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Effect of calabash chalk geophagy on the epididymis of adult male Wistar rats

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Abstract

Context: Calabash chalk is also known as poto in English. It is a naturally occurring mineral and is chiefly composed of fossilized seashells as constituent. It is traditionally consumed by communities for pleasure and also constitutes major remedy for morning sickness in pregnancy.
Objective: This study is aimed at determining the effect of calabash chalk on the body weight and epididymis of adult male Wistar rats.
Materials and method: Twenty (20) rats weighing between 180 and 200g were divided into four groups and five rats were assigned to each (A-D). Group A served as control, animals in this group were given rats feed and water only. Group B animals were administered 100mg/kg of dissolved calabash chalk daily. Group C animals were administered 200mg/kg of dissolved calabash chalk. Group D animals were administered 400mg/kg of dissolved calabash chalk. All experimental animals were allowed free access to regular feed and water and were sacrificed on the 56th day of research. Epididymal tissues were harvested for assessment of sperm parameters and histological processing.
Results: The results show no statistical significant differences (P>0.05) in the sperm parameters across all groups. Also, there were no significant changes in the contents and histoarchitecture of the epididymes of the treatment groups when compared with the control group.
Conclusion: We could conclude that Calabash Chalk has no deleterious effects on the sperm quality and histoarchitecture of the epididymis of adult Wistar rats.

Keywords: Calabash chalk, geophagy, epididymis histoarchitecture, sperm quality

Introduction

Calabash chalk is also known as poto in English, nzu by the Igbos, ndom by the Efiks/Ibibios of Nigeria, and Mabele by the Lingala of Congo.[1] It is available in difference sizes with varying mineral compositions in different regions. The commonest type consumed in Africa contains essential elements like as phosphorus, potassium, magnesium, copper, zinc, manganese, and iron in various proportion as demonstrated by Ekong et al.[2] Geophagia is a common practice associated with religious beliefs as part of a normal diet.[3,4] An examination had shown that geophagia is not restricted to any age group, race, sex, regions, but highly prevalence among the world's poorer and tribally oriented people.[5] Calabash chalk is a natural substance derived from fossilized sea shells but can be manufactured artificially by heating the mixture of clay, sand, wood ash and salt.[1]

Most Africans irrespective of gender and ages ingest calabash chalk for the purpose of recreation also, most pregnant women claimed that it reduces vomiting, over-salivation, and nausea during pregnancy.[3] It is believed to negatively affect hemoglobin concentrations, red blood cell counts, erythrocyte sedimentation rate, platelet count in

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Wistar rats.[6] According to Moses et, al calabash chalk causes some gastrointestinal ailments such as nausea, ulcers, and gastritis due to histomorphological changes in the stomach and esophagus caused by calabash.[7]

Calabash chalk considered to be harmful in women of reproductive age as it causes histological abnormalities in the rats' uterus by affecting growth rate and create histological alterations in the uterus such as chronic endometriosis, endometrial hyperplasia and presence of inflammatory cells, the hyperplastic and metaplastic effect could be due to the presence of lead in calabash chalk.[8]

Materials and methods

Experimental animals: Twenty Wistar rats weighing between 180g and 200g were procured and housed in the Department of Anatomy Animal House unit for a period of two weeks for acclimatization. They were kept in clean, well-ventilated aluminium cage at room temperature and they were fed a conventional laboratory feed and water liberally.

Experimental design: The twenty (20) rats were assigned into four groups, each with five animals. Group A served as the control group and the animals were given regular feed and water. Group B served as low dose group and the animals were given 100mg/kg of calabash chalk, feed and water. Group C served as medium dose group and it animals received 200mg/kg of calabash chalk, feed and water. Group D served as the high dose group and the animals were administered with 400mg/kg of calabash chalk, feed and water.

Sacrifice of animals: At the end of the research, all twenty rats were weighed and sacrificed under chloroform anaesthesia. Thereafter, a ventral abdominal wall incision was made and developed into the peritoneal cavity and extended to the groin to expose testes and epididymes. In preparation for regular histology procedures, epididymes were removed, weighed, and preserved in Bouin solution. Bouin solution is a preparation of 5% acetic acid, 9% formaldehyde, and 1.5% picric acid in aqueous solution.

Histological Analysis: The tissues were dehydrated in ascending grades of alcohol (ethanol), cleared in xylene and embedded in paraffin wax. The deparaffinised sections were stained routinely with Haematoxylin and Eosin and digital photomicrographs of the tissue sections were taken at various magnifications.

Statistical Analysis: Data were analyzed using descriptive and inferential statistics. All values were presented as the standard error of mean (SEM) for five rats each of the four groups. The significance of the difference in the means of all parameters were determined using one-way analysis of variance (ANOVA; 95% confidence interval). All statistical analyses were carried out using Statistical Package for Social Sciences (SPSS) software (V20, USA).

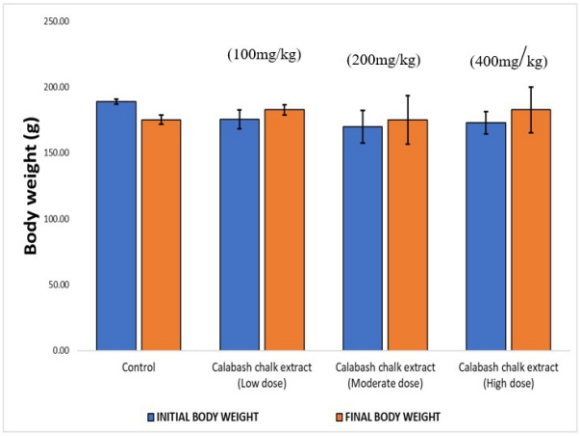

Figure 1: Chart showing the comparison between the initial and final body weight

There were no statistical significant differences (P>0.05) of body weights in all the groups, when the initial body weights were compared to the final body weights.

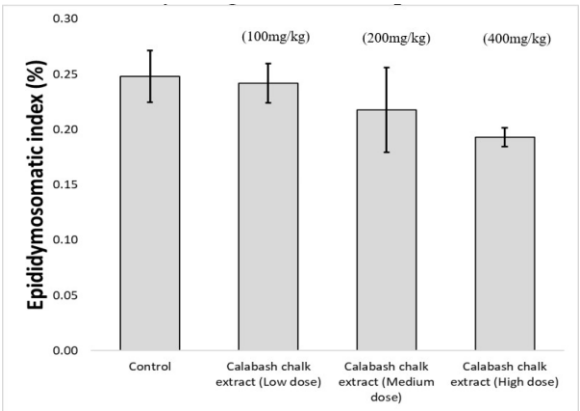

Figure 2: Chart showing the Epididymosomatic index of the different groups

There were no statistically significant differences (P>0.05) in epididymosomatic index across the groups.

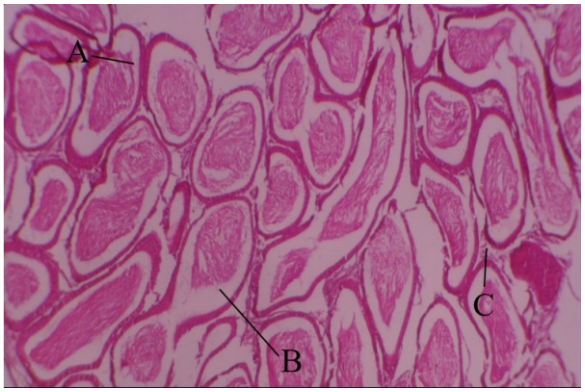

Plate 1. Control Rat epididymis. Composed of normal epididymal architecture:
A. ductules lined by columnar epithelium, filled with B. spermatozoa, C. interstitial space (H&E x 40)

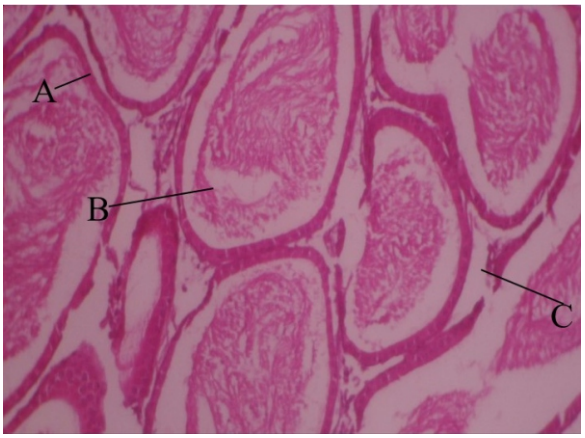

Plate 2. Control epididymis. Composed of normal epididymal architecture:
A. ductules lined by columnar epithelium, filled with B. spermatozoa, C. interstitial space (H&E x 100)

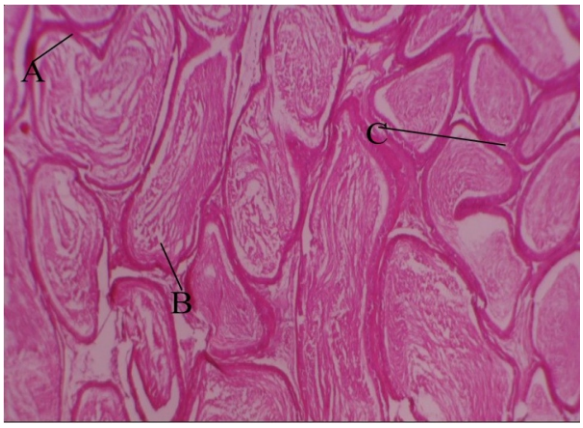

Plate 3. Rat epididymis given low dose Calabash Stone showing normal architecture:
A. ductule, remarkably filled with B. spermatozoa, C. interstitial space (H&E x 40)

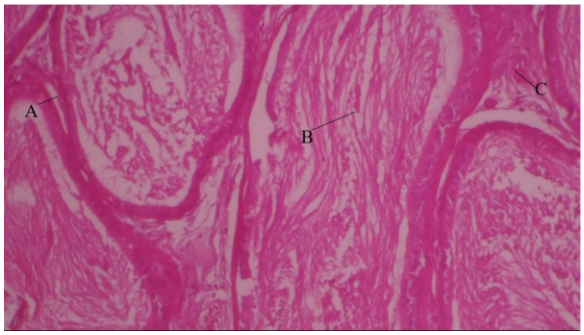

Plate 4. Rat epididymis given low dose Calabash Stone showing normal architecture:
A. ductule, remarkably filled with B. spermatozoa, C. active interstitial congestion (H&E x 100)

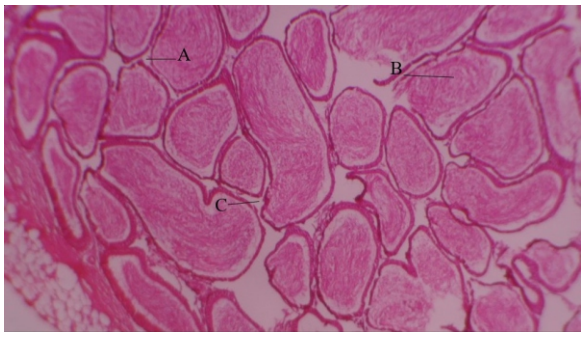

Plate 5. Rat epididymis given medium dose Calabash Stone showing normal architecture:
A. ductule, remarkably filled with B. spermatozoa, C. interstitial space (H&E x 40)

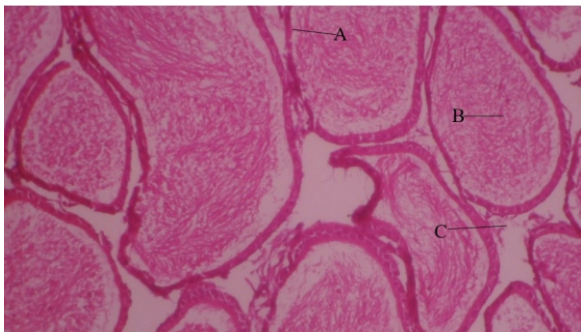

Plate 6. Rat epididymis given medium dose Calabash Stone showing normal architecture:
A. ductule, remarkably filled with B. spermatozoa, C. interstitial space (H&E x 100)

Result

The results obtained from the experiment are shown below. Figures 1 is the chart showing changes in the body weight of rats before and after treatment while figure 2 showed the result of the epidimosomatic index of the treated and the control rat groups.

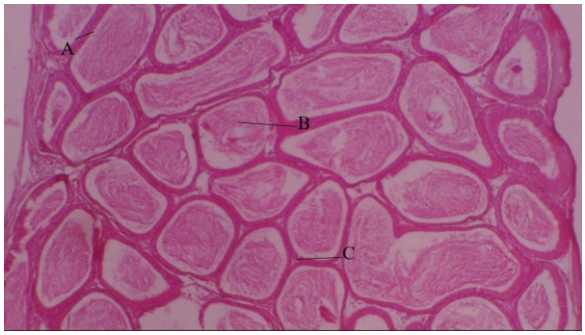

Plate 7. Rat epididymis given high dose Calabash Stone showing normal architecture:
A. ductule, remarkably filled with B. spermatozoa, C. interstitial space (H&E x 40)

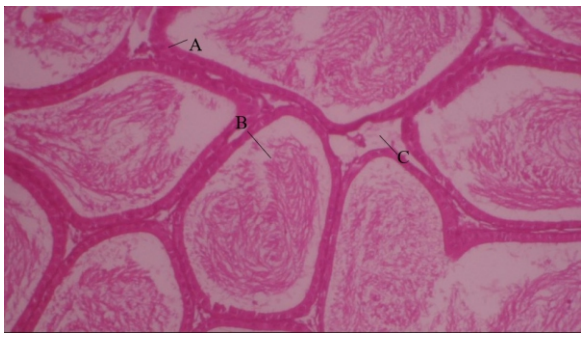

Plate 8. Rat epididymis given high dose Calabash Stone showing normal architecture:
A. ductule, remarkably filled with B. spermatozoa, C. interstitial space (H&E x 100)

Similarly, plates 1 to 8 showed the photomicrographs rats epididymes across all groups at x40& x100 magnifications.

Discussion

The epididymis is a crucial part of the male reproductive system where spermatozoa are matured and stored there until they are released into the vas deferens. Organ weight changes are vital parameter for evaluating experimental animals as such changes often indicate one of the early signs of toxicity.[9] In this study, there was no significant difference in the mean body weight and epididymosomatic indices of the test group compared with the control group. Although calabash chalk is known to contain different element of which some are harmful to health, the result of this study did not show significant difference in the body or organ weight of treated animals when compared with the control. The lack of difference in the mean body weight and epididymosomatic indices indicates lack of, or insignificant effect of the calabash chalk on the epididymis and body weight. It could also be asserted that the concentration of harmful elements in the chalk may not be significant to the extent of being injurious to the body, this might also be related to the chemical composition of soil where the chalk was harvested. This contradict earlier result by Ekong et al., who asserted that Calabash chalk is a common kaolin clay that contains major pollutants such as chromium, nickel, titanium, rubidium, lead, tin, and arsenic acid which are hazardous to the human body and should be avoided at all cost.[10] The lack of appreciable weight gain could be due to the low nutritive values of the chalk as previous work by Trckova et al., has shown that stoppage of calabash chalk ingestion restores body weight in young piglets that were earlier treated with calabash chalk.[11] This is however different from the assertion made by Ekanem et, al., who reported that consumption of calabash chalk causes increase in mean body weight of experimental animals.[12]

Observations from histological results show no changes in epididymal architecture, as the epithelial lining of the epididymes remained intact in all the treatment and the control groups. Also, the contents of the epididymes were remarkably filled with spermatozoa across all groups of the experimental animals.

Conclusion

The findings of this study suggest that calabash chalk does not cause any significant changes in body weight, the nutritive value may be low as there were no significant weight gain at the end of the research across the groups of experiment. Also, there were no alteration in the epididymal lining and contents of the Wistar rats as the histology of the epididymes were not distorted at the end.

Limitation

Finance was the major limitation during the course of this study. Secondly, Inadequate facility and dearth of man-power which necessitated the processing of the tissues in University of Benin Teaching Hospital.

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Conflict of interest
There is no conflict of interest.

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