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Original Article

DETERMINATION OF ELEMENTAL COMPOSITION AND MEDIAN LETHAL DOSE OF CALABASH CHALK

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ABSTRACT

Calabash chalk is a geophagic material consumed mostly for its emetic function, though other reasons are not rule out. A report of a sample analysis revealed toxic metals/metalloids, as well as persistent organic pollutants. This lead to this study to investigate the elemental composition of a sample from southern Nigeria, and to determine its median lethal dose. Non-salted calabash chalk was analyzed for the presence of some major and trace elements using inductively coupled plasma-mass spectrometry, and their concentrations was determined by inductively coupled plasma-quadrupolar mass spectrometry. The 'limit test' in the 'up and down' procedure was used to determine the median lethal dose (LD50) with up to 5000 mg/kg body weight of calabash chalk suspension on mice. Analysis results revealed magnesium (1100±100 mg/kg±SD), aluminium (160000±15000 mg/kg±SD), potassium (5500±500 mg/kg±SD), calcium (160±14 mg/kg±SD), titanium (11000±1000 mg/kg±SD), vanadium (125±10 mg/kg±SD), chromine (130±10 mg/kg±SD), manganese (40±5 mg/kg±SD), iron (15000±1500 mg/kg±SD), cobalt (4.1±0.2 mg/kg±SD), nickel (25.5±1.3 mg/kg±SD), copper (15.5±0.7 mg/kg±SD), arsenic (11.5±0.8 mg/kg±SD), silver (0.50±0.03 mg/kg±SD), cadmium (0.76±0.04 mg/kg±SD), antimony (0.42±0.02 mg/kg±SD), barium (200±10 mg/kg±SD), thallium (0.33±0.02 mg/kg±SD), lead (57±3 mg/kg±SD) and zinc (<100mg/kg). No mortality and/or toxicity was recorded with up to 5000 mg/kg body weight of the single dose treatment of calabash chalk. In conclusion, calabash chalk contains many biological beneficial, as well as adverse elements depending on their concentrations in the chalk, as well as their bioavailability. Though this chalk may be relatively less toxic in acute usage, the opposite may apply in chronic usage; hence, its use is discouraged.

1. Introduction

Calabash chalk is also known as poto in English, la craie in French, and mabele in Lingala of Congo. In Nigerian languages, it is called nzu in Igbo and ndom in Efik/Ibibio. The chalk is commercially available, and may be sold in blocks, as large pellets, and in powder forms [1]. It may be packaged in a clear plastic bag with or without labelling, or sold without any packaging, and in a variety of forms. Reports show that the ones commonly ingested in Africa contains phosphorus, potassium, magnesium, copper, zinc, manganese, and iron [2,3]. The chalk could be naturally occurring or artificially formulated. The naturally occurring chalk is chiefly made up of fossilized seashells, while the artificial form may be prepared from clay and mud which may be mixed with other ingredient including sand, wood ash and sometimes salt. The resulting product is moulded and then heated to produce the final product [1].

Calabash chalk is generally made up of aluminium silicate hydroxide, which is a known member of the kaolin clay group, with the formula: Al2 Si2 O5 OH4 [4]. Several other contaminants which could be poisonous to the body have also been reported [4, 5, 6, 7]. These includes; metals, metalloids and persistent organic pollutants [4]. The metals include; iron, aluminium, potassium, titanium, barium, chromium, zinc, manganese, nickel, rubidium, copper, tin and lead as shown in Table 1 [4], and the metalloid being arsenic [4, 5. 6. 7]. The organic pollutants as reported include alpha lindane, endrin, endosulphan II and p, pI-dichloro diphenyl dichloroethane (DDD) [4].

Not much researches have reported the effect of calabash chalk on the biological system. Reports on animal models revealed sinusoidal enlargements and fragmented liver parenchyma, and depletion of red blood cells [8, 9, 10]. Ekong et al [11, 12] reported oedema and haemorrhages in the mucosa of the stomach, as well as acanthosis, hyperkeratosis, and koilocytic changes in the mucosa of the oesophagus, and the alteration of growth rate and de-mineralization of the femur bone. A recent report on the chalk showed anxiogenic potentials and cerebral cortical alteration [13].

The calabash chalk is reported to be eaten by pregnant and post-partum women, as well as children across societal class, and in different societies. This study thus investigated the elemental composition of the chalk obtained from the southern region of Nigeria and its median lethal dose using mice.

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MATERIALS AND METHODS

Two large blocks of calabash chalk (non-salted) obtained naturally from the same source and prepared by roasting in fire was obtained from a local market in Ikot Omin, Calabar. It was chopped into small pieces and grounded into fine powder with the aid of a manually operated grinder. The calabash chalk was then analysed for some major (potassium, calcium, magnesium) and trace (iron, copper, aluminium, cobalt, manganese, nickel, barium, chromine, lead, cadmium, titanium, vanadium, zinc, arsenic, silver, antimony and thallium) elements, using the flame atomic absorption spectrometer (Unicam-939).

Analysis of the calabash chalk

Trace and major elements concentrations were measured using inductively coupled plasma-mass spectrometry (ICP-MS) on totally digested samples. One hundred milligram samples were totally dissolved by successive additions of nitric acid (HNO3) and hydrochloric acid (HCl) mixture, hydrogen fluoride (HF), and perchloric acid (HClO4) in Teflon vessels using a heating block (Digiprep, SCP Science). Ultra pure reagents were used (Normatom grade, VWR, France for HNO3, and HCl, ''for trace metal analyses'', Baker, SODIPRO, France). The solutions were evaporated to dryness, retaken 3 times in 2 mL of HNO3 and then diluted with 50 mL of MilliQ water. The concentrations were determined by inductively coupled plasma-quadrupolar mass spectrometry (ICP-QMS) (X Series, Thermo-Electron, France).

To correct for instrumental drifts and plasma fluctuations, all solutions were spiked with rhodium (Rh) and rhenium (Re) standard solutions (SPEX, SCP Science, France) to a final concentration of 10 mg/L for Rh and 1 mg/L for Re. To minimise isobaric interferences, analysis with the Collision Cell Technology (CCT) introducing a supplementary gas mixture of H2 (7 %) and He (93 %) was applied for Fe, Mn, and the 6 metals studied (Cd, Cr, Cu, Ni, Pb, Zn). The solutions were weighted at each step of the dilution and spiking operations. The data quality was controlled with lake sediment reference material SL1 (from the International Atomic Energy Agency, Vienna). The values obtained agreed within 10% of the certified values.

Treatment for the median lethal dose (LD50)

The 'limit test' in the 'up and down' procedure was used to determine the median lethal dose (LD50). Twenty-five female albino mice were divided into 5 groups of 5 mice each. The animals were handled according to the ethical guidelines of the National Institute of Health (NIH) of the United States. A maximum of 5 animals per group were sequentially administered with calabash chalk suspension up to a test dose of 5000 mg/kg [14, 15, 16] as shown in Table 3.

Forty grams of the calabash chalk was dissolved in 1000 mL of distilled water in a glass jar. Since calabash chalk is partially miscible with water, it was administered as suspension, stirred prior to the administration. Groups 1-5 were administered respectively, 1000, 2000, 3000, 4000 and 5000 mg/kg body weight of calabash chalk suspension (Table 3). The limit test involved dosing one animal with up to 5000 mg/kg body weight. When the animal survived, two additional animals were dosed. When both animals survived, the LD50 was said to be greater than the limit dose and the test was terminated (i.e. carried to full 14-day observation without dosing of further animals).

RESULTS

The presence and quantities of the different metallic elements in the calabash chalk is presented in Table 2. Analysis revealed the following; magnesium (1100±100 mg/kg±SD), aluminium (160000±15000 mg/kg±SD), potassium (5500±500 mg/kg±SD), calcium (160±14 mg/kg±SD), titanium (11000±1000 mg/kg±SD), vanadium (125±10 mg/kg±SD), chromine (130±10 mg/kg±SD), manganese (40±5 mg/kg±SD), iron (15000±1500 mg/kg±SD), cobalt (4.1±0.2 mg/kg±SD), nickel (25.5±1.3 mg/kg±SD), copper (15.5±0.7 mg/kg±SD), arsenic (11.5±0.8 mg/kg±SD), silver (0.50±0.03 mg/kg±SD), cadmium (0.76±0.04 mg/kg±SD), antimony (0.42±0.02 mg/kg±SD), barium (200±10 mg/kg±SD), thallium (0.33±0.02 mg/kg±SD). lead (57±3 mg/kg±SD), zinc (<100mg/kg).

The mice were administred a calabash chalk suspension of 40 grams dissolved in 1000 mL distilled water, and the groups were administered a maximum of 5000 mg/kg body weight calabash chalk suspension. At 5000 mg/kg body weight of the single dose treatment of calabash chalk, no mortality and/or toxicity was recorded. Therefore, calabash chalk may have a median lethal dose (LD50) of over 5000 mg/kg body weight (Table 3).

Table 1: Energy dispersive X-ray fluorescece spectroscopy (EDXRF) analysis of calabash chalk samples as reported by Dean et al (2004)

Element	Within sample variation Mean±sd (mg/kg) (n = 5)	Between sample Variation Mean±sd (mg/kg) (n = 5)	Comparison of two experimental means t-Test significant
Aluminum (Al)	8856±176	8630±152	No
Potassium (K)	1618±25	1372±63	Yes
Titanium (Ti)	8052±134	7230±98	Yes
Chromine (Cr)	52.5±1.6	49.6±5.9	No
Manganese (Mn)	24.1±2.2	24.0±2.1	No
Iron (Fe)	14 770±86	14 402±155	No
Nickel (Ni)	15.5±0.8	15.0±1.3	No
Copper (Cu)	1.8 ±0.5	3.1 ±0.5	No
Zinc (Zn)	26.9±0.9	25.6±1.8	No
Arsenic (As)	Nd	nd	
Rubidium (Rb)	13.4±0.2	12.0±0.6	Yes
Strontium (Sr)	85.9±1.1	78.2±3.8	No
Yttrium (Y)	17.4±0.3	19.8±1.6	No
Zirconium (Zr)	355.3±1.4	337.7±24.2	No
Niobium (Nb)	72.9±0.62	67.1±2.6	Yes
Cadmium (Cd)	nd	nd	
Tin (Sn)	6.2 ±0.7	6.2 ±0.7	No
Antimony (Sb)	nd	nd	
Barium (Ba)	226.5±6.0	230.9±5.9	No
Cerium (Ce)	266.2±7.4	243.6±9.6	No
Mercury (Hg)	nd	nd	
Lead (Pb)	42.5±1.2	36.4±2.8	No

nd- Arsenic, cadmium, antimony and mercury were not detected in the samples analyzed

Dean et al (2004)

Table 2: Analysis of calabash chalk showing the concentration of different elements

Elements analyzed	Concentration of elements (mg/kg±SD)
Magnesium (Mg)	1100±100
Aluminium (Al)	160000±15000
Potassium (K)	5500±500
Calcium (Ca)	160±14
Titanium (Ti)	11000±1000
Vanadium (V)	125±10
Chromine (Cr)	130±10
Manganese (Mn)	40±5
Iron (Fe)	15000±1500
Cobalt (Co)	4.1±0.2
Nickel (Ni)	25.5±1.3
Copper (Cu)	15.5±0.7
Arsenic (As)	11.5±0.8
Silver (Ag)	0.50±0.03
Cadmium (Cd)	0.76±0.04
Antimony (Sb)	0.42±0.02
Barium (Ba)	200±10
Thallium (Tl)	0.33±0.02
Lead (Pb)	57±3
*Zinc (Zn)	<100mg/kg

Results are presented as Mean ± Standard deviation

*A precise figure for zinc (Zn) concentration could not be arrived at because of some analytical problems. So Zn concentration can be said to be lower than 100 mg/kg.

Table 3: Calabash chalk treatment for the determination of median lethal dose (LD50) in mice

Groups n=5	Dose of calabash chalk suspension (mg/kg)	Mice mortality
1	1000	Non
2	2000	Non
3	3000	Non
4	4000	Non
5	*5000	Non

***Ld_{50} is over 5000 mg/kg, and the administration was discontinued**

DISCUSSION

The calabash chalk is reported to be eaten by pregnant and post-partum women, as well as children across societal class, and in different societies [17]. Thus, it poses a risk to the consumers due to the various inherent toxic constituents [4, 5, 7, 18]. This study, thus investigated the elemental composition of the chalk and its median lethal dose using mice.

Analysis of the chalk revealed the following minerals; iron, magnesium, potassium, manganese, calcium, copper, zinc among others. These minerals are known to be beneficial in the biological systems of both plants and animals [19, 20, 21, 22, 23].

The presence of these minerals portrays the chalk as useful for nutritional purposes, which may justify its consumption.

Other elements present, and whose effects on biological system is uncertain includes; barium and titanium [24, 25]. However, the presence of metals such as aluminium, lead, arsenic, chromine, vanadium and cadmium in the chalk depending on its composition and bioavailability, may however not be beneficial. The harmful/toxic nature of these metals have also been established [26, 27, 28]. Thus, the usefulness of the chalk may not supercede the potential toxic effects due to the presence of these adverse elements. However, it is reported that the bioavailability of these elements in similar chalk samples may not result in any serious consequences [29], a situation that may also apply in this study. Hence, calabash chalk may play a dual role in the biological system due to its mixed benefical and adverse chemical composition.

The calabash chalk sample in this study exhibited concentration values for trace elements in soil sample generally [30, 31], which indicates its soil-like nature. However, this is at variance with a previous report where the concentration values for trace elements was below the average [29]. The clay-rich calabash chalk sample in this study also showed probably significant absorption potential for other pollutants not reported. Previous reports has shown the likely presence of microbes, as well as persistent organic pollutants [4, 29].

The composition of the chalk in this study is to an extent, at variance with that of previous studies [4, 29]. Their report on calabash chalk's composition did not include cobalt, vanadium, arsenic, antimony and silver. Also, the concentration of the elements in the present study was higher compared with these other studies [4, 29]. Another report by Campbell [5], showed that the calabash chalk contained both arsenic and lead. The actual concentration of these elements were not provided.

The difference between this study and the previously reported ones could be due to differences in the environment where both chalks were obtained. In the study by [32], the samples were obtained in Jos and Zaria in northern Nigeria, while Dean et al [4] obtained theirs in a retail store in Newcastle upon Tyne, with its origin not known. Campbell [5] also did not revelaed the origin of the calabash chalk analyzed. In the present study, the chalk was obtained in southern Nigeria which is replete with crude oil and other natural mineral reserves [32, 33, 34]. These mineral deposits could have influenced the concentration of the reported elements found in the calabash chalk of this study.

Though the actual quantities of arsenic in calabash chalk has not been reported previously, the quantity of lead as reportedly contained in the chalk is approximately 40 mg/kg [4], an amount that high when food safety levels is not supposed to exceed 1 mg/kg [35, 36]. Consumption of the chalk sample would result in an undesirable increase in lead intake because as high as 60g of calabash chalk, with an average of 20g can be consumed a day [29]. The lead level involved may amount to about 4.5 fold the safety guideline, before even taking into account any additional exposure from other sources [37]. A report also has it that young adults with higher blood lead levels were more likely to have major depressive disorder (MDD) or panic disorder [38].

Exposure to the higher levels of lead and arsenic in the present study by pregnant and breast feeding women, poses a risk to the mental development of their developing unborn babies and breast feeding infants, respectively [5, 7, 18]. The high lead level may also result cancers of the urinary bladder, lungs and skin [7], because lead, a toxic metal is known to induce a broad range of physiological, biochemical, and behavioural dysfunctions in laboratory animals and humans [39], including central and peripheral nervous systems [40], haematopoietic system [41], cardiovascular system [42], kidneys [43], liver [44], and reproductive systems [45, 46]. However, the nervous system damage is considered the most serious. Neurological and visual alterations (including retina) have been reported with low lead concentration particularly in the developing nervous system [40}.

In this study, calabash chalk showed the LD50 of over 5000 mg/kg body weight. This indicates that calabash chalk has low acute toxicity hazard because of the high LD50 of over 5000 mg/kg body weight. Thus, this chalk may be classified under 'Hazard Category 5' in the Globally Harmonised Classification System for Chemical Substances and Mixtures (GHS) [14, 15, 16].

CONCLUSIONS

Analysis of calabash chalk has shown several metals and metalloid with many being biologically beneficial, and some either of unknown function or adverse. Though it may be relatively non-toxic in acute usage, the chronic usage may be toxic; hence, its consumption is discouraged.

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