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

**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, bis-cryptolepine, cryptoquinidine, cryptospirepine, 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 Ugboagu A, Chukwuma E.C,
Manufacturers' instructions.

**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.

**Results**

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 in a dose-related manner.

**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].
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
The results from this study demonstrate that administration inconsistency seen in this study could be dose-dependent. Though this feedback seems to be maintained (GnRH), do not function correctly when testosterone levels synthesis and release of gonadotropin-releasing hormone seen in association with testosterone suppression suggests production of more testosterone. The normal level of FSH expected to be consequently accompanied with increase in testosterone suppression. Suppression of testosterone is treated rats is in attendant with the normal FSH level. The increase in LH observed in the testosterone level, and a rise in LH concentration, but a...
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Source of Support: Nil. Conflict of Interest: None declared.

How to cite this article: Ajayi AF, Akhigbe RE. Antifertility activity of Cryptolepis sanguinolenta leaf ethanolic extract in male rats. J Hum Reprod Sci 2012;5:43-7.