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Ethanolic Leaf Extract of *Chrysophyllum albidum* on sperm analysis, hormonal profile, SOD and testicular histology of adult male wistar rats.

Onyeka C. Anna*, Fabunmi O. Oladapo^, Aligwekwe A. Ugochukwu, Ofoego U. Chikere, Leko Bankole, Anthony O. Anthony^

Department of Human Anatomy. Faculty of Basic Medical Sciences.

^Department of Physiology. Faculty of Basic Medical Sciences.

Madonna University Okija, Elele Campus. River State. Nigeria.

*Corresponding author: Tel: + 234-(0)805-491-9134. E-mail - annachi67@yahoo.com

ABSTRACT

The purpose of the present study was to evaluate the effects of ethanolic leaf extract of *Chrysophyllum albidum* on hormones and sperm analysis of laboratory rats. Fifteen male rats weighing 230-250g were completely randomised into three groups: A-C. Animals in Group A (control) were orally administered with 0.5ml of distilled water once daily for 21 days while those in Groups B and C, received 500 and 1000mg/kg body weight of the extract respectively), and effect of the treatment on the testes was investigated. Significant increase ($p<0.05$) were recorded in the body weights and also the weight of the testis. The treatment caused increase in motility, morphology, and number of spermatozoa in cauda epididymidis. Histologically, testes in rats treated with the plant extract showed no alterations in the seminiferous tubules when compared to the control. However hormonal assay showed significantly reduced levels ($p<0.05$) of FSH, LH and Testosterone in experimental groups while there was increase in the activity of SOD, which was dose dependent. The results thus suggest that *C. albidum* treatment does not cause suppression of spermatogenesis but decrease the hormonal profile.

Keywords*: Chrysophyllum albidum*, gonadotropic hormones, testosterone, testes

INTRODUCTION

Chrysophyllum albidum in South-western Nigeria is a fruit called “agbalumo” and popularly referred to as “udara” in South-eastern Nigeria. The plant often grows to a height of 36.5m though it may be smaller (Bada, 1997), several other components of the tree including the roots and leaves are used for medicinal purposes (Adewusi, 1997; Bada, 1997). 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). The leaf has antiplatelet and hypoglycemic properties (Adebayo *et al*., 2010). The root bark has anti-fertility effects (Onyeka *et al*., 2012a). Stem bark extracts has antimicrobial effects (Adewoye *et al*., 2011), Anti-plasmodial (Adewoye *et al*., 2010). The seed cotyledon has been reported to possess anti-hyperglycemic and hypolipidemic effects (Olorunnisola *et al*., 2008).

Testosterone, follicle-stimulating hormones and luteinizing hormones are very important hormones involved in human reproduction and development. These are vital in the production and maturation of sperm cells and promotion of secondary sexual characteristics (Rahman, 2001). In the male, LH acts on the Leydig cells of the testes, this is responsible for the production of testosterone, an androgen that exerts both endocrine activity and intratesticular activity on spermatogenesis (Nielsen *et al*., 2001).

Anti-androgens exert their anti-fertility effect by their action on the hypothalamus-pituitary-gonadal axis or direct hormonal effect on reproductive organs, resulting in the inhibition of spermatogenesis (Shibeshil *et al*., 2006). The study therefore is aimed at providing information on the effects of the ethanolic

leaf extract on the spermatogenic and hormonal profile in rats.

MATERIALS AND METHODS

Experimental Animals: Matured fifteen healthy male albino rats weighing 230-250 were obtained from the University of Port Harcourt animal house. The rats were fed with vital feed (grower pelletized) and water was available *ad libitum* throughout the period of acclimatization. Its cages were contained in a well-ventilated standard housing conditions (temperature: 28-31^{0}C; photoperiod: 12h natural light and 12h dark; humidity: 50-55%).

Preparation of ethanolic leaf extract of *Chrysophyllum albidum:* The leaves of *Chrysophyllum albidum* was obtained from the garden of Agriculture Development Project (ADP) Asaba, Delta state and were identified in Madonna University, Department Of Pharmacognosy, with batch number MAU/cognosy/36/001. The leaves were dried for 5 days under direct sunlight, grounded into a powder form, and weighted. The grounded leaves were socked in absolute alcohol in a beaker for 24 hours. The mixtures were then filtered into storage containers using a Whatman no. 1 filter paper and cotton wool and left to stand for some minutes.The solvents were then put into a soxhelt extractor in order to separate the extract from the ethanol. The extracts were then transferred into an oven where it was allowed to dry completely. The oven was regulated at a temperature below room temperature in order to prevent denaturing of the contents of the extract. The ethanolic leaf extract of *C. albidum* were then stored in a dry clean container.

Administration of extract: The matured male rats were divided into three groups of five, the control group A, received orally, 0.5ml of distilled water while those Groups B and C were orally administered ethanolic leaf extract of *C. albidum* containing 500 and 1000mg/kg body weight respectively. The administration was done once daily for 21 days, using oropharyngeal cannula.

Specimen collection: On the 22nd day, the rats from each groups was anaesthetized in chloroform. Under anaesthesia, their blood was also collected and poured into EDTA bottle to preserve them. The blood samples were centrifuged and the serum was collected and poured into plane bottles, ready for analysing.

Estimation of sperm count, motility and morphology: The testes and epididymis were delivered via an abdominal section. The testes from each rats were remove, weighed with an electronic balance (GH-300. Mettle, USA).The samples collected were fixed in Bouin's fluid and processed by the usual method for paraffin embedment and stained with Hematoxylin and Eosin (H & E). 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 Protocol: Cage- side examination were conducted daily to detect signs of toxicity (loss of hair, behavioural 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 labelled antibodies. A solution of Tetramethyl- benzidine 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).

Determination of superoxide dismutase (SOD) activity: Superoxide Dismutase activity was determine by its ability to inhibit the auto-oxidation of epinephrine determined by the increase in absorbance at 480nm as described by (Sun and Zigman, 1978). The reaction mixture (3ml) contained 2.95ml, 0.05M sodium carbonate buffer initiate the reaction. The reference cuvette contained 2.95ml buffer, 0.03ml of substrate (epinephrine) and 0.02ml of water. Enzyme activity was calculated by measuring the change in absorbance at 480nm for 5 minutes.

Statistical analysis: Data obtained from this study was analyzed using the statistical package of social science (SSPS) version 17.0 for windows. Analysis of variance (ANOVA) was done at 0.05 level of significance which $p< 0.05$ was said to be statistically significant. The result obtained thereof is represented as mean± SEM.

RESULTS

Body and testes Weight: Significant body weight gain was observed in the treated group C, when compared to the control group ($P<0.05$) as seen in (Table i). Moreso, there was an increase in the testicular weight of the treated groups as seen also in table ii.

Table i: Body weight (mean ± SEM)

Groups	Initial weight	Final weight	Difference
A (Control)	230.0 ± 1.99	251.6 ± 2.89	21
B (500mg of *C. albidum*)	243.9 ± 0.93	266.3 ± 0.75	22
C (1000mg of *C. albidum*)	250.1 ± 0.55	278.0 ± 0.40	28*

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

Table ii: Weight of the Testes after sacrifice (mean ± SEM)

	Group A	Group B	Group C
Right testes	0.95 ± 0.007	1.23 ± 0.096*	1.39 ± 0.005*
Left testes	1.05 ± 0.012	1.33 ± 0.129*	1.39 ± 0.032*

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

Epididymal Sperm parameters: Data in table iii showed that the administration of 500 and 1000mg/kg extract of ethanolic did show marked increase in sperm count from the control. However, there is significant increase in the sperm motility of 500 and 1000mg/kg ethanolic extract of *C. albidum* when compared to the control

Table iii: Semen parameters (mean ± SEM)

Parameters	Group A	Group B	Group C
Sperm count (10^6/ml)	51.6 ± 1.06	62.5 ± 2.02	69.5 ± 7.09*
Sperm motility (%)	74.8 ± 1.01	76.0 ± 1.26	80.4 ± 1.73*
morphology (% Normal)	70.3 ± 1.70	75.0 ± 2.89	79.6 ± 2.83*

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

Hormone level and SOD: Significant ($p < 0.05$) decrease in FSH, LH, and T levels of the treated groups compared to the control as shown in table iv. Conversely, there was also significant ($p < 0.05$) increase in the activities of SOD level in the high dose (treated) group compared to control as seen in table iv.

Table iv: Hormonal profile of the testes

Parameters	Group A	Group B	Group C
FSH (miu/ml)	O.94 ± 0.02	0.75 ± 0.05*	0.63 ± 0.06*
LH (miu/ml)	1.64 ± 0.02	1.26 ± 0.01*	1.18 ± 0.05*
T (ng/ml)	8.78 ± 0.07	7.02 ± 0.06*	3.86 ± 0.34*
SOD (u/ml)	266.0 ± 1.87	309.8 ± 4.20*	433.2 ± 2.04*

Key: *(p<0.05) significantly different from the control. FSH = Follicle Stimulating Hormone, LH = Luteinizing Hormone. T= Testosterone, SOD= Superoxide dismutase

Histological Analysis of the Testes: The control group have numerous seminiferous tubules that are small and more rounded in shape. They are tight and closely packed interstitium, with Ledig cells, spermatocytes in their lumens. The administration of the ethanolic leave extract of *C. albidum* to albino Wistar male rats did not show any appreciable gross and histological changes of the testes.

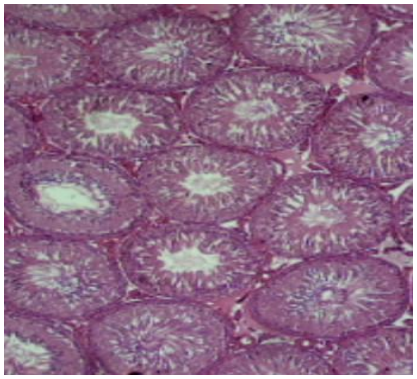

Fig 1: Cross-section of testes from a control rat showing normal spermatogenesis and histological structure of the seminiferous epithelium and intertubular spaces. The lumen is full of spermatids and spermatozoa (H and E; 100 x)

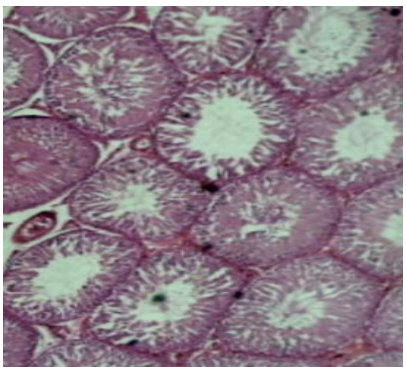

Fig 2: Cross section of the testes from a rat treated with 500mg/kg extract of *C. albidum*, showing evidence of spermatocytes within the varying tubular lumens. (H and E; 100 x)

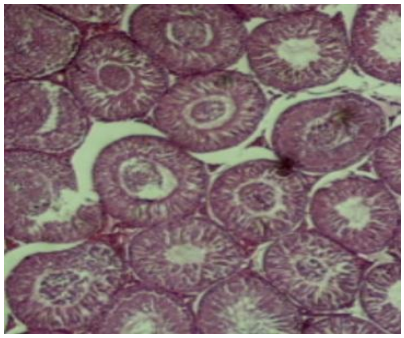

Fig 3: Testicular cross-section from a rat treated with 1000mg/kg bw extract of leave of *C. albidum*, showing normal spermatogenesis (H and E; 100 x)

DISCUSSION

Results indicated that extract of *C. albidum* caused increase in body weight and testes weights. The increase in the testes and body weight observed with the extract may be due to increase in the functional ability of the organ (Ashafa *et al*., 2009). Testicular size and weight is the best primary assessment of spermatogenesis. Increases in testicular weight, which is known to be mostly related to the number of spermatozoa present in the tissue (Shams Lahijani *et al*., 2008). Data from sperm count and motility showed a significant increase ($P<0.05$) in treated groups compared with the control group, the possibility may be due to the extract influence directly on the matured and stored sperm in epididymis, due to the free radical scavenging moiety of *C. albidum* as an anti-oxidant.

In this study, pituitary gonadotropins (FSH and LH) following treatment with *C. albidum* extract were reduced. This implies that the plant acted directly on the anterior pituitary to inhibit synthesis of gonadotropins. The low T obtained in the treated groups may not be due to destruction of the Leydig cells but a reflection of the complex hormonal interplay at the level of the hypothalamic-pituitary-

testicular axis. The extract could disrupt the functioning of the LHRH receptor or its interaction with LHRH resulting in diminished LH release. Studies in animals and humans have shown that when T levels decrease, LH levels do not increase as would be expected (Van, 1983; Maneesh et al., 2006).

However, the data in the present study do not support the notion that enhancement of fertility is due to the increase in testosterone production or secretion for this reasons. First, *C. albidum* did not decrease the weights of testes, which is an androgen-dependent tissue. Secondly, the fertility-enhancing properties of *C. albidum* extract in healthy male rats may not have any correlation with serum reproductive hormone including testosterone. Since a minimum level of blood androgen is thus required, among others for the maintenance of the normal histology of the testes, motility and fertilizing capacity of the spermatozoa (Yakubu *et al*., 2012).

Living systems are therefore protected from ROS by antioxidant enzymes (SOD, CAT, LPO etc.) and other endogenous antioxidant sources (Nilesh *et al*., 2010). SOD has been reported as one of the most important enzymes in the enzymatic antioxidant defence system. It is the only enzyme that employs the superoxide anions as a substrate and produces the hydrogen peroxide as a metabolite and thus diminishing the toxic effect caused by this radical (Curtis and Mortiz, 1972). The significant increase in the levels of SOD could be attributed to the antioxidant properties of *C. albidum* (Onyeka *et al*., 2012b). 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 anti-androgenic effect.

CONCLUSION

In conclusion, results presented here clearly suggest that *C. albidum* leave extract reduced serum levels of gonadotrophins probably by affecting hypothalamic-pituitary-gonadal axis.

The reduced levels of FSH, LH and T do not have effect on the testicular spermatogenesis for 21 days. However there was an increase in the SOD enzyme activities above the control level thereby improving its antioxidative capacity.

Conflict of interest statement: We declare that we have no conflict of interest.

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