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Research Article

Effect of Calabash Chalk on the Walls Of the Uterine Body (Corpus Uteri) of Adult Female Wistar Rat

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Abstract

Calabash chalk is a naturally occurring minerals, predominantly consumed by pregnant women for its antiemetic effects, it is prepared from clay and mud made up of lead, arsenic, sand and wood ash. This study was aimed at establishing the effect of Calabash chalk on the wall of the uterine body. Sixteen Adult female wistar rats weighing between 150-200g were assigned into four groups. Group A animals served as the control and received food and distilled water only, while group B, C and D served as experimental groups that received 1000 mg/kg, 2000 mg/kg and 3000 mg/kg of aqueous extract of calabash chalk respectively. The extract administration lasted for 28days. On day 29 the animals were anaesthesized using chloroform vapour and were sacrificed. The blood was aspirated by cardiac puncture for hormonal Analysis and uterus were harvested, weighed and fixed in 10% buffered formalin for histological examination. Data was Analysed using SPSS version 25. Results from this present study showed a significant increase in Body weight of group C (P = 0.028), while Group B (p = 0.192) and group D (p = 0.065), showed a non significant decrease, when compared to group A. It was also noticed that there was a non significant increase in relative uterine weight of Groups B (P = 0.828) and C (P = 0.828) when compared to group A, while Group D (P = 0.004) showed a significant increase. The study also present that there was a non significant decrease in Estrogen level in groups B, C and D when compared to Group A, Progesterone results showed a significant decrease in groups B,C and D when compared to group A. Histological findings showed a mild, moderate to severe damage to the walls of the uterine cavity in groups B,C and D respectively when compared to the control group. This could account for certain levels of miscarriage experienced among pregnant women due to poor implantation of the foetus on the uterine wall, further investigation should be carried out on the incidence of miscarriage among pregnant women that consumes Calabash chalk, to establish the etiology of miscarriage, suggested by this scientific investigation.

Keyword: Uterus; Calabash Chalk; Wistar Rats; Miscarriage; Lead

Introduction

Geophagia (synonymous geophagy, geophagism and geotragia) is the deliberate consumption or crave for eating earth, soil, chalk, or clay, which is an ancient practice common to both animals and humans (especially pregnant women) [36]. The practice is not limited to any geographic region or sex, but has become a common habit cutting across all social classes [36]. It is highly prevalent in situations like poverty and famine to suppress appetite [36], and

in some psychiatric conditions like pica, compulsive indiscriminate eating of nonnutritive substances [23,36].

The constituent of calabash chalk as reported by [9], show that Aluminium silicate hydroxide- $Al_2Si_2O_5(OH)_4$, is the major component of Calabash chalk, and thus; a member of the Kaolin clay group. However, multi-elemental analysis has been to contain about 22 elements in calabash chalk with lead having high concentration [9]. In addition, other metals identified were Iron, aluminium, potassium, titanium, chromium, manganese, zinc, and nickel as well as the metalloid arsenic [9,13]. There are two types of chalk, which are; salted and non salted form. Calabash chalk is used as antiperspirant and facial powder when grounded [3], Calabash chalk is also used in facial masks and soaps, Although this chalk is consumed by both sexes and by many age groups, the prevalence of chalk consumption is greater among women, especially during pregnancy. Also Consumption of calabash chalk is believed to have diverse ethnobotanical uses; where it is used as antacid, antidiarrheal, contraceptive [10], nutritional supplement [9], wound healing and skin beautification agent [36]. It is also used for the treatment of skin diseases, fetal growth and wellbeing during pregnancy [36], and sociocultural activities [28].

Both forms of chalk (salted and Non salted) have shown fragmented liver parenchyma and hepatic sinusoidal enlargement as well as decrease in red blood cell in experimental animals [11]. Depletion of red blood cells [3,11], Oedema with haemorrhage in the mucosa of stomach, hyperkeratosis, and Koilocytic changes in the mucosa of the oesophagus. Geophagical materials when consumed and comes in contact with digestive fluids, have the potential of releasing clinical or sub-clinical toxic effects on an individual and pregnant women gravitate more toward Calabash chalk consumption in humans [36,37]. Calabash chalk is a geophagic material, popularly consumed in West Africa countries for pleasure and by pregnant women as a cure for morning sickness. People engage in geophagia for a variety of reasons including religious beliefs, medicinal purposes, or as part of a regular diet. Although this chalk is consumed by both sexes and by many age groups, the prevalence of chalk consumption is greater among women, especially during pregnancy. Health risks of consuming calabash chalk include; the alteration of the normal concentration of (hematological parameters) hemoglobin, red blood cell counts and erythrocyte sedimentation rate; alteration of growth rate and de-mineralization in the femur bone [5], fragmentation of the parenchymal cells, and dilation of sinusoids of the liver due to treatment, lead and arsenic contamination in pregnant and breastfeeding women [17], causes hypertrophy and hyperplasia of cells in all the cortical layers, with less demonstrated Nissl and higher, and increased body weight in pregnant rats [12] Another report from [11].

Reproduction is vital for the continuation of the human species [6]. The uterus, also known as the womb, is a hollow, pear-shaped organ that is responsible for a variety of functions such as gestation (pregnancy), menstruation, and labor and delivery [8]. The uterus functions by nurturing the fertilized ovum, which passes through the fallopian tube. The ovum then implants into the endometrium, where it receives nourishment from blood vessels, which exclusively developed for this purpose [4]. The uterus has three tissue layers which include the following: Endometrium: the inner lining and consists of the functional (superficial) and basal endometrium. The functional layer responds to reproductive (implantation) hormones. When this layer sheds, this results in menstrual bleeding. If there is damage to the basal endometrium, this can result in the formation of adhesions and fibrosis (Asherman syndrome). Myometrium: the muscle layer and is composed of smooth muscle cells. Serosa/Perimetrium: the thin outer layer composed of epithelial cells [4].

Implantation is defined as the process by which the embryo attaches to the endometrial surface of the uterus and invades the epithelium and then the maternal circulation to form the placenta. Before the initiation of implantation, however, both embryo and endometrium should embark on an elaborated process in a time- and location-specific manner [25,31]. To achieve successful implantation, the uterine body will have to undergo structural and functional remodeling. Estrogen and progesterone are the master hormones mediating these changes. Estrogen and progesterone bind to their respective nuclear receptors. The progesterone receptor exists in two isoforms, PR-A and PR-B, and the estrogen receptor also exists in two isoforms, as ERα and ERβ [22,26]. The International Federation of Gynecology and Obstetrics [20]. Asserted that cancer of the corpus uteri, typically referred to as endometrial cancer, arises from the epithelia lining of the uterine cavity and the new round –up of evidence shows endometrial cancer is the sixth most common malignant disorder worldwide,

and this is attributed to greater rate of obesity and more sedentary lifestyles.

Materials and Methodology

Location and duration of the study

The practical was carried out in the Department of Anatomy, faculty of Basic medical sciences, Nnamdi Azikiwe Univeristy, College of Health Science, Nnewi Campus, Anambra State, Nigeria. They were made to acclimatize for a period of two weeks after which the test substance was administered for 28 days.

Ethical approval

Ethical approval was obtained from the ethical committee of the Faculty of Basic Medical Sciences, Nnamdi Azikiwe University, College of Health Science, Nnewi Campus for animal based research**.** The study was reviewed and approved by the Department of Anatomy research committee.

Calabash chalk procurement and extraction

Blocks of Calabash chalk (Nzu) will be purchased from a Local market (Nwko Market) in Ihiala local government area, Anambra State, Nigeria. The Block form of calabash chalk will be grounded into powered form using a manually operated grinder, after which it will be macerated in distilled water for 24hours, after which it will be filtered using whatman filter paper, the extract will then air-dried. The resulting extract was then stored in a refrigerator at 4°C until futher use of the experiment.

Acute toxicity study

The median lethal dose (LD_{50}) of Calabash chalk (*Nzu*) will be carried out in the department of Physiology, Faculty of Basic Medical Science, Nnamdi Azikiwe University, Nnewi Campus. This will be determined using the method of [24]. In this study, thirteen rat will be used, they received the extract via oral route and it was carried out in two phases.

Phase I

A total of nine (9) rats will be employed for the study and three rats will be allocated for each group.

- Group 1 received 10 mg/kg per rat
- Group 2 received 100 mg/kg per rat
- Group 3 received 1000 mg/kg per rat

The animals will be monitored for 24hours for morbidity and mortality. The rats remained normal after 24 hours of observation in phase 1 then the study proceeded to the second phase were the four rats were employed for the study comprising one rat per group.

Phase II

- Group 1 received 1200 mg/kg per rat
- Group 2 received 1600 mg/kg per rat
- Group 3 received 2900 mg/kg per rat
- Group 4 received 5000 mg/kg per rat.

The animals were monitored for another 24 hours for morbidity and mortality.

$LD_{50} = \sqrt{a \times b}$

A–maximum dose with 0% mortality

B–minimum dosed with 100% mortality

$LD_{50} – \sqrt{a}$ X b.

Experimental animals

Sixteen adult female wister rat will be used for this study. The wister rat was purchased from a local farm (Gaberiels farm) in Nnewi and moved to the site of experiment, The animals was maintained at room temperature and fed with growers mesh manufactured by Top feed PLC, twice daily and allowed distilled water *ad libitum.* They were housed in well ventilated cages with suitable environmental conditions and clean surroundings. Before administration of Calabash chalk extract, the wister rat will be allowed to acclimatize to the new environmental conditions of the animal house of College of Health Sciences Okofia, Nnewi Campus for a period of two weeks. They will be cared for, according to the ethical guidelines for the use of animals in experimental study.

Experimental design

The Sixteen adult female wister rat of 150g - 200g will be randomly assigned to four groups A, B, C and D of four rats in each group. Group A will served as the control group while groups B,C, and D, will serve as the experimental groups.

Administration of the extract

The experimental groupings will be divided into four groups, which were groups A,B,C and D, groups. The Group A (control) received only water and food pellets, Group B received low dose of aqueous extract of Calabash chalk at 1000 mg/kg. Group C received moderate dose of 2000 mg/kg of aqueous extract of Calabash chalk. Group D received a high dose of 3000 mg/kg. Extract administration lasted for a period of 28 days will be the animals administered with the extract by oral application using a stainless cannula and group A is the control group. Animals will be caged according to the layout.

Sample collection

The animals was anaesthetized with diethyl ether in an enclosed container, after 24 hours of the last administered dose of the Calabar chalk extract, ocular puncture was done by method of, blood was collected and analyzed for Hormones. The uterus was harvested and fixed in 10% Formalin as a preservative in a container before taking to histopathologist for histological examination.

Hormonal analysis

AccuBind enzyme-linked immunoabsorbent assay (ELISA) microwells for Estradiol and Progesterone were purchased from Monobind Inc. Lake Forest CA 92630, USA with respective product code 4925-300 for Estradiol Test and 4825-300 for Progesterone. The serum samples were collected in the morning to reduce the influence of diurnal variations in hormones.

Estradiol test

The reagents, serum references and control were brought to room temperature before the assay. The microplate's wells for each serum references were formatted with control and tests specimen assayed in duplicates. 0.025 ml of the serum of the control or specimen was pipetted, into the assigned well. 0.050 ml of the estradiol biotin reagent were added to all the wells. The microplates were swirled gently for 20-30 seconds to mix and then covered and incubated for 30 minutes at room temperature. 0.050 ml of estradiol enzyme reagent was added directly on top the reagent dispensed in all the wells and the microplates were gently swirled again for 20-30 seconds to mix. The wells were covered and incubated for 90 minutes at room temperature. The contents of the microplates were discarded by decantation or aspiration. If decanting blot the plate dry with absorbent paper. 350UL of wash buffer was added and then decantation and aspiration was done. This process was repeated two additional times for a total of three washes. 0.100 ml of substrate solution was added to all the wells in the same order to minimize reaction time difference between wells without allowing the plate to shake. Incubation was done at room temperature for 20 minutes and 0.05 ml of stop solution added to each well and gently mixed for 15-20 seconds. The absorbance in each well was read at 450nm using a reference wavelength of 620-630 nm. The results were read within 30 minutes of adding the stop solution. A dose response curve was used to ascertain the concentrations of estradiol in unknown specimen using the computer data reduction software designed for ELISA assay (AccuBind procedure, 2019).

Progesterone test

The reagents, serum references and control were brought to room temperature before the assay. The microplate's wells for each serum references were formatted with control and tests specimen assayed in duplicates. 0.025 ml of the serum of the control or specimen was pipetted, into the assigned well. 0.050 ml of the Progesterone Enzyme Reagent were added to all the wells. The microplates were swirled gently for 10-20 seconds to mix at room temperature. 0.050 ml of Progesterone Biotin reagent was added to all the wells and the microplates were gently swirled again for 10-20 seconds to mix. The wells were covered and incubated for 60 minutes at room temperature. The contents of the microplates were discarded by decantation or aspiration. If decanting, blot the plate dry with absorbent paper. 350UL of wash buffer was added and then decantation and aspiration was done. This process was repeated two additional times for a total of three washes. 0.100 ml of substrate solution was added to all the wells in the same order to minimize reaction time difference between wells without allowing the plate to shake. Incubation was done at room temperature for 20 minutes and 0.05 ml of stop solution was added to each well and gently mixed for 15-20 seconds. The absorbance in each well was read at 450nm using a reference wavelength of 620-630 nm. The results were read within 15 minutes of adding the stop solution. A dose response curve was used to ascertain the concentrations of progesterone in unknown specimen using the computer data reduction software designed for ELISA assay (Accu Bind procedure, 2019).

Tissue processing

Tissue sections were produced by normal histochemical methods of fixation, dehydration, clearing, impregnation,

embedding, sectioning, mounting, and staining. The micrographs of relevant stained sections were subsequently taken with a light microscope.

Fixation

After weighing the organs, a small part (0.8 um) was cut from each of them and immediately fixed in Bouin's fluid in order to preserve various constituents of the cells in their normal micro anatomical position and to prevent autolysis and putrefaction.

Dehydration

After fixation, the tissues were transferred and dehydrated in ascending grades of alcohol (50%, 70%, 90%, 95% and 100% or absolute alcohol once for 2 hours each but twice in absolute alcohol). The tissues were placed in ascending grades of alcohol to prevent distortion and distortion to the cell structure which would have happened if directly placed in absolute alcohol. However, sufficient time was allowed in sufficient alcohol to enable complete dehydration.

Clearing

The tissues were cleared twice in xylene for 1-2 hours each time. This was to avoid over exposure in the clearing agent, which will make them brittle. Xylene was used as the clearing agent as it does not only remove alcohol but is equally miscible with paraffin used in embedding.

Impregnation

The tissues were placed in molten paraffin wax at a constant temperature of 56°C (3°C above the melting point of paraffin wax used) in an oven and were passed through two changes of paraffin wax in the oven, 4 hours each. This was done to replace the clearing agent with molten paraffin wax and can also be referred to as infiltration. The tissues were subsequently removed from the oven and embedding in paraffin wax.

Embedding

This a process of burying the tissue in molten paraffin wax. The paraffin becomes a solid firm structure when it is cold. This forms a support medium for the tissue during microtomy. The tissue were then immersed in molten paraffin wax at a constant temperature of 36°C to 60°C on an oven of paraffin bath changing it twice for 2-4 hours each time. They were left to cool and solidify in metallic embedding moulds. The tissue block obtained were casted unto the wooden blocks for sectioning.

Sectioning

This was done using a Rotatory Microtome. The tissue blocks were mounted on wooden blocks with the microtome knife and blocks positioned accurately. Sections were made at 5 microns each. The ribbons of sections were floated in warm water bath (37°C) to straighten them. The best ribbons were picked with forceps and placed in albuminized slides. The slides were labeled using diamond pencils and transferred to a slide rack. They were then placed in a oven to keep the specimens warm before staining.

Staining

The tissuese were stained using Ehrlch's Heamatoxylin and Eosin stains. The staining procedures is as follows: The slides treated with paraffin wax were cleared in xylene for 3 minutes, and were rehydrated in descending grades of alcohol, absolute alcohol for 2 minutes to remove xylene, 90% alcohol for 2 minutes, 70% alcohol for 2 minutes, 30% alcohol for 2 minutes and then rinsed in water for 1 minute each. The tissues were stained by immersing the aqueous solution of heamatoxylin for 30 minutes and then rinsed in water to remove excessive stains. The tissues were subsequently differentiated in 1% acid alcohol for 1 minute. This process called Bluing gave the tissues their characteristic blue background. The tissues were then stained in aqueous eosin for 10 minutes. The tissues were now immersed in ascending grades of alcohol as follows; 50%, 70%, 90% and absolute alcohol for 1 minute each and then cleared in xylene for 1 minute. Staining gives contrasting colours to different elements of the cells or tissues, thus making them conspicuous and easy to study.

Mounting

The slides were removed from the rack through their edges with the aid of forceps and placed on the filter papers. Blotting was done in one direction on the filter papers using the index finger and few drops of xylene were placed on the slides to make the wet, A drop of Dibutyphthalate xlene (DPX) mountant was placed on the slide which was laid in the middle to minimize the likelihood of trapping air bubbles. The slides were quickly inverted over cover slip and then brought down horizontally until the mountant made contact.

Data analysis

The data collected was statistically evaluated using Analysis of variance (ANOVA). The analysis was performed using Microsoft Office Excel (version 2013) and Statistical package for Social

Sciences (SPSS). The mean difference was considered significant when p-value is less than 0.05. Data for Organ weight was analyzed using One-way ANOVA, followed by Post hoc LSD test. While body weight was analyzed using Student dependent T-test.

Material

Calabash chalk

Electronic weighing balance (NAPCO Precision Instruments JA-410).

Oral cannula

Wistar Rats(16)

Distilled water

10% Buffered formalin

Standard Cages

Cotton wool and Hand gloves

Beakers and Measuring cylinder

5ml hypodermic syringe

Animal weighing balance (CAMRY LB11)

Diethyl ether

Vital top feed (Jos, Nigeria)

Dissecting kits

EDTA container

Micro-hematocrit centrifuge SH120

Capillary Tube

Micro haematocrit Reader

Plasticine and Neubaur Counting Chamber

Haemocytometer

Plain Container

Spectrophotometer (Model 721).

Result

Physical and behavioural observations

During the period of acclimatization, all rats feed normally. All rats were healthy, with smoothly laid hairs on their skin, pinkish eyes, normal skin colour and they increased significantly in size and weight. After the commencement of experiment, group A, B, C and D showed no observable clinical signs.

		Mean	±STD	BWD	P-value	T-value
Group A (control)	Initial body weight (g)	167.00	±4.83	38.50		-3.234
	Final body weight (g)	205.50	±19.21			
Group B (1000 mg/kg of calabash chalk)	Initial body weight (g)	154.25	±14.45	18.00	0.192[a]	-1.680
	Final body weight (g)	172.25	±26.85			
Group C (2000 mg/kg of calabash chalk)	Initial body weight (g)	133.50	±6.13	18.25	0.028*	-4.025
	Final body weight (g)	151.75	±4.78			
Group D (3000 mg/kg of calabash chalk)	Initial body weight (g)	145.50	± 6.32	27.50	0.065[a]	-2.861
	Final body weight (g)	118.00	±14.75			

Table 1: Shows the effect of calabash chalk on the body weight.

Data was analyzed using t-test and values were considered significant at $p < 0.05$. STD: Standard deviation; BWD: Body weight difference * means significant; [a]: not significant.

Aggressiveness was observed significantly in group C and D, other signs observed are, restlessness, dizziness, reduced food and water intake.

Effect of calabash chalk on the body weight of the animals

The initial and final body weight of the different groups were measured, recorded and analyzed. Result from table 2, showed that Group A had a mean initial body weight of 167.00g with a standard deviation of ±4.83 and a mean final body weight of 205.5g with a standard deviation of ±19.21. Group B had a mean initial body weight of 154.25g with a standard deviation of ±14.45 and a mean final body weight of 172.25g with a standard deviation of ±26.85. Group C had a mean initial body weight of 133.50g with a standard

deviation of ±6.13, and a mean final body weight of 151.75g with a standard deviation of ±4.78, Group D had a mean initial body weight of 145.50g with a standard deviation of ±6.32 and a mean final body weight of 118.00g with a standard deviation of ±14.75. This result revealed a statistically significance ($P < 0.05$) increase in the body weight of group C (0.028), and statistically non significant ($p > 0.05$) increase in group B (0.192) and D (0.065) when initial weight was compared to final weight.

Figure 1: Bar chart showing the effect of calabash chalk on the body weight.

Figure 1 shows the graphical illustration of the body weight changes. A graph of the body weight was plotted against the animal groups. From the above bar chart, Group A had a mean initial body weight of 167.00g with a standard deviation of ±4.83 and a mean final body weight of 205.5g with a standard deviation of ±19.21. Group B had a mean initial body weight of 154.25g with a standard deviation of ±14.45 and a mean final body weight of 172.25g with a standard deviation of ±26.85. Group C had a mean initial body weight of 133.50g with a standard deviation of ±6.13, and a mean final body weight of 151.75g with a standard deviation of ±4.78, Group D had a mean initial body weight of 145.00g with a standard deviation of ±6.32 and a mean final body weight of 118.50g with a standard deviation of ±14.75.

Effect of calabash chalk on the relative uterine weight of the animals

Table 3, Shows the effect of calabash chalk on the relative organ of the animals. Group A, had a relative organ weight of 0.03g with a standard deviation of ±0.01. Group B had a relative organ weight of 0.04g with a standard deviation of ±0.01. Group C had a relative organ weight of 0.05g with a standard deviation of ±0.00. Group D had a relative organ weight of 0.028g with a standard error deviation of ±0.01.The result revealed a statistically insignificant increase ($p > 0.05$) in the relative uterine weight in-groups B, C and D had an increase but was significant at group D and not significant at groups C compare to group A.

		MEAN	±STD	P-Value	F-Value
Relative uterine weight (g)	Group A (control)	0.03	±0.01		
	Group B (1000 mg/kg of calabash chalk)	0.0	4±0.01	0.828	16.48
	Group C (2000 mg/kg of calabash chalk)	0.05	±0.00	0.828	
	Group D (3000 mg/kg of calabash chalk)	0.028	±0.01	0.004*	

Table 2: Shows effect of calabash chalk on the relative uterine weight.

Data was analyzed using ANOVA followed by post hoc LSD multiple comparison and values were considered significant at $p < 0.05$.

Graph showing the effect of calabash chalk on the relative uterine eight of animals

Figure 2 Shows the graphical illustration of the relative organ weight. A graph of the relative organ weight were plotted against the animal groups. Group A, had a relative organ weight of 0.03g with a standard deviation of ±0.01. Group B had a relative organ

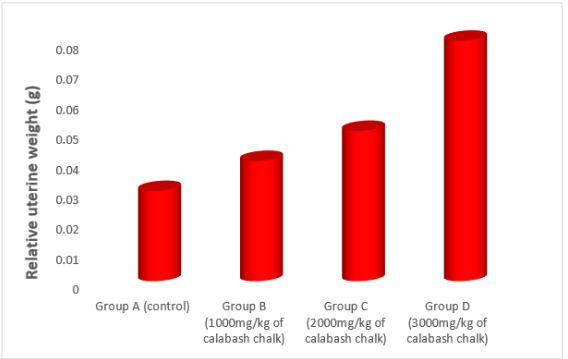

Figure 2: Bar chart showing the effect of calabash chalk on relative uterine weight of the animals.

weight of 0.04g with a standard deviation of ±0.01. Group C had a relative organ weight of 0.05g with a standard deviation of ±0.00. Group D had a relative organ weight of 0.028g with a standard error deviation of ±0.01.

Effect of calabash chalk on estrogen and progesterone

Table 4, Shows the effect of calabash chalk on the estrogen and progesterone level of the animals. Group A, had an estrogen level of 150.00 pg/ml with a standard deviation of ±50.00. Group B had an estrogen level of 95.00 pg/ml with a standard deviation of ±5.00. Group C had an estrogen level of 100.00 pg/ml with a standard deviation of ±0.00. Group D had an estrogen level of 100.00 pg/ml with a standard deviation of ±0.00. The result revealed a statistically insignificant decrease (*p>0.05*) in estrogen level in groups B, C, and D compared to group A.

		Mean	±STD	p-Value	F-Value
Estrogen (pg/ml)	Group A (control)	150.00	±50.00		
	Group B (1000 mg/kg of calabash chalk)	95.00	±5.00	0.104	3.19
	Group C (2000 mg/kg of calabash chalk)	100.00	±0.00	0.147	
	Group D (3000 mg/kg of calabash chalk)	100.00	±0.00	0.147	
Progesterone (pg/ml)	Group A (control)	60.00	±0.00		
	Group B (1000 mg/kg of calabash chalk)	25.00	±5.00	0.001*	144.92
	Group C (2000 mg/kg of calabash chalk)	21.00	±1.00	0.000*	
	Group D (3000 mg/kg of calabash chalk)	30.00	±0.00	0.020*	

Table 3: Shows the effect of calabash chalk on estrogen and progesterone level.

Data was analyzed using ANOVA followed by post hoc LSD multiple comparison and values were considered significant at $p < 0.05$.

It also shows the effect of calabash chalk on the progesterone level of the animals. Group A, had a progesterone level of 60.00 pg/ml with a standard deviation of ±0.00. Group B had a progesterone level of 25.00 pg/ml with a standard deviation of ±5.00. Group C had a progesterone level of 21.00g with a standard deviation of ±1.00. Group D had an estrogen level of 30.00 pg/ml with a standard deviation of ±0.00. The result showed a significant decrease ($p < 0.05$) in groups B, C, and D compared to group A.

Graph of the effect of calabash chalk on the estrogen level

Graph of the effect of calabash chalk on the progesterone level

Figure 3 and 4 shows the graphical illustration of effect of calabash chalk on the estrogen and progesterone level of the animals. A graph of estrogen and progesterone were plotted against

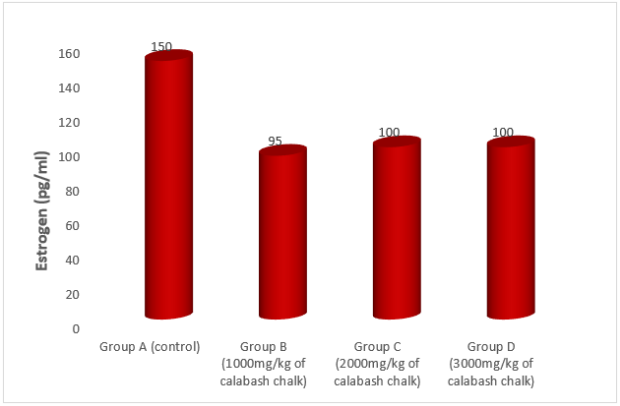

Figure 3: Bar chart showing the effect of calabash chalk on estrogen level.

Figure 4: Bar chart showing the effect of calabash chalk on progesterone level.

the animal groups respectively. Group A, had an estrogen level of 150.00 pg/ml with a standard deviation of ±50.00. Group B had an estrogen level of 95.00 pg/ml with a standard deviation of ±5.00. Group C had an estrogen level of 100.00 pg/ml with a standard deviation of ±0.00. Group D had an estrogen level of 100.00 pg/ml with a standard deviation of ±0.00.

It also shows the effect of calabash chalk on the progesterone level of the animals. Group A, had a progesterone level of 60.00 pg/ml with a standard deviation of ±0.00. Group B had a progesterone level of 25.00 pg/ml with a standard deviation of ±5.00. Group C had a progesterone level of 21.00g with a standard deviation of ±1.00. Group D had an estrogen level of 30.00 pg/ml with a standard deviation of ±0.00.

Histological micrographs

Plate 1: (control): A Photomicrograph section of uterus presents normal uterine tissue with stratum basal(SB), stratum functionalis (SF) and endometrial gland (EG). The stratum functionalis is lined by simple cuboidal epithelium (SCE) the overall features appear normal. (x400)(H/E).

The micrograph Plate 1 presents the photomicrograph of the uterine uterus of group A which received only feed and water for 28 days. The uterus shows normal uterine tissue with stratum basal(SB), stratum functionalis (SF) and endometrial gland (EG). The stratum functionalis is lined by simple cuboidal epithelium (SCE) the overall features appear normal.

Plate 2: (low dose): A Photomicrograph section of uterus administered with low dose of calabash chalk presents mild effect on the uterine tissue with mild congestion of the blood vessel (CBV) and thickening of the epithelia cell (EC) with mild cluster of the inflammatory cell (IC). x400) (H/E).

The micrograph plate 2, when compared to plate 1 (Control), presents mild effect on the uterine tissue with mild congestion of the blood vessel (CBV) and thickening of the epithelia cell (EC) with mild cluster of the inflammatory cell (IC).

Plate 3: (medium dose): A Photomicrograph section of uterus administered with medium dose of calabash chalk show moderate effect on the uterine tissue with moderate thickening of the epithelia layer (EL) with moderate cluster of the inflammatory cell (IC). X 400) (H/E).

The micrograph plate 3 when compared to plate 1 (Control), presents moderate effect on the uterine tissue with moderate thickening of the epithelia layer (EL) with moderate cluster of the inflammatory cell (IC).

Plate 4: (high dose): A Photomicrograph section of uterus administered with high dose of calabash chalk shows moderate to severe effect on the uterine tissue with moderate thickening of the epithelia layer (EL) moderate focal area of hemorrhage (H) with moderate cluster of the inflammatory cell (IC). x400) (H/E).

The micrograph plate 4, when compared to plate 1 (Control) presents moderate to severe effect on the uterine tissue with moderate thickening of the epithelia layer (EL) moderate focal area of hemorrhage (H) with moderate cluster of the inflammatory cell (IC).

Discussion

The term geophagy is applied to the recurrent intentional eating of soil (calabash chalk) with multifactorial motivation. Geophagists are generally defined, gender (women), age (children), physical status (e.g. pregnancy, lactation, postpartum), social status (people exposed to significant nutritional deficiency), and culture, but lost awareness of traditional medical meaning of this practice, is changing these consumption patterns and increasing health risks [38]. Moreover, although the holistic anthropological perspective recognizes soil consumption as mineral supplementation under certain circumstances, we should consider how the living environment has changed and is changing along with diet, nutrition requirements and habits. therefore, benefits-to-risks ratio of cultural behaviors initiated centuries ago based on traditional medical practices requires deep revision and assessment [38]. Knowledge on minerals metabolism, bioavailability and interactions is required to properly assess the role of geophagy in a balanced and safe intake of micronutrients, most important, the chemistry of geophagic clays are uncontrolled, variable, and difficult to standardize [38], However, this study was aimed at establishing the effect of calabash chalk on the uterine body wall, and biochemical hormones. Hormones such as estrogen and progesterone are key in structural and functional remodeling of the uterus for successful implantation; a decrease in these hormones will affect implantation.

In this present study, the experimental results revealed a significant and dose dependent reduction in weight gained by animals as a result of inclusion of aqueous extract of Calabash chalk in their diet that lead to the reduction in their food intake. The highest increase in body weight was in Group A, which served as the control group (which received animal feed and distilled water only), but there was a decrease in body weight of Groups B, C and D which received 1000 mg/kg and 2000 mg/kg and 3000 mg/kg of aqueous extract of Calabash chalk respectively, when compared to group A, This study is in line with the work done by [7]. who reported that Calabash chalk contains high amount of lead

that can lead to alteration and reduction of body weight, this work agrees with the report of [33] who reported that Calabash chalk (that contains lead) exposure resulted in dose-specific weight gain in adult wistar rats accompanied by alteration of DNA methylation. This study disagrees with the study made by [13] who reported that there is no difference in the weights. This study disagrees with the work of [13] who reported that there is a significant decrease in body weight and insignificant decrease in the weight and length of both right and left femur bones of animals treated with calabash chalk. This study agrees with the work of [35] who asserted that there is reduction in body weight gain in the their study on lead -induced adverse effects on the reproductive system of rats with particular reference to histopathological changes in uterus. The mechanism of action for the reduction in body weight after exposure to lead (found in calabash chalk) can be attributed to it's influence on feeding behaviour via Central Nervous system or secretion of growth hormone [19]. Another reason for The increase in body weight of animals in control group could be physiological as the animals were only exposed to water and feed throughout the experiment. The reduction in body weight could be due to the disruption in the absorption of nutrient by the chalk, since it contains Kaolin which has been reported to coat the lining of the gastrointestinal tract [5], This coating prevents the absorption of beneficial nutrients which results to poor nutrition and loss of weight. This is in line with [29], who reported a reduction in weight in the treated group when compared to control.

Findings from this study revealed that there was a statistically insignificant increase ($p > 0.05$) in the relative uterine weight in group B, C, but group D had a statistically significant increase. The significance increase in group D could be pathological which may be due to inflammation of the organ. This study contradicts the work of [14], who observed a progressive decline in uterine weight in animals exposed with lead, This study also contradicts the work of [13], who reported that there was no difference in the weight of spleen in animals treated with calabash chalk. The different between this study and those that disagrees with it can be attributed to the difference in dose and duration of calabash chalk exposure.

It also present a statistically insignificant decrease ($p > 0.05$) in estrogens level in groups B, C, and group D, compared to group A. Progesterone levels showed a statistically significant decrease ($p < 0.05$) in groups B, C and D compared to group A, the mechanism that could lead to this may be connected to the presence of lead in calabash, this study agrees with the work of [29], who reported that a reduction in follicles stimulating hormone levels of animals treated with calabash chalk. This study also agrees with the work of [39], who conducted a research on effect of chronic exposure to lead on estrogen action in the prepubertal rat uterus and reported that there is an interaction of lead with the difference mechanism of estrogen action in the uterus at various levels. This study agrees with [16], who reported a decrease in the plasma progesterone concentration in animals exposed with lead. This study agrees with that of [35] 5 who reported a marked reduction in progesterone levels following exposure to animals to lead and it is attributed to increasing in activities of five Beta-reductase, a progesterone metabolising enzyme in liver and uterus homogenates [1], According to the study done by [40], who reported that elevated serum heavy metals (lead and cadmium) may contribute to recurrent spontaneous abortion.

Histopathological findings from this present study revealed mild to moderate effect on the uterine tissue wall (Mild to moderate congestion of the blood vessels, thickening of the epithelial layer and cluster of the inflammatory cells), in both group B and C respectively when compared to plate I. In group D, it showed severe effect on the uterine wall, (moderate to severe on thickening of the epithelial layer, focal area of hemorrhage and cluster of the inflammatory cells), when compared to plate I,. this study agrees with the work of [29], who reported that the Histological examination of the ovaries showed severe deterioration of the ovarian follicles, necrosis and follicular atresia, on the research conducted on Histological and hormonal studies of calabash chalk on ovarian function in adult female wistar rats. This study is also in line with the work of [27], who reported the adverse effect of lead on the reproductive system (uterus) which caused chronic endometriosis with sloughing of the epithelial lining and severe vacuolar degeneration in the lining of endometrial cell, Histopathological studies on uteri of different treatment groups in the present study revealed its dose-dependent deleterious effects on myometrium. Cystic degeneration of goblet cells and sloughing off of endometrial lining epithelial cells was observed in uteri following exposure of lead (found in calabash chalk). Degenerative and inflammatory changes in uterus including decrease in height of columnar cells and undistinguished areas of blood vessels, lymphatics and connective tissues have been documented by [32]. In utero or gestational exposure to lead has

been reported to cause necrosis of uterine glands and destruction of uterine lining cells [15]. Importance of endometrial glands and their secretions in maintaining estrous cycles, conceptus survival and growth at the peri-implantation stage has been reported by [18]. Therefore, lead may produce deleterious effect on in-utero physiological functions by altering uterine glandular secretions like enzymes, growth factors, cytokines, lymphokines, hormones and transport proteins essential for development of conceptus [34]. [21] reported that low-to-moderate lead exposure may increase the risk of spontaneous abortion.

Conclusion

This study reveals the dangerous effect of calabash chalk to the uterine body wall, which could account for certain levels of spontaneous abortion (miscarriage) experienced among pregnant women due to poor implantation of the foetus on the uterine wall, These alterations have been shown to be the leading cause of infertility in adults female Wistar rats of which their tissues are similar to that of human. Hence, proper monitoring, education, and regulation of the product is needed. This research will help create awareness on the risks involved in the consumption of calabash chalk. Women especially of reproductive age should be discouraged from consuming this substance.

Recommendations

The pathological Effect observed in this study must be taken into consideration by the general public especially the pregnant women and young children of Reproductive age.

Further investigation should be carried out on the incidence of spontaneous abortion (miscarriage) among pregnant women that consumes calabash chalk, to establish the etiology of spontaneous abortion (miscarriage), suggested by this scientific investigation.

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