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Effect of Ethanol Root Bark Extract of *Salacia lehmbachii* Loes on Male Reproductive Hormones in Albino Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Author GAE designed the study, wrote the protocol and the first draft of the manuscript. Author ADE managed the experimental process. Author GCA did the literature searches and statistical analyses of the study. Authors FVU and AE edited the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: *Salacia lehmbachii* Loes is a herb found commonly in Southeastern Nigeria where it is used by the locals for the treatment of many ailments especially malaria and the Nigerian population has a relatively large youth segment. This research was carried out to evaluate the effects of ethanol extract of *S. lehmbachii* root bark on reproductive hormones in sexually mature male Albino rats.
Place and Duration of Study: Department of Pharmacology, Faculty of Basic Medical Sciences, University of Calabar, Nigeria; between September, 2015 and November, 2015.

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Methodology: The extract was derived from Soxhlet extraction of petroleum ether-defatted plant powder using ethanol. Twenty four mature male rats weighing between 230-250 g were randomly divided into four groups (n=6), labeled 1 to 4. The animals in group 1 (Control) had 2 mL of distilled water, the vehicle for the extract while those in groups 2, 3 and 4 received 250, 500 and 750 mg/kg body weight of the extract respectively. Administration was orally via a gastric cannula and daily between 9 to 10 am for six weeks. At the end of the experimentation period, rats were anaesthetized in a chloroform fume cupboard and blood collected by cardiac puncture into plain sample bottles. The blood samples were allowed to clot for 2 hours and the clotted blood centrifuged at 3000 rpm for 10 minutes to obtain sera which were used to determine the concentrations of testosterone, luteinizing hormone, follicle stimulating hormone, progesterone and estradiol using the enzyme linked immunosorbent assay technique.
Results: There was a significant (P<.05) reduction dose dependently in serum levels of testosterone, luteinizing hormone, follicle stimulating hormone and progesterone in treated rats compared to control while estradiol levels were increased.
Conclusion: The ethanol root bark extract of *S. lehmbachii* depresses male reproductive hormones and thus may impair male fertility in albino rats.

Keywords: Salacia lehmbachii; root bark; ethanol extract; fertility; male hormones.

1. INTRODUCTION

The use of herbal preparations in the treatment of common ailments is common practice globally. Because these remedies are considered natural, many people use them as the main therapeutic approach to diseases. However, like other therapeutic agents, herbal medicines have inherent toxic potential depending on many factors which include dosage, exposure parameters, age and animal species [1]. *S. lehmbachii,* a small flowering tree of about 3 meters in height is a popular herb in southeastern Nigeria used by the locals to treat a number of ailments. The common names of the plant are 'eba-enang-enang' (the people of Akwa Ibom and Cross River States) and 'ara-mmanu'(the Igbos). The plant originated from West Tropical Africa and also found in Southeastern Nigeria (Akwa Ibom State, Cross River State and Imo State) and Cameroon especially the South west province of Bakassi forest reserve. The plant is one of the 52 species in the genus Salacia belonging to family, Celastraceae [2]. The observed pharmacological actions of the plant include analgesic and antipyretic, nephroprotective effect, anti-abortifacient effect and antioxidant activity [3,4,5,6]. The antimalarial action of the plant has been scientifically established (Essien AD, University of Calabar, Calabar, Nigeria, unpublished results). This finding has confirmed the claims by herbalists that the plant is effective in the treatment of malaria, an endemic disease in Nigeria and other tropical and subtropical Communities, the geographical regions immediately bordering the tropics [7].

Reproductive health has received increased attention in recent times especially with the observed global decline in fertility particularly in male population [8,9,10]. Demographics of Nigerian population with a relatively large youth segment, suggests that a significant proportion of patients with malaria that are likely to use herbal treatment would be in the reproductive age group. The effect of therapeutic agents on fertility should thus be a matter for consideration by health care providers in disease management processes. Screening of therapeutic agents for safety and effect on reproductive function is therefore of great importance. On the basis of this awareness, this study to determine the effect of ethanol root bark extract of *Salacia lehmbachii* on male reproductive hormones in male albino rats was conducted.

2. MATERIALS AND METHODS

2.1 Collection and Identification of Plant Material

The roots of *S. lehmbachii* were purchased from Watt market, a local market in Calabar, capital of Cross River State, Nigeria. The plant was authenticated by the Department of Botany of the University of Calabar where a Voucher Specimen with herbarium number 688 was deposited.

2.2 Preparation of the Extract

The roots were washed with water to remove dirt and dried in their lengths in an electric oven, thermostatically controlled at 40℃, for 12 hours. The bark was obtained by striking the dry roots

on a hard surface and the pieces obtained were pulverized into a coarse powder with a manual grain mill (Corona®, Columbia). The root bark powder was stored in an airtight container. Eight hundred grammes of the root bark powder was Soxhlet extracted in a two-staged process starting with petroleum ether (99.9%, Sigma Chemical Limited, USA) at 65℃ as the solvent for twelve hours to remove fats. The petroleum ether residue was dried, weighed and re-extracted with ethanol at 60℃ for 72 hours to give ethanol extract solution which was evaporated to dryness using a rotatory evaporator at a reduced temperature of 45℃ in-vacuo. The solid extract was weighed (the yield), put in a clean specimen bottle and preserved in a refrigerator at a temperature of -4℃), until required for this work and further experiments.

2.3 Experimental Animals

Twenty-four **s**exually mature male albino rats *(Rattus norvegicus)* weighing between 230-250 g were purchased from the animal house of the Department of Pharmacology, University of Calabar and used for this study. They were housed in plastic cages with wire gauzed top and sawdust on the floor of the cage as bedding. Each cage contained six rats which were branded with diluted picric acid for proper identification. The animals were acclimatized for fourteen days to normal laboratory conditions (relative humidity: 50±5%; temperature: 28±2℃ and 12 hours of light-dark cycle) before the start of the experiment and maintained at the same conditions throughout the experimentation period. They were fed with standard rat chow (Agro-Feeds, Calabar) and water (Water board, Calabar) *ad libitum.* The guidelines on Care and Use of Laboratory animals were observed [11]. The experimental protocols were examined and approved by the appropriate ethics committee.

2.4 Animal Treatment

Twenty four rats were randomly divided into four groups of six rats per group. The groups were labeled 1 to 4. Rats in group 1 were the Control rats and had 2 mL of distilled water (vehicle) while those in groups 2, 3 and 4 received 250, 500 and 750 mg/kg body weight of the ethanol root bark extract of *S. lehmbachii* respectively. Administration was orally, via a gastric cannula carried out daily between 9 to 10 am to play down the effect of circadian rhythm and lasted for six weeks. The selected doses were percentiles of median toxic dose (TD_{50}) earlier determined in our laboratory. At the end of the experimentation period, the rats were anaesthetized in a chloroform fume chamber and blood collected by cardiac puncture. To obtain serum, the blood was put in plain sample bottles, left for 2 hours to clot and then centrifuged at 3000 rpm for 10 minutes for the serum to separate. Serum was then extracted with syringe and 21 G hypodermic needle and emptied into a clean tube for storage at -20℃. Stored frozen serum was used within 12 hours of preparation.

2.5 Hormonal Assay

Sera from the treated rats and controls were used for estimation of hormones by the ELISA (enzyme-linked immunosorbent assay) technique using kits from Life Science Inc, USA. Each standard and sample was done in duplicate. The ELISA technique is based on competitive binding of hormones on immobilized antibodies. Determination of hormone levels followed previously described methodology for testosterone [12], leutinizing hormone [13], and follicle stimulating hormone [14].

2.6 Data Analysis

SPSS version 20.0 software was used for data processing and values obtained from descriptive statistics expressed as means ± standard error of mean (SEM). The data was analyzed adopting one-way ANOVA with Turkey's multiple comparison post hoc tests to compare the level of significance between results from control and treated groups. Differences were considered significant at *P* value of $P < .05$ [5].

3. RESULTS

The extract significantly reduced ($P<.05$) the serum levels of testosterone in a dose dependent manner in the treated rats compared to control as reflected in Fig. 1. Low doses (250 and 500 mg/kg body weight) of the extract produced significant ($P<.05$) reductions in the levels of LH and FSH while high dose (750 mg/kg body weight) caused a significant rise ($P<.05$) in the levels of the two hormones (Fig. 1). Serum progesterone levels were significantly reduced ($P<.05$) in a dose dependent pattern in treated rats compared to control (Fig. 2). The concentration of estradiol was increased significantly ($P<.05$) in all treated rats compared to control (Fig. 2). The levels of all hormones at the highest dose of the extract were significantly ($P<.05$) different from other doses (250 and 500 mg/kg body weight).

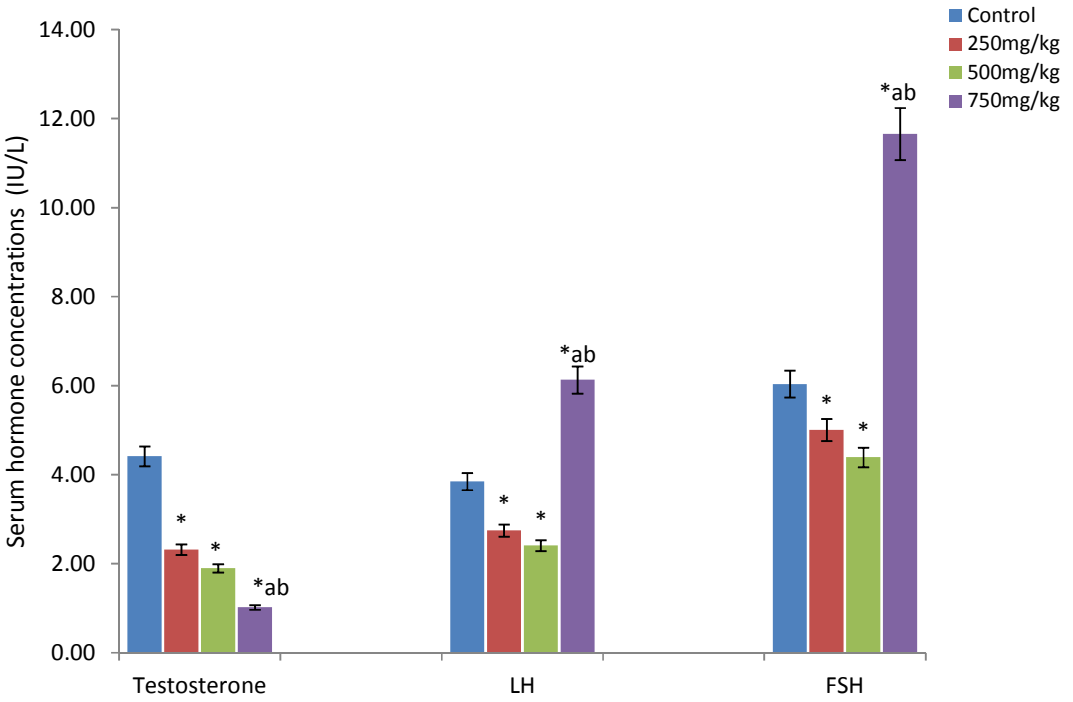

Fig. 1. Effect of ethanol root bark extract of *S. lehmbachii* on rat serum levels of testosterone, leutinizing hormone (LH) and follicle stimulating hormone (FSH)

Values are expressed as mean ± SEM. n=6
**significantly different from control (P<.O5)*
a = significantly different from 250 mg/kg ESL (P<.O5)
b = significantly different from 500 mg/kg ESL P<.O5)

4. DISCUSSION

Male reproduction is regulated by the hypothalamo- pituitary axis involving the hypothalamus, anterior pituitary gland and the testes [15]. The hormones primarily involved are the gonadotrophins (follicle stimulating hormone, leutinizing hormone) and testosterone. Progesterone and estradiol also play vital roles. Gonadotropin-releasing hormone (GnRH) from the hypothalamus stimulates the anterior pituitary gland to release FSH and LH which act on the testes. Leutinizing hormone stimulates the synthesis and secretion of testosterone while FSH induces spermatogenesis in the testes [16,17]. Testosterone in males is necessary for the development and maintenance of normal sexual characteristics, and also nocturnal and nonerotic penile erections [18,19].

The reduced levels of LH and testosterone by low doses of the extract observed in this study may be due to an inhibitory effect on hypothalamic activity. It may also be a direct effect on the pituitary gland whereby reduced levels of LH leads to a decrease in Leydig cell activity in the testicles which then manifests as a drop in serum testosterone levels. The reduction in serum testosterone may in turn decrease androgen-dependent parameters like mating behaviour and maintenance of spermatogenesis, signaling the fact that at those doses, the extract could impair male fertility.

The significant increase of LH at a dose of 750 mg/kg body weight can be regarded as a natural negative feedback response, whereby reduced testicular (target organ) function, caused by the high dose of extract, provokes an increase in LH (negative feedback) in a bid to boost target organ function. This dose of the extract may have depressed testicular function to a critical level or even threatened to cause testicular failure, such that the natural negative feed back mechanism was mobilized in an effort to boost testosterone production which was markedly reduced at this dose. These explanations for the reduction of LH at low doses and increase at high doses are also applicable to the observed changes in serum FSH levels.

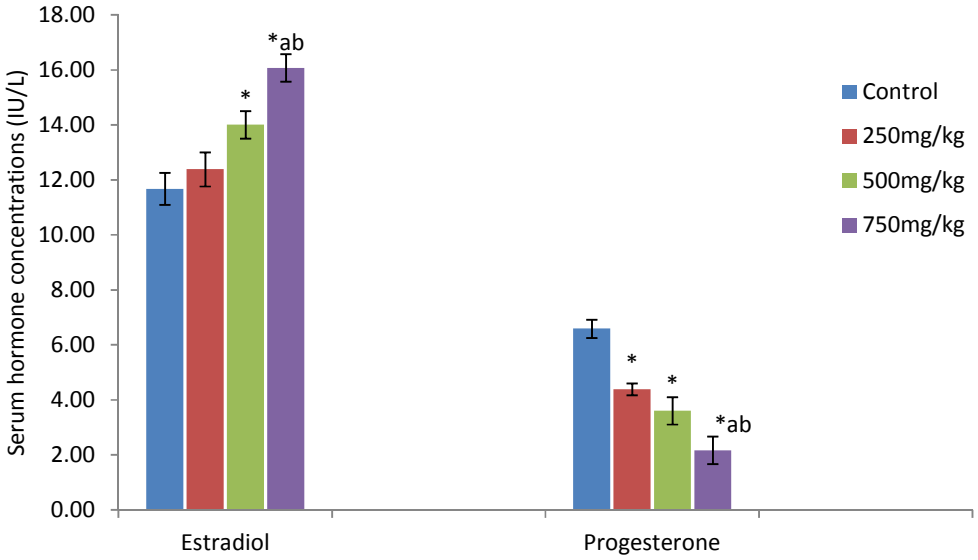

Fig. 2. Effect of ethanol root bark extract of *Salacia lehmbachii* on rat serum levels of estradiol and progesrerone

Values are expressed as mean±SEM. n=6;
**significantly different from control (P<.O5)*
a = significantly different from 250 mg/kg ESL(P<.O5)
b = significantly different from 500 mg/kg ESL P<.O5)

While the reduction in FSH levels at low doses (250, 500 mg/kg body weight) is likely to also be from the depressive effect of the extract on hypothalamo-pituitary activity, the rise at a high dose (750 mg/kg body weight) may be a response to severe depression of the spermatogenic function of the sertoli cells of the testicles or testicular failure. More FSH is produced through a negative feedback mechanism in a bid to revive the depressed function. High FSH is usually an indicator of testicular failure in clinical practice.

Progesterone is necessary for the biosynthesis of testosterone by the Leydig cells of the testis. It is also useful in spermiogenesis and capacitation of sperm cells [20]. The reduction of serum levels of progesterone in this study may thus be one pathway for inhibition of testosterone synthesis by the extract. Estradiol, an estrogen with androgen-independent inhibition of release of gonadotropins was also found to be increased in the treated rats [20]. This increase may possibly be the pathway by which the extract exerted its inhibitory effect on release of gonadotropins also found in our results.

This depressant effect on male reproductive hormones by extracts of *S. lehmbachii*, a plant with proven antimalarial properties is not perculiar. Other workers have shown similar action following administration of some other herbs with antimalarial action. Examples include *Azadirachta indica*, *Creptolepsis sanguinolenta* and *Carica papaya* seed [21,22,23]. Such herbs thus need to be evaluated for their effect on reproductive hormones before approval for use or should be used with caution in the reproductive age group.

5. CONCLUSION

In conclusion, the doses of ethanol root bark extract of *S. lehmbachii* used in this study and for the duration indicated above produce a dose dependent reduction in the serum levels of male sex hormones and can thus impair male fertility. Further studies are however necessary to evaluate its effect on sperm parameters and other fertility indices to validate this assertion.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The authors hereby declare that guidelines on Care and Use of Laboratory animals in research were followed and also specific national laws where applicable. The experimental protocol was approved by the appropriate ethical committee.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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