Evaluation of Neuro-pharmacological Effects of Calabash Chalk on *Swiss albino* Mice

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Bachelor of Pharmacy (Hons.)

Department of Pharmacy
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Declaration

It is hereby declared that

1. The thesis submitted is my own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material, which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all main sources of help.

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Approval

The thesis/project titled "Evaluation of Neuro-pharmacological Effects of Calabash Chalk on *Swiss albino* mice" submitted by Luluin Maknun Shova (15346003) of Summer, 2015 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy (Hons.) on September 30, 2019.

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Ethics Statement

Ethical Permission has been achieved from the Department of Pharmacy, Jahangirnagar University.

Abstract

In some countries in Asia and Africa, calabash chalk consumption is common which creates safety concerns. The study aims to evaluate the neuro-pharmacological effect of this chalk in mice. Twenty-four female *Swiss albino* mice weighing 22-28 grams were taken and were randomly divided into 4 groups. The chalk were ingested by oral gavage at the doses of 200, 400 and 600 mg/Kg/day. A control group was treated with distilled water. Four neuro-pharmacological tests were performed. These were: OFT (Open Field Test), EPM (Elevated Plus Maze), HCT (Hole Cross Test), HBT (Hole Board Test). The results showed that there has been a decrease in the locomotor activity and exploratory behavior. It indicates a probable impairment at a neuromuscular level due to a reduction of locomotive activities as a result of calabash chalk consumption. Therefore, necessary caution must be taken while consuming calabash chalk.

Keywords: Calabash Chalk*, Swiss albino* Mice, Locomotion, Neuromuscular

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Table of Contents

Declaration ..... **ii**

Approval ..... **iii**

Ethics Statement ..... **iv**

Abstract/Executive Summary ..... **v**

Acknowledgement ..... **vi**

Table of Contents ..... **vii**

List of Tables ..... **ix**

List of Figures ..... **ix**

List of Acronyms ..... **x**

Chapter 1 Introduction **1**

1.1 Background of the Study 1

1.2 Chemical Constituents of Calabash Chalk 2

1.3 History of Geophagy 4

1.4 Territory of Practice 5

1.5 Hypothesis Behind Geophagy 5

1.6 Geophagy And Religious Beliefs 6

1.7 Geophagy And Cultural View 7

1.8 Benefits of Calabash Chalk 7

1.9 Adverse Effects of Calabash Chalk 8

1.10 Aim 9

1.11 Objectives 9

Chapter2 Method 10

2.1 Research Design 10

2.2 Animal Grouping 11

2.3 Preparation and Administration of Sample 11

2.4 Laboratory Tests 12

2.5 Statistical Analysis 14

Chapter 3 Result and Discussion 15

3.1 Measurement of Body Weight 15

3.2 Hole Board Test 16

3.3 Hole Cross Test 17

3.4 Open Field Maze Test 18

3.5 Elevated Plus Maze Test 19

Chapter 4 Conclusion 21

4.1 Conclusion 21

4.2 Limitations of the Study 22

4.3 Recommendation 22

4.4 Future Research Plan 22

References 23

List of Tables

Table 1: Analysis of Calabash Chalk Showing the Concentration of Element 3

Table 2: Evaluation of Neuro-pharmacological Effects of Calabash Chalk by Hole Board Test..... 16

Table 3: Evaluation of Neuro-pharmacological Effects of Calabash Chalk by Hole Cross test..... 17

Table 4: Evaluation of Neuropharmacological Effects of Calabash Chalk by Open Field Maze test .. 18

Table 5: Evaluation of Neuropharmacological Effects of Calabash Chalk by Elevated Plus Maze test 20

List of Figures

Figure 1: Calabash chalk in different shapes 2

Figure 2: Research Design 10

Figure 3: Animal Grouping 11

Figure 4: Comparison of body weight among the control and the treated groups of mice..... 15

List of Acronyms

OFT	Open Field Test
EPM	Elevated Plus Maze
HCT	Hole Cross Test
HBT	Hole Board Test

Chapter 1

Introduction

1.1 Background of the Study

Intake of soil and chalk is defined as practice of geophagy (M. B. Ekong et al., 2014). Although the practice occurs worldwide almost in every country and is not restricted within age, sex, race, region and occupation, it is more commonly practiced by rural and tribal people than urban societies (Owhorji, Okon, Nwankwo, & Osim, 2019). Moreover, the purpose of taking geophagic material is often related with medicines, regular diet and religious beliefs (M. B. Ekong, John, Mbadugha, Bassey, & Ekanem, 2012).

According to Nyanza, Joseph, Premji, Thomas & Mannion (2014), etiology of geophagy continues to be difficult to track down. Geophagic practice has been influenced largely by cultural, psychological, physiological and socioeconomic factors. In addition, geophagy has contentious and indecisive health impacts, health advantages and harmful effects. The danger connected with contaminated soil intake relies on dose, frequency and bioavailability. On the other hand, gastroenteritis, nausea, diarrhea and vomiting have long been treated with substances that have clay constituents.

One of them is the consumption of a blend of clay and cray, called a calabash chalk (M. B. Ekong et al., 2014). It is a geophagic substance that is frequently eaten as a remedy for nausea and for the sake of enjoyment in Nigeria & other Western African nations (M. B. Ekong et al., 2012). The material has distinct names such as Calabar rock in English, Nzu by the Nigerian tribe Igbo, Ndom by the Efik/Ibibio in Nigeria and Argile/La Craie by French. Ebumba, Poto and Ulo- these names are also used elsewhere (Owhorji et al., 2019).

The chalk is naturally composed of fossilized shells but can be ready artificially. It is made by combining clay and mud, with other components like wood ash, sand and occasionally-salt. Molding and then heating this combination is done to generate the finished item. It can be obtained in a number of shapes, including dust, molded shapes and blocks. Although the calabash chalk was originally collected from Africa but it can be found in ethnic shops and markets in the United Kingdom (M. B. Ekong et al., 2012).

Figure 1: Calabash chalk in different shapes

In the UK, Calabash Chalk has been linked to immigrants from West Africa and Southern Asia and is basically imported from Nigeria and marketed in ethnic stores. The migration of mankind from communities where geophagy is practiced results cultural transference to developed countries where many people do not believe in soil and clay consumption (Wariso & Uket, 2018).

1.2 Chemical Constituents of Calabash Chalk

Calabash chalk usually consists of aluminum silicate hydroxide, a common representative of the kaolin clay group, with the formula: $Al_2Si_2O_5(OH)_4$. A number of other contaminants

have also been recorded that could be harmful for the body. These include; metals, metalloids and persistent organic pollutants. The metals include iron, manganese, potassium, aluminium, copper, zinc, barium, chromium, nickel, titanium, rubidium, lead, tin and arsenic. Moreover, organic pollutants are present such as-alpha lindane, endosulphan II and p, endrin, pI-dichloro diphenyl dichloroethane (DDD) (M. Ekong, Peter, & Ekanem, 2016).

The concentration of elements present in the calabash chalk are shown in the following table:

Table 1: Analysis of Calabash Chalk Showing the Concentration of Element

Elements analyzed	Concentration of elements (mg/kg±SD)
Magnesium (Mg)	1100±100
Aluminium (Al)	160000±15000
Potassium (K)	5500±500
Calcium (Ca)	160±14
Titanium (Ti)	11000±1000
Vanadium (V)	125±10
Chromine (Cr)	130±10
Manganese (Mn)	40±5
Iron (Fe)	15000±1500
Cobalt (Co)	4.1±0.2
Nickel (Ni)	25.5±1.3
Copper (Cu)	15.5±0.7
Arsenic (As)	11.5±0.8
Silver (Ag)	0.50±0.03
Cadmium (Cd)	0.76±0.04
Antimony (Sb)	0.42±0.02
Barium (Ba)	200±10
Thallium (Tl)	0.33±0.02
Lead (Pb)	57±3
*Zinc (Zn)	<100mg/kg

(M. Ekong et al., 2016)

High amount of lead present in the calabash chalk poses health hazards and affects different systems in the body of humans and animals such as central and peripheral nervous system, haematopoietic system, cardiovascular system and reproductive system. Neurological and visual changes with low doses of lead were also reported. However, harm to the nervous system is viewed as the most serious effect *(ibid)*.

According to UK Food Standard Agency, the amount of lead present in Calabash chalk ranges from 8.2 mg/kg to 16.1 mg/kg. Moreover, the American Academy of Pediatrics have reported that lead above 10 μg/dl in blood concentration is toxic for children (Wariso & Uket, 2018).

1.3 History of Geophagy

In ancient period, clay and soil consumption was not unusual. The first definition of geophagy was found in a textbook which was written by Kos Hippocrates (460-377 BC). Over the years, the textbook had been a basis of medical practices. Therefore, geophagia were acquainted to Greek and Roman doctors (Woywodt & Kiss, 2002).

On the contrary, textbooks written by Roman, Greek and Arab people were used in the middle period. One of the most commonly used medical textbook was collected by the Persian Ibn Sina (980-1037 AD) where geophagia was mentioned in detail *(ibid).*

Finally, in the 16th and 17th centuries, the word pica was first cited in a surgical job context. More data about pica was collected from medical disquisition. In addition, the British Library define a notable spectrum on geophagy. Researchers who traveled overseas in the 17th century also mentioned about geophagy*(ibid)*.

1.4 Territory of Practice

1.4.1 Territory of Geophagic Practice

Geophagy is performed and socially endorsed only in certain cultures, communities and classes of individuals. Over the years, these categories included young individuals, African slaves, pregnant women; and immigrants from India, Africa, Pakistan, Great Britain and African American women in the United States (Henry & Cring, 2014).

1.4.2 Territory of Calabash Chalk Practice

The Calabash chalk is a geophagic material that is frequently used for pleasure in Nigeria and other countries of West Africa and for nausea by pregnant women. In Sub-Saharan African nations such as Nigeria, Tanzania, Rwanda, Kenya, Ghana and South Africa, the use of calabash chalk among pregnant women is common. The practice of geophagy differs between and within nations, but is estimated to be between 10 to 75 percent (Nyanza, Joseph, Premji, Thomas, & Mannion, 2014).

1.5 Hypothesis Behind Geophagy

Three overall physiological reasons have been developed in relation to human regarding etiology of geophagy. The brief explanation of three hypothesis are given below:

Hypothesis 1: Nutrient Deficiency

The hypothesis suggests that individuals consume soil to compensate for the mineral nutrient inadequacies especially iron, zinc and calcium. In short, the minerals will be referred as nutrients collectively. The people who practiced geophagy however do not relate their behavior with salt shortage and geophagic land is not usually salty with only a few exceptions. Moreover, anemia is often related with geophagy because soil consumption can

treat iron deficiency. However, anemia can also be triggered by infection and loss of blood. Some researches propose that geophagy in animals was motivated by sodium deficiency (Author, Young, Sherman, Lucks, & Pelto, 2011).

Hypothesis 2: Protection

The hypothesis suggests that the soil is consumed as a medicine to decrease the short-term depression and long-term impacts of toxic chemicals, bacteria and pathogens. Many food crops generate harmful chemicals-tannins and glycoalkaloids can cause gastrointestinal disorder, swelling and muscle pain. Hazardous pathogens usually include bacteria, viruses and parasites transmitted through food and water. There are two methods under this hypothesis by which geophagic soil can be defensive: by decreasing the permeability of the stomach lining to toxins and by immediately linking to toxins and pathogens. Here, soil can bind and strengthen mucosal layer and thus increase the mucosal secretions of the intestinal walls. Secondly, it can immediately bind to toxins, parasites and other pathogens. The process can either make them unabsorbable or prevent their respiration *(ibid).*

Hypothesis 3: Non-Adaptive

The hypothesis suggests that eating earth has no advantage. Instead, individuals practice this because they do not have food to consume or suffer from mineral deficiency *(ibid*).

1.6 Geophagy and Religious Beliefs

Soil consumption is strongly adopted by religious beliefs of people. Geophagy is a marvel that is deeply distinct representing spiritual belief, social exercise, psychiatric distress and psychological need. According to Jakari (2015), a trust in soil's spiritual and magical strength may come from other fundamental factors. The practice of earth-eating is a form of many religious practices and services were reported by Hunter (1973). He also cited some examples about religious beliefs including- Muslims ate a brown, plain and rectangular cake consisting

of powder taken from the grave of the Prophet as a remedy for all illnesses. Moreover, Christians took white stones to the nursing mothers from Bethel Helm Grotto.

1.7 Geophagy and Cultural View

Most evidence of soil consumption is obtained in Africa where it is recognized culturally by women, especially during pregnancy and young people (Geissler, 2016). Deliberate earth-eating can be motivated by social intensions. The practice of geophagy will be depicted as a socially acceptable rehearsal which is firmly recognized by people, family and community. In addition, earth-eating comes into processes of advancement from adolescent to adult and on the societal level as a social exercise. Moreover, some women consumed the clay deliberately and passionately because they believed that soil intake will produce fair skin color of their offspring. Woywodt and Kiss (2000) mentioned the perception of South African women that consumption of geophagic soil would increase their beauty (Jakari, 2015).

1.8 Benefits of Calabash Chalk

Little information is available regarding the health advantages of geophagic clay including calabash chalk. These are:

- Ingesting clay is an accepted medication for gastrointestinal diseases. In addition, clay is widely used in the treatment of vomiting, diarrhoea and nausea (Jakari, 2015).
- Frankel (1997) mentioned that, a number of geophagists claim that their pica helps in reducing heartburn and nausea.
- Mahaney and Krishnamani (2002) found that monkeys that frequently consume soil have considerably lower parasite loads.
- It also acts as an immunological adjuvant capable of performing as a vaccine (Jakari, J. M. 2015).

- Nzu or calabash chalk, particularly in pregnant females can certainly decrease morning illness. The taste of chalk can prevent dizziness.
- It can also be used as a facial masks and soaps(M. B. Ekong et al., 2012).

1.9 Adverse Effects of Calabash Chalk

Calabash chalk impacts various organs and mechanisms of our body as stated below:

- Severe intake of calabash chalk can increase anxiety and the perception of pain in mice (Owhorji et al., 2018).
- Lead and arsenic present in calabash chalk can cross the blood-brain barrier to trigger various consequences including neurotoxicity (Cleveland et al., 2008; Ekong et al., 2014).
- Calcium, magnesium, potassium, iron and aluminium present in calabash chalk can cross the blood-placental membrane thus they can enter to the growing fetus and can impact the metabolism and growth (Ekanem, Ekong, Eluwa, Igiri, & Osim, 2015).
- When geophagic substances such as calabash chalk are eaten and reach the digestive system, the impacts on individuals may be detrimental (M. B. Ekong et al., 2012).
- Numerous studies on the health hazards of calabash chalk shown that it adversely affects the blood. It changes the standard hemoglobin concentration, the RBC and the sedimentation rate of erythrocytes in the body (M. B. Ekong et al., 2012).
- Calabash chalk can change the growth rate and trigger bone demineralization (M. B. Ekong et al., 2012).
- Calabash chalk contains large quantity of lead. Overconsumption of lead during pregnancy may also impact the offspring. It could trigger neurological damage, decrease IQ and increase the danger of cognitive development (M. B. Ekong et al., 2012).

1.10 Aim

The aim of the study is to evaluate the neuropharmacological changes of *Swiss albino* mice after ingestion of calabash chalk.

1.11 Objectives

The objectives of the study are:

- to perform neuro-pharmacological parameters
- to observe changes in the behavioral pattern
- to determine possible locomotor activities on mice model

Chapter2

Method

Twenty-four female *Swiss albino* mice weighing 22-28 gram (approximately) were procured from the animal house of the Department of Pharmacy, Jahangirnagar University. At room temperature, these mice were nurtured and provided adequate food and water. Three weeks of intense acclimatization care was provided before the administration of calabash chalk. Moreover, body weight of each mouse were recorded every day within the three weeks. The research was conducted for 21 days. Here, calabash chalk solution was provided every day for three weeks by oral gavage. In addition, ethical permission was taken from the Jahangirnagar University.

2.1 Research Design

The following steps are performed to conduct the research on mice model:

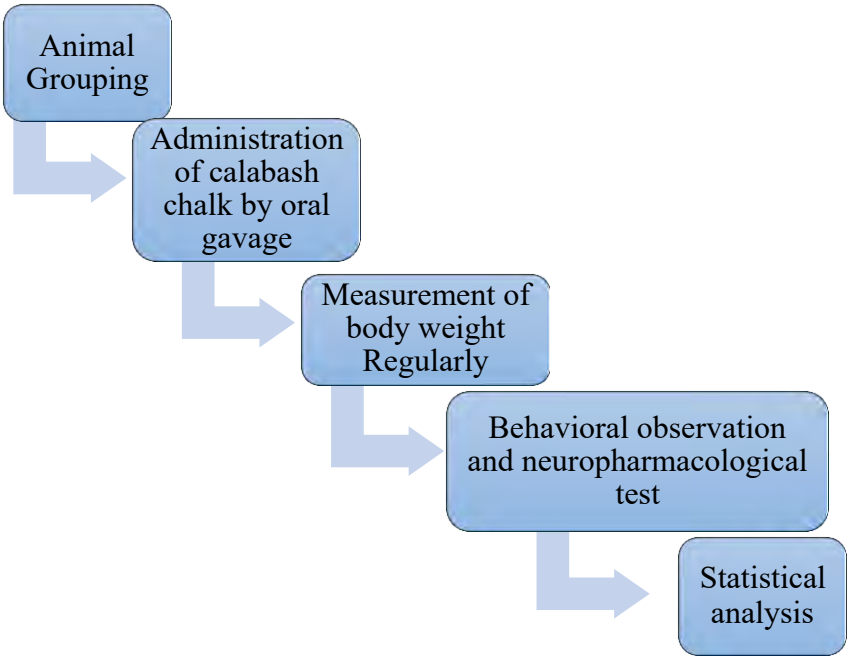

Figure 2: Research Design

2.2 Animal Grouping

Twenty-four female *Swiss albino* mice (n=24) were split into four groups. Each group has six mice and were designated as group 1, 2, 3 and 4. Then, the dose of 200 mg/kg, 400mg/kg and 600 mg/kg was prepared for group 2, 3 and 4 respectively. Here, group 1 was taken as a control group and group 2, 3 and 4 were the treatment groups.

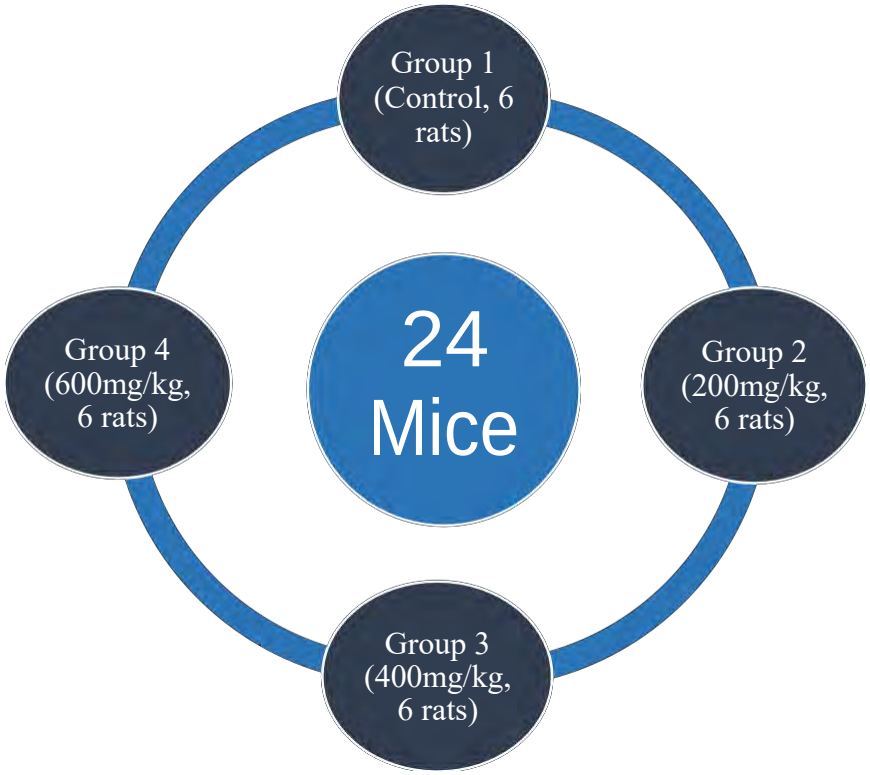

Figure 3: Animal Grouping

2.3 Preparation and Administration of Sample

Calabash chalk has been gathered from the Sylhet, a region of Bangladesh. It has been sun-dried for three days to ensure a reduction in moisture. The dried chalk was stored in sterile

polythene bags, sealed and was carefully carried to laboratory. Finally, the sample was broken in mortar and pestle and sieved to make the powder fine and smooth.

A stock solution of sample is prepared with distilled water. In 500 ml of distilled water, 20 gm of prepared powder was added and appropriately stirred before the administration of calabash chalk. Here, the control group (Group 1) was only provided with everyday food on distilled water. On the other hand, treatment group (Group 2, 3 and 4) were provided with1ml sample solution from three different doses (200mg/kg, 400mg/kg and 600 mg/kg) by oral gavage. Moreover, body weight was recorded on a daily basis and record kept on the data sheet.

2.4 Laboratory Tests

Four experiments were conducted on treated mice to evaluate the behavioral pattern due to consumption of calabash calk has been provided below:

2.4.1 OPEN FIELD TEST (OFT)

The OFT was formulated by Calvin S. Hall in 1932 for the study of animal emotion. Open area was used with measurements of 7×7 feet and food set in the center. Hall noticed how animals gently reached to meals in circular motion. However, the inclination of animals to discover a new area reduced when meal was withdrawn. OFT is now commonly used in many science fields including psychopharmacology and neuroscience. There are some parameters present in OFT include shape and size of the open-field area, duration, apparatus, level of illumination, food in the open field etc. At the beginning of the experiment, the animal is put in the central area, usually for a duration of five minutes and factors such as horizontal locomotion, rate of vertical operation and grooming are assessed. The amount of anxiety is measured by the ratio of central entries and the open field test periphery (Belovicova, Bogi, Csatlosova, & Dubovicky, 2017).

2.4.2 HOLE BOARD TEST

In 1970, the hole board was first used which comprises of a sealed area with holes in where the animal can do head-dipping. Factors such as- number and period of head-dipping are determined for the measurements of neophilia. A variety of research have shown that head-dips and locomotives can vary autonomously. In particular, high head-dipping number is perceived to be indicating neophilia, while low rates are presumed to arise from absence of neophilia or are presumed to represent a strong anxiety condition in the animal. Moreover, the hole-board experiment is presently being used in behavioral pharmacology.

The hole-board device comprised of a wooden, gray container sized 68 cm × 68 cm. The length of ceilings were 40 cm and by using a metal stand, the box was lifted 28cm. Four holes were cut into the device floor. Using black masking tape, the floor of the box had been marked into one central area and four outer areas. The device was placed in a small experiment space with dimmed white light. A subject was put in one corner of the device, at the start of the trial. It lasted 10 minutes for every trial. The walls along with floors were cleansed with 70% alcohol between each trial (Brown & Nemes, 2008).

2.4.3 HOLE CROSS TEST

A hyper-emotional reaction to a new setting is the most consistent change in behavior. A partition having a size of 30cm × 20cm × 14cm was fixed in the center of the cage. Wood is used to make the board. A 3 cm diameter hole created in the middle of the box from a height of 7.5 cm. Subject was put in the middle of the cage on one side. Over a span of 3 minutes, at 0, 30, 60, 90 and 120 minutes after the administration, the number of passages through the hole was counted (Shahed-Al-Mahmud & Lina, 2017).

2.4.4 ELEVATED PLUS MAZE (EPM) TEST

The EPM was identified as an easy technique for evaluating rodent's anxiety reactions. It is a Y shaped device with an open alley and enclosed alley. Two open and two closed arms are organized in such a way that produces a plus shape. The analysis of rodent anxiety is measured by using the ratio of duration of stay on the open arms to the closed arms. If the mice spent more time in open arms, it will indicate less anxiety measurements. Moreover, it will take ten minutes to complete the test.

Mouse elevated plus maze is built with stainless steel and then painted black. It has four arms measuring 30cm length and 5cm width. A metal leg is attached to each of the arms that lifted the arms up to 40 cm from the surface level. The mouse maze is smaller compared to the rat elevated plus maze and is easily movable (Walf & Frye, 2007).

2.5 Statistical Analysis

SPSS was used for data input and analysis. One-way analysis of variance (ANOVA) was applied for the statistical analysis of all data, followed by Independent Samples T-tests. All results were considered as significant at $p<0.05$.

Chapter 3

Result and Discussion

3.1 Measurement of Body Weight

Significant changes were observed in body weight gain between the control and the treated groups. No significant differences were observed within the three different doses (Figure 4).

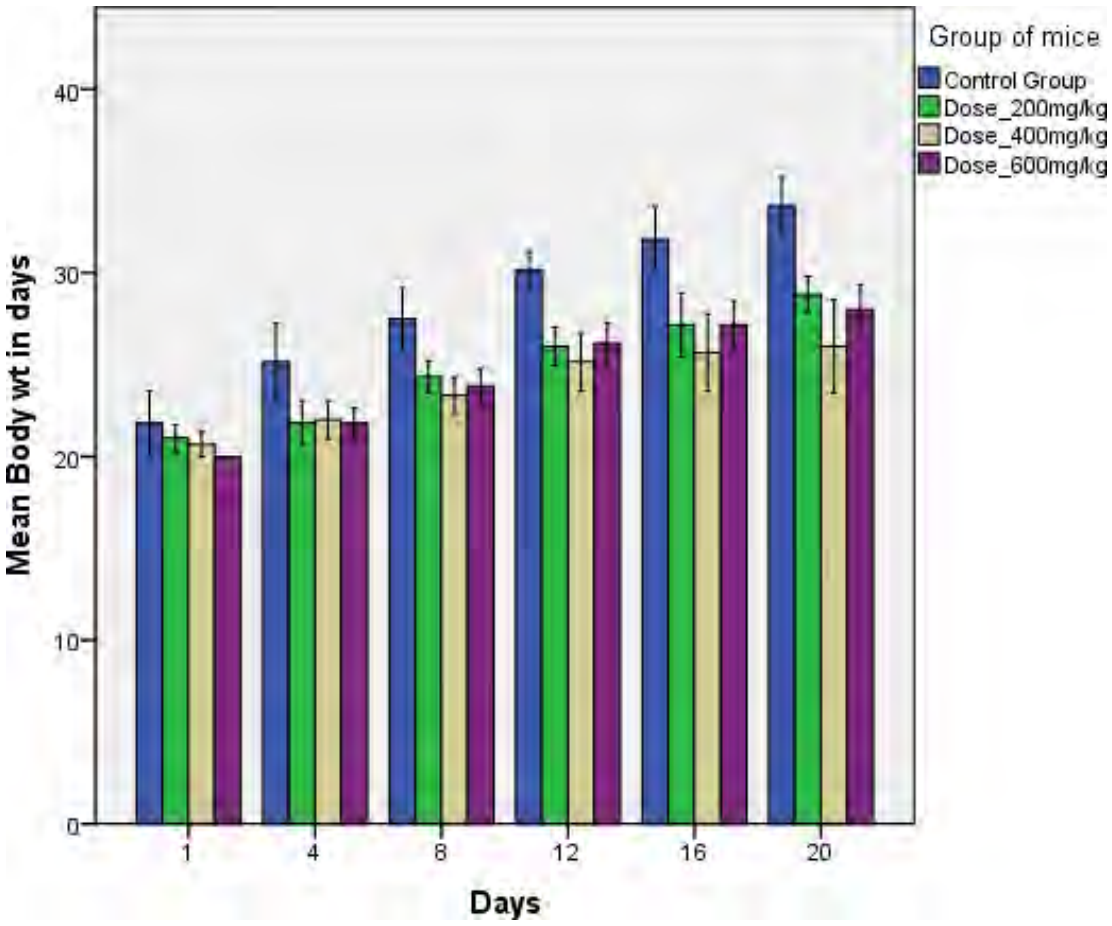

Figure 4: Comparison of body weight among the control and the treated groups of mice

The results showed body weight gain in control group, but no difference existed in the body weight gains between three treatment groups. Significant changes has been also observed between group 1 (control) and group 2, 3 and 4 (treatment groups).

After treating 21 days with calabash chalk, no significant impact on the weight of swiss albino mice was observed. However, the treatment group showed a slight increase in weight compared to the control group. The three treatment group did not show any significant change in weights among themselves. On the other hand, from day 1 to 21, individual group of mice gained weight.

According to Ekanem, Ekong, Eluwa, Igiri, & Osim (2015), there were no morphological changes on the mice used in both experimental groups. The control group showed higher percentage of increase in weight compared to the treatment groups and this may have occurred due to the elemental composition of the calabash chalk. As mentioned earlier, a number of elements such as calcium, potassium are known to be able to cross blood-placental membrane which can have an impact on metabolism.

3.2 Hole Board Test

Evaluation of neuropharmacological test using hole board are given below:

Table 2: Evaluation of Neuropharmacological Effects of Calabash Chalk by Hole Board Test

Group (n=6)	Number of area crossed	Number of head dipping	Number of defecations
Group 1 (Control)	6.50±0.43	35.33±1.78	2.00±0.52
Group 2 (Dose 200mg/kg)	5.00±0.37*	29.00±0.58**	1.17±0.48
Group 3 (Dose 400mg/kg)	4.00±0.36***	20.83±2.04***	0.50±0.22*
Group 4 (Dose 600mg/kg)	3.00±0.45***	16.00±2.42***	0.33±0.21*

Results were presented as mean ± standard error of means.
***, **, * denotes the significance level p at 0.001, 0.01 and 0.05, respectively.

In the hole board test, the treatment groups (group 3 and 4) were significantly lower in number of area crossing compared to the control group. In case of head dipping group 3 and

group 4 were treated with 400mg/kg and 600mg/kg were significantly lower than group 1 respectively.

Treatment groups (group 3 and 4) has lower frequency both in terms of hole crossing and head dipping when compared with the control group. However, if you do not take control group into consideration, we can see that the changes among three treatment groups are small. However, among groups 2, 3 and 4 head-dipping parameters show a significant decrease with increased dose. According to Brown & Nemes (2008), head dipping is usually high in first trial, then decreases in the next 2 trials and remains stable afterwards, Therefore, with decrease in fearfulness, there was a decrease in head-dipping. We are assuming that when exposed to a novel apparatus, the state of anxiety is normal.

3.3 Hole Cross Test

Evaluation of neuropharmacological effects using hole cross device are given below:

Table 3: Evaluation of Neuropharmacological Effects of Calabash Chalk by Hole Cross test

Group (n=6)	Number of crossing hole
Group 1 (Control)	7.33±0.67
Group 2 (Dose 200mg/kg)	6.00±1.00
Group 3 (Dose 400mg/kg)	3.00±0.68***
Group 4 (Dose 600mg/kg)	1.67±0.56***

Results were presented as mean ± standard error of means.
***, **, * denotes the significance level at 0.001, 0.01 and 0.05, respectively.

In the hole cross test, frequency of crossing hole were significantly lower in treatment groups (group 3 and 4) when compared with control group.

Mice treated with 200mg/kg have not shown significant change in hole crossing when compared with the control group but the frequency was higher among the mice treated with higher dose, with 400mg/kg and 600mg/kg. The hole crossing numbers showed a sharp decline with the increase of dose.

This test is one the most common tests that is used to examine the exploratory behavior of animals. It has been proved that a number of drugs results in a decrease curiosity in the animals. Result shows significantly ($p < 0.01$) decreased locomotion of the animals in hole cross test. It has also been proved that muscle weakness and sedation is caused by CNS depressant which ultimately limits the performance of animals (Moniruzzaman, Sharoti Bhattacharjee, Rahman Pretty, & Sarwar Hossain, 2016).

3.4 Open Field Maze Test

Evaluation of neuropharmacological effects using open field test are given below:

Table 4: Evaluation of Neuropharmacological Effects of Calabash Chalk by Open Field Maze test

Group (n=6)	Line Crossing	Groom	Rearing	Stretch Attend	Central Square Entry	Central Square Duration	Defecation	Urination
Group 1 (Control)	252±19	11.00± 0.58	26±2.2	6.83± 1.14	3.33± 0.67	7.33±0.56	2.50±0.43	0
Group 2 (Dose 200mg/kg)	209.67±22	7.50± 0.67	20.00± 4.04	11.67± 1.02**	1.33± 0.42	2.50± 0.89***	2.67±0.42	0.67± 0.33
Group 3 (Dose 400mg/kg)	105.80±4.16***	2.20± 0.58***	12.40± 1.54***	16.04± 0.40***	0.20± 0.20	0.80±0.80***	1.60±0.68	0.20± 0.20
Group 4 (Dose 600mg/kg)	74.50±13.84***	1.33± 0.50***	4.67± 1.31***	14±0.36***	0.33±0.21	1.33±0.84***	1.17±0.48	0

Results were presented as mean ± standard error of means.
***, **, * denotes the significance level at 0.001, 0.01 and 0.05, respectively.

In the open field maze test, if the control group is compared with treatment groups (group 2, 3 and 4), there is significant changes observed in frequency of line crossing, grooming, rearing, stretch attending and central square entry and duration.

The parameters in this test showed large deviation among groups. Most of the parameters excluding stretch attended and urination showed a gradual decrease with increased dose. According to Wariso & Uket (2018), measurements of exploratory behavior and locomotive operation are often observed by behavior such as the frequency of line crossing. A low frequency of this behavior indicates a high anxiety level and thus a decrease in exploratory and locomotive experience. Group C, which was fed 20% calabash chalk showed a significantly lower quantity than control group and Group B, which was fed 10% calabash chalk. This indicates a probable impairment of neuromuscular activity.

The decreased locomotive activity can be attributed to the demineralizing effect of calabash chalk on the bone. An increased rearing frequency exhibits improved exploratory behavior. The outcome indicates that the group of 20 percent had a much-reduced frequency compared to control group. However, the control group and 10% group showed no significant difference between them. This suggests 20% calabash chalk reduces locomotive and exploratory behavior. Results from the center square operations in the open field maze indicated that the center square operations for the high-dose group were considerably lower than the low-dose team and control represented a decreased locomotive exploratory activity.

3.5 Elevated Plus Maze Test

Evaluation of neuropharmacological effects using elevated plus maze are given below:

Table 5: Evaluation of Neuropharmacological Effects of Calabash Chalk by Elevated Plus Maze test

Group (n=6)	Open area	Closed area
Group 1 (Control)	0.67±0.33	9.33±0.33
Group 2 (Dose 200mg/kg)	1.67±0.66	3.67±0.62***
Group 3 (Dose 400mg/kg)	3.83±0.98*	3.00±0.36***
Group 4 (Dose 600mg/kg)	4.83±0.60***	2.17±0.31***

Results were presented as mean ± standard error of means.

***, **, * denotes the significance level at 0.001, 0.01 and 0.05, respectively.

In the elevated plus maze test, Group 3 and 4 were significantly higher than control group and group 2 in the frequency of passing open area. On the other hand, in the frequency of passing closed area, there were significant decrease in three different treated groups when compared with control group.

If we look at open area, we'll find that there is no significant difference between group 3 and 4 if we do not take control group into consideration and exposure to open area decreased with increased dose. In the closed area parameter, group 2,3 and 4 did not show any significant difference. In addition, a substantial reduction was noted with enhanced dose relative to the control group in the frequency and duration to the closed area. (Walf & Frye, 2007), when results compared to control, both low and high dose groups showed lower close arm frequency and duration in the elevated maze experiment. Less time was spent in the close arm. Frightened mice are unwilling to open and prefer darker and more confined spaces, so these findings indicate that calabash chalk in mice has reduced anxiety.

Chapter 4

Conclusion

4.1 Conclusion

After conducting four different tests on the treatment groups, it was found that there has been a decrease in the locomotor activity and exploratory behavior. It indicates a probable impairment at a neuromuscular level due to a reduction of locomotive activities as a result of calabash chalk consumption. There were three different doses that were applied on mice and with the increase in dose the mice showed lower frequency in almost every parameter. Moreover, some components of calabash chalk such as arsenic, lead, aluminum are known to

cross the blood brain barrier and can have a negative impact on different parts of brain. In pregnant women, the propensity to consume calabash chalk is very common. Therefore, calabash chalk consumption should be checked and monitored while pregnant women and children are consuming it.

4.2 Limitations of the Study

A couple of limitations were faced while conducting the study. Despite these limitations, the outcome of this study was significant. Limitations of the study are given below:

1. The study was carried out in a time span of 21 days. One of the variables that contributed in the outcome of the result is time period. If time had varied, the outcome of the study could have been different.

2. Neuro-pharmacological tests were done once only. The results would have been more reliable and accurate if we could conduct the test for three times.

3. Four tests were conducted for behavioral observation. Other available tests could be performed for further findings.

4.3 Recommendation

There needs to be further studies on this topic. An extensive study on this topic will provide us thorough understanding of the impact.

4.4 Future Research Plan

Further scopes of study include a study on general association of geophagy and neuro-pharmacology. Along with a plan to do histopathology of brain on mice, we will also be studying the effect of calabash chalk on various organs of mice.

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