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Effect of aqueous extract of the aerial parts of C*aralluma dalzielii* N. E. Brown on the testosterone levels of male Wistar rats

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Abstract
The aqueous extract of the aerial parts of *Caralluma dalzielii* (family Asclepiadaceae) has been used traditionally for the treatment of many ailments including infertility, convulsion, stomach problems and as aphrodisiac. The aim of the study was to evaluate the effect of the extract on testosterone level in male Wistar rats. Male Wistar rats were allotted into four groups of five rats each. Three doses of the extract (150, 300 and 600 mg/kg) and one distilled water control group were used for the assessment. The administration was done orally in all groups for 28 days. On the 29th day, blood samples were withdrawn via cardiac puncture for testosterone level assay. The testes were excised and fixed in Bouin's solution for histopathological study. There was a significant (p<0.001 at 300 mg/g and p<0.05 at 150 and 600 mg/kg) rise in testosterone level among the test groups compared to the control. Histopathological examination showed no distortion in the testicular tissues. In conclusion, the extract of *Caralluma dalzielii* caused an increase in testosterone level which may be responsible for its use as an aphrodisiac in the traditional setting.

Keywords: *Caralluma dalzielii*, testosterone, testis, Wistar rats, aphrodisiac

INTRODUCTION

The use of traditional medicine for the treatment of various ailment is as old as 4000-5000 B.C [1]. This may be because it has remained the most affordable and easily accessible source of treatment in the primary health care system of resource poor communities. Though contemporary medicine may exist side-by-side with traditional medicine, the later has often maintained its popularity for historical and cultural reasons [2]. Such traditional medicines among others is the use of the decoction of *Caralluma dalzielli* for the treatment of infertility and as aphrodisiac.

Caralluma dalzielii N.E. Brown (Asclepiadaceae) is a cactus-like shaped plant distributed across the Sahel [3]. It can grow up to 1 meter high. It is commonly known as mosque stalk and 'Karan massallaci' amongst the Hausa tribe of Nigeria. It is used traditionally to treat infertility, diabetes, leprosy, rheumatoid arthritis, severe pain on epigastrium [4] and as aphrodisiac [5,6].

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Previous researches have validated its use as anti-inflammatory [4] and antiulcer [7], but none as an aphrodisiac.

The incidence of male sexual dysfunction is growing, and as such, the need for more and rapid search of plants with aphrodisiac abilities is important [8]. In Northern Nigeria, the use of herbal products as aphrodisiac is common. Aphrodisiacs are substances which are used to treat sexual dysfunction or that can enhance sexual activity [9]. They act by altering specific neurotransmitters or sex hormone levels in the body. Such hormones include the testosterone concentration in the serum [10]. *Caralluma dalzielii* has been regarded as a stimulant for sexual activity however, this has not been scientifically proven. The aim of the study was to evaluate the effect of aqueous extract of the aerial parts of *Caralluma dalzielii* on serum testosterone level in male Wistar rats.

EXPERIMENTAL

Plant collection, identification and preparation. The fresh plants of *C. dalzielii* were obtained from a traditional medicine practitioner in Bodinga, Sokoto state Nigeria in April 2017. It was identified and authenticated by Dr. H. E. Mshelia in the Department of Pharmacognosy and Ethnopharmacy, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University Sokoto, Nigeria. A voucher specimen was stored in the herbarium of the same department (Pcg/UDUS/Asdy/001). The aerial parts of *C. dalzielii* was air dried under shade for a period of one month and pulverised. Five hundred grams (500 g) of the dried plant powder was macerated in 2 L of distilled water at room temperature for 48 h. It was then filtered using Whatman filter paper No.42 (125mm) and the filtrate evaporated to dryness over a water bath at 60°C.

Experimental animals. Twenty-eight adult male Wistar rats weighing 150-200 g obtained from the animal facility centre of Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Nigeria were used for the test. The animals were acclimatised under standard laboratory conditions for 2 weeks before the commencement of the study. Standard commercial chow and water were given *ad libitium* to the animals. Housing conditions were maintained at 23±2ºC at 12 h day/ night light cycles. The study was approved by the animal research ethical committee of the Department of Pharmacology and Toxicology, Usmanu Danfodiyo University, Sokoto (PTAC/Cd/OT/003-18). The care and handling of the animals were according to the established public health guidelines for care and use of laboratory animals.

Experimental design. The rats were allotted into four groups of seven animals each. Group I served as control receiving 5 ml/kg of distilled water, Group II received 150 mg of *C. dalzielii* extract, Group III received 300 mg of *C. dalzielii* extract while Group IV received 600 mg/kg of *C. dalzielii* extract. The oral administration of the extract and distilled water was daily by gavage using metal oropharyngeal canula for a period of 28 days. On the 29th day, the rats were anaesthetised with chloroform. Blood samples were collected via cardiac puncture for testosterone level assay. The testes were immersed in Bouin's fixative for histological analysis.

Testosterone assay. Testosterone radioimmunoassay test kit was purchased from DRG Diagnostics GmbH, Germany. Serum samples were assayed by using the procedure described by the producers. This assay was based on the principle of radioimmunoassay of competitive binding between the sample serum and the standards for a constant amount of the antisera [11].

Testicular histology. Testes tissues were washed through graded concentrations of ethanol saturated with lithium carbonate. The tissues were passed through 90% and absolute alcohol and xylene for different durations

before they were transferred into two changes of molten paraffin wax for 1 h each in an oven at 65ºC for infiltration. They were subsequently embedded and serial sections cut using rotary microtome at 5 microns. The tissues were fixed into albumenised slides and allowed to dry on hot plate for 2 min. The slides were dewaxed with xylene and passed through absolute alcohol; 70% alcohol, 50% alcohol and then to water for 5 min. The slides were then stained with haematoxylin and eosin (H&E). The slides were mounted in Canada balsam. The sections were examined using light microscope to investigate the spermatogenesis process and other changes [12,13]. Photomicrographs were taken using X40 objectives.

Statistical analysis. The results of the study are expressed as the mean ± S.E.M. The results were analysed using Graph Pad Prism version 6 software. Comparison in all the groups was made using one-way analysis of variance (ANOVA) followed by Dunnett's post-hoc test. Differences were considered significant at $p \leq 0.05$.

RESULT AND DISCUSSION

Figure 1 showed that the aqueous extract of *Caralluma dalzielii* significantly increased testosterone level at all doses. The highest level was noted at 300 mg/kg. In men, testosterone is the principal secreted androgen and a hormonal marker for androgenism [14,15]. It is also the major male gonadal hormone produced from the testis by the interstitial cells of Leydig. Adequate testosterone level is essential for the maintenance of normal sexual desire, nocturnal penile tumescence and non-erotic penile erections [16]. It is also required for the initiation and maintenance of spermatogenesis and general wellbeing of the male reproductive system [17]. This implies that any alteration in the level of this hormone would directly affect spermatogenesis and possibly desire for sexual activity. The significant increase in serum testosterone observed in our study suggests that the extract may possess ability to enhance libido and spermatogenic activity of the male rats. Furthermore, histopathological evaluation of the testes is regarded as the most sensitive assessment of the harmful effects of substances on spermatogenesis and a good pointer to reproductive toxicity [18]. Tissue sections taken from the testes of *C. dalzielii* treated rats showed no gross abnormalities at all dose levels (Figure 2).

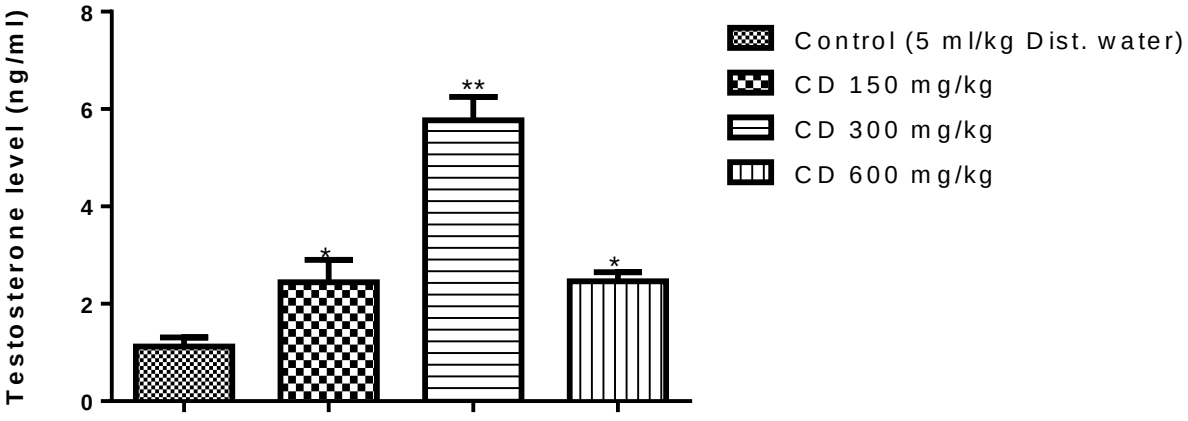

Figure 1. Effect of *Caralluma dalzielii* on serum testosterone level in rats.
CD= *Caralluma dalzielii*. Values are presented as mean ± S.E.M. **p< 0.001; *p<0.05

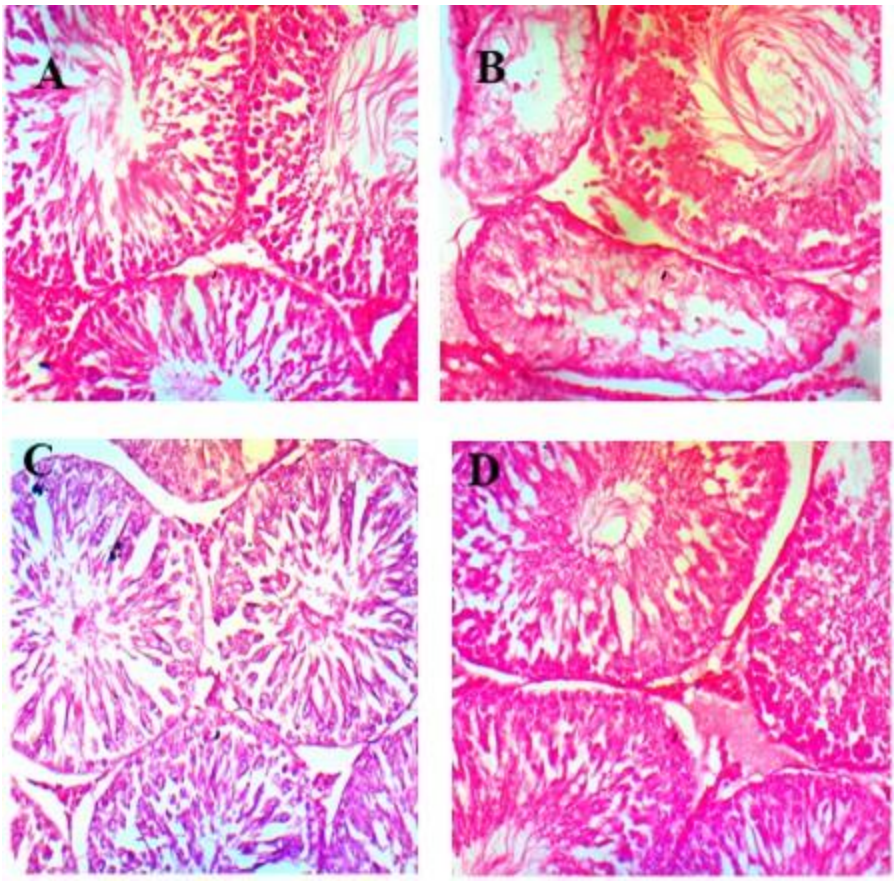

Figure 2. Effect of *Caralluma dalzielii* on histology of testes of rats treated with different doses of *Caralluma dalzielii* aqueous extract.
Photomicrograph of control (A), 150 mg (B), 300 mg (C) and 600 mg/kg of *Caralluma dalzielii* aqueous extract showing regular seminiferous tubule and Leydig's interstitial cells

The photomicrograph showed regular seminiferous tubule, interstitial cells of Leydig and spermatogenic cells. This implies that the extract may not be toxic to the testes on constant administration. However, further studies are needed to evaluate the effect of the extract on sperm motility, sperm count and other sexual function parameter, first in rats and subsequently in humans.

In conclusion, the aqueous extract of the aerial parts of *Caralluma dalzielii* caused an increase in serum testosterone level in the treated rats and hence could be promoted as a sexual booster. In addition, histological results of the testes did not produce any deleterious effect in the organ. This in part, validates the claim by the traditional healers that aqueous extract of *C. dalzielli* is an aphrodisiac.

Conflict of interest. The authors declare that there is no conflict of interest.

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