Antifertility activity of *Cryptolepis sanguinolenta* leaf ethanolic extract in male rats

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ABSTRACT

BACKGROUND: Complementary medicine has grown over time with more botanicals emerging and remaining integral parts of medicare. Such botanicals include *Cryptolepis sanguinolenta*. **AIM:** This study investigated the effect of Cryptolepis sanguinolenta leaf ethanolic extract on male reproductive system using rat model. **MATERIALS AND METHODS:** Control and treated rats were maintained on control diet. Treated rats also received graded doses of the extract. **RESULTS:** When compared with the controls, *Cryptolepis sanguinolenta* treatment led to significant testosterone suppression associated with consequent significant rise in luteinizing hormone (LH) and decrease in sperm count. Treatment with *Cryptolepis sanguinolenta* did not result in significant attenuation of follicular stimulating hormone (FSH) levels and testicular morphometry. Sperm viability, motility, and morphology were also comparable in all groups. **CONCLUSION:** These results suggest that *Cryptolepis sanguinolenta* possesses anti-androgenic and anti-spermatogenic properties with potential anti-aphrodisiac activity.

KEY WORDS: *Cryptolepis sanguinolenta,* FSH, LH, sperm, testes, testosterone

INTRODUCTION

The use of plants in the management of illnesses has been since time antiquity, and has continuously grown over time. Though western medicine has influenced the use of herbal remedies, most rural communities still practice complementary medicine as they are readily and cheaply available healthcare alternatives.[1] Complementary medicine co-exists with the medicare of most societies and is based on the use of natural and local products related to the people's perspective on the world and life.[2,3] Plants thus remain a major constituent of life in many communities in the world[4,5] and their utilization in medicare is still well-disseminated around the world.[6-9]

Cryptolepis sanguinolenta is one of the commonly used plants for its anti-malarial[10-16] and anti-diabetic activities, particularly in Nigeria and Ghana.[17-20] It has also been reported to have anti-cancer,[21] anti-microbial,[22-28] anti-thrombotic,[29] and anti-inflammatory potentials.[30,31] The biological activities of its different morphological parts have been attributed to its alkaloid constituents. Cryptolepine, an alkaloid, is the major bioactive principle of the plant.[32] In addition to cryptolepine, other minor alkaloids and their salts that have been isolated include the hydrochloride and the 11-hydroxy derivatives of cryptolepine, cryptoheptine, iso- and neo-cryptolepine, quindoline, biscryptolepine, cryptoquindoline, cryptospirolepine, cryptosanguinolentine, cryptotakienine, and cryptomisrine.[33-37]

Though the therapeutic efficacy of *C. sanguinolenta* extract in the treatment of a plethora of human illnesses has been established, it is pertinent to evaluate its effects on other systems. This study consequently sought to determine the effect of ethanolic extract of *C. sanguinolenta* leaf on male reproductive profile in experimental paradigm.

MATERIALS AND METHODS

Plant material

Fresh leaves of *C. sanguinolenta* were obtained from Womirere, Iresi, Osun state and identified by Ugbogu A, Chukwuma E.C,

and Shasanya O.S, Forestry Herbarium, Ibadan, Nigeria where a specimen has been deposited (voucher number FHI.108847).

Preparation of extract

C. sanguinolenta leaves air-dried and milled. 526 g of the milled leaves was extracted in 65% v/v ethanol. After the 3rd day, the leaf extract was separated from the leaf with a cloth sieve. For absolute separation of the leaf from the extract, filter paper was used to sieve the extract into a bottle. The extract was then taken to the laboratory for the process of evaporation. The evaporation process involved the total removal of ethanol and water with which the extraction took place from the extract. The extract was concentrated using a rotary evaporator at 40°C. 0.1 g/ml stock solution was then used for the experiment.

Animal

Experiment was performed with male albino rats of Wistar strain of comparable weight. The animals were allowed to acclimatize to the laboratory condition (12:12h light/dark cycle at 25°C ± 2) for 2 weeks and fed on rat chow and water without restriction. The study was approved by the ethical committee of the department, and all procedures were in accordance to the National Institute of Health Guidelines for the Care and Use of Laboratory Animals (NIH, department of Health and Human services publication no. 85-23, revised 1985).

Experimental design

Rats were randomly divided into 4 equal groups. The control was given 1 ml of distilled water (vehicle for extract). Group I, II, and III were given 50, 150, and 250 mg/kg of the extract, respectively. The vehicle and extract were administered orally for 21 days. After the experimental period, blood samples were collected from each rat into plain bottles via cardiac puncture for hormonal assays, and testes were removed from post-euthanized rats.

Ethics

This study was approved by the ethics committee. All animals received humane care in compliance with the institution's guideline and criteria for humane care as outlined in the National Institute of Health Guidelines for the Care and Use of Laboratory Animals.

Determination of testicular morphometry

The testes were excised, blotted with tissue paper, and weighed. The length and diameter were also measured.

Determination of FSH, LH, and testosterone

Serum FSH, LH, and testosterone concentrations were estimated by the enzyme-linked immunosorbent assay (ELISA) using standard assay kits following the manufacturers' instructions.

Estimation of sperm quality/semen analysis

The testes were removed along with the epididymis. The caudal epididymis was separated from the testis and lacerated to collect semen onto the microscope slide.[38] Sperm characteristic was evaluated as described by Woode *et al*.[39] using 2.9% sodium citrate as buffer and 10 mL phosphate buffer saline for sperm motility and count, respectively. Briefly, sperm motility was examined under the microscope, and sperm count was carried out in the improved Neubauer hemocytometer and calculated. Sperm viability was evaluated using the eosin-nigrosin stain technique. 2 drops of the stain was mixed with semen. A thick smear was prepared, air-dried and examined under microscope. Live/viable sperm cells were unstained while dead/non-viable sperm cells appeared stained. The live and dead sperm were counted and the percentage of each calculated. Sperm morphology was done using 2 drops of Walls and Ewas stain and air-dried and examined under the microscope. The normal sperm cells were counted and the percentage calculated.

Histological study

Testicular tissues were transferred into 10% formalin after being fixed in Bouin's fluid for 6h. They were dehydrated with varying percentage of ethanol; sections were cleared in xylene and embedded in molten wax. Thin sections were cut (5 μm), stained with hematoxylin and eosin, and microscopically analyzed.

Statistical analysis

Results are expressed as Mean ± SEM ($n = 6$). The difference between the means was determined by one-way Analysis of Variance (ANOVA) complemented with unpaired t-test. In all statistical tests, a value of $P < 0.05$ was considered significant.

RESULTS

Testicular morphology was comparable in all groups. Though testicular weight, length, and diameter were altered following *Cryptolepis sanguinolenta* leaf extract administration, the morphometric changes were not statistically significant [Figure 1].

FSH was statistically similar in all groups while LH was significantly raised in the treated groups when compared with the control. On the other hand, testosterone was significantly reduced in the treated groups when compared with the control in a dose-related manner. The rise in LH and fall in testosterone observed in the treated groups were statistically comparable across the treated groups [Figure 2].

Sperm motility, viability, and morphology was not statistically different across all groups, however, sperm count was statistically reduced in the treated groups when

compared with the control. Similar to LH and testosterone changes, sperm count was reduced in a dose-dependent manner in the treated groups. Treatment of animals with 50 mg/kg of the extract showed the highest reduction in testosterone level, sperm count and a higher rise in LH concentration. However, hormonal and sperm count changes observed across the treated groups were not statistically different [Figure 3].

Histomorphological observations revealed that administration of the extract did not cause any alteration in the testicular tissues of rats treated with 50 and 150 mg/kgBW of the extract though rats treated with 250 mg/kgBW of the extract showed mild distortion of the seminiferous tubules.

DISCUSSION

Plants and their products are integral parts of medicare. They are also a major source of most formulated drugs in western medicine. None of these forms of therapy are, however, without side effects ranging from mild to severe. Though the side effects of a drug could be used for therapeutic purposes in other conditions, it is necessary to evaluate the effects of medicinal plants, their products, and formulated drugs commonly used in the treatment of

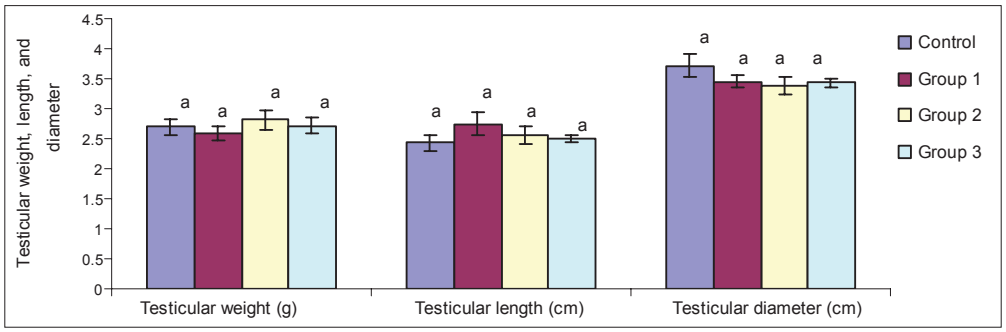

Figure 1: Effect of administration of ethanolic extract of *Cryptolepis sanguinolenta* leaf on testicular morphometry Bars carrying same letters, a, as controls on each variable are statistically not different at *P*<0.05

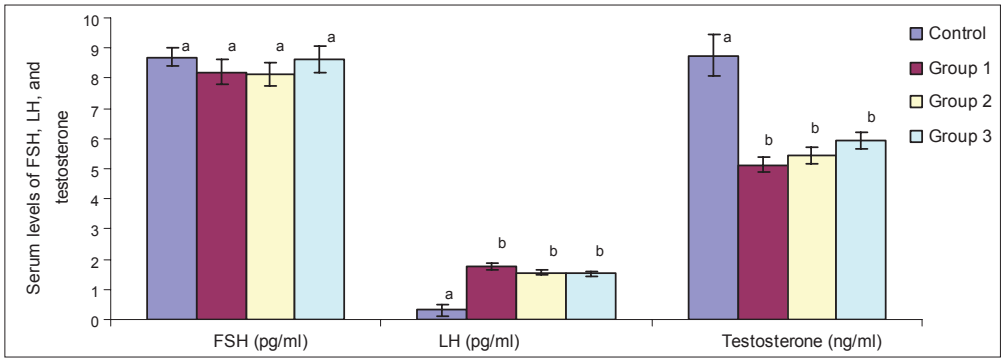

Figure 2: Effect of administration of ethanolic extract of *Cryptolepis sanguinolenta* leaf on male reproductive hormones Bars carrying same letters, a, as controls on each variable are statistically not different at *P*<0.05

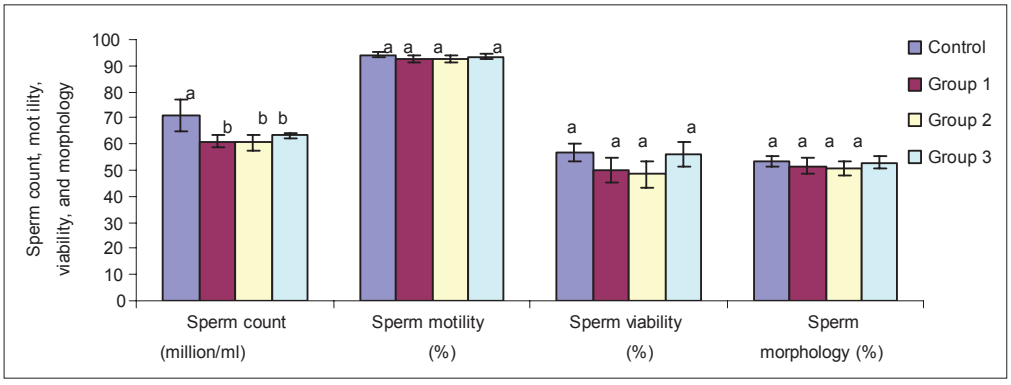

Figure 3: Effect of administration of ethanolic extract of *Cryptolepis sanguinolenta* leaf on sperm profile Bars carrying same letters, a, as controls on each variable are statistically not different at *P*<0.05

ailments on other organs/systems to determine their side effects/adverse effects and possibly their beneficial effects on other pathological conditions. This led us to study the pharmacological effect of *C. sanguinolenta* on the male reproductive system. This seems to be the first study to account for the influence of *C. sanguinolenta* on the male reproductive indices.

The male reproductive system is a complex that consists of the hypothalamus, anterior pituitary gland, the testes,[40] and intimately related organs like prostate gland, seminal vesicle and bulbourethral glands. These structures work together to maintain the potentness, fertility, and male secondary sexual characteristics. Results from this study showed that *C. sanguinolenta* did not alter testicular morphometry. This suggests that the plant extract does not affect the gross anatomy of testes. This is in conflict with the previous study of Ansah *et al*.[41] that documented reductions in testes weights following *C. sanguinolenta* treatment, especially in animals treated with 1000 mg/kgBW of the extract. The inconsistency seen in this study could be dose-dependent.

The results from this study demonstrate that administration of *C. sanguinolenta* caused a significant reduction in testosterone level, and a rise in LH concentration, but a normal FSH level. The increase in LH observed in the treated rats is in attendant with the *C. sanguinolenta*-induced testosterone suppression. Suppression of testosterone is expected to be consequently accompanied with increase in LH and FSH concentrations in an attempt to stimulate the production of more testosterone. The normal level of FSH seen in association with testosterone suppression suggests that the hypothalamic cells, which are responsible for the synthesis and release of gonadotropin-releasing hormone (GnRH), do not function correctly when testosterone levels decrease.[42] Though this feedback seems to be maintained for LH release as it increased following testosterone decline. A potential mechanism through which *C. sanguinolenta* may reduce testosterone levels is its aromatization into estradiol or impairment of the conversion of one or more of its precursors. Low testosterone levels have been associated with decreased reproductive ability.[42] Hence, it appears that the anti-fertility potential of *C. sanguinolenta* is mediated at all 3 levels of the male reproductive axis: The hypothalamus, pituitary, and testes.

Sperm characteristics are important reproductive indices as they account for male fecundity. The observation that low sperm count induced by *C. sanguinolenta* treatment in animals is associated with testosterone suppression is consistent with previous findings.[41] A possible explanation for these observations could be attributed to the alkaloids contained in the botanical extract. Alkaloids-containing *C. sanguinolenta* has been documented to possess anti-muscarinic,[43] α-adrenoceptor antagonistic,[30] or/and cytotoxic[21,44] activities. Anti-muscarinic agents have been reported to reversibly impair male fertility by an unknown mechanism.[45,46] a-adrenoceptor antagonists have been documented to inhibit sperm emission[47,48] via inhibition on both the neutrally-evoked contractions on vas deferens and sperm transport from the caudal epididymis to the distal vas deferens.[49-51] Its cytotoxic effect may cause a direct destructive effect on the sperm cells,[52] with consequent reduced sperm count. However, the botanical did not cause any alteration in the sperm motility, viability, and morphology. The distortion of the seminiferous tubules observed in *C. sanguinolenta* treatment could also be ascribed to its cytotoxic activities.

In conclusion, we have demonstrated that the ani-fertility activity of *C. sanguinolenta* is associated with its anti-muscarinic,α-adrenoceptor antagonistic and cytotoxic effects. This study suggests that *C. sanguinolenta* treatment exerts quantitative ani-androgenic and ani-spermatogenic effect.

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