Quest Journals
Journal of Medical and Dental Science Research
Volume 3 ~ Issue 1 (2016) pp:16-24
ISSN(Online) : 2394-076X ISSN (Print):2394-0751
www.questjournals.org

Research Paper

Semen Quality in Male Albino Rats Fed With Various Concentrations of *Pausinystalia Yohimbe* Bark Powder (Burantashi)

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Received 22 December, 2015; **A**ccepted 23 January, 2016

ABSTRACT:- The effects of Pausinystalia yohimbe bark powder (burantashi) on semen quality and quantity was investigated in whole adult male wistar rats administered graded concentrations of the powder in combination with feed in the order 2%, 4%, 6%, 8% and 10% for thirty days. Results obtained indicate significant ($P<0.05$) and dose dependent increase in testicular weights, sperm counts and motility. There was also a dose dependent increase in primary, secondary and total sperm abnormalities following chronic consumption of burantashi while semen $_{P}H$ and liquefaction were not significantly affected when compared to the control. In conclusion, the use of burantashi for cases of erectile dysfunction though has no obvious deleterious effects on semen quality and quantity; it however does increase the percentage of abnormal and immature spermatozoa and may therefore impair fertility in rats. If the results are applicable to humans, then long term consumption of burantashi may also impair fertility.

KEYWORDS:- Burantashi bark powder, *Pausinystalia yohimbe*, Semen, Testicular,

I. INTRODUCTION

Pausinystalia yohimbe has today become a plant of interest because of its constituent alkaloids (Oliver-Bayer, 1986) of which a major portion is an active compound called yohimbine (Tyler, 1993). It is established that yohimbe extract in sufficient dosages provides comcomitant and adrenoceptor blockage thereby enhancing erections than yohimbine alone (Dhir and Kyulkarni, 2007). In Nigeria, the powder resulting from the grinding the bark of the African tree, *Pausinystalia yohimbe* is called burantashi, a name derived from the Hausa language of the north which literally means " penis, get up" and attempts to explain the effect of the powder to penile erection in men. Burantashi is also a common additive to barbecued meat (suya) in Nigeria where it is commonly consumed.

Pausinystalia yohimbe is an evergreen tree belonging to family Rubiaceae. The plant is native to South, West and Central Africa where it is commonly found in the forest and jungles of Cameroun, Congo, Gabon, Nigeria and Equatorial Guinea (Duke, 1985; en.wikipedia.org/wiki/Pausinystalia). The tree usually grows up to 30 meters high and possesses a heavily fissured grey-brown coloured bark usually spotted with lichen. The erect stems branch extensively, with ovate or elliptical leaves. Apart from the popular use of Burantashi to boost erection, it has also been used traditionally as tonic for the management of exhaustion, chest pain, skin disorders and inflammations (en.wikipedia.org/wiki/Pausinsystalia).

The widespread consumption of burantashi and the fact that infertility among men and women has become a global challenge, call for the scientific evaluation of this popular meat additive, so as to effectively evaluate its impact on reproductive functions. The focus in this current paper is semen quality. This work was therefore designed to evaluate the effects of Pausinystalia yohimbe bar extract (burantashi) on semen quality in adult male albino rats.

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II. MATERIALS AND METHODS

2.1 *Collection of plant stem*

Pausinystalia yohimbe stem bark (burantashi) was obtained from a local herbal practitioner in Lafia, Nasarawa state and was authenticated at the department of forestry, college of Natural Resources and Environmental Management, Michael Okpara University of Agriculture, Umudike. A voucher number MOUAU/CVM/VPP/14/028 was assigned to the sample which was thereafter deposited at the departmental herbarium. The collected stem bark was dried under shade for 14 days and was thereafter ground into powder using an electric powered locally fabricated mill.

2.2 *Preparation of extract and bark powder*

Fifty (50) grams of this powdered material was introduced into the extraction chamber of the soxhlet extractor and extraction was done using methanol as solvent. Extraction temperature was maintained at 70^0C for 48 hours. At the end of the period, the ethanol was evaporated at low temperature in an electric oven to obtain a crude extract which weighed 8.75g and represented a yield of 17.50%. and was hereafter referred to as burantashi ethanol extract (BEE) The remainder (Larger portion) of the powdered material hereafter referred to as burantashi bark powder (BBP) was preserved dried for mixture with feed at various concentrations.

2.3 *Animals*

A total of forty eight (48) adult male rats and twenty five mice (30 – 40g) obtained from the Animal production unit of the College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, were used for the study. The animals were housed under specific pathogen free (SPF) conditions one in a metabolic cage with 13 H/11 H light/dark schedule and were provided standard feed and water *ad libitum*. Experiments were conducted in compliance with NIH guidelines for Care and Use of Laboratory Animals (Pub. No. 85-23, Revised 1985), as reported by Akah *et al.*, (2009). The study was carried out in the Physiology Laboratory of the Department of Physiology and Pharmacology, Michael Okpara University of Agriculture, Umudike.

2.4 *Acute Toxicity (LD_{50}) Determination*

An initial pilot LD_{50} was carried out on five mice to ascertain the dose design to be used for the acute toxicity determination. Twenty five mice were then divided into five groups of five mice each and each group was assigned a particular dose level of BEE. Groups 1, 2, 3, 4 and 5 received Intraperitoneal administrations of 250, 500, 1000, 2000 and 3000mg/kg body weights respectively. The mice were thereafter returned to their cages, allowed free access to feed and water and were monitored for signs of toxicity including deaths within a period of 24 hours. Acute toxicity value was then determined by Karber's arithmetic method described by Enegide *et al*., (2013).

2.5 *Effects of Burantashi (BBP) on Semen Quality and Quantity in Rats*

Forty eight adult male rats were divided into 6 groups of 8 rats and were given varying concentrations of BBP in normal feed following the order:

Group A: Normal feed and water and served as the control
Group B: 2% BBP plus feed combination and water
Group C: 4% BBP plus feed combination and water
Group D: 6% BBP plus feed combination and water
Group E: 8% BBP plus feed combination and water
Group F: 10% BBP plus feed combination and water

The animals were allowed to feed freely on these diets for a period of thirty (30) days. At the end of the period, the animals were sacrificed for collection of semen (from vas deferens/epididymis) for determination of semen parameters. Body and testicular weights were also determined using a compact electronic scale (model EL20001, China).

2.6 Semen Analysis

2.6.1 *Sperm motility*

A drop of semen was placed on a glass slide and 9.2% sodium citrate was added drop wise. A cover slide was then placed on the smear of the diluted semen and viewed under a microscope at a magnification of x40. Only sperm cells moving in straight forward direction were included in the motility count, while those moving in circles, backward direction or showing pendular movements were excluded. The motility was then scored as percentage.

2.6.2 ***Sperm morphology and Live/Dead ratio***

This was carried out following the method reported by Oyeyemi *et al*., (1996). About 2-3 drops of semen sample was placed on a clean glass slide and 1-2 drops of eosin nigrosine stain added and mixed. A thin smear was of the mixture on the slide and viewed for both morphology and estimation of live-dead ratio. The dead sperm cells took up the stain and appeared pinkish or purplish while the live ones did not take up the stain. Live/dead spermatozoa were estimated from 100 sperm cells counted and expressed as percentages.

2.6.3 Testicular volume, Sperm concentration and total counts

Testicular volume was determined by the water displacement method described by Akusu, (1981). For total counts, semen dilution was made in the ratio of 1:200 in dilution fluid. A drop was placed and allowed to run into the counting chamber of the neubauer haemocytometer for counts.

2.7 Statistical analysis

Results were expressed as Means+ standard error of mean (SEM) and analyzed using one way Analysis of variance. P-values less than 0.05 at 95% level of significance were considered significant.

3.0 RESULTS

3.1 ***Acute toxicity***

Deaths were recorded in most groups within 24 hours of acute toxicity study. All animals that died had serious signs of toxicity with aggression, convulsions, lifting of fore limbs followed by fall backwards before eventual death. Deaths were recorded in some groups as shown in the table 1 below:

Table 1:Mortality (LD_{50}) effect of *burantashi* bark extract in mice

Group	Dose Mg/kg	No of Dead mice	% mortality	Dose Difference (Dd)	Mean Death (Md)	DdxMd
1	250	0	0	250	0.5	125
2	500	1	20	500	2	1000
3	1000	3	60	1000	4	4000
4	2000	4	100	1000	4.5	4500
5	3000	5	100	-	-	-

$$LD_{50} = LD_{100} - \frac{\sum (Dd \times Md)}{N}$$

Where LD_{50} = Dose that killed 50% of a given population

LD_{100}= Dose that killed 100% of a given population

$\sum (Dd \times Md)$ = Summation of Dose Difference and mean deaths

N= Number of animals in each group

$$LD_{50} = LD_{100} - \frac{\sum (Dd \times Md)}{N}$$

$$= 3000 - \frac{9625}{5}$$

$$= 3000 - 1925$$

LD_{50} = 1075 mg/kg

3.2 ***Effects of BBP on body weight of the treated rats***

The supplementation of *burantashi* diet was found to increase the net body weight in dose related manner as used in this study as compared to the control in spite of equal food intakes with the group F (10% BBP) recording the highest percentage weight gain of 102.47% and the control group which took no BBP recording the least (Table 2). Gain in weight was significantly improved in all rats treated with BBP (groups B to F) when compared to the control (group A) ($P< 0.05$).

Table 2: Effect of BBP on body weights

Group	Initial weight (g)	Final weight (g)	Percent weight gain
A. CONTROL	191.80 ± 14.82	232.78 ± 16.70	21.78 ± 3.15
B. 2 % BBP	175.29 ± 8.14*	256.69 ± 3.75	48.23 ± 5.66*
C. 4 % BBP	133.83 ± 13.75*	213.99 ± 15.12	64.10 ± 6.72*
D. 6 % BBP	106.16 ± 4.63*	195.64 ± 6.48*	85.23 ± 4.77*
E. 8 % BBP	125.40 ± 7.86*	219.59 ± 9.96	77.24 ± 6.29*
F. 10 % BBP	112.30 ± 9.36*	220.46 ± 9.27	102.47 ± 11.99*

**p<0.05 when compared to control*

3.3 *Effect of BBP on weight of testicular and relative testicular weights*

The weights of the testes and relative testicular weights were also significantly affected by the treatment except for animals in group B. Testicular and relative testicular weights were significantly higher in groups C to F, while that of group B was not statistically significant ($p < 0.05$) when compared to the control (Table 3).

Table 3: Effect of BBP on testicular and relative testicular weights

Group	Weight of testes	Relative testicular weight
A. Control	5.21 ± 0.11	2.29 ± 0.11
B. 2 % BBP	5.60 ± 0.26	2.18 ± 0.10
C. 4 % BBP	5.81 ± 0.41	2.73 ± 0.12*
D. 6 % BBP	6.18 ± 0.26*	3.16 ± 0.11*
E. 8 % BBP	6.81 ± 0.16*	3.13 ± 0.08*
F. 10 % BBP	7.46 ± 0.18*	3.42 ± 0.14*

**p<0.05 when compared to control*

3.4 *Effect of BBP diet on the morphology of sperm cells (Primary and Secondary Sperm Abnormalities)*

Sperm examination showed that the sperm abnormalities (head and tail) were more frequent in all rats treated with BBP than those of the control group. The percentages of occurrence of loose heads were significantly more pronounced at doses of 8% *burantashi* (13.50 ± 3.27) and 10% *burantashi* (15.88 ± 2.60) when compared to the control (3.75 ± 0.77). At 10% *burantashi* diet there was a recognized pattern of occurrences of primary abnormalities notably loose heads, knobbed heads and stumped tails compared to the control ((Table 4)). Statistical analysis showed no particular increase in frequencies of specific primary abnormality in the burantashi treated rats. Sperm shape with bent tail secondary abnormalities significantly increased ($p < 0.05$) at 8% and 10% *burantashi* treated rats while coiled tail secondary abnormalities were statistically significant at doses of 6%, 8% and 10% BBP treatment compared with the control group (Table 5). No significant difference was observed in frequency of occurrence of specific secondary abnormalities.

Table 4 Effect on primary sperm abnormalities

Group	Abnormal head	Loose head	Knobbed head	Stumped tail	Total no of 1° abnormality	% abnormality
A. Control	2.50 ± 0.50	3.75 ± 0.77	0.38 ± 0.26	0.63 ± 0.32	7.25 ± 1.34	7.25 ±1.34
B. 2 % BBP	3.00 ± 0.38	6.38 ± 0.78	0.38 ± 0.26	1.75 ± 0.41	11.50 ± 1.11	11.50 ±1.11
C. 4 % BBP	9.00 ± 0.91*	2.75 ± 0.49	2.13± 0.64*	1.13 ± 0.35	15.00 ± 1.12*	15.00±1.12*
D. 6 % BBP	5.25 ± 0.96*	4.88 ± 0.90	3.75± 0.67*	3.25±0.73*	17.25 ± 1.33*	17.25±1.33*
E. 8 % BBP	3.63 ± 0.71	13.50±3.27*	0.63 ± 0.32	1.38 ± 0.53	19.13 ± 3.40 *	19.13±3.40 *
F. 10 % BBP	3.25 ± 0.53	15.88±2.60*	2.63± 1.07*	5.38±1.74*	27.13 ± 0.67*	27.13± 0.67*

**p<0.05 when compared to control*

Table 5: Effect of BBP on secondary sperm abnormalities

Group	Pro Cyto	Distal Cyto	Bent Tail	Coiled Tail	Looped Tail	Total No Of 2° Abnormality	% 2° Abnormality
A. Control	0.00 ± 0.00	0.50 ± 0.19	2.13±0.48	0.50 ± 0.19	1.00±0.38	4.13±0.77	4.13±0.77
B. 2%BBP	0.00 ± 0.00	1.50 ± 0.42	1.75±0.75	2.13±0.35*	3.75±1.00*	9.50±1.43*	9.50±1.43*
C. 4%BBP	0.00 ± 0.00	3.63±0.63*	2.50±0.33	1.88±0.51	2.00±0.60	10.00±1.39*	10.00±1.39*
D. 6%BBP	0.25±0.16*	1.75±0.49*	3.25±0.59	3.75±0.53*	3.13±0.88*	11.88±0.61*	11.88±0.61*
E. 8%BBP	0.00 ± 0.00	1.00±0.38	4.63±0.73*	3.50±0.82*	2.63±0.63	12.13±0.67*	12.13±0.67*
F.10%BBP	0.13 ± 0.13	1.25±0.31	7.25±0.70*	3.63±0.63*	2.00±0.60	14.13±1.09*	14.13±1.09*

*p<0.05 when compared to control

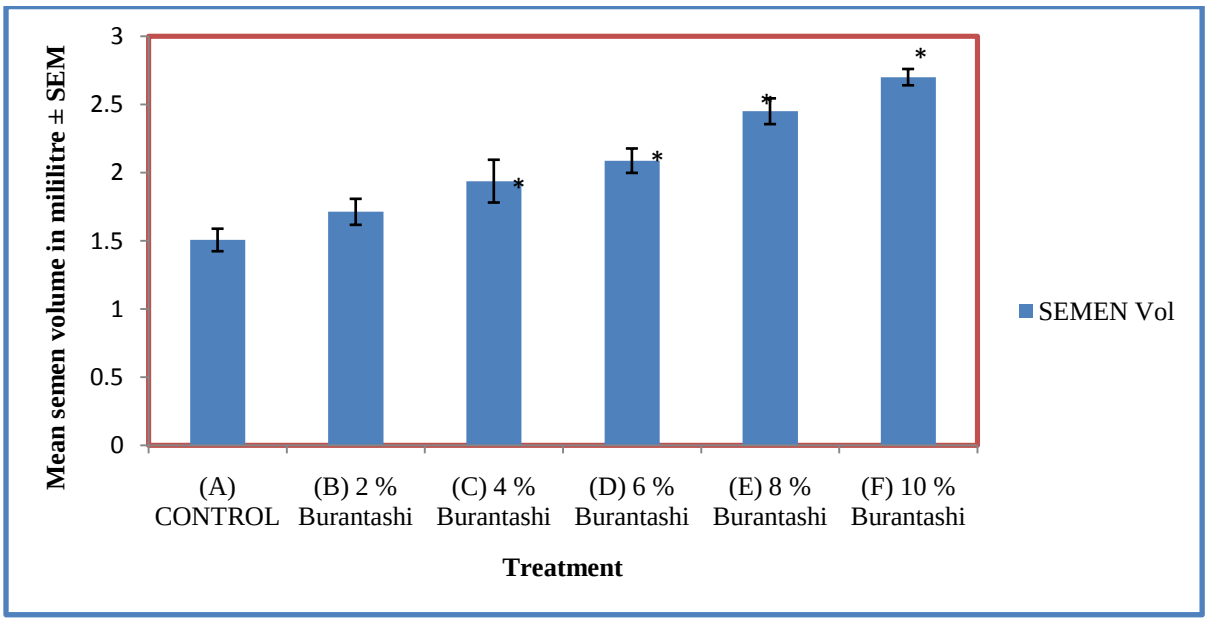
Table 6: Effect on total sperm abnormalities

Group	% total abnormality
A. Control	11.38 ± 1.94
B. 2 % Burantashi	21.00 ± 2.07*
C. 4 % Burantashi	25.00 ± 2.10 *
D. 6 % Burantashi	29.13 ± 1.72*
E. 8 % Burantashi	31.25 ± 3.23*
F. 10 % Burantashi	41.25 ± 0.80*

*p<0.05 when compared to control

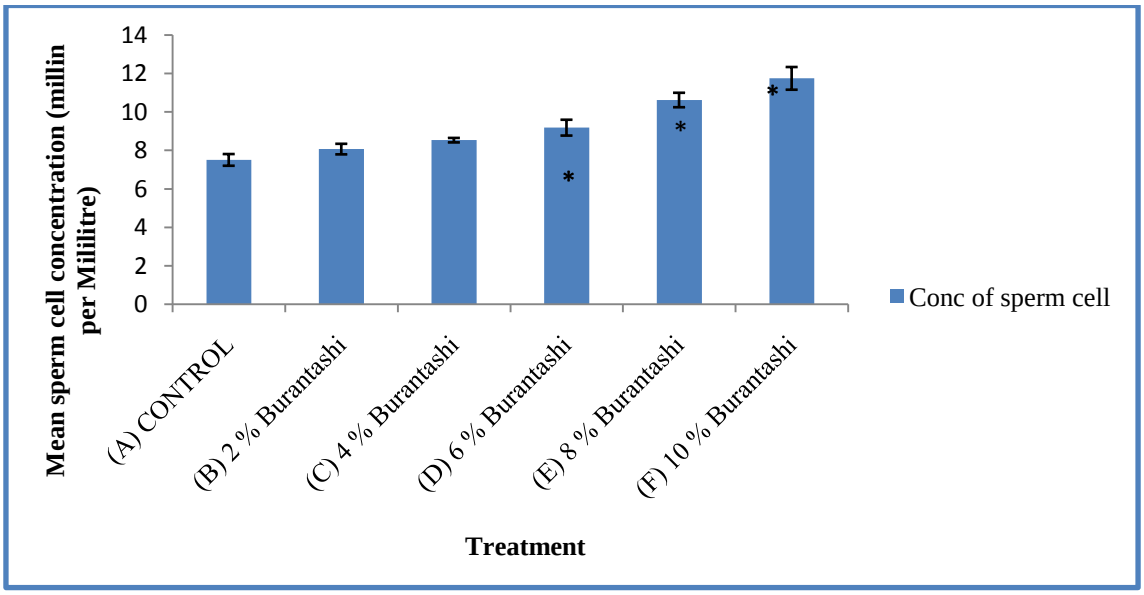
3.5 ***Effect of different doses of buratashi diet on sperm quality; Semen Volume (ml), Sperm Count (million cells/ml) and Sperm Motility (%), Live-dead (%), $_{P}H$ and Liquefaction (mins).***

The **semen volume** followed a similar but well pronounced trend with group F having the significantly highest value, followed by group E, group D and group C (Fig. 1), While that of group B was not significantly (P > 0.05) different from that of the control. Sperm motility followed same trend as the volume (Fig. 3). **Life-Dead Ratio** was significantly higher in groups C, D, E and F (P < 0.05), while that of group B was not significantly different from control (Fig. 4). A less comparable and weaker trend was noted in the **$_{P}H$** (Fig. 5) and **Liquefaction** (Fig. 6), where the pattern of change with the control was found to be non-significant, decreased only in group B compared to control in the parameter of liquefaction.



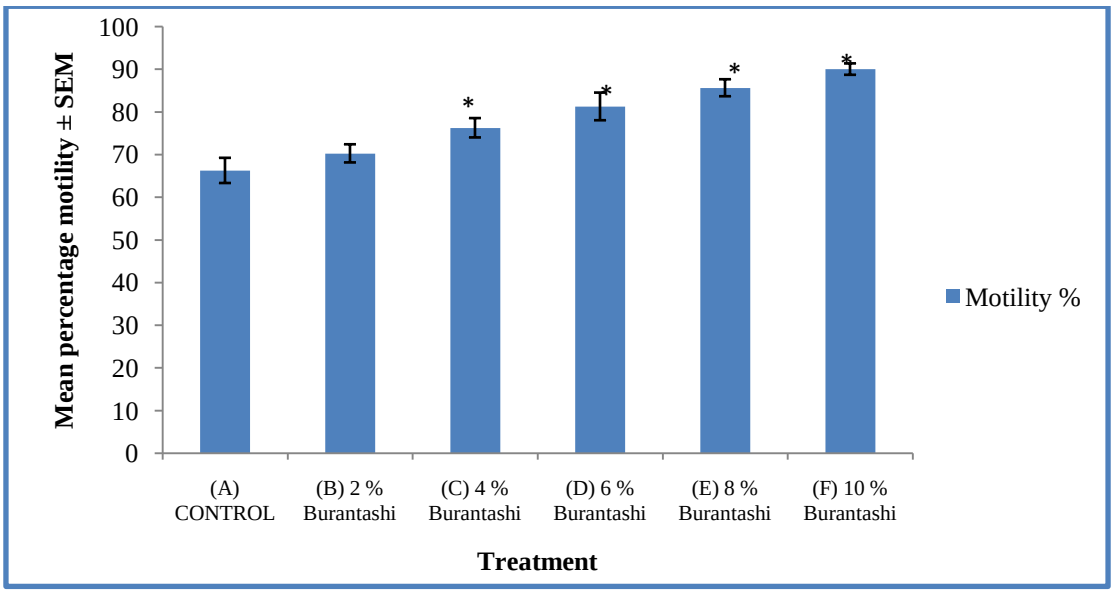
*p<0.05 when compared to the control

Figure 1-Effect of *burantashi* of different doses (0%, 2%, 4%, 6%, 8%and 10%) for 30 days treatment on sperm volume (ml) showing significant increase (p < 0.05) in treatment groups C (1.94 ± 0.16), D (2.09 ± 0.09), E (2.45 ± 0.09), F (2.70 ± 0.06).



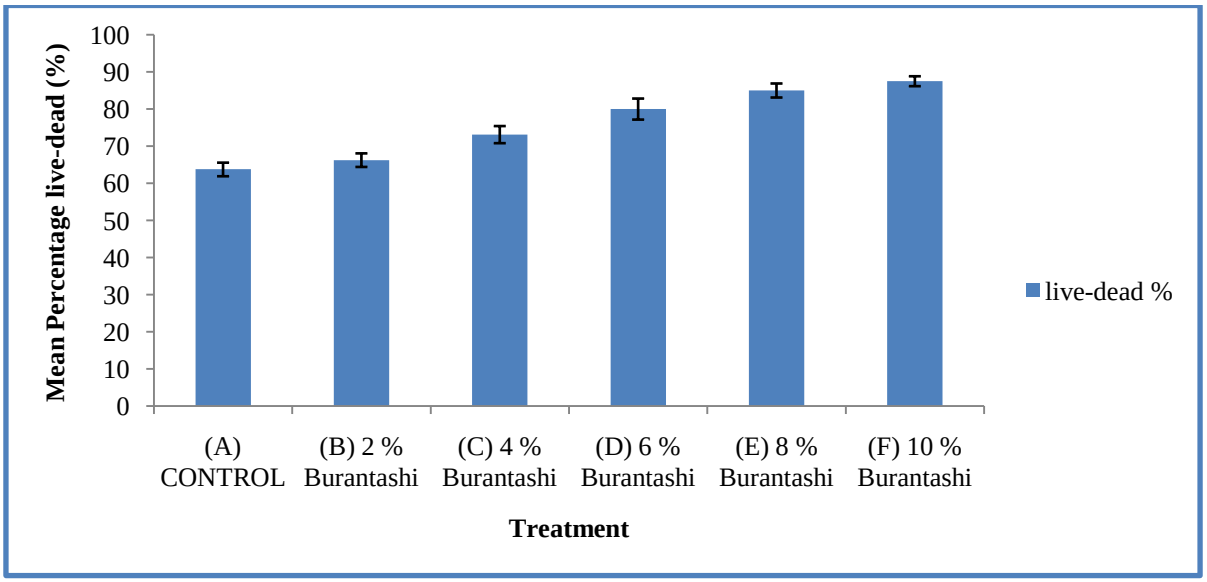
*p<0.05 when compared to the control

Figure 2-Effect of different doses of *burantashi* on semen concentration (million/ml) of treated rats showing statistically increased sperm counts at 6% *burantashi* (9.19 ± 0.41), 8% *burantashi* (10.63 ± 0.38) and 10% *burantashi* (11.75 ± 0.59) when compared to the control (7.51 ± 0.30).



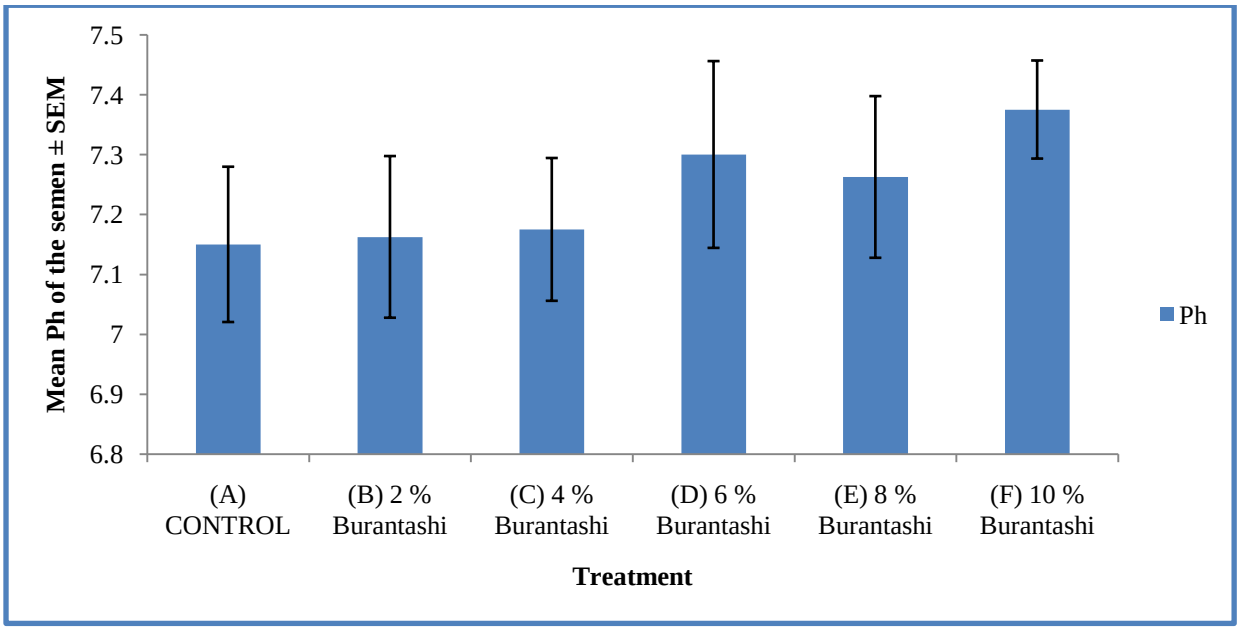
***p<0.05 when compared to the control**

Figure 3-Effect of *burantashi* of different doses (0%, 2%, 4%, 6%, 8%and 10%) for30 days treatment on sperm motility showing significant increase ($p < 0.05$) in treatment groups C (76.25 ± 2.27), D (81.25 ± 3.24), E (85.63 ± 1.99), F (90 ± 1.34).



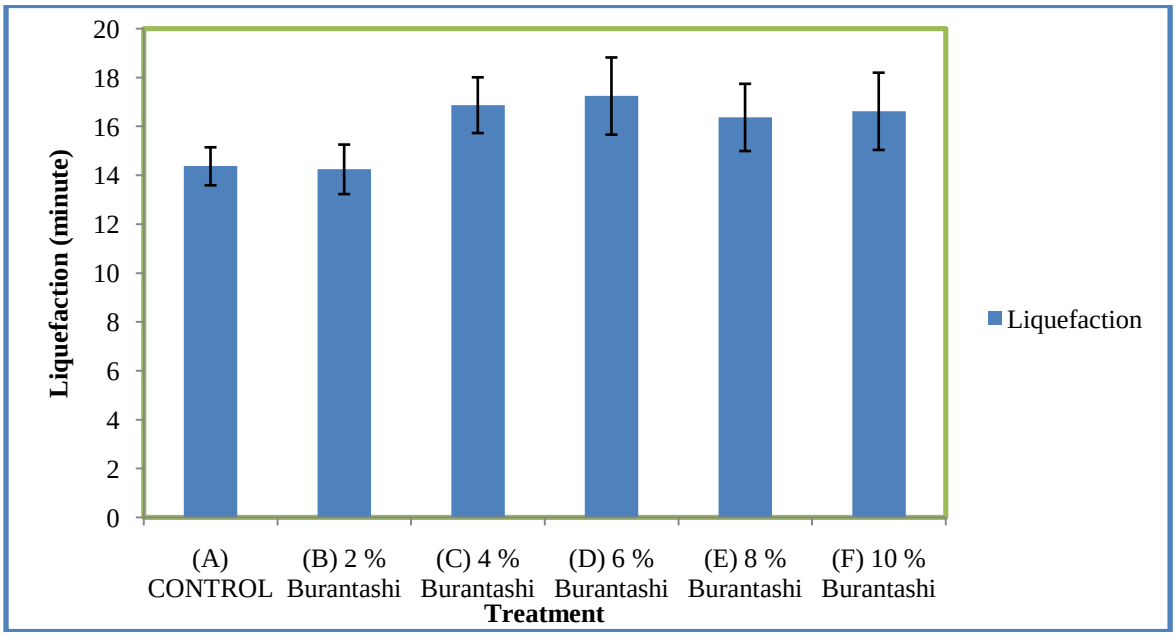
***p<0.05 when compared to the control**

Figure 4-Effect of different doses of *burantashi* on percentage livability of sperm cells of treated rats showing statistically increased live-dead ratio at 4% *burantashi* (73.10 ± 2.30), 6% *burantashi* (80.00 ± 2.83), 8% *burantashi* (85.00 ± 1.89) and 10% *burantashi* (87.50 ± 1.34) when compared to the control (63.75 ± 1.83).



*p<0.05 when compared to the control

Figure 5-Effect of different doses of *burantashi* on semen PH in experimental rats. The values of groups D (7.30± 0.16) and F (7.38 ± 0.08) were higher than the rest of the groups but showed non-significant difference compared with the control group (7.15 ± 0.13).



*p<0.05 when compared to the control

Figure 6-Effect of different doses of *burantashi* on liquefaction of semen (minutes) in experimental rats. The values of groups C (16.88 ± 1.14) and D (17.25± 1.58) were higher than the rest of the groups but showed non-significant difference compared with the control group (14.38 ± 0.78).

IV. DISCUSSION

Administration of BBE induced some level of toxicity and caused death of some mice within few hours of administration, while chronic consumption of BBP increased semen volume and sperm motility, concentration, liveability, primary and secondary liveability but did not significantly affect PH and liquefaction. The toxicity level revealed by the LD50 value (1075mg/kg) of Pausinystalia yohimbe bark extract is indicatiove of some degree of toxicity associated with the use of the extract and could be as a result of high amounts in the extract of bioactive substances including yohimbine (Tyler, 1993), alkal;ids, flavonoids, terpenoids, resins, tannins and saponins (Okonkwo, 2012), some of which have been implicated in toxicity when taken beyond tolerable limits. Burantashi has been reported to contain the alkaloid yohimbine, which at high doses produces serious toxicity signs and death due to its ability to excessively raise the mean arterial blood pressure of treated animals (Ajayi *et al*., 2003). The implication of this is that excessive and constant consumption of burantashi can be toxic to the consumers and may contribute to the increasing number of persons with high blood pressure in Nigeria.

The observed increase in testicular weights after chronic consumption of BBP in the experimental rats suggests that BBP may have increased the number of germ cells of the germinal epithelia and serum levels of testosterone. Increase in germ cells and rise in testosterone level are reported to be strongly associated with increase in testicular weight. Testosterone is responsible for the growth, structural integrity and functional activities of sex organs (Takashi *et al*., 1982). BBP may have induced more sperm production, accounting for the observed rise in testicular weights and the increase in sperm counts and semen concentrations in the treated rats. The relationships between testicular weights, testosterone levels and sperm concentration have been reported (Skinner, 1975; Guyton and Hall, 1996). The unidirectional and progressive movement of sperm cells in all treated animals is suggestive of high amounts of protein and fat in BBP and agrees with Oyeyemi *et al*., (1998), who reported that adequate nutrition with high percentage of protein increases motility of sperm cells and semen concentration, since spermatozoa having more energy will move more rapidly with vigor within any medium and for a long time.

Long term consumption of BBP did not significantly affect semen PH and Liquefaction (time for the gel of semen to become more liquid) as these parameters did not significantly differ from the control and fall within the normal values as prescribed by WHO (7.2 – 7.8 for PH and 20 minutes for liquefaction). Metabolic activities in the body result in either acidosis or alkalosis which ultimately affects the PH of the body. It is likely therefore that BBP did not affect the body metabolism of the treated rats in such a manner that could lead to acidosis or alkalosis. The increase in both primary and secondary sperm abnormalities in BBP treated rats,

particularly in group F (treated with 10%) suggests that high doses of BBP may affect negatively the functional integrity of the testes leading to increase in number of abnormal sperm cells and may be a good indicator of the dysfunction at the testicular level and may further give information on maturation arrest which is associated with shedding of germ cells from spermatogonia to spermatocytes and spermatids in semen as reported by Ariagno *et al*., (2002).

In conclusion, the use of burantashi for cases of erectile dysfunction though has no obvious deleterious effects on semen quality and quantity; it however does increase the percentage of abnormal and immature spermatozoa and may therefore impair fertility in rats. If the result is applicable to humans, then long term consumption of burantashi may also impair fertility. The relationships between this current work, hormonal profile and histological changes in male rats treated with BBP shall be adequately elucidated in subsequent publications.

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