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Effects of Crude Aqueous Extract of *Ocimum gratisssimum* Leaves on Testicular Histology and Spermiogram in the Male Albino Rat (*Wistar strain*)

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Abstract: The effects of the aqueous leaf extract of *Ocimum gratisssimum* on testicular histology and spermiogram were investigated using 18 male albino rats. The rats were randomly divided into 3 groups: A-C, each containing 6 rats. Fifty grams of fresh leaves of *Ocimum gratisssimum* were rinsed, thoroughly blended in 100 mL of distilled water and filtered through filter paper. A 10×10^{-4} and 5×10^{-4} mL kg^{-1} of the filtrate were administered to groups A and B, respectively thrice weekly for 5 weeks. Group C served as control and received distilled water. Histopathology revealed mild to severe congestion and edema as well as germinal tissue erosion of the seminiferous tubules. Spermatozoa motility, concentration, live-dead ratio and semen volume did not show significant changes ($p>0.05$). However, differences in spermatozoa abnormalities between control and tested groups were highly significant ($p<0.01$). Mean differences in sperm abnormalities between groups A (28.5), B (13.75) and the control were also greater than calculated Least Significant Difference (LSD) value of 2.146. These findings indicate antifertility properties of *Ocimum gratisssimum*.

Key words: Aqueous extract, ocimum, testicular histology, spermiogram, reproduction, rat

INTRODUCTION

Ocimum gratisssimum (Linn) is an annual herbaceous plant commonly found throughout India and in some other places including West Africa especially, Nigeria (Ibe and Nwufo, 2005). In India, it is called Tulsi (Ahmed *et al*., 2002), while in Nigeria, it is frequently called Ahuji by the Igbos, Daidoya by the Hausas and Effinrin-nla by the Yorubas. *Ocimum gratissimum* belongs to a larger family of plants known as Labiaceae (Singh and Singh, 2009). The plant is known to posses many beneficial effects ranging from medicinal to nutritive. Parts of the plant (e.g. leaves, seeds and roots) have been used extensively in the ancient system of Indian medicine known as Ayurveda (Kirtikar and Basu, 1935). In Nigeria also, different parts of the plant are used often as a broad spectrum relief and cure of various ailments and diseases. Decoctions of the leaf have been used in the treatment of mental illness (Offiah and Chikwendu, 1999) and epilepsy (Kirtikar and Basu, 1935). The plant has also been found to be effective in the treatment of diarrhea and dysentery (Prakash and Gupta, 2005). The leaf extract has been reported to be curative for respiratory diseases, earache, vomiting and gastric upsets in children (Ahmed *et al*., 2002). In African folklore medicine, the plant has been reported to possess mosquito repellant and poultice-like properties (Kelm and Nair, 1998). The leaves of the plant are also used as a condiment in cooking. Recent investigations in medicine have also shown that administration of fresh leaves of the plant will significantly reduce blood sugar level in hyperglycaemic patients (Agarwal *et al*., 1992; Chattopdhyay, 1999) and serum total cholesterol in hypercholesterolaemic patients (Sarkar *et al*., 1994). In view of the fact, that the plant is widely prescribed for use and consumed by man, the present study was undertaken to investigate possible effects of the leaf extract on testicular histology and spermiogram of rats.

MATERIALS AND METHODS

Eighteen male albino rats (*Wistar strain*) were obtained from the Animal house of the Faculty of Veterinary Medicine, University of Ibadan, Nigeria for the study. The rats weighed between 280-374 g. They were kept in plastic cages within the experimental animal unit and fed with commercial rat cubes (Ladokun Feeds, Nigeria Ltd.). They were allowed unrestricted access to clean fresh water in bottle dispensers *ad libitum*. The rats were allowed to stabilize for a period of 7 days following which they were randomly divided into 3 groups (A-C) containing 6 rats each. Group C served as control and was

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given water instead of the extract. Fresh leaves of Efinrin (*Ocimum gratissinum*) were collected from the Teaching and Research Farm of the University of Ibadan, Nigeria. The leaves following authentication at the herbarium of the Botany department of the same University were washed in distilled water and allowed to drain for an hour on the laboratory bench at room temperature (27°C). Fifty grams of the leaves were then chopped and blended in 100 mL of distilled water. The crude aqueous extract was obtained following filtration of the blended mixture into a conical flask through filter paper. This process of extract production was repeated weekly as each preparation was preserved at 4°C in a refrigerator to test rats once daily during the study, which lasted 5 weeks. The filtrate was administered orally with the aid of a modified Tuberculin syringe, which was used to depress the posterior part of the tongue. The dosage of the filtrate administered to group A rats was 10×10^{-4} mL kg^{-1} while, group B received 5×10^{-4} mL kg^{-1}. At the end of the 5th week, the rats were sacrificed by cephalic dislocation and the testicles and epididymides were dissected out through a lower abdominal incision. The epididymides were separated from the testes by blunt dissection. The epididymides were cut open longitudinally and with gentle pressure on the serosa, a drop of semen was expressed on a pre-warmed (37°C) slide. Semen examinations were done using methods described by Zemjanis (1970). Briefly, a drop of Sodium citrate buffer (2.9%) was added to the expressed semen and cover-slip was applied to evaluate motility under ×40 of microscope. The semen sample was also stained with Eosin-Nigrosin to evaluate live-dead ratio. This same sample was used to estimate sperm abnormalities. The epididymides were then submerged in a graduated test-tube containing 5 mL of Formol saline. Semen volume was roughly evaluated as the measure of displacement of Formol saline. The entire epididymis was then crushed in Formol saline and this mixture was used to evaluate spermatozoa concentration using the improved neubar chamber. Following separation of the epididymides, the testicles were fixed in 10% buffered formalin in labeled bottles. Tissues were processed routinely and embedded in paraffin wax as described by Junqueira and Carneiro (1980). The data obtained were subjected to Analysis of variance (ANOVA) and where results are significant ($p<0.05$), the Least Square Difference (LSD) test was conducted (Lewis, 1984).

RESULTS

The results of the parameters investigated in the study are presented as follows. For ease of presentation, A and B represents experimental groups of rats, which received 10×10^{-4} and 5×10^{-4} mL kg^{-1} of the extract, respectively while, C served as the control group. Table 1 shows the mean values for semen parameters during the study. The differences in the mean values obtained in groups A-C, respectively for spermatozoa motility (62.50±2.50, 72.50±2.50, 95.00±0.00) and concentration (67.50±3.12, 79.25±1.89, 101.50±2.96), live-dead ratio (83.75±3.23, 93.75±1.66, 98.00±0.00) and semen volume (0.15±0.03, 0.15±0.03, 0.20±0.00) during the study were not significant ($p>0.05$). The differences in the mean values for spermatozoa abnormalities for groups A-C, respectively (i.e., 64.5±5.92, 49.75±2.87, 36.00±1.41) were however, highly significant ($p<0.01$). The mean differences in sperm abnormalities (i.e., 28.5 for A and 13.75 for B) were also greater than the calculated LSD value of 2.146. Table 2 shows that the differences in the percentage of the various abnormalities observed in the study are significant ($p<0.05$). In groups A-C, head abnormalities from the 1st to the 5th week varied from 1.00-2.05, 1.00-1.60 and 1.00-0.50%, respectively. Midpiece abnormalities in the groups vary from 4.00-5.75, 3.00-4.00 and 2.75-2.75% for groups A-C, respectively. Similarly, Tail abnormalities increased from 9.00% in the 1st week to 11.50% in the 5th week for group A and 6.75-9.50% for group B, while it ranged between 4.75% in the 1st week to 5.00% in the 2nd and 3rd week and 4.75% in the 4th and 5th week for group C. Figure 1 and 2 shows the testicular histology of treated rats in the study. Evidence of germinal erosion of the seminiferous tubules with mild interstitial edema was observed in group A rats, while severe congestion and edema at the interstitium of the seminiferous tubules characterize group B rats. Figure 3 shows the germinal epithelium of control rats with no visible lesions.

Table 1: Mean values ±SD for Semen parameters during the study

Parameters	A	B	C	p-value
Sperm motility (%)	62.50±2.50	72.50±2.50	95.00±0.00	>0.05
Livability (%)	83.75±3.23	93.75±1.66	98.00±0.00	>0.05
Semen volume (mL)	0.15±0.03	0.15±0.03	0.20±0.00	>0.05
Sperm concentration $\times10^7$ cell mL^{-1}	67.50±3.12	79.25±1.89	101.50±2.96	>0.05
Spermatozoa abnormalities	64.50±5.92	49.75±2.87	36.00±1.41	<0.05

Table 2: Percentage (s) of Spermatozoa abnormalities during the study

Type of sperm abnormality (%)	Week (s)	Group A (10×10^{-4} mL kg^{-1})	Group B (5×0^{-4} mL kg^{-1})	Group C (distilled water)
Head abnormalities	1	1.00	1.00	1.00
	2	1.25	1.00	1.00
	3	1.75	1.25	0.50
	4	1.75	1.50	1.00
	5	2.05	1.60	0.50
Midpiece abnormalities	1	4.00	3.00	2.75
	2	4.25	3.50	2.50
	3	5.25	3.50	2.50
	4	5.25	3.50	2.50
	5	5.75	4.00	2.75
Tail abnormalities	1	9.00	6.75	4.75
	2	9.50	7.25	5.00
	3	9.75	7.75	5.00
	4	10.50	9.00	4.75
	5	11.50	9.50	4.75

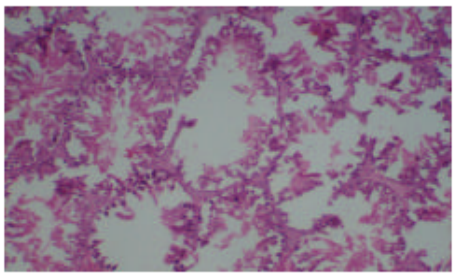

Fig. 1: Germinal tissue erosion of the seminiferous tubules and mild interstitial oedema (×400). Micrograph showing seminiferous tubule from Group A rat

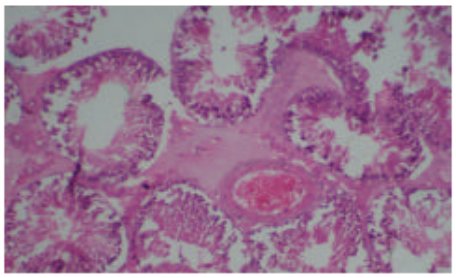

Fig. 2: Severe congestion and oedema at the interstitium (×100). Micrograph showing the seminiferous tubule fom Group B rat

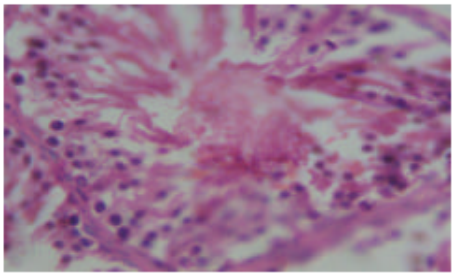

Fig. 3: Normal germinal epithelium of the seminiferous tubule showing no visible lesion (×400). Micrograph showing seminiferous tubule from Group C rat (control)

DISCUSSION

The discovery that some of the common vegetables consumed by man and animals as food and concoction possess deleterious systemic effects appears to be growing in recent times (Oyeyemi *et al*., 2007). The findings in the present study with *Ocimum gratisssimum* leaves indicated an increasing level of damage on the seminiferous tubules of experimental rats, from the lower (i.e., 5×10^{-4} mL kg^{-1}) to the higher (10×10^{-4} mL kg^{-1}) dosages, where the lesion is characterized by erosion of germinal tissue and interstitial edema (Fig. 1). Earlier, a severe degeneration of spermatogenic element with disturbance in spermatogenesis and reduction in activity of GTP, a marker of Sertoli cell function was reported in rabbits treated with extracts of *Ocimum* (Reghunandan *et al*., 1997). The histological integrity of the entire testis is fundamental to the production of fertile spermatozoa (White, 1933). Therefore, any factor (s), which damages the testicles such as infections, toxic agents, malnutrition or heat will result in the production of subfertile spermatozoa (Noakes *et al*., 2001). Although, the semen parameters investigated in the study occupies a central position in male fertility studies, the differences observed in the groups were not significant ($p>0.05$), except for sperm abnormalities ($p<0.01$). This suggests that the testicular damage caused by Ocimum leaf extract, as revealed by histology, within the duration of the study did not cause any significant change in semen parameters other than sperm abnormalities. Increases in percentage of sperm abnormalities have most frequently been observed as one of the earliest indicators of testicular pathology (Noakes *et al*., 2001). Hence, the increase observed in the percentage sperm abnormalities in both treatment groups (A and B) can be said to be due to the administration of the extract, which has been reported to disrupt spermatogenesis (Kasinathan *et al*., 1972). Even the response in sperm abnormalities appears to be dose

and duration related. This is further supported by the higher percentages of abnormality obtained in the latter weeks of the study and with higher dosage of the extract. Earlier, Seth *et al.* (1981) have reported a dose dependent effect on the weight of the testes and significant reduction in sperm count and motility. The observation in this study indicates that with higher frequency of administration and longer duration of exposure, testicular pathology may increase, so also spermatozoa abnormalities with an increased tendency to infertility. It is possible that once the exposure is discontinued, there may be gradual improvement/reduction in sperm abnormalities with complete testicular healing occurring some later time (Ahmed *et al.*, 2002).

CONCLUSION

From the observations in this study, we conclude that the crude aqueous extract of *Ocimum gratisssimum* leaves possess antifertility factors. This is similar to reports from earlier studies (Knatak and Gogate, 1992; Ahmed *et al.*, 2002). However, because of its clear evidence as a very useful plant for food and its medicinal products (Rastogi *et al.*, 2007; Sharma *et al.*, 2001), more research efforts are required on the plant.

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