Gestational Toxicity of Calabash Chalk (Nzu) in Wistar Rats

Abstract

Objective: Calabash chalk (Nzu) is a geophagic material, consumed by many pregnant women and breastfeeding mothers as remedy for morning sickness in most African countries. The present study aims to evaluate the impact of Calabash chalk consumption in pregnant Wistar rats.

Materials and Methods: Median lethal dose of Calabash chalk was initially determined in Wistar rats to be >5000 mg/kg po using Lorke's method. Thereafter, 24 pregnant Wistar rats were divided randomly into 3 groups (n = 8) and orally administered 0, 400, or 800 mg/kg of Calabash chalk from gestation day 0 to 20. Maternal body weights were monitored during sample administration. Fetuses were delivered under anesthesia by cesarean section and pregnancy outcome was assessed. Results: Calabash chalk exposure inhibited maternal weight gain. Uterine implantations were absent in 85% of Calabash chalk exposed rats, and the number of implantation sites were decreased (P = 0.0262) in the rest, compared to control. Uterine weight and the number of fetuses formed in uterine horns of Calabash chalk exposed pregnant rats were decreased (P = 0.0204) when compared with control. In addition, there was resorption of pregnancy, abortion (58%), and stillbirth (5%) in Calabash chalk exposed rats, and fetuses delivered were sluggish and pale. Most of these effects observed were dose-dependent. Conclusion: The results suggest that Calabash chalk has a negative relationship with maternal health and pregnancy outcome.

Keywords: Abortion, Calabash chalk, gestation, Nzu, pregnancy outcome

Introduction

Geophagia (synonymous geophagy, geophagism, and geotragia), the deliberate consumption or crave for eating earth, soil, chalk, or clay is an ancient practice common to both animals and humans.1-2 The practice is not limited to any geographic region or sex, but has become a common habit cutting across all social classes.2-3 It is highly prevalent in situations like poverty and famine to suppress appetite,1-2 and also in some psychiatric conditions like pica, compulsive indiscriminate eating of nonnutritive substances.2-4,5

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 ethnobotanical uses. It is used as antacid, antidiarrheal,6 contraceptive,7 nutritional supplement,8 wound healing, and skin beautification agent.2 It is also used for the treatment of skin diseases,9 fetal growth and wellbeing during pregnancy,10 and sociocultural activities.10 In general, pregnant women gravitate more toward Calabash chalk consumption in humans.2,11 They use Calabash chalk as remedy for morning sickness and to satisfy their increased appetite and other peculiar desires.

Being an earth material, Calabash chalk is not unlikely to be contaminated with microorganisms or heavy metals, which may even increase with processing and or storage. Studies on the analysis of Calabash chalk are currently very few, but the available data indicate that it contains heavy metals whose qualitative and quantitative compositions vary with respect to the geographical location of the chalk.6,12 However, it is believed that aluminum forms one of the major...
constituents, probably because of the presence of kaolin clay group in most Calabash clay. Others include, lead, arsenic, and chromium.[6,12] Recently, the Texas Department of State Health Services and Food and Drug Administration has raised concerns of potential health risks from Calabash chalk consumption, especially by pregnant and breastfeeding women in view of lead and arsenic contamination.[13] This is important especially as pregnancy is a delicate and vulnerable period, and is generally unsafe for many chemicals.[14] An empirical evaluation of the safety or toxicity profile of the consumption of Calabash chalk during pregnancy is thus justified as currently, not much is known about its effect on pregnancy.

The present study aims to determine the effect of Calabash chalk (obtained from Umuahia North in the southeastern part of Nigeria) on maternal and fetal health indices following its exposure throughout the period of gestation in Wistar albino rats.

Materials and Methods

Sample collection and preparation

Natural Calabash chalk was excavated from a local mining site at Ogbanzu in Ohia, Umuahia North Local Government Area, Abia State, a southeastern state of Nigeria in October, 2016 with the assistance of a local miner and commercial dealer of Calabash chalk in the area. The sample was oven-dried at 110°C, powdered with a mortar and pestle and stored until used for the experiment. The sample was dissolved in distilled water and administered during the experiment.

Acute toxicity study (median lethal dose determination) of Calabash chalk

Three-graded doses of Calabash chalk (1000, 2500, or 5000 mg/kg) were administered to overnight fasted groups of rats (n = 3 per group) by oral gavage. They were observed closely for mortality, behavioral changes and other symptoms of toxicity over a period of 24 h.[15]

Study design

Twenty-four sexually mature female Wistar albino rats of body weight 150–170 g and twelve adult male rats of body weight 180–200 g were used in this study. The animals were obtained from the Animal House of our institution. They were maintained with standard rodent diet and water was given ad libitum under natural lighting condition and ambient temperature of 25°C ± 3°C. Animals were handled in accordance with international guidelines and experimental procedures followed the approved guideline of the Ethical Committee on Animal Studies of our institution (UPH/CREC/ERA/328).

Female rats in the estrous phase were mated with male rats (2:1) and examined for the occurrence of pregnancy. The presence of a copulatory plug in female animals confirms pregnancy and the day of observation represents gestation day 0 (GD0). Estrous phase was determined by evaluation of vaginal smear as described by Marcondes et al.[16] Vaginal smears of animals in estrus phase had predominantly cornified cells.[16] Pregnant rats were separated and divided randomly into three groups (I, II, and III) containing eight rats each. Group I rats were given distilled water (1 ml) and served as control. Groups II and III were administered Calabash chalk (400 or 800 mg/kg) once daily. The sample was administered orally by oral gavage from GD0 to GD20. The animals were deeply anesthetized with diethyl ether (Loba-chemie PVT, Ltd., India) and laparotomized on GD20 (the average gestation period of rats). Fetuses were delivered and their number and physical characteristics in each animal group were recorded. The two horns of the uterus were equally examined to determine the number of implantation sites and presence of resorption sites. After examination, the uterus was weighed and recorded. Before animals sacrifice, maternal body weights of control and experimental rats were recorded on GD0, GD10, and GD20.

Statistical analysis

Data are expressed as a mean ± standard error of mean. Data were analyzed by one-way analysis of variance followed by Student’s t-test for comparison between control and the treated groups using GraphPad Prism Version 5 software (GraphPad Software Inc., San Diego, CA, USA). Values of P < 0.05 were considered statistically significant.

Results

Acute toxicity study (median lethal dose determination)

There was no mortality after treatment with Calabash chalk, even at the concentration of 5000 mg/kg. In addition, there were no observable behavioral changes or signs of toxicity in all the animals [Table 1]. Thus, lethal dose (LD₅₀) is >5,000 mg/kg, po in rats.

Effect of Calabash chalk exposure on pregnancy indices

There was statistically significant increase in body weight of rats in control (P < 0.0001) and experimental groups (P = 0.0022, P = 0.0005) at GD10 when compared to GD0 [Figure 1]. Body weight of control rats at GD20 was also higher (P = 0.0026) compared to GD10, but weights of experimental rats at GD20 were not different compared to their weights at GD10 (P = 0.1479, P = 0.7497) [Figure 1].

| Table 1: Oral treatment with Calabash chalk over 24 h causes no acute toxicity in rats |
| Dose (mg/kg) | Mortality | Behavioral change | Other signs of toxicity |
|--------------|-----------|-------------------|------------------------|
| 1000         | 0         | None              | None                   |
| 2500         | 0         | None              | None                   |
| 5000         | 0         | None              | None                   |
| n=3 per group |           |                   |                        |
Furthermore, whereas all control rats showed the presence of fetuses and implantation sites in their uteri, these were observed only in 6 (75%) of 400 mg/kg Calabash chalk exposed rats [Table 2]. In addition, the number of fetuses in Calabash chalk exposed mothers were fewer ($P = 0.0084, P < 0.0001$) when compared to control rats [Tables 2 and 3]. Besides, 2 (5%) of the fetuses in 400 mg/kg Calabash chalk treated rats were not alive and were delivered as stillbirths [Table 2]. Number of implantation sites in the Calabash chalk treated rats were also lower ($P = 0.0262, P = 0.0004$) compared to control rats [Tables 2 and 3]. Furthermore, the number of fetuses in the control animals was the same compared to the number of implantation sites, whereas the number of fetuses was fewer in the Calabash chalk exposed animals. This corresponded to 19.2 and 57.9% abortions, respectively, in the exposed rats [Table 2]. Furthermore, resorption of pregnancy was observed in the exposed rats, whereas control rats had zero resorption [Tables 2 and 3]. Macroscopical examination of the fetuses after delivery showed that fetuses in all the three groups were active and morphologically normal. However, fetuses of Calabash chalk (800 mg/kg) exposed mothers appeared weak, sluggish and pale [Table 2]. Furthermore, uterine weights of Calabash chalk exposed rats were decreased ($P = 0.0204$) relative to control rats [Figure 2].

**Discussion**

Median LD$_{50}$ of the sample was determined initially to select suitable (safe) dose levels to be used for the study. LD$_{50}$ is a fundamental acute toxicity index which measures the potential of a compound to cause lethality in animals and is an important determinant of the safety margin or therapeutic index of a drug.$^{[17]}$ The acute toxicity study showed that Calabash chalk produced no mortality at 5000 mg/kg (i.e., LD$_{50} > 5,000$ mg/kg), indicating that the sample is likely to have a high safety margin.$^{[15]}$ The doses used in this study (400 and 800 mg/kg) were much lower than the obtained LD$_{50}$ value and deliberately selected in consideration of the sensitivity of pregnancy period to chemicals exposure.

From the results, it was observed that Calabash chalk treatment inhibited weight gain in pregnant rats in a dose-dependent manner over the dose range used. Furthermore, its administration resulted in dose-dependent reductions in number of implantations, as well as number of fetuses delivered. All these are serious negative indices of pregnancy, which can independently affect the 

![Figure 1: Maternal Calabash chalk (Nzu) exposure inhibits gestational weight gain in Wistar albino rats. Data are expressed as mean ± standard error of mean (n = 8 per group). *P < 0.01, GD10 versus GD0; **P < 0.001, GD10 versus GD0; ***P < 0.001, GD10 versus GD0; ¶P < 0.01, GD20 versus GD10; GD: Gestation day](image1)

![Figure 2: Maternal Calabash chalk (Nzu) exposure reduces uterine weight in Wistar albino rats. Data are expressed as mean ± standard error of mean (n = 8 per group). *P < 0.05](image2)

| Table 2: Effect of calabash chalk (Nzu) exposure on pregnancy indices in Wistar albino rats |
|-------------------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
| **Dose (mg/kg)**     | **Number of rats with implantations in uterus** | **Number of implantation sites (a)** | **Number of live pups delivered (b)** | **Number of still births (c)** | **Resorption (a-b-c)** | **Abortifacient activity** | **Physical characteristics of pups delivered** |
|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|
| Control              | 8                     | 75 (10, 9, 11, 10, 8, 7, 10) | 75 (10, 9, 11, 10, 8, 7, 10) | 0 (0, 0, 0, 0, 0, 0, 0) | 0 (0, 0, 0, 0, 0) | 0 | Active, normal in morphology |
| 400                  | 6                     | 47 (0, 2, 0, 11, 8, 8, 10, 8) | 38 (0, 0, 9, 6, 7, 8, 8) | 2 (0, 0, 0, 0, 0, 0) | 7 (0, 2, 0, 2, 2, 1, 2) | 19.15 | Active, normal in morphology |
| 800                  | 2                     | 19 (0, 0, 0, 0, 0, 9, 10) | 8 (0, 0, 0, 0, 0, 0, 0) | 0 (0, 0, 0, 0, 0, 0) | 11 (0, 0, 0, 0, 0, 9, 2) | 57.89 | Weak, normal in morphology, and pale in appearance |

$n=8$ per group
Table 3: Maternal exposure of calabash chalk (Nzu) in Wistar albino rats affects implantation, and number of fetuses formed

| Dose (mg/kg) | Number of implantation sites | Number of live pups delivered | Number of resorption |
|--------------|------------------------------|------------------------------|----------------------|
| Control      | 9.38±0.46                    | 9.38±0.46                    | 0.00±0.00            |
| 400          | 5.88±1.59**                  | 4.75±1.42**                  | 0.88±0.35            |
| 800          | 2.38±1.56***                 | 1.00±1.00***                 | 1.38±1.12            |

Data are expressed as mean ± standard error of mean, *n = 8 per group. *P < 0.05, **P < 0.001, ***P < 0.0001

overall wellbeing of the developing embryo or fetus.[18–20] Implantation of the embryo follows egg fertilization and is essential for embryo development and sustenance of pregnancy. The presence of implantations was observed only in 2 (25%) of the rats that received 800 mg/kg, whereas implantations were retained in six animals (75%) that were administered 400 mg/kg of Calabash chalk. It does appear that Calabash chalk possibly deteriorates or dissolves implantation after pregnancy, i.e., preventing embryo growth. Further, among the exposed rats in which pregnancy progressed, there was 57.9% loss of pregnancy and 5% fetal death (in utero), providing evidence that Calabash chalk has the potential of inducing abortion in animals. This can obviously interfere with the normal sustenance of pregnancy and may support the reported local use of the agent by young girls of reproductive age in rural African communities as contraceptive.[7] Furthermore, Calabash chalk treatment caused a reduction in the uterine weight. This may result from alteration of reproductive hormone secretion and/or activity and can contribute to the poor sustenance of pregnancy that was observed in the treated rats. In addition, it was observed that the fetuses delivered by pregnant rats that were treated with Calabash chalk appeared pale, suggestive of anemia. These results strongly indicate that Calabash chalk consumption during pregnancy can result in negative effects.

Conclusion

Gestational exposure of Calabash chalk causes negative maternal health consequences and pregnancy outcome in rats.

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Conflicts of interest

There are no conflicts of interest.

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