



Original Article

Antifertility Effects of Ethanolic Root Bark Extract of *Chrysophyllum albidum* in Male Albino Rats

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Summary: The present study was conducted to investigate the antifertility activity of the ethanol root bark extract of *Chrysophyllum albidum* on sperm parameter and hormonal levels in rats. Eighteen male rats were divided into three groups of six animals each. The first group (A) received distilled water and served as control. The second and third group (B & C) of animals were administered the ethanol root bark extract daily at 100mg/kg body weight and 200mg/kg decrease in the caudal epididymal sperm count, motility and sperm morphology was observed compared with the control. Serum gonadotrophins and testosterone were measured and *C. albidum* extract also caused a dose related significant reduction ($p < 0.05$) of serum testosterone, Luteinizing hormones and FSH concentrations in all treatment groups as compared to the control. The result showed that ethanol extract of the root bark of *C. albidum* suppresses the hormonal levels and sperm production in rats and deserves to be further investigated as a potential male contraceptive agent.

Industrial relevance: The unique advantages of this antifertility option is that they are safer, reliable, affordable, long-lasting, acceptable and can be taken without consulting a health worker in comparison to pharmaceutical drugs that are expensive and have negative side effect.

Keywords: *Chrysophyllum albidum*; Infertility; testosterone; gonadotrophin; testis

INTRODUCTION

Fertility regulation comprising contraception and management of infertility forms an important component of reproductive health (Allag *et al.*, 2002). Although many types of contraceptive agents are available but these have several side effects, there are several medicinal plants known to possess male contraceptive properties either by suppressing spermatogenesis or by spermicidal action (Paul *et al.*, 2006). There is a growing interest in search of male contraceptive of natural origin with least side effect (Sharma *et al.*, 1996).

Although very few contraceptives have been developed from plant extracts, their potentiality has not been determined accurately, and their mode of action has been beyond our knowledge until now because there are many problems in assessing plant extract including

batch to batch variation and a lack of definite active portion of the extract used for the development of herbal contraceptives (Sathiyaraj *et al.*, 2010). Fertility regulation with plants or plant preparations has been reported in the ancient literature of indigenous systems of medicine (Sathiyaraj *et al.*, 2010). However, the search for an oral antifertility agent that is active, affordable, safe with effective plant preparation is needed as contraceptive for male.

Chrysophyllum albidum belongs to the family Campodeoidea. It is widely distributed in the low land rain forest zones and frequently found in villages (Madubuike and Ogbonnaya, 2003). It is often called the white star apple and distributed throughout the southern part of Nigeria (Idowu *et al.*, 2006). In South-western Nigeria, the fruit is called “agbalumo” and popularly referred to as “udara” in South-eastern

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Nigeria. It is a plant which has been used in traditional/alternative medicine in Nigeria to treat health problem, various parts of this herb have been proved to have a wide range of therapeutic effects. Phytochemical profile shows it contains an array of biologically active substances that include alkaloids, tannin, saponin, phenol and flavonoid (Okoli and Okere 2010). It is rich sources of natural antioxidants have been established to promote health by acting against oxidative stress related disease such infections as; diabetics, cancer and coronary heart diseases (Burits & Bucar, 2002). The bark is used for the treatment of yellow fever and malaria while the leaf is used as an emollient and for the treatment of skin eruption, stomachache and diarrhea (Adisa, 2000; Idowu et al., 2006). Cotyledons from the

seeds of *C. albidum* are used as ointments in the treatment of vaginal and dermatological infections in Western Nigeria and also possess anti-hyperglycemic and hypolipidemic effects (Olorunnisola et al., 2008). Antimicrobial activity (Idowu et al., 2003), anti-nociceptive, anti-inflammatory and antioxidant activities (Idowu et al., 2006) and antiplatelet effect (Adebayo et al., 2010). People take the same herbs for other medicinal purposes, ignorant of their anti-fertility effects. The present study was conducted to investigate the antifertility effect of the ethanolic root bark extract of *C. albidum* on the hormonal levels (pituitary-testicular axis) and caudal epididymal fluid parameters in wistar rats.



Figure 1: Plant of *Chrysophyllum albidum*

MATERIALS AND METHODS

Collection of plant materials: The root bark of the *Chrysophyllum albidum* was obtained from Ekeakpara Village in Osisioma Ngwa LGA, Abia State in July 2011. It was authenticated in the Department of Botany, University of Nigeria, Nsukka. A voucher specimen has been preserved in the laboratory for future reference. The ethanolic extraction process was carried out in the Pharmacognosy Department of the Faculty of Pharmacy, Madonna University, Elele, River State.

Preparation of the plant extract. The method of Atangwho et al., 2010 was used. Root bark of *C. albidum* was separated from the root of plant collected. The sample was washed in tap water and was chopped into bits with a knife on a chopping board. The bits were dried in an uninhabited room for four weeks at room temperature. Dried samples were ground into powder mechanically, using manual grinder. The fine powdered

root bark was kept in airtight containers at room temperature until the time of use. 281.6g of plant powder was soaked in 1400ml of 99% ethanol and kept in refrigerator at 4°C for 48 hours. The mixture was then vigorously shaken intermittently for additional 2 hours, to allow for complete extraction. The resulting mixture was rapidly filtered through Whatman No 1 filter paper and later with cotton wool to obtain a homogenous filtrate. These filtrates were then concentrated *in vacuo* at low temperature (37- 40°C) to about one tenth the original volume using a rotary evaporator. The concentrates were allowed open in a water bath (40°C) for complete dryness yielding 67g (8.6%) of brown gummy substance. The extract was later reconstituted in normal saline (0.90% NaCl) at a concentration of 1 g/ml before administration. The extract was then refrigerated at 2- 8°C until use.

Determination of LD₅₀. The LD₅₀ was determined using the fixed-dose procedure described by Walum

(1998). Briefly, the *Chrysophyllum albidum* root bark was given at one of the three fixed doses at a time to 5 male wistar rats.

Experimental Animals Eighteen albino rats (males only) of wistar strain weighing about 100-200g were obtained from the animal house of University of Nigeria, Nsukka. The rats were housed in cages under standard conditions (12h light / 12 h dark, 25°C ±2°C) and were kept for 15 days for acclimation prior to experimentation. The animals were maintained on pelletized Growers feed obtained from Vital Feeds, Port Harcourt, Rivers State, Nigeria, and tap water *ad libitum*. After 15 days of acclimation, they were randomly divided into 3 groups with 6 animals in each group. The daily dose of the extract was prepared and administered to each animal for 3 weeks. The treatment schedule of each group was as follows:

Group I: Control rats given distilled water.

Group II: The rats were treated with *Chrysophyllum albidum* ethanolic root bark extract (100 mg/kg body wt) for 21 days.

Group III: The rats were treated with *Chrysophyllum albidum* ethanolic root bark extract (200 mg/kg body wt) for 21 days.

The extract and distilled water were administered once a day by gavage using a canula and a syringe. At the end of the experiments, the rats were sacrificed a day after the last dose of administration of the extract or distilled water. All sacrifices were done under mild anaesthesia with intra-peritoneal ketamine hydrochloride at a dose titrated against consciousness starting with 0.01 ml. Laparotomy was done, and the testes delivered per abdomen. The caudal epididymis neatly excised and the epididymal fluid obtained by the swim up technique immediately analyzed. Blood samples were taken from the left ventricle, centrifuged at 1000 g, 25°C for 10 min in an angle head centrifuge. Blood sera were separated and immediately assayed for LH, FSH and T levels.

Estimation of sperm count, motility and morphology. The spermatozoa were obtained by making small cuts in caudal epididymis and vas deferens placed in 1ml of modified Krebs Ringer-bicarbonate buffer (pH 7.4). The sperm suspension was evaluated for sperm content, percent motility. The percent motility was determined by the progressive and non-progressive movements of sperms observed under a compound microscope. The sperm count was determined under a Neubauer haemocytometer. To evaluate the spermatozoa abnormalities, the sperm suspension was stained with eosin; smears were made on slides, air dried and made permanent.

General Protocols

Cage- side examination were conducted daily to detect signs of toxicity (loss of hair, behavioral abnormalities, dead rats, salivation, refusal of feed, weight loss and

chew jaw movement). All procedures in this study conformed to the guiding principles for research involving animals as recommended by the Declaration of Helsinki and the Guiding Principles in the care and use of Animals and approved by the Department Committee on the Use and Care of Animals.

Serum Testosterone assay. Serum T was assayed from blood obtained from left ventricular puncture. The samples were assayed in batches from a standardized curve using the enzyme linked immunosorbent assay (ELISA) method (Tietz, 1995). The microwell kits used were from Syntro Bioresearch Inc., California USA. Using 10 µl of the standard, the samples and control were dispensed into coated wells. 100 µl T conjugate reagent was added followed by 50 µl of anti-T reagent. The contents of the microwell were thoroughly mixed and then incubated for 90 min at room temperature. The mixture was washed in distilled water and further incubated for 20 min. The reaction was stopped with 100 µl of 1N hydrochloric acid. Absorbance was measured with an automatic spectrophotometer at 450 nm. A standard curve was obtained by plotting the concentration of the standard versus the absorbance and T concentration was determined from the standard curve.

Luteinizing hormone assay. The BioCheck LH ELISA is based on the principle of a solid phase enzyme-linked immunosorbent assay (Clinical Guide to Laboratory Tests, 1995). The assay system utilizes sheep polyclonal anti-LH for solid phase (microtiter wells) immobilization, and a mouse monoclonal anti-LH in the antibody enzyme (horseradish peroxidase) conjugate solution. The test sample was allowed to react simultaneously with the antibodies, resulting in the LH molecules being sandwiched between the solid phase and enzyme-linked antibodies. After 45 min incubation at room temperature, the wells were washed with water to remove unbound labeled antibodies. A solution of Tetramethylbenzidine was added and incubated for 20 min, resulting in the development of a blue colour. The colour development was stopped with the addition of HCl, and the resulting yellow colour measured spectrophotometrically at 450 nm. The concentration of LH is directly proportional to the colour intensity of the test sample.

Follicle stimulating hormone assay. This assay was carried out using double antibody radio immuno-assay. A rat recombinant FSH {I125} from Amersham, UK was used. The sensitivity of the assay was 0.9ng/ml (Clinical Guide to Laboratory Tests, 1995).

Statistical Analysis. All biochemical results were expressed as Mean±SD significant differences among the groups were determined by one-way analysis of variance (ANOVA) followed by Bonferroni post-test or unpaired Student's t-test using the SPSS statistical

analysis program. Statistical significance was considered at $p < 0.05$.

RESULTS

Body Weight. Significant body weight gain was observed in all the experimental groups mostly the group B when compared to the control group ($P < 0.005$) (Table 1).

Epididymal Sperm. The sperm of the control rats had normal counts, motility, and morphology (Table 2). In *C. albidum* ethanolic root bark extract, the treated rats' caudal epididymal sperm parameters showed evidence of dose dependent toxicity. The sperm concentration decrease in the treated groups, with the animals treated with 200mg/kg bodyweight *C. albidum* ethanolic root bark extract having the lowest value (11.3 ± 0.8) compared with those that received 100mg/kg body weight of the extract (14.7 ± 1.5) and the control group (62 ± 0.3). The sperm motility was very much inhibited in both group B and group C animals (Control: 76 ± 5.7 ; low dose group: 50 ± 7.0 ; High dose group: 42 ± 2.0). There was no significant difference in the percentage of normal spermatozoa in the animals exposed to 200mg/kg body weight of *C. albidum*

(54.7 ± 0.9) compared with animals that received a lower dose of 100mg/kg bodyweight (54.3 ± 1.3), however there was a significant difference between the treated groups and the control animals (84.3 ± 0.7). The reduction of sperm concentration and sperm motility were significantly ($P < 0.005$) higher in plant extract 200 mg/kg body wt (Group C) treated animals when compared to 100 mg/kg body wt (Group B) and control animals.

Hormone levels. Serum T levels in groups B and group C tended to be lower ($p < 0.05$) than that of the group A viz 8.51 ± 0.005 and 1.12 ± 0.01 and 10.52 ± 0.04 ng/ml for groups B, C and A, respectively (Table 3). The mean serum LH concentrations of rats in groups B and C were 1.75 ± 0.02 and 1.12 ± 0.01 mIU/ml respectively; these values were significantly different when compared to the control 3.45 ± 0.20 ($p < 0.05$). The mean serum FSH levels of experimental animals follow similar pattern to those of serum LH, groups B (0.21 ± 0.005) and group C (0.14 ± 0.01) showed a significant decrease compared to of control 1.51 ± 0.14 mIU/ml. (Table 3)

Table 1. Effect of ethanolic root bark extract of *C. albidum* on body weight of rats (Mean \pm SEM).

Groups (n=6)	Body weight (g)		WD (g)
	Initial	Final	
A (control)	135 \pm 14.5	148 \pm 14.25	13
B (100mg)	138 \pm 15.26	184 \pm 5.84	46*
C (200mg)	137 \pm 17.37	171 \pm 13.61	34*

*($p < 0.05$) significantly different from the control

WD: Difference between the final and initial body weight of the rats

Table 2. Summary of mean sperm parameters

Semen Parameters	Group A	Group B	Group C
	(control)	(low dose <i>C. albidum</i>)	(high dose <i>C. albidum</i>)
Sperm Concentration ($\times 10^6$)	80.0 \pm 2.9	25.7 \pm 0.7 *	17.0 \pm 1.3*
Sperm motility (%)	76 \pm 5.7	50 \pm 7.0 *	42 \pm 2.0*
Motile count ($\times 10^6$)	62 \pm 0.3	14.7 \pm 1.5*	11.3 \pm 0.8 *
Sperm morphology (% normal)	84.3 \pm 0.7	54.7 \pm 1.3*	54.3 \pm 0.9*

*($p < 0.05$) significantly different from the control

Table 3. Effects of *C. albidum* on hormone profile of male albino wistar rats. Values given represent the Mean±SEM.

Groups	Group A	Group B	Group C
	(Control)	(low dose <i>C. albidum</i>)	(high dose <i>C. albidum</i>)
FSH	1.51±0.14	0.21±0.005*	0.14±0.01 *
LH	3.45±0.20	1.75±0.02*	1.12±0.01*
Testosterone	10.52±0.04	8.51±0.005*	1.12±0.01*

*($p < 0.05$) significantly different from the control

DISCUSSION

The findings of the present study showed that the ethanolic root bark extract of *C. albidum* could significantly alter the fertility potential of male rats. The fact that there was significant increase in body weight ($p < 0.05$) on treated animals does not rule out the possibility of a systemic toxicity at the doses treated due to behavioral alterations observed within the treated groups which showed progressive decrease in agility. Furthermore the significant increase in body weight ($p < 0.05$) indicates that the extract may have toxic effect on the rats. The analysis of caudal epididymal fluids of these treated rats revealed a concomitant decrease in the sperm concentration which may be due to the inhibition in spermatogenesis which has been reflected here by the low count. Another possibility of low sperm concentration by the extract treatment may be due to oxidative stress (Ghosh *et al.*, 2002b). The present study also reveals a significant decrease in sperm motility which focuses the direct effect of the extract on matured and stored sperm in epididymis, also increased abnormal sperm morphology was observed. The sera of animals treated with the ethanolic root bark extract of *C. albidum* showed a significant decrease in the level of assayed gonadotrophins (FSH and LH) compared to control. This indicates interference of pituitary-testicular axis. However, it has also been shown that the testosterone level in serum and plasma correlate with sperm concentration and sperm motility (Carropo *et al.*, 2003). Two possible hypotheses may be proposed to explain the antigonadal activities of the herbal agent. One hypothesis is that the active ingredient(s) of the extract may alter the pituitary gonadotropins hormones i.e. luteinizing hormone and follicle stimulating hormone (Kusemuji *et al.*, 2010). Low levels of these hormones decrease endogenous testosterone secretion from the testis depriving developing sperm of the signal required for normal maturation and also it suppress testicular steroidogenesis and spermatogenesis (Kusemuji *et al.*, 2010) since the pituitary-testicular axis is a central regulatory conduit for testicular function that culminates in the production of spermatozoa (Cheng *et al.*, 2010). Besides hormonal alteration, the alternative hypothesis is that the effective ingredient(s) may induce

oxidative stress in testicular tissue and stored germ cells leading to generation of free radical products, as they exert a detrimental effect on spermatogenesis (Gosh *et al.*, 2002b). Also, for male contraception, it is not necessary to stop spermatogenesis, but rather to eliminate the fertilizing ability of the spermatozoa by causing changes in the morphology or in the function of the sperm (Niketani *et al.*, 2000). The exact agent(s) responsible for all these effects is/ are not clear and might be substance(s) present in *C. albidum* extract, which lead to its antispermatic effect.

CONCLUSION

This study demonstrated that the root bark of ethanolic extract of *C. albidum* produces a pronounced inhibition of serum testosterone and gonadotrophins concentration. These extract had antifertility effect on the testes of albino wistar rats and could be further investigated for possibility of developing a cheap, acceptable and easy available male contraceptive.

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