertility, 1982. 28(2): p. 91-95.

[7] Swan, S.H., E.P. Elkin, and L. Fenster, Have sperm densities declined? A reanalysis of global trend data. Environmental health perspectives, 1997. 105(11): p. 1228.

[8] Osser, S., P. Liedholm, and J. Ranstam, Depressed semen quality: a study over two decades. Systems Biology in Reproductive Medicine, 1984. 12(1): p. 113-116.

[9] Irvine, S., et al., Evidence of deteriorating semen quality in the United Kingdom: birth cohort study in 577 men in Scotland over 11 years. Bmj, 1996. 312(7029): p. 467-471.

[10] Plas, E., et al., Effects of aging on male fertility? Experimental Gerontology, 2000. 35(5): p. 543-551.

[11] Petrelli, G. and I. Figa-Talamanca, Reduction in fertility in male greenhouse workers exposed to pesticides. European journal of epidemiology, 2001. 17(7): p. 675-677.

[12] Wdowiak, A., L. Wdowiak, and H. Wiktor, Evaluation of the effect of using mobile phones on male fertility. Annals of Agricultural and Environmental Medicine, 2007. 14(1): p. 169-172.

[13] Carlsen, E., et al., Declining semen quality and increasing incidence of testicular cancer: Is there a common cause? Environmental health perspectives, 1995. 103(Suppl 7): p. 137.

[14] Ochsendorf, F., Sexually transmitted infections: impact on male fertility. Andrologia, 2008. 40(2): p. 72-75.

[15] Kaufman, J.M. and A. Vermeulen, The decline of androgen levels in elderly men and its clinical and therapeutic implications. Endocrine reviews, 2005. 26(6): p. 833-876.

[16] Sikka, S.C., Relative impact of oxidative stress on male reproductive function. Current medicinal chemistry, 2001. 8(7): p. 851-862.

[17] Riley, A., Yohimbine in the treatment of erectile disorder. The British journal of clinical practice, 1993. 48(3): p. 133-136.

[18] Morales, A., Yohimbine in erectile dysfunction: the facts. International journal of impotence research, 2000. 12: p. S70-S74.

[19] Gonzales, G., et al., Effect of Lepidium meyenii (Maca) on spermatogenesis in male rats acutely exposed to high altitude (4340 m). Journal of Endocrinology, 2004. 180(1): p. 87-95.

[20] Abu, A., D. Ochalefu, and A. Ibrahim, Aqueous ethanolic extract of Cochlospermum planchonii rhizome enhances spermatogenesis in male albino rats. African Journal of Biotechnology, 2012. 11(53): p. 11636-11639.

[21] Nocerino, E., M. Amato, and A.A. Izzo, The aphrodisiac and adaptogenic properties of ginseng. Fitoterapia, 2000. 71: p. S1-S5.

[22] Hong, B., et al., A double-blind crossover study evaluating the efficacy of Korean red ginseng in patients with erectile dysfunction: a preliminary report. The Journal of urology, 2002. 168(5): p. 2070-2073.

[23] Brown, D., Herbs from the African rainforest: living & working in Yorubaland, SW Nigeria, I.i.o.T. Agriculture, Editor. 2013: Oyo, Nigeria.

[24] Smith, J.F., et al., The use of complementary and alternative fertility treatment in couples seeking fertility care: data from a prospective cohort in the United States. Fertility and sterility, 2010. 93(7): p. 2169-2174.

[25] Aromatics, A. Afrodisiacs – African Aphrodisiacs. 2014 [cited 2014 18 June]; Available from: http://africanaromatics.com/afrodisiacs-african-aphrodisiacs/.

[26] Odesanmi, O.S., et al., Nutritional prospect of an aphrodisiac Microdermis keayana. Journal of Medicinal Plants Research, 2012. 6(7): p. 1187-1190.

[27] Agunbiade, O.M. and T. Ayotunde, Ageing, sexuality and enhancement among Yoruba people in south western Nigeria. Culture, health & sexuality, 2012. 14(6): p. 705-717.

[28] Ojekale, A., et al., Long term effects of aqueous stem bark extract of Cissus populnea (Guill. and Per.) on some biochemical parameters in normal rabbits. African Journal of Biotechnology, 2007. 6(3).

[29] Akomolafe, S.F., et al., Inhibitory Effect of Aqueous Extract of Stem Bark of Cissus populnea on Ferrous Sulphate- and Sodium Nitroprusside-Induced Oxidative Stress in Rat's Testes In Vitro. ISRN Pharmacol, 2013. 2013: p. 130989.

[30] Kone, W., et al., Traditional medicine in North Côte-d'Ivoire: screening of 50 medicinal plants for antibacterial activity. Journal of ethnopharmacology, 2004. 93(1): p. 43-49.

[31] Geidam, M., G. Adoga, and F. Sanda, Effects of aqueous stem bark extract of Cissus populnea on some serum enzymes in normal and alloxan induced diabetic rats. Pakistan Journal of Biological Sciences, 2004. 7(8): p. 1427-1429.

[32] 32. Moody, J., et al., Anti-sickling potential of a Nigerian herbal formula (ajawaron HF) and the major plant component (Cissus populnea L. CPK). Phytotherapy Research, 2003. 17(10): p. 1173-1176.

[33] Atawodi, S., et al., Indigenous knowledge system for treatment of trypanosomiasis in Kaduna state of Nigeria. Journal of Ethnopharmacology, 2002. 79(2): p. 279-282.

[34] Uchendu, C., O. Ojo, and M.I. Choudhary, Effects of methanolic root extract of Cissus populnea on isolated uterine muscle of the rat. Journal of Tropical Medicinal Plants, 2009. 10(2).

[35] Soladoye, M. and E. Chukwuma, Quantitative phytochemical profile of the leaves of Cissus populnea Guill. & Perr.(Vitaceae)-an important medicinal plant in central Nigeria. Archives of Applied Science Research, 2012. 4(1).

[36] Ibrahim, H., et al., Anthraquinones of Cissus Populnea Guill &Perr (Amplidaceae). African Journal of Traditional, Complementary and Alternative Medicines, 2011. 8(2).

[37] Osibote, E., et al., Electrochemical sensors, MTT and immunofluorescence assays for monitoring the proliferation effects of cissus populnea extracts on Sertoli cells. Reprod Biol Endocrinol, 2011. 9: p. 65.

[38] Smith, H., et al., Effect of Cissus populnea on gonads, accessory organs and on carbon terachloride induced rate liver peroxidation. Nigerian Quarterly Journal of Hospital Medicine, 2004. 10(1): p. 59-62.

[39] 39. Smith, H. and O. Ogunfeibo, Effects Of Cissus Populnea (Ogbolo) On Mainstream Smoked Weaned Rabbit. Nigerian Quarterly Journal of Hospital Medicine, 2008. 14(2): p. 195-198.

[40] Oremosu, A., et al., Effects of Cissus populnea and Panax ginseng on Flutamide-Induced Testicular Defect in Pre-Pubertal Male Rats. British Journal of Medicine & Medical Research, 2013. 3(1): p. 173-181.

[41] Silber, S.J., Microsurgical TESE and the distribution of spermatogenesis in non-obstructive azoospermia. Human Reproduction, 2000. 15(11): p. 2278-2284.

[42] Orth, J.M. and M.P. McGuinness, Development of postnatal gonocytes in vivo and in vitro, in Function of somatic cells in the testis. 1994, Springer. p. 321-344.

[43] Muanya, C. and O. Odukoya, Lipid Peroxidation as Index of Activity in Aphrodisiac Herbs. Journal of Plant Sciences, 2008. 3(1).

[44] Sönmez, M., G. Türk, and A. Yüce, The effect of ascorbic acid supplementation on sperm quality, lipid peroxidation and testosterone levels of male Wistar rats. Theriogenology, 2005. 63(7): p. 2063-2072.

[45] Sheehan DC, H.B., Enterline HT, Theory and Practice of Histotechnology. 2nd ed. 1987, Columbus, OH: Battelle Press.