

APHRODISIAC ACTIVITY OF AQUEOUS AND HEXANE ROOT EXTRACTS OF AFRICAN LABURNUM (*CASSIA SIEBERIANA* DC.) IN ALBINO RATS

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ABSTRACT

The root of *Cassia sieberiana* is among the medicinal plants used to enhance sexual libido in Northern Nigeria without any scientific proof. This study therefore, was undertaken to evaluate the aphrodisiac activity of aqueous and hexane root extracts of the plant on albino rats. Rats were randomized into eight groups of five each. *C. sieberiana* extracts at doses of 100, 200 and 400 mg/kg body weight were orally administered once daily for 21 days and Sildenafil citrate was used as positive control. Sexual behaviour parameters were monitored and effect of the extracts on serum reproductive hormones and cholesterol were determined using standard procedure. Acute and sub-acute toxicity study was also carried out to determine the effects of the extracts on liver and kidney function indicators. The extracts significantly ($P < 0.05$) increased mount and intromission frequencies but significantly decreased mount and intromission latencies in a dose dependent manner. Only the dose of the extracts at 400mg/kg of the extracts prolonged ejaculatory latency. Hormonal assay shows significantly higher ($P < 0.05$) testosterone, luteinizing hormone and follicle stimulating hormone levels compared to the control. No significant ($P < 0.05$) effect on the concentration of prolactin was observed. Thus, the results of the study suggest that the extract of *C. sieberiana* exert significant aphrodisiac activity at the administered doses.

Keywords: *C. sieberiana*, Hormonal assay, Aphrodisiac, Sexual behaviour.

INTRODUCTION

Aphrodisiacs are agents or compounds known to enhance erectile function, sexual desire, performance, and overall enjoyment (Wani *et al.*, 2011; Ehigiator and Ozolua, 2024). These substances often act by crossing the blood-brain barrier, stimulating the medial preoptic area (mPOA) of the anterior hypothalamus in the central nervous system, or modulating the influence of the sympathetic nervous system (Patel *et al.*, 2011). Some aphrodisiacs improve the nutritional value of foods, thus contributing to better health and enhanced sexual performance, while others function physiologically to increase blood flow to the penile area, thereby improving erection and addressing sexual dysfunction (Salmon, 1983; Kayode and Yakubu, 2016).

Sexual inadequacy is a common condition that affects the sexual life of millions of men globally, with a prevalence of (36%) among Nigerian males (Muhammad *et al.*, 2023). Historically, many locally accepted plants/preparations were used as an alternative to improve sexual performance. These preparations were frequently displayed and advertised by itinerant herbalists in the market, bus

stations or places of worship. One such plant, *Cassia sieberiana* DC. (Caesalpiniaceae), commonly known as African laburnum (*Margaa* in Hausa), is widely used by the people of Northern Nigeria for its reputed aphrodisiac effects. However, scientific validation of its efficacy remains lacking. This study aims to evaluate the effects of aqueous and hexane root extracts of *C. sieberiana* on the sexual behaviour of male albino rats.

MATERIALS AND METHODS

Standard Aphrodisiac Drugs

Sildenafil citrate, estradiol and progesterone were obtained from Concord Pharmacy Ltd, No. 29 Aliyu Jodi Road, Sokoto.

Plant collection and identification

Cassia sieberiana (roots) was obtained from a nearby forest, Dajin Adarawa, Wamakko Local Government, Sokoto State, Nigeria. A Taxonomist authenticated the plant species in the Botany unit, Biological Science Department, Usmanu Danfodiyo University, Sokoto. A voucher specimen (UDUH/ANS/0242) was deposited at the Herbarium of the University.

Experimental animals

Healthy, male rats (*Rattus norvegicus*), 2–2.5 months old weighing between 180–200g and females, 1.5–2 months old weighing between 155–165g were obtained from the animal house, Usmanu Danfodiyo University Sokoto, Nigeria. The animals were kept in well-aerated laboratory cages in well-ventilated rooms under supervision in the animal house with free access to feed (Vital Agricultural Feed Nigeria Ltd) and water *ad libitum*. The animals were kept in the same environment for two weeks to acclimatize.

Preparation of the Plant Extracts

The preparation of the extract was carried out according to the method of Hassan *et al.* (2013). The roots of *C. sieberiana* were cleaned with distilled water, shade-dried in the laboratory and ground to fine powder using pestle and mortar. An equal amount of the dried powder (500g) was macerated separately using 2 L of normal hexane and 2 L of distilled water for 72 hours and the resulting mixture was filtered with muslin cloth first and then with filter paper Whatman No. 1. The filtrates were concentrated using a rotary evaporator (RE –52A, Shanghai Ya Rong Biochemistry Instrument Factory, China) and then evaporated to dryness. The dried extracts were stored in plastic containers and kept at room temperature.

Animal Grouping and Extract Administration

A total of eighty (80) albino rats made up of an equal number of both sexes were used for this study. Forty (40) male albino rats were completely randomized into eight groups (I–VIII) of 5 rats each. Rats in group I (normal control) and II (positive control) were administered orally once daily with 1mL of distilled water and a standard drug, Sildenafil citrate (5mg/kg body weight) respectively using a metal oropharyngeal cannula. Rats in groups III, IV, and V were orally administered with 100, 200 and 400mg/kg body weight of the aqueous extract of *C. sieberiana* respectively. Rats in groups VI, VII, and VIII were orally administered 100, 200 and 400mg/kg body weight of the hexane extract of *C. sieberiana* respectively. The oral administration of the extract was carried out daily using an orogastric tube (cannula) daily for 21 days.

Preparation of female rats

Female rats were housed in separate cages with food and water *ad libitum*. The female rats were artificially brought to oestrus by administering estradiol benzoate 10 µg/100 g body weight orally 48 h before mating and progesterone injected subcutaneously at a dose of 0.5 mg/100 g 6 h before the mating (Szeclman *et al.*, 1991; Yakubu *et al.*, 2005). Receptivity was tested by pairing with a normal male rat before experimental testing and the female rat which was sexually active was termed sexually receptive female.

Mating behaviour test

The test was carried out on the 22nd day after the extract administration by adopting the methods described by Yakubu and Akanji, (2011). The investigation was conducted between 19 and 22 h in the same laboratory under a dim light. Each male rat was placed individually in a rectangular plastic transparent cage 10 minutes before the introduction of a primed female to get acclimatized to the cage environment. The sexually receptive female was silently introduced into the cage with one female to one male ratio. Sexual behaviours of a male with a female were observed from the cage side preceptive and pre-copulatory behaviours. Sexual behaviour parameters were monitored for 30 minutes using camera Amkov Digital Camera (5.0 fitted MP CMOS Sensor) and direct observation from the cage side.

Measurement of Sexual Parameters

According to the standard and basic procedures described by Yakubu and Akanji, (2011), the following male sexual parameters were calculated for the observatory period: mount frequency (MF),

intromission frequency (IF), and ejaculation frequency (EF). The female rats were also observed for the presence of vaginal plugs. In addition, other standard parameters of sexual behaviour obtained through manual data acquisition using a stopwatch included mount latency (ML), intromission latency (IL), ejaculation latency (EL) and post-ejaculatory interval (PEI).

Measurement of sexual organ weight

After the sacrifice, the rats were thereafter quickly dissected and organs like testis, seminal vesicles, and prostate glands were carefully removed and their weight was recorded.

Hormonal analysis

The serum testosterone, follicle-stimulating hormone (FSH), luteinizing hormone (LH) and prolactin concentration were determined quantitatively using the Microplate Enzyme Immunoassay kit as described in the manufacturer's test procedure (Accu-Blind ELISA Microwells, Product code: 53225–300, Monobind Inc., USA).

Statistical Analysis

All data are presented as mean ± Standard Error of Mean (SEM). Values were analyzed using Instat software (Version U.S.A). Statistical significance of the difference between means was carried out using a one-way analysis of variance (ANOVA). P<0.05 was considered statistically significant.

RESULTS

Effects of Aqueous and Hexane Extract of Roots of *C. sieberiana* in Sexual Behaviour of Male Albino rats.

Oral administration of aqueous and hexane extracts of *C. sieberiana* root for 21 days enhanced sexual behaviour in male rats in a dose-dependent manner (Table 1). The aqueous extract significantly increased mounting frequency (MF), intromission frequency (IF), and ejaculation latency (EL), while the hexane extract significantly influenced MF and ejaculation frequency (EF), but not IF at 100 mg/kg. Neither extract significantly affected EF at 100 and 200 mg/kg. Both extracts significantly reduced mount latency (ML) and intromission latency (IL) at higher doses. Sildenafil citrate also significantly increased MF, IF, and EL, and decreased ML and IL. Overall, the aqueous extract showed superior aphrodisiac activity compared to the hexane extract.

Table 1: Effect of Doses of Aqueous and Hexane Extract of roots of *Cassia sieberiana* root on Mating Behaviours of Rats

| Treatment groups | Doses (mg/kg body weight) | Mount Frequency (MF) | Intromission Frequency (IF) | Ejaculation Frequency (EF) | Mount Latency (in Sec) | Intromission Latency (in Sec) | Ejaculation Latency (in Sec) |
|---|-----------------------------|----------------------|-----------------------------|----------------------------|------------------------|-------------------------------|------------------------------|
| Group I (Negative Control) | Distilled Water | 4.0±0.51 | 3.2±0.37 | 2.0±0.20 | 63.4±4.34 | 86.0±5.84 | 272.8±6.11 |
| Group II (Positive Control) | Sildenafil citrate (5mg/kg) | 11.0±0.71* | 9.2±0.86** | 3.4±0.34* | 37.2±2.38** | 51.4±3.11** | 392.4±9.83** |
| Group (III-V) (Aqueous Extracts) | 100 | 6.8±0.74* | 6.00±0.45* | 2.6±0.68 | 49.8±1.16** | 69.8±4.49** | 317.6±3.67** |
| | 200 | 9.2±0.49** | 8.2±1.07** | 2.6±0.24 | 41.6±3.78** | 60.2±2.99** | 345.4±7.59** |
| | 400 | 10.4±0.93* | 7.6±0.51** | 3.0±0.55* | 39.6±2.32** | 57.8±5.27** | 372.6±15.43** |
| Group VI-VIII (Hexane Extracts) | 100 | 6.4±0.51* | 5.8±0.66 | 2.2±0.37 | 54.4±1.81* | 69.2±2.15* | 307.2±4.73* |
| | 200 | 7.4±0.58** | 6.4±0.51* | 2.4±0.25 | 52.0±2.92* | 70.2±4.36* | 298.2±5.05* |
| | 400 | 6.6±0.51* | 6.2±0.66* | 2.8±0.37* | 44.2±2.44** | 67.4±3.36* | 321.2±4.65** |

Values are expressed as Mean± SEM (n= 5). Significantly different from control: *P<0.05, **P<0.01. One-way ANOVA followed by Dunnett Multiple Comparison Test

Effect of oral administration of the Aqueous and Hexane Extract of Roots of *C. sieberiana* in Reproductive Hormone in Rats.

The effect of *C. sieberiana* on reproductive hormones is presented in **Table 2**. The result showed that the aqueous extract significantly (P < 0.05) increased testosterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) levels at all doses. The hexane

extracts also significantly increased testosterone and LH at 200 and 400 mg/kg, and FSH at all doses. Sildenafil citrate similarly elevated testosterone, LH, and FSH levels. However, neither extract significantly (P > 0.05) affected prolactin levels compared to the normal control group.

Table 2: Effect of Doses of Aqueous and Hexane Root Extracts of *C. sieberiana* on Reproductive Hormones in rats

| Treatment Groups | Doses (Mg/kg body wt.) | Testosterone (ng/ml) | FSH (mIU/ml) | LH (ng/ml) | Prolactin (ng/ml) |
|--|--|----------------------|--------------|-------------|-------------------|
| Group I (Negative Control) | Distilled Water | 13.87±0.23 | 1.20±0.03 | 1.29±0.07 | 1.51±0.08 |
| Group II (Positive Control) | Sildenafil Citrate (5mg/kg body weight.) | 14.68±0.74* | 1.63±0.05* | 1.61±0.14* | 1.51±0.03 |
| Group III-V (Aqueous Extracts) | 100 | 15.87±0.49* | 1.69±0.07** | 1.48±0.06 | 1.34±0.11 |
| | 200 | 17.15±0.22** | 1.98±0.05** | 2.24±0.12** | 1.39±0.08 |
| | 400 | 18.24±0.20** | 2.22±0.14** | 2.57±0.09** | 1.28±0.07 |
| Group VI-VIII (Hexane Extracts) | 100 | 13.42±0.76 | 1.59±0.04* | 1.19±0.09 | 1.39±0.06 |
| | 200 | 16.29±0.46** | 1.61±0.15* | 1.59±0.07* | 1.43±0.05 |
| | 400 | 15.15±0.77* | 1.65±0.11* | 1.62±0.08* | 1.30±0.06 |

Values are expressed as Mean \pm SEM (n= 5). Significantly different from control: * $P < 0.05$, ** $P < 0.01$. One-way ANOVA followed by Dunnett Multiple Comparison Test. FSH-Follicle Stimulating Hormone, LH-Luteinizing Hormone.

DISCUSSION

Mating Behaviour Study

The administration of aqueous and hexane extracts of *C. sieberiana* root significantly enhanced sexual behaviour in male rats, with the aqueous extract showing greater efficacy across all tested doses. Mount frequency (MF) and intromission frequency (IF), which are important indicators of sexual arousability, vigour, and potency (Yakubu and Akanji, 2011), were significantly increased. Specifically, MF reflects sexual motivation, while IF indicates erectile efficiency and the ease of initiating ejaculation (Yakubu and Akanji, 2011). The significant rise in these parameters suggests that *C. sieberiana* possesses aphrodisiac properties, enhancing libido, performance, and vigour.

These findings are supported by previous research on aphrodisiac plants such as *Myristica fragrans* (Tajuddin *et al.*, 2005), *Fadogia agrestis* (Yakubu *et al.*, 2007), *Pseudarthria viscida* (Christopher *et al.*, 2015), and *Tapinanthus globiferus* (Ashafa *et al.*, 2023).

Additionally, the extracts significantly reduced mount latency (ML) and intromission latency (IL), both of which are inversely related to sexual motivation (Yakubu and Akanji, 2011). These reductions imply enhanced sexual drive and arousability in the treated male rats, aligning with findings by Ratnasoorinya *et al.* (2007), Yakubu and Afolayan (2008), Christopher *et al.* (2015), and (Ashafa *et al.*, 2023).

The significant increase in ejaculation latency (EL) observed in extract-treated animals suggests prolonged coital duration, further indicating heightened sexual motivation. Pelvic thrusting behaviour during intromission and ejaculation, also noted in the treated rats, is a sign of proper penile-vaginal contact, potentially stimulating lordosis in females (Agmo, 1997; Yakubu and Akanji, 2011). These findings are consistent with the report by Zade *et al.* (2013), affirming the sexual function-enhancing effects of *C. sieberiana* extracts. The aqueous extract, especially at 200 and 400 mg/kg, demonstrated superior aphrodisiac activity compared to the hexane extract. Interestingly, Watcho *et al.* (2007) reported an opposite trend for *Mondia whitei*, where the hexane extract was more potent than the aqueous form. This may be attributed to phytochemical variability between species and solvents. It is possible that the bioactive aphrodisiac compounds in *Cassia sieberiana* are more polar and thus more efficiently extracted in water compared to hexane, whereas *Mondia whitei* may contain more non-polar bioactive constituents.

Effect on Hormone Levels

The administration of *C. sieberiana* root extracts significantly increased testosterone, luteinising hormone (LH), and follicle-stimulating hormone (FSH) levels in a dose-dependent manner, while prolactin levels remained unaffected. These hormones are closely linked to male sexual behaviour, as androgens influence libido and erection through both central and peripheral nervous systems (Moreli *et al.*, 2006). Testosterone enhances sexual function and orgasm intensity (Grahl *et al.*, 2007; Morales, 1996), while LH and FSH, produced by the anterior pituitary, regulate testosterone synthesis and spermatogenesis (Achard *et al.*, 2009; Wistuba *et al.*, 2007). Increases in LH and FSH, therefore, lead to elevated testosterone levels (Yakubu *et al.*, 2007), potentially

explaining the improved sexual behaviour observed. Similar hormonal effects were noted in studies involving *Zanthoxylum leprieurii* and *Piper guineense* (Kpomah *et al.*, 2012), and *Citrullus colocynthis* (Chakraborty *et al.*, 2018). While prolactin typically suppresses LH and FSH and is linked to reduced sexual function when elevated (Paick *et al.*, 2006), no significant changes in prolactin were found in this study, differing from Kpomah *et al.* (2012).

Conclusion

Based on the findings of this study, the aqueous and hexane root extracts of *Cassia sieberiana* significantly enhanced sexual behavior in male albino rats, likely through stimulation of testosterone, LH, and FSH. The aqueous extract exhibited greater efficacy compared to the hexane extract. These results provide scientific evidence supporting the traditional use of *C. sieberiana* as an aphrodisiac in Northern Nigeria.

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