Nig. J. Anim. Prod. 2019, 46(2):47 - 54 Nigerian Journal of Animal Production

Effects of aqueous leaf extract of *Acalypha wilkesiana* on semen morphology and characteristics in male Wistar albino rats

[1]Olukunle, J. O., [2]Jacobs, E. B., [1]Adeleye, O. E., [1]Oyewusi, J. A., [1]Adenubi, O. T. and [1]Durotoye, L. A.

[1]Department of Veterinary Physiology and Pharmacology, College of Veterinary Medicine, Federal University of Agriculture, PMB 2240, Abeokuta, Ogun State.

[2]Department of Veterinary Medicine and Surgery, College of Veterinary Medicine, Federal University of Agriculture, PMB 2240, Abeokuta, Ogun State.

***** *Correspondence author: drfaks@yahoo.com; 234-810-184-6078*

Abstract

To study the effect of the aqueous leaf extract of Acalypha wilkesiana (Euphorbiaceae) on some reproductive parameters of male Wistar albino rats, three groups (A, B and C) of six rats each were treated with 400, 800 and 1600 mg/kg/day of leaf extract of Acalypha wilkesiana, respectively for 28 days while 5 mLs/kg of distilled water was given to the control group D. At the end of the experimental period, animals were sacrificed and sperm characteristics, histology of the testes and epididymis were assessed. Sperm motility (80.00±7.0, 75.00±2.9), sperm count (82.75±2.7, 76.50±3.8) and sperm live/dead (96.00±2.0, 94.50±1.7) were not significantly reduced (P<0.05) at the dosages of 400 and 800 mg/kg Acalypha wilkesiana leaf extract, respectively. did not have any significant reduction (P<0.05) in, but significant (P<0.05) reductions in sperm count (71.50±3.5) was observed at 1600 mg/kg dose. Also, significant (P<0.05) increase in morphological abnormalities of the spermatozoa were only observed in the 1600 mg/kg treated group of rats. Histopathological evaluation of the testis and revealed varying degrees of degeneration and necrosis of the germinal epithelia cell of the seminiferous tubules and spermiostasis, whereas no significant lesion was seen in the epididymis. The high dose of the leaf extract of Acalypha wilkesiana may have significant anti-spermatogenic effects on adult male Wistar rats.*

Keywords: *extract, leaf extract of Acalypha wilkesiana,* rats, sperm.

Introduction

Acalypha wilkesiana also called copperleaf or Jacob's coat and 'jinwini, eela or aworoso' among the Yorubas of West Africa, belongs to the plant family Euphorbiaceae and is a large fast growing evergreen coarse textured shrub whose heart shaped leaves are available in varying mottled color combinations of green, purple, orange, pink or white.

Historically believed to be a native of Asia, it has been reported with pain killing activities (Burkhill, 1985), antidiarrheal (Oluyemi *et al*., 1998), antimicrobial activities against gram +ve bacteria and other species of bacteria (Akinyemi *et al*., 2006). It is also claimed to have antifungal activity effective in treating eczema, and *Tinea pedis, Pityriasis versicolor, Candida intetrigo, Impetigo contagia* and *Tinea coporis* infections (Oyelami *et al*., 2003). It exhibits antitumor and antiinflammatory activities when used in combination with other plants, as employed empirically by traditional healers in southwest Nigeria to treat breast tumors and inflammation (Bussing *et al.,* 1999). *A. wilkesiana* has hemolytic activities in vitro and has been used as antihypertensive agent with diuretic properties (Kokko, 1984; Ikewuchi *et al.,* 2008). Recently it has also been reported that the chemical

constituents of the crude extracts of this plant has antiplasmodial effects along with analgesic activities (Udobang *et al.,* 2009). Alkaloids, tannins, saponins, flavonoids, anthraquinones and glycosides have been reported to be some of the phytochemical constituents of this plant (Akinyemi *et al*., 2006; Oladunmoye, 2006; Hirano *et al*., 1989). In addition, Akinde (1986) and Adesina *et al*. (2000) have also reported presence of Gallic acid, corilagin, gerenin, quercetin 3-0 rutinoside, sequiterpenes, monoterpenes, triterpenoids and polyphenols.

There have been various reports on the antispermatogenic and antifertility effect of antimalarial agents. Chloroquine, an antimalarial drug, has been discovered to have negative effects on sperm motility and fertility as a whole (Okanlawon *et al*., 1993); quinine is also known to inhibit spermatogenesis (Osinubi *et al*., 2004). Furthermore, pyrimethamine was observed to arrest spermatogenesis and cause infertility in a dose-dependent manner. Cessation of administration of the aforementioned drugs resulted in full restoration to normal fertility status (Awoniyi *et al.,* 1993; Consentino *et al*., 1990). Many medicinal plants have also been reported to have antispermatogenic effects. *Alstonia boonei*, a tropical plant, reputed in traditional medicine to have antimalarial, antipyretic, analgesic and anti-inflammatory properties (Ojewole, 1984; Olajide *et al*., 2000) was reported to cause dose dependent changes in the body weight, organ weights and sperm characteristics in male rats. *Azadirachta indica*, another medicinal plant with very potent antiplasmodial activities in mice, has also been reported to cause mass atrophy of spermatogenic elements and Leydig cells (Gbile, 1986).

There has not been any documented report on the effect of the leaf extract of *Acalypha wilkesiana* on sperm parameters. This study was therefore carried out to investigate the effects of the administration of aqueous leaf extract of *Acalypha wilkesiana* on the sperm characteristics and morphology of male Wistar rats.

Materials and methods

Animal management

Twenty-four adult male Wistar rats weighing between 170-200g were used for this study. The rats were housed in the Experimental Animal Unit of the College of Veterinary Medicine, University of Agriculture, Abeokuta, Ogun State, Nigeria. They were kept in well-ventilated metal cages at ambient temperature of 26 ± 4^{o}C and a period of 12-hour light and 12hour darkness was maintained. The rats were fed standard ration (Vital Feeds Limited, Ibadan) and clean water *ad libitum*.

Plant material

The leaf of *Acalypha wilkesiana* was harvested within the campus of the Federal University of Agriculture, Abeokuta, Ogun State. Identification and authentication was done at the Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria.

Plant extraction

The plant extraction was done as described by (Iwu and Igboko, 1982; Iwu 1985). Briefly, the leaf of *Acalypha wilkesiana* was air dried, pulverized, finely sieved and 500g of the plant was soaked in 1 litre of distilled water for 24 hours after which it was filtered. Thereafter, the filtrate was evaporated to dryness and 1g of it was dissolved in 20 mLs of distilled water to give a concentration of 50 mg/mL.

Experimental procedure

The rats were randomly assigned into four groups (A-D) of 6 rats each. The rats in groups A, B and C were given aqueous extract of the leaf of *Acalypha wilkesiana* at 400, 800 and 1600mg/kg body weight,

respectively once daily orally for 28 days. Group D rats were the control to which 5 mLs/kg distilled water was administered orally once daily for 28 days. Thereafter, the rats were euthanized by placing them in a glass chamber containing cotton wool soaked in diethyl ether till they lost consciousness followed by cervical dislocation. A ventral midline abdominal incision was then made using a scalpel blade size 14 to expose the abdominal organs. The testis and epididymis of each rat were identified, carefully removed and a small incision was made on the caudal epididymis to squeeze out the semen content on a glass slide for semen evaluation. The testis and epididymis were thereafter preserved in Bouins fluid for histopathological evaluation.

Statistical analysis

Data were subjected to ANOVA package of SAS (1999) and means were separated using DMRT of the same software at P<0.05

.

Results

Acalypha wilkesiana leaf extract at the dosages 400 mg/kg and 800 mg/kg did not cause any significant change in the percentage life sperm cells/ death ratio (96.00±2.0, 94.50±1.7), sperm motility (80.00±7.0, 75.00±2.9), and sperm count of the treated rats when compared with the control (Table 1). However, at the dosage of 1600mg/kg, there was a decrease in the sperm count of the Wistar rats following oral administration of aqueous extract of *A. wilkesiana* leaves. The decrease in sperm count value of (71.50±3.5) in the group dosed with 1600mg/kg is statistically significant at (P<0.05) when compared with the control (Table 1).

At the dose of 400mg/kg, statistically significant (P<0.05) increases were observed in the values of bent tail (3796.3±274.9) and curved tails (3519.0±268.2) when compared to the control (Table 2).

400mg/kg	80.00±7.0	96.00±2.0	82.75±2.7
800mg/kg	75.00±2.9	94.50±1.7	76.50±3.8
1600mg/kg	85.00±5.0	96.50±0.9	71.50±3.5*
Control	93.75±1.3	98.00±0.0	129.00±5.0

Table 1 : Effects of *Acalypha wilkesiana* aqueou s extract on serum characteristic of rats

ACALYPHA WILKESIANA	SPERM MOLITY	LIVE/DEAD	SPERM COUNTS

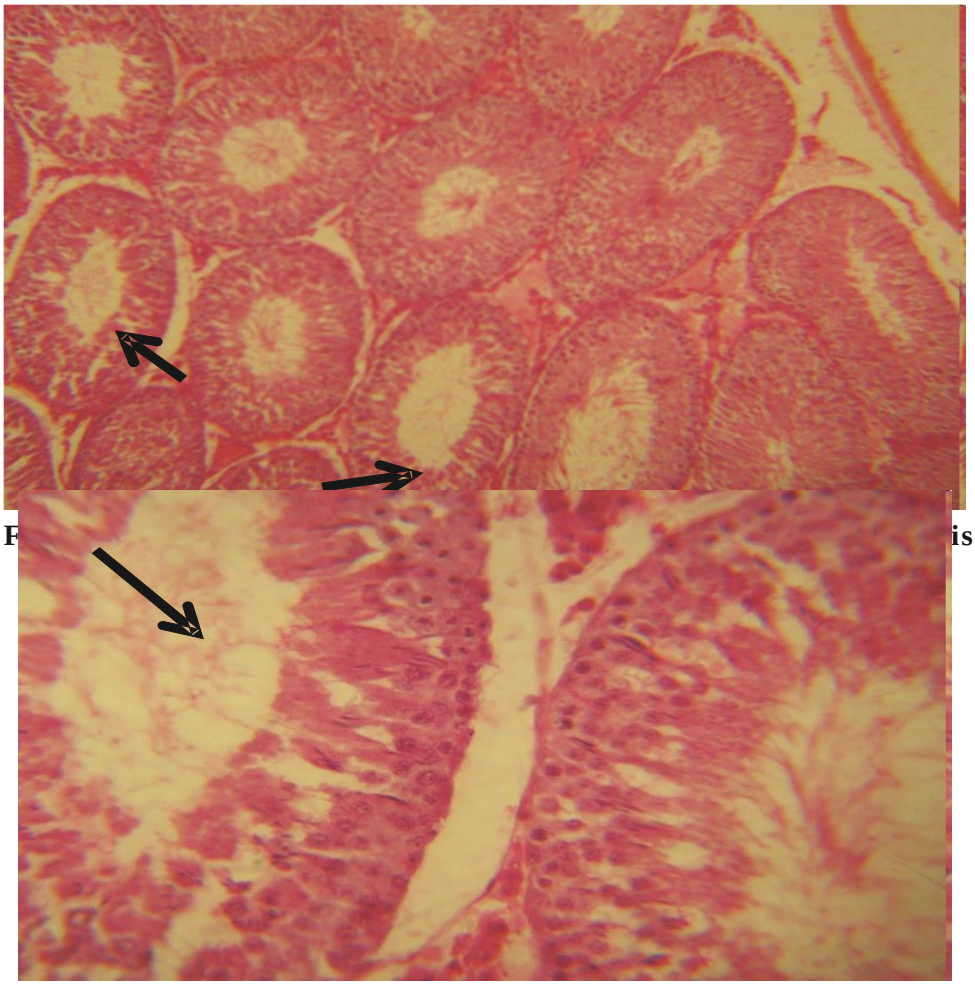
* Superscripted items are significant at P <0.05.

There was no significant (P<0.05) change in the morphology of spermatozoa in the group treated with 800mg/kg when compared with the control except in the curved tail (3517.25±269.2) which is statistically higher (P<0.05) than the control (Table 2). However, at 1600mg/kg dose, there were significant increases (P<0.05) in the values observed in headless tail (239.3±27.3), tailless head
(239.25±27.6), bent tail (4157.0±475.45), curved tail (4099.3±260.0) and curved midpiece (4557.5±259.3) compared to the Control. Others like rudimentary tail (18.50+2.5), bent midpiece (4379.00+388.0) and looped tail
(16.00+2.9) did not exhibit any statistically significant (P<0.05) changes when compared to the control values (Table 2). Histopathology of the testis and epididymis rats administered 400 and 800 mg/kg doses of aqueous leaf extract of *A. wilkesiana* revealed no significant lesion, except the group dosed with 1600mg/kg which had various degrees of lesions in the testis including spermiostasis, degeneration and necrosis of the germinal epithelial cells of the seminiferous tubules (Figures 1 and 2). At 1600mg/kg body weight, the following lesion was observed:

Table 2: Effects of *Acalypha wilkesiana* aqueous extracts on serum morphology of rats (Mean ± Standard Error of Mean)

Acalypha wilkesiana (mg/kg)	Headless Tail	Tailess Head	Rudimentary Tail	Bent Tail	Curved Tail	Bent Mid Piece	Curved Mid Piece	Looped Tail	Coiled Tail	Total Abnormalty
400	191.25±62.3	118.75±56.5	11.0±4.1	3796.3±274.9*	3519.00±268.2*	2769.3±323.8	3102.00±260.9	18.50±2.5	11.00±4.1	406.3±2.4
800	164.00+47.7	188.50+60.6	16.00+2.9	3546.00+259.4	3517.25+269.2*	3216.8+408.1	3256.5+28.6	16.00+2.9	11.00+5.8	403.75+24
1600	239.3+27.3*	239.25+27.6*	18.50+2.5	4157.0+475.4*	4099.3+260.0*	4379.00+388.0	4557.5+259.3*	16.00+2.9	11.00+4.0	403.75+2.4
Control	116.25±54.9	68.75±47.5	8.50±4.8	2492.00±279.0	2767.00±290	3494.25±240.0	2696.25±300.6	13.50±4.8	6.00±2.9	403.75±2.4

* Superscripted items are significant at P <0.05.



F **is,**

Figure 2: Enlarged photomicrograph of the testis showing degeneration and necrosis of the germinal epithelial cells of the seminiferous tubules (arrows). Hand E. X 200.

spermiostasis of the germinal epithelial cells of the seminiferous tubules (arrow) H & E, X450.

Discussion

Administration of the doses of aqueous leaf extract of *Acalypha wilkesiana* (400, 800 and 1600 mg/kg) did not cause any change in progressive sperm motility, percentage sperm live/dead ratio and sperm count. Some secondary morphological sperm abnormalities such as headless tail, bent tail and curved mid-piece were significantly higher in the 1600mg/kg treated rats, this may be due to the presence of high level of the phytochemicals with antispermatogenic tendency in the extract of *Acalypha*

wilkesiana used at extra- pharmacological dosage. Also there were obvious testicular lesions observed at 1600mg/kg dose of the extract (Figures 1 and2) which are sequela of the effects of the antispermatic phytochemicals.

The leaf extract of *Acalypha wilkesiana* is believed to have a wide safety margin. It was observed that oral administration of extract of *A. wilkesiana* up to 3000mg/kg with LD50 of 2828.34 mg/kg to rats neither showed mortality nor any apparent signs of weakness in the animals (Olukunle *et al*., 2015). However, in this study, three doses; 400, 800 and 1600mg/kg were tested. There were usually multiple doses of 400mg/kg of the extract mostly used by herbal practitioners.

Sperm motility depends on the coordinated propagated flagella wave under acetylcholinesterase control (Nelson, 1972). Fructose utilization and glucose oxidation are important means by which spermatozoa derive energy for their motility. The reduction in the progressive sperm motility of the treated rats seen in this study could be due to the acetylcholinesterase inhibition and glucose lowering properties of the species of this plant (Nelson, 1972).

Since several studies have reported the antifertility effects of antimalarial agents, this result is in consonance with previous studies on chloroquine, quinine and quinacrine all of which have been reported to inhibit Leydig cell steroidogenesis and fertility in the male (Adeeko and Dada, 1998).

Conclusion and recommendation

In conclusion, this study shows that daily oral administration of 1600mg/kg body weight of *Acalypha wilkesiana* leaf extract for a period of 28 days has tendency to exhibit anti-spermatogenic effects in adult male Wistar rats as seen with most other anti-malarial herbs. Further studies aimed at elucidating the mechanism of activity of *Acalypha wilkesiana* extract would be worthwhile.

References

Adesina, S. K., Idowu, O., Ogundaini, A., Oladimeji, H., Olugbade, T. A., Onawunmi, G. O., Akinde, B. E. and Odeyemi, O. O. 1987. Extraction and Microbiological Evaluation of oils from the leaves of *Acalypha Wilkesiana*. *Nigerian Med. Journal* 38: 147-158.

Akinde, B. E. and Odeyemi, O. O. 1987. Extraction and microbiological evaluation of the oils from the leaves of *Acalypha wilkesiana*. *Nigerian Medical Journal* 17:163–165.

Akinyemi, K. O., Oluwa, O. K. and Omomigbehin 2006. *African Journal of Traditional Complimentary and Alternative Medicines*, 3, 4-12.

Awoniyi, C. A., Chandrashekar, V., Hurst, B. S., Kim, W. K., Schlaff, W. D., 1993. The effects of chronic administration of pyrimethamine on spermatogenesis and fertility in male rats. *Journal of Andrology*. 14 (3): 174-179.

Burkill, H. M. **1985**. The Useful Plants of West Tropical Africa, Vol. 2. 2nd ed. Royal Botanic Garden, Keaw, London, p. 127.

Bussing, A., Stein, G. M., HerterichAkinpelu, I., Pfuller, U. **1999**. Apoptosis-associated generation of reactive oxygen intermediates and release or pro-inflammatory cytokines in human lymphocytes and granulocytes by extracts from the seeds of *Acalypha*

wilkesiana. *Journal of Ethnopharmacology* 66, 301–309.

Cosentino, M. J., Ruth, E., Fried, J. 1990. Pyrimethamine; An approach to the development of a male contraceptive. *Proceedings of National Academy of Science*. USA, 87: 1431-1435.

Gbile, Z. O. 1986. Medicinal plant research in Nigeria. Proceedings of workshop on state of medicinal research in Nigeria. Sofowora, A. (Ed). Ife University Press. 21.

Hirano, T., Kokka and Akiba M. 1989. Effects of Synthetic and natural flavonoids on Ng, K-ATPase; Aspect of the structure relationship and action mechanism *Life Science*; 45: 1111-1117.

Ikewuchi, J. C., Anyadiegwu, A., Ugono, E. Y. and Okungbowa, S. O. **2008**. Effects of *Acalypha Wilkesiana* Muell Arg on Plasma Sodium and Potassium Concentration of Normal Rabbits. *Pakistan Journal of Nutrition* 7 (1): 130-132, 2008.

Kokko, J. P. 1984. Site and Mechanism of action of Diuretics. *American Journal of Medicine*77:11-17

Nelson, L. **1972**. Quantitative evaluation of sperm motility control mechanisms. *Biology of. Reproduction.* 6:319-324.

Ojewole, J. A. O. 1984. Studies on the pharmacology of Echitamine, an alkaloid from the stem bark of *Alstonia boonei*. L *International Journal Crude Drug Research* 22: 121-143.

Okanlawon, A. O., Noronha, C. C., Ashiru, O. A. 1993. An investigation into the effect of chloroquine on fertility of male rats. *West. African. Journal of Medicine*. 12:118-121.

Olajide, O. A., Makinde, J. M. Okpako, D. T. and Awe, S. O. 2000. Studies on the anti-inflammatory and related pharmacological properties of the aqueous extract of *Bridelia ferruginea* stem bark. *Journal of Ethnopharmacology* 71:153-160.

Osinubi, A. A., Akinola, J. T., Agbaje, M. A., Okanlawon, A. O., Noronha, C. C. 2004. Effects of short term administration of quinine on the seminiferous tubules of SpragueDawley rats. *Nigeria Journal of Health and Biomedical Sciences* 3(1): 1-7.

Oladunmoye, M. K. 2006. Comparative Evaluation of Antimicrobial Activities and Phytochemical screening of two varieties of *Acalypha wilkesiana*. *Trends in applied sciences Research* 1(5); 538-541.

Olukunle J. O., Jacobs, E. B., Ajayi, O. L. and Biobaku, K. T.. 2015. Toxicological evaluation of the aqueous extract of Acalypha wilkesiana in Wistar albino rats. Journal of Complementary and Integrative Medicine (DGJCIM)10(1): 12(1):53-56, 2014. http://www.degruyter.com/view/jcim.2015. 12 .issue-1/jcim -2.

Onawunmi, G. O. 2003. Clinical evaluation of Acalypha ointment in the treatment of superficial fungal

skin diseases. *Phytotherapy Research* 17, 555–557.

Oyelami, O. A., Onayemi, O., Oladimeji, F. A., Ogundaini, O. A., Olugbade, T. A., Pais, M. 2000. Antimicrobial constituents of the leaves of *Acalypha wilkesiana* and *Acalypha hispida*. *Phytotherapy Research* 14, 371–374.

Udobang, J. A., Nwafor P. A. and Okokon J. E. 2009. Analgesic and antimalarial activities of crude leaf extract and fractions of *Acalypha wilkesiana*. *Journal of Ethnopharmacology* 127 (2010) 373–378.

Vogler, B. K. and Ernst, E. **1999**. "Aloe Vera: A Systematic Review of Its Clinical Effectiveness". *British Journal of General Practice* **49** (447): 823–8. PMID10885091

Received: 12th July, 2018
Accepted: 9th February, 2019