Sperm parameters of male Wistar rats treated with *Anacardium occidentale* L. leaf extract

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Abstract

Objective: *Annacardium occidentale* is a medicinal plant with several biological properties. Phytochemical screening of its leaf and stem bark was reported to be rich in alkaloids, polyphenols, tannins and saponins. Many plant extract with these phytochemicals are reputed for their antifertility activities. This study was therefore designed to investigate the effects of *Annacardium occidentale* leaf extract (AOLE) on sperm parameters of male rats.

Methods: Fifteen male rats were randomly assigned to 3 groups (n=5). Group 1 served as the control and received 0.5 ml of the vehicle (distilled water). Groups 2 and 3 received 50 and 200 mg/kg respectively (daily, p.o) for 6 weeks. All rats were sacrificed by cervical decapitation. Organ weights and sperm analysis (motility, viability, count, volume and morphology) were determined.

Results: AOLE had no effect on total body weight, visceral organ weights and weights of testes, caudal epididymis, seminal vesicle and prostate gland. Also sperm viability, sperm count, sperm volume and the percentage of sperm cells with abnormal morphology was not affected by AOLE treatment.

Conclusion: AOLE at doses administered caused a slight decline in sperm motility but had no effect on other male reproductive parameters.

Keywords: *Annacardium occidentale*, sperm, antifertility, phytochemicals

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Paramètres de sperme de rats Wistar mâles traités avec *Anacardium occidentale* L. extrait de feuille

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Resume
Objectif: *Annacardium occidentale* est une plante médicinale avec plusieurs propriétés biologiques. criblage phytochimique de sa feuille et la tige écorce a été signalé à être riche en alcaloïdes, les polyphénols, des tanins et des saponines. Beaucoup extrait de plante avec ces phytochimiques sont réputés pour leurs activités de stérilisants. Cette étude a donc été conçue pour étudier les effets de l'extrait de feuille *Annacardium occidentale* (AOLE) sur les paramètres du sperme de rats mâles.

Méthodes: Quinze rats mâles ont été assignés au hasard à 3 groupes (n = 5). Le groupe 1 a servi de témoin et a reçu 0,5 ml du véhicule (eau distillée). Les groupes 2 et 3 ont reçu 50 et 200 mg / kg, respectivement (par jour, p.o.) pendant 6 semaines. Tous les rats ont été sacrifiés par décapitation cervicale. Le poids des organes et l'analyse du sperme (motilité, la viabilité, le nombre, le volume et la morphologie) ont été déterminées.

Résultats: AOLE n'a eu aucun effet sur le poids total du corps, le poids et le poids des testicules organes viscéraux, épididyme caudal, des vésicules séminales et de la glande de la prostate. Aussi la viabilité des spermatozoïdes, le nombre de spermatozoïdes, le volume de sperme et le pourcentage de spermatozoïdes avec une morphologie anormale n'a pas été affectée par le traitement AOLE.

Conclusion: AOLE à des doses administrées a provoqué une légère diminution de la motilité des spermatozoïdes, mais n'a eu aucun effet sur d'autres paramètres reproducteurs mâles.

Mots-clés: *Annacardium occidentale*, sperme, anticonceptionnel, phytochimiques

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INTRODUCTION

Annacardium occidentale L. is a plant that belongs to the family Anacardiaceae. It is a medium sized tree with evergreen spreading branches and grows to a height of 10-12 m. The plant is native to South America, majorly Brazil and Mexico. Portuguese adventurers dispersed it in the seventeenth century to Africa, India and the Far East, adopting into their language a Brazilian vernacular name *Caju* (**1**). The plant is now known in all European countries and in Nigeria as Cashew (2).

The different parts of *A. occidentale* plant are beneficial and have been used for various purposes (3). The wood is reddish brown, moderately hard and termite-resistant. It is seldom in large pieces and is used in boat-building, for boxes, chests, mortars, house, fence-posts and firewood. The bark and leaf has astringent properties and contains 9–23% tannin and is used in tanning in Senegal and Gabon (**1**). Bark and leaf infusions are used to relieve toothache, sore gums, dysenteric conditions, disease known in Yoruba (south-west Nigeria) as éfu (thrush), symptomized by a white tender tongue, and for another disease known as kolobo or ishanu, a more serious condition with a black tongue (4). In Congo the bark-infusion is taken for urethral discharge and stomach pain by women. The sap may be expressed and taken for diarrhea, used as dye for fishing net, gum for book binding and has physiological action in the treatment of leprosy. It is obnoxious to insects and has practical application in the treatment of wood against decay and termites (**1,3**).

Biological properties attributed to *A. occidentale* such as larvicidal (5), antifungal (3,6) and antibacterial potentials (7,8) have been reported. Also, medicinal properties such as cytotoxicity against tumor cell lines (9), anti-inflammatory (10,11), analgesic (10), hypoglycemic and antidiabetic (12,13,14) potentials have been documented. The leaf extract however possess potent antibacterial and antifungal activity against broad spectrum of human pathogens (3,8,15,16). Recently, Jothi *et al.* reported that both the aqueous and methanol extracts of the leaf have potent antibacterial activity against periodontal pathogens (17). Its use traditionally in Nigeria as an antimalarial is well documented (18,19). The liquid obtained from the shell of cashew nut also have larvicidal, molluscicidal, antifungal, antibacterial, anti-inflammatory, antimicrobial, astringent, diuretic, hypoglycemic and other medicinal properties (3,8).

Phytochemical screening of *A. occidentale* leaf and stem bark has been reported to be rich in alkaloids, triterpenoids, polyphenols, tannins and saponins (15). Many medicinal plants with antibacterial and antimalarial actions have been reported to be rich in these phytochemicals and are reputed for their antifertility actions. These include *Alstonia Boonei (*20*), Azadirachta indica* (21) and *Gossypium herbaceum* (22)**.** With this background knowledge of phytochemical constituents of *A. occidentale* and its use as an antibacterial and antimalarial agent, we therefore sort to evaluate it effects on sperm parameters of experimental rats.

MARETIALS AND METHODS

Annacardium occidentale

Fresh leaves of *A. occidentale* were obtained from the Botanical garden, University of Ibadan and were authenticated at the herbarium of Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria where Voucher specimen number FHI-108362 was assigned to the specimen. The leaves were shade dried and then pulverized. The crude methanol extract were prepared by maceration of 5g of powdered leaves in 100ml methanol (80% v/v) at room temperature for 72 hours, with daily shaking. The extract obtained was concentrated to a black residue on a rotary evaporator at 40^{o}C. The methanol extract of seeds of *A. occidentale* (MEAO) obtained was concentrated to dryness by lyophilization and preserved in refrigerator before use.

Experimental Animals

Adult male Wistar rats (180-200g) were housed in well ventilated wire mesh cages in the Animal House of the Department of Physiology, College of Medicine, University of Ibadan with constant 12-h light 12-h dark cycle. They were fed standard rat feed and clean water *ad libitum* and were allowed two weeks of acclimatization.

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 (2002) as amended (23).

Experimental Design

A. occidentale leaf extract (50 and 200mg/kg *b.w.*) was administered daily (p.o.) for 6 weeks to two experimental groups, while a control group concurrently received vehicle (distilled water) for same duration. Administered doses used were beneficial doses as reported in

literature (13,14). Each group was made up of 5 male rats of Wistar strain. At the end of week 6, all male rats were sacrificed by cervical decapitation. Reproductive and visceral organs were excised, cleared of fat and connective tissue and weighed to the nearest milligram.

Sperm Analysis

Sperm characteristics analysis was performed on spermatozoa samples collected from the caudal epididymis using Olympus research microscope (Olympus, Japan) under X40 microscope objectives. Progressive motility was assessed immediately. A drop of diluted sperm suspension (5µl) was placed on a pre-warmed slide and two drops of warm 2.9% sodium citrate was added and covered with cover slip. Progressive forward motility was examined and scored to the nearest 10 (24). Viability study (percentage of live spermatozoa) was done using eosin/nigrosin stain. The motile (live) sperm cells were unstained while the non-motile (dead) sperms absorbed the stain. The stained and unstained sperm cells were counted and an average value for each was recorded from which percentage viability was calculated. Sperm count was done under the microscope with the aid of the improved Neubauer hemocytometer. Counting was done in five Thoma chambers (25). The epididymis was immersed in 5 ml normal saline in a measuring cylinder and the volume displaced was taken as the volume of the epididymis (26).

Sperm morphology was evaluated by staining the sperm smears on microscope slides with two drops of Walls and Ewa stain after they were air-dried. The slides were examined under the microscope under oil immersion with X100 objectives. The abnormal sperm cells were counted and the percentage calculated according to the method described by Wyrobek and Bruce (27).

Statistical Analysis

Data were expressed as mean ± standard error of mean (SEM). Comparisons were made by using Student's t-test at p=0.05.

RESULTS

There were no changes in the total body weight of rats during treatment with either dose of *A. occidentale* extract (Table 1). Also weights of visceral organs viz lungs, liver, heart, kidney and spleen were not significantly different when either *A. occidentale* treatment dose was compared with the control (Table 2). Total epididymal weight significantly increased by 200 mg/kg *A. occidentale* treatment, but no changes were observed in the caudal epididymal weight in both treatment groups when compared with the control. Also, no effect was observed on the weights of testes, seminal vesicle and prostate gland (Figure 1). Most of the sperm of the control rats had normal motility, viability, count and volume. In the *A. occidentale* extract treated rats, sperm motility showed a slight decline in progressive forward motility. The percentage of sperm cells with abnormal morphology were however not significantly different when treatment groups was compared with the control (Figure 2).

DISCUSSION

The effects of *A. occidentale* treatment on male sperm parameters were assessed in this study. *Anacardium occidentale* at doses administered had no effect on the weight of testis, seminal vesicle and prostate gland. Antihyperglycemic and renal protective properties of this plant at similar doses have reported. The total epididymal weight of *A. occidentale* (200mg/kg) treated rats was significantly increased when compared with the control rats. However, the caudal epididymal weight was not affected. Also, no significant effect was observed on sperm viability, sperm count, sperm volume and sperm morphology. Lack of effect on *A. occidentale* on sperm parameters and weight of sex and accessory sex organs suggests that *A. occidentale* leaf extract does not interfere with fertility in male rats.

Many medicinal plants with antibacterial or antimalarial potentials such as *Quassia amara* (28), *Gossypium herbaceum* (22) and *Azadirachta indica* (21) were reported to have antifertility potentials. Antimalarial or antibacterial use of these medicinal plants may have been limited by their antifertility charecteristics. However, *A. occidentale* has also been reported to have antimalarial properties and its use for malarial therapy in southwest Nigeria has been documented (18). Unlike majority of these plants with antimalarial properties, results from this study showed that *A. occidentale* appears to have minute antifertility effect, save the slight decline observed in the sperm motility of the treated rats. Also, earlier reports from literature indicated that *A. occidentale* treatment had no effect on the cyto-architecture of rat testis (29). The authors also reported no evidence of apoptotic bodies in the testis and therefore concluded that *A. occidentale* had no effect on histology of rat testis. In another study, sub acute

toxicity and genotoxicity study after 30 day exposure to *A. occidentale* crude extract produced no toxicity symptoms up to 2000 mg/kg (30). Also Edet *et al*. reported no effect on histoarchitecture of brain and kidney when rats were treated with *A. occidentale* leaf extract up to 2000 mg/kg (31).

CONCLUSION

A. occidentale at doses administered had no toxicity on male reproductive parameters. In the light of continued search for an ideal antimalarial drug with minimal side effect, further research on the antimalarial properties of *A. occidentale* is therefore recommended.

Conflict of interest: No conflict of interest was declared.

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Table 1: Total body weights (g) of *A. Occidentale* treated-rats.

	WEEK 1	WEEK 2	WEEK 3	WEEK 4	WEEK 5	WEEK 6	SACRIFICE
Control	190±4.5	200±5.5	220±8.9	222±6.6	232±3.7	236±2.4	250±5.5
50mg/kg	186±4	192±7.3	210±5.5	212±4.9	226±2.4	234±2.4	240±3.2
200mg/kg	192±4.9	196±5.1	208±7.3	212±3.7	224±2.4	230±3.2	238±3.7

Values are mean ± SEM, n = 5, g = gram *A. Occidentale* treatment had no effect on the total body weight of treated rats when compared with the control

Table 2: Visceral organ weights (g) of *A. Occidentale* treated-rats.

Organ	Lungs (g)	Liver (g)	Heart (g)	Kidney (g)	Spleen (g)
Control	1.48± 0.04	6.54 ± 1.31	0.6 ± 0.09	0.68 ± 0.05	1.01 ± 0.05
50mg/kg	1.53 ± 0.2	6.99 ± 0.5	0.73 ± 0.05	0.69 ± 0.05	0.91 ± 0.13
200mg/kg	1.55 ± 0.11	7.25 ± 0.4	0.75 ± 0.06	0.71 ± 0.04	1.2 ± 0.11

Values are Mean ± SEM, n = 5, g = gram. Comparisons made with the values of the corresponding control group (student'sttest) showed no significant changes.

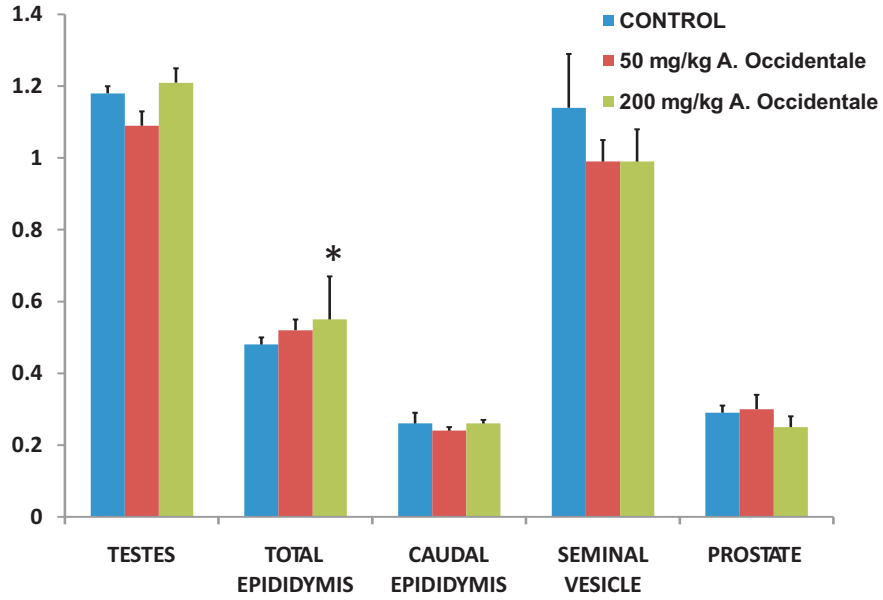

Figure 1: Weight (g) of sex and accessory sex organs of *A. Occidentale* treated-rats
A. Occidentale treatment had no effect on weights of caudal epididymis, seminal vesicle and prostate gland. Total epididymal weight was however increased by 200 mg/kg treatment

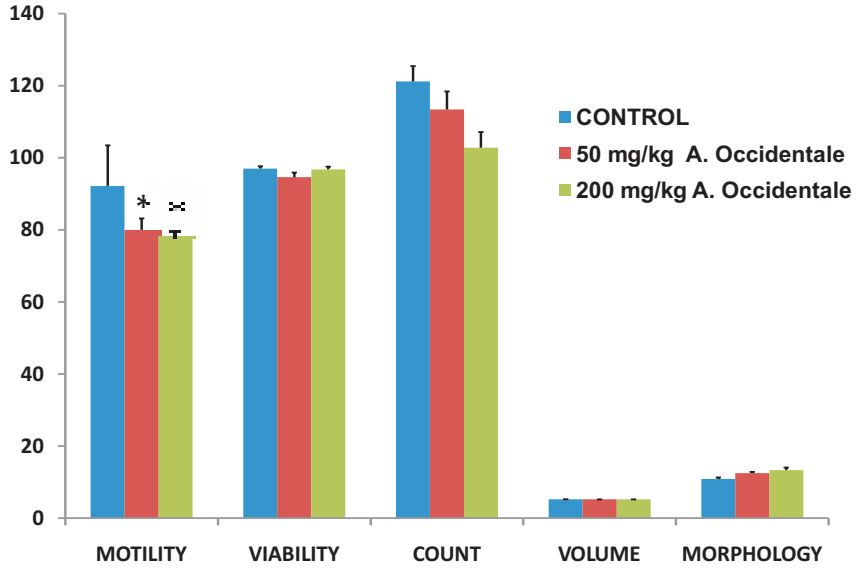

Figure 2: Sperm variables of *A. Occidentale* treated-rats
The sperm progressive forward motility declined in both *A. occidentale* treated groups No effect was observed on sperm viability, count, volume and morphology