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

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**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


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 established 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, venereal diseases, intestinal parasites,
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 College of Medicine, Ikeja, Lagos, Nigeria 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% formal 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 haematoxylin and eosin stains. They were examined under light microscopes (CETI, UK) at magnifications ranging from 400 to 1000.

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 for abnormalities in head, neck, mid-piece or tail regions.

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

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>C. populnea</th>
<th>Addyzoa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Motility (%)</td>
<td>8.75 ± 4.75</td>
<td>28.75 ± 5.82</td>
<td>13.75 ± 4.43</td>
</tr>
</tbody>
</table>
| Morphology (%)      | normal: 70 ± 7.5  
abnormal: 30 ± 7.5 | normal: 77.5 ± 4.66  
abnormal: 22.5 ± 4.63 | normal: 77.5 ± 4.66  
abnormal: 22.5 ± 4.63 |
| Total count (x10⁶)  | 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

<table>
<thead>
<tr>
<th>Organ weights</th>
<th>Control</th>
<th>C. populnea</th>
<th>Addyzoa</th>
</tr>
</thead>
</table>
| 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).

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 1](https://example.com/figure1.jpg) Photomicrograph of cross section of testis from control showing normal tubular outline and complement of germ cells. H&E ×400

![Figure 2](https://example.com/figure2.jpg) 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 × 400

![Figure 3](https://example.com/figure3.jpg) 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 x1000
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