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Research Paper

Determination of the Elemental Composition of Calabar Clay and The Pharmacological Action of Its Crude Solution On Uterine Contraction

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ABSTRACT: *This study was conducted to determine the elemental composition of Calabar clay and its contractile activity of the uterus of the albino rat. Ten (10), 8 week-old non pregnant albino rats were used for the experiment.The rat was stunned by a blow on the head and the jugular vein was severed subsequently the abdomen was opened with scalpel blade and the uterus was located at the ventral part of the abdomen (pelvic cavity).it was carefully removed and transferred to a petri dish containing aerated dejalonsolution,it in to about 1cm stripes. The ends of the uterine stripe were tied with a thread to the bent end of the stylus tube while the other end was attached to a frontal lever. The tissue was mounted in to 75ml organ bath containing aerated dejalonsolution.The tissue was allowed to stayed for about 30min for the tissue to stabilize at 37^0C.Normal contractions were recorded at sweep 0.2m/sec on the physiograph screen after which the calabar clay suspension was added at a range contraction in to 75ml tissue bath and allowed to act on the tissue for 30 seconds,it is then washed off by draining with fresh dejalon solution, the tissue was allowed to recover and stabilize for 2 minutes to attain baseline of contraction as shown on the physiograph. The dose of calabar clay suspension was increased subsequently from 100mg/ml, 200mg/ml and 400mg/ml with insulin syringe, the organ bath was washed after each addition of calabar clay suspension and standard drugs with a fresh warmth dejalonsolution.The same procedure was carried out for standard drugs (oxytocin, propranolol, prazosin, acetylcholine, atropine, indomethacin) to obtain uterine stripes and procedure was repeated for calabar clay suspension and standard drugs three times. The result from this study that the exposure of the non-pregnant rat uterine stripes to the crude suspension of Calabar clay produced significant ($P< 0.05$) dose dependent decrease in amplitude (height) of contraction of the uterine stripes.*

KEYWORDS: *Calabar clay, Uterine strip, Physiograph, Amplitude of contraction.*

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I. INTRODUCTION:

Calabar clay is a geophagic material consumed by pregnant and nursing mothers in Nigeria and other African countries (Abrahams, P.W.;*et al*. 2013).Scientists are now trying to figure out this practice and place some kind of label of it. Sometimes it is referred to as "Geophagy" which is the practice of eating clay including soil and chalk. This practice is neither new nor outdated and can be associated with religious belief, medication or as part of regular diet (Moses, E.B.; *et al*. 2012). The eating practice of Calabar clay has being placed under the umbrella of "Pica" which is when people crave things that are not normal food. On the other hand, this has evolved as a cultural practice among African women and later being used as medicinal substance by pregnant women for protective mechanism against morning sickness (Moses, E.B.; *et al*. 2012).Calabar clay also known as Calabar stone is identified with different names in Nigerian languages such as "Farankasa" in Hausa, "kattamanada" in Kanuri, "laddidaneri" Fulfulde, Nzu in Igbo, and Ndom in Efik/Ibibio (Abrahams, P.W.; *et al*. 2013).Traditionally the clay has numerous uses including direct consumption of the beneficiated form with the belief that it neutralizes toxins and cleanses the internal body system. Pregnant and nursing mothers rub this clay on their bodies and that of the newborn to reveal their fertile state and purify the skin of the infant, the beneficiated form is used to make facial designs and decoration during traditional marriage rites (Abrahams, P.W.; *et al*. 2013).Calabar clay is a known member of kaolin group, chiefly made up of Aluminium silicate hydroxide ($Al_2Si_2O_5(OH)_4$). It can be naturally occurring or artificially prepared compound. The naturally occurring type resulted from gradual deposition of fossilized seashells while the artificial form may be prepared

from clay or mud which may be mixed with other ingredients like sand, wood ash and sometimes salt. The resulting product is molded and then heated to produce the final product (Food Standards Agency, 2008). Research conducted on the clay proximate analysis revealed that it contained several elements and other contaminants which could be poisonous to the biological system. These include metals, metalloids and persistent organic pollutants (Food Standards Agency, 2008).Calabarchalk is commercially available, and may be sold in blocks, as large pellets, and in powder forms. It may be packaged in a clear plastic bag with or without labelling, or sold without any packaging, and in a variety of forms (Dean et al., 2004).Calabar clay contains of many biologically body required as well as adverse elements depending on their concentration in the chalk as well as their bioavailability. Though the chalk may be relatively less toxic in acute usage, the Parameters, Edema and hemorrhages of gastrointestinal tract, enlarged and fragmented liver, and alteration of growth rate and de-mineralization of the femur bone; hence its consumption should be discouraged (Dean et al., 2004).Calabar clay is richer in both micro and macro elements and the habit of eating Calabar clay by pregnant women is increasing these days, this can either be as a response to mineral deficiencies or an instinctual effort to "protect against harm from toxins and pathogens." Research has shown that the demand for minerals by the body goes up very high during pregnancy, some of these mineral deficiencies are calcium, iron, copper, and magnesium which are essential requirements for normal body function but even more critical during pregnancy this might explain the uncontrollable impulse that many women feel when it comes to craving Calabar clay during pregnancy.Only a few researcheswere conducted on elemental composition and it's toxicity on laboratory animals and no research was reported on the pharmacological action of the clay on the contractile activity of the uterus. Hence there is a need to analyze the elemental composition of calabash clay and to investigate the health risk associated with the ingestion of the compound and its activity on uterine contraction. This work was designed to determine the elemental composition of Calabar clay and its contractile activity of the uterus of the albino rat.This study will contribute to the knowledge required to resolve experimentally the dangers associated with the consumption of Calabar clay by pregnant/nursing mothers where it prolongs consumption may pose them or their children to the deleterious effect of heavy metals and organic pollutants present.

II. STUDY AREA:

The study was conducted in Maiduguri, Borno State which lies between latitude 10.20N and 13.40N longitude 9.80E and 14.40N with an area of 69,436 sq km located in the Northeastern corner of Nigeria sharing borders with Niger to the North, Chad to the Northeast and Cameroon to the east (Musa and Pindar, 2005). The State has Sahel vegetation in the North and a Sudan Savanna in the South.

III. EXPERIMENTAL ANIMALS

Ten (10), 8 week-old non-pregnant albino rats were used for the experiment. They were purchased from Sandakyarimi zoological garden Maiduguri Borno State and kept at Veterinary Physiology Laboratory University of Maiduguri, Borno State. The temperature of the room was maintained at 36oC to 37oC with 12-hour light and 12-hour dark cycle. The animals were cared for according to the international regulations governing the use and care of laboratory animals. They were housed in wire cages and maintained on standard feeds (please specify the type marsh/pellets and company). Drinking water was allowed ad libitum. The animals were allowed to acclimatize for (3 to 5 days) and each animal was weighed before the commencement of the experiment. Two rats were sacrificed each day for the experiment. The number used was following regulation concerning the use of laboratory animals for the experimental model in university of Maiduguri.

3.1 PREPARATION OF CALABAR CLAY SUSPENSION MIXTURE:

Blocks of salted Calabar clay were purchased from a local market in Maiduguri Monday market.

To prepare the stock solution 1gram of the Calabar clay suspension was measured and dissolved in 10ml of distilled water which was equivalent to 100mg/ml.

3.2 PREPARATION OF PROPRANOLOL

40mg of propranolol was dissolved in 10ml of distilled water to give a concentration of 4mg/ml used as the stock solution

3.3 PREPARATION OF OXYTOCIN

One ampule of oxytocin containing 10 IU/ml was diluted with 10ml 0f water to obtain the stock solution at 1IU/ml.

3.4 PREPARATION OF ACETYLCHOLINE

10mg of acetylcholine crystals were dissolved in 1ml of distilled water to obtain 10mg/ml Calabar clay suspension, then 9ml 0f distilled water was added to make up to 10ml which is equivalent to1mg/ml. 1ml was then measured and 9ml of distilled water was added to made-up 100ug/ml, 1ml was finally measured and 9ml of distilled water was added to obtain 10ug/ml as used stock solution.

3.5 PREPARATION OF INDOMETHACIN

One capsule of indomethacin 4mg was dissolved in 10ml of distilled water to obtain the stock solution at 4mg/ml.

IV PREPARATION OF PHYSIOLOGICAL SOLUTION

All the salts were individually weighed using ADA 120/C chemical balance but calcium chloride was added last to avoid precipitation of solution. The composition of dejalon solution is as follows

Nacl	9.0g/l
Kcl	0.42g/ l
$NaHCO_2$	0.50g/ l
$CaCl_2$	0.06g/l
$C_6H_{12}O_6$	0.50g/l
H_2O (Distilled)	1litre

The solution is usually prepared and used on each day whenrunning the experiment.

4.1 EQUIPMENT

Organ bath and accessories
Physiograph and accessories
Dissecting kit
Sensitive weighing balance
Physiologic solution(dejalon solution)
Distilled water and extract
Standard drugs (oxytocin,propranolol,indomethacin,atropine,prazosin)

4.2 TISSUE PREPARATION

4.2.1 RAT UTERUS STRIPE

The rat was stunned by a blow on the head and the jugular vein was severed subsequently the abdomen was opened with scalpel blade and the uterus was located at the ventral part of the abdomen (pelvic cavity).it was carefully removed and transferred to a petri dish containing aerated dejalonsolution,it in to about 1cm stripes.

4.2.2 TISSUE MOUNTING

The ends of the uterine stripe were tied with a thread to the bent end of the stylus tube while the other end was attached to a frontal lever. The tissue was mounted in to 75ml organ bath containing aerated dejalonsolution.The tissue was allowed to stayed for about 30min for the tissue to stabilize at 37^0C.

4.2.3 PHYSIOGRAPH RECORDING

Normal contractions were recorded at sweep 0.2m/sec on the physiograph screen after which the Calabar clay suspension was added at a range contraction into 75ml tissue bath and allowed to act on the tissue for 30 seconds, it is then washed off by draining with fresh dejalon solution, the tissue was allowed to recover and stabilize for 2 minutes to attain baseline of contraction as shown on the physiograph. The dose of Calabar clay suspension was increased subsequently from 100mg/ml, 200mg/ml and 400mg/ml with insulin syringe, the organ bath was washed after each addition of Calabar clay suspension and standard drugs with a fresh warmth dejalon solution. The same procedure was carried out for standard drugs(oxytocin, propranolol, prazosin, acetylcholine, atropine, and indomethacin) to obtain uterine stripes and procedure was repeated for Calabar clay suspension and standard drugs three times. The same procedure was carried out for the combined effect of Calabar clay suspension and standard drugs with a fixed concentration of acetylcholine at 1ug/ml, oxytocin 1IU/ml while increasing the concentration of Calabar clay solution and replicates were obtained.

V. RESULTS

Exposure of the non-pregnant rat uterine stripes to the crude suspension of Calabar clay, Indomethacin , combined Calabar Clay suspension with oxytocin, and combined Calabar Clay suspension with atropineproduced significant ($P< 0.05$) dose-dependent decrease in amplitude (height) of contraction of the uterine stripes. The mean (±SD) amplitude of contraction decrease as shown in Table I, 3, 4, 5, 8, and 9respectively.

Table 1. Mean (±SD) Amplitude of contraction of rat uterine stripes exposed to the graded concentration of Atropine at bath temperature of 37^0C.

Bath concentration (mg/ml)	normal response	atropine response
100	2.80± 1.20	2.15± 1.63
200	2.00± 1.41	1.25 ± 0.61
400	1.65± 1.20	0.85± 0.92

Table 3. Mean (±SD) Amplitude of contraction of rat uterine stripes exposed to the graded concentration of indomethacin at bath temperature of 37^0 C

Amplitude of contraction of Calabar Clay North suspension with oxytocin

Bath concentration (mg/ml)	normal response	Calabar Clay susp. + oxytocinresponse
100	2.85± 0.21	2.15± 0.35
200	2.95± 1.06	2.5± 1.13
400	3.00± 0.42	1.80± 0.28

Table 4. Mean (±SD) Amplitude of contraction of rat uterine stripes exposed to degraded concentration of the crude aqueous suspension of Calabar Clay + oxytocin at bath temperature of 37^0C.

Amplitude of contraction of Calabar Clay South suspension with oxytocin

Bath concentration (mg/ml)	normal response	Calabar Clay susp. +oxytocin response
100	2.50± 0.49	1.90± 4.20
200	2.60± 0.71	1.75± 0.92
400	2.65± 0.35	1.10± 0.28

Table 5. Mean (±SD) Amplitude of contraction of rat uterine stripes exposed to degraded concentration of the crude aqueous suspension of Calabar Clay South + oxytocin at bath temperature of 37^0C.

Amplitude of contraction of Calabar Clay North suspension +propranolol

Bath concentration (mg/ml)	normal response	Calabar Clay susp. response
100	2.00±0.14	0.80± 0.14
200	1.20± 0.28	0.65± 0.35
400	1.15± 0.35	0.45± 0.07

Table 8. Mean (±SD) Amplitude of contraction of rat uterine stripes exposed to the graded concentration of the crude aqueous suspension of Calabar Clay with atropine at bath temperature of 37^0C

Amplitude of contraction of Calabar Clay South suspension + atropine

Bath concentration (mg/ml)	normal response	Calabar Clay susp +atropine response
100	0.95± 0.49	0.70± 0.42
200	0.80± 0.72	0.50± 0.28
400	0.75± 0.35	0.30± 0.28

Table 9 Mean (±SD) Amplitude of contraction of rat uterine stripes exposed to the graded concentration of the crude aqueous suspension of Calabar Clay South with atropine at bath temperature of 37^0C.

The Calabar Clay suspension combined with exogenous oxytocin caused antagonistic effect of the crude suspension of Calabar Clay, but less in magnitude compared to propranolol which is a beta adrenergic receptor antagonist. Table 2 shows the mean (±SD) amplitude of the contraction of the normal and combination of Calabar Clay suspension with oxytocin exposed uterine stripes.

Exposure of the non-pregnant rat uterine stripes to the oxytocin produced significant ($P<0.05$) dose dependent increase in amplitude (height) of contraction of the uterine stripes. The mean (±SD) amplitude of contraction of the normal and oxytocin exposed stripes Increases as shown in Table 2.

Table 2. Mean (±SD) Amplitude of contraction of rat uterine stripes exposed to the graded concentration of oxytocin at bath temperature of 37^0C.

Bath concentration (mg/ml)	normal response	oxytocin response
100	2.75± 0.33	5.85± 0.21
200	2.00± 0.42	6.35±1.20
400	1.75± 0.35	6.90± 1.56

Amplitude of contraction of indomethacin alone

Bath concentration (mg/ml)	normal response	indomethacin response
100	4.00± 0.28	2.80± 0.28
200	3.70± 0.14	3.25± 1.06
400	3.80± 0.28	3.65± 1.20

Exposure of the non-pregnant rat uterine stripes to propranolol, and Calabar Clay suspension, combined with propranolol produced significant($P < 0.05$) dose dependent blocking effect of propranolol (decrease in amplitude) of contraction of the uterine stripes as shown inTable 6, and 7.

Table 6. Mean (±SD) Amplitude of contraction of rat uterine stripes exposed to the graded concentration of the crude aqueous suspension of Calabar Clay +propranolol bath temperature of 37^0C.

Amplitude of contraction of Calabar Clay South solution +propranolol

Bath concentration (mg/ml)	normal response	Calabar Clay susp. + propranolol response
100	1.15±0.35	1.00± 0.14
200	1.02± 0.21	0.80± 0.35
400	0.95± 0.35	0.60± 0.42

Table 7. Mean (±SD) Amplitude of contraction of rat uterine stripes exposed to the graded concentration of the crude aqueous suspension of Calabar Clay South +propranolol bath temperature of 37^0C.

Amplitude of contraction of Calabar Clay North suspension + atropine

Bath concentration (mg/ml)	normal response	Calabar Clay susp. + atropine response
100	1.15± 0.21	1.00± 0.28
200	0.95± 0.07	0.75± 0.07
400	0.85± 0.49	0.60± 0.57

NB:
i. Normal response means with Calabar clay suspension only.
ii. All the responsis are measured in centimetre (cm).

5.1 RESULT OF ELEMENTAL ANALYSIS
Sample of was submitted to department of soil science faculty of agriculture university of Maiduguri for determination of macro elements and another sample was sent to department of chemistry Adamawa state university Mubi for micro elements. The following results were obtained.

Macro elements	N(%)	P(%)	K(%)	Ca2+(%)	Mg2+(%)	Na+(%)	Hardner
	8.00	21.6	0.81	3.78	34.19	0.22	3.15
Micro elements	Cd(mg/kg)	Zn(mg/kg)	Fe(mg/kg)	Ni(mg/kg)	Cr(mg/kg)	Pb(mg/kg)	Cu(mg/kg)
	0.08	0.04	9.41	1.21	0.27	4.49	0.62

VI. CONCLUSION

Table 1 represents the mean± SD on the amplitude of contraction of uterine stripes of non-pregnant rat at graded concentration of crude aqueous suspension of Calabar Clay (100mg/ml), 0.2ml,0.4ml,0.6ml produced significant ($P<0.05$) with decreased amplitude of contraction when data were subjected to statistical analysis. Table 2 represents the mean ± SD on amplitude of contraction of uterine stipes of non-pregnant rat at graded concentration of atropine sulphate, the amplitude of contraction has greatly reduced with prolong refractory period. Table represents the mean ±SD on the amplitude of uterine stripes of non-pregnant rat at graded concentration of oxytocin. Oxytocin produced marked increase amplitude of contraction with change in normal base line already established. Propranolol at graded concentration of100mg/ml, 200mg/ml and 400mg/ml shown significant decrease on amplitude of contraction compared to crude aqueous solution of Calabar Clay on uterine stripes. Combination of Calabar Clay suspension with standard drugs known to reduced smooth muscle contraction have shown significant ($P<0.05$) when data were subjected to statistical analysis, this is well

represented on a graph attached to appendix.The result of this experiment revealed that the crude aqueous suspension of Calabar Claycontains active substance capable of decreasing the amplitude of contraction of **non-pregnant** rat uterine stripes. Effect of Calabash Chalk on the Histomorphology of the Gastro-Oesophageal Tract of Growing Wistar Rats earlier reported by *Moses, E.B.; et al. (2012)*. Their study revealed that Calabash chalk caused histological changes to the stomach and the oesophagus that may lead to other pathophysiological conditions. Calabar Clay caused a concentration- dependent inhibition of acetylcholine-induced contractility of the isolated detrusor muscle which was statistically significant this was in support of this study that statistically **produced significant inhibition** of rat uterine **stripes oxytocin** - induced contraction. The result also support the study of Patacchini*et al,* **1990** which found that in isolated rat urinary bladder Calabar Clay at concentration of 10 to 300 µm was effective in desensitizing the bladder to the contractile effects of capsqicin although in that study the antagonistic effect could have at least partly been due to mechanical effect of undissolvedCalabar Clay on the rat urinary bladder muscle. The of this study also support studies that have reported a relaxant effect of Calabar Clay on other types ofisolated smooth muscle (Itthipannichpong*et al.,2003*).

The result of elemental analysis of Calabar Clay show significant value ofMagnessium, Potasium, Calcium, Cromium, Lead, Iron, Zinc, Nickel, Copper, and Cadnium, which agreed with the study conducted by Ekong at al., 2015.The present study has shown that Calabar Clay inhibits the contractility of isolated rat uterus. The results suggest that this effect is due to an agonistic effect on beta adrenergic receptors.

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