

Full Length Research Paper

Effect of aqueous leaf extracts of *Leptadenia hastata* Pers (Decne) on spermiogram and testicular histology in albino rats (Wistar strain)

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This study was designed to determine the effects of prolonged oral administration of aqueous leaf extract of *Leptadenia hastata* Pers (Decne) on sperm concentration, sperm motility and testicular histology in Wistar rats. Rats were given graded doses (200, 400, 600 and 800 mg kg⁻¹) of aqueous leaf extract of *L. hastata* orally for 28 days. Weekly changes in sperm concentration and motility as well as testicular weight and histology were monitored. Sperm concentration, motility and testicular weights did not show significant change ($p > 0.05$) among the groups, but a significant difference ($p < 0.05$) exist between control group and the group that had the highest dose (800 mg/kg) of *L. hastata*. Histopathological studies revealed marked depletion of spermatozoa in the testes and epididymis of rats that received 800 mg/kg of *L. hastata*. Prolonged administration of aqueous leaf extract of *L. hastata* at 800 mg/kg caused a reduction in total sperm concentration, sperm motility and a reduction in testicular weight in rats.

Key words: Fertility, *Leptadenia hastata*, spermiogram, rats, testis.

INTRODUCTION

Medicinal plants have been used in many parts of Africa and other parts of the world for the treatment of diseases (Algier et al., 2005). Some studies have shown the anti-fertility/spermicidal potentials of some medicinal plants (Huffman, 2003; Khillare and Singh, 2014). Fertility regulating plants and plant products have been traditionally used to control birth in different parts of Africa

(Kidane et al., 2014). *Leptadenia hastata* Pers Decne (*Njara* in Kanuri, *Yadiya* in Hausa, *Sabato*, *Kusubi* in Fulani and *Hagalhadjar* in Arabic) belongs to the family Asclepiadaceae (Thomas, 2012). It is a perennial voluble plant, light green in colour with creeping latex stems which could climb a height of up to 10 cm (Garba et al., 2013). In most parts of Northern Nigeria, the plant is

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commonly used as a spice (Ibrahim et al., 2012) and also for the treatment of hypertension and skin diseases (Dambatta and Aliyu, 2011). Other reported medicinal uses of this plant include treatment of prostate cancer and rheumatism (Mathieu and Meissa, 2007). Furthermore, the plant has been shown to have anti-inflammatory and antibacterial activities (Nikiema et al., 2001; Magassouba et al., 2007). Earlier studies on the effects of *L. hastata* leaf extract on the reproductive system in rats showed that the leaf extract had anti-androgenic effects (Bayala et al., 2011). Equally, *L. hastata* leaf extracts has shown abortifacient effects in albino rats (Garba et al., 2013; Omeh et al., 2016). Consumption of the leaves and stems of *L. hastata* by donkeys, horses and dromedary camels resulted in a disruption in fertility status of these animals (Kasonia, 1998; Bayala et al., 2011). In certain parts of West Africa, cases of infertility has been reported in sheep and goats fed with pastures containing *L. hastata* (Arbonnier, 2000). In the light of the foregoing, this study was designed to determine the effect of aqueous extract of *L. hastata* leaves on sperm characteristics and histology of the testes and epididymis of rats.

MATERIALS AND METHODS

Plant material

The leaves of *L. hastata* were collected in Maiduguri, a city in North eastern Nigeria. The leaves were identified and authenticated by a taxonomist in the Department of Biological Sciences, University of Maiduguri, Nigeria and a voucher specimen (number 306) was prepared and deposited at the Department of Veterinary Physiology and Pharmacology Laboratory, University of Maiduguri. The leaves were washed with distilled water, and air dried at room temperature (26°C). The leaves were pulverized and stored in an air tight container until required for the experiment. The extraction of the aqueous extract was done as described by Lin et al. (1999). Briefly, 500 g of pulverized leaves of *L. hastata* was extracted in 1000 mL distilled water at 60°C for 72 h using Soxhlet extractor (Gallenkamp, England). The aqueous extract was then concentrated in a water bath at 50°C. This gave a total mean extract weight yield of 24.1% w/w of extract which was further oven dried at 40°C and maintained in a desiccator (Gallenkamp, England) until a constant weight was obtained.

Experimental animals

Sixty male albino rats (Wistar) weighing between 145 and 175 g obtained from the Animal House, Faculty of Veterinary Medicine, University of Maiduguri were used for the study. The rats were maintained in cages under the following conditions: The temperature of the room was set at $25 \pm 1^\circ\text{C}$ and <30% relative humidity with a 12 h light/dark cycle. Pelleted commercial feed (ECWA, Nigeria Plc., Jos, Nigeria) and water were provided *ad libitum*. The rats were allowed 21 days to stabilize before the commencement of the experiment. This research (PGA/04/07663) was approved by the animal welfare Committee of the Faculty of Veterinary Medicine, University of Maiduguri, Nigeria. The animals were handled according to the international guiding principles for biomedical research involving animals (Council for International

Organizations of Medical Sciences, CIOMS, 1985).

Experimental design

The sixty rats were randomly divided into 5 groups of 12 rats each. Group 1 received distilled water and served as control group. Rats in Groups 2, 3, 4 and 5 were orally administered with the aqueous leaf extract of *L. hastata* at 200, 400, 600 and 800 mg/kg respectively for 28 consecutive days. Water was provided *ad libitum*. Three rats in each group were sacrificed by cephalic dislocation on days 7, 14 and 21 of the experiment. Upon euthanasia of the remaining rats using 100 mg/kg sodium pentobarbital on day 28 of the experiment; the testes and epididymis were harvested for the determination of testicular weight, epididymal sperm count and sperm motility.

Organ collection and sperm collection and analysis

A scrotal incision was made to exteriorize the testes and epididymis. These were then removed and cleared of connective tissue and fat. To prepare the sperm suspension, epididymal sperm was obtained by homogenizing the *cauda epididymis* in a pre-warmed beaker containing 2 ml of physiological saline maintained at 37°C. Sperm characteristics were determined as previously described by Raji et al. (2005). A drop of sodium citrate buffer (2.9%) was added to earlier expressed epididymal fluid on a glass slide. The glass slide was covered with a cover-slip and sperm motility was evaluated under a light microscope at x40 magnification. Sperm concentration (expressed as million/ml of suspension) was done using the improved Neubauer's haemocytometer under a light microscope at x400 magnification (Raji et al., 2005).

Histopathology

Tissues were processed routinely and embedded in paraffin wax as described previously by Bancroft and Stevens (1991). Briefly, serial sections (5 μ thick) were prepared, stained with H&E dye and examined under a light microscope (Olympus, Japan).

Statistical analysis

Data obtained were expressed as mean \pm SEM and subjected to one way analysis of variance (ANOVA) to determine the differences between the experimental groups using GraphPad Instat software. P value was considered significant at $p < 0.05$.

RESULTS

Effect of *L. hastata* on organ weight, sperm count and testicular sperm motility

There was a non-significant ($p > 0.05$) decrease in the testicular weight across all groups, however, significant decrease ($p < 0.05$) was observed between rats in the control group and rats that received 800 mg kg^{-1} (Figure 1). The testicular weights returned to near normal values on day 21 in all the experimental groups (Figure 1). There was a significant decrease ($p < 0.05$) in the testicular sperm count in all the groups with a more pronounced

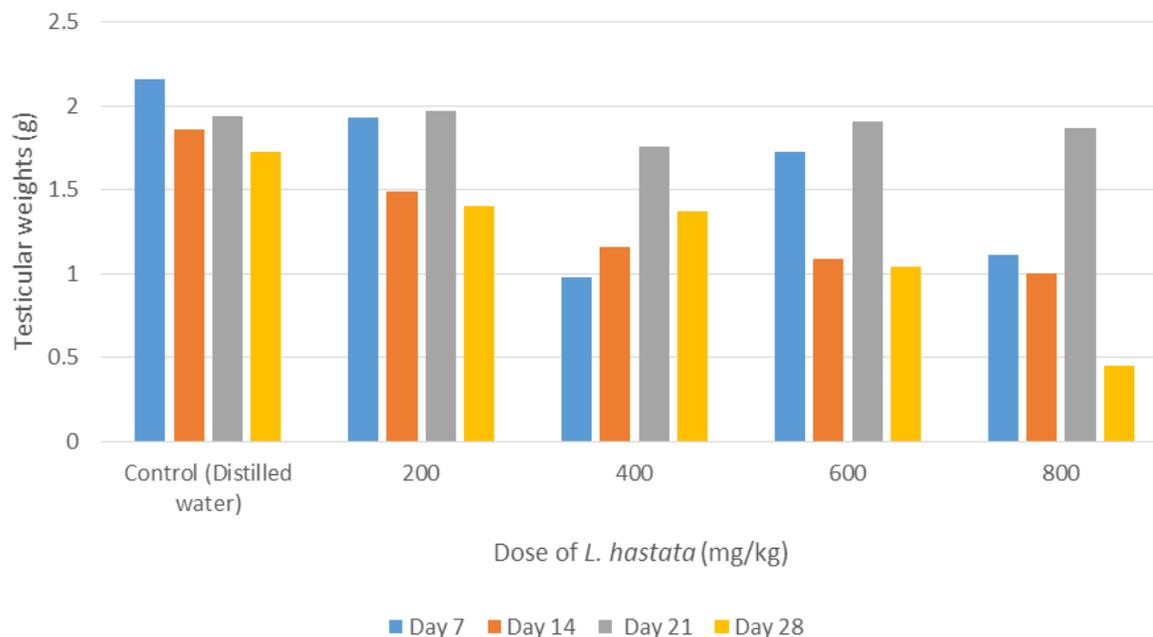


Figure 1. Effects of the aqueous extract of *L. hastata* leaf on testicular weight in Wistar albino male rats.

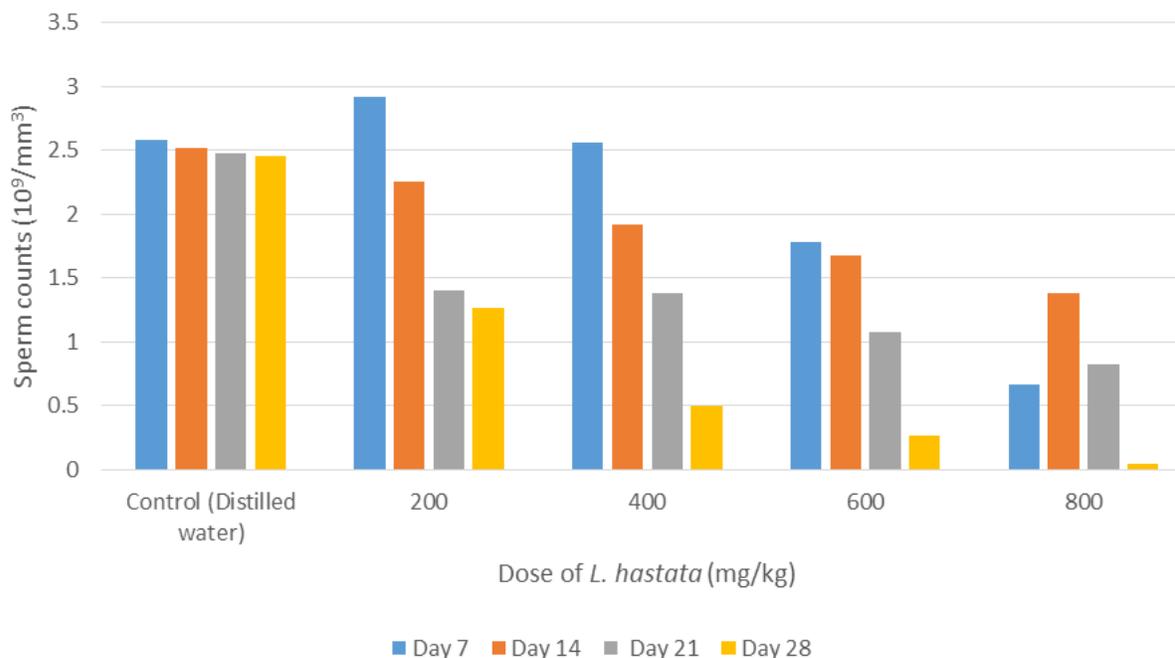


Figure 2. Effects of the aqueous extract of *L. hastata* leaf on testicular sperm counts in Wistar albino male rats.

decrease in rats that were given 800 mg/kg⁻¹ (Figure 2). There was a slight decrease ($p>0.05$) in sperm motility. However, significant ($p<0.05$) decrease in sperm motility was observed on day 21 as compared to other days (Figure 3).

Histopathology

Histopathological studies on sections of the epididymis of the control group showed numerous regularly spaced seminiferous tubules with all cells of the spermatogenic

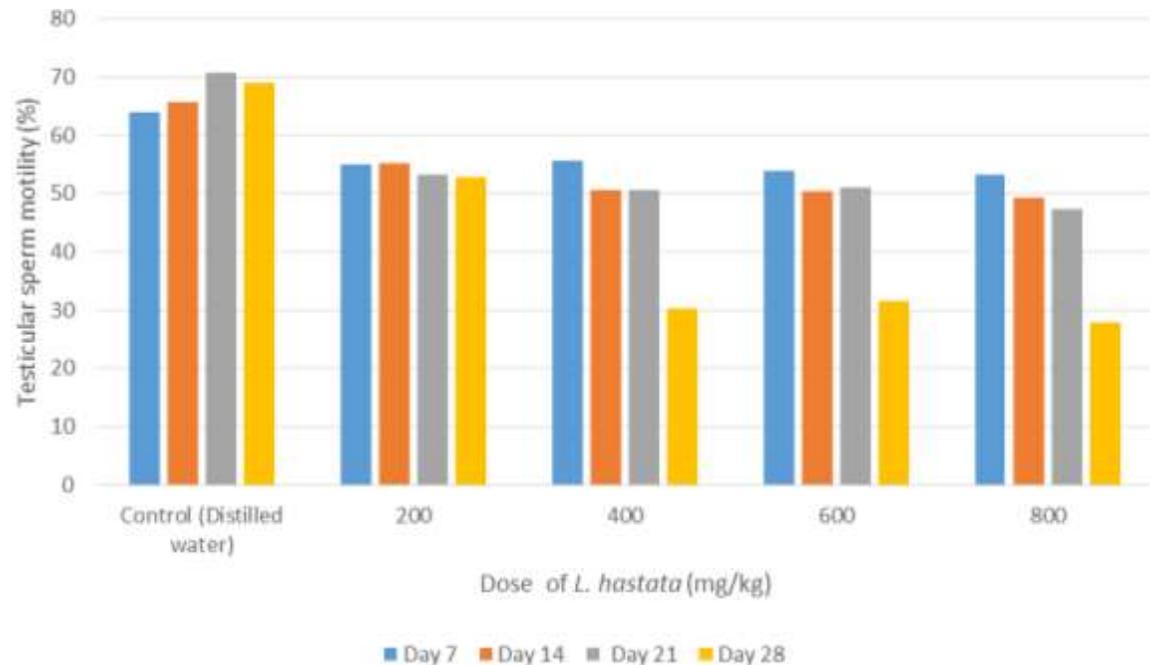


Figure 3. Effects of the aqueous extract of *L. hastata* leaf on testicular sperm motility in Wistar albino rats.

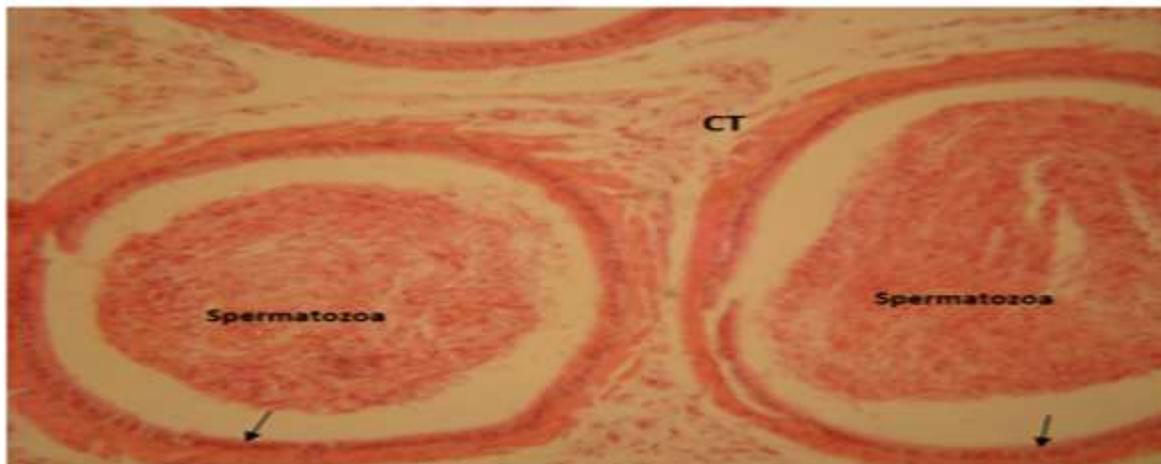


Figure 4. Photomicrograph of epididymis of rat in the control group showing numerous spermatozoa, ciliated columnar cells (arrowed) and connective tissue (CT) H&E x400.

series and interstitial spaces that appear normal (Figure 4). In the groups that were treated with higher doses of the extract, there was significant depletion of spermatogonia and spermatozoa in the epididymis (Figure 5).

DISCUSSION

The male reproductive system is made up of the testis,

epididymis and other accessory sex organs and together their main function is to produce spermatozoa as well as to provide an environment for the growth and maturation of spermatozoa. Compounds that alter the testicular and epididymal form and function could have a profound effect on the quality and quantity of spermatozoa (Obianime et al., 2010). Some plants are known to possess some components that could cause damaging systemic effects in both humans and animals (Oyeyemi et al., 2007). Results from this study show that prolonged

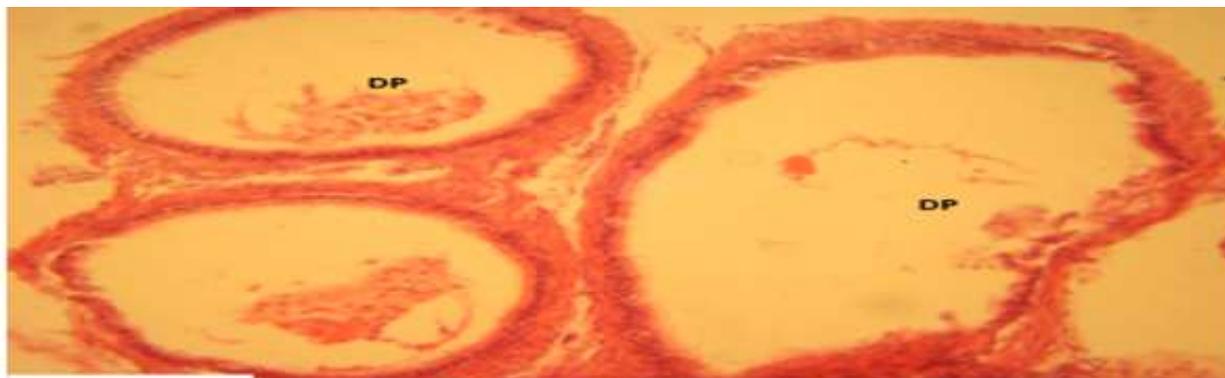


Figure 5. Photomicrograph of rat epididymis treated with 800 mg/kg of extract showing marked depletion of spermatozoa (DP) H&E x400.

administration of aqueous *L. hastata* leaf extract to male rats caused reductions ($p > 0.05$) in testicular weight, sperm counts and sperm motility. The extract of *L. hastata* caused a decrease in the weight of testes as well as a decline in some biomarkers used for the evaluation of spermatogenesis in rats. Although these parameters are important in male fertility, the differences observed were not significant ($p > 0.05$). Reduction in testicular weight as observed in this study may have been caused by the direct effect of some constituents of *L. hastata* on the histoarchitecture of the testes. Other authors have reported similar results while using other plants (Khoury and El-Akawi, 2005; Bernhoft et al., 2010; Bayala et al., 2011; Halvaei et al., 2012). It is likely that the biological activity of *L. hastata* leaf extracts on the testis may be due to one or more of the phytochemical components in the aqueous leaf extract of *L. hastata*. Some components in leaves of plants, such as alkaloids have been reported to cause a decrease in sperm production and also cause testicular atrophy (Bernhoft et al., 2010). Bello et al. (2011) had earlier detected phenolic glycosides, tannins, flavonoids, proanthocyanidins, alkaloids and saponins in *L. hastata* leaves. Furthermore, oral administration of *L. hastata* has been reported to cause a reduction in the weights of accessory sex organs in rats (Bayala et al., 2011). Two modes of action are postulated on how a component in *L. hastata* leaves exert their effect. Firstly, the components of *L. hastata* could inhibit androgen binding to an appropriate receptor (Andersen et al., 2002; Long et al., 2003) and secondly, due to the inhibition of enzymes involved in the production of sex hormones, such as, 5α -reductase and aromatase (Corradi et al., 2009). The identification of this active principle responsible for this activity was however not within the scope of this study. A separate study will be required to elucidate on the active principle responsible for this effects. Other workers have reported similar results in testicular weight reduction accompanied by decreased serum testosterone levels, impaired spermatogenesis and a decrease in sperm parameters in a dose

dependent manner in male rats treated with other plants (Raji and Bolarinwa 1997; Raji et al., 2003; Chaturvedi et al., 2003; Pankajakshy and Madambath, 2009; Bazrafkan et al., 2010).

The blood-epididymal barrier selectively regulates the flow of substances in and out of the epididymal lumen (Hinton and Howards, 1981). Larger molecular weight substances cannot pass easily through this barrier (Uchendu et al., 2000). Some active principles such as alkaloids in the aqueous leaf extract of *L. hastata* could have cross the blood-epididymal barrier and thus alter the microenvironment of the *cauda epididymis*, leading to the inhibitory effects on metabolic pathways and consequent reduction in sperm viability (Verma and Chinoy, 2001). The impairment of epididymal function due to compromised blood-testes and blood-epididymis barriers as reported in the present study is also supported by previous studies (Hinton and Howards, 1981; Bayala et al., 2011). It is plausible that the effects attributed to *L. hastata* in this study could have been due to its direct mechanism on the testes.

Results of this study also revealed some slight decrease ($p > 0.05$) in testicular weights, sperm count, and sperm motility. Earlier studies have shown that *L. hastata* can cause a reduction in the weights of accessory sex organs as well as a reduction and inhibition of action of testosterone propionate (Bayala et al., 2011). Furthermore, *L. hastata* has been shown to significantly reduce the levels of phosphatase acid and fructose in seminal vesicle and prostate glands (Bayala et al., 2012). The results of this study and earlier similar studies show that continuous administration of *L. hastata* in male rats could have some anti-androgenic effects and therefore could likely affect reproduction in this species.

Conclusion

This study has shown that prolonged administration of *L. hastata* at low dosages does not cause any significant

change in either testicular weight, sperm motility or sperm concentration but it may cause a decrease in these parameters when administered at higher doses.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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