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Comprehensive Journal of Medical Sciences Vol. 3(1), pp. 20-27, December 2016.
ISSN-2135-6848
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RESEARCH ARTICLE

ACUTE TOXICITY STUDY AND HORMONE PROFILE ENHANCING ACTIVITY OF *Pausinystalia yohimbe* BARK POWDER (BURANTASHI) IN INVIVO EXPERIMENTAL ANIMAL MODELS

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Accepted April 2016.

The acute toxicity and effects on hormone profile and testicular weights of *Pausinystalia yohimbe*, Burantashi bark powder (BBP) was evaluated in groups of adult mice assigned varying doses of BBP extract and 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 an acute toxicity value of 1075mg/kg body weight with deaths occurring in some groups of the treated mice. Significant (P<0.05) and dose dependent increase in testicular weights and hormone levels were also observed with 10% burantashi-feed combination raising testosterone, follicle stimulating hormone and luteinizing hormone levels from 5.25±0.27mg/ml, 0.77±0.03mIU/ml, and 9.38±0.58mIU/ml respectively in the control to 9.40±0.54ng/ml, 1.41±0.02mIU/ml and 21.19±1.13mIU/ml respectively. Similar results were also obtained following treatment with lower concentrations of burantashi except for follicle stimulating hormone which was not significantly affected. In conclusion, the results suggest that burantashi could be lethal at doses beyond 1000mg/kg but may be used are low to moderate doses to enhance reproductive activities in males since the functional integrity of the male reproductive organs depends on adequate bioavailability of testosterone and other male hormones.

KEY WORDS: Burantashi Bark Powder, Follicle Stimulating hormone, Luteinizing hormone, Testosterone

INTRODUCTION

Current global statistics reveal decline in sperm counts and cases of infertility amongst many males (Li et al., 1991, Swan et al., 1997), a problem which is attributable to lifestyle and diets (Mitchellet al., 2001, Hammoud, et al., 2008), use of antibiotics (Whan et al., 2006), and selective serotonin reuptake inhibitors such as citalopram, escitalopram, fluoxetine (Safarinejad, 2008), and various chemicals (Mortimer, 2013). For reasons of cost, effectiveness and accessibility, there seem to be growing dependence on alternative medicines involving the exploitation of plants to treat infertility cases in males. Several medicinal plants have scientifically been studied for their anti-infertility potentials to verify local claims and also as deliberate efforts at sourcing for new agents with better activities and less or no side effects. Some of the medicinal plants that have shown positive activities in the management of infertility in males include *Panax ginseng*, *Panax quinquefolius* and *Lepidium meyenii* which improved sexual desire and sustained libido. Other plants which were found to treat infertility via improving sperm parameters include *Astragalus racemous*, *Withinia somnifera* and *Acanthopanax senticosus*. *Pausinystalia yohimbe* is one the medicinal plants currently under study and is reported to be of value in the management of sexual dysfunction (Dhir and Kulkarni, 2007).

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*). *Pausinystalia yohimbe* usually grows up to 30 meters high and possesses a heavily fissured grey-brown colored bark usually spotted with lichen. The erect stems branch extensively, with ovate or elliptical leaves. The tree is popularly known to contain yohimbine, an alkaloid which has been extensively used for sexual erectile dysfunctions (Tyler, 1993). The bark extract has also been used traditionally as tonic for the management of exhaustion, chest pain, skin disorders and inflammations (en.wikipedia.org/wiki/Pausinsystalia).The bark contains active compounds which are alkaloids (Oliver-Beyer, 1986). A major portion of the alkaloids is the active compound called yohimbine, which is also known by other names such as aphrodine, quebrachine or corynine (Tyler, 1993). Yohimbine is widely distributed over the counter as an herbal aphrodisiac. Yohimbe plant does not just contain yohimbine but also 55 other alkaloids and accounts for 1-20% of total alkaloids among which is corynanthine, an adrenergic blocker (Doxey et al., 1984). The use of yohimbe extract in sufficient doses may provide concomitant adrenoceptor blockage, enhancing erections in the process in a manner better than yohimbine alone (Dhir and Kulkarni, 2007).

In our previous study, we reported that the use of burantashi for cases of erectile dysfunction though had no obvious deleterious effects on semen quality and quantity; it however increased the percentage of abnormal and immature spermatozoa and may therefore impair fertility in rats (Ogwo et al., 2015). This current was designed to investigate the effect of the agent on hormone profile and testicular to body weight ratios of male albino rats with a view to complementing previous work and also provide the much needed data on the activity of the extract on hormone profile.

MATERIALS AND METHODS

Collection of plant stem and preparation of bark powder

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. The resulting powdered material hereafter referred to as BBP was preserved dried for mixture with feed at various concentrations.

Animals

A total of twenty five and forty eight (48) adult male rats 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). While the mice were used for acute toxicity study, the rats were rats were used for the hormone profile study. The study was carried out in the Physiology Laboratory of the Department of Physiology and Pharmacology, Michael Okpara University of Agriculture, Umudike.

Effect of chronic consumption of BBP on the hormone profile of male rats

The forty eight adult male rats were divided into 6 groups of 8 rats each and were given diets containing varying concentrations of BBP in 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 to collect blood into plain tubes. Sera obtained from the clotted blood were assayed for their hormone content. Testosterone, Luteinizing hormone (LH) and Follicle stimulating hormone (FSH) assays were done using enzyme immune assay kits, following procedures outlined by the commercial kit producer, Syndro Biosearch Inc. California, USA. Body and testicular weights were also determined using a compact electronic scale (model EL20001, China).

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.

RESULTS

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	45000
5	3000	5	100	-	-	-

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

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

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

× (Dd x Md) = Summation of Dose Difference and mean deaths
N= Number of animals in each group

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

=3000 ï 9625
5

= 3000 ï 1925

LD_{50} = 1075 mg/kg

Effects of BBP on body and testicular weights of 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 6 (10% BBP) recording the highest percentage weight gain of 102.47% and the control group which took no BBP recording the least (Table 1). Gain in weight was significantly improved in all rats treated with BBP (groups 2 to 6) when compared to the control (group 1) ($P< 0.05$). Weights of testes and relative testicular weights were also significantly ($p< 0.05$) increased (Table 2 Table 1: Effect of BBP on body weights

Effect of BBP on Testosterone levels of treated rats

The results recorded in figure 1, showed that there is a pronounced increase in the testosterone levels of experimental groups when compared with control however this increase is markedly significant ($p<0.05$)just after 2% burantashi treatment (6.49 ± 0.42ng/ml).The serum testosterone level was dose dependent with the control having the lowest value (5.25 ± 0.27ng/ml), followed by Group B (6.49 ± 0.42ng/ml), Group C (6.91 ± 0.33ng/ml), Group D (7.93 ± 0.47ng/ml), Group E (8.61 ± 0.47ng/ml) and Group F (9.40 ± 0.54ng/ml).

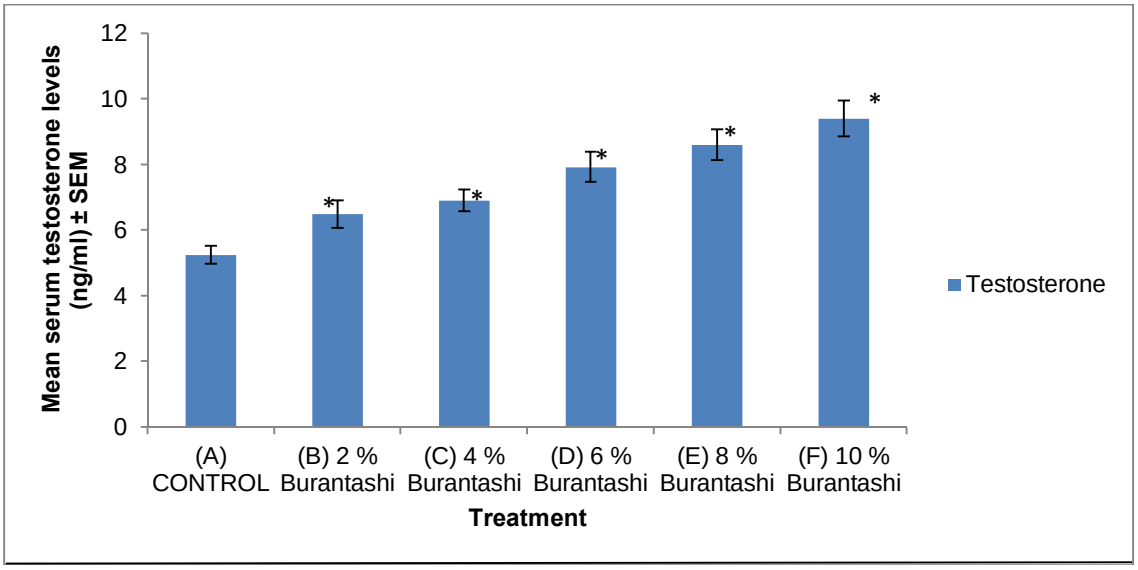
Group	Initial weight (g)	Final weight (g)	Percent weight gain
A. CONTROL	191.80 ± 14.82	232.78 ± 16.70	21.78 ± 3.15
B. 2 % BBP	175.29 ± 8.14*	256.69 ± 3.75	48.23 ± 5.66*
C. 4 % BBP	133.83 ± 13.75*	213.99 ± 15.12	64.10 ± 6.72*
D. 6 % BBP	106.16 ± 4.63*	195.64 ± 6.48*	85.23 ± 4.77*
E. 8 % BBP	125.40 ± 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*

Table 2: Effect of BBP on testicular and relative testicular weights

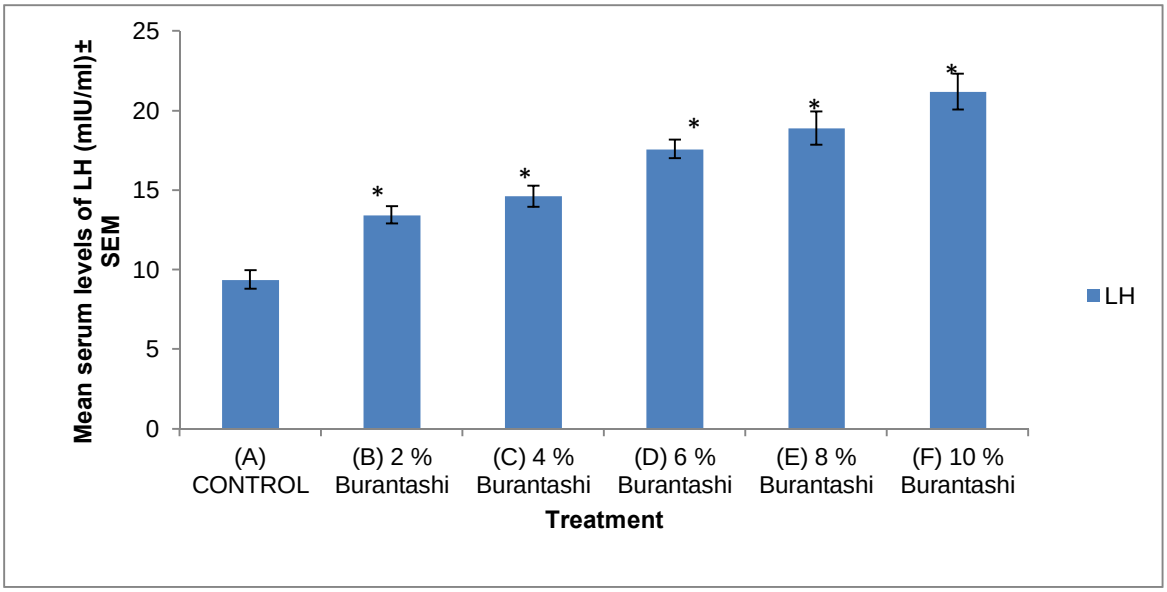
Group	Weight of testes	Relative testicular weight
A. Control	5.21 ± 0.11	2.29 ± 0.11
B. 2 % BBP	5.60 ± 0.26	2.18 ± 0.10
C. 4 % BBP	5.81 ± 0.41	2.73 ± 0.12*
D. 6 % BBP	6.18 ± 0.26*	3.16 ± 0.11*
E. 8 % BBP	6.81 ± 0.16*	3.13 ± 0.08*
F. 10 % BBP	7.46 ± 0.18*	3.42 ± 0.14*

**p<0.05 when compared to control*

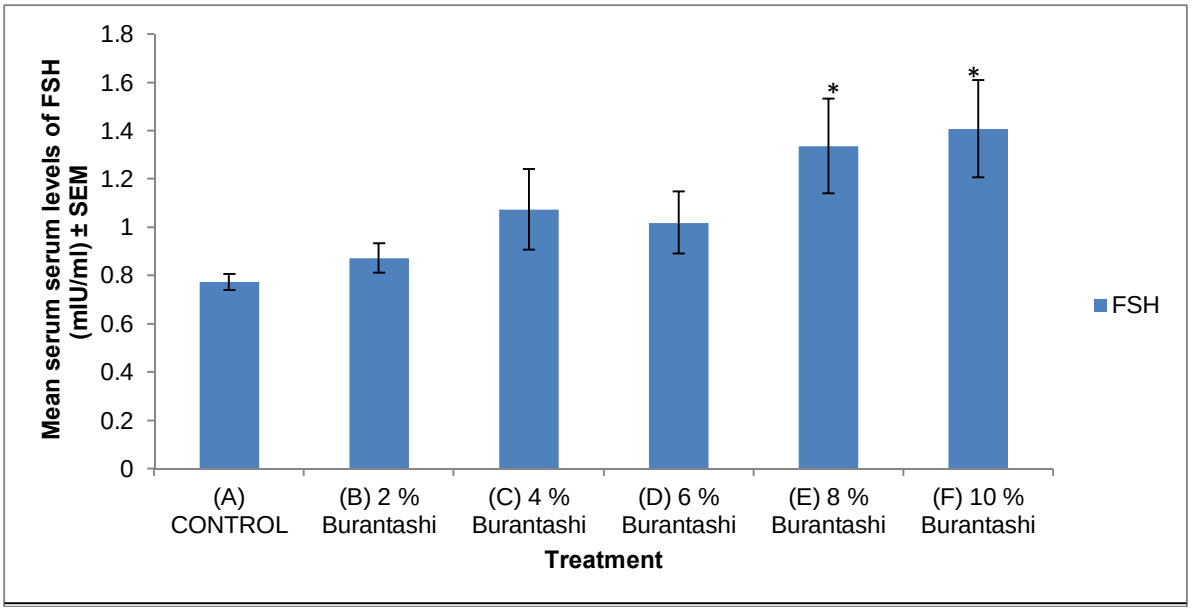


P values < 0.05 when compared to the control

Figure 1- Effects of burantashi of different doses for 30 days of oral administration on serum testosterone level of adult male rats. (Values are Mean ± S.E.M, n= 8 in each group).



P values < 0.05 when compared to the control



P values <0.05 when compared to the control

Effect of BBP on luteinizing hormone levels of treated rats

Serum luteinizing hormone levels also followed similar trend as testosterone increasing significantly with increasing doses of BBP. Groups B, C, D, E and F had values13.43 ± 0.55mIU/ml, 14.62 ± 0.65mIU/ml,

17.58 ± 0.59mIU/ml, 18.89 ± 1.03mIU/ml and 21.19 ± 1.13mIU/ml respectively) when compared to the control group A which had 9.38 ± 0.58mIU/ml (Fig. 2).
Figure 2- Effects of burantashi of different doses for 30 days of oral administration on serum LH levels of adult male rats. (Values are Mean ± S.E.M, n= 8 in each group).

Effect of BBP on Follicle Stimulating Hormone levels of treated rats

Treatment with BBP did not significantly affect the Follicle Stimulating Hormone (FSH) levels in all experimental groups at lower doses with groups B, C and D having 0.87± 0.06mIU/ml, 1.07± 0.17mIU/ml and 1.02± 0.30mIU/ml respectively. However at 8% and 10% treatments, FSH levels of 1.34 ± 0.20mIU/ml and 1.44 ± 0.02mIU/ml were observed and were quite significant when compared to the control (0.77 ± 0.03mIU/ml) (Fig. 3)
Figure 3- Effects of burantashi of different doses for 30 days of oral administration on serum FSH level of adult male rats. (Values are Mean ± S.E.M, n= 8 in each group).

DISCUSSION

Acute toxicity of BBP and its effects on body weights and testosterone, luteinizing and Follicle stimulating hormones concentrations in experimental male albino rats were studied and result obtained revealed an acute toxicity value of 1075mg/kg and a dose dependent increase in hormone profile following thirty days treatment. Intraperitoneal administration of BBP extract at doses up to 1075mg/kg was observed to cause toxicity in the animals used. The animals manifested toxicity signs in the form of tremor, restlessness, convulsions and eventual deaths. This suggests that BBP may contain toxic phytochemical agents which may be present 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). This finding is in line with the OECD guideline for acute toxicity studies. OECD (2001), had reported that mortality is the expected end point of acute toxicity and non observation of mortality within a population treated with a dose range at which mortality is expected indicates tolerance or lack of acute toxicity. In this case however, deaths were recorded in various study groups, suggesting presence of toxicity.
Although the exact mechanism by which BBP increases body weight requires further evaluation, results from this work suggest possible stimulation of the building up of more protein molecules, evidenced by its ability to enhance testicular and body weights. Okonkwo, (2012) had demonstrated direct effect of burantashi on protein components in the body. The observed increase in body weight may also be as a result of the rise in testosterone level following treatment with BBP. Testosterone has been implicated in the growth of testicular tissues (Takahashi et al., 1982) and as an anabolic steroid hormone, favors the development of more muscular tissues which directly enhances body weight.

The fact that BBP raised testosterone levels in all treated rats gives credence to the local use of the substance for the treatment of erectile dysfunction and to enhance sexual performance. Testosterone is reported to be a major player in the enhancement of sexual drive (Guyton and Hall, 1996). It is reported that BBP in sufficient dosage provides concomitant and adrenoceptor blockage thereby enhancing erections (Dhir and Kyulkarni, 2007). The rise in testosterone, follicle stimulating hormone (FSH) and luteinizing hormone (LH) levels in all experimental rats following 30 days administration of various concentrations of BBP suggests that BBP increases spermatogenesis and may be of value in raising sperm counts in males. Testosterone acts together with FSH to regulate spermatogenesis. FSH binds to specific receptors attached to sertoli cells in the seminiferous tubules causing these cells to grow and secrete various spermatogenic substances (Guyton and Hall, 1996). The exact mechanism by which BBP increases male reproductive hormones is not quite understood, however the presence of flavonoid in BBP (Okonkwo, 2012) may offer some explanation. Flavonoid is reported to enhance reproductive functions in males and has been used to treat male reproductive problems (Yuan et al., 2014). In a similar work

carried out by (Da- Nian et al., 2000), flavonoid was found to significantly increase the testosterone, FSH and LH levels, testicular weights and other reproductive indices in all treated male rats. These results obtained for BBP suggest fertility enhancing property. Similar conclusion was drawn on the effects of *Fumaria parviflora* leaves extract on reproductive parameters in adult male rats (Mehran et al., 2013).

In conclusion, the use of burantashi may be of value in the management of erectile dysfunction and may stimulate more production of sperm cells having been found to enhance both testicular weights and male reproductive hormones in male albino rats

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