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Research Paper

Effect of Chronic Consumption of Calabash Chalk Diet (Nzu) On Locomotor Activities in Swiss White Mice.

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ABSTRACT*: There are safety concerns as regards the consumption of Calabash chalk which is common practice in some localities in Africa and Asia. The effect of chronic Consumption of calabash chalk on locomotive activities in the Swiss White Mice was carried out using the Open Field Maze Test (OFM).The OFM provided simultaneous assessment of locomotion, exploration and anxiety using parameters such as recording of number of line crossing, frequency of walling, frequency of rearing and frequency of center square entry. A total of forty-five (45) healthy Swiss White Mice (SWM) randomly divided into groups A, B and C; were used for this work. Group A, which served as a control, was fed the normal rodent chow and water ad libitum while group B and C were fed with low dose (LD) and had high dose (HD) of the Calabash Chalk extracts mixed with rodent chow respectively. The result of the OFM showed a general reduction in locomotor activities among animals in the test groups following chronic consumption of calabash chalk diet. This may be linked to the possible neurotoxic or arthropathic effect following long chronic exposure to lead found which is a component of calabash chalk .It is imperative that caution be exercised in the human consumption of calabash chalk.*

KEY WORDS: *Locomotion, Calabash chalk, Swiss White Mice.*

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I. INTRODUCTION

Calabash Chalk is identified by different names such as Calabar Stone in English, La Craie or Argile by the French, Mabele Lingala in Congo, Nzu by the Igbo tribe of Nigeria, Ndom by the Efik/Ibibio of Nigeria. It is also known as Ebumba, Poto and Ulo in other places[1].

Geophagia is the practice of eating the earth, including soil and chalk[1]. This practice is associated with religious beliefs, medication or as part of a regular diet[2]. A review of literature clearly indicates that geophagia is not limited to age group, race, sex, geophagic region or period, though today the practice is most obviously common amongst the world's poorer or more tribally oriented people and is, therefore, particularly extensive in the tropics[3]. Calabash chalk is a geophagic material popularly consumed in Nigeria and other West African countries for pleasure, and by pregnant women as a cure for nausea[1]. Though native to Africa, Calabash chalk is now available in the United Kingdom (UK) in ethnic stores and markets[4]. In the UK, it is known to be associated with immigrants from south Asia and West Africa[3] with the latter consuming calabash chalk that has been imported from Nigeria and sold in ethnic shops. The migration of people from societies where geophagia is especially prevalent results in a cultural transfer of the practice to countries that many would consider to be not typically associated with this deliberate consumption[1]. In some developed countries, concern has been expressed about this consumption- not only in the UK[1&5], but also in Canada[6&1] and USA[1] because of the lead content.

The UK Food Standard Agency had reported presumably a total lead concentration in Calabash chalk to range from 8.2 mg/kg to 16.1 mg/kg, Kaolin being the underlying mineral and organic pollutants in it[7]. Authorities such as the American Academy of Pediatrics defined lead poisoning as blood levels higher than 10µg/dl for children[8] while the Center for Disease Control has set the standard elevated blood lead level for adults to be 25 micrograms per deciliter of the whole blood[9].

Lead poisoning causes a variety of symptoms and signs which vary depending on the individual and the duration of lead exposure[10]. Lead poisoning may be acute (from intense exposure of short duration) or chronic (from repeat low-level exposure over a prolonged period) but the latter is much more common[11]. The symptoms predominantly affect the central nervous system include insomnia, delirium, cognitive deficits, tremor, hallucinations and convulsions [12]. Lead accumulates and substitute's calcium in bone tissues and the resultant effect is that of disruption of mineralization, alteration of compositional properties and bone formation mechanisms, as well as the gradual depletion of bone minerals [13-15]. Lead exposure is a potential aggravating factor for the development of hypertension and a risk factor for the development of heart disease [16]. Lead (Pb) induces adverse effects when it chronically accumulates in the body, including effects on the nervous and cardiovascular systems [17]. The study by Simoes et al demonstrated for the first time in an animal model of a controlled, low dose chronic lead exposure that cardiovascular changes, such as arterial hypertension, are accompanied by impaired autonomic control of the cardiovascular system, as characterized by reduced baroreflex sensitivity and a sympathovagal imbalance [17]. Lead has no essential role in the body, and lead poisoning accounts for about 0.6% of the global burden of the disease [18] Since Calabash chalk consumption is a common practice in Nigeria, with the reports that lead, one of its constituents lowers intelligent quotient in children and damages brain cells in mice [19], it becomes necessary to find out possible effects on locomotion using white Swiss mice as experimental models.

II. MATERIALS AND METHODS

Subjects: A total of forty five Swiss white mice were procured from the Animal House of the Physiology Department of College of Medicine and Health sciences, Abia State University, Uturu, Nigeria. Proper animal acclimatization and home caging management was maintained with well-designed feeding cages. Rat chow and calabash chalk were procured and adequate clean water made available for drinking and swimming. Also provided was the Morris water maze and liquid milk to make the water opaque. Activities of the animals were monitored with a digital video recording device.

Methods: The animals were randomly divided into three groups of 15 and identified as group A, group B and group C respectively. In preparing the rodent chow and Calabash chalk mixture, 10% Calabash chalk diet was gotten by mixing a portion of ground calabash chalk with 9 portions of rodent chow and 20% Calabash chalk diet was constituted in same manner by adding 2 portions of ground calabash chalk to 8 portions of rodent chow. The 10% Calabash chalk diet was termed low dose (LD) while 20% Calabash chalk diet was termed high dose (HD). Group A, used as the control, was fed with only rodent chow, water *ad libitum*. Group B was fed LD Calabash chalk diet while Group C received HD Calabash chalk diet. Equal feeding portions of the different diet compositions were presented to the corresponding three groups as already described and the animals allowed to feed freely for thirty days with liberal water intake.

Test for Locomotion: The open field (OF) apparatus was constructed from white plywood with 72 x 72 cm floor and 36 cm walls. One of the walls is made up of clear Plexiglas floor. The lines divide the floor into sixteen 18 x 18 cm squares from which a central square is marked at the middle of the open field[20]. Each mouse was scooped up in a small plastic container from its home cage and placed at the center square of the OF, then allowed to explore the apparatus for about 5 minutes while observing the behaviors (frequency of line crossing, frequency of walling, frequency of rearing, frequency of center square entry and grooming frequency) after which it is returned to its home cage. The OF was cleaned with 70% ethyl alcohol and permitted to dry between trials.

Statistical analysis: Data entry and analysis were done using SPSS viewer (13.0). Descriptive statistics such as mean, standard deviation (SD) and range were used for data analysis. Data are reported as mean ± SD. Multiple regression analysis was used for analysis. P value of 0.001 was used as a criterion for reporting statistical significance.

III. RESULTS

The frequency of line crossing for groups A,B and C were 139.1±3.95, 80.24±44.34 and 27.4±2.74 for every 5 minutes respectively thus showing a significantly ($p<0.001$) lowest frequency in group C compared to that of group A and B. The frequency of line crossing in Group C was also significantly ($p<0.001$) lower than that seen in group B. The mean frequencies of walling in the OF apparatus for groups A, B and C were 20.1±3.21, 24±1.79 and 14±1.06 for every 5 minutes respectively. The frequency of walling of group C was significantly ($p<0.001$) lower than both groups A and B. The mean frequency of rearing for groups A, B and C were 10.7±3.06, 5.88±1.01 and 1.3±0.57 per 5 minutes respectively with both groups B and C significantly ($p<0.001$) lower than group A. The frequency of center square entry for groups A, B and C were 3.4±0.72, 1±0.32 and 0.2±0.13 per 5 minutes thus indicating a significantly ($p<0.001$) lower frequency in both groups B and C compared to the control group A, however, group B was significantly ($p<0.001$) higher than in group C which id the high dose group.

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IV. DISCUSSION

Behaviours such as number of line crossing and the frequency of rearing are measures of locomotor activity and exploratory behavior. A high frequency of these behaviours indicates increased locomotor and exploratory activity and a low level of anxiety. The frequency of line crossing measures horizontal locomotor activity. The frequency of the 20% calabash chalk fed group (group C) was significantly lower than the 10% calabash chalk fed group (group B) and control group (group A) thus, showing possible impaired neuromuscular activity. Another possible reason for the reduced locomotor activity may be linked to its demineralizing effect on bone as concluded in a study by.[19] Such adverse effect on bone may lead to degenerative changes around the joints leading to arthropathies which pose discomfort especially during movement of joints associated with locomotion. The reduction in bone mineralization can lead to an increase in bone fragility and ostheoporosis .[13] Lead has also been implicated in reduction of bone strength. [13&21] A high frequency of rearing shows increased exploratory behavior. The result shows that the 20% group had a significantly lower frequency than the control. There was however no significant difference between the 10% and the control. This shows that calabash chalk at 20% reduced exploratory locomotor activity as well as horizontal locomotor activity.

Exploratory behavior refers to the tendency to investigate a novel environment. It is considered a motivational behavior however, not clearly distinguishable from curiosity. The results of the center square activities in the open field maze showed that the center square activities for the high dose group were significantly lower than the low dose group and the control thus depicting a reduced exploratory locomotor activity.

V. CONCLUSSION

The decrease in the locomotor activity and exploratory behavior in the test groups shows that calabash chalk consumption reduces locomotive activities in mice which could either be as a result of an impairment at the neuromuscular level or possible bone arthroparthy due to the demineralizing effects of lead. Caution, therefore need to be exercised in the consumption of calabash chalk among pregnant women and Children who are usually in the habit of consuming it.

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