ACTA SCIENTIFIC MEDICAL SCIENCES

Volume 3 Issue 5 May 2019

Research Article

Protective Effect of Aqueous Extract of the Bark of *Boswellia dalzielii* on Flutamide-induced Testicular Reprotoxicity in Wistar Rat

Seno Akpobasaha, Joseph Okike Ezugworie and Nto Johnson Nto*

Department of Anatomy, Faculty of Basic Medical Sciences, College of Medicine, University of Nigeria Enugu Campus, Enugu, Nigeria

***Corresponding Author:** Nto Johnson Nto, Department of Anatomy, Faculty of Basic Medical Sciences, College of Medicine, University of Nigeria Enugu Campus, Enugu, Nigeria.

Received: May 10, 2019; **Published:** May 29, 2019

Abstract

The study is to evaluate the protective effect of aqueous extract of the bark of *Boswellia dalzielii* on flutamide-induced testicular defect in wistar rats. Sixteen male wistar rats were randomly divided into four groups (A-D) of 4 rats each. Group A (normal control group) received a daily dose of 5ml of normal saline only. Group B (positive control group) recieved a daily 10mg/kg body weight of flutamide. Group C (protective group I) and Group D (Protective group II) recieved low and high dose of aqueous extract of *Boswellia dalzielii* for 7 days after which 10mg/kg body weight of flutamide and aqueous extract of *Boswellia dalzielii* were administered simultaneously for the next 14 days. There was a significant ($p<0.05$) dose dependent increase in the body weight in the protective groups. The protective groups (C and D) showed increase in the level of testosterone level compared to the normal control (group A). Testicular sections of protective groups showed increase in number and volume of germinal epithelium similar to the controls. The result of this present study suggests that the aqueous extract of the bark of *Boswellia dalzielii* can protect the testis from the effects of flutamide.

Keywords: *Boswellia dalzielii*; Flutamide; Wistar Rat

Background of Study

Plant materials provide an important source of nourishment and are used in traditional medicine for the treatment of different diseases [1]. Many of these plant materials used in phytotherapy have been screened for their medicinal properties [1,2]. *Boswellia dalzielii* is consumed locally and is employed in traditional medicine [1,3].

B. dalzielii is a tree species in the genus *Boswellia*, abundant in the Savannah region of West Africa [1]. It grows up to 13m high, with characteristically pale papery bark, peeling and ragged [1]. The bark secretes a whitish exudate which dries readily and is friable. The exudate is a fragrant and it is burned alone or with other fragrant resins to fumigate clothing and rooms to drive out flies and mosquitoes. The bark-decoction is consumed locally in Northern Nigeria for its antipyretic, anti-snake venom, antiulcer, antimalarial, aphrodisiac and fertility potentials, and is also used as an antiseptic wash for sores [1,4,5]. The main antioxidants isolated from the stem bark of *B. dalzielii* are protocatechuic and gallic acids, others include 4'-Methoxy-(E)-resveratrol 3-O-rutinoside, incensole and b-sitosterol3. Its biologically active compounds include: cholesterol, flavonoid, tanins , glycosides, alkaloids, anthracene and saponins [1,4,6].

Flutamide is a nonsteroidal antiandrogen which is used primarily to treat prostate cancer [7]. It acts as a selective antagonist of the androgen receptor. Exposure to flutamide has been reported to compromise the function of the epididymis and sperm quality [2,8]. The aim of this study is to evaluate the protective effect of aqueous extract of the bark of *B. dalzielii* on flutamide-induced testicular defect in male wistar rat.

Materials and Methods

Collection of Plant material

Fresh stem bark of *B. dalzielii* was harvested fresh from its natural habitat in Kano State, Northern Nigeria and authenticated at the Department of Botany, University of Nigeria Nsukka. Voucher number UNH252.

Preparation of extract

The stem bark of *B. dalzielii* was air dried and pulverized using a mortar and pestle and sieved to fine powder. The powdered stem bark was soaked in water for 24hours and filtered using white muslin cloth to remove debris. The resultant filtrate was allowed to evaporate to dryness which was stored in a sealed plastic container until required [1,6].

Experimental animals

Sixteen adult male Wistar rats were procured from the animal house of the Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, University of Nigeria Nsukka. The rats were housed in cages in the animal house of Anatomy Department Faculty of Basic Medical Sciences University of Nigeria, Enugu campus. The rats were acclimatised for two weeks and maintained under standard environmental conditions. The animals had free access to standard livestock pellets and water.

Experimental design

Sixteen adult male Wistar rats were randomly divided into 4 groups of 4 rats each. The period of treatment was 21 days and administrations were done orally.

Group A (normal control group) received a daily dose of 5ml of normal saline only. Group B (positive control group) was given a daily 10mg/kg body weight of flutamide. Group C (protective group I) was given 100mg/kg body weight of aqueous extract of *B. dalzielii* for 7 days after which 10mg/kg body weight of flutamide and 100mg/kg body weight of aqueous extract of B. dalzielii were administered simultaneously for the next 14 days. Group D (Protective group II) was given 300mg/kg body weight of aqueous extract of *B. dalzielii* for 7 days after which 10mg/kg body weight of flutamide and 300mg/kg body weight of aqueous extract of *B. dalzielii* were administered simultaneously for the next 14 days.

Sample collection

At the end of the treatment, each rat was anaesthetized with 25% urethane at a dose of 0.6 ml/100g. The blood was collected via the left ventricular cardiac puncture for hormonal assay and the testes were rapidly dissected for processing and light microscopic study.

Hormonal assay

Blood was collected from each rat via the left ventricular cardiac puncture and kept in non-heparin vacuum container which was span at 2500rpm for 10min using a bio-centrifuge (MSE, O-5122A, Germany). The levels of free serum testosterone, luteinizing hormone (LH) and follicle stimulating hormone (FSH) were measured with ECOBAS-6000 hormone analyzing machine [1,9].

Histological study

The harvested testes were fixed in Modified Davidson's Fluid (MDF) for 24hours [1,10]. Standard protocol was followed in processing the tissue for microscopic examination [1,11]. Paraffin sections were cut at 3µm thickness [1,10] and stained with haematoxylin and eosin (H and E).

Statistical analysis

Data collected was analysed using Statistical Package for Social Science (SPSS) version 2.0. One-way analysis of variance (ANOVA) and student t-test were used to study variations between groups. Statistical significance was considered at p-value <0.05. Data was reported as mean ± standard deviation.

Results

Body weight

The percentage increase in body weight was observed (Table 1) to be significant high ($p<0.05$) in the treatment groups (C and D) compared to the controls (A and B). The percentage increase in body weight in the treatment groups (C and D) was dose dependent.

GROUPS	Day 1 (g)	Day 22 (g)	Percentage Weight change (%)
A	239.9 ± 0.2	254.3 ± 0.6	6.0
B	250.9 ± 0.3	258.1 ± 0.2	2.9
C	221.8 ± 0.5	244.9 ± 0.4	10.4
D	180.5 ± 0.3	206.1 ± 0.1	14.2

Table 1: Body Weight of experimental models.

Weight of testis

The weight of the testis in the treatment groups (C and D) was statistically ($p>0.05$) not different from the controls (A and B).

Hormonal assay

The treatment groups (C and D) showed increase in the level of testosterone level (table 3) when compared to the normal control (group A), this was however, not statistically significant ($p> 0.05$). The negative control (group B) showed significantly high ($p<0.05$) testosterone level compared to all other groups. The serum FSH and LH level the treatment groups were low in the treatment groups.

Group	Right Testis (g)	Left Testis (g)
A	1.65 ± 0.35	1.65 ± 0.35
B	1.60 ± 0.71	1.60 ± 0.14
C	1.60 ± 0.00	1.60 ± 0.14
D	1.60 ± 0.14	1.65 ± 0.71

Table 2: Weight of the testis of experimental models.

GROUPS	TESTOSTERONE	FSH	LH
A	0.10 ± 0.00^{a}	13.50 ± 0.70	10.0 ± 1.41
B	1.35 ± 0.71	13.00 ± 0.00	11.5 ± 0.71
C	$0.30 \pm 0.0^{0}a$	3.90 ± 0.14^{Aa}	9.1 ± 0.14^{a}
D	0.20 ± 0.0^{a}	3.00 ± 1.41^{Aa}	9.5 ± 0.71^{Aa}

Table 3: The levels of testosterone, FSH and LH of the experimental models.

Testicular histology

The light microscopic study showed that the testis of the normal control (Plate I) had an apparently normal testicular histomorphology and cellular composition. Seminferous tubules well defined, presence of spermatogenic cells, Sertoli cells and spermatozoa within the seminiferous tubules and also obvious are the interstitial cells of Leydig. Photomicrograph of testis of the negative control (Plate II) showed distortion of most of the seminiferous tubules, loss of spermatogenic cells and Sertoli cells, reduction of spermatozoa within the lumens of the seminiferous tubule and hypertrophy of interstitial cells of Leydig. Testicular sections of treatment groups (Plate III and IV) showed increase in number and volume of germinal epithelium similar to the controls.

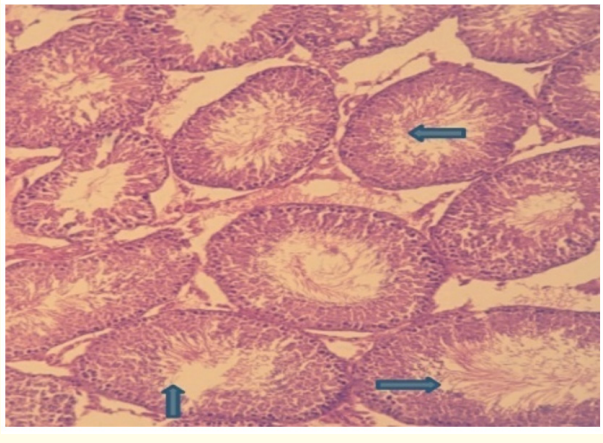

Plate I

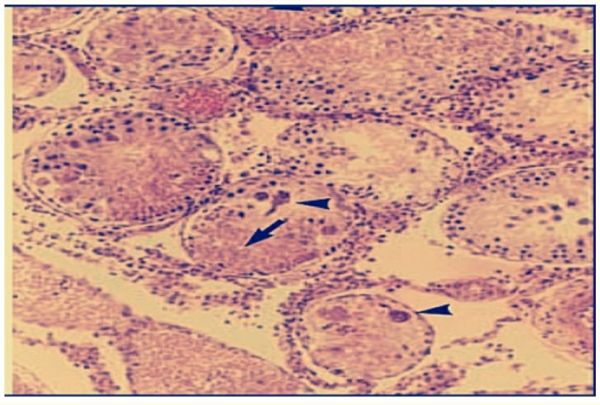

Plate II

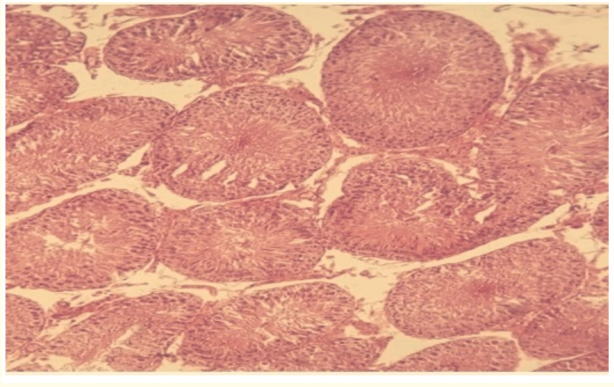

Plate III

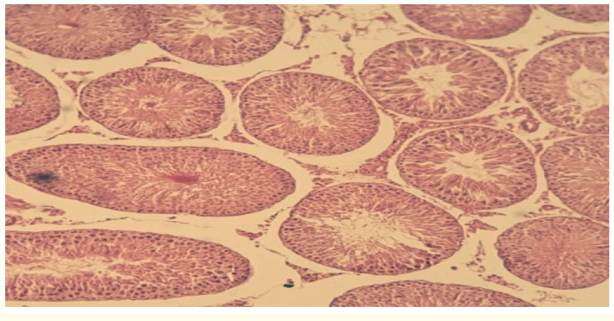

Plate IV

Discussion

This present study evaluated the protective effect of aqueous extract of the bark of *B. dalzielii* on flutamide-induced testicular reprotoxicity in male wistar rat. The results showed that flutamide administration caused a significant increase in serum testosterone

level. This agrees with the finding of Hassan., *et al*. 2018 [2]. The light microscopy study also showed that there was loss of spermatogenic and Sertoli cells, reduction of spermatozoa within the lumens of the seminiferous tubule and hypertrophy of interstitial cells of Leydig in the flutamide treated group. This was similar to the findings of Oremosu., *et al*. [11]. The high serum testosterone level following flutamide administration may be caused by hypertrophy of the Leydig cells resulting from flutamide induced toxicity [11], altered functions of Sertoli cells and the consequent disruption of the hypothalamo-pituitary gonadal axis [2,11].

B. dalzielii has been reported to have aphrodisiac and fertility potentials [1]. The biologically active compounds in *B. dalzielii* include: cholesterol, flavonoid, tanins , glycosides, alkaloids, anthracene and saponins [1,4,6]. Flavonoids, tanins and saponins has been indicated to enhance sexual function and fertility [1,12]. Protocatechuic acid is one of the major antioxidants isolated from the stem bark of *B. dalzielii* [3,13].

The administration of aqueous extract of the bark of *B. dalzielii* before inducing testicular toxicity resulted in: a significant dose dependent increase in body weight, no alteration in the weight of the testis, elevated serum testosterone level and decreased the serum luteinizing hormone and follicle stimulating hormone levels compared to the normal control, and an increase in number and volume of germinal epithelium compared the controls.

Testosterone, a steroidal androgen, is anabolic and increases protein in skeletal muscle cells [11,14]. The increased serum testosterone level will result in increased metabolism and consequently increased muscle mass and body weight [2,11,14]. The non-altered weight of the testis suggests that sperm production and fertility were not affected, this is supported by light microscopy study which showed increase in size of seminiferous tubules, number and volume of germinal epithelium and Leydig cells. Morphological changes in testicular histo-architecture has been associated with increased testosterone level [2,11,15].

The findings of this study shows that *B. dalzielii* can protect the testis from the adverse effects of flutamide. This may be as a result of presence protocatechuic acid in the aqueous extract of the stem bark of *B. dalzielii* [3,13]. Protocatechuic acid has been reported to possess free radical-scavenging capacity and protects tissue against oxidative damage [13]. Oxidative stress is a major factor in the aetiology of male infertility [16,17]. Protocatechuic acid may have counteracted oxidative damage in the testes from flutamide administration.

Conclusion

The result of this present study suggests that the aqueous extract of the bark of *B. dalzielii* can protect the testis from the reprotoxic effects of flutamide.

Bibliography

1. Ezugworie JO., *et al*. "The Histological and Hormonal Effect of Aqueous Extract of Boswellia diazielii on the Testis of Male Wistar Rat". *International Journal of Pure and Applied Bioscience* 6.2 (2018): 31-36.

2. Hassan LA., *et al*. "Protective Effect of Aqueous Extract of Cyperus Esculentus on the Flutamide Induced Testicular Defect of Male Wistar Rats". *SJAMS* 6.6 (2018): 2391-2395

3. Alemika Taiwo E., *et al*. "Antibacterial phenolics from Boswellia dalzielii". *Nigerian Journal of Natural Products and Medicines* (2006).

4. Adoum AO. "Determination of toxicity effects of some savannah plants using brine shrimp test (BST)". *Applied Science* 2.3 (2008): 1-5.

5. Nwinyi FC., *et al*. "Evaluation of the Aqueous Extract of Boswellia dalzielii Stems Bark for Antimicrobial activities and Gastrointestinal Effects". *African Journal of Biotechnology* 3 (2004): 284-288.

6. Abdulaziz Hassan HS and Usman MA. "Preliminary Phytochemical and antispasmodic studies of the stem bark of BoswelliaDalzielli". *Nigeria Journal Pharmaceutical Science* 8 (2009): 1-6.

7. Elks J. "The Dictionary of Drugs: Chemical Data: Chemical Data, Structures and Bibliographies". Springer. (2014): 573.

8. Perobelli JE., *et al*. "Impairment on Sperm Quality and Fertility of Adult rats after Antiandrogen Exposure during Prepuberty". *Reproductive Toxicology* 33 (2012): 308-315.

9. Atlar MR., *et al*. "Laboratory Manual Experimental Microbiology. Mosby-Year Book Inc. (1995).

10. Finbarrs-Bello., *et al*. "Cognitive and Immunochemistry Study on the Effect of Neostigmine on Ketamine Induced Wistar Rats". *International Journal of Development and Research* (2015): 4781-4784.

11. Oremosu AA., *et al*. "Effect of Cissuspopulneal and Panax ginseng on Flutamide-Induced Testicular Defect in Pre-Pubertal Male Rats". *British Journal of Medicine and Medical Research* 3 (2013): 173-181.

12. Atuadu VO., *et al*. "Effect of Ethanolic Rind Extract of Citrullus lanatus on the Hpg Axis and Sexual Behavior of Male Wistar Rats". *International Journal of Pure and Applied Bioscience* 6 (2018): 1-7.

13. Liu C., *et al*. "In vivo protective effect of protocatechuic acid on tert-butyl hydroperoxide-induced rat hepatotoxicity". *Food and Chemical Toxicology* 40 (2002): 635-641.

14. Powers M. "Performance-Enhancing Drugs". Principles of Pharmacology for Athletic Trainers (2nd ed). Slack Incorporated 345.

15. Ang HH., *et al*. "Sexual arousal in sexually sluggish old male rats after oral administration of Eurycomalongifolia". *Journal of Basic and Clinical Physiology and Pharmacology* 15 (1997): 3-40.

16. Aitken RJ and Roman SD. "Antioxidant systems and oxidative stress in the testes". *Oxidative Medicine Cellular Longevity* 1 (2008): 15-24.

17. Latendresse JR., *et al*. "Fixation of the Testes and Eyes Using a Modified Davidson's Fluid: Comparison with Bouin's Fluid and Conventional Davidson's Fluid". *Toxicology and Pathology* 30 (2002): 524-533.

Volume 3 Issue 6 June 2019