Original Article

Maternal Geophagy of Calabash Chalk on Foetal Cerebral Cortex Histomorphology

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Abstract

***Background*: Calabash chalk, a kaolin-base substance is a common geophagic material mostly consumed by pregnant women. This study investigated its effect on the histomorphology of the foetal cerebral cortex.**

***Methods:* Twelve gestating Wistar rats were divided equally into groups 1 and 2. On pregnancy day seven (PD7), group 2 animals were administered 200 mg/kg body weight of calabash chalk suspension, while group 1 animals served as the control and received 1 ml of distilled water, by oral gavages and for 14 days (PD7-PD20). On PD21, the dams were sacrificed, and the foetuses removed, examined for gross malformations, weighed and culled to two foetuses per mother. Their whole brains were excised, weighed and preserved using 10% buffered formalin, and routinely processed by haematoxylin and eosin, and Luxol fast blue methods.**

***Results:* The foetuses showed no morphological change, but their mean body weights was higher ($p=0.0001$). Histomorphological sections of the cerebral cortex showed hypertrophy and hyperplasia of cells in all the cortical layers, with less demonstrated Nissl and higher ($p=0.001$) cellular population compared with the control group.**

***Conclusion:* Calabash chalk cause body weight increase and histomorphological changes in the cerebral cortex of foetuses.**

Keywords: *chalk, maternal, cerebral cortex, body weight, morphology, rats*

Introduction

Calabash chalk geophagy, a common practice in Nigeria and some other sub-saharan African countries (1), is also practiced in developed countries (2). The chalk which is a mixture of clay and chalk is mostly consumed by women and children, with pregnant and postpartum women craving more for it due to their emotional states; usually attributed to feelings of misery, homesickness, depression and alienation (3).

Calabash chalk is also known as calabash clay, Calabar stones, poto, la craie or argile in French, nzu in Igbo and ndom in Efiks/Ibibios of Nigeria, and mabele in Lingala of Congo. It is naturally occurring, but there are artificially formulated forms as well (4). The naturally occurring forms is chiefly made up of fossilised seashells, while the artificial forms may be prepared from clay and mud, which may be mixed with other ingredients including sand, wood ash and sometimes salt. The resulting product is moulded and then heated to produce the final product (4).

Calabash chalk is generally made up of aluminium silicate hydroxide, a member of the kaolin clay group, with the formula: $Al_2 Si_2 O_5 OH_4$ (5). It also contains several other substances which could be poisonous to the body depending on their bioavailability (5–9). These includes; metals, metalloids and persistent organic pollutants (5,9).

There are limited reports on the biological effects of calabash chalk. Some reports on rat model revealed sinusoidal enlargements and fragmented liver parenchyma, as well as depletion of red blood cells population (10–12). Reports by Ekong et al (13,14) showed oedema and haemorrhages in the mucosa of the stomach, acanthosis, hyperkeratosis and koilocytic changes in the mucosa of the oesophagus, as well as demineralisation in the femur bone and alteration of the animals' growth rate. Another report show that the chalk causes cerebral cortical changes in dams (15), this is despite the reported lethal dose (LD_{50}) that is greater than 5000 mg/kg body weight (9). These reports presents a disturbing trend that may also affect the foetus. Hence, this study was to investigate the effect of maternal geophagy of calabash chalk on the histomorphology of the foetal cerebral cortex.

Materials and Methods

Eighteen mature Wistar rats consisting of 12 females and 6 males were used. The female rats were divided into groups 1 and 2 of 6 rats each; group 1 was the control, while group 2 was the test group. The male rats only serve for mating purpose, and this was allowed during the proestrous phase of the female rats' oestrous cycle.

Two blocks of non-salted calabash chalk was obtained from a local market in Calabar, Nigeria. They were chopped into small pieces and grounded into fine powder with the aid of a manually operated grinder. Forty g of the calabash chalk powder was dissolved in 1000 ml of distilled water in a glass jar. Since the calabash chalk was partially miscible with water, it was administered as a suspension, stirred prior to the administration. Two hundred mg/kg body weight of the calabash chalk suspension was administered to the gestating rats in group 2 (chalk group), while those in the control group were administered distilled water. All treatments which were by oral gavages were carried out on pregnancy days 7–20.

All the dams (six per group) were sacrificed on pregnancy day 21 (PD21). The foetuses were removed, examined for gross malformations, and weighed. The foetuses were then culled to two foetuses per mother rat, making a total of 12 foetuses per group. Their whole brains were excised, weighed and preserved using 10% neutral buffered formalin.

Forty eight hours after fixation, the cerebral cortex from each foetus was excised from the whole brain and routinely processed for histological observations using the haematoxylin and eosin (H & E), and the Kluver's luxol fast blue for Nissl substance and myelin. The cellular population in the H & E stained sections were quantified using ImageJ™ software.

Statistical analysis using Mann-Whitney rank-test sum was applied by means of Primer of Biostatistics (version 3.01) software. All results were regarded as significant at $p < 0.05$.

Results

There were no morphological changes on the foetuses in both experimental groups. The mean body weights of the foetuses in the chalk group whose mothers were administered 200 mg/kg body weight of calabash chalk suspension was significantly ($p < 0.001$) higher than the body weights of the foetuses in the control group (Table 1).

Histomorphologic sections of the cerebral cortex in the control group showed a varying population of prominent neurons and glia, and these were less distinct from each other in all the six cortical layers. Layer one, the outer marginal layer, showed sparse population of small-sized cells. Layer 2, the cortical plate, showed a dense population of slightly larger-sized cells. Layer 3, the subcortical plate, showed a sparse population of cells with their sizes similar in appearance to those of the cortical plate. Layers 4–6 (intermediate, subventricular and ventricular zones) whose cell sizes were similar in size to the cortical and subcortical plates were not easily distinguishable (Figure 1). The section of

Table 1: Comparison of the body weights of the foetuses

Group (n = 12)	Foetus (g)
Control group	0.000328014±99.9752%
Chalk group (200 mg/kg of calabash chalk)	0.000612142±99.9388%*

Results are presented as median ± interquartile range; U = 128; z = 3.23316; $p < 0.001$
*Significantly different from control group at $p < 0.05$

Table 2: Comparison of the cellular population of the cerebral cortical sections

Group (n = 12)	Cellular density
Control group	3.69801e-07±100%
Chalk group (200 mg/kg of calabash chalk)	1.61282e-05±99.9984%

Results are presented as median ± interquartile range; U = 144; z = 4.15692; $p < 0.001$
*Significantly different from control group (1) at $p < 0.05$

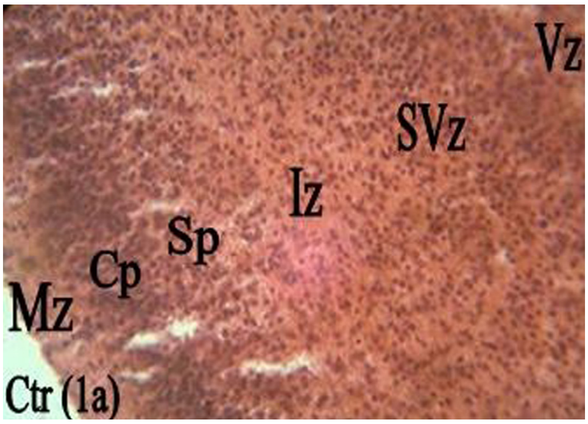

Figure 1: Ctr (1a) - Section of the foetal cerebral cortex of the control group showing six cortical layers: Mz (marginal layer), Cp (cortical plate), Sp (subcortical plate), Iz (intermediate plate), SVz (subventricular zone), and Vz (ventricular zone) (H. & E. Å~400).

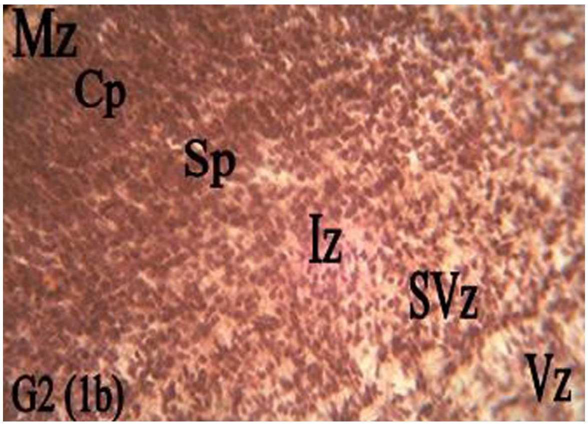

Figure 2: G2 (1b) – Section of the foetal cerebral cortex of the chalk group whose mothers received 200 mg/kg of calabash chalk suspension showed hyperplasia and hypertrophy of cells in all the layers, especially in the cortical plate (H. & E. Å~400).

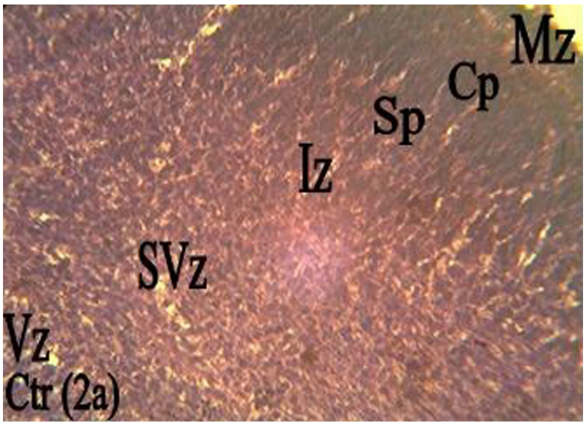

Figure 3: Ctr (2a) - Section of the foetal cerebral cortex of the control group showing six cortical layers: Mz (marginal zone), Cp (cortical plate), Sp (subcortical plate), Iz (intermediate plate), SVz (subventricular zone), and Vz (ventricular zone). The Nissl substance was deeply demonstrated throughout the entire layers (Luxol Fast Blue. Å~400).

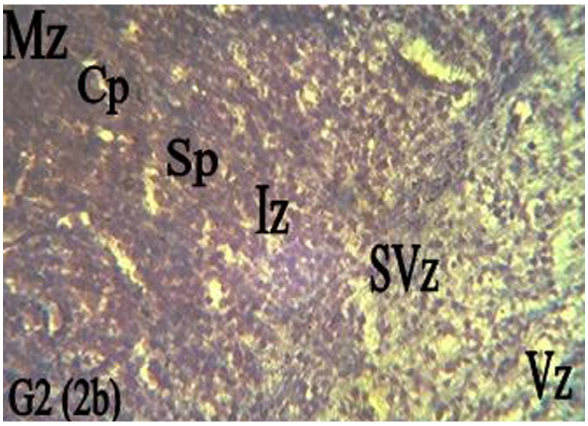

Figure 4: G2 (2b)- Section of the foetal cerebral cortex of the chalk group whose mothers received 200 mg/kg of calabash chalk suspension showed less deeply demonstrated Nissl substance throughout the entire layers compared with the control group (Luxol Fast Blue. Å~400).

the cerebral cortex in the chalk group showed hyperplasia and hypertrophy of cells in all the cortical layers. In this section, layers 1–3 were prominent, but layers 4–6 were indistinguishable. There was higher cellular density in the molecular, cortical and subcortical plates. The intermediate, subventricular, and ventricular zones blended imperceptibly, compared with the control group (Figure 2).

The Nissl substances were deeply and densely demonstrated throughout the entire section, though myelin staining was not prominent (Figure 3). The Nissl substances were less deeply demonstrated with less intervening myelin especially at the deeper layers compared with the control group (Figure 4). The cellular population density of the section of the foetal cerebral cortex of group 2 was significantly ($p < 0.001$) higher than the control group (Table 2).

Discussion

Calabash chalk is reported to contain several adverse elements (5,9), which are known to be detrimental to health. Pregnant women have a predilection for consuming it especially in Nigeria and other sub-Saharan African countries. Thus, this study investigated the effect of maternal calabash chalk consumption on the developing foetus and the histomorphology of its cerebral cortex.

The results revealed that the mean foetal body weight was higher in the chalk group whose mothers were administered the calabash chalk suspension compared with the control group, and this may be attributed to the elemental composition of the calabash chalk. Elements such as calcium, potassium, magnesium, aluminium and iron amongst others have been reported as constituents of calabash chalk (5,9,16). Most of these elements are known to cross the blood-placental membrane (17), thus, having access to the developing foetus and may influence their metabolism and development. This could be a possible reason for the higher foetal body weights in the test group of this study.

Other elements such as lead and arsenic contained in the calabash chalk (5,9) are known to cross the blood-placentral membrane, and accumulates throughout gestation, usually resulting in adverse effects (18). Adverse effects such as neurological and visual alterations in the developing nervous system (19), and decline in children's IQ scores have been ascribed to lead (20). Arsenic on the other hand, causes damage to many tissues and organ systems including the nervous system (21). Thus, the higher body weight in the test group of this study may not be beneficial.

Histomorphologically, the foetuses in the chalk group whose mothers received 200 mg/kg of calabash chalk showed hypertrophy and hyperplasia of cells of the cerebral cortex, which was significant compared to the control group. Cellular hypertrophy and hyperplasia are usual mechanisms by which tissues cope with trauma initiated physically or by chemical reaction (22). The result suggests that calabash chalk or its constituents were able to cross the blood-placental membrane (23–25), as already reported in the body weight increase, and to initiate trauma to the developing cerebral cortex. The invasion of this brain area by the elemental constituents of calabash chalk, could pose a threat to the integrity of the developing brain. However, as neurons are not known to proliferate when traumatised except in specific areas of the brain, astrocytes and microglia may be the likely cells involved, as they proliferate when the brain tissue is traumatised (26,27).

There was less Nissl substance staining in the chalk group compared with the control group, an indication that the calabash chalk effect may also alter the neuronal integrity. Nissl substances are large granular bodies found in neurons (28), which are the rough endoplasmic reticulum and are the site of protein synthesis (28–30). Nissl substances show changes under various physiological and pathological conditions, hence in injured neurons, chromatolysis may result (31,32). Alterations in the distribution pattern of Nissl substances influence their metabolic activities (33,34), as well as protein synthesis.

The cerebral cortex is the outermost part of the cerebrum of the mammalian brain that develops from the most anterior part of the neural plate, after it folds and closes to form the neural tube (35). The cortex plays a key role in memory, attention, perceptual awareness, thought, language and consciousness (36). Thus, cortical changes as seen in this study may affect these functions, which may lead to severe body impairment and/or other health challenges.

In conclusion, calabash chalk may cause body weight increase and histomorphological changes in the cerebral cortex of foetuses.

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Conflict of Interest

None.

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Authors' Contributions

Conception and design: TBE, MBE
Analysis and interpretation of the data: AOI
Drafting of the article: MBE
Critical revision of the article for important intellectual content: TBE, EEO
Final approval of the article: TBE, MAE
Provision of study materials or patients: MAE
Statistical expertise: EEO
Obtaining of funding: MBE
Collection and assembly of data: MBE

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References

1. Kawai K, Saathoff E, Antelman G, Msamanga G, Fawzi WW. Geophagy (soil-eating) in relation to anemia and helminth infection among HIV–infected pregnant women in *Tanzania*. *Am J Trop Med Hyg*. 2009;**80(1)**:36–43.

2. Abrahams PW, Follansbee MH, Hunt A, Smith B, Wragg J. Iron nutrition and possible lead toxicity: an appraisal of geophagy undertaken by pregnant women of UK Asian communities. *Appl Geochem*. 2006;**21**:98–108. doi: 10.1016/j.apgeochem.2005.09.015.

3. Henry J, Kwong AM. Why is geophagy treated like dirt? *Deviant Behav*. 2003;**24**:353–371. doi:10.1080/713840222.

4. Agency warns of the dangers of traditional remedy for morning sickness [Internet]. London (GB): Food Standards Agency; 2002 [cited 2012 May 12]. Available from: http://tna.europarchive.org/20110116113217/http://www.food.gov.uk/news/pressreleases/2002/oct/calabash.

5. Dean JR, Deary ME, Gbefa BK, Scott WC. Characterisation and analysis of persistent organic pollutants and major, minor and trace elements in Calabash chalk. *Chemosphere*. 2004;**57(1)**:21–25. doi: 10.1016/j.chemosphere.2004.05.023.

6. Campbell H. Calabash chalk (calabar stone, la craie, argile, nzu, mabele) [Internet]. Belfast (IE): Department of Health, Social Service and Public Safety; 2002 [cited 2011 May 1]. Available from: http://www.docstoc.com/docs/54253409/calabashchalk-(calabar-stone-La-Argile-Nzu-Mabele).

7. Calabash chalk may pose health risk for pregnant and breastfeeding women [Internet]. Ontario (CN): Health Canada; 2007 [cited 2014 June 16]. Available from http://www.hc-sc.gc.ca/ahc-asc/media/advisories-avis/_2007/2007_136-eng.php.

8. Dooley EE. The beat. Environ *Health Perspect*. 2010;**118**:A200-A201. doi: http://dx.doi.org/10.1289/ehp.118-a200b

9. Ekong MB, Peter AI, Ekanem TB, Osim EE. Determination of elemental composition and median lethal dose of calabash chalk. *J Basic Appl Res International*. 2015; **5(2)**:83–89.

10. Ekong MB, Akpantah AO, Ibok OS, Eluwa MA, Ekanem TB. Differential effects of calabash chalk on the histology of liver of adult Wistar rats. *Internet J Health*. 2009 [cited 2012 Feb 8]; 8(2). Available from: http://www.ispub.com/journal/the_internet_journal_of_health/volume_8_number_2_12/article/differentia_effects_of_calabash_chalk_on_the_histology_of_the_liver_of_adult_wister_rats.html.

11. Akpantah AO, Ibok OS, Ekong MB, Eluwa MA, Ekanem TB. The effect of calabash chalk on some haematological parameters in female adult Wistar rats. *Turk J Hematol*. 2010;**27(3)**:177–181. doi: 10.5152/tjh.2010.25.

12. Ekong MB, Ekanem TB, Abraham KE, Akpanabiatu MI, Peter AI, Edagha IA. Effects of calabash chalk on hematology indices and histomorphology of the spleen of growing Wistar rats. *Instasci J Med Sci Clin Res*. 2012;**2(1)**:1–7.

13. Ekong MB, Ekanem TB, Sunday AO, Aquaisua AN, Akpanabiatu MI. Evaluation of calabash chalk effect on femur bone morphometry and mineralization in young Wistar rats: A pilot study. *Intern J Appl Basic Med Res*. 2012;**2(2)**:107–110. doi: 10.4103/2229-516X.106352.

14. Ekong MB, John EE, Mbadugha CC, Bassey EI, Ekanem TB. Effect of calabash chalk on the histomorphology of the gastro-oesophageal tract of growing Wistar rats. *Malays J Med Sci*. 2012;**19(1)**:30–35.

15. Ekong MB, Peter AI, Ekanem TB, Eluwa MA, Mbadugha CC, Osim EE. Calabash chalk's geophagy affects gestating rats' behavior and the histomorphology of the cerebral cortex. *Intern J Brain Sci*. **2014**;2014. doi: http://dx.doi.org/10.1155/2014/394847.

16. Abrahams PW, Davies TC, Solomon AO, Trow AJ, Wragg A. Human geophagia, calabash chalk and undongo: mineral element nutritional implications. *PLoS One*. 2013;**8(1)**:e53304. doi: 10.1371/journal.pone.0053304.

17. Lockless SW, Zhou M, MacKinnon R. Structural and thermodynamic properties of selective ion binding in a K+ channel. *PLoS Biol*. 2007;**5(5)**:e121. doi: 10.1371/journal.pbio.0050121.

18. Halsted JA. Geophagia in man: Its nature and nutritional effects. *Am J Clin Nutr*. 1968;**21(12)**:1384–1393.

19. Bressler J, Kim KA, Chakraborti T, Goldstein G. Molecular mechanisms of lead neurotoxicity. *Neurochem Res*. 1999;**24(4)**:595–600. doi: 10.1023/A:1022596115897.

20. Canfield RL, Henderson CR, Cory-Slechta DA, Cox C, Jusko TA, Lanphear BP. Intellectual impairment in children with blood lead concentrations below 10 µg per deciliter. *N Engl J Med*. 2003;**348(16)**:1517–1526. doi: 10.1056/NEJMoa022848.

21. Frumkin H, Thun MJ. Arsenic. *CA Cancer J Clin*. 2001;**51(4)**:254–262. doi: 10.3322/canjclin.51.4.254.

22. Kumar V, Abbas AK, Fausto N. *Robbins and Cotran: pathologic basis of disease*. Philadelphia (PA): Elsevier Saunders; 2005.

23. Goyer RA. Transplacental transport of lead. *Environ Health Perspect*. 1990;**89**:101–105.

24. Zadorozhnaja TD, Little RE, Miller RK, Mendel NA, Taylor RJ, Presley BJ, et al. Concentrations of arsenic, cadmium, copper, lead, mercury, and zinc in human placentas from two cities in Ukraine. *J Toxicol Environ Health A*. 2000;**61(4)**:255–263. doi: 10.1080/00984100050136571.

25. Vahter M. Effects of arsenic on maternal and fetal health. *Annu Rev Nutr*. 2009;**29**:381–399. doi: 10.1146/annurev-nutr-080508-141102.

26. Roumier A, Pascual O, Bechade C, Wakselman S, Poncer JC, Real E, et al. Prenatal activation of microglia induces delayed impairment of glutamatergic synaptic function. *PLoS One*, 2008;**3(7)**:e2595. doi: 10.1371/journal.pone.0002595.

27. Tambuyzer BR, Ponsaerts P, Nouwen EJ. Microglia: Gatekeepers of central nervous system immunology. *J Leuk Biol*. 2009;**85(3)**:352–370. doi: 10.1189/jlb.0608385.

28. Kiernan JA. *Barr's the human nervous system: an anatomical viewpoint*. Philadelphia (PA): Wolters Kluwer/Lippincott Williams and Wilkins; 2009.

29. Young B, Heath J. *Wheaters functional histology*. London (GB): Churchill Livingstone; 2000.

30. Snell RS. The neurobiology of the neuron and neuroglia. *Clinical neuroanatomy for medical students*. Baltimore (MD): Lippincott Williams and Wilkins; 2001. p. 33–72.

31. Singh I. *Textbook of human neuronanatomy*. New Delhi (IN): Jaypee Brothers Medical Publishers; 2002.

32. Lowe J, Cox G. Neuropathological technique. In J. D. Bancroft and A. Stevens, editors. *A theory and practice of histological techniques*. Edinburgh (GB): Churchill Livingstone; 1992. p. 343–378.

33. Macsween RNM, Whaley K. Cell injury and death. *Muri's textbook of pathology*. London (GB): ELBS. 1992; p. 805–810.

34. Bancroft JD, Gamble M. *Theory and practice of histological techniques*. London (GB): Churchill Livingstone; 2007.

35. Sadler TW. *Langman's medical embryology*. New Delhi (IN): Wolters Kluwer; 2010.

36. Kandel ER, Schwartz JH, Jessell TM. *Principles of neural science*. New York (NY): McGraw-Hill; 2000.