SUB-ACUTE HEPATOTOXICITY OF PAUSINYSTALIA YOHIMBE BARK EXTRACT (BURANTASHI) IN MALE ALBINO RATS (RATTUS NOVERGICUS)

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ABSTRACT

Background: Burantashi (Yohimbe in English) (Pausinystalia yohimbe = Corynanthe yohimbe) (Family Rubiaceae) is a true aphrodisiac used as a possible treatment for organic, psychogenic and substance induced erectile impotence and other male sexual dysfunctions. It increases blood flow to erectile tissues and exerts its erectogenic effects through a central action and peripheral autonomic nervous system effects.

Aim: This study was carried out to examine the hepatotoxicity and cellular deleterious effects of Pausinystalia yohimbe bark ethanolic extract used as burantashi at varying concentrations.

Materials and Methods: This study was experimental in design following 30 days oral administration of burantashi at 2 days intervals in 30 male albino rats (Rattus novergicus), with an average weight of 202.26g and were assigned to 5 groups of 6 rats per cage. The rats were housed in a well-ventilated wire-bottom steel cage with ambient temperature of 27±2°C. Food and water were provided ad libitum. All rats were sacrificed by Chloroform (anesthetization), excised organs were grossed and cut at 3-5mm, while 3-5µm sections were obtained using the rotary microtome and subsequently stained with Mayer’s haematoxylin and eosin method to demonstrate the general tissue structure of the liver in particular and some selected internal organs. Sections were examined using the light microscope.

Results: The results showed a progressive reduction in body weight of rats treated with P. yohimbe bark extract. Gross examination revealed that liver weight (LW) was significantly (p<0.05) reduced in a dose-related manner. Hepatocellular injury as a result of dose-related hepatotoxicity was observed in rats treated with higher doses. Meanwhile, selected organs (pancreas, spleen, testes, lungs and heart) showed no alterations in their weights and the histological sections taken from the were normal limits.

Conclusion: This study suggests that indiscriminate consumption of burantashi for a long duration may exert similar hepatotoxic effects in man.

Keywords: Burantashi, hepatotoxicity, impotence, Yohimbine and P. yohimbe
INTRODUCTION

Most sufferers of impotence or erectile dysfunction (ED) tend to make use of sex enhancers in order to augment their sexual ability. One of such locally prepared sex enhancers known in Nigeria is burantashi. It is locally produced from an evergreen forest tree that is native to the South Western Nigeria as well as Cameroon, Gabon and the Congo Republic. Botanically, this plant is known as (Pausinystalia yohimbæ = Corynanthe yohimbæ) (Family, Rubiaceae). The dried stem bark is widely used in North Eastern Nigeria for the treatment of ED and as an aphrodisiac. Yohimbine is the principal indole alkaloid derived from the bark of the Yohimbe tree. Yohimbine is also an appetite suppressant, and decreases food intake in both lean and obese mice. The stem bark extract of this plant is called burantashi in Hausa, apidogun in Yoruba and yohimbe in English language. Detailed information about the plant has been documented.

Burantashi is a true aphrodisiac used as a possible treatment for organic, psychogenic and substance induced erectile impotence and other male sexual dysfunctions. It increases blood flow to erectile tissues and may increase testosterone levels. Currently, it is assumed that yohimbine (the active ingredient in burantashi) exerts its erectogenic effects through a central action while Kaplan et al. suggested that its effect on male sexual performance is possibly related to its peripheral autonomic nervous system effects. Furthermore, yohimbine has only a modest effect on psychogenic erectile dysfunction and none on organic erectile dysfunction. In response to the foregoing, it is important to examine the side effects that may be associated with the indiscriminate consumption of burantashi used as treatment option, which is always available as a self-prescribing herbal therapy without a safe-dose regimen and may be without a standard preparatory procedure. Conversely, in view of the multifunctional morphological makeup of the liver, it becomes paramount to critically examine this special and important organ that is constantly exposed to indiscriminate consumption of herbal preparation almost on a daily basis.

It is imperative to mention that there is a dearth or no reliable clinical studies available to guide the administration of yohimbine bark used as burantashi for the purpose of sexual enhancement and performance. Again, there is a necessity for the present study as there appears to be paucity of documented information especially on hepatotoxic and cellular deleterious effects of burantashi on the liver and some selected organs. Therefore, this study was carried out to examine the histological effects of oral administration of P. yohimbæ bark ethanolic extracts (burantashi) on the liver of albino rats at varying concentrations.

MATERIALS AND METHODS

This study was conducted at the Department of Medical Laboratory Science, School of Basic Medical Sciences and Department of Pharmacognosy, Faculty of Pharmacy, University of Benin, Benin City, Nigeria between August and October, 2014.

Plant Collection and Extraction

Preparation and extraction method by Odigie and Odigie was adapted in the present study, with some modification. The dried chopped pieces of P. yohimbæ bark were obtained from Hausa quarters at Aduwawa, Benin City, Nigeria. It was authenticated at the Faculty of Pharmacy, University of Benin, Benin City. The plant barks were pulverized into coarse form using a household mortar and pestle. About 482.8g of the powdered particles were soaked in 1L of 99% ethanol. The mixture was left on laboratory bench for 24hrs with occasional agitation. The mixture was later extracted using a soxhlet-extractor to obtain the ethanolic extract. The extract was concentrated over a water bath at a temperature range of 45°C to 50°C to obtain 36.6g ethanol extract. The dried powdered extract was kept in an amber glass container in the laboratory for experimental use.

Experimental animals

Thirty inbred male albino rats with mean weight of 202.26g were housed in a well-ventilated wire-bottom steel cage with ambient humidity and temperature of 27±2°C with 12 hr light/dark cycle. The rats had been selectively assigned according to age and weight to 5 groups of 6 rats per cage at the animal house of the Faculty of Pharmacy, University of Benin, Nigeria and were allowed to acclimatize for a period of 2 weeks. They were maintained on rat chow and water ad libitum. The animal study was used in accordance with National Institute of Health (NIH) Guide for the care and use of laboratory Animals.
Experimental design

Following a pilot study and based on the indiscriminate consumption of burantashi in Nigeria, dose regimens were chosen and extrapolated
developed by Ajiboso et al.
for the present study. The method described by Ajiboso et al. was used to determine body weights of experimental rats. Meanwhile, Odigie and Odigie method was used for physical measurement according to behavioral signs of acute toxicity by close monitoring and observation. A hand towel was used to pick the rats one at a time while oral cannula was used to administer appropriate concentrations of the extract to the rats in group (B, C, D and E) in the order of 100mg/ ml, 200mg/ ml, 300mg/ ml and 400mg/ ml by oral administration for 30 days at 2 days intervals, while group A served as the untreated group. At termination, the rats were anaesthetized using cotton wool soaked in chloroform vapour. The liver and some selected organs (pancreas, spleen, testes, lungs and heart) were excised and grossed. They were cut at 3-5mm and processed histologically by the automatic tissue processor (Heston ATP7000 tissue processor-Germany). Thereafter, 3-5µm of sections were obtained using digital rotary microtome (Heston ERM 4000 Germany). Mayer’s haematoxylin and eosin stained sections were examined by the Swift® binocular microscope® (Olympus England) with an in built lighting system at x10 and 40 magnifications. Hepatotoxicity scoring was based on the severity of hepatocellular damage observed while the average score for hepatocellular indices was derived as a result of double blind reported scores from two or more pathologists in agreement.

Statistical Analysis

Data was presented in Means ± S.E.M and analyzed using one way ANOVA and student’s t-test. Significance was determined at p <0.05 using Statistical Package for Social Sciences (SPSS) version 18.0 (Inc Chicago, Illinois, USA).

RESULTS

There was a significant reduction observed in organ weight (liver) of the high dose treated rats (group D and E) that was thought to be dose-related (Table 1). Histology sections of the treated rat’s liver revealed a dose-dependent hepatocellular injury as a result of increase in sinusoid, distortion of kupffer cells and vacuolation of the central vein (Figure 1).

<table>
<thead>
<tr>
<th>Organ</th>
<th>Untreated A</th>
<th>Test B</th>
<th>Test C</th>
<th>Test D</th>
<th>Test E</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0mg/ ml</td>
<td>100mg/ ml</td>
<td>200mg/ ml</td>
<td>300mg/ ml</td>
<td>400mg/ ml</td>
</tr>
<tr>
<td>Liver</td>
<td>7.20 ± 0.10</td>
<td>6.80 ± 0.20</td>
<td>6.20 ± 0.22</td>
<td>5.82 ± 0.26</td>
<td>5.22 ± 0.21</td>
</tr>
<tr>
<td>Pancreas</td>
<td>0.60 ± 0.18</td>
<td>0.61 ± 0.11</td>
<td>0.60 ± 0.24</td>
<td>0.62 ± 0.21</td>
<td>0.62 ± 0.26</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.68 ± 0.32</td>
<td>0.64 ± 0.42</td>
<td>0.68 ± 0.12</td>
<td>0.72 ± 0.04</td>
<td>0.78 ± 0.16</td>
</tr>
<tr>
<td>Testes</td>
<td>1.62 ± 0.14</td>
<td>1.62 ± 0.24</td>
<td>1.64 ± 0.40</td>
<td>1.64 ± 0.44</td>
<td>1.62 ± 1.41</td>
</tr>
<tr>
<td>Lung</td>
<td>1.06 ± 0.12</td>
<td>1.00 ± 0.21</td>
<td>1.02 ± 0.22</td>
<td>1.12 ± 0.26</td>
<td>1.24 ± 0.64</td>
</tr>
<tr>
<td>Heart</td>
<td>0.52 ± 0.12</td>
<td>0.48 ± 0.18</td>
<td>0.50 ± 0.22</td>
<td>0.54 ± 0.66</td>
<td>0.58 ± 0.61</td>
</tr>
</tbody>
</table>

Values were represented as mean ± S.E.M of 6 replicates organs; values in parenthesis indicates gross reduction of organ weights with respect to the untreated organs (p< 0.05).
Fig. 1: Revealed the following results represented on plates $A_1$ and $A_2$ to $E_1$ and $E_2$

**Plate 1:** Showed untreated rat liver section ($A_1$ and $A_2$) with normal cyto-architecture which composed of portal triad surrounded by hepatocytes and sinusoids.

**Plate 2:** Mild dose ($B_1$ and $B_2 = 100\text{mg/ml}$) showed negligible signs of hepatotoxic effects with vacuolated central vein.

**Plate 3:** High dose ($200\text{mg/ml}$) showed portal congestion and dilatation, activation of peri-portal lymphocytes and vacuolated central vein in high doses.

**Plate 4:** Section of the high dose ($300\text{mg/ml}$) showed portal congestion, peri-portal lymphocytosis and kupffer cells activation.

**Plate 5:** Section of the high dose ($E_1$ and $E_2 = 400\text{mg/ml}$) showed enlarged hepatocyte, kupffer cells activation and piece meal of lysed red blood cells within the vacuolated central vein with compressed or congested sinusoid (Mayer's H&E x 400 and PAS X 400)
However, histological appearances of some selected organs (pancreas, spleen, testes, lungs and heart) were within normal limits when compared with the untreated group (Table 1). In addition, (Table 2) showed hepatotoxicity analysis of *P. yohimbe* at varying concentrations treated in male albino rats. Although, varying concentration of burantashi (100mg/ml to 400mg/ml) had no significant effect on selected organ weight (pancreas, spleen, testes, lungs and heart) (p< 0.05); while hepatocellular effect of ethanolic extract of burantashi on organ and body weight in adult male albino rats showed that oral administration of the extract at a dose of 300mg/ml and 400mg/ml (group D and E) for 30 days at 2 days intervals significantly reduced the liver and body weight in experimental rats (Table 3).

Although, varying concentration of burantashi (100mg/ml to 400mg/ml) had no significant effect on selected organ weight (pancreas, spleen, testes, lungs and heart) (p< 0.05); while hepatocellular effect of ethanolic extract of burantashi on organ and body weight in adult male albino rats

<table>
<thead>
<tr>
<th>Cage</th>
<th>Dose in mg/kg</th>
<th>Effect on the sinusoid</th>
<th>Inflammatory/ cellular infiltration</th>
<th>Inflammatory cell/ distortion of bile duct</th>
<th>Hepatocellular necrosis/ enlarged hepatocyte</th>
<th>Congested/ Distorted/ enlarged central vein</th>
<th>Vacuolation of the central vein</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>B</td>
<td>100</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>C</td>
<td>200</td>
<td>±</td>
<td>-</td>
<td>-</td>
<td>±</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>D</td>
<td>300</td>
<td>±</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>E</td>
<td>400</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>++</td>
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</tbody>
</table>
showed that oral administration of the extract at a dose of 300mg/ml and 400mg/ml (group D and E) for 30 days at 2 days intervals significantly reduced the liver and body weight in experimental rats (Table 3). These findings are in agreement with the observations by which reductions in the number of renal corpuscle in the kidneys of rats in a similar study were prominent. In addition, Eweka et al. reported that
degenerative and atrophic changes were observed in the kidneys of rats that received the higher doses of the ground stem bark of 
Pausinystalia yohimbe
Similarly, greater hepatotoxicity was observed in the high dose treated animals in the present study thereby suggesting some level of similarity in the results obtained in Eweka et al. though from different organs in the present study.

### Table 3: Empirical and physical measurement of male albino rats treated with oral administration of burantashi for 30 days at 2 days intervals

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose in mg/kg body weight in rats</th>
<th>Mean weight before administration of burantashi</th>
<th>Mean weight after administration of burantashi</th>
<th>Physical Weight loss / gain</th>
<th>Activities / or dullnes</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0</td>
<td>170.33 ± 2.1</td>
<td>172.10 ± 1.8</td>
<td>↑</td>
<td>-</td>
</tr>
<tr>
<td>B</td>
<td>100</td>
<td>190.00 ± 2.3</td>
<td>189.23 ± 3.1</td>
<td>↓</td>
<td>±</td>
</tr>
<tr>
<td>C</td>
<td>200</td>
<td>193.32 ± 1.8</td>
<td>190.56 ± 2.7</td>
<td>↓</td>
<td>±</td>
</tr>
<tr>
<td>D</td>
<td>300</td>
<td>200.64 ± 1.2</td>
<td>193.32 ± 1.1</td>
<td>✈</td>
<td>+</td>
</tr>
<tr>
<td>E</td>
<td>400</td>
<td>220.16 ± 1.4</td>
<td>211.12 ± 1.5</td>
<td>✈</td>
<td>++</td>
</tr>
</tbody>
</table>

**Key**

↑ → Slight increase in weight  
↓ → Slight weight loss  
⇑ → Marked increase in weight  
⇓ → Severe weight loss  
+ → Presence of features  
± → Intermediate features  
++ → Marked presence of features  
- → Absence of features

### DISCUSSION AND CONCLUSION

Gross abnormalities and histopathological changes were not observed in selected organs (pancreas, spleen, testes, lungs and heart) of albino rats investigated. However, the results obtained from Mayer's haematoxylin and eosin stained liver sections showed that oral administration of 
Pausinystalia yohimbe bark extract (burantashi) resulted in varying degrees of cyto-architectural distortion of the hepatic cells when compared to the untreated sections (Figure 1).
Therefore, this study suggests that *Pausinystalia yohimbe* bark ethanolic extract may be safe on the following organs: pancreas, spleen, testes, lungs and heart at oral doses ranging from 100mg/ml to 400mg/ml but could be hepatotoxic at the same dose and concentration leading to distortion of cyto-architecture of the liver. Nonetheless, further studies are necessary to corroborate these findings.

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