THE ANTIMALARIAL HERBAL *CRYPTOLEPSIS SANGUINOLENTA* (LINDL.) SCHLTR IS ANTIANDROGENIC.

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Abstract

The aqueous root extract of *Cryptolepis sanguinolenta* (Periplocaceae), a popular West African antimalarial herbal had previously been reported to reduce sperm count in the caudal epididymis and fertility in male mice. The objective of the present study was to examine further, the effects of the extract on male reproduction. Using the rodent Hershberger bioassay, mounting behaviour, serum hormones and the chick comb method for androgenic activity, the effects of cryptolepis on male reproduction were studied. Cryptolepis extract (62.5 – 1000 mg/kg) decreased libido as measured by the mounting behaviour studies. The extract also decreased serum testosterone in male mice at all doses tested. Additionally, it inhibited testosterone-induced growth of male accessory organs and disrupted the arrangement of the seminiferous tubules in the testes. In the chick comb method for androgenic activity, the effects of the aqueous extract were similar to cyproterone. These results confirm the anti-androgenic activity of the aqueous extract of *Cryptolepis sanguinolenta*.

Keywords: cryptolepis, testosterone, anti-androgenic, mounting behavior.

1. INTRODUCTION

Malaria is endemic in the West Africa. In the rural communities, where health facilities are limited, the use of herbal medicines for the treatment of malaria is widespread. Among the herbal remedies used for the treatment of malaria in the region, *Cryptolepis sanguinolenta* (Periplocaceae) is very popular [1, 2]. The aqueous root extract of the plant and its major alkaloid, cryptolepine (Figure 1) have diverse biological actions [1-6]. We reported previously that cryptolepis, the aqueous root extract of the plant reduced sperm count in the caudal epididymis and fertility in male mice and inhibited foetal development and reproduction in female mice [3]. Recent evidence also suggests that the ethanolic leaf extract of the plant caused reduced fertility in male rats [7]. Notwithstanding the differences in the part of the plant used in the two studies, the vehicle for extraction and the animal species used [3, 7], the studies are complementary and together suggest strongly that the antimalarial plant could adversely affect reproduction and foetal development in mammals. It is known that some therapeutic drugs, conventional and herbal alike, used at their therapeutic doses exert negative effects on male fertility [8, 9].

![Figure 1: Cryptolepine, the major alkaloid from Cryptolepis sanguinolenta](http://www.urpjournals.com)
2. MATERIALS AND METHODS

2.1 Plant material.
Cryptolepis sanguinolenta roots were obtained and authenticated at the Centre for Scientific Research into Plant Medicine, Mampong-Akwapim, Ghana, where it is used as one of the agents for the treatment of malaria. The roots of the plant were prepared to simulate the traditional method of preparation. Briefly, dried cryptolepis roots (1 kg) was milled and extracted by boiling with 10 litres of distilled water for 30 minutes. The solution was filtered and the filtrate after cooling was freeze-dried to obtain cryptolepis, a yellowish-brown powder. The percentage yield was 9.3% w/w. Cryptolepis was freshly prepared in water and administered by gavage to the experimental animals.

2.2 Animals
ICR mice (20-30 g) and Sprague-Dawley rats (100 -120 g) were purchased from Noguchi Memorial Institute for Medical Research, University of Ghana, Legon, Accra, Ghana and maintained in the animal house of the Department of Pharmacology, College of Health Sciences, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana. They were housed in stainless steel cages (34 x 47 x 18 cm) with soft wood shavings as bedding, and fed with normal commercial pellet diet (GAFCO, Ghana) and given water ad libitum.

Day-old single comb white leghorn chicks, Gallus domesticus were obtained from Asamoah and Yamoah farms at Kegya, Kumasi, Ghana and kept in the animal house of the Department of Pharmacology, KNUST for 14 days for acclimatization. All animals were humanely handled throughout the experiment in accordance with internationally accepted principles for laboratory animal use and care (EEC Directive of 1986: 86/609 EEC). Additionally, all animal experiments were approved by the departmental ethics committee.

2.3 Chemicals
The following chemicals were obtained from the indicated sources. Testosterone propionate (JinLing Pharmaceutical, China), cyproterone acetate (Bayer Pharmaceuticals, Germany), ELISA kit for testosterone, prolactin, follicle stimulating hormone (FSH) and leutinizing hormone (LH) (Fortress Diagnostics Limited, United Kingdom).

2.4 Effect of Cryptolepis sanguinolenta Extract on mounting behaviour in mice.

To quantify mounting behaviour, experiments were designed as previously described by Lawler [13] to measure the libido of the male mice [14, 15]. Mount is defined as the male assuming the copulatory position but failing to intromit and an attempted mount is an incompetent mount in which the orientation is wrong, such as mounts of the female’s head or side. Male mice were treated with saline (control group) or cryptolepis (62.5-1000 mg/kg, p.o.) for fourteen days. On day fourteen, male mice were placed individually in a plexiglas cage (60 x 75 x 20 cm). After 15 minutes of acclimatization, a non-estrous female was introduced into the arena and the numbers of mounts, anal sniffs, penile licks, attempted mounts were recorded during a 15-minute observation period.

2.5 Effect of cryptolepis extract on serum hormone levels in mice.

To measure the effect of cryptolepis on serum hormone levels, five groups of male mice (n=10) were treated with either distilled water or cryptolepis (62.5, 100, 500, 1000 mg/kg p.o.) for 14 days. Mice were sacrificed on day 14 and blood was collected into vacutainer tubes. The blood was centrifuged at 500 g for 15 min and serum was collected and stored at -20 °C. Hormone assays were carried out as described by the manufacturer by competitive enzyme immunoassay.

2.6 Effect of cryptolepis extract on testicular histology in mice.

Testicles were taken from the experiment above for histological studies. Transverse sections were made for testicles at each dose level. Sections were fixed in formalin solution (10 % neutral buffered, Sigma Co. Ltd). They were later embedded in paraffin and stained with hematoxylin and eosin. Digital images were acquired with a camera mounted on a Nikon E400 microscope using x4 and x10 objectives.

2.7 Effect of cryptolepis extract on rat male accessory sex organs in the Hershberger Bioassay

Male Sprague-Dawley rats, 45 days old were castrated and kept for two weeks to allow for recession of androgen dependent tissues. To determine the effects of cryptolepis on androgen dependent tissues, castrated rats were grouped into 4 (n=6). Group 1 received distilled water and groups 2, 3, 4 received 62.5, 500, 1000 mg/kg p.o. cryptolepis respectively for 10 days. Castrated rats in all groups received 4 mg/kg of testosterone propionate s.c. 30 minutes daily before the administration of cryptolepis and distilled water. On the 11th day all rats were euthanized and seminal vesicles, ventral prostate, glans penis, Cowper’s glands and levator ani-bulbo cavernous muscle, kidney, liver and adrenal glands were carefully dissected and weighed.

2.8 Evaluation of cryptolepis extract in Chick comb method for androgenic activity

The Chick comb method [16] was used with modification to evaluate the androgenic activity of the aqueous extract of cryptolepis. On Day 14 chicks were randomly assigned to one of ten groups (n = 8) and treated as follows; group 1 served as control receiving only distilled water. Group II, III, IV, received 0.2, 0.5, 1 mg/kg i.m of testosterone propionate respectively for five days. Group V, VI, VII received 3, 10, 30 mg/kg p.o. of cyproterone acetate for 5 days. Group VIII, IX, X received cryptolepis 62.5,100,1000 mg/kg p.o for 5 days. Prior to the beginning of the assay, the length and height of each chick comb was determined. Twenty four hours after the last drug administration, the growth of the comb was estimated as the product of the mean change in length and height.

In another experiment to determine the effect of cryptolepis on testosterone-induced growth of the chick comb, single comb white leghorn chicks were grouped into five (n=6) and treated as follows:

Group 1: Distilled water only
Group II: 0.7 mg/kg testosterone i.m
Group III: 300 mg/kg cryptolepis p.o. followed by 0.7 mg/kg testosterone i.m after 30 minutes
Group IV: 600 mg/kg cryptolepis p.o. followed by 0.7 mg/kg testosterone i.m. after 30 minutes
Group V: 10 mg/kg cyproterone p.o. followed by 0.7 mg/kg testosterone i.m. after 30 minutes

2.10 Statistical Analysis
All results were analyzed with graph pad prism version 5. Results were presented as either mean ± SEM and analyzed with one-way analysis of variance (ANOVA) using Neuman-Keuls post hoc test.

3. RESULTS AND DISCUSSION
The aqueous root extract of Cryptolepis sanguinolenta has been used as an anti-malarial agent for many years in the West Africa region. Our previous report on the effect of aqueous root extract of cryptolepis on reproduction and foetal development in mice [3] warranted further studies into the potential reproductive toxicity of the plant. Our report on the effect of the aqueous extract on ovulation in rabbits [17] and a recent report on the effect of the ethanolic leaf extract in male rats [7] are contributions to the effect of the plant on reproduction in mammals. In Ghana, traditional medical practitioners claim sexual benefits for male users of the aqueous root extract of the plant. In the present study, we investigated the claim by the traditional medical practitioners in the mounting behaviour study in mice [13]. In contrast to the claim by traditional practitioners, treatment of mice with cryptolepis for 14 days caused a decrease in mounting behaviour relative to controls (Figure 2). Mounting and attempted mounts were significantly lower than controls particularly at 62.5 and 1000 mg/kg (p < 0.001) (Figure 2). Cryptolepis did not however reduce penile licks (Figure 2).

Male mice were treated with cryptolepis for 14 days. Serum FSH, LH, prolactin and testosterone were then measured. Cryptolepis caused a significant (p < 0.05) reduction in serum testosterone in treated animals at all doses (Table 1) Cryptolepis treatment did not affect serum prolactin and its effect on serum FSH was inconsistent. At doses of 62.5 - 500 mg/kg, cryptolepis treatment increased serum LH, though not statistically significant but at 1000 mg/kg, it reduced serum LH (Table 1).

Testicles were extracted from mice for histological studies after 14-day treatment with cryptolepis. Transverse sections were made for testicles at each dose level. The photomicrograms of the testes showed that cryptolepis disrupts the histarchitecture of the testes particularly at 1000 mg/kg (Figure 3).

Figure 3: Photomicrogram of tranverse sectioning of testes showing arrangement of seminiferous tubules after 14-day treatment with Cryptolepis sanguinolenta. A) Control (B) 62.5 mg/kg of cryptolepis (C) 100 mg/kg cryptolepis (D) 500 mg/kg cryptolepis (E) 1000 mg/kg cryptolepis

It is known that a drop in testosterone particularly androgen withdrawal such as orchidectomy leads to atrophy and marked apoptosis in male animal reproductive organs [18] and cyproterone, a testosterone antagonist has been reported to show multiple inhibitory effects on testicular weight and spermatogenesis evoking apoptosis in epididymis of male rats [18, 19]. The myriad of effects of cryptolepis on male reproduction observed in the present and previous studies [3] may be linked to a fall in testosterone levels observed in the present study. Libido is primarily dependent on testosterone levels in males. Most
likely, the reduction in mounting behaviour parameters (Figure 2) is related to the reduction in testicular and epididymal weight, epididymal sperm number and fertility as reported previously [3] and correlates well with the decreased testosterone levels observed in the present study (Table 1).

When cryptolepis was administered to castrated Sprague-Dawley rats receiving testosterone propionate, there was significant reduction in the growth of androgen-dependent accessory organs particularly at the highest dose of 1000 mg/kg (Table 2). According to the OECD guidelines [20] on anti-androgenic assays, a substance is deemed anti-androgenic if it significantly inhibits growth in at least two of the male accessory organs. In the Hershberger’s assay [20], cryptolepis affected almost all the male accessory organs. The guidelines also stipulate that agents that affect the prostate and seminal vesicles are likely to be interacting with 5α-reductase or its product dihydrotestosterone (DHT) as DHT is responsible for prostatic growth. Comb growth in chicks is highly androgen dependent. Testosterone is converted to dihydrotestosterone which is responsible for the rapid comb growth. In this study, cryptolepis dose dependently delayed comb growth in chicks (Table 3 and Figure 4). Similar effects were seen with cyproterone acetate.

Table 1: Effects of Cryptolepis on serum hormone levels

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Control</th>
<th>Testosterone 200 μg/kg</th>
<th>Testosterone 50 μg/kg</th>
<th>Testosterone 1 mg/kg</th>
<th>Testosterone 3 mg/kg</th>
<th>Testosterone 10 mg/kg</th>
<th>Testosterone 30 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prolactin (ng/ml)</td>
<td>2.63±0.613</td>
<td>0.95±0.588*</td>
<td>0.02±0.201*</td>
<td>0.004±0.004*</td>
<td>0.42±0.1488*</td>
<td>0.37±0.1333</td>
<td>0.28±0.0853</td>
</tr>
<tr>
<td>LH (mIU/ml)</td>
<td>14.5±4.032</td>
<td>15.23±0.432</td>
<td>15.67±0.078</td>
<td>14.89±0.114</td>
<td>15.45±0.034</td>
<td>14.5±0.114</td>
<td>15.45±0.034</td>
</tr>
<tr>
<td>FSH (mIU/ml)</td>
<td>12.9±4.3.417</td>
<td>7.11±3.4501</td>
<td>17.61±2.386</td>
<td>11.1±0.012</td>
<td>3.74±5.6130</td>
<td>8.10±3.011</td>
<td>9.89±2.221</td>
</tr>
<tr>
<td>Testosterone (ng/ml)</td>
<td>6.67±0.781</td>
<td>5.67±1.001</td>
<td>8.01±3.011</td>
<td>9.89±2.221</td>
<td>3.08±0.5510</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Results presented as mean±SEM. Statistical analysis is by one way ANOVA using Neuman-Keuls post hoc test. * means p < 0.05.

Table 2: Effects of Cryptolepis on rat male accessory sex organs in the Hershberger Bioassay

<table>
<thead>
<tr>
<th>Organ</th>
<th>Initial weight</th>
<th>Final weight</th>
<th>Glans Penis</th>
<th>Seminal vesicles</th>
<th>Ventral prostate</th>
<th>Levator-anibulacervenous muscle</th>
<th>Cowpers gland</th>
<th>Adrenal Gland</th>
<th>Liver</th>
<th>Kidneys</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testosterone only</td>
<td>101.3±8.822</td>
<td>100.8±15.24</td>
<td>0.1629±0.0095</td>
<td>0.4105±0.0119</td>
<td>0.1333±0.0081</td>
<td>0.4447±0.0371</td>
<td>0.0366±0.0043</td>
<td>0.0352±0.0023</td>
<td>4.808±4.452</td>
<td>0.7460±0.0454</td>
</tr>
<tr>
<td>Testosterone + cryptolepis (100 mg/kg)</td>
<td>101.2±7.031</td>
<td>101.9±8.661</td>
<td>0.1578±0.0098</td>
<td>0.3036±0.0575</td>
<td>0.125±0±0101</td>
<td>0.3476±0.0126</td>
<td>0.030±0.0043</td>
<td>0.0336±0.0023</td>
<td>4.918±3.794</td>
<td>0.7418±0.4674</td>
</tr>
<tr>
<td>Testosterone + cryptolepis (500 mg/kg)</td>
<td>125.6±11.98</td>
<td>132.6±7.019</td>
<td>0.1428±0.00093</td>
<td>0.2428±0.0449*</td>
<td>0.1345±0.0103</td>
<td>0.3794±0.0150</td>
<td>0.02366±0.0435</td>
<td>0.03346±0.00025</td>
<td>4.724±0.2511</td>
<td>0.7462±0.3598</td>
</tr>
<tr>
<td>Testosterone + cryptolepis (1000 mg/kg)</td>
<td>123.4±10.91</td>
<td>132.6±7.019</td>
<td>0.1326±0.01062</td>
<td>0.2366±0.0435*</td>
<td>0.0853±0.0163*</td>
<td>0.2870±0.02730*</td>
<td>0.02578±0.0035</td>
<td>0.03346±0.0025</td>
<td>4.770±0.4250</td>
<td>0.7262±0.04267</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SD. Statistical analysis is by one-way ANOVA using Newman-Keuls post-hoc test. * means p<0.05.

Table 3: Effects of cryptolepis, cyproterone and testosterone on the chick comb

<table>
<thead>
<tr>
<th>Dose</th>
<th>% weight change</th>
<th>% change in length</th>
<th>% change in height</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>16.2± 3.2</td>
<td>38.9± 6.730</td>
<td>144.4±53.79</td>
</tr>
<tr>
<td>Testosterone 200 μg/kg</td>
<td>24.6±1.97</td>
<td>66.93±6.130*</td>
<td>188.9±36.18</td>
</tr>
<tr>
<td>Testosterone 50 μg/kg</td>
<td>25.0±2.97</td>
<td>78.50±9.448**</td>
<td>408.3±13.07**</td>
</tr>
<tr>
<td>Testosterone 1 mg/kg</td>
<td>25.8±1.11</td>
<td>107.8±12.60***</td>
<td>420.8±90.47**</td>
</tr>
<tr>
<td>Cyproterone 3 mg/kg</td>
<td>20.5±2.50</td>
<td>34.18±6.283†††</td>
<td>72.22±19.56†††</td>
</tr>
<tr>
<td>Cyproterone 10 mg/kg</td>
<td>24.3±2.87</td>
<td>26.86±5.399†††</td>
<td>50.56±11.07†††</td>
</tr>
<tr>
<td>Cyproterone 30 mg/kg</td>
<td>19.0±1.54</td>
<td>24.61±7.249†††</td>
<td>35.42±25.50†††</td>
</tr>
<tr>
<td>Cryptolepis 62.5 mg/kg</td>
<td>17.7±5.18</td>
<td>39.37±6.570†††</td>
<td>109.7±20.35†††</td>
</tr>
<tr>
<td>Cryptolepis 100 mg/kg</td>
<td>18.5±2.01</td>
<td>37.77±6.599†††</td>
<td>77.78±18.09†††</td>
</tr>
<tr>
<td>Cryptolepis 1000 mg/kg</td>
<td>18.2±4.25</td>
<td>38.67±4.324†††</td>
<td>88.89±19.08†††</td>
</tr>
</tbody>
</table>

Results is presented as mean ± SEM. Statistical analysis is by one way ANOVA using Newman-Keuls post-hoc test. ** means p < 0.01, *** p < 0.001, when compared to control. †† means p < 0.01, ††† means p < 0.001 when compared to testosterone (1 mg/kg).

Figure 4: Effects of cryptolepis and cyproterone acetate on testosterone propionate induced comb growth. Results presented as mean ± SEM. Statistical analysis is by one way ANOVA. * means (p < 0.05) when compared to control. † means (p < 0.05) when compared to testosterone propionate.
acetate, which is a known anti-androgen. The present findings indicate strongly that cryptolepis is a testosterone antagonist. Considering the claimed traditional use of cryptolepis as an aphrodisiac in the face of the present findings, we reason that cryptolepis may not necessarily affect sexual behaviour and libido by enhancing testosterone levels but may have a direct effect on the carvenosum of the penile organ. Based on the classification of aphrodisiacs, [21] the activity of cryptolepis may be related to increased potency by promoting erection through vasodilation. Blockade of α-adrenergic pathway has been shown to be a mechanism by which some aphrodisiacs improve erectile dysfunction [22] probably by causing vasodilation. Theoretically, muscarinic receptor antagonists can delay ejaculation thereby prolonging sexual activity. Cryptolepine, the main alkaloid in the aqueous extract of Cryptolepis sanguinolenta is reported to be an α-adrenoceptor antagonist with preferential α-2-adrenoceptor blocking activity [23, 24] and non-selective muscarinic activity [25] which may give scientific credence to its traditional claims as an aphrodisiac.

Traditionally, it is the ethanolic extract popularly called ‘bitters’ that is mostly used as a sex enhancer. Although the effects of alcohol on sexual behaviour are quite controversial, it is widely known that at the early stages it enhances sexual activity. This could partly account for the acclaimed aphrodisiac effects of cryptolepis. Thirdly, male sexual dysfunction or weakness may be secondary to other medical diseases. The use of cryptolepis as a male sex enhancer may not necessarily be due to a direct aphrodisiac effect but rather its ability to treat sexual problems secondary to other medical conditions. For instance, there is a high correlation between leucocytospermia and reduced fertility in men. In the absence of clinical symptoms, the origin of the leucocytes as well as their role in infertility is unknown. However, research from several fertility clinics show that selective COX-2 inhibitors may enhance sperm numbers and quality in treated males as well as increased pregnancy rates in women with treated partners [26, 27, 28]. Cryptolepis is a selective COX-2 inhibitor [29] and hence may be very beneficial to male infertility associated with leucocytospermia independent of the effect on testosterone reported in the present study.

CONCLUSION
We conclude from the present study that the aqueous root extract of the antimalarial herbal Cryptolepis sanguinolenta is potently anti-androgenic. The study also shows that the anti-androgenic activity is species independent.

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