

Spermatogenic and Androgenic Effect of the Root of *Boscia Senegalensis* (Per) Plant

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ABSTRACT

More than 90% of male infertility is due to low sperm count, poor sperm quality or both. *Boscia senegalensis* locally known as Anza in Sokoto State, north-western Nigeria is used traditionally in treating fertility related problems. The plant root was washed and air dried pulverized into a dry powder and extracted with methanol. Eighty matured male rats were divided into four groups (A-D) of 20 rats each. Rats in group A, B and C were administered orally with 100mg/kg, 200mg/kg and 300mg/kg body weight respectively of the extract daily until the end of the experiment. Rats in group D served as a control and were given distilled water. Five rats from each group were anaesthetized with chloroform and sacrificed on day 0, 10, 20 and 30 of treatment. And for each batch the Sperm Concentration and Testosterone were determined. The data were analysed using a one-way analysis of variance (ANOVA). The extract exhibited an inhibitory effect on the testosterone concentration at the 10th and 20th day of treatment at all dose levels. However, after the 30th day of treatment, the serum testosterone concentration ($P < 0.001$) and epididymal sperm count ($P < 0.01$) increased significantly at the 300mg/kg body weight dose. In conclusion, the extract of *Boscia senegalensis* may possess a significant androgenic and spermatogenic effect on a prolonged oral administration in a white albino rat which suggests that it may be useful in the management of male infertility problems.

Keywords: androgenic, spermatogenic, sperm count, testosterone and methanolic extract.

INTRODUCTION

Infertility is a common problem, affecting perhaps one couple in six, the majority of whom now seek medical care [1]. A number of studies suggest that male problems represent the commonest single defined cause of infertility [2] and more than 90% of male infertility is due to low sperm count, poor sperm quality or both [3]. The total sperm count is one of the major indices of fertility in males [4]. Spermatogenesis is a biological process of gradual transformation of germ cells into spermatozoa over an extended period of time within the boundaries of the seminiferous tubules of the testis. It involves a complex inter play between the structural element of the testis and the endocrine system [5] especially follicle stimulating hormone (FSH), luteinizing hormone and testosterone. Follicle stimulating hormone stimulates spermatogenesis and luteinizing hormone stimulates synthesis and release of testosterone. Generally, testosterone causes a direct stimulation of spermatogenesis and this impact is significant [6].

Herbal medicine is the most popular form of traditional medicine [7]. The use of medicinal plants is probably the mother of all healing practices known to man and it is an established fact that most of the pharmaceutical drugs originated from researches into plants [8]. The World Health Organization recommends that these researches should be encouraged especially in countries where access to the conventional treatment is not adequate. Presently, only folkloric information is available in the local communities in Sokoto State, Nigeria on the use of *Boscia senegalensis* in the treatment of low sperm count in men. And there is a drift from the synthetic drugs to natural plants in the treatments of infertility [9]. Therefore, there is need for more pharmacological research to ascertain the efficacy and safety of these more affordable, accessible and natural treatment options [9]. This has necessitated the assessment of the effects of prolonged oral administration of alcoholic extract of roots of *B. senegalensis* on rats so as to ascertain the folklore use. *Boscia senegalensis* locally known as Anza in Sokoto State, north-western Nigeria is used traditionally in treating fertility related problems among others. It belongs to the family Capparidaceae.

MATERIAL AND METHOD

Plant Preparation

The root of *Boscia senegalensis* per (capparaceae) was collected from Tureta Local Government Area of Sokoto State in August, 2011. The plant was authenticated by Mallam Muhammed Musa of Biological Science Department, Ahmadu Bello University, Zaria with voucher number 900537 deposited at the departmental herbarium. The plant root was washed and air dried pulverized into a dry powder using mortar and pestle and extracted with methanol for six hours using Soxhlet extractor.

Experimental Animal

Sexually matured male albino rats (weighing between 180-200g and about 12-30 weeks of age) were obtained from the animal unit of the Faculty of Pharmaceutical Sciences, UDUS and kept at a constant 12 hours light/dark cycle and maintained at 35°C for 2 weeks for acclimatization to the pharmacology laboratory. The animals were fed with rats' pellets (vital feeds) and had free access to tap water *ad libitum*, before the commencement of the experiment.

Animal Treatment

Eighty matured male rats were divided into four groups (A-D) of 20 rats each. Rats in group A, B and C were administered orally through an oesophago-gastric tube [10] with 100mg/kg, 200mg/kg and 300mg/kg body weight respectively of the extract daily until the end of the experiment. Rats in group D served as a control and were given distilled water. Five rats from each group were anaesthetized with chloroform and sacrificed on days 0, 10, 20 and 30 of treatment. And for each batch the Sperm Concentration and Testosterone were determined.

Sperm Concentration Determination

The Cauda epididymis were carefully dissected out. The adherent fat, blood vessels and connective tissue were cut away. The sperm was released by cutting the cauda epididymis longitudinally with a pair of fine pointed scissors, compressed into 2ml of formal saline. Using a white blood cell pipette, a 1:100 dilution was made using 0.025% of homogenizer (trypsin). Later, a 1:20 dilution of the homogenized semen was made using sodium bicarbonate formalin as diluting fluid and mixed well. A Pasteur pipette was used to fill an improved Neubauer chamber with the diluted semen, 3-5 minutes was allowed for the spermatozoa to settle. The sperm concentration per ml homogenate was done using a hemocytometer [11][12]. The Total sperm count per homogenate was determined using the formula:

Sperm Count per Homogenate = Volume of (homogenate) x (count in 16 squares) x (0.05 x 100).
Testosterone Determination

On the zero, 10th, 20th and 30th day of treatment following anesthesia with chloroform and sacrifice, blood samples were collected through cardiac puncture for hormonal analysis. The analysis of serum testosterone was carried out using immunoassay (ELISA) method [13]. The FSH and LH were not carried out due to logistic reasons.

Statistical Analysis

The data were analysed using a one-way analysis of variance (ANOVA) and the comparison between the control and the experimental/groups were done using the turkey-krammars test. The level of significant was set at $P < 0.05$. The data were expressed as mean $\pm$ SD.

RESULTS

Sperm Count

Treating various groups of rats for 30 days using distilled water as control and doses of 100, 200 and 300mg/kg body weight produced a cauda epididymal sperm counts which were not significantly different ($P > 0.05$) from the control at all doses of the extract studied on the 10th and the 20th day of treatment (table 1 and figures 1&2). However, the observation on the 30th day treatment revealed a significant increase ($P < 0.01$) in sperm count at 300mg/kg body weight of rat when compared to the control groups of rats (table 1 and figure 3)

Table 1: Effect of daily administration of the extract on the epididymal sperm count (25x 10⁶) of male albino rats

Days of Treatment	Distilled water (D)	Dose (mg/kg)/ Group		
		100 (A)	200 (B)	300 (C)
0	2.77±0.89	3.00±1.66	6.22±1.50	4.99±0.98
10	3.18±1.38	3.68±1.41	5.18± 2.70	5.02±0.48
20	12.06±2.72	9.40±2.86	7.70±1.44	10.16±2.98
30	9.12±4.33	13.12±3.65	9.22±1.81	18.12±1.55*

Values expressed as mean ±SD n = 5, g = gram, p < 0.05, * = significant values

Testosterone Concentration

The testosterone concentration in rats treated with the extract observed on the 10th day was significantly reduced (P<0.001) for all doses when compared with the control group (table 2). The observation made on the 20th day of treatment revealed that the testosterone concentration was significantly reduced (P<0.01) on the treated group at the doses of 100 and 200mg/kg body weight when compared to the control group but no significant difference (P>0.05) was observed on the testosterone concentration between the treated and control group at dose 300mg/kg body weight of rat (table 2). But on the 30th day of treatment, the testosterone concentration was significantly increased (P<0.001) at all doses 100, 200 and 300mg/kg body weight when compared with the control groups (table 2).

Table 2: Effect of Daily Administration of the Extract on the serum testosterone Concentration (ng/dl) in male albino rat.

Days of Treatment	Distilled water(D)	Dose (mg/kg)/Group		
		100 (A)	200 (B)	300 (C)
0	0.99±0.01	3.026±1.10*	1.44±0.55	1.04±0.22
10	2.08±1.08	1.504±1.28	3.50±0.954*	1.08±1.06*
20	2.80±0.72	0.4426±0.18*	0.89±0.1905*	1.81±1.06*
30	1.11±0.86	4.011±0.72*	4.64±0.4581*	9.25±1.51*

Values expressed as mean± SD, n = 5, g = gram, p < 0.05, * = significant values

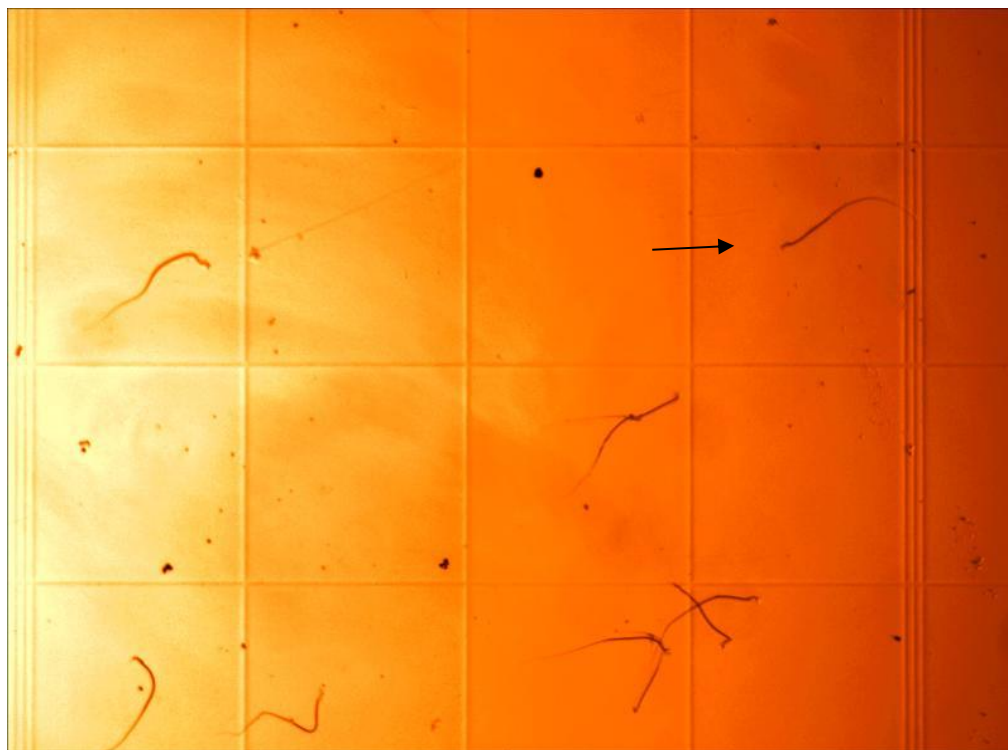


Figure 1: A photomicrograph showing the effect after the 30th day oral administration of distilled water on the male rat epididymal sperm count showing normally distributed spermatozoa.

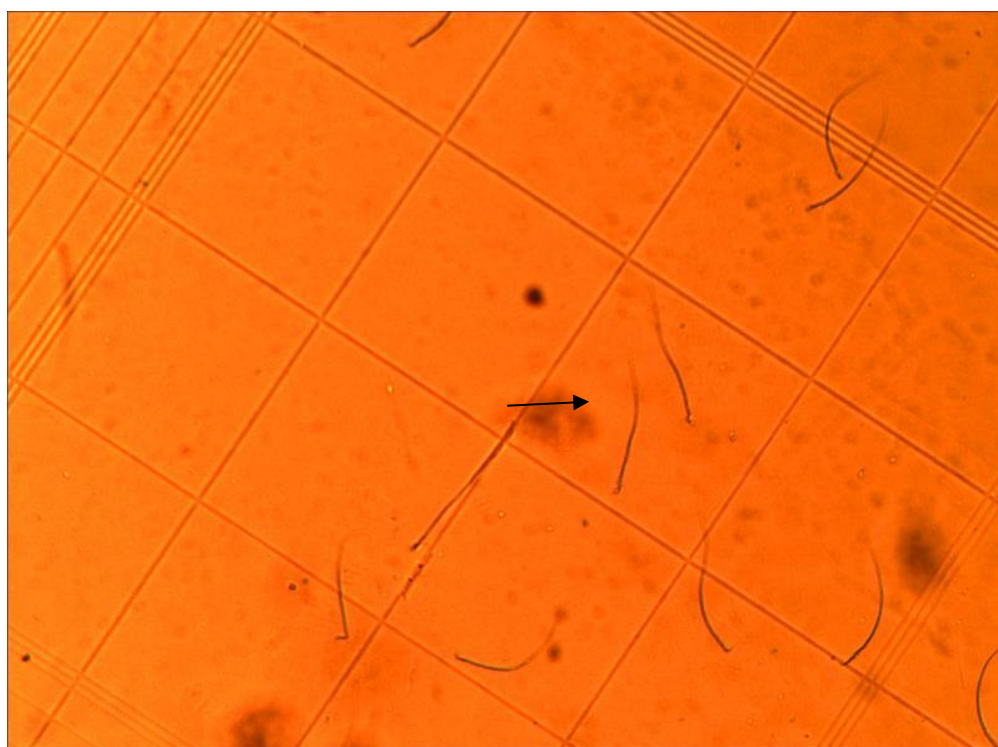


Figure 2: A photomicrograph showing the effect after the 30th day oral administration of 200mg/kg of crude root extract of *Boscia senegalensis* on the male rat epididymal sperm count showing sparsely distributed spermatozoa.

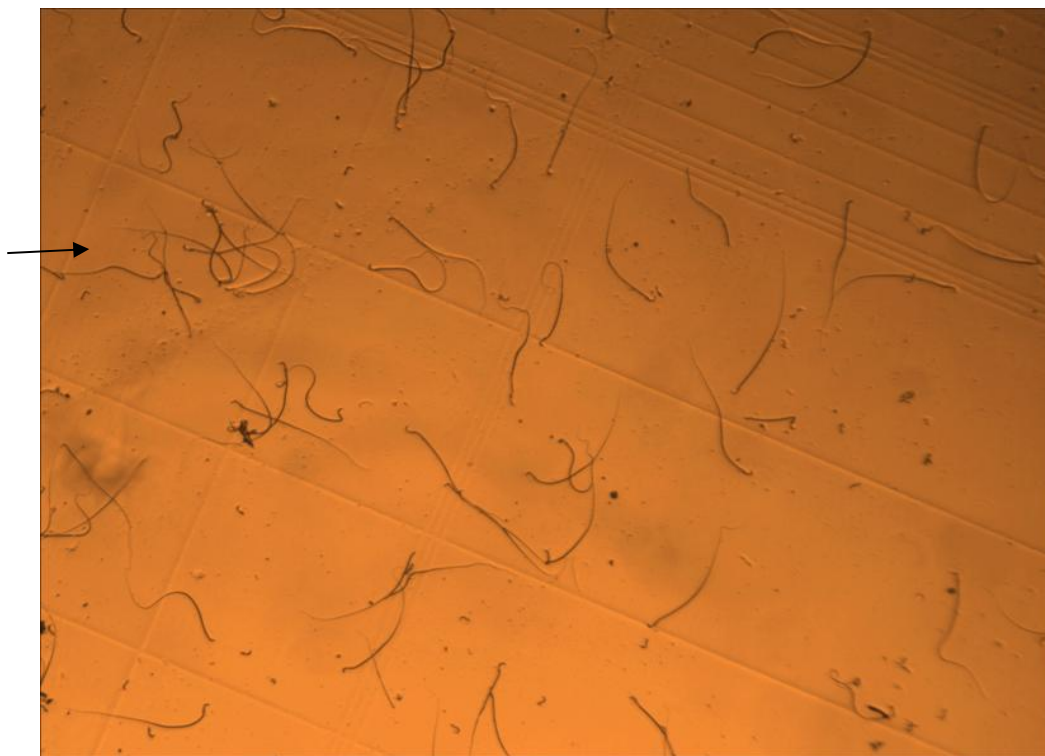


Figure 3: A photomicrograph showing the effect after the 30th day oral administration of 300mg/kg crude root extract of *Boscia senegalensis* on the male rat epididymal sperm count showing densely distributed spermatozoa.

DISCUSSION

The study examined the androgenic and spermatogenic effects of *Boscia senegalensis* in male albino rat. The extract exhibited an inhibitory effect on the testosterone concentration at the 10th and 20th day of treatment at all dose levels. However, after the 30th day of treatment, the serum testosterone concentration ($P < 0.001$) and epididymal sperm count ($P < 0.01$) increased significantly at the 300mg/kg body weight dose. The significant effect of the extract observed at day 30 of treatment on both sperm count and testosterone concentration was probably due to increased spermatogenesis after at least 2 cycles of the seminiferous epithelium; one cycle being 12-13 days [14][15]. It has been noted that epididymal sperm head count as indicator of spermatogenesis do not provide information on sperm cell motility, fertilizing potential or genetic integrity when biological active substance has been administered therefore any substance being evaluated for its effect on spermatogenesis should be administered for prolonged period to effect sperm cell production to the extent that the effect may be quantified by sperm head counts [16]. Therefore, the observation may suggest that the increasing effects of the extract on the serum testosterone concentration and sperm count only occur on a prolonged oral administration. This fact was supported by the previous findings in a prior investigation of the toxicity of the plant *Boscia senegalensis* on the testis of male rats (in press) [17] where there was a significant increase in the relative weight of the testis, area of the interstitial space and the diameter of the seminiferous tubules when compared to the control group, observed to be highest at 500mg/kg body weight dose suggesting the highest androgenic and spermatogenic effect of the extract at this dose. The phytochemical screening of *Boscia senegalensis* in the same prior study (in press) [17] generally revealed the presence of phytosteroid and triterpenoids and more specifically the presence of saponnins which may be

contributory to the endogenous effect of *Boscia senegalensis* on testosterone level [18] and subsequently action on spermatogenesis.

In conclusion, the extract of *Boscia senegalensis* may possess a significant androgenic and spermatogenic effect on a prolonged oral administration in a white albino rat which suggests that it may be useful in the management of male infertility problems. Though further research is recommended in the following areas

- Determination of the effect of the plant extract on leuteinizing hormone and follicle stimulating hormone.
- Isolation and characterization of the active principles
- Mating of the extract treated rats with fertile female to determine pregnancy rates to establish a correlation between androgenic, spermatogenic effects and fertility.

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