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Serum Lipid Profile and Electrolyte Concentration in Rats Administered Calabash Chalk

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Authors' contributions

This work was carried out in collaboration between both authors. Author ALA designed the study, wrote the protocol and the first draft of the manuscript. Author NMO managed the literature review, managed the analyses of the data and performed the statistical analysis. Both authors read and approved the final manuscript.

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ABSTRACT

Introduction: Geophagia is the act of consumption of earth's materials such as clay, soil or chalk-like stones. It is a common and sometimes compulsive practice among pregnant women in Eastern part of Nigeria. The study aims at investigating the effects of calabash chalk (geophagic clay) on serum lipid profile and electrolytes in rats in order to understand the possible effects of calabash chalk in human.
Place and Duration of Study: Research work was carried out in department of Biochemistry, Faculty of Life Sciences, University of Ilorin, Ilorin, Nigeria; between March and August, 2015.
Methods: Processed calabash chalk (PCC) and unprocessed calabash chalk (UCC), weighing 400 g each was pulverized separately into powder and sieved. The sieved powder (200 g of UCC and PCC each) was mixed with 1 L of distilled water and stirred continuously to form a suspension, which was filtered. The filtrate was evaporated to obtain 20 g of processed and 15 g of unprocessed calabash chalk respectively. The required doses of 150, 250 and 500 mg/kg body

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weight were prepared using distilled water and administered to rats daily for twenty-eight days. Lipid profile and biochemical parameters were analyzed from blood collected from animals on 29th day.

Results: The results obtained, showed that both processed calabash chalk (PCC) and unprocessed calabash chalk (UCC) at all the doses, produced significant reduction on lipid profile. There was significant increase in bilirubin level at 500mg, while albumin and urea concentration were significant reduced at 150 mg/kg b.w when compared with the control.

Conclusion: In view of the above results, ingestion of calabash chalk could cause imbalance in lipid and electrolyte profile which may constitute a serious health risk especially among pregnant women.

Keywords: Calabash chalk; geophagia; lipid profile; electrolyte concentration.

1. INTRODUCTION

Geophagia, the act of eating soil, clay and chalk, is an old human practice that is still common but commonly overlooked in many cultures [1], especially among pregnant women where it is consumed as a palliative for vomiting and morning sickness [2]. This practice of eating geophagic materials (also referred to as Pica) is associated with religious beliefs, medicinal and dietary purposes [3]. For some pregnant women, eating clay could be a compulsive action, in view of the craving to consume the geophagic materials in pregnancy [4,5].

Calabash Chalk is found in Eastern part of Nigeria and other West African communities. It is also sold in ethnic stores and markets in the United States of America and the United Kingdom [6,7]. Different names have been ascribed to this chalk, such as; Calabar stone (English), La Craie or Argile (French), Mabele by Lingala (Congo), Nzu by Igbo (Nigeria) and Ndom by the Efik in Nigeria [8]. Calabash Chalk is also known according to language/locality as Argile, Calabar stone, Calabash clay, Ebumba, Lacraie, Mabele, Ndom, Poto and Ulo. Nzu is a commonly adopted term used for naming these geophagical material in Nigeria [9].

Geophagic clay materials consumed in Nigeria are mainly hydrated silicates of Aluminium, Sodium, Calcium, Magnesium, or/and Iron. An assessment of heavy metals contamination of calabash chalk, based on the index of geo-accumulation (I{geo}) shows that Cr, Cu, Zn, Co, and Ni (all with I{geo} < 1) did not contaminate the clay materials. On the contrary, they are highly contaminated by As and Cd (I{geo} = >5), and are moderately to strongly contaminated by Pb (I {geo} = 2-3) [7].

The chemical structure of calabash chalk (Nzu) as described by Abrahams et al. [10] is 6-(2H-indazol-4-yl)-1-methyl-N-(3-methylsulfonylpropyl) pyrazolol [3,4-d] pyrimidin-4-amine, with a molecular weight of 385.44 daltons. Most of the calabash chalk samples are clay-rich soil materials that have been dried and/or baked into block or spherical unit [9]. The processed calabash chalk (PCC) is prepared using clay, wood-ash and salt. The soil/clay is mixed with common salt (sodium chloride), mold and then heated in a hot furnace to reduce the risks of contamination from pathogens [11]. Calabash chalk that has not undergone any processing but sold and consumed in the natural state is referred to as unprocessed calabash chalk (UCC). Calabash chalk is available in a variety of forms including powder and solid molds.

Consumption of geophagic materials have been associated with micronutrient deficiency, heavy metal poisoning and anaemia in pregnancy [4,12,13].

2. MATERIALS AND METHODS

2.1 Calabash Chalk

Processed and unprocessed calabash chalks were obtained from “Kubwa village” market, Kubwa, Abuja, Nigeria.

2.2 Laboratory Animals

Thirty-five healthy albino rats (*Rattus novergicus*) of both sexes weighing 170 ± 20 g were obtained from the animal holding unit, Department of Biochemistry, University of Ilorin, Ilorin, Nigeria. All the animals were kept in plastic cages that provided access to rat pellets (Bendel Feeds and Flour Mills Ltd., Ewu, Nigeria) and clean water *ad libitum*.

2.3 Reagents

Assay kits for serum lipid profile, electrolytes, urea and creatinine were products of Randox Laboratories Ltd., United Kingdom. Other reagents used, which were of analytical grades, were prepared with distilled water and kept in clean air-tight reagent bottles.

2.4 Methods

2.4.1 Preparation of aqueous calabash chalk

Processed calabash chalk (PCC) and unprocessed calabash chalk (UCC), weighing 400 g each was pulverized separately into powder, with ceramic mortar and pestle, and sieved through a nylon mesh. 1000 ml of distilled water was added to 200 g of each powder calabash chalk (processed and unprocessed) and stirred continuously, using magnetic stirrer, for 3 hours at room temperature. The suspension was then filtered using Whatman filter paper to remove debris. The filtrate was evaporated in a water bath at 40℃ to obtain 20 g of processed and 15 g of unprocessed calabash chalk respectively. The required doses of 150, 250 and 500 mg/kg body weight were prepared from this residue daily for twenty-eight days using distilled water.

2.4.2 Administration of calabash chalk to rats in 28 days study

Thirty-five albino rats were randomly divided into seven groups (A-G) of 5 rats each. Group 1 (control) received 10 ml/kg body weight of distilled water, groups B, D and F received 150, 250 and 500 mg/kg b.w of unprocessed calabash chalk (UCC) respectively, while groups C, E and G were administered 150, 250 and 500 mg/kg b.w of processed calabash chalk (PCC) respectively.

All the animals in each group were administered extract and distilled water (control) orally for 28 consecutive days using oral cannula.

Group A: Control group – received distilled water
Group B: received 150 mg/kg body weight unprocessed calabash chalk.
Group C: received 150 mg/kg body weight processed calabash chalk.
Group D: received 250 mg/kg body weight unprocessed calabash chalk.
Group E: received 250 mg/kg body weight processed calabash chalk.
Group F: received 500 mg/kg body weight unprocessed calabash chalk.
Group G: received 500 mg/kg body weight processed calabash chalk.

2.4.3 Preparation of serum

The rats were individually weighed and then anaesthetized with diethyl ether on the 29th day. They were dissected to expose the heart and blood was collected by cardiac puncture into labelled plain tubes. The blood was centrifuged at 3000 rev/min for 10 minutes to obtain serum which was collected using a Pasteur pipette into clean, dry sample bottles and kept in refrigerator at -4℃ until required for assay.

2.5 Serum Electrolyte Analysis

2.5.1 Electrolyte analysis

The effect of daily administration of aqueous extract of Calabash Chalk (Nzu) for twenty-eight days on the serum electrolyte concentration of the rats was determined using the method described by Callahan [14].

2.5.2 Lipid profile analysis

The level of triacylglycerol was determined using the method described by Tietz [15]. The level of cholesterol was determined using enzymatic saponification procedure as described by Allain et al*.* [16]. The HDL level was determined using the method described by Bachorik [17]. The LDL Cholesterol level was calculated using the formulae as described by Friedewald et al*.* [18]. The Friedewald equation is as follows:

LDL Cholesterol (mmol/l) = Total Cholesterol – [VLDL] – [HDL-Cholesterol].

The value of VLDL Cholesterol was determined using the formulae described by Friedewald *et al.* [18]. The equation is as follows:

$$\text{VLDL (mmol/l)} = \frac{\text{TRIGS}}{2.2}$$

2.5.3 Determination of biochemical parameters

Serum albumin, globulin, bilirubin, urea and creatinine were determined by standard procedures in an auto analyzer using biochemistry kits (Randox laboratories UK).

2.6 Statistical Analysis

Data were expressed as mean ± standard error of mean (SEM) of five replicates. Statistical analysis was done using Graphpad Prism version 5.0 (Graphpad software). The differences between the means were compared using analysis of variance (ANOVA) followed by Student's t-test. *P* = .05 was considered statistically significant.

3. RESULTS

3.1 Serum Lipid Profile

Table 1 and 2 showed a significant decrease in the serum total cholesterol in the groups administered with 150 and 250 mg/kg b.w. of processed calabash chalk (PCC). A significant reduction in serum total cholesterol was also recorded in the group administered with 250 and 500 mg/kg b.w. dose of unprocessed calabash chalk (UCC). Significant decrease in the serum triacylglycerol, high density lipoprotein (HDL) and very low density lipoprotein (VLDL) was also recorded at all the doses of processed and unprocessed calabash chalk administered.

3.2 Effect of Administration of Processed Calabash Chalk (PCC) on Biochemical Parameters in Rats

Table 3 showed that there is a significant reduction in concentration of albumin at 150 and 500 mg/kg b.w while bilirubin concentration at 500 mg/kg b.w was significantly elevated when compared with the control. The Urea concentration showed significant reduction only in group administered with 150 mg/kg b.w, when compared with the control. The Creatinine concentrations showed no significant difference in all groups when compared with the control.

Table 1. The serum lipid profile of albino rats administered with processed calabash chalk (PCC)

Lipid profile (mg/dL)	Control (mg/kg b.w)	150 mg/kg b.w	250 mg/kg b.w	500 mg/kg.bw
Total cholesterol	360.33 ± 27.01	134.82 ± 3.72*	234.80 ± 49.78*	317.33 ± 8.22
Triglyceride	546.25 ± 75.86	248.60 ± 38.89*	160.04 ± 23.47*	299.80 ± 28.13*
HDL	134.82 ± 12.67	50.98 ± 5.12*	66.06 ± 20.43*	104.07 ± 10.67
LDL	151.86 ± 39.56	33.40 ± 8.45*	123.86 ± 51.68	104.53 ± 16.68*
VLDL	106.56 ± 19.27	49.72 ± 7.78*	32.01 ± 4.70*	59.96 ± 7.29*

*Values are mean ± SD of 5 replicates. * significantly different from control at p < 0.05*

Table 2. The serum lipid profile of albino rats administered with unprocessed calabash chalk (UCC)

Lipid profile (mg/dL)	Control (mg/kg b.w)	150 mg/kg b.w	250 mg/kg b.w	500 mg/kg.bw
Total Cholesterol	360.33 ± 27.01	382.45 ± 31.85	90.25 ± 6.01*	280.50 ± 48.85*
Triglyceride	546.25 ± 75.86	368.23 ± 50.56*	252.17 ± 31.80*	224.28 ± 42.85*
HDL	134.82 ± 12.67	24.65 ± 3.71*	32.04 ± 5.66*	63.00 ± 2.85*
LDL	151.86 ± 39.56	308.08 ± 30.54	26.18 ± 10.66*	157.54 ± 45.34
VLDL	106.56 ± 19.27	73.65 ± 20.11*	50.44 ± 6.34*	44.88 ± 8.57*

*Values are mean ± SEM of 5 replicates. * Significantly different from control at p < 0.05*

Table 3. Effect of administration of processed calabash chalk (PCC) on biochemical parameters in rats

Dose (mg/kg b.w)	Albumin (mg/dl)	Globulin (mg/dl)	Bilirubin (mg/dl)	Urea (mg/dl)	Creatinine (mg/dl)
Control	4.93 ± 1.89	87.62 ± 1.71	0.33 ± 0.08	75.25 ± 7.87	0.73 ± 0.43
150 PCC	2.56 ± 0.78	98.75 ± 2.81	0.81 ± 0.09	50.49 ± 6.26*	0.82 ± 0.23
250 PCC	5.36 ± 1.31	99.50 ± 2.59	0.33 ± 0.09	65.14 ± 4.71	1.42 ± 0.45
500 PCC	1.82 ± 0.56*	100.02 ± 1.23	1.32 ± 0.44*	79.70 ± 22.8	1.15 ± 0.17

*Values are mean ± SD of 5 replicates. * Significantly different from control at p < 0.05*

3.3 Effect of Administration of Unprocessed Calabash Chalk (UCC) on Biochemical Parameters in Rats

Table 4 showed that there is no significant difference in all the biochemical parameters measured except for urea with a significant reduction at 500 mg/kg b.w when compared with the control.

3.4 Electrolyte Profile

3.4.1 Effect of processed calabash chalk on electrolyte profile

The different doses of PCC did not produce any significant difference in the concentrations of Na^+, K^+, Cl^-, HCO_3^-, Ca^{2+} and PO_4^{3-} (Table 5) when compared with the control.

3.4.2 Effect of unprocessed calabash chalk on electrolyte profile

The different doses of UCC did not produce any significant difference in the concentrations of Na^+, K^+, Cl^-, HCO_3^-, Ca^{2+} and PO_4^{3-} (Table 6) when compared with the control.

4. DISCUSSION

The act of eating dirt, clay or soil (Geophagia), has been reported in many regions of the world either as a peculiar behavior of isolated individuals or a cultural practice of particular societies [19].

In terms of health risk assessment of calabash chalk, heavy metals such as Arsenic, Lead and Chromium was not detectable in the calabash chalk used in this study as documented in our earlier report [11]. However, this is in contrast to the review of Lar et al*.* [7] that documented the presence of heavy metals such as As, Cd and Pb in some of the geophagic clays obtained from different regions in Nigeria. Mineral or elemental content of a soil may vary from one region/ locality to another depending on the nature/ geology of the soil and proximity to industrial waste disposal site.

Alterations in the concentration of serum lipids, such as total cholesterol, triacylglycerol, high density lipoprotein, low density lipoprotein and very low density lipoprotein, following administration of a drug/herb can give useful information on the effects of such substance on lipid absorption and metabolism [20].

The significant reduction compared with the control, in total cholesterol, triglycerides, HDL, LDL and VLDL at 150 and 250 mg/kg b.w after administration of processed Calabash Chalk (PCC) suggest that calabash chalk or some of the additives used in the processing could disrupt

Table 4. Effect of administration of unprocessed calabash chalk (UCC) on biochemical parameters in rats

Dose (mg/kg b.w)	Albumin (mg/dl)	Globulin (mg/dl)	Bilirubin (mg/dl)	Urea (mg/dl)	Creatinine (mg/dl)
Control	4.93 ± 1.89	87.62 ± 1.71	0.33 ± 0.08	75.25 ± 7.87	0.73 ± 0.43
150 UCC	4.93 ± 1.89	87.47 ± 0.04	0.42 ± 0.07	58.60 ± 4.88*	1.37 ± 0.35
250 UCC	3.79 ± 0.80	88.90 ± 2.00	0.35 ± 0.14	78.03 ± 9.91	1.55 ± 0.60
500 UCC	3.79 ± 0.63	91.26 ± 2.35	0.37 ± 0.11	55.85± 5.46*	0.27 ± 0.18

*Values are mean ± SEM of 5 replicates. * Significantly different from control at p < 0.05*

Table 5. Serum electrolytes concentration (mmol/L) of rats fed with processed calabash chalk (PCC)

Dosage (mg/kg b.w)	Na^+ (mmol/L)	K^+ (mmol/L)	Cl^- (mmol/L)	HCO_3^- (mmol/L)	Ca^{2+} (mmol/L)	PO_4^{3-} (mmol/L)
Control	43.80 ± 1.71	2.62 ± 0.20	19.00 ± 1.14	10.00 ± 0.71	2.40 ± 0.10	1.80 ± 0.06
150 PCC	43.20 ± 1.02	2.70 ± 0.18	20.40 ± 0.93	12.80 ± 1.50	1.91 ± 0.25	1.52 ± 0.09
250 PCC	43.00 ± 1.41	2.24 ± 0.24	20.00 ± 1.04	10.80 ± 0.58	1.35 ± 0.17	1.38 ± 0.16
500 PCC	46.00 ± 0.71	1.88 ± 0.28	17.75 ± 0.85	10.00 ± 0.71	1.44 ± 0.10	1.18 ± 0.35

Values are mean ± SEM of 5 replicates

Table 6. Serum electrolytes concentration (mmol/L) of rats administered unprocessed calabash chalk (UCC)

Dosage (mg/kg b.w)	Na^+ (mmol/L)	K^+ (mmol/L)	Cl^- (mmol/L)	HCO_3^- (mmol/L)	Ca^{2+} (mmol/L)	PO_4^{3-} (mmol/L)
Control	43.80 ± 1.71	2.62 ± 0.20	19.00 ± 1.14	10.00±0.71	2.40 ± 0.10	1.80 ± 0.06
150	46.60 ± 0.87	3.16 ± 0.13	20.80 ± 0.73	8.40 ± 0.60	2.35 ± 0.10	1.42 ± 0.09
250	42.20 ± 0.49	1.84 ± 0.27	20.80 ± 0.37	11.20± 0.73	1.30 ± 0.08	1.30 ± 0.07
500	46.80 ± 1.11	1.96 ± 0.30	19.60 ± 1.44	9.60 ± 1.21	1.34 ± 0.21	1.16 ± 0.13

Values are mean ± SEM of 5 replicates

absorption of lipids in rats or possibly decrease the synthetic pathway for cholesterol and lipoproteins. All the lipid profile parameters are also reduced (though not significant) at the highest dose of 500 mg/kg b.w (Table 1). The unprocessed calabash chalk (UCC) also caused significant reduction in the lipid profile parameters at 250 and 500 mg/kg b.w, when compared with the control, except LDL with a marginal increase at 500 mg/kg b.w (Table 2).

Effects of processed and unprocessed calabash chalk (PCC and UCC) on biochemical parameters were analysed to investigate the outcome of consumption of these geophagic materials on some liver and kidney functions. After administration of PCC, albumin and bilirubin were significantly reduced at 500 mg/kg b.w, while urea was significantly reduced at 500 mg/kg b.w (Table 3). The reduction in Albumin and bilirubin suggest possible effects of PCC, at 500 mg/kg b.w, on inhibition of synthetic function of the liver as it relates to albumin and bilirubin production [21]. Decrease in serum albumin level in rats, after administration of 500 mg/kg b.w PCC, could adversely affect colloidal osmotic pressure, which serves to maintain a normal blood volume and normal water content in the interstitial fluid and the tissues [22]. The albumin fraction is the most important in maintaining this normal colloidal osmotic or oncotic pressure in blood. Decrease in serum albumin concentration, may lead to fluid accumulation in interstitial space and the tissues, leading to edema which could significantly affect women consuming this clay during pregnancy.

Unprocessed calabash chalk (UCC) did not produce any significant change in the concentration of the biochemical parameters except urea, which showed significant reduction at 150 and 500 mg/kg b.w when compared with the control (Table 4).

Both PCC and UCC did not produce any significant change in all the electrolytes measured except for sodium with a mild (not significant) increase at 500 mg/kg b.w when compared with the control (Tables 5 and 6). The increase in level of sodium at 500 mg/kg of calabash chalk may be attributed to the common salt (sodium chloride) added as an additive in processing the calabash chalk. The electrolyte imbalance observed at the highest dose of 500 mg/kg b.w of both PCC and UCC is similar to other studies [7,23,24] that reported various electrolyte imbalance especially iron, in human subjects after consuming geophagic materials.

5. CONCLUSION

The results of this study showed that both processed calabash chalk (PCC) and unprocessed calabash chalk (UCC) at all the doses, produced significant reduction on lipid profile. There was significant increase in Bilirubin level at 500 mg, while albumin and urea concentration were significant reduced at 150 mg/kg b.w when compared with the control. Regular consumption of calabash chalk might pose health risk and should be discouraged especially during pregnancy.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Authors hereby declare that the "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, and all experiments were examined and approved by the ethics committee of Biochemistry Department, University of Ilorin, Nigeria.

COMPETING INTEREST

Authors have declared, no competing interests exist.

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