

### Effects of *Lippia multiflora* leaf extract on the semen of Wistar rat

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#### Abstract

To investigate the effect of *Lippia multiflora* (Verbanaceae) on the reproductive activity of male Wistar rats, three groups (A,B and C) of rats were treated with 400, 800 and 1600 mg/kg/day of *Lippia multiflora* leaves extract respectively for 28 days while 5ml/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. *Lippia multiflora* leaf extract caused a significant reduction ( $P<0.05$ ) in sperm motility, a statistically significant dose dependent reduction in the sperm count and a significant ( $P<0.05$ ) dose dependent increase in morphological abnormalities of the spermatozoa of the treated rats. Histopathological evaluation of the testis and epididymis revealed varying degrees of degeneration and necrosis of the germinal epithelia cell of the seminiferous tubules, spermiostasis and complete epididymal aspermia. *Lippia multiflora* leaf extract has significant anti-spermatogenic effects on adult male Wistar rats which could impair reproductive activities in these rats.

**Keywords:** extract, *Lippia multiflora*, rats, sperm.

#### Introduction

*Lippia multiflora* is a herbaceous plant of the genus *Lippia* which belongs to the family Verbanaceae (Jigam *et al.*, 2009; Owolabi *et al.*, 2009). *L. multiflora* is a stout woody, perennial and aromatic shrub mainly distributed throughout tropical Africa, South and Central American countries (Pascual *et al.*, 2001). In Northern Nigeria, *L. multiflora* is commonly found along river beds. It is called bush tea, healer herb, Bunsurun fadama or 'godon kada' (Hausa) (Kunle, 2000). The Yoruba names are 'Efinrin gogoro', 'Efinrin odan' or 'Efinrin Ajase' according to the specific area (Owolabi *et al.*, 2009)..

Some researchers have reported several known compounds and secondary metabolites in the plant. Such metabolites which are known to belong to special classes of organic compounds that possess

potent pharmacological activities and have been reported in literature include, essential oils, lignins, cellulose, tannins, starch, oxalates, flavonoids, saponins, glycosides, peptides, caffeine, terpenes and alkaloids (Kunle *et al.*, 2002; Kunle *et al.*, 2003; Jigam *et al.*, 2009). However, very few of these compounds have been isolated and characterized from the plant.

The *Lippia* species have a long history of traditional medicinal application some of which have scientific validation. *L. multiflora* has been used in many traditional and herbal medicines to treat bronchial inflammation, malaria fever, conjunctivitis, gastro-intestinal disturbance, enteritis, coughs and colds (Pascual *et al.*, 2001), and possesses hypotensive, fatigue-relieving, and diuretic properties (Kanco *et al.*, 2004). In an *in vitro* antimalarial study by Valentin *et al.* (1995), the oil of *L. multiflora* was found to be active against cultures of *Plasmodium falciparum* (FcB1-Columbia

chloroquine-resistant strain and F32-Tanzania chloroquine-sensitive strain) parasite. When tested on a synchronized culture, the essential oil inhibited growth mostly at the trophozoite-schizont stage, indicating a potential effect on the first nuclear division of the parasite (Valentin *et al.*, 1995).

There have been various reports on the anti-spermatogenic and anti-fertility effect of anti-malarial agents (Adeeko & Dada, 1998; Awoniyi *et al.*, 1993). Chloroquine, an anti-malarial drug, has been discovered to have negative effects on sperm motility and fertility as a whole (Okanlawon *et al.*, 1993) while quinines have negative effects on spermatogenesis (Osinubi *et al.*, 2004). In addition, pyrimethamine was observed to cause spermatogenic arrest and infertility in a dose-dependent manner and upon cessation of drug administration, all animals returned to normal fertility status (Awoniyi *et al.*, 1993).

Many medicinal plants have also been reported to have anti-spermatogenic effects. *Alstonia boonei*, a tropical plant, reputed in traditional medicine to have anti-malarial, anti-pyretic, analgesic and anti-inflammatory properties (Olajide *et al.*, 2000) was reported to cause dose dependent changes in the body weight, organ weights and sperm characteristics of male rats. *Azadirachta indica*, another medicinal plant with very potent anti-plasmodial activities in mice, has also been reported to cause mass atrophy of spermatogenic elements and Leydig cells (Gbile, 1986).

There is a dearth of information on the effect of *Lippia multiflora*, a potent anti-malarial herb on sperm parameters. This study was therefore carried out to investigate the effects of the administration of aqueous extract of *Lippia multiflora* leaves on the spermogram of male Wistar rats.

## **Materials and Methods**

### ***Experimental Animals***

Twenty four adult male Wistar rats (mean body weight- 180.0±3.33g) were used for this study. The rats were housed in the Experimental Animal Unit of the College of Veterinary Medicine, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria. They were kept in well-ventilated metal cages at ambient temperature and a period of 12 hour light and 12 hour darkness was maintained. The rats were fed standard ration (Vital Feeds Limited, Ibadan) and clean water *ad-libitum*.

### ***Plant material***

Plant extraction was done as described by Iwu *et al.*, (1990). Briefly, the leaves of *Lippia multiflora* was purchased from Kuto Market in Abeokuta, Ogun State. Identification and authentication was done at the Forestry Research Institute of Nigeria (FRIN).

### ***Plant extraction***

The plant extraction was done as described by (Iwu and Igboko, 1982 and Iwu 1985). Briefly, the leaf of *Lippia multiflora* 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 20mls of distilled water to give a concentration of 50mg/ml.

### ***Experimental procedure***

The rats were randomly divided into four groups (A-D) of 6 rats each. The rats in groups A, B and C were given aqueous extract of *Lippia multiflora* leaf extract at 400mg/kg, 800mg/kg and 1600mg/kg body weight respectively once daily orally for 28 days. Group D rats were the control to which 5ml/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 chloroform 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 Bouin's fluid for histopathology evaluation.

#### **Microscopic Examination of Semen**

The microscopic examination of semen includes assessing sperm concentration, motility, vitality, total sperm count and secondary morphological abnormalities and these were done as described by (Blom, 1973; Mortimer, 1994 and WHO, 1987). Briefly, the sperm concentration was determined by harvesting epididymis into a graduated test tube containing 5.0ml of distilled water to obtain a final volume (volume of distilled water + epididymis). The epididymis in distilled water was properly crushed with the pair of mortar and pestle. A drop of the solution formed was placed into haemocytometer with a cover slip. The haemocytometer is made up of 25 counting chambers and sperm cells were counted in the 5 diagonal and inner chambers. Sperm concentration =  $A \times 1000 \times 5$  sperm cells/ml where A is the number of sperm cell counted.

The sperm morphology involved counting the abnormalities present in the sperm cells (spermatozoa). Mixing a drop of sperm from the epididymis on a slide with a drop of Wells and Awa stain did this. The smear of the mixture was then made on the slide and observed under high power (x 40) microscope lens. The sperm abnormalities considered were tailless

head, headless tail, rudimentary tail, bent tail, curved tail, curved midpiece and bent midpiece.

Motility was evaluated within 30minutes of collection and was assessed by placing a drop of well-mixed semen under a 22 X 22-mm coverslip. Using a laboratory counter, 10 random fields were counted on a microscope and scored, counting at least 200 spermatozoa.

#### **Statistical Analysis**

The mean and standard error of mean were calculated for all the sperm parameters. Analysis of variance (ANOVA) was used to establish any significant difference in all the stated parameters. P value less than 0.05 was considered significant in all cases.

#### **Results**

At the dose of 1,600mg/kg, there was a significant ( $P < 0.05$ ) decrease in the progressive sperm motility and percentage sperm live/dead ratio of the male Wistar rats treated with the aqueous extract *Lippia multiflora* leaves when compared with the control (Table 1). In addition, sperm count of the Wistar rats following oral administration of 800mg/kg and 1600mg/kg doses of the aqueous leaf extract of *Lippia multiflora* was significantly reduced in a dose-dependent manner when compared with the control (Table 1).

At doses of 400mg/kg and 800mg/kg, there was no significant change in the total number of abnormal spermatozoa in the treated groups compared with the control. However at 1600mg/kg, there was a significant ( $P < 0.05$ ) increase in the secondary spermatozoa abnormalities when compared with the control group (Table 2). These abnormalities include headless tails, tailless heads, bent tails, curved tails and curved mid-piece. However at doses of 400mg/kg and 800mg/kg, significant

**Table I: Effects of *Lippia multiflora* leaf extract on sperm characteristics of male Wistar rats.**

<i>Lippia multiflora</i> (Dose)	SPERM MOLITY (%)	LIVE/DEAD (%)	SPERM COUNTS (x10 <sup>6</sup> /mL)
400mg/kg	92.50+1.4	93.25+2.8	78.50+2.5
800mg/kg	77.50+6.3	98.50±0.9	68.00+2.9*
1600mg/kg	60.00±0.0*	48.00+0.0*	60.50+1.4*
Control	93.75±1.3	98.00±0.0	129.00+5.0

Values are mean ±standard error of mean.  
Asterisked values are significant at  
\* P <0.05

(*P*<0.005) increase occurred only in bent tails (Table 2).

Histopathology of the testis and epididymis of rats administered different doses of aqueous leaf extract of *Lippia multiflora* revealed varying degrees of degeneration and necrosis of the germinal epithelia cell of the seminiferous tubules, spermiostasis and a complete absence of spermatocytes in the epididymis (figures I & II).

**Discussion and Conclusion**

Administration of aqueous extract of *Lippia multiflora* leaf caused a reduction in progressive sperm motility, percentage sperm live/dead ratio, sperm count, and also

increased some secondary morphological sperm abnormalities.

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 as reported by Olajide et al. (1999).

The significant decrease in the sperm count supported by the various degrees of degeneration in the histologic sections of the testis and epididymis, suggests that *Lippia multiflora* leaf extract is capable of

**TABLE II: Effects of *Lippia multiflora* leaf extract on sperm morphology of male Wistar rats (mean ± standard error of mean)**

<i>Lippia multiflora</i>	HEAD LESS TAIL	TAILES S HEAD	RUDI MEN TAR Y TAIL	BENT TAIL	CURVED TAIL	BENT MID PIECE	CURVED MID PIECE	LOOP ED TAIL	COIL ED TAIL	TOTA L ABNO RM
400mg/k g Group A	163.75+ 47.6	191.3+6 2.3	11.0+ 4.1	3471.3+2 49.9*	3047.00+2 39.7	3596.00+2 42.8	3822.00+2 58.8	8.50+4. 8	8.50+ 4.8	401.3+ 1.3
800mg/kg Group B	217.00+ 55.4*	166.50+ 48.6	11.00 +5.8	3721.5+2 83.2*	3227.00+5 73.9	3822.0+28 8.1	3763.00+2 59.2	11.0+5. 8	6.00+ 2.9	402.5+ 1.4
1600mg/k g Group C	266.00+ 31.8*	211.50+ 0.3*	16.25 +4.7	3777.5+3 20.1*	4269.5+33 .3*	3836.5+22 1.9	4826.5+28 5.5*	16.00+ 2.9	16.00 +2.9	400.00 +0.00
Control	116.25± 54.9	168.75± 47.5	8.50± 4.8	2492.00± 279.0	2767.00±2 90	3494.25±2 40.0	2696.25±3 00.6	13.50± 4.8	6.00± 2.9	403.75 ±2.4

Values are mean ±standard error of mean.  
Asterisked values are significant at \* P <0.05

**Photomicrographs of the testis and epididymis of rats treated with *L. multiflora***

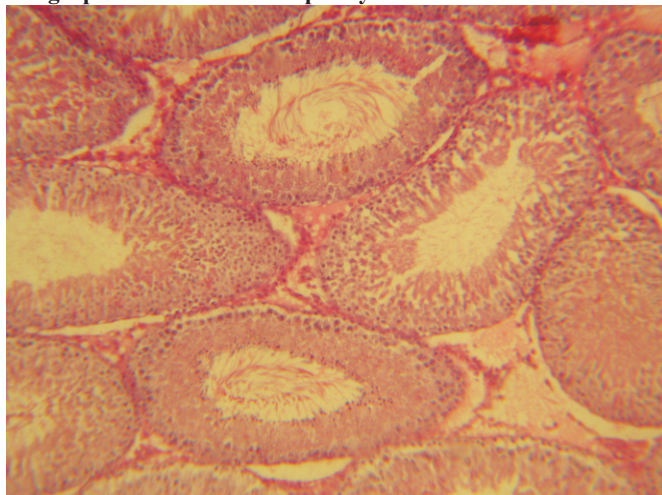


Fig I : Showing section of testis with mild spermiostasis, degeneration and necrosis of the germinal epithelial cells of the seminiferous tubules. H & E X 300.  
Effects of 28days administration of 1600mg/kg dose aqueous extract of *L. multiflora*

permeating the blood-testis barrier (Cooper, 1992).

Since several studies have reported the antifertility effects of antimalarial agents, the outcome of this study is in consonance with the previous studies (Adeeko & Dada, 1998; Olajide *et al.*, 2000 and Gbile, 1986). In conclusion, this study shows that daily oral administration of 400-1600mg/kg body weight of *Lippia multiflora* leaf extract for a period of 28 days may have significant anti-spermatogenic effects on adult male Wistar rats as seen with most other anti-malarial herbs. Further studies aimed at elucidating the activities of *Lippia multiflora* leaf extract would be worthwhile.

**References**

**Adeeko, A.O., Dada, O.A. 1998.** Chloroquine reduces the fertilizing capacity of epididymal sperm in rats. *African J. of Med. and Medical Sci.*

27:63-68.

**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. *J. Androl.* 14 (3): 174-179.

**Blom, E. 1973.** The ultrastructure of some characteristic sperm defects and a proposal for a new classification of the bull spermogram. *Mord Vet. Med.* 25: 283-5.

**Cooper, T.G. 1992.** The epididymis as a site of contraceptive attack. In Niechslag, E. and Habenicht, U.F (eds). *Spermatogenesis, Fertilization, Contraception, Molecular, Cellular and Endocrine Events in Male Reproduction.* Springer-Verlag, Berlin, 419-460.

**Gbile, Z.O. 1986.** Medicinal plant research in Nigeria. Proceedings of workshop on state of medicinal research in

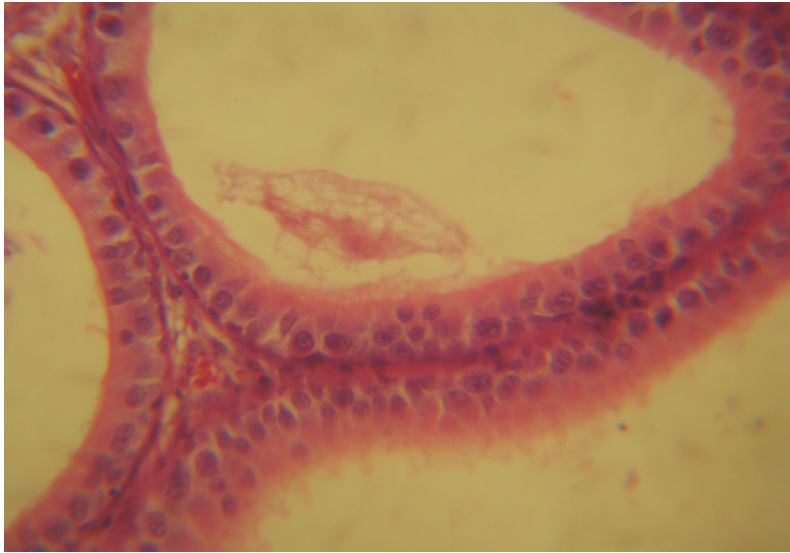


Fig II: Showing section of epididymis with total absence of spermatocytes. H & E. X 250. Effects of 28days administration of 1600mg/kg dose aqueous extract of *L. multiflora*.

- Nigeria. Sofowora, A. (Ed). Ife University Press. 21.
- Iwu, M. and Igboko, O. 1982.** Flavonoids of *Garcinia Kola* seeds. *J. Nat. Prod.* 45, 650-651
- Iwu, M. 1985.** Antihepatotoxic constituents of *Garcinia Kola* seeds. *Experientia* 41, 699-700.
- Jigam A.A., Akanya H.O., Ogbadoyi E.O., Dauda B.E.N., Egwim C.E. 2009.** *In vivo* antiplasmodial, analgesic and anti-inflammatory activities of the leaf extract of *Lippia multiflora* mold. *J Med Plants Res.* 3(3): 148-154
- Kambu, K. 1990.** Elements de phytotherapie compagne plants medicinales Africaines CRP-Kinshasa 20-22, 38-39, 40-41, 51-62.
- Kanco C., Koukoua G., N'Guessan Y.T., Fournier J., Pradère J.P., Toupet L. 2004.** Contribution à l'étude phytochimique de *Lippia multiflora* (Verbenaceae). *C R Chimie.* 7: 1029-1032.
- Kunle O.F. 2000.** Phytochemical and microbiological studies of the leaf of *Lippia multiflora* Mold., Family Verbenaceae. Unpublished Ph.D dissertation of the Ahmadu Bello University, Zaria, Kaduna State, Nigeria.
- Kunle O.F., Jegede I.A., Ibrahim H., Okogun J.I. 2002.** Pharmacognostic studies on the leaf of *Lippia multiflora* Moldenke. *JOPAT.* 7(1&2): 40-45.
- Kunle O., Okogun J., Egamana E., Emojevwe E., Shok M. 2003.** Antimicrobial activity of various extracts and carvacrol from *Lippia multiflora* leaf extract. *J. Phytomedicine.* 10: 59–61.
- Mortimer D. 1994.** Clinical relevance of diagnostic procedures. In: Practical Laboratory Andrology. New York, NY: Oxford University Press. 241-264
- Nelson, L. 1972.** Quantitative evaluation of sperm motility control mechanisms. *Biol. Reprod.* 6:319-324.

- Okanlawon, A.O., Noronha, C.C., Ashiru, O.A. 1993. An investigation into the effect of chloroquine on fertility of male rats. *W. Afri. J. Med.* 12:118-121.
- Olajide, O.A., Awe, S.O., Makinde, J.M., Morebise, O. 1999. Evaluation of the anti-diabetic property of *Morinda lucida* leaves in streptozotocin-diabetic rats. *J. Pharm. Pharmacol.* 51:1321-1324.
- Olajide, O.A., Makinde, J.M. Okpako, D.T., 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 Sprague-Dawley rats. *Nigeria J. of Health and Biomedical Sci.* 3(1): 1-7.
- Owolabi M.S., Ogundajo A., Lajide L., Oladimeji M.O., Setzer W.N., Palazzo M.C. 2009. Chemical Composition and Antibacterial Activity of the Essential Oil of *Lippia multiflora* Moldenke from Nigeria. *Rec Nat Prod.* 3(4): 170-177.
- Pascual M.E., Slowing K., Caretero E., Mara K.D., Villar A. 2001. *Lippia*: Traditional uses, chemistry and pharmacology. A Review. *J. Ethnopharmacol.* 76:201-214
- Valentin A., Pelissier Y., Benoit F., Marion C., Kone D., Mallie M., Bastide J.M., Bessière J.M. 1995. Composition and antimalarial activity in vitro of volatile components of *Lippia multiflora*. *Phytochem.* 40: 1439-1442
- World Health Organization. 1987. WHO Laboratory Manual for Examination of Human Semen and Semen-Cervical Mucus Interaction, 2nd ed. London: Cambridge University Press; p1-10.

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