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Aqueous root extract of *Lecaniodiscus cupanioides* restores the alterations in testicular parameters of sexually impaired male rats

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ABSTRACT

Objective: This study aimed to investigate the effects of aqueous root extract of *Lecaniodiscus cupanioides* (*L. cupanioides*)on the alterations in the testicular parameters of paroxetine-treated rats. **Methods:** Group A rats which is the control received distilled water orally for 5 d. Groups B, C, D, E and F consisted of paroxetine-induced sexual dysfunction rats. In addition, Groups C, D, E and F rats were orally treated with 25, 50 and 100 mg/kg body weight of the extract and 7.14 mg/kg body weight of PowMax once daily for 5 d respectively. **Results:** Paroxetine-induced sexual dysfunction resulted into significant ($P<0.05$) reduction in the levels of testicular protein, sialic acid, glycogen and cholesterols. These decrease were dose dependently reversed by aqueous root extract of *L. cupanioides*. The decrease in the specific activities of acid and alkaline phosphatases, lactate dehydrogenase and gamma-glutamyl transferase in the testes of paroxetine-treated rats were significantly ($P<0.05$) reversed. Testicular testosterone level decreased significantly ($P<0.05$) in sexually impaired rats. This decrease was significantly prevented by aqueous root extract of *L. cupanioides*. All these alterations brought about by the administration of the extract (25 and 50 mg/kg body weight) compared significantly ($P<0.05$) with the reference drug, while the 100 mg/kg body weight of the extract compared significantly ($P<0.05$) with the control. **Conclusions:** The results of this study showed that aqueous root extract of *L. cupanioides* restored the alterations in the testicular function parameters of sexually impaired rats. Thus supporting the use of the plants in the management of sexual dysfunction in the folkloric medicine of Nigeria.

1. Introduction

Medicinal plants are used from ancient times and only true natural medicines have been found useful in several ways. Medicinal plants and their phytochemical constituents are used for different therapeutic purposes and as precursor for the synthesis of drugs[1]. The use of herbs is very common in developing countries, particularly in rural settings. However, during the last decade, an increase in the use of plants has been observed in metropolitan areas of developed countries[2]. In Nigeria, several botanicals such as *Tribulus terrestris*, *Fadogia agrestis*, *Massularia accuminata*, *Bulbine natalensis*, and *Cnestis ferruginea*[3-7] have been reported to enhance sexual performance in rats and some compounds like yohimbine were reported to be responsible for this activities.

Lecaniodiscus cupanioides Planch. ex Bth. (*L. cupanioides*) (Sapindaceae) is a shrub widely distributed throughout deciduous and non-deciduous rain forests[8]. It is known as aaka or akika (Yoruba), kaafi-nnamaa-zaaki (Hausa) and okpu (Igbo) in Nigeria. Parts of the plant have various applications in folk medicine for the treatment of boils, burns, wounds, oral hygiene, fever and abdominal swelling caused by liver abscess[8]. The decoction of the root of the plant is claimed among the Yoruba people of Nigeria to

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control epilepsy and to enhance penile erection. The plant has also been reported to have central nervous system depressant activity[9] and analgesic activity[10].

Despite the acclaimed sexual enhancing potentials of the root decoction of this plant, there is dearth of information on the effects of this plant on the testicular function parameters as related to sexual enhancement. This study thus investigated the effects of aqueous root extract of *L. cupanioides* on the testicular function of sexually impaired rats.

2. Materials and methods

2.1. Plant materials

Dried roots of *L. cupanioides* were bought from herb sellers at Oja tuntun market, Ilorin, Nigeria. The plant was identified and authenticated by Mr Bolu Ajayi, a botanist in the Department of Plant Biology, University of Ilorin, Ilorin, Nigeria.

2.2. Rats

Thirty healthy Wistar male rats weighing (156.24±3.22) g were housed in clean aluminum cages contained in well ventilated housing conditions (temperature of (22±3) °C; photoperiod of 12 h; and humidity of 45%-50%). The animals were used according to the Guide for the Care and Use of Laboratory Animals[11] and in accordance with the principles of Good Laboratory Procedure (GLP)[12].

2.3. Assay kits

Assay kits for lactate dehydrogenase, gamma-glutamyl transferase, acid phosphatase and alkaline phosphatase were obtained from Randox Laboratories, Co-Atrim, UK, whereas that for testosterone was obtained from Immunometrics (UK) Ltd., London. All other reagents used were of analytical grade and were prepared in volumetric flask using all glass-distilled water.

2.4. Preparation of extract

Briefly, dried roots of *L. cupanioides* were pulverized in a blender (Mikachi Blender with Mill, Model. MK-1830, China) and the resulting powder weighing 50 g was extracted in 200 mL distilled water for 48 h at room temperature. The extract was filtered with Whatman No. 1 filter paper and the resulting filtrate was concentrated on steam bath to give a yield of 4.98 g of the residue (brownish-black slurry). This was reconstituted in distilled water to give the required doses of 25, 50 and 100 mg/kg body weight before administration to experimental animals.

2.5. Experimental design

Normal sexually experienced male rats and sexually impaired male rats were employed for this investigation. Sexual impairment was induced and confirmed in rats as described by Chan *et al*[13]. A total of thirty male rats were completely randomized into six groups (A-F) with each group comprising of five animals. Those in the Group A (normal sexually experienced rats) were administered with distilled water only. The Group B, C, D, E & F consisted of sexually impaired rats and they were treated with 7.14 mg/kg of PowMax (a reference herbal drug), 25, 50 and 100 mg/kg body weight of aqueous root extract of *L. cupanioides* daily for 5 d, respectively.

2.6. Preparation of testicular supernatants

The rats were sacrificed by placing them in a jar containing wool soaked in diethyl ether. Testes excised from the rats were immersed in ice-cold 0.25 mol/L sucrose solution to maintain the integrity of the organ. The testes were blotted with tissue paper, cut thinly with sterile scalpel blade and then homogenized in ice-cold 0.25 mol/L sucrose solution at a mass-to-volume ratio of 1:5. The homogenates were centrifuged at 800 r/min for 10 min at 4 °C; the resulting supernatant was frozen at -20 °C until use for the biochemical assays.

2.7. Determination of biochemical parameters

The testicular activities of lactate dehydrogenase, gamma-glutamyl transferase, acid phosphatase, alkaline phosphatase and testosterone concentration were determined respectively by the methods as described previously[14-18]. The concentrations of testicular protein, glycogen, cholesterol and sialic acid were estimated using the methods as described previously[19-22].

2.8. Statistical analysis

Analysis of variance (ANOVA) followed by Tukey-Kramer test for differences between means was used to detect any significant differences ($P<0.05$) between the variables used in this study using StatPlus, 2011 (AnalystSoft Inc., Alexandria, USA).

3. Results

There were significant ($P<0.05$) reductions in the

concentrations of protein, cholesterol, sialic acid and glycogen in the testes of the paroxetine-treated rats (Table 1). Administration of the aqueous root extract of *L. cupaniodes* at the doses of 25, 50 and 100 mg/kg body weight produced significant ($P<0.05$) increases in the levels of protein, cholesterol, sialic acid and glycogen when compared to the paroxetine-treated rats (Table 1). However, only 100 mg/kg body weight treated-group compared significantly ($P<0.05$) with the control. PowMax, the reference drug, produced similar pattern of increase, however, it did not compare significantly with the control (Table 1).

The level of testosterone in the testes of paroxetine-treated rats decreased significantly ($P<0.05$) (Table 1). This decrease was completely attenuated by 100 mg/kg body weight of the aqueous root extract of *L. cupanioides* (Table 1). Although the doses of 25 and 50 mg/kg body weight of the aqueous root extract of *L. cupanioides* significantly attenuated the testosterone levels when compared with the paroxetine-treated rats, these attenuations did not compare with the control (Table 1).

Specific activities of alkaline phosphatase, acid phosphatase, lactate dehydrogenase and gamma-glutamyl transferase decreased significantly ($P<0.05$) in the testes of the paroxetine-treated rats when compared with the control (Table 2). These decreases were significantly reversed by the administration of the aqueous root extract of *L. cupanioides* when compared with the paroxetine-treated rats (Table 2). Only 100 mg/kg body weight reversed the decrease at the levels significant ($P<0.05$) to the control. PowMax also produced similar reversal but this was not significant when compared with the control (Table 2).

4. Discussion

Testicular protein, cholesterol, sialic acid and glycogen are indices of functional capacity of the testes[23-25]. Testicular proteins are required for spermatogenesis and sperm maturation[26]. Thus, the significant reduction in the concentration of testicular protein in paroxetine-treated rats could impair sperm maturation[26]. The restoration of the concentration of testicular protein following the administration of the aqueous root extract of *L. cupanioides* could enhance sperm maturation, indicating the androgenic potential of the plant.

The significant reduction in the testicular sialic acid in paroxetine-treated rats could hinder sperm motility, since sialic acid facilitates the movement of sperm, reducing friction among the spermatozoa in the testes[27]. Thus, the reversal of paroxetine-mediated reduction in the testicular sialic acid concentration could restore the normal sperm motility. Testicular glycogen provides carbohydrate reserves for seminiferous tubular cells. The level of glycogen is directly proportional to steroid hormones[28]. The decrease in glycogen levels in paroxetine-treated rats may result in

Table 1
Levels of some testicular parameters and testicular testosterone concentration in sexually impaired rats following the administration of aqueous root extract of *Lecaniodiscus cupanioides*.

Group	Protein (mg/mL)	Sialic acid (mg/g)	Glycogen (mg/100 mg glucose)	Cholesterol (mmol/L)	Testosterone (nmol/L)
Distilled water (control)	8.88±0.02^a	4.59±0.07^a	3.87±0.02^a	9.72±0.10^a	21.00±1.68^a
Paroxetine-treated	2.88±0.01^b	1.72±0.01^b	1.13±0.01^b	2.02±0.03^b	17.50±0.46^b
Paroxetine + PowMax	6.42±0.05^c	3.25±0.03^c	2.02±0.04^c	5.32±0.07^c	17.50±1.62^b
Paroxetine + 25 mg/kg body weight of extract	5.18±0.01^d	2.02±0.02^d	1.83±0.01^c	5.01±0.05^c	18.40±2.24^c
Paroxetine + 50 mg/kg body weight of extract	6.12±0.03^c	2.87±0.01^e	2.72±0.02^d	6.80±0.01^d	19.38±0.48^a
Paroxetine + 100 mg/kg body weight of extract	7.76±0.05^a	3.66±0.03^c	3.04±0.01^a	8.02±0.12^a	22.25±1.62^a

Data are mean±SD of five determinations. Values carrying superscripts different from the control for each parameter are significantly different ($P<0.05$).

Table 2
Specific activities (IU/mL) of some testicular enzymes in sexually impaired rats following the administration of aqueous root extract of *Lecaniodiscus cupanioides*.

Group	Alkaline phosphatase	Acid phosphatase	Lactate dehydrogenase	Gamma-glutamyl transferase
Distilled water (control)	7.78±0.30^a	8.86±0.40^a	3.48±0.04^a	32.86±2.43^a
Paroxetine-treated	1.13±0.47^b	2.56±0.01^b	0.83±0.01^b	16.75±0.54^b
Paroxetine + PowMax	4.86±0.52^c	6.22±0.16^c	1.78±0.01^c	28.85±2.07^c
Paroxetine + 25 mg/kg body weight of extract	3.97±0.11^d	3.22±0.07^d	1.29±0.02^c	20.60±1.35^d
Paroxetine + 50 mg/kg body weight of extract	5.31±0.31^c	5.96±0.04^c	2.03±0.05^d	25.24±3.11^c
Paroxetine + 100 mg/kg body weight of extract	6.28±0.10^a	7.28±0.19^a	2.98±0.04^a	31.47±0.49^a

Data are mean±SD of five determinations. Values carrying superscripts different from the control for each parameter are significantly different ($P<0.05$).

reduction in carbohydrate reserve. The attenuation of this decrease by *L. cupanioides* indicates the protective potential of the plant.

Cholesterol, a precursor of steroid hormone, is required for normal testicular activity[25]. Thus, the significant ($P<0.05$) reduction in the level of testicular cholesterol in paroxetine treated rats could decrease androgen concentration. The attenuation of paroxetine-mediated reduction in the concentration of testicular cholesterol by the aqueous root extract of *L. cupanioides* could restore androgen concentration via enhanced steroidogenesis[29]. Reports have shown that medicinal plants with aphrodisiac properties increase the levels of testicular cholesterol[30,31].

Testicular alkaline phosphatase is involved in the intra and intercellular transport, which is needed for the metabolic reactions to channelize the necessary inputs for steroidogenesis[32], showing its importance in steroidogenesis[33]. The decreased alkaline phosphatase in paroxetine-treated rats could compromise the transport of necessary materials needed for spermatogenesis. *L. cupanioides* administration significantly attenuated the paroxetine-mediated decrease, indicating the capability of the extract to enhance intra and intercellular transport of materials needed for steroidogenesis.

The decrease in acid phosphatase, an important enzyme in the physiology of sperm, in the testes following the administration of paroxetine could decrease steroidogenesis. Acid phosphatase activity has been shown to rise when testicular steroidogenesis is increased[34]. The attenuation of paroxetine-mediated decrease in acid phosphatase following the administration of the aqueous root extract of *L. cupanioides* shows the capability of the plant extract to enhance the process of steroidogenesis.

Lactate rather than glucose is the preferred substrate for glycolysis in primary spermatocyte and lactate is generated from glucose in the Sertoli cells under the influence of follicle stimulating hormone[35]. Thus, the significant decrease in testicular lactate dehydrogenase could result in energy reduction, which may halt spermatogenesis by preventing the transformation of spermatocyte to spermatozoa. Hence, the attenuation of paroxetine-mediated reduction in the activity of lactate dehydrogenase following the administration of the aqueous root extract of *L. cupanioides* is an indication of protective and androgenic potentials of the extract.

The significant decrease in gamma-glutamyl transferase, a "marker" enzyme of Sertoli cell function of the testis in paroxetine treated rats, could result in Sertoli dysfunction and consequently alteration in spermatogenesis[36]. The ability of the extract to reverse the decrease mediated by paroxetine treatment indicates its capability to enhance spermatozoa production.

Testosterone is a marker of androgenicity[37]. Thus, the significant reduction in the levels of testicular testosterone in paroxetine-induced sexual dysfunction in rats could be due to direct toxic effect of the drug on the gonads or an indirect effect on the pituitary gland[38]. The enhanced level of testicular testosterone following the treatment of sexually impaired rats with the aqueous root extract of *L. cupanioides* could help maintaining body shape, increasing muscle mass/strength, physical function and erectile function of rats[39,33].

In conclusion, the aqueous root extract of *L. cupanioides* restored the alterations in the testicular functional parameters of paroxetine-treated rats. This study thus supports the use of the plants in the management of sexual dysfunction in the folkloric medicine of Nigeria.

Conflict of interest statement

We declare that we have no conflict of interest.

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