



Effect of Calabash Chalk on the Liver Enzymes, Blood Glucose Level and Clotting Time of Female Wistar Rats

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ABSTRACT

Calabash chalk is a natural 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. Calabash chalk which contains some heavy metals is generally taken by women and children, with pregnant and postpartum women craving more for it to subsidize nausea and other early morning sicknesses. This study takes into account some impacts of the Calabash chalk to the liver, blood glucose level and clotting time. For the study, 25 female wistar rats were used and they were assigned into five (5) groups (A-E) of 5 rats each and housed in five standard cages. Group A served as Positive control (received just feed and water) Group B, C, D and E received 200, 400, 800 and 1600mg/kg of the Calabash chalk extract respectively. The result revealed that calabash chalk caused a significant decrease on the AST (Aspartate aminotransferase) level of group C which was administered 400mg/kg and ALP (Alkaline phosphatase) level of group E which was administered 1600mg/kg while there was a significant increase in the ALT (Alanine transaminase) level of group D and E which were administered 800 and 1600mg/kg respectively, and the liver weight of group D which was administered 800mg/kg decreased significantly. It had no significant increase on the blood glucose levels of group C, D and E which were administered 400, 800 and 1600mg/kg respectively, but caused a significant increase on group B which was administered 200mg/kg. The calabash chalk had no significant effect on the clotting time of all the experimental groups.

INTRODUCTION

1.1 Background of Study

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. (Akpantah *et al.*, 2010).

Calabash chalk geophagy, a common practice in Nigeria and some other sub-Saharan African countries, is also practiced in developed countries. 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. (Akpantah *et al.*, 2010).

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 (Akpantah *et al.*, 2010). The naturally occurring forms is chiefly made up of fossilized 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 molded and then heated to produce the final product (Abrahams *et al.*, 2006).

Calabash chalk is generally made up of aluminum silicate hydroxide, a member of the kaolin clay group, with the formula: $Al_2Si_2O_5(OH)_4$. It also contains several other substances which could be poisonous to the body depending on their bioavailability. These include; metals, metalloids and persistent organic pollutants (Abrahams *et al.*, 2006).

In most African countries, Calabash chalk or Calabash clay is one of the most common geophagic materials. Popularly called Nzu in Nigeria, Calabash chalk is known as La Craie or Argile in French, and Mabele in Congo. It is found and obtained usually from the soil (mining pits), and occurs naturally as a light brown to almost white solid which is relatively soluble in water. Calabash chalk is marketed in the raw form or processed by combining clay, sand, wood ash or salt; packaged as powder, molded blocks or pellets and is readily available in open shops. It is consumed by many because it is believed to have various ethno botanical uses. It is used as antacid, antidiarrheal, contraceptive, nutritional supplement, wound healing, and skin beautification agent. (Ekong *et al.*, 2010). It is also used for the treatment of skin diseases, fetal growth and

wellbeing during pregnancy,] and sociocultural activities. In general, pregnant women gravitate more toward Calabash chalk consumption in humans. They use Calabash chalk as remedy for morning sickness and to satisfy their increased appetite and other peculiar desires. (Akpantah *et al.*, 2010).

METHODOLOGY

The study was carried out in the department of Physiology, Nnamdi Azikiwe University in the faculty of Basic Medical Science; the rats were housed in cages of the animal house of the College of Health Science, Nnamdi Azikiwe University, Nnewi campus. Ethical approval was obtained from the Faculty of Basic Medical science ethical committee, Faculty of Basic Medical Science, College of Health Science, Nnamdi Azikiwe University, Nnewi campus. Rats handling and treatments conform to the rodent handling and restraint (JOVE science education) manual.

The study lasted for five (5) weeks which included: two (2) weeks of acclimatization of the Wister rats and three (3) weeks of extract administration during which the animals' weight was checked and recorded.

Twenty (25) young female rats of the Wister strain weighing between 110-140g were used for the study. They were allowed to acclimatize for period of fourteen (14) days. The experimental animals were assigned into five (5) groups (A-E) of 4 rats each and housed in five standard cages.

Group A served as Positive control (received just feed and water), Group B received 200mg/kg of the Calabash chalk extract, Group C received 400mg/kg of the Calabash chalk extract, Group D received 800mg/kg of the Calabash chalk extract, and Group E received 1600mg/kg of the Calabash chalk extract.

The experiment lasted for 21 days, after which the animals were anesthetized using chloroform in an enclosed container for two minutes and blood samples collected via ocular puncture as described by Parasuraman *et al.* (2017) after which liver enzymes, clotting time and blood glucose levels were determined.

RESULT

Table 1 revealed a significant increase in the body weight in groups A, B, C, and D while group E had a non-significant increase when the initial weight was compared to the final weight.

Table 1: Effect of Calabar Chalk on the weight of Rats

	Initial weight (g)	Final weight (g)	P-value	T-value
	MEAN±SEM	MEAN±SEM		
Group A (control)	117.79±9.23	171.52±9.23	0.005*	-3.087
Group B (200 mg/kg of Calabash chalk)	122.84±4.75	159.20±8.02	0.048*	-3.238
Group C (400 mg/kg of Calabash chalk)	116.31±4.63	162.40±6.60	0.003*	-9.228
Group D (800 mg/kg of Calabash chalk)	130.73±4.37	166.55±5.71	0.028*	-4.016
Group E (1600 mg/kg of Calabash chalk)	126.59±10.05	156.06±12.70	0.248 ^a	-1.614

Table 2 revealed a non-significant increase in groups C, D, and E ($p=0.402$, $p=0.230$, $p=0.306$) while group B ($p=0.002$) had a significant increase compared to group A.

The clotting time result demonstrated a non-significant increase in groups B and C ($p=0.667$, $p=0.885$) while groups D and E ($p=0.352$, $p=0.395$) had a non-significant decrease compared to group A.

Table 2: Effect of Calabar Chalk on the Glucose level and Clotting Time of Rats

	Glucose level (mg/dl)	Clotting time (seconds)
	MEAN±SEM	MEAN±SEM
Group A (control)	90.33±2.72	50.00±12.58
Group B (200 mg/kg of Calabash chalk)	111.660±2.02*	55.00±5.77 ^a
Group C (400 mg/kg of Calabash chalk)	94.66±1.45 ^a	51.66±9.27 ^a
Group D (800 mg/kg of Calabash chalk)	96.66±3.28 ^a	39.00±5.56 ^a
Group E (1600 mg/kg of Calabash chalk)	95.66±6.06 ^a	40.00±2.88 ^a
F-value	5.38	0.82

Table 3 result revealed a non-significant decrease in AST level in groups B and E ($p=0.319$, 0.119), group D ($p=0.848$) indicated a non-significant increase and group C ($p=0.040$) showed a significant decrease compared to group A.

The ALT result reported a non-significant increase in-group B ($p=0.803$) and group C ($p=0.709$) had a non-significant increase, and groups D and E ($p=0.018$, $p=0.005$) had a significant increase compared to group A.

The ALP result demonstrated a non-significant decrease in groups B and D ($p=0.418$, $p=192$), group C ($p=0.054$) had a non-significant increase and group E ($p=0.001$) had a significant decrease compared to group A.

The relative liver weight showed a non-significant decrease in groups B, C, and E ($p=0.729$, $p=0.897$, $p=0.310$) and group D ($p=0.021$) had a significant decrease compared to group A.

Table 3: Effect of Calabar Chalk on Liver Enzymes (AST, ALT, ALP) and Organ Weight.

	Aspartate Transaminase (IU/L)	Alanine Transaminase (IU/L)	Alkaline Phosphatase (IU/L)	relative liver weight (g)
	MEAN±SEM	MEAN±SEM	MEAN±SEM	MEAN±SEM
Group A (control)	46.33±10.26	23.66±2.30	439.06±100.83	3.87±0.46
Group B (200 mg/kg of Calabash chalk)	41.00±4.00 ^a	24.33±0.57 ^a	393.83±91.42 ^a	3.75±0.05 ^a
Group C (400 mg/kg of Calabash chalk)	34.33±0.57 [*]	22.66±1.52 ^a	555.96±42.32 ^a	3.82±0.27 ^a
Group D (800 mg/kg of Calabash chalk)	47.33±8.02 ^a	31.00±5.19 [*]	364.16±28.61 ^a	2.97±0.07 [*]
Group E (1600 mg/kg of Calabash chalk)	37.66±2.88 ^a	33.00±4.00 [*]	89.78±19.76 [*]	3.52±0.05 ^a
F-value	2.38	6.54	20.63	2.55

DISCUSSION

There are limited works on the impact of Calabash chalk on the liver, blood glucose and clotting time.

The mean body weight change of the rats in Groups A B, C and D (Table 1) were significantly ($P<0.05$) high. This result is in variance with the study carried out by (Chinko *et al.*, 2022) which reported a reduction in weight amongst the experimental animals when compared to the control. It is possible that the doses administered were not enough to effect such changes. Studies by Akpantah *et al.*, 2010 and Moses *et al.*, 2012 showed that abrasive quartz content in calabash chalk interfered with their normal nutrient absorption which may have played a role in appetite suppression and reduction of lipid profile (Alli and Nafiu, 2017) which was evident in group E where increase in weight was not statistically significant.

There was no significant increase in blood glucose levels of groups C, D, and E While there was a significant increase in group B compared to the control group A. The result suggests that the arsenic content of the Calabash chalk may have caused the significant increase on the glucose level in group B as a study done by Ana *et al*, 2005 showed that Arsenic could influence diabetes development.

There was no significant effect of the calabash on the clotting time across all groups. Which suggest that calabash chalk had no effect on the clotting time. However, no studies have been done to support this claim and hence further research id recommended.

There was a significant decrease in the AST levels of group C when compared to the control group. A study done by Mauro and Diana *et al*, showed that AST levels are expected and that however, low AST may be due to Vitamin B6 deficiency in rare cases.

There was significant increase in the ALT levels of group D and E when compared with the control group. This may suggest some degree of hepatic damage with increasing doses of calabash chalk.

There was a significant decrease in ALP level in Group E which may be due to inactivation of the enzyme molecules by the active components in the calabash chalk.

There was a significant decrease in the weight of the liver of Group D when compared with the control group. A decrease in organ weight may reflect loss of function mass associated with atrophy (Giboney *et al.*, 2005). A study by Ekong *et al.*, 2008 and Nafiu *et al.*, 2016 reported that some of the liver enzymes of wistar rats were significantly affected following administration of calabash chalk.

The study showed that calabash chalk had no adverse effects on the clotting time and body weight but it may however cause significant damage to the liver and cause an increase in the blood glucose level when administered for a long period. Further studies are however recommended.

Ethical Approval

Ethical approval was obtained from the Ethical Committee of Nnamdi Azikwe University, Nnewi Campus.

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