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Evaluation of the effects of calabash chalk on the haematological profile of Wistar rats

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Abstract

Calabash chalk is a naturally geophagic mineral composed mainly of fossilized seashells or an artificial preparation of a combination of clay, mud, and sand. It is one of the widely consumed earth materials in the practice of geophagia. The present study is aimed at evaluating the effects of oral administration of calabash chalk on haematological parameters and body weight using Wistar rat models. Ten (10) healthy female mice and twenty (20) adults female Wistar rats weighing 20-30 g and 160 -200 g were used for the determination of the LD50 and the experimental study respectively. Locally sourced calabash chalk was orally administered to the Wistar rats at 400, 600 and 800 mg/kg respectively for twenty-eight (28) days. Haematological parameters were determined using a standard automated procedure. The result of the study shows that there was a significant decrease in red blood cell count, haemoglobin concentration, packed cell volume, total white blood cell count, neutrophils, lymphocytes, mid-range absolute and platelet counts among the experimental groups compared to the control ($p<0.05$). Also, there was a significant reduction in the percentage of weight changes among the experimental animals compared to the control ($p<0.05$). The present study has shown that despite the wide safety margin of calabash chalk, it can depress haematopoiesis by reducing the red blood cell, white blood cell and platelet counts.

Keywords: body weight; calabash chalk; haematology; platelet; red blood cells; white blood cells

Introduction

Calabash chalk also referred to as calabash clay or calabash stone is a naturally geophagic mineral composed mainly of fossilized seashells or an artificial preparation of a combination of clay, mud, sand and wood (Akpantah *et al*., 2010; Moses *et al*., 2012). They occur as pellets or large blocks predominantly found in West Africa, especially in Nigeria where they are known by local names such as *nzu* (Igbo), *eko* (Bini), *ndom* (Efik/Ibibio). They are known as *mabele*, *shilè* and *umcako* in the Congo, Ghana and South Africa respectively (Dean *et al*., 2004; Ekong *et al*., 2012; Owhorji *et al*., 2019). Calabash chalk remains one of the widely

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consumed earth minerals in the practice of geophagia with reasons for its deliberate consumption ranging from age-long unproven medicinal benefits to habits, cultural beliefs and cravings. In Nigeria, they are mostly consumed by pregnant women to reduce nausea and vomiting as well as over-salivation (Abrahams *et al*., 2013; Ogbuagu *et al*., 2019). Besides use by pregnant women, it has been noted that the consumption of calabash chalk is not limited to sex, age group or geographic region although the practice seems to be more common among poorer and culturally oriented people in Africa and Asia (Abrahams *et al*., 2013; Gomes and Rautureau, 2021). The consumption of calabash chalk has been theorized to be associated with the protection of the stomach, malnutrition, anaemia, taste and mental satisfaction (Al-Rmalli *et al*., 2010; Mishra and Roy, 2015).

Calabash chalk is principally made up of aluminium silicate hydroxide ($Al_2Si_2O_5OH_4$), a member of the kaolin clay group with different samples from various locations having varying content of minerals alongside other organic pollutants such as arsenic, chromium, endrin, silicon and alpha lindane (Ekong *et al*., 2012; Moses *et al*., 2012; Abrahams *et al*., 2013). Some of the health benefits attributed to the consumption of calabash chalk include the treatment of stomach disorders. It is said to bind with stomach contents and serves to reduce gastric pains and heartburns (Ogbuagu *et al*., 2019). In the cosmetic industry, it is used as an antiperspirant, facial powder and eyeliners (Popoola *et al*., 2013).

Several reports documenting experimental evidence of some of the hazardous effect of calabash chalk has shown it to cause alteration in the growth of the femur bone (Ekong *et al*., 2012), gastrointestinal disorders (Moses *et al*., 2012), alterations in the cerebral cortex of foetuses (Ekanem *et al*., 2015), ovarian, uterine and gestational toxicity and (Oyewopo *et al*., 2017; Aprioku and Ogwo-Ude, 2018; Opara and Nwagbaraocha, 2018), alteration in lipid and electrolyte profile (Alli and Nafiu, 2017), haematoxicity and hepatotoxicity (Akpantah *et al*., 2010; Ogbuagu *et al*., 2017). Despite the negative health effects of consumption of calabash chalk, the consumption has continued unabated. More so, the paucity of data regarding its effect on full blood count prompted the need for this research in other to bridge this knowledge gap. The present study is therefore aimed at the evaluation of the effect of oral administration of calabash chalk on haematological parameters and body weight changes using Wistar rat models.

Materials and Methods

Procurement and Identification of Calabash Chalk

Salted calabash chalk was procured from Choba market, Port Harcourt, Nigeria and authenticated at the Department of Geology, University of Port Harcourt. Chemical components of the calabash chalk were identified using X-Ray Diffraction Method (Olympus, TERRA-575, USA) at the Department of Pure and Applied Chemistry, University of Port Harcourt.

Experimental animals

Ten (10) healthy female mice and twenty (20) adults female Wistar rats weighing 20-30 g and 160 -200 g respectively were sourced from the animal house of the Department of Pharmacology, University of Port Harcourt and used for this study. The animals were allowed four weeks of acclimatization before the start of the study under standard animal husbandry conditions: the temperature at 25-29 °C under natural light/dark cycle. They were allowed normal rat chow and water *ad libitum*. The mice and the Wistar rats were used for the determination of the LD_{50} and the experimental study respectively.

Determination of LD_{50}

An acute toxicity test of calabash chalk was done using Lorke's method as previously described (Lorke, 1983; Chinedu *et al*., 2013). The test was carried out in two phases. In the first phase, six (6) female mice were divided into three (3) groups of two (2) animals each and were administered with an aqueous mixture of calabash chalk at 50, 500 and 5000 mg/kg respectively. Similarly, in the second phase, three (3) female mice were grouped into three (3) groups of one (1) mouse each and administered with an aqueous mixture of calabash chalk at 4000, 8000 and 1600 mg/kg respectively. During these phases, the animals were monitored for signs of toxicity and eventual mortality.

The LD_{50} was calculated as follows:

$LD_{50}= \sqrt{(D_0 \times D_{100})}$

Where D0 = Highest dose that gave no mortality.

D_{100} = Lowest dose that produced mortality.

The LD_{50} of the calabash chalk was determined to be 2,828.427mg/kg.

Experimental design

Twenty (20) female mice (160-200 g) were randomly divided into four groups (4) of five (5) animals each and treated as follows:

Groups	Name	Treatment
1	Control	Distilled water
2	Low Dose	400 mg/kg of calabash chalk
3	Medium Dose	800 mg/kg of calabash chalk
4	High Dose	1600 mg/kg of calabash chalk

The oral administration of an aqueous mixture of calabash chalk lasted for twenty-eight (28) days during which the animals were allowed access to standard rat chow and water *ad libitum*.

Blood collection and assay

Animals were anaesthetized using cervical dislocation and blood was collected by cardiac puncture. Blood samples were transferred into EDTA bottles for haematological assay (Automatic Haematology Analyzer, Mindray, China).

Ethical considerations

This study was carried out in line with the highest ethical standards concerning animal experiments. Animals were housed under standard conditions and were humanely sacrificed at the end of the experiment. The research design and protocol were approved by the University of Port Harcourt Research Ethical Committee.

Statistical analysis

The mean and standard error of mean were determined using SPSS version 25. The one-way ANOVA followed by an LSD post hoc analysis was used to determine the difference in means among the groups. The difference in means was considered significant at $P<0.05$.

Results

Table 1 shows the composition of the various chemical compounds found in calabash chalk. Kaolinite was found to be the most predominant compound (44.7%) and followed closely by quartz (38.3%).

Table 1. Chemical composition of calabash chalk

Chemical Compound	Chemical formula	Amount (%)	Peaks matched	Peaks in range	Number of peaks
Kaolinite	$AL_2H_4O_9Si_2$	44.7%	55	91	506
Quartz	O_2Si	38.2%	14	14	98
PN (NH)	HN_2P	7.6%	6	6	74
	H_6MgO_6Si	5.3%	18	18	374
	$ALO_{25}Li_{25}O_2SiO_{75}$	3.8%	12	12	102
Caesium	Cs	0.4%	6	6	42

Table 2 shows the elemental composition of the sample of calabash chalk. Oxygen (52.38%) and silicon (31.18%) were the predominant elements followed by aluminium (9.35%).

Table 2. Elemental composition of calabash chalk

Element	Amount (weight %)
Oxygen (O)	52.38%
Silicon (Si)	31.89%
Aluminium (Al)	9.35%
Phosphorus (P)	3.92%
Nitrogen (N)	3.54%
Hydrogen (H)	1.03%
Magnesium (Mg)	0.83%
Cesium (Cs)	0.42%

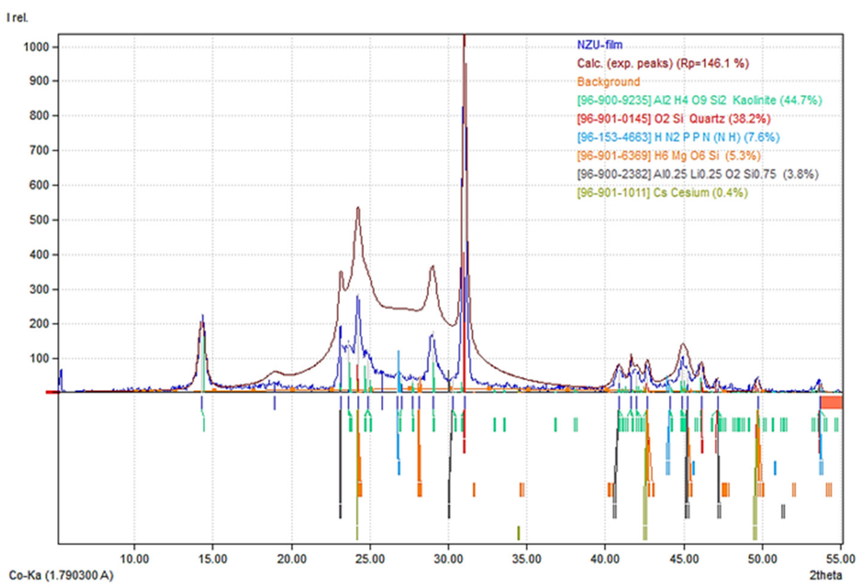

Figure 1. Diffraction pattern of the chemical components of calabash chalk

Table 3 shows the effect of oral 28 days administration on the mean values of red blood cell count (RBC), haemoglobin concentration (HB), packed cell volume (PCV), mean corpuscular volume (MCV), and mean corpuscular haemoglobin (MCH) of Wistar rats. The result of the study shows that there was a significant decrease in RBC, HB and PCV among the experimental groups (Groups 2-4) compared to the control ($p<0.05$).

Table 3. The effect of calabash chalk on haemoglobin concentration, packed cell volume, red blood cell and red blood cell indices of Wistar rats

Parameters	Control (distilled water) (n=5)	400 mg/kg (n=5)	800 mg/kg (n=5)	1600 mg/kg (n=5)
RBC ($\times 10^{12}$/L)	7.63±0.21	6.84*±0.13	6.41*±0.18	7.00±0.13
HB (g/dl)	14.56±0.13	12.74*±0.35)	12.46*±0.33	13.46*±0.33
PCV (%)	46.64*±0.96	42.96*±0.81	40.50*±1.22	42.30*±0.96
MCV (fL)	61.13±0.95	62.87±1.42	63.35±2.18	59.08±0.59
MCH (pg)	19.11±0.43	18.61±0.29	18.99±0.33	18.87±0.33
MCHC(g/dL)	31.27±0.53	29.68±0.91	29.68±1.06	31.94±0.22

The result is given as mean± standard error of mean
*Significantly different compared to control ($p<0.05$)

Table 4 shows the effect of oral 28 days administration of calabash chalk on total white blood cells (WBC), lymphocyte and neutrophil counts, mid-range absolute count (MID), platelet count (PLT) and the plateletcrit (PCT). The result indicates that there was a significant decrease in the mean values of WBC, neutrophils, lymphocytes, MID and among the experimental rats (Groups 2- 4) compared to the control group (Group 1) ($p<0.05$).

Table 4. Effect of calabash chalk on total white blood cells, white blood differentials and platelets of Wistar rats

Parameters	Control (distilled water) (n=5)	400 mg/kg (n=5)	800 mg/kg (n=5)	1600 mg/kg (n=5)
WBC ($\times 10^9$/L)	7.34±1.23	5.60*±0.46	5.24*±0.71	5.12*±0.24
Lymphocytes ($\times 10^9$/L)	5.60±0.43	4.50*±0.45	3.90*±0.48	2.94*±0.23
Neutrophil ($\times 10^9$/L)	1.24±0.23	0.68*±0.04	0.94±0.22	0.58*±0.05
MID ($\times 10^9$/L)	0.64±0.11	0.40*±0.03	0.40*±0.07	0.32*±0.02
Platelets	860.60±43.48	715.61*±46.67	659.60*±33.63	743*.00±46.67
PCT	0.85±0.12	0.61*±0.04	0.58*±0.04	0.64*±0.04

The result is given as mean± standard error of mean
*Significantly different compared to control ($p<0.05$)

Figure 2 shows the effect of oral 28 days administration of calabash chalk on the percentage of body weight changes of Wistar arts. There was a significant reduction in the percentage of weight changes among the experimental animals compared to the control ($p<0.05$).

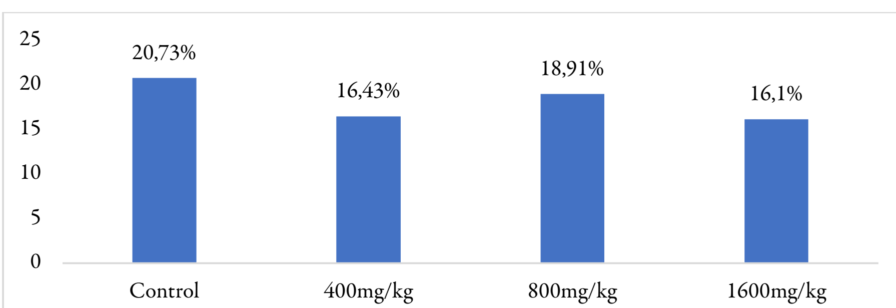

Figure 2. Effect of calabash chalk on body weight of Wistar rats

Discussion

The deliberate consumption of calabash chalk is one of the common forms of geophagia. The present study evaluated the effect of calabash chalk on haematological parameters and body weight changes of Wistar rats. Results from this study showed that our sample of calabash chalk as determined by X-Ray diffraction contains significant quantities of kaolinite (44.7%, quartz (38.2%), PN(NH) (7.6%), and caesium (0.4%). Also, the elemental analysis indicated the rich presence of oxygen, silicon, aluminium, phosphorus, nitrogen, hydrogen, magnesium, and caesium. This is similar to the elemental composition reported in other studies (Dean *et al.*, 2004; Abrahams *et al.*, 2013; Ekong *et al.*, 2015; Ebana *et al.*, 2021) with more major and trace elements observed, although with slight variations. However, Dean *et al*. (2004), Abraham *et al*. (2013), and Ekong *et al.* (2015) did not observe the presence of silicon and caesium. These could be attributed to the environmental differences from where the calabash chalk was sourced.

Results obtained from the haematological evaluation of the female Wistar following the oral administration of an aqueous mixture of calabash chalk show a significant reduction in the mean values of RBC, HB and PCV among the experimental groups (Groups 2-4) compared to the control ($p<0.05$). This could be attributed to the presence of kaolinite (44.77%) as a major component of calabash as observed in this study (Table 1). Kaolinite $Al_2Si_2O_5(OH)_4$ is one of the most abundant and common clay geomaterials on earth with several useful pharmaceutical applications (Awad *et al.*, 2017). However, kaolin ingestion has been associated with iron deficiency anaemia (Geissler *et al.*, 1998; Von Garnier *et al.*, 2008). Kaolinite reduces the bioavailability of iron for absorption (Mogongoa *et al.*, 2011). The negatively charged surface of kaolinite can exchange and adsorb cations (Fe^{2+} and Fe^{3+}) in the duodenum, where iron absorption occurs (Von Garnier *et al.*, 2008). The production of red blood cells remains the single largest consumer of iron in the body, hence any impairment in iron absorption leading to a reduction in iron will automatically affect erythropoiesis (Papanikolaou and Pantopoulos, 2017; Rishi and Subramaniam, 2017). Also, parasitic infections due to kaolin consumption have been shown to cause iron loss, further reducing the amount available for erythropoiesis (Mogongoa *et al.*, 2011). Furthermore, the presence of quartz (Table 1) in a significant quantity (38.20%) could be another probable cause of iron loss leading to the observed reduction in RBC, PCV and HB of animals exposed to calabash chalk. Quartz has been known to cause intestinal irritations and abrasion and consequent reduction of the absorption of vital micro and macro elements required for erythropoiesis (Von Garnier *et al.*, 2008). Similar studies showing possible erythropoietic depression due to calabash chalk have been reported elsewhere (Patterson and Staszak, 1977; Akpantah *et al.*, 2010; Ogbuagu *et al.*, 2017; Rishi and Subramaniam, 2017).

The present study also indicates that there was a significant decrease in the mean values of WBC, neutrophils, lymphocytes, MID, PCT and platelet count among the experimental rats compared to the control group ($p<0.05$) (Table 4). WBCs are very important components of the immune system which help the body to fight against infections. This result shows that calabash chalk may have depressed immune reactions in some way. This may be due to the excessive phagocytosis of foreign toxins and parasites found in calabash chalk (Dean *et al.*, 2004). Also, the presence of abrasive quartz limits intestinal absorption of vital nutrients required for leucopoiesis. However, Ogbuagu *et al*. (2017) and Akpantah *et al*. (2010) reported higher WBCs and its differentials. The disparity could be due to the source of the calabash chalk used in the present study as different sources of calabash chalk are likely to have different toxins and parasites. Platelets are often activated as a result of bleeding or injury as they clump together and plug up at bleeding sites (Minors, 2007; Clemetson, 2012). The observed decrease in the mean platelet count and plateletcrit (PCT) could be due to possible haemorrhage in the stomach of the animals caused by calabash chalk (Moses *et al.*, 2012).

As the Wistar continued to gain weight throughout the experiment, the percentage of body weight changes was found to reduce among the experimental animals compared to the control ($p<0.05$). It is possible that calabash chalk interfered with the normal nutrient absorption (Akpantah *et al.*, 2010; Moses *et al.*, 2012) or it may have played a role in appetite suppression and reduction of lipid profile (Alli and Nafiu, 2017) among the Wistar rats.

Conclusions

The present study has demonstrated the effect of calabash chalk on the haematological profile of Wistar rats. It shows that despite the wide safety margin of calabash chalk, it has the potential to depress haematopoiesis and reduce body weight. Oral administration of calabash chalk reduced the red blood cell, white blood cell, platelet counts and body weight at 400, 800 and 1600 mg/kg.

Authors' Contributions

All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

The research design and protocol were approved by the University of Port Harcourt Research Ethics Committee (UPH/CEREMEAD/REC/MM76/018).

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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