Full Length Research Paper

Fertility enhancing effects of aqueous stem bark extract of *Lophira lanceolata* in male Spargue dawley rats

E. U. Etuk * and A. A. Muhammad

Department of Pharmacology, College of Health Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria.

Accepted 1 September, 2009

Lophira lanceolata stem bark extract is commonly used by herbalists in Sokoto state for the treatment of fertility related problems in males. Recently the sexual stimulatory effect of the plant in male rats has been reported. The present study examined the effect of 7 days oral administration of 100, 200 and 300 mg/kg body weight of the plant stem bark extract on fertility indices (Sperm motility, Sperm number and Sperm morphology) in male Sprague dawley rats. The extract produced a significant and dose dependent increase (p < 0.05) in the sperm number of the rats. There was no significant change in the sperm motility and morphology. There was a decrease in the blood level of Follicle stimulating hormone (FSH) but no significant increase in the levels of Leutinizing hormone (LH) and Testosterone. Testicular histology examination revealed increased spermatogenesis. *L. lanceolata* may have the potentials of being developed into a male fertility enhancing drug.

Key words: Lophira lanceolata, fertility enhancer, sperm number, testes.

INTRODUCTION

Infertility is a common problem, affecting perhaps one couple in six; the majority of whom now seek medical care (Glazener et al., 1987). Although diagnostic problems make it difficult to establish the extent of the male partner's contribution with certainty, a number of studies suggest that male problems represent the commonest single defined cause of infertility (Van den Eede, 1995). More than 90% of male infertility cases are due to low sperm counts, poor sperm quality, or both. In 30 to 40% of cases of sperm abnormalities, the cause is unknown (Stephen et al., 1996). The management options available for the treatment of infertility in males include the use of drugs and a variety of surgical procedures (Purvis and Christiansen, 2008). The total sperm count, motile sperm count and with normal morphologic features has been reported as the indices of fertility in males (Smith et al., 1977; Small et al., 1987).

The use of plant extracts as fertility enhancer in animals is now in the increase because of the shifting of attention from synthetic drugs to natural plant products (Dada and Ajilore, 2009). Plant that were once considered of no value are now being investigated, evaluated and developed into drugs, with little or no side effects (Adedeji et al., 2009). *Lophira lanceolata* which is known locally as "Namijin kande" in Hausa is a very popular medicinal plant among herbalists in Sokoto State, Northwestern Nigeria because of its acclaimed effect in curing fertility related problems in men. The efficacy of the plant as a sexual stimulant and its toxicity profile has been reported through our investigation (Unpublished). The focus of the present study is to examine the fertility enhancing properties of the plant stem bark extract in male Sprague dawley rats through analysis of sperm number, sperm motility and morphology.

MATERIALS AND METHODS

Collection and identification of plant material

L. lanceolata was identified by an Herbalist from its natural habitat in Fakon Idi area in Sokoto North Local Government Area of Sokoto State, Nigeria in June 2008. The plant parts were collected and further authentication was carried out by a Botanist in the Department of Biological Sciences, Usmanu Danfodiyo University where a Voucher Specimen was deposited.

Preparation of the extract

The plant stem bark was washed and air-dried to a constant weight in the Pharmacology laboratory. The dried material was pulverized

^{*}Corresponding author. E-mail: etuk2005@yahoo.co.uk.

Treatment group (mg/kg)	Absolute wt of testes (g)	Relative wt of testes (%)	Absolute wt of epididymis (g)	Relative wt of epididymis (%)
Control	2.75 ± 0.24	0.01 ± 1.5	0.22 ± 0.1	0.182 ± 0.01
100	2.83 ± 0.27	0.01 ± 1.7	0.23 ± 0.2	0.183 ± 0.01
200	2.10 ± 0.15	0.01 ± 2.0	0.22 ± 0.1	0.191 ± 0.01
300	2.76 ± 0.03	0.01 ± 0.0	0.21 ± 0.1	0.176 ± 0.02

Table 1. Effect of L. lanceolata stem bark extract on weights of testes and epididymis in male Sprague dawley rats.

n = 5; g = gram; P < 0.05; wt = weight.

Table 2. Effect of *L. lanceolata* stem bark extract on epididymal sperm count, morphology and motility.

Treatment Group (mg/kg)	Sperm Count (x 10 ⁶)	Abnormal sperm morphology (%)	Active motile sperm (%)
Control	38.2 ± 1.43	2.3	28.2
100	62.1 ± 2.18*	2.9	27.0
200	107.4 ± 3.30*	2.8	29.8
300	112.1 ± 2.80*	2.8	27.5

n = 5; g = gram; P < 0.05; * = significant values.

into a dry powder using a mortar and pestle. About 250 g of the powder was extracted with 3.75 L of distilled water. The filtrate was concentrated in an electric Oven at $50 \,^{\circ}$ until a semisolid residue was obtained. The percentage yield of the extract was calculated.

Experimental animals

Adult male Sprague dawley rats weighing between 180 - 200 g were obtained from the experimental animal unit of the Faculty of Veterinary Medicine of Ahmadu Bello University, Zaria and used for this study. The animals were housed in cages and maintained in the animal Facility of the Department of Pharmacology, Usmanu Danfiodiyo University Sokoto for 14 days before the commencement of the drug treatment. They were given free access to rat feed (Nemeith livestock feeds Ltd. Nigeria) and tap water under normal laboratory environmental conditions. The study protocol was approved by the Institutional Ethical Committee.

Animal treatment

Twenty Sprague dawley rats were randomly selected and divided into four groups (n = 5), labeled A to D. The animals in the test groups (A, B and C) were treated orally with 100, 200 and 300 mg/kg body weight of the extract respectively for 7 days. The doses selection was based on the previous work on the extract conducted in our laboratory. Those in group D that served as normal control were given equivalent volume of distilled water. All the animals were given free access to food and water during the treatment period.

On the 8th day, the weight of each rat was measured. The animals were anaesthetized with chloroform and sacrificed. Blood samples collected through cardiac puncture for hormonal analysis. The hormone analysis (Testosterone, Follicle stimulating hormone, and Leutenizing hormone) was carried out using Immunoassay (ELISA) method (Yakubu et al., 2007). The testes and epididymis from the rats were carefully dissected out and weighed independently. From the epididymis, sperm were collected, mounted on a slide and their motility assessed immediately under the microscope at ×10 objective. The motility assessment was expressed as

percentage motile forms. The slides were later stained with Carbol Fuschin and the sperm number and morphology were examined (Parven et al., 2003).

Statistical analyses

The data obtained from this study were expressed as mean \pm standard error of mean (Mean \pm SEM) and analyzed using analysis of variance (ANOVA). Further comparison between groups was performed using Duncan's multiple range test. All differences were considered significant at 5% level, which is p < 0.05.

RESULTS

The aqueous extraction of L. lanceolata stem bark produced a dark brown colour residue which was tasteless with no peculiar smell. The percentage yield was 34.2% w/w. The extract showed no significant effect (p > 10.05) on the testicular and epididymal weights of the animals after 7 days treatment with various doses (Table There was a significant (p < 0.05) and dose 1). dependent increase in epididymal sperm number when compared to the control (Table 2). But the sperm motility and morphology remained unchanged. The extract produced a decrease in the blood level of Follicle stimulating hormone (FSH) and a non significant increase in the levels of Leutinizing hormone (LH) and Testosterone (Table 3). The histology of the testis revealed increase spermatogenesis among the extract treated rats (Figures 1 and 2).

DISCUSSION

This study examined the effect of oral administration of L.

Table 3. Effect of extract on different hormones after	7 days treatment period.
--	--------------------------

Treatment (mg/kg)	Testosterone (ng/ml)	FSH (miu/ml)	LH (miu/ml)
Control	0.16 ± 0.02	0.13 ± 0.02	0.08 ± 0.01
100	0.20 ± 0.03	0.08 ± 0.03	0.11 ± 0.01
200	0.21±0.04	0.10 ± 0.01	0.13 ± 0.01
300	0.22 ± 0.03	0.09 ± 0.04	0.11 ± 0.02

n = 5. Mean ± SEM; FSH = Follicle stimulating hormone; LH = leutinizing hormone; Miu = milli international unit; ng = nanogramme.



Figure 1. Photomicrograph of rat testis in the control group showing Spermatozoa and Sertoli cells (x40) (Ziehl Nelson).

lanceolata stem bark extract on sperm indices in male Sprague dawley rats. The total sperm count, motile sperm count and with normal morphologic features has been reported as the indices of fertility in males (Smith et al., 1977; Small et al., 1987).

Administration of 100 - 300 mg/ kg body weight of aqueous extract of *L. lanceolata* for 7 days increased the sperm number from $38.2 \pm 1.43 \times 10^6$ to $112.1 \pm 2.80 \times 10^6$ in the male rats. In a study carried out by Small et al.

(1987) among the male partners of 1074 infertile couples; it was shown that the cumulative pregnancy rates were more than double when the sperm count in the male doubled following treatment. This illustrates clearly the relevance of sperm number in male fertility. This plant extract has almost produced a three fold increase in the sperm count of the rats after 7 days treatment. This result is very significant. However, it should be noted that the present result is obtained from animal studies; more



Figure 2. Photomicrogragh of rat testes showing increased spermatogenesis after administration of 300mg/Kg of the extract (×40) (Carbol fuschin stain).

investigations should be carried out and extended to humans. Animal experimental results are not always reproducible in humans.

Herbs have been used since the beginning of time to aid in many different ailments. Of these ailments, fertility has been enhanced and even corrected by the use of certain herbs (Ramsey, 2000). The effect of the extract in increasing the sperm number may be due to the increase in blood testosterone level as shown in this study (Table 3). Testosterone is a male hormone that has significant impact on spermatogenesis (Lee et al., 2001). This study also indicated a reduce level of FSH after 7 days treatment with the extract. An apparent relationship was reported between low Inhibin B levels together with elevated FSH levels and decrease sperm density as indicative of a high risk of infertility (Lee et al., 2001). In this case, a reverse may improve fertility. The increase in sperm count following the administration of the extract was further collaborate by increased spermatogenesis seen in the testicular histology. Based on this result, this extract has the potentials of being developed into a male fertility enhancing agent. A similar investigation is in progress to assess the effect on female fertility.

REFERENCES

- Adedeji OS, Farimi GO, Ameen SA, Olayemi JB (2006b). Effects of bitter kola (*Garcinia kola*) as growth promoter in Broiler Chicks from day old to four weeks old. J. Anim. Vet. Adv., 5(3): 191–193.
- Dada AA, Ajilore VO (2009). Use of ethanol extracts of *Garcinia kola* as fertility enhancer in female catfish *Clarias gariepinus* broodstock. Int. J. Fish. and Aquacul., 1 (1): 005-010.

- Glazener CM, Kelly NJ, Weir MJ (1987). The diagnosis of male infertility-prospective time specific study of conception rates related to seminal analysis and post-coital sperm-mucus penetration and survival in otherwise unexplained infertility. Hum. Reprod., 2: 665-671.
- Lee PA, Coughlin MT, Bellinger MF (2001). Inhibin B: Comparison with Indexes of Fertility among Formerly Cryptorchid and control men. The J. Clin. Endo. Met., 86 (6): 2576-2584.
- Odebiyi O and Sofowora EA (1979). Phytochemical screening of Nigerian medicinal plants- part 1. 2nd OAU/STRC. Inter- Africa Symposium on Trade Pharmacoceia and African medicinal plants. Pub. No. 115 Lagos, 216 pp.
- Parveen S, Suwagmani D, Chandra PK, Perreira BMJ (2003). A comprehensive evaluation of the reproductive toxicity of *Quassia amara* in male rats. Repro. Toxicol., 17(1): 45-50.
- Purvis K, Christiansen E (2008). Infection in the male reproductive tract. Impact, diagnosis and treatment in relation to male infertility. Int. J. Androl., 16(1): 1-13.
- Ramsey L (2000). Fertility enhancing herbs. Online publ. http://www.suite101.com/print_article.cfm/preconception/47212.
- Small DR, Collins JA, Wilson EH, Wrixon W(1987). Interpretation of semen analysis among infertile couples. CMAJ. 136(8): 829-33.
- Smith KD, Rodriguez-Rigau LJ, Steinberger E (1977). Relationship between Indices of semen analysis and pregnancy rate in infertile couples. Fertil Steril. 28(12):1314-9.
- Steven RL, David HB, MZ, Barry W, Hussein A, Brian C, Harry F, Patricia B. (1996). Abnormal sperm morphology is highly predictive of pregnancy outcome during controlled ovarian hyperstimulation and intrauterine insemination. J. Assisted Reprod. Genet. 13(7): 569-572.
- Van den Eede B (1995). Investigation and treatment of infertile couples: ESHRE guidelines for good clinical and laboratory practice. Hum. Reprod., 10: 1246-1271.
- Yakubu MT, Akanji MA, Oladiji AT (2007). Effect of oral administration of aqueous extract of *Fadogia agestis* (Schweinf. Ex.Hiern) stem on some testicular function indices of male rats. J. Ethnophamacol., 115(2): 288-292.