

Morphometric Study of Testes of Adult Wistar Rats Treated With Aqueous Extract of *Boswellia Dalzielii* Stem Bark

Emmanuel A. O.¹ Adediji, J. A.^{2*}

¹(Department of Anatomy, Faculty of Basic Medical Sciences, University of Benin, Benin City Nigeria.)

²(Centre for Training Community Health Officers, University of Benin Teaching Hospital, Benin City, Nigeria.)

Abstract: This study was designed to determine the morphometric changes induced by aqueous extract of *Boswellia dalzielii* stem bark on the testes of Adult Wistar Rats. Thirty six (36) male Adult Wistar Rats weighing between 199-290g were randomly divided into four (4) groups of nine(9) rats each containing five(5) experimentals and four (4) controls. The experimental rats in all the four groups were administered 200mg/kg of the plant extract for different period. Group 1,2 3 and 4 were sacrificed on the 2nd, 4th, 6th and 8th weeks of the experiment.

The morphometric studies showed a very significant increase in the seminiferous tubules of those treated with the plant extract for six and eight weeks when compared to the control ($p < 0.01$) however, insignificant increase in the interstitial cell of Leydig when compared to the control ($p > 0.05$) was observed.

The present study indicated that the aqueous extract of *Boswellia dalzielii* stem bark significantly increased the size of the seminiferous tubule which suggest that it may have the ability to enhance the activities of the cells within the seminiferous tubule.

Keywords: Morphometric, *Boswellia dalzielii*, stem bark, testes, aqueous extract

I. Introduction

Herbal medicine is the use of herbs for their therapeutic and medicinal values.

Herbalism is becoming more mainstream as improvements in analysis and quality control along with advances in chemical research show their value in the treatment and prevention of disease¹.

Boswellia dalzielii (B. dalzielii) hutch is from the genus *Boswellia* and the family of Burseraceae. It is a tree plant abundantly found in North- western Nigeria and very common among the locals as a potent source of ethnomedicine².

Boswellia dalzielii has various names which is been identified for. It is commonly known in English as the frankincense tree while the French calls it bouquet. In Nigeria, the Hausa speaking people calls it Hano or Harrabi,³ Fulfulde calls it juguli, the Kanuri people calls it kafi- dukkan. In Niger, the fula-fulfulde calls it adakehi gorki while in Ghana, it is generally called pianwogu⁴.

The genus *Boswellia* has about twenty five (25) species spread all over the world but the specie B. dalzielii is basically found in the West-African region, concentrated more in Nigeria⁵.

B. dalzielii is a tree plant of about 13m high of the wooden savanna⁴. It has a characteristic smooth papery patches which on rapping exudes a whitish fragrant resin.

The gum resin gotten from B. Dalzielii is used along with other medicines as a stomachic and for the treatment of venereal diseases⁶. The roots and barks are used for antidote to arrow poisons. The fresh bark is eaten in Adamawa State of Nigeria to induce vomiting after a few hours and thus relieve symptoms of giddiness and palpitation⁴. The extract from it leaves is used for the treatment of diarrhoea in poultry³ and humans².

The resin gotten from B. Dalzielii in combination with *Steganotaenia aralia* have anti-inflammatory activity due to the presence of Acetylketoboswellic acid (KABA)⁷.

The aqueous extract of *Boswellia dalzielii* was used to investigate its effect on the liver of albino rats and found that there was no significant effect on total protein and albumin content within the short period of five (5) days the investigation was carried out³.

Phytochemical screening of the extract of B. Dalzielii was investigated and tannin was detected, it was also investigated for antimicrobial effect using 200mg/kg and there was no antimicrobial effect on the tested organisms. The result obtained shows that the extract probably contains active ingredient that could be developed for gastrointestinal problems⁶.

A further investigation was carried out on the anti-diarrhoe effect of *Boswellia dalzielii* stem bark extract in albino rats on castor induced diarrhoea using graded doses of 100, 200 and 300mg/kg before induction in diarrhoea with castor oil. The extract produced a significant inhibition of the castor induced diarrhoea².

The aqueous stem bark of B. Dalzielii possesses anti-inflammatory effect which may be related to anticholinergic mechanism².

A further investigation was carried out by Etuk et al (2006)² on the weight of the various organs in the body. The organs were the liver, kidney, heart and lungs, a significant decrease in the weight was observed. It was suggested that the prolonged oral administration of very high dose of the aqueous stem bark extract of *B. Dalzielii* may be associated to increased risk of toxicity².

The Boswellic acid which is a component of the resin from the bark of *Boswellia dalzielii* have shown some promises as a treatment for asthma and various inflammatory conditions and in West Africa, the bark have been reported to be used for the treatment of fever, rheumatism and gastrointestinal problems.

Boswellia dalzielii have been proven to be very effective (bacteriostatic) when the stem bark is grounded and mixed with alcohol to heal this *lingua villosa*⁸.

I. I Aims And Objectives

Various works have been done on the plant to know its uses and effects on various organs of the body but there was no research done studying its effect on the testes. This investigation is aimed at determining the morphometric effects of aqueous extract of *Boswellia dalzielii* stem bark on the testes.

II. Materials and Method

II.I Plant Collection and Authentication

The stem bark of *Boswellia dalzielii* hutch was collected from its natural habitat of Portiskum, Yobe state which is located in the North-Western part of Nigeria in the month of November, 2009. The plant was identified and authenticated by Prof. S.S Sanusi, a botanist in the department of Biological Sciences, University of Maiduguri.

II.II Preparation of Plant Extract

The method described by Nwinyi et al, (2004)⁶ was adopted. The plant stem bark was cleaned, cut into pieces and air-dried under shade for seven (7) days avoiding direct sunrays on it. After which, it was pulverised to powder. 200g of the powder was cold macerated with 1.5litres (1500ml) of water for twenty four (24) hours. The macerated product was filtered and the filtrate was evaporated to dryness at a constant range of temperature between 50-60°C.

On every experimental day, a fresh solution was prepared using the dry powder at the required concentration before administration.

II.III Animals and Diet

Male albino rats weighing between 199g to 290g were gotten from the national Research Institute, Vom, Plateau State and were kept in a well ventilated cages to acclimatized for three (3) weeks before investigation began.

II.IV Experimental Design

Thirty six (36) male albino rat ranging from 199g and 290g were used for the experiment. They were divided into fours of nine rats each containing five (5) experimental rats four (4) control groups. The experimental rats were given 200mg/kg/p.d of body weight while the control were given comparable volume of distilled water. Groups 1, 2, 3 and 4 were sacrificed on the 2nd, 4th, 6th and 8th weeks respectively through cervical dislocation. The testes were immediately removed, measured, and weighed⁹.

II.V Morphometry Study

The morphometric studies of the testes was carried out where the diamtres of the seminiferous tubule and the interstitial cells of Leydig were taken using stage and ocular micrometres according to the method described by Anissimov, 2010¹⁰ and Caprette, 2005¹¹.

II.VI Statistical Analysis

Data collected were expressed as mean±SEM and analysed using the Instat software programme. One way analysis of variance (ANOVA) with Dunnette test was used to determine the level of significance at confidence of 95% (p value <0.05 was considered significant).

II.VII Phytochemical Test

The plant stem bark was subjected to phytochemical screening to test for its' chemical constituents and their proportions in the plant.

II.VIII Materials

The following material were used during the course of the study; tripple beam balance, knife, beakers, measuring cylinder, disposable syringe, vernier calliper, metre rule, razor blades, forceps, pins, clipboard, specimen bottles, cages, drinkers, oven, paraffin, slide glasses, stage and occular micrometre, chemical balance.

IV. Results

IV.I Behavioural Observation

All through the period of the investigation, there was no observable changes of behaviour as a result of the administration of the plant extract of *Boswellia dalzielii*.

IV.II Phytochemical Result

Table 1 shows the phytochemical screening of the aqueous extract of the stem bark of the plant yielded the following constituents with its' proportional percentage.

Table 2 shows the mean weight of the rats treated with aqueous extract of *Boswellia dalzielii* in comparism with the control. There was an initial reduction in weight but later stabilized. The change of weight was not significant ($p>0.05$)

Table 3 shows the length, diametre and the weight of the testes comparing the mean values of treated rats with the control. The length and diametre of the treated rats showed no significant variation when compared to the control ($p>0.05$). the weight of the testes for rats treated with the aqueous extract of the plant for 2nd, 4th and 6th showed no significant decrease or increase when compared to the control ($p>0.05$) while the rats treated with the extract for 8wks showed a significant increase in the right testes only, ($p<0.05$).

IV.III Morphometric Studies

Table 4 shows the mean diametres of the seminiferous tubule and the mean diametre of the Leydig cells (interstitial cells) of rats treated with the aqueous extract of *Boswellia dalzielii* stem bark measured in microns. Rats treated with the extract for 2 and 4 weeks did not show any significant increase in the size of their seminiferous tubule when compared with the control ($p>0.05$) while the rats treated with the extract for 6 and 8 weeks showed a very significant increase in the size of the seminiferous tubule when compared to the control ($p<0.01$). The mean diametre of the Leydig cells in rats treated with the plant extract did not show any significant change when compared to the control ($p>0.05$).

Morphometric studies shows that Leydig cell still retained it size considerably while there was very significant increase in the size of the seminiferuos tubule of the rats treated with the aqueous extract of *B.dalzielii* for 6 and 8weeks. A reduction in size could be as result of degeneration of the spermatids¹² and seminiferous tubule¹³ but an increase in sertoli cells could suggest increase in their activities¹³.

V. Tables

Table 1: Result for the phytochemical screening of aqueous extract of *Bosweelia dalzielii* stem bark.

Constituents	Proportion(%)
Pentose	2
Ketose	4
Monosacharrides	2
Phlobatannins	4
Tannins	10
Combined reducing sugar	3

Table 2: Effect of *Boswellia dalzielii* aqueous extract on the mean body weight of rats.

Group	Duration (weeks)	Initial weight (g)	Final weight (g)	Weight difference (g)	Percentage weight diff(%)
Control		133.25±22.531	177.38±18.248	44.13	24.88
I.	2	211.50±30.695	207.13±25.248	4.37	02.11
II.	4	151.85±9.320	18.45±16.27	29.60	16.31
III.	6	185.05±9.517	220.90±12.081	35.85	16.23
IV.	8	204.95±18.407	234.88±15.710	29.93	12.71

Values are expressed as Mean±SEM of 29 rats in treated groups and 16 rats in the control. ($p>0.05$).

Table 3: Effect of *Boswellia dalzielii* aqueous extract on the length, diameter and weight of treated rats compared with the control.

Group	Duration (Weeks)	Length of Testes(cm)		Diameter of Testes(cm)		Weight of Testes(g)	
		R/T	L/T	R/T	L/T	R/T	L/T
Control		1.84±0.065	1.87±0.047	1.13±0.018	1.18±0.014	1.068±0.006	1.161±0.056
I	2	1.75±0.065	1.83±0.063	1.140.038	1.16±0.027	1.108±0.030	1.178±0.026
II	4	1.80±0.083	1.73±0.206	1.08±0.011	1.15±0.050	1.279±0.071	1.284±0.081
III	6	1.83±0.032	1.94±0.036	1.18±0.011	1.16±0.010	1.192±0.110	1.251±0.084
IV	8	1.93±0.038	1.91±0.041	1.14±0.021	1.14±0.006	1.320±0.012*	1.242±0.056

Values are expressed as Mean±SEM of 20 rats in treated groups and 16 in the control. *=P<0.05.

Table 4: Effect of *Boswellia dalzielii* on the diameter of seminiferous tubule and diameter of the Leydig cell.

Group	Duration (weeks)	Diameter of seminiferous tubule(µm)	Diameter of Leydig cell(µm)
Control		211.0±10.97	5.63±0.63
I	2	254.3±7.22	6.06±0.40
II	4	253.3±3.88	5.50±0.10
III	6	277.0±17.21**	6.69±0.21
IV	8	278.2±9.06**	6.44±0.12

Data are expressed as Mean±SD of 20 rats in treated groups and 16 in control group. *=significant; **=very significant.

VI. Conclusion

This study showed that the aqueous extract of the stem bark of *Boswellia dalzielii* significantly increased the size of the seminiferous tubule which suggests that it may have the ability to enhance the activities of the cells within the seminiferous tubule.

VII. Recommendation

The morphometric study was restricted to the size of the Leydig and seminiferous tubule. Further studies should be carried out on the sperm count and testosterone level in the body. It should also extend to the female reproductive organs to see the effect of the plants' aqueous extract on the ovary.

References

- [1] Hawkins EB, Ehrlich SD and Phoenix A. What is herbal medicine? History of Herbal Medicine. University of Maryland Medical Centre. 2007. <http://www.umm.edu.com>.
- [2] Etuk EU, Agaie BM, Onyeyili PA, and Otta CU Anti-diarrhoea effect of *Boswellia dalzielii* stem Bark Extract in Albino Rats; Journal of Pharmacology and Toxicology. 2006. 1(6); Academic Journals Inc, USA; Pp 591-596.
- [3] Aliyu R, Gatsing D, Jaryum KH. The effect of *Boswellia dalzielii* (Burseraceae) aqueous extract on rat liver function; Asian Journal of Biochemistry; 2006 Academy Journals.
- [4] Burkhill HM. Burseraceae; Useful Plants of West Tropical Africa; Royal Botanic Gardens, 1985. Kew <http://www.aluka.org>.
- [5] Burfield T. Frankincense; A Brief Catch up; Samara Nature Intelligence; http://www.aroma_connection.org/ecologicalcultural_sustainability. Posted January 1, 2009 as assessed on 21 December, 2009.
- [6] Nwiyi FC, Binda L, Ajoku GA, Aniagu SO, Enwerem NM, Orisadipe A, Kubarawa D, Gamaniel KS. Evaluation of the aqueous extract of the *Boswellia dalzielii* stem bark for antimicrobial activities and gastrointestinal effects; African Journal of Biotechnology. 2004. Vol 3(5); Pp 284-288.
- [7] Alemika TE. Bioactive Compounds from *Boswellia dalzielii* and *Steganotaenia araliacea*; African Journals of Traditional Complementary and Alternative Medicines. 2009. Vol 2, number 2. Pp 177-205.
- [8] Herbal Granny. Herbal Treatment for Hairy Tongue. 2009. <http://www.herbalgranny.com> as assessed on 12th Jan, 2010.
- [9] Drury, R.A.B, (1973); General Tissue Processing; General Staining Procedures; Carletons' Histological Technique; 4th Edition; London. Oxford University Press. Pp 100-132.
- [10] M. Anissimov. How large is a Micrometre? 2010. <http://www.wisegEEK.com>, as assessed on April 5, 2010.
- [11] D.R. Caprette, Measurement with the Light Microscope <http://www.caprette@rice.edu>. Last updated on May 5, 2005 as assessed on April 5, 2010.
- [12] J.M. Bartlett, JB Kerr and RM Sharpe. The effect of selective destruction and regeneration of rats' Leydig cells on the testicular distribution of testosterone and morphology of the seminiferous epithelium. Journal of Andrology. 2009. vol 7; issue 4; pp240-258.
- [13] T.W. Jacks, S.A. Asala, and J. Prasad. Testicular Enhancement Activity of Aqueous Extract of *Pausinystalia Macroceras* stem bark in Wistar Rats; Journal of Anatomy Sciences. 2007. vol 1; pp 3-6.