Fertility enhancing effects of methanolic leaf extract of *Dracaena arborea* in albino rats (*Rattus norvegicus*)

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ABSTRACT: The effects of methanolic extract of *Dracaena arborea* on mean testicular weight, mean cauda epididymal sperm reserve, and testicular morphology were evaluated. A total of sixty mature male Sprague Dawley rats were divided into three equal groups. The first group (A) received distilled water while the other two groups (B and C) received orally the methanolic leaf extracts of *Dracaena arborea* in two doses (100 and 500 mg/kg, respectively) daily for 84 days. Following oral administration of the extract, mean testicular weight, mean cauda epididymal sperm reserve, and testicular morphology were determined on days 28, 42, 56, 70, and 84. The extract produced a significant and dose-dependent increase ($P < 0.05$) in the sperm number. There was also a significant increase ($P < 0.05$) in the mean testicular weights on days 70 and 84 of the extract administration. The testicular morphology remained unchanged while further testicular histology examination revealed increased spermatogenesis. It was concluded that the methanolic leaf extract of *D. arborea* has fertility enhancing properties.

Keywords: spermatogenesis; sperm number; testicular weight; morphology

Infertility has been a recurring problem among male and female individuals. Today, orthodox medicine has almost exceeded its limits in resolving problems of infertility. This is why the use of phytomedicine is becoming a main stay in the treatment of infertility. It has been reported that alternative medicines have proven efficacious in the treatment of female infertility (Rabia et al., 2008; Gaware et al., 2009).

Locally grown plants have been used worldwide as supplements and therapies for several ailments. In recent times, extracts of *Nigella sativa, Lophira lanceolata, Cochlospermum planchonii, Kaempferia parviflora*, among others, have been reported to enhance fertility (Chaturapanich et al., 2008; Al-Sa'aidi et al., 2009; Etuk and Muhammad, 2009; Abu et al., 2012). More than 90% of male infertility cases are due to low sperm counts, poor semen quality or both. The causes of 30–40% of the cases of sperm abnormalities cannot be accounted for (Lindheim et al., 1996). The active principles such as phenols, alkaloids, saponins, and most especially flavonoids are known to have estrogenic (Das et al., 2004) and androgenic (Yousef et al., 2005) activity.

Dracaena arborea is a member of the family *Agavaceae* and is locally called "Okono" in Akwa Ibom State (Ajibesin et al., 2008). Here the plant is very popular because of its acclaimed aphrodisiac effect and its fertility enhancing potentials. It is commonly grown in residential compounds as a panacea for witchcraft and has been reported to have activity against pests (Epidi and Udo, 2009). Patients with preterm labor (PTL) that could lead to preterm birth (PTB) are reported to be at risk despite of vaginal progesterone administration (Sharami et al., 2010). But traditionally, this plant is believed to resolve several problems of infertility, including preterm birth. The objective of the present study is to investigate the fertility enhancing effects of methanolic leaf extract of *D. arborea* through the evaluation of sperm number, testicular weight, and morphology.

MATERIAL AND METHODS

Plant

Fresh leaves of *D. arborea* were collected in the period of January–April from Abak Itenge, Abak Local Government of Akwa Ibom State, Nigeria. The plants were identified by Mr. Ozioko from the Department of Botany, University of Nigeria, Nsukka where the voucher specimen was deposited.

Extraction

The leaves of *D. arborea* were dried at room temperature under shade, and then pulverized in the Department of Crop science, University of Nigeria. Following this, 1000 g of the pulverized plant was placed in a bottle containing 3600 ml of analytical methanol. This was vigorously shaken and left for 48 h for efficient extraction. The shaking was repeated each day and the extract was then passed through Whatman filter papers. The filtrate was evaporated at 40°C and the percentage yield of *D. arborea* was obtained. The extract was then stored in a refrigerator at 4°C.

Animals

Sixty male Sprague Dawley rats weighing 180–200 g were used for this study. They were obtained from the experimental animal unit of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka and then kept in the animal house of the Department of Veterinary Obstetrics and Reproductive Diseases, University of Nigeria, Nsukka where they were allowed to acclimatize for 14 days. They were fed standard rat ration and fresh water *ad libitum*.

Animal treatment

The sixty male Sprague Dawley rats were randomly divided into three groups (A, B, and C) of 20 rats each. The animals in the test groups (B and C) were treated orally with 100 mg/kg and 500 mg/kg of the crude extract respectively for 84 days. The animals in group A (control) received equivalent volume of distilled water for 84 days. On days 28, 42, 56, 70, and 84, four rats from each group were anaesthetized with chloroform and sacrificed. The testes were dissected out and weighed independently. The mean testicular weights were later determined. Testicular samples were collected for histology and the slides stained with H and E stain.

Epididymal sperm reserve

Epididymal sperm concentration was determined using the haemocytometric method according to Amman and Almquist (1961).

Histological procedures

Following the collection of testicular samples, the organs were promptly fixed in Bouins fluid to preserve the structure and molecular composition of the testes. Further histological preparations were carried out as described by Igwebuike and Eze (2010), and stained with hematoxylin and eo-

Table 1. Mean cauda epididymal sperm reserve ($\times 10^6$) of male Sprague Dawley rat exposed to graded doses of *Dracaena arborea* extract

Days	Groups		
	A (distilled H_20)	B (100 mg/kg)	C (500 mg/kg)
28	99.50 ± 2.33[a]	187.55 ± 1.10[b]	247 ± 1.94[c]
42	111 ± 1.55[a]	199 ± 1.71[b]	254 ± 1.49[c]
56	123 ± 1.32[a]	208 ± 1.04[b]	261 ± 0.91[c]
70	129 ± 0.91[a]	225 ± 1.75[b]	270 ± 0.91[c]
84	144 ± 1.89[a]	233 ± 1.11[b]	280 ± 1.11[c]

[a–c]significant differences at $P < 0.05$ across the row (across the group)

Table 2. Mean testicular weights of male Sprague Dawley rat exposed to graded doses of *Dracaena arborea* extract (in g)

Days	Group		
	A (distiled H_20)	B (100 mg/kg)	C (500 mg/kg)
28	3.11 ± 0.19[a]	3.05 ± 0.21[a]	3.57 ± 0.11[a]
42	3.03 ± 0.09[a]	3.20 ± 1.13[ab]	3.45 ± 0.11[b]
56	3.05 ± 0.21[a]	3.13 ± 0.18[a]	3.57 ± 0.11[a]
70	2.86 ± 0.13[a]	3.51 ± 0.17[b]	3.61 ± 0.09[b]
84	2.86 ± 0.13[a]	3.45 ± 0.16[b]	3.62 ± 0.08[b]

[a–c]significant differences at $P < 0.05$ across the row (across the groups)

sin for light microscopy. Photomicrographs were captured using a Moticam® digital camera (Motic China Group Co., Ltd., Xiamen, China) including Motic® Images Plus 2.0 software.

Data analysis

The data were statistically analyzed using One-Way Analysis of Variance (ANOVA) and significant means were separated by the Least Significant Difference (LSD) method. The level of statistical significance was taken as $P = 0.05$.

RESULTS

The result of this study revealed that the treatment with methanolic leaf extract of *Dracaena arborea* led to a significant and dose-dependent increase ($P < 0.05$) in the sperm number (Table 1). Significant increase ($P < 0.05$) in the mean testicular weight on days 70 and 84 of the treatment was observed (Table 2). Histology of the testis revealed normal seminiferous tubules with increased spermatogenesis among the extract-treated rats (Figures 1 and 2).

DISCUSSION

The present study shows that the methanolic leaf extract of *D. arborea* at the doses of 100 and 500 mg/kg enhanced the fertility of male albino rats. It has been reported that sperm number and normal testicular histology are indices of fertility (Al-Sa'aidi et al., 2009; Etuk and Muhammad, 2009).

From Table 1 it can be seen that methanolic extract of *D. arborea* induced a dose-dependent increase in the sperm number on days 28, 42, 56, and 84 of the treatments. The sperm numbers of the treatment groups were significantly higher

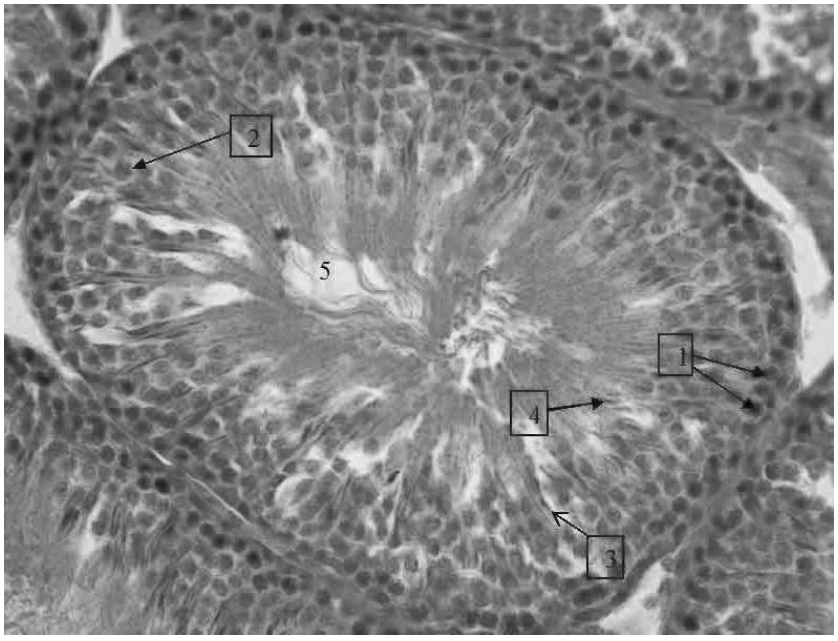

Figure 1. Photomicrograph of the testes of a Sprague Dawley rat of group A (control) stained with H and E (×40). Seminiferous tubules showing: 1 = spermatogonia, 2 = primary spermatocytes, 3 = early spermatid, 4 = late spermatid, 5 = lumen

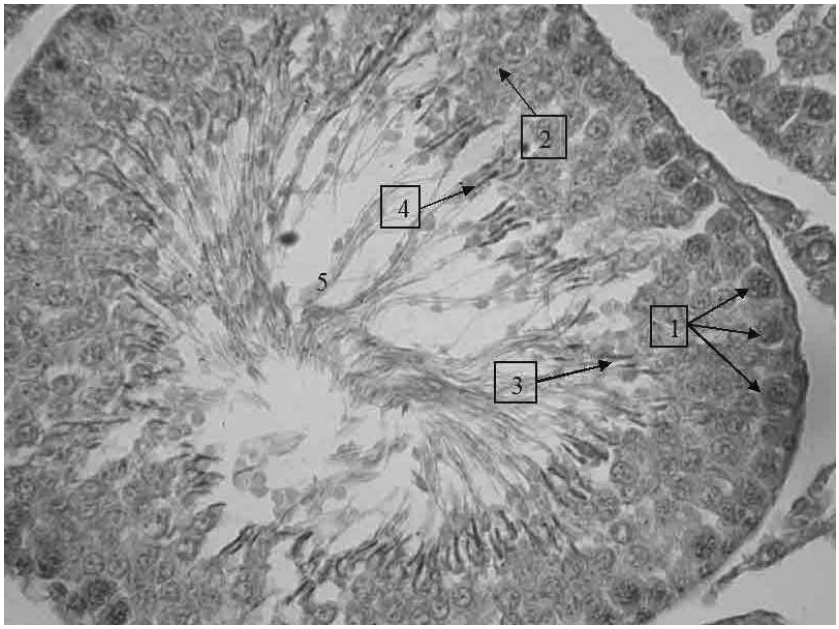

Figure 2. Photomicrograph of the testes of a Sprague Dawley rat of group C stained with H and E (×40). Seminiferous tubule showing: 1 = spermatogonia, 2 = primary spermatocyte, 3 = early spermatid, 4 = late spermatid, 5 = lumen with spermatozoa

($P > 0.05$) than the sperm number of the control. At the dose of 500 mg/kg, the sperm numbers of groups A, B, and C were 144 ± 1.89, 233 ± 1.11, and 280 ± 1.11, respectively. *D. arborea* extract has produced almost a threefold increase in the sperm count of the rats after 84 days of treatment.

Table 2 shows that the methanolic extract of *D. arborea* led to a significant increase ($P > 0.05$) in mean testicular weight on days 70 and 84. Although there were gradual increases in the mean testicular weight in groups B and C, this was insignificant when compared with the control on days 28, 42, and 56. The gradual increase in the mean testicular weight of the treated rats is probably due to the increased activity in their testes. This may include increased testosterone secretions (Dalrymple et al., 1968; O'Keane et al., 1986). Testicular oedema can cause increases in the mean testicular weight as a result of increasing testosterone levels (Corbier et al., 1978; Maddocks and Sharpe, 1989) but in this investigation the testes were not oedematous as demonstrated in the tissue sections.

Figures 1 and 2 show normal testicular tissues but in Figure 2 there is an increased spermatogenic activity. The lumen of seminiferous tubules of Figure 2 demonstrates increased spermatozoa when compared to that of Figure 1. This accounts for the sperm number in groups B and C.

The increase in sperm number of the treatment group is due to increased production of testosterone and probably of follicle-stimulating hormone (FSH) (Al-Sa'aidi et al., 2009). Testosterone and FSH are reported to be responsible for spermatocytogenesis and spermatogenesis in the seminiferous tubules. Testosterone alone is responsible for maturation of spermatozoa (McLachlan et al., 2002). The significant increase in sperm number recorded in this study reveals the potential of the plant to cure male fertility problems, especially those related to hormonal levels, sperm count, and sperm viability. Although animal experimental results are not always reproducible in humans, the testimonies of traditional herbalists and the animal experimental results recorded in this study prove that the experiment may yield similar or related result in humans. Notice that sperm number increased with increasing length of the treatment. It is important to take into account the duration of therapeutic administration because it influences the desired effects (Kreek, 1996).

In this study, the increased sperm count and testicular weight following administration of the methanolic leaf extract of *D. arborea* as well as normal testicular morphology with increased spermatogenesis show that the extract has fertility-enhancing ability.

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