



Neutral Effect of Coffee Senna (*Senna occidentalis* (L.) Link Leguminosae) Leaf Ethanol Extract on Reproductive Parameters in Male Wistar Rats

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ABSTRACT: Many antimalarial remedies are known for reproductive toxicity in male. Also, several plants are known to have antifertility action in both sexes. The aim of this study is to evaluate the effect of ethanol extract of *Senna occidentalis* (L.) Link Leguminosae leaf (EESO) on male reproductive parameters such as sperm count, motility, morphology and the histology of testes in Wistar rats by employing standard procedures. The extract (50% ethanol) was orally administered to male Wistar rats daily, at 250, 500 and 1000mg/kg with distilled water as control, for 20 days after which reproductive parameters were performed on the epididymal sperm and testes. The results show that all features were consistent with normal histology of the testes in the treated and untreated groups, the sperm count (SC), motility (SM) and morphology (MP) were also comparable with the control. The plant has been shown to be devoid of the traditional sperm toxicity associated with antimalarial agents.

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Senna occidentalis (L.) Link (Leguminosae) formerly known as *Cassia occidentalis* L. is a prominent member of the Leguminosae family. The common names of this plant include Coffee Senna, Coffee Weed, and Negro coffee among others. It is locally referred to as Sanga-sanga or Rai dore (Hausa) Akidi agbara (Ibo) and Abo rere (Yoruba) in Nigeria (Isah *et al.*, 2018). It grows in more than 50% of the continents and is found distributed in countries like Sri Lanka, India and the United States of America. It is also found in African countries like Nigeria and Ghana. It is an annual/perennial herb which grows up to 0.60-1.50 m in height and at altitudes of up to 1500 m (Ali *et al.*, 2019; Kaur *et al.*, 2014). Reported ethnomedicinal uses of *S. occidentalis* leaf show that it prevents leucorrhoea and also possesses febrifugal, purgative and diuretic properties. It is employed in the treatment of cough, rheumatism, jaundice and it is popular for its relief of many skin ailments (Vijayalakshmi *et al.*, 2013). Phytoconstituents such as physcion, 4, 4, 5, 5-

tetrahydroxy-2,2-dimethyl-1,1-bianthraquinone, helminthosporin, quercetin, questin, xanthorin, occidentalol-I, occidentalol-II, cassiaoccidentals A, B and xanthorin have been reportedly isolated from the leaf (Ali *et al.*, 2019; Yadav *et al.*, 2009). Among the biological activities that have been reported for the plant are hypoglycemic, hepatoprotective, anti-inflammatory, antimalarial, immunosuppressant, anti-atherosclerogenic, hypolipidemic and antipyretic activities (Nde *et al.*, 2022; Ali *et al.*, 2019; Uzzi and Grillo, 2013; Yadav *et al.*, 2009). It is one of the plants which have aphrodisiac function and among the five categories of aphrodisiac, this plant with others like *Cannabis sativa* L. (Cannabaceae), *Strychnos nuxvomica* L. (Loganiaceae) and *Myristica fragrans* Houtt. (Myristicaceae) causes improvement of ejaculatory functions (Chauhan *et al.*, 2014; Singh and Mukherjee, 1998). Female antifertility activity of the plant has also been established in animal model (Nworgu *et al.*, 2019). Plants like *Azadirachta indica*

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A. Juss (Meliaceae) and *Ocimum gratissimum* L. (Lamiaceae) have both male and female antifertility activities (Oguejiofor *et al.*, 2020; Sripriya *et al.*, 2011; Upadhyay *et al.*, 1993; Upadhyay *et al.*, 1990). Effect of some co-generic species like *S. tora* (L.) Roxb. *S. podocarpa* (Guill. & Perr.) Lock and *S. alata* (L.) Roxb on male reproductive parameters has also been evaluated (Akinsomisoye *et al.*, 2021; Khan and Mali, 2017). The objective of this study is therefore to evaluate the effect of ethanol extract of *S. occidentalis* leaf (EESO) on reproductive parameters such as sperm concentration (SC), motility (SM) and morphology (MP) as well as its action on the histology of the testes (HT).

MATERIALS AND METHODS

Identification and Collection of plant material: Identification and authentication of *S. occidentalis* were carried out by Dr. A.T. Oladele of the Department of Forestry and Wildlife Management, University of Port Harcourt, Nigeria. A voucher specimen number NDUP236 was assigned and the voucher sample of the leaf was deposited at the herbarium of the Department of Pharmacognosy and Herbal medicine, Niger Delta University, Bayelsa, Nigeria. Collection of the leaves of *S. occidentalis* was done at the Niger Delta University, Bayelsa State, Nigeria.

Processing of *S. occidentalis* leaf sample: The leaves of *S. occidentalis* were dried in an oven at 40°C for 72 h. and thereafter pulverized. Marceration of 500g of the pulverised plant sample was carried out using 50% ethanol for 72 h. Decantation and filtration of the extract were done, followed by concentration *in vacuo* at 30°C. Re-extraction of the marc was done using the same solvent. The batches of extract obtained were added together to obtain a yield of 18.6 %w/w.

Animals: Male Wistar rats (148.3±2.31g) were bought from the Animal House of the Department of Pharmacology & Toxicology, Faculty of Pharmacy, Niger Delta University, Bayelsa State, Nigeria. The rats were housed in animal cages, kept in a well-ventilated room (12 hr. light/ 12 hr. darkness) and fed with standard diet and water was freely made available. Handling of the rats was done in accordance to the guidelines of the NIH.

Reproductive effect *S. occidentalis* leaf ethanol extract: Four groups of twenty-four male Wistar rats which comprised six each were used in this study. Those in Groups I - III received 250, 500 and 1000 mg/kg EESO, respectively, while group IV which functioned as control, was administered distilled water; the medium of the extract dissolution. A

twenty-day extract administration was used in this study and the animals were allowed to go through an overnight fasting after the last dose. This was followed by anaesthetizing them by using chloroform, the rats were sacrificed; the organs of attention (epididymis and testes) were carefully removed and used for reproductive assessment.

Epididymal sperm count: The freshly excised cauda epididymis was carefully placed in a beaker containing normal saline (1:9) and spermatozoa sample was collected by using a sharp needle to puncture it and allowing them to swim out into the normal saline (Opuwari and Monsees, 2020). A 1 in 20 dilution of sperm was done by using semen fluid after which SC was performed with the aid of improved Neubauer haemocytometer. This was represented as 10⁶cells/ml. Determination of the vitality of sperm (SM and MP) was also performed microscopically at x10 objective using standard method (Opuwari and Monsees, 2020). A drop of semen sample was carefully placed on a clean slide, covered with a coverslip and observed under a binocular microscope. Active, sluggish, non-motile, abnormal and normal spermatozoa were observed, counted and recorded in percentages.

Routine histological preparation: The excised testes were fixed in buffered formalin solution and the tissues were processed with hematoxylin & eosin stain. The preparation of the tissue slides was carried out in the Histology Laboratory, Department of Pathology, Niger Delta University Teaching Hospital Complex, Bayelsa State. The photomicrographs of the tissues were taken at x 100 objectives

Data analysis: GraphPad Prism version 6.0.0 for Windows (GraphPad Software, www.graphpad.com) was employed for the analysis of the obtained data. Data were analyzed with one-way ANOVA (analysis of variance) and Tukey's multiple comparison test. Data were indicated as mean ± standard error of the mean, percentages and a *P*-value less than 0.05 was set as statistically significant.

RESULTS AND DISCUSSION

The epididymal sperm count, SM and MP showed no significant difference between the untreated and treated rats (Table 1). This is at variance with *A. indica* and *O. gratissimum* leaves that have antifertility actions in both male and female rats.

The result of the HT as observed in the treated animals did not exhibit any notable deformity or distortion in the cell architecture when juxtaposed with the untreated ones

Table 1: Effect of *S. occidentalis* leaf ethanol extract on sperm concentration/motility and morphology in male Wistar rats

	Total sperm count/x million cells/ml ± SEM	Sperm Motility (%)			Morphology (%)	
		Active	Sluggish	Non active	Normal	Abnormal
0	1.72 ± 0.23	11	17	72	58	42
250mg/kg	1.75 ± 0.31	10	18	72	56	44
500mg/kg	1.76 ± 0.21	9	15	73	55	45
1000mg/kg	1.76 ± 0.25	9	16	75	56	44

Values are mean ± S.E.M., n = 6 animals per group

Every stage of spermatogenesis cycle was intact and spermatozoa were abundant in the seminiferous tubule lumen in the treated Groups I-III, as well as in the rats

of the control Group IV. Also, the aphrodisiac action reported in ethnomedicine can be justified by the intact leydig cells (Plates 1-3).

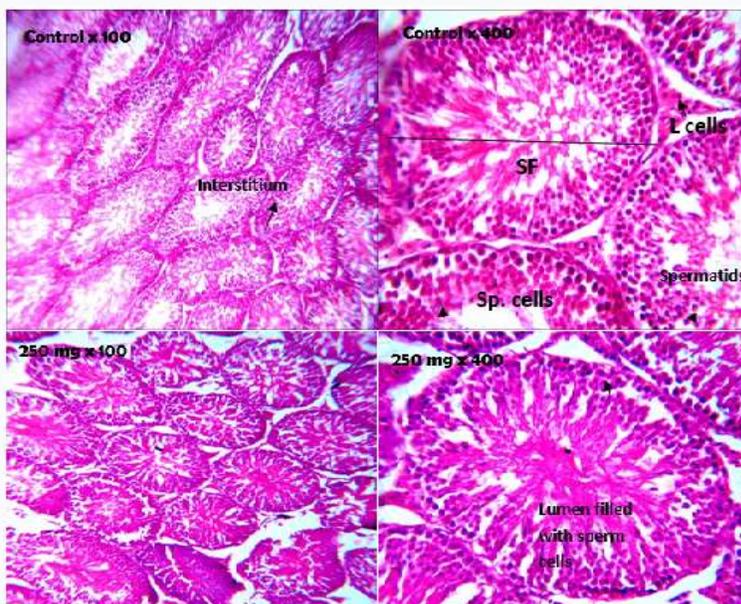


Plate 1: Transverse section of rat testis showing control group and group administered with 250mg/kg *S. occidentalis* ethanol extract showing seminiferous tubules epithelium containing spermatogonia and sertolic cells, and lumen filled with flagella (sperm cells). The interstitial cells show normal histology displaying leydig cells. Features are consistent with normal histology of the testis – Normal Testis

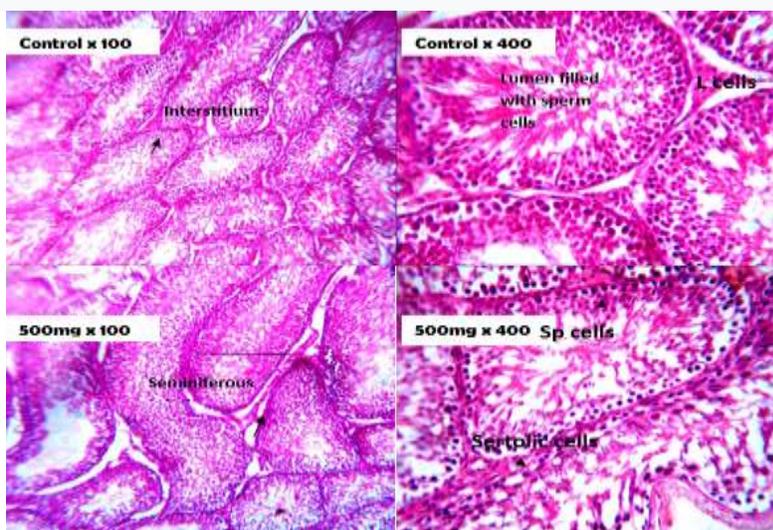


Plate 2: Transverse section of rat testis showing control group and group administered with 500mg/kg *S. occidentalis* ethanol extract showing seminiferous tubules epithelium containing spermatogonia and sertolic cells, and lumen filled with flagella (sperm cells). The interstitial cells show normal histology displaying leydig cells. Features are consistent with normal histology of the testis – Normal Testis

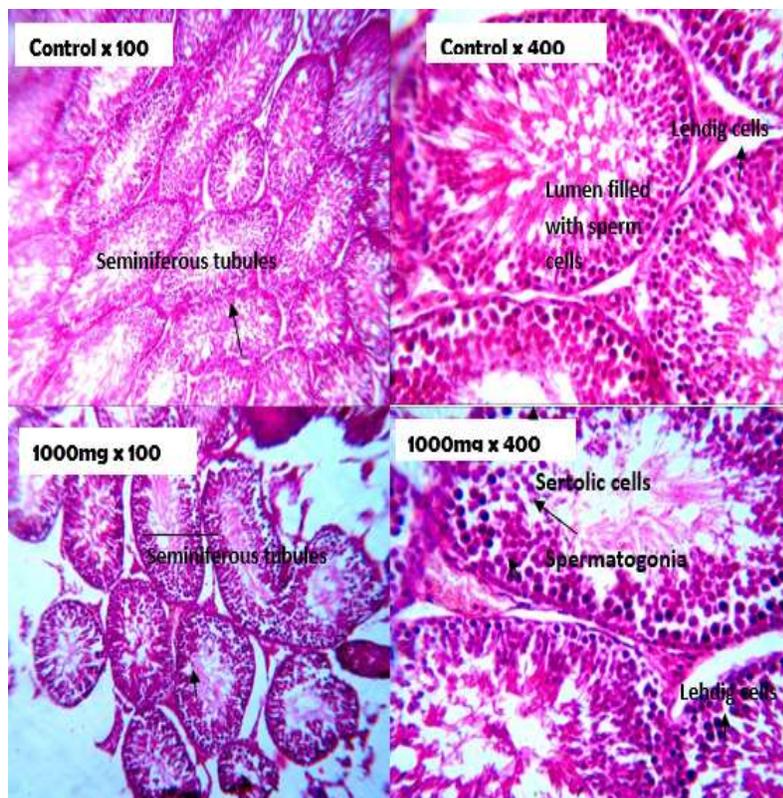


Plate 3: Transverse section of rat testis showing control group and group administered with 1000mg/kg *S. occidentalis* ethanol extract showing seminiferous tubules epithelium containing spermatogonia and sertolic cells, and lumen filled with flagella (sperm cells). The interstitial cells show normal histology displaying leydig cells. Features are consistent with normal histology of the testis – Normal Testis

Several antimalarial agents have been implicated in male antifertility effects, with many of them inducing reproductive toxicity especially on spermatozoa (Adewole and Attah, 2020). This is not limited to synthetic drugs like chloroquine and Artesunate, but also to some medicinal plants such as *A. indica* and *O. gratissimum* which are employed as antimalarial remedies (Erhirhie, 2016; Akinsomisoye and Raji, 2011). Given this result, utilization of *S. occidentalis* leaf therefore, as a remedy for malaria should be devoid of the traditional reproductive toxicity and this makes it an advantage for further development into a suitable dosage form as well as for commercialization.

Conclusion: The leaf of *S. occidentalis* has a potential to be developed as an exceptional antimalarial drug shorn of the expected reproductive toxicity outcome. This drug should be safe for young male adults of child bearing age.

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