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The Effects of Calabash Chalk on the Female Reproductive Organs: An In-Vivo Study

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ABSTRACT

The present study investigated the effects of Calabash Chalk on the histoarchitecture of the ovary and the uterus of adult wistar rats. Twenty one female wistar rats weighing between 180-200g were used for this study. All the animals were housed properly and fed with feed and water *ad libitum*. Daily vaginal smear analysis was done on all the rats for three consecutive estrous cycles to ascertain the state of the estrous cycle of each rat so as to ensure that they were all at the same phase (proestrous) of the estrous cycle before administration commenced on each rat. The rats were divided into three Groups: Group A (control) was given distilled water, groups B and C were given 500mg/kg and 1000mg/kg body weight of Calabash chalk respectively orally for fourteen days. On day 14, the animals were euthanized by cervical dislocation and the ovary and uterus were excised and taken for histopathological examination. The results revealed that there was no appreciable change in mean weight across the treatment groups ($p<0.05$). The histological examination of groups B and C showed normal histology of the ovaries. Also there was no noticeable change in the histological features of the uterus. The findings suggest that Calabash chalk has no adverse effect on the histology of the ovary and uterus.

Keywords: Calabash Chalk, Ovaries, Geophagy, Histology, Vaginal smear

INTRODUCTION

Geophagia, a term used interchangeably with Geophagy is the practice of earth eating or eating soil-like substrates like clay, chalk etc. it is seen among animals and in some humans It occurs in non-human animals where it may be a normal or abnormal behaviour, and also in humans, most often in rural or preindustrial societies among children and pregnant women.[1,2] Geophagia is a common practice seen almost all over the world in various cultures.[3] The practice of geophagy has been reported earlier among native Americans[3] and among the Otomacs tribe.[4] Africa has not been left out in these practices of soil eating as reported in Gabon, Cameroun and Equitorial Guinea where "Calaba" as its popularly known in those areas was consumed for pleasure or to suppress hunger.[5,6] Reports have also revealed that most men who ate clay did so because they believed it increased sexual prowess, while some females claimed that eating clay helped pregnant women to deliver with ease.[7] In Haiti, geophagia was often seen among the poor who made pastries from soil, salt, and vegetable shortening and consumed to keep soul and body together even though it had little or no nutritional value.[8] However, long-term eating of those locally made delicacies have been reported to cause stomach pains and malnutrition.[9] In Nigeria, the most consumed geophagic material is Calabash chalk also known as calabash stones or calabash clay in English. It is called nzu in Igbo, ndom among the Efiks/Ibibios and eko among the Edos of Nigeria. Calabash chalk is a naturally occurring substance made up of fossilized sea shells but is also artificially constituted. This is achieved using a combination of clay and mud, with other ingredients such as sand, wood ash and sometimes salt. These substances are mixed together, moulded and passed through heat in order to get a solid form. It is available in different forms such as moulds, powder, and blocks.[10]

The major component of calabash chalk is aluminum silicate hydroxide [$Al_2 Si_2 O_5 OH_4$ (5)] which comes from the kaolin clay group. It is known to have a very high concentration of lead and other constituents such as aluminum, persistent organic pollutants, silicon, alphalindane, endrin, endosulfan2, arsenic and chromium.[11] These substances have been reported to be

harmful to the body depending on their bioavailability.[10-14] Calabash chalk geophagy cuts across both gender and different ages. However, it is mostly seen among women especially during pregnancy, with the claim that it prevents nausea and excessive salivation as well as vomiting [15]. Calabash chalk is also used in making facial masks and soaps. [16] In this study, the effects of Calabash Chalk on the histoarchitecture of the ovary and the uterus of adult wistar rats were investigated.

MATERIALS AND METHODS

Collection and Preparation of Sample: Calabash chalk was obtained from a local market in Elele, Rivers State, Nigeria. The sample was oven-dried at 110°C and crushed to powdered form using a mortar and pestle and stored until use. The sample was dissolved in distilled water and administered during the experiment.

Acute toxicity study (determination of median lethal dose) of Calabash chalk: The rats were divided into three groups (n=3) and fasted overnight after which three-graded doses of Calabash chalk (1000, 2500, and 5000 mg/kg) were administered to them orally using a gavage. They were closely observed for behavioral changes, mortality and other signs of toxicity for 24 hours (Lorke, 1983).

Study design: Twenty one (21) sexually mature female Wistar rats weighing between 180–200 g were used for the study. The animals were housed in a cross-ventilated room under standard conditions of temperature and illumination (temperature 25±2.0°c, 12hr light /12hr dark cycle) and were fed with standard rat feed. Animals were handled in accordance with international guidelines and experimental procedures. Animals were divided into three groups of seven rats each. Rats in Group A were given distilled water (1 ml) and served as control. Groups B and C were administered Calabash chalk (500 and 1000 mg/kg respectively) once daily. The sample was administered orally by oral gavage for 14 days and laparotomized.

Determination of Estrous cycle: Estrous phase was determined by evaluation of vaginal smear as described by Marcondes *et al.*[17]

Statistical analysis: Data were expressed as a mean ± standard error of mean. Data were analyzed by one-way analysis of variance comparison between control and the treated groups using SPSS. Values of $P < 0.05$ were considered statistically significant.

RESULTS

Acute toxicity study (determination of median lethal dose): No cases of mortality were observed after treatment with Calabash chalk, even at the concentration of 5000 mg/kg. Also, there were no observable behavioral changes or signs of toxicity in all the animals (Table 1). Hence, lethal dose (LD_{50}) is >5,000 mg/kg in rats.

Table 1: Oral treatment with Calabash Chalk over 24 hr had no Toxic Effect in the Rats

Dose (mg/kg)	No of Mortality	Observed Behavio ral change	Other toxicity signs
1000	0	Nil	Nil
2500	0	Nil	Nil
5000	0	Nil	Nil

*n=3

Table 2: Effect of Calabash Chalk on the Body Weight of Female Wistar Rats

GROUPS (n=5)	Initial weight(g)	Final weight (g)
A (Control)	162.6±10.72	176.0±15.09
B1(500g of Calabash chalk)	155.3±7.42	162.6±7.68
B2(1000g of Calabash chalk)	153.3±3.52	164.0 ± 4.0

The effects of calabash chalk on the histology of the uterus and ovary revealed no appreciable change in the endometrial lining and glands of uterus, and follicular and stroma cells of ovary, as illustrated in Plate 1 – 6 below

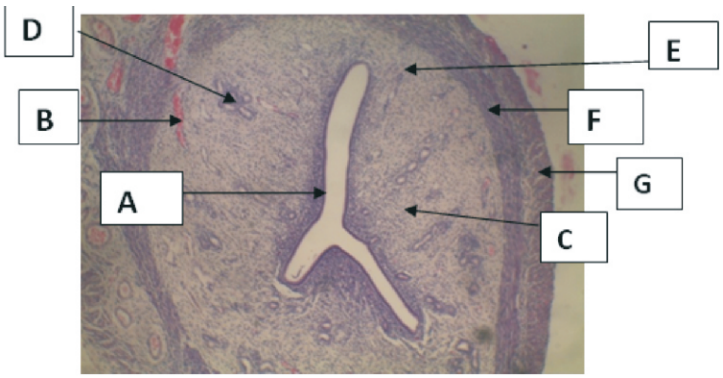

Plate 1: Photomicrograph of the uterus showing a section of Group A (control) (H&E x125). Showing(A)simple cuboidal cells lining the endometrium,(B)blood vessels are seen,(C)stroma with stromal cells,(D)endometrial glands,(E)endometrium,(F)myometrium,(G)perimetrium ,it is in diestrous stage .

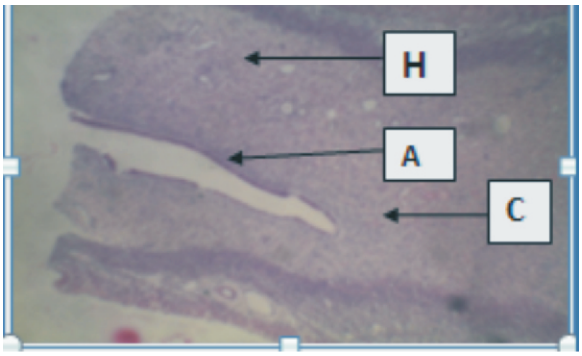

Plate 2: Photomicrograph of the uterus showing a section of Group B treated with 500mg/kg of calabash chalk (H&E x125). Showing (A)simple cuboidal cells lining the endometrium,(C) stroma (H) myometrium consists of inner circular and outer longitudinal layer, it is in diestrous stage .

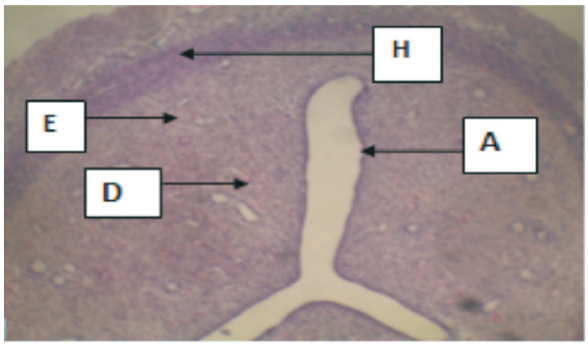

Plate 3: **P**hotomicrograph of the uterus showing a section of Group C treated with 1000g/kg of calabash chalk (H&E x125). **S**howing (A)simple cuboidal cells lining the endometrium,(D) endometrial gland, (E) endometrium, it is diestrous stage (H) myometrium consisting of inner circular and outer longitudinal layer.

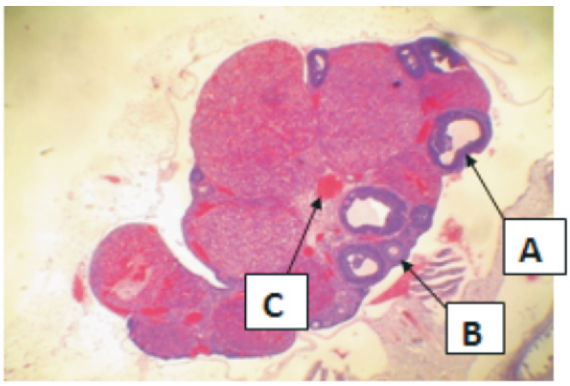

Plate4: Photomicrograph of the ovary showing a section of Group A (control) (H&E x125). (A) collapsing graffian follicle after ovulation,(B)growing follicle,(C) ovarian stroma containing large blood vessels engorged with blood.

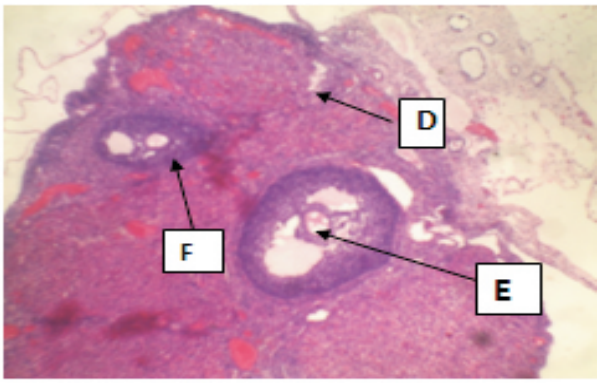

Plate 5: Photomicrograph of the ovary showing a section of Group B treated with 500mg/kg of calabash chalk (H&E x125).showing(D) atretic graffian follicle, (E)maturing follicle, mural granulosa cells are dying off (F)graffian follicle in the process of becoming atretic.

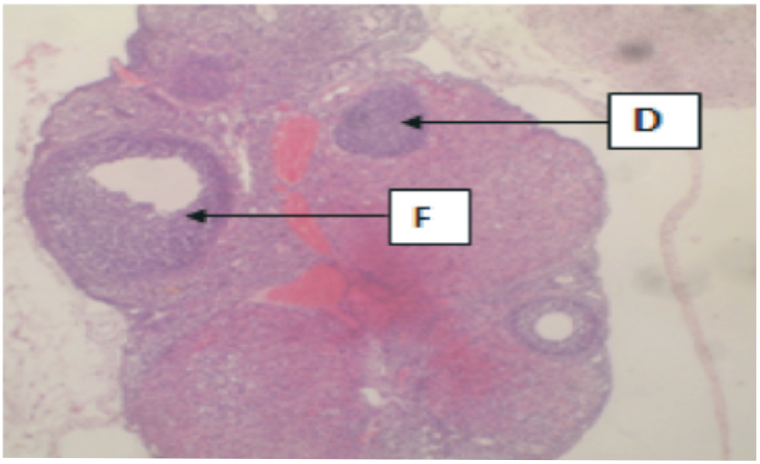

Plate 6: Photomicrograph of the ovary showing a section of Group C treated with 1000mg/kg of calabash chalk (H&E x125). Showing (D)atretic follicles (F) Graffian follicle in the process of becoming atretic .

DISCUSSION

The present study investigated the effects calabash chalk on the histoarchitecture of the ovary and uterus of wistar rats and also looked at the effects on their body weights during the period of study.

The results show that there was a slight increase in the body weight of the rats though not significant. Previous studies have reported endometrial hyperplasia and presence of inflammatory cells in the uterus [18] and structural changes in the ovaries as well as ovarian hormones [19]. However, in the present study, the histological observation of the groups administered with calabash chalk showed the uterus with normal simple cuboidal cells lining the endometrium,the endometrial gland within the endometrium which was in the diestrous stage, the myometrium consisting of inner circular and outer longitudinal layer. The ovaries also showed growing follicles and collapsing graffian follicle after ovulation, the ovarian stroma containing large blood vessels engorged with blood, atretic graffian follicle with no ovum, maturing follicles, mural granulosa cells that were dying off and graffian follicle in the process of becoming atretic. All these indicated the normal features of the ovary and uterus.

CONCLUSION

This study did not reveal any visible histopathological effect on the uterus and ovaries. Therefore, consumption of calabash chalk up to 1000mg/kg body weight could not lead to a pathological change in the structures of the ovary and uterus. Thus, it may not be unsafe to the uterus and ovary of a Wistar rat.

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