NISEB Journal Vol. 14, No. 2, June, 2014
Printed in Nigeria

1595-6938/2014
(2014) Nigerian Society for Experimental Biology
http://www.nisebjournal.org

Effect of Ethanolic Root Extract of *Cissus populnea* on Testicular Antioxidant Status, Fertility and Some Sexual Performance Parameters in Rats

Okolie, N.P.,[†] *Ogunsemoyin, I. and Uanseoje, S.*
Department of Biochemistry, Faculty of Life Sciences,
University of Benin,PMB 1154, Benin City. Nigeria

Abstract
The study was carried out to investigate the effect of ethanolic root extract of Cissus populnea (ERCP) on some sexual performance parameters in rats, to see if there are any merits in its ethno-medicinal use as an aphrodisiac. One group of 6 sexually-mature male rats was given by gastric intubation, the equivalent of 270mg of extract/kg body weight/day for 21 days, while another group received vehicle, and served as control. Both groups were maintained ad libitum on normal rat feed and drinking water. At the end of 21 days, each male rat was paired with 3 sexually-mature, oestrous female rats and assessed for mounting frequency, intromission time, ejaculation frequency and fertility (% of pregnant females/group). The male rats were subsequently sacrificed under chloroform anaesthesia, and their testes were weighed and analysed for total protein, antioxidant vitamins and some antioxidant enzymes. Results obtained revealed that the extract treatment brought about significant increases in mounting frequency and ejaculation latency, while significantly reducing intromission latency ($p<0.05$). In addition, the antioxidant vitamins ß-carotene, ascorbic acid and α-tocopherol, as well as the activities of superoxide dismutase, SOD and catalase were significantly elevated in the testes of the extract-treated rats when compared to controls ($p<0.05$). The ERCP-treated rats also had significantly higher testicular weight, higher testicular total protein and higher fertility than controls ($p<0.05$). These results strongly suggest that ERCP possesses significant testicular antioxidant-enhancing, libido-promoting and fertility-boosting properties, which may account at least in part, for its aphrodisiac use in ethno-medical practice.

Keywords: *Cissus populnea*; Testicular Antioxidants; Sexual Performance, Fertility.

Introduction

Erectile dysfunction (ED) is the most common type of sexual dysfunction in men. It refers to persistent inability to maintain a penile erection strong enough to permit copulation (1, 2)**.** Several predisposing factors have been associated with ED. These include advancing age, stress and ill-health. Treatment strategies depend on the underlying cause, and may involve hormonal, chemical and surgical interventions. The discovery of sildenafil (Viagra), vardenafil and tadalafil has revolutionalised the treatment of ED so much that they are considered as primary drugs of choice (3). These drugs are type5 inhibitors of phosphodiesterase, an enzyme which degrades cGMP whose presence is necessary for maintenance of penile tumescence. Thus they potentiate erection by sustaining circulatory levels of cGMP generated by the nitric oxide-induced stimulation of adenylate cyclase. However the use of these drugs is often associated with some undesirable side effects such as headache, nasal congestion, flushing, upset stomach and visual impairment (4, 5). Other important constraints include the issue of affordability/cost of treatment especially amongst low income earners, and the fact that Viagra^R and its generics are contra-indicated in men with a history of hypertension or cardiovascular disease. The side effects and limitations of these orthodox treatments have tended to generate increasing interest in herbal alternative remedies, which being natural, are safer, cheaper and associated with minimal side effects. Indeed herbal preparations occupy a very important place in the treatment of a variety of diseases in the developing and some developed countries (6).

Cissus populnea Guill & Perr is a woody semi-climber which belongs to the family *Amplidaceae* (*Vitaceae*). It is common in the savannah areas of Africa, where it may attain a height up to 3 meters. All parts of the plant produce a mucilaginous exudate, which is exploited in some Nigerian homes for thickening of soups and sauces (7). In Ondo State, South-Western Nigeria, aqueous and ethanolic root extracts of the plant are used for the treatment of erectile dysfunction, and for boosting fertility and sexual performance. To date, no investigations have been carried out to ascertain the veracity of androgenic properties associated with root extracts of the plant. The present study is aimed at determining the sexual performance and fertility-enhancing effects of ethanolic root extract of *Cissus populnea* in rats.

[†]*Corresponding Author: E-mail: ajino@uniben.edu Tel.: +2347031677944*

MATERIALS AND METHODS

Preparation Cissum populnea root extract

Fresh roots of *Cissus populnea* were obtained from a local herb vendor in Igbokoda village, Ilaje Local Government area, Ondo State. The roots were extracted by the method of Sofowora (8). The samples were washed, peeled and sliced to thin pieces using a clean knife edge. 50g of the sliced roots were transferred to a 250-ml beaker containing 150 ml of ethanol diluted 1:5 (v: v) with water. The beaker and contents were allowed to stand at room temperature (about 27^{o}C) for 48 hrs for optimum extract yield. After decanting the first extract, the roots were re-suspended in 75 ml of the solvent for a further 24 hrs, after which the two extracts were pooled. To determine the concentration of the extract, 1ml was evaporated to dryness in a pre-weighed glass dish, and the mass of the resultant residue was obtained by difference. The extract was stored refrigerated in a sealed glass flask and used within a few days.

Experimental Animals and treatment

Twelve sexually-mature male albino rats were obtained from the Faculty of Pharmacy, University of Benin. The animals were housed in pairs in clean metal cages and acclimatized on grower's mash (Guinea Feeds, Benin City) for 2 weeks prior to commencement of the experiment. The rats were subsequently weighed and assigned to two groups (6rats/group). One group received by gastric intubation, 270mg extract/kg body weight/day for 21 days, while members of the second group received an equivalent volume of vehicle (solvent) for the same period, and served as control. The two groups had access to feed and drinking water *ad libitum*, and were weighed weekly for adjustments in treatments. The study was carried out in strict compliance with the ethics in Guidelines and Specifications on Experimental Animal Care (9).

Assessment of sexual performance parameters

At the end of 21 days, each male rat was matched with 3 sexually-mature, estrous female rats in a separate cage. Prior to this, each female rat was made receptive (estrous) by subcutaneous injection of 10µg estradiol in peanut oil 48 hrs before subcutaneous injection of 500µg of progesterone. The sexual matching was started 8 hrs after the progesterone injection, using the most receptive females. The male rats were carefully monitored for mounting frequency, intromission latency and ejaculation latency. The number of females that mated in each group was recorded, and these were subsequently monitored for signs of pregnancy.

After the sexual experiment, the male rats were sacrificed under chloroform anaesthesia, and their testes were dissected out, weighed and rinsed in ice-cold physiological saline. Tissue homogenates of the testicular samples were prepared in ice-cold physiological saline in the ratio of 1:4 (gram: volume) using a pre-chilled hand mortar and pestle. The homogenates were centrifuged at 17000rpm for 20 minutes, and the supernatant fractions kept refrigerated prior to analysis. All samples were analysed within 4days for superoxide dismutase, SOD; catalase, ascorbic acid, ß-carotene and α-tocopherol.

Enzyme and antioxidant vitamin assays

SOD activity in the testicular extract was estimated by the method of Misra and Fridovich (10), while catalase was assayed colorimetrically according to the method of Sinha (11).

Ascorbic acid was estimated colorimetrically by the method of Roe & Keuther (12) as modified by Nino & Shah (13). To 2.5ml of tissue extract and 2.5ml of standard ascorbic acid was added 1.0ml of freshly-prepared 20% TCA. The mixture was centrifuged for 10minutes at 2500rpm. Blank containing 2.5ml of TCA was similarly treated. Then 0.4ml of dinitrophenyl hydrazine-thiourea (DTCS) reagent was added separately to 1.2ml of each supernatant fraction. The tubes were capped, vortexed and incubated at 37^{o}C for 3hrs. Thereafter 2ml of 12M H_2O_4 was added to each of the test, standard and blank tubes. On cooling to room temperature, the absorbance of the test and standard tubes were read at 520nm against reagent blank. The concentration of ascorbic acid in the test was calculated by reference to standard absorbance and concentration of standard.

ß-carotene and vitamin E (α-tocopherol) were estimated spectrophotometrically after prior precipitation of proteins with absolute ethanol, followed by extraction of the vitamins in petroleum ether according to the method of Jakutowicz *et al* (14). For ß-carotene, 0.5ml of absolute ethanol was added to 1.0ml of the extract. On shaking, 4.0ml of petroleum ether (boiling range=60-80^{o}C) was added to the mixture. The absorbance of the clear ether layer was read directly at 450nm, and the level of ß-carotene was calculated after extrapolation from a beta-carotene standard curve.

The estimation of α-tocopherol followed the same steps as for ß-carotene, except that after the extraction with petroleum ether, 2.0ml of 0.4% bathophenanthroline (4,7-diphenyl-1,10-phenanthroline) was added, and absorbance was read at 536nm. The a-tocopherol content of each sample was calculated after extrapolation from standard calibration curve.

Estimation of protein

The protein contents of the extracts from the testicular homogenates were determined by the Biuret method of Gronall *et al* (15) as modified by Henry *et al* (16), using bovine serum albumin, BSA as standard.

Statistics

The results obtained in the various assays for treated rats and controls were expressed as Mean ± SD, and analysed for significant differences using Student's t-test. P values less than 0.05 were taken as significant.

Results

The effect of administration of ERCP on testicular weight, testicular total protein and testicular levels of the antioxidant enzymes SOD and catalase is shown on Table 1. The rats given ERCP for 21 days had significantly higher weight of testes and higher testicular total protein than control rats which received vehicle alone ($P<0.05$). In addition, the activities of SOD and catalase were significantly higher in the testes the ERCP-treated rats when compared with rats in the control group ($P<0.05$)

Table 2 shows results obtained in the analysis of the effect of ERCP administration on testicular levels of ß-carotene, ascorbic acid and α-tocopherol. The rats given ERCP for 21 days had significantly higher testicular contents of these vitamins when compared to untreated rats (control) ($P< 0.05$).

The ethanolic root extract of *C. populnea* produced significant increases in mounting frequency and intromission in the male rats ($P< 0.05$).The extract-treated rats also produced significantly higher percentage of fertility in oestrous female rats than untreated controls ($P< 0.05$). These results are shown in Table 3.

Table 1: Effect of daily administration of ethanolic root extract of *C. populnea*, ERCP at a dose of 270mg/kg body weight for 21 days, on testicular weight, testicular total protein, and testicular activities of SOD and catalase of rats.

Parameter	Control rats	ERCP-treated rats
Weight of testes (g)	3.23 ± 0.09^{a}	3.37 ± 0.15^{b}
Total protein content of testes (g/dL of extract)	1.92 ± 0.30^{a}	2.85 ± 0.37^{b}
Catalase (U/mg protein)	5.30 ± 1.15^{a}	9.58 ± 2.04^{b}
SOD (U/mg protein)	2.45 ± 0.48^{a}	3.26 ± 0.45^{b}

Results are expressed as Mean ± SD (n=6). For each parameter, values that have different superscripts across differ significantly ($P<0.05$).

Table 2: Effect of daily administration of ethanolic root extract of *C. populnea*, ERCP at a dose of 270mg/kg body weight for 21 days on testicular levels of ß-carotene, ascorbic acid and α-tocopherol in rats.

Parameter	Control rats	ERCP-treated rats
ß-carotene (µg/g)	19.92 ± 1.55^{a}	24.75 ± 0.38^{b}
Ascorbic acid (mg/g)	33.00 ± 4.72^{a}	54.89 ± 7.09^{b}
A-tocopherol (mg/g)	18.38 ± 3.02^{a}	41.72 ± 6.97^{b}

Results are expressed as Mean ± SD (n=6). Values that have different superscripts across differ significantly ($P<0.05$)

Table 3: Effect of daily administration of ethanolic root extract of *C. populnea*, ERCP at a dose of 270mg/kg body weight for 21 days on some sexual performance parameters of male rats.

Parameter	Control rats	ERCP-treated rats
Mounting frequency	2.00 ± 0.45^{a}	2.83 ± 1.66^{b}
Ejaculation latency (sec)	21.00 ± 4.46^{a}	29.09 ± 5.90^{b}
Intromission (sec)	12.67 ± 2.72^{a}	8.33 ± 1.71^{b}
Fertility (% pregnancy in mated females)	37.50	62.50

Mean ± SD (n=6). Values that have different superscripts across differ significantly ($P<0.05$)

Discussion

The sexual performance-enhancing properties of herbal aphrodisiac remedies have continued to engage the attention of researchers. These studies usually involve investigating the effects of the herbal extracts on standard parameters like fertility, sperm count, sperm motility, fertility, intromission latency and mounting frequency; as well as their effects on associated biochemical indices such as testicular antioxidant status and testicular testosterone levels. The purpose is to establish empirical evidence for the claims made by habitual users on the sexual performance-enhancing potencies of these plants. Our results demonstrate that administration of ethanolic root extract of *Cissus populnea* at a dose of 270mg/kg body weight to male rats significantly increased the testicular antioxidant status with respect to SOD, catalase, and the antioxidant vitamins. In addition, the extract-treated rats had significantly higher testicular protein and testicular weight. An increase in testicular antioxidant status is linked to enhanced sexual performance, increased fertility and sperm motility and viability**.** Several studies have demonstrated the importance of testicular antioxidants in androgenesis (17, 18, 19). Antioxidants promote sperm viability by protecting it from the damaging effects of free-radical mediated membrane lipid peroxidation (20). Although the phytochemical composition of the extract used in this study was not analysed, it is known that roots of *Cissus populnea* are rich in alkaloids, saponins, tannins and flavonoids (21). The presence of flavonoids may be responsible for the enhancement of testicular antioxidant status in the extract-treated rats, and the higher androgenic effects seen in this group with respect to fertility and sexual performance. Indeed the potentiating effects of plant extracts on sexual performance have been attributed to the presence of flavonoids, tannins and steroids in these extracts (22, 23, 24, 25). Increases in ejaculatory latency and mounting frequency, with evidence of reduced intromission are standard indicators of improved sexual performance (3).

Muthu and Krishnamoorthy (26) have reported that the androgenic effect of *M. pruriens* in rats is associated with increases in testicular and epidydymal total protein, total cholesterol, and testestorone. This is in agreement with the observed significant increases in testicular total protein and testicular weight seen in the present study. In various animal species and in humans, studies have established a positive correlation between testis size and daily sperm output (27, 28, 29, 30, 31). In male rats, the androgenic effect of root extract of *Mondia whitei* has also been linked to elevation of testicular protein, sperm density and testicular testosterone level (32). Thus the increases in testicular weight and testicular total protein seen in this study are positive androgenic factors linked to increased sperm production and fertility.

In contrast, Ojekale *et al* (33) reported that stem bark extract of *Cissus populnea* had no significant effect on sperm parameters in human male volunteers continuously given the extract at a dose of 150mg/kg body weight for 72 days. In the present study, we used the root extract, which is the form consumed in Ondo State of Nigeria. It is very likely that the active androgenic principles of the plant resides more in the root than the aerial/apical regions. Such differences in phytochemical compositions between roots and stem of the same plant have been reported (34). Further investigations are on-going to ascertain the detailed phytochemical characteristics of the root and stem back extracts of this plant.

Conclusion

Our results demonstrate that ethanolic root extract of Cissus populnea exerts significant sexual performance and fertility-enhancing effects in rats. If animal-to-man extrapolation is permitted, these results may explain the basis of the use of root extracts of this plant as an aphrodisiac in some parts of Nigeria.

References

1. NIH: Consensus Development Panel on Impotence. National Institute of Health Consensus Conference: impotence. J. Am. Md. Assoc. 270:83-90. 1993.
2. Beckman TJ, Abu-Lebdeh HS, Mynderse LA: Evaluation and medical management of erectile dysfunction**.** *Mayo Clinic Proceedings* 81:385–390. 2006.
3. Santosh BT, Chitma HR, Rabbani G, Jafar M: Effect of *Leptadenia reticulate* on stress-modulated sexual behavior of male rats. Inter. Res. J. Pharm. 2:27-36. 2011.
4. Fazio L, Brock G: Erectile dysfunction: Management update. Can. Med. Assoc. J. 170:1429-1437. 2004
5. Kim ED, Brosman SA: Erectile dysfunction. Htpp://emedicine.medscape.com.article/444220-overview. 2011
6. Abdullah, A: The Conservation of Medicinal and Aromatic Plants: Hamdad Medicus. Hamdad Foundation Press, New York, 12-14pp. 2006.
7. Ibrahim H, Mdau B, Ilyas M: Anthraquinones of *Cissus populnea* Guill and Perr (Amplidaceae). Afr. J. Complem. Altern. Med. 8:140-143. 2011.
8. Sofowora A: Medicinal plants and traditional medicine in Africa. John Wiley & Sons, New York. 27-29pp. 1982.

9. Animal Care and Use Program: In: Guidelines for Care and Use of Animals in Scientific Research (8th Edition). National Academic Press, Washington. 11-103pp. 2011.
10. Misra HP, Fridovich I: The role of superoxide dismutase in the autooxidation of epinephrine and a single assay for superoxide dismutase. J. Biol. Chem 247: 3170-3175. 1972.
11. Sinha KA: Colorimetric assay of catalase. Anal. Chem. 47: 389-394. 1972.
12. Roe JH, Keuther CA: The determination of ascorbic acid in whole blood and urine through the 2,4-dinitrophenylhydrazine. J. Biol. Chem. 147:399. 1943.
13. Nino HV, Shah W: Vitamins. In: Tietz, N.W. (ed). Fundamentals of Clinical Chemistry. 2nd edition. W.B. Saunders, Philadelphia, pp 547-550. 1986.
14. Jakutowicz K, Tomick *ZI,* Leokadia L: Determination of total plasma tocopherol in the presence of carotenes. Pol. Arch. Weter. (Warszawa) 20*:*45–57. *1977.*
15. Gornall AG, Bardawill CJ, David MM: Determination of serum proteins by means of the Biuret reaction. J. Biol. Chem. 177:751-766. 1949.
16. Henry RJ, Cannon DC, Win JW: Method of protein determination in plasma. Clin. Chem. 20:1362-1363. 1974.
17. Ghlissi Z, Atheymen R, Boujbiha MA *et al:* Antioxidant and androgenic effects of dietary ginger on reproductive function of male diabetic rats. Inter. J. Food Sci. Nutr. 64: 974-978. 2013.
18. Khaki A, Fathiazad F, Nouri M, Khaki, AA, Khamenehi HJ, Hamadeh M: Evaluation of androgenic activity of *Alium cepa* on spermatogenesis in the rat. Folio Morphologica 68:45-51. 2009.
19. Memudu AE, Akinrinade ID, Ogundele OM, Duru F: Investigation of the androgenic activity of ginger (*Zingiber officinale*) on the histology of the testis of adult Sprague dawley rats. J. Med. Sci. 3: 697-702. 2012.
20. Bansal AK, Bilaspuri GS: Impacts of oxidative stress on semen functions. Vet. Med. Inter. 2011:686137. 2011.
21. Soladoye MO, Chukwuma EC: Phytochemical analysis of the stem and root of *Cissus populnea* (*Vitaceae*) – an important medicinal plant in Central Nigeria. Phytologia Balcanica 18:149-153. 2012.
22. Shukla VN, Khanuja PS: Chemical, pharmacological and botanical studies on *Pedalium murex*. J. Arom. Plant Sci. 26: 64-96. 2004.
23. Das S, Parveen S, Kundra CP, Pereira BM: Reproduction in male rats is vulnerable to treatment with the flavonoid-rich seed extract of *Vitex negundo*. Phytother. Res. 18: 8–11. 2004.
24. Malini T, Vanithakumari G: Anti-fertility effects of beta-sitosterol in male albino rats. J. Ethnopharmacol. 35**:** 149–153. 1991.
25. Barry TN: The role of condensed tannins in the nutritional value of *Lotus pedunculatus* for sheep. Rates of body and wool growth. Br. J. Nutr. 54**:** 211–217. 1985.
26. Muthu K, Krishnamoorthy P: Evaluation of androgenic activity of *Mucuna pruriens* in male rats. Afr. J. Biotechnol.10:15017-15019. 2011.
27. Berndtson WE, Igboeli G, Parker WG: The numbers of Sertoli cells in mature Holstein bulls and their relationship to quantitative aspects of spermatogenesis. Biol. Reprod. 37: 60-67. 1987.
28. Berndtson WE, Thompson TL: Changing relationships betweentestis size, Sertoli cell number and spermatogenesis in Sprague-Dawley rats. J. Androl. 11:429-435. 1990.
29. Amann RP: Sperm production rates. *In*: *The Testis*. Johnson AD, Gomes WR and VanDemark PT.(eds). Academic Press, New York. pp 433-482. 1970
30. Johnson L, Zane RS, Petty CS, Neaves WB: Quantification of the human Sertoli cell population: its distribution, relation to germ cell numbers, and age-related decline. Biol. Reprod. 31:785-795. 1984.
31. Ugwu SOC, Onyimonyi AE, Foleng H: Testicular development and relationship between body weight, testis size and sperm output in tropical boars. Afr. J. Biotechnol. 8:1165-1169. 2009.
32. Watcho, P.S., Kamtchouing, P., Skeng, S.D., Moundipa, P.F., Tantchou, J,., Essame JL, Koueta N: Androgenic effect of *Mondia whitei* roots in male rats. Asian J. Androl. 6:269-272. 2004.
33. Ojekale AB, Lawal, OA, Lasisi, AK, Adeleke TI: Phytochemistry and spermatogenic potentials of extract of *Cissus populnea* (Guill and Per) stem bark. TSW Hol. Health Med. 1: 176 – 182. 2006.
34. Benhammonou N, Ghambaza N, Benabdelkader S, Atik-Bekkara F, Kadifkova, PT: Phytochemicals and antioxidant properties of extracts from the root and stems of *Anabasis articulata*. Inter. Food Res. J. 20: 2057-2063. 2013.