

Geophagy: Histopathological Perspective of an Unusual Craving for Clay

A Project Submitted

By

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Dedicated to:

My beloved parents and my sisters, who always support and inspire me to face every challenge of life

Certification Statement

This is to certify that the project titled “Geophagy: Histopathological perspective of an unusual craving for clay” submitted in order to fulfill the partial requirements for the degree of Bachelor of Pharmacy from the department of Pharmacy, BRAC University under the supervision of Dr. SharmindNeelotpol, Assistant Professor, Department of Pharmacy, BRAC University and proper referencing have been made where the language, concept and writing of others are used.

Signed,

Countersigned by the supervisor,

Acknowledgement

First of all, I am obliged to our Creator, the most merciful and the most gracious, Allah. With the help of His grace I am able to finish this project work. Now coming to an end of this thesis I am aware that this work would not have been possible without the help and guidance of many people.

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Abstract

Due to the increasing number of industries many toxic and heavy metals are getting mixed with soil. Since many women have the tendency of eating a special type of clay, which is known as calabash chalk, it has become very important to find its effect on health.. So this study aimed to find out the effect of calabash chalk on different organs of *Swiss albino* rats. The importance becomes double when it is about pregnant women as the fetus can be affected by any change in the organ of the mother. To find out the effect of calabash chalk on different organs this study was performed. To study was conducted for 70 days and with a total of 24 rats (male and female) albinowistar rats weighing 135-185g were kept in laboratory. These 24 rats were divides into four groups according to dose. The groups were- control group, groups treated with 0.5ml, 1.0ml and 1.5ml of the suspension made with calabash chalk. The rats that were in control group were receiving only normal saline and rest of the groups were receiving the suspension according to the dose. The effect of temperature was tried to keep to the possible extent. After 70 days the rats were sacrificed and at that time some specific organs were collected. The organs that were taken were- liver, kidney, brain, lung and ovary. The collected organs were immediately kept in plastic container containing formalin. After that these were taken for the preparation of making gross which is taking sections from the organs, blocks and from those blocks slides were prepared carefully. After this, the slides were examined using microscope and photographs were taken. The changes in the organs were not much significant but still there was a slight change in the organs of those rats.

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Chapter1

Introduction and Background of Study

1.1. Introduction and Background of Study:

Some of the pregnant women have found with a strong desire for eating of a special kind of soil, calabash chalk and the practice is called geophagy. Basically, this is the most common form of pica, involving non-food and non-nutritive substances (Al-Rmalli et al. 2010). It can be either deliberate or can be accidental. There are so many concepts regarding this issue some of which reports about its' benefits and some of which talk about the harmful effects for human body. This practice is going on from generation to generation, among different cultures.

This practice is existing on the earth since the ancient times. Hippocrates first described geophagy in 460-377 BC. Because of the cultural transfer the exact area where people have this tendency cannot be said but the prevalence of this has been observed in Asia, South America and parts of Europe. Sikor consumption is also seen in women of Bangladesh residing in UK although the prevalence of this tendency is no clearly defined but the tendency is mostly practiced by pregnant women (Al-Rmalliet al.2010). The reason can be they are ashamed of this practice and so; they do not want to disclose this to people. Although it is very difficult to translate the information about calabash chalk consumption in number but the availability of it in both UK and in Bangladesh is a clear indication of its demand. The above evidences show that, this practice is existing on the earth since a long period.

At the beginning it was thought that the major minerals in this clay are- kaolin, illite and quartz which were very low in carbon and is essentially salt free. With time it was seen that, it contains- Fe, Zn and Mn which can contribute to normal daily uptake level or to excess amount of these. Beside these, there may be elements like- As, Pd, Cd (Abrahams et. al. 2013) which can be toxic for human health. As we know there are different substances in the clay they can show different effects in our body. If the cadmium is in excess amount it can lead to toxicity, carcinogenic effects, and kidney damage. Potassium rich soils have been associated with hyperkalemia and hypokalemia myopathy (Njiruet al.2011).The exact purpose of ingestion of the soil is difficult to figure out.Previously religion, beauty, detoxification, prevention of birthmarks;relaxation and making babies slide easily at birth were thought to be the reasons of its consumption. Beside these, there are differenttheories about the consumption of this ice or chalk that are really important to think about

- Firstly, this can be a gustatory mistake (welsh et al, 2011). (People without having any specific reason may have this only for their mental satisfaction or the reason can only be its' taste. They might feel better taking this at the time of pregnancy.
- Sometimes this can appear as a reason of anemia and malnutrition. Although this can be a reason there is no evidence about this yet (welsh et al, 2011). if the this a reason of anemia or malnutrition the women taking iron or mineral supplements are supposed to have decreased level of calabash chalk consumption but till now there is no report found on this .
- There is a third theory about consumption of calabash chalk which assumes to have some protective action of this in case of stomach (welsh et al, 2011).

The color, pleasant taste and smell contribute a lot in this type of consumption of soil (Nirjuet al.2011). When a substance has a different taste and the smell also differs it's very common that people find it tasty and gets the mental satisfaction of having something different. As it is known that, in case of pregnancy different changes go on within the body of a woman. At that time they find some kind of smell and taste pleasant which is not normally liked by people. This can be because of different reasons among which relief from nausea and vomiting tendency can be one.

There are certain factors predisposing pregnant women to geophagy. Among these factors cultural expectation is a common one. In some communities if the pregnant women do not consume this soil it is considered to be strange. Even because of the cultural belief 32% people consider it as the reason of their pregnancy (Al-Rmalliet al.2010).

Another factor of this practice can be physiological need for additional micronutrients. Since, in pregnancy the nutritional demand increases to a higher level. As a consequence, a craving for the soil also develops. This is mainly for the substances like-calcium, zinc, and iron. The physiological demand may also increase because of frequent childbearing, the additional physiological burden of parasite disease, physical loading from farm labor and so on.

Next, boosting our immune system may be another reason for geophagy. In pregnancy it is supposed that the immune system becomes suppressed although there remains a need for certain substances for the protection of vulnerable fetus. The fetus needs some extra protection as it is more vulnerable to any hindrances. Beside it can also be assumed that, exposure to the soil also

makes the fetus exposed to microorganisms which may also give the fetus some immunity when the former produces Immunoglobulin A antibodies. It is very useful in prevention of attachment of bacteria and some virus on mucosal services in addition to this clay contains a high level of cation-exchange capacity which can absorb the plant toxins (e.g. tannins, glycoalkaloids and phytotoxins). During the rapid cell division, which is notably pregnancy and childhood binding of clay with the mucosa makes it less permeable to toxins and pathogens (Nirju et al. 2011).

If we talk more about the factors, as the clay contains kaolin and smectite, both of which are used in modern pharmaceuticals to reduce nausea and vomiting, it can be assumed that it is very helpful in reducing gastrointestinal upset. Additionally, it can also be used in case of heartburn. As we know that heartburn is caused by the hydrochloric acid in the stomach the alkaline nature of the clay can prove to be effective in reducing this (Nirju et al. 2011).

There are three main hypotheses about geophagic practice-hunger hypothesis, micronutrient deficiency, protection from toxins and pathogens. Hunger hypothesis assumes that deficiency of food is responsible for consumption of non-food substances. The micronutrient hypothesis is related with deficiencies of micronutrient and which is mainly responsible for the geophagic practice. Finally the protection hypothesis assumes that pica is helpful in reduction of harmful effects of plant chemicals or microbes (Zakari et al. 2015).

Geophagy has different effects on our physiological system. The effects can be either positive or even negative depending on the pattern of consumption. Because determining the correct dose of different components in the clay is almost impossible (Nirju et al. 2011). The same amount of clay from different sources can provide us with different amount of toxic and non-toxic substances. So it is very difficult to define the exact amount of substances in the clay.

Studies show that there might have relationship between geophagy and anemia (Rmalli et al. 2010). Whether geophagy is associated with benefits or risks it is still a matter of discussion. As because some studies show that they can be important mineral supplement especially in poor countries where there is deficiency for iron (Nirju et al. 2011). Whereas other studies reflect their harmful effects in different organs. Despite of the micronutrient supply this geophagic tendency is related with interference of bioavailability of micronutrients, ingestion of heavy metals which

may adversely affect different organs of the body. Beside these, gastrointestinal problem and risk of ingestion of geohelminths is also there (Nirjuet al.2011).

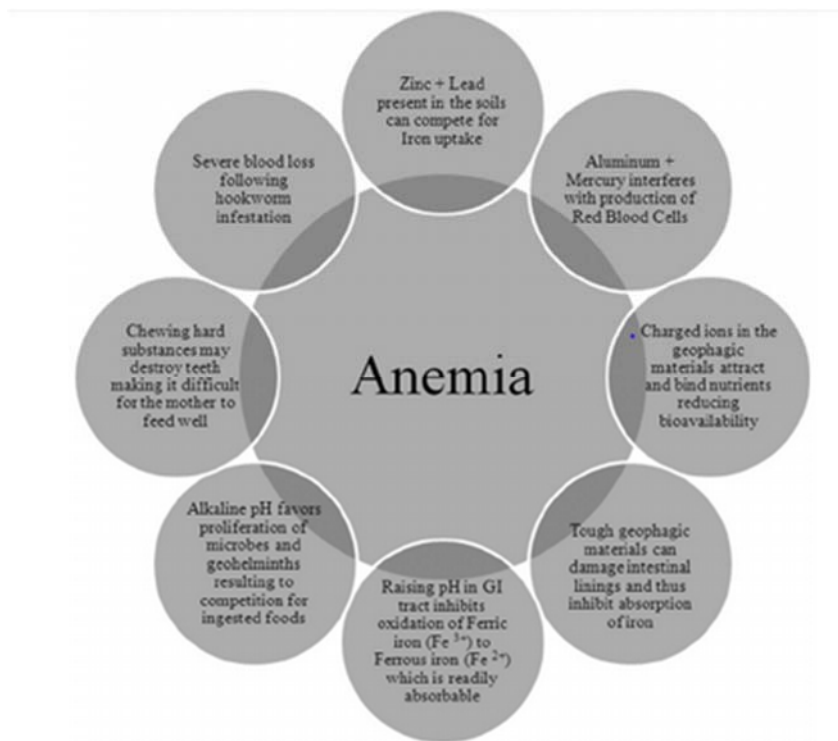


Figure 1.1: Mechanisms through which GiP could lead to anemia (Njiru et al. 2011)

Since geophagy is somehow related with iron levels in the body study about it may bring an assessment of the impact of geophagic practice on iron status which can be helpful for the programs related with reducing iron deficiency (Harvey et al.2000). If we talk about the amount of consumption of geophagic clay –the lower amount was one gram to a maximum of three hundred grams per day. The highest consumption was 150 grams in 15 minutes which was reported by Vermeer and Frate, (1979). If the elemental content exceeds the estimated amount, it can be harmful for health.

Data from different sources show that poorer and tribally oriented people like- people of Nigeria are mostly vulnerable to this practice (Abrahams et al.2013).It can be assumed that because of their lack of knowledge and cultural belief they hold the craving for the soil. Along with these, it can also be assumed that they are confined with the superstition that this soil is helpful in

reduction of different types of diseases. Although these assumptions drawn about geophagy are not exactly accurate and the exact reason of this is still unknown(Al-Rmalli et al. 2010).When cultural transfer occurs then it can also be found in some developed countries as well. Therefore, some developed countries are already concerned about the consumption of this soil (Abrahams et al.2013).

Table 1.1: prevalence of GiP In Selected Developing Countries (Njiru et al.2011)

Country (Ref)	GiP Prevalence (%)
1. Sudan(22)	14.5
2. Tanzania(15)	29
3. Zambia(14)	31.8
4. Latin America(20)	33.5
5. Namibia(36)	44
6. Kenya(19)	45.7
7. Nigeria(21)	50
8. Kenya (18)	1.2
9. Tanzania(23)	63.7
10. Kenya(16)	73
11. Kenya(17)	73
12. Uganda(24)	84

Before the revolution of industries geophagy was considered to be safer as there was less chance of contamination of the soil with toxic substances from the industries (Njiru et al.2011). As we know toxin usually contains substances which are poisonous when toxin goes into our body along with the clay they can become a reason for many harmful effects.

The scenario of consumption of calabash chalk in Bangladesh is very interesting as it is mainly prevalent among the pregnant women (Al-Rmalliet al.2010). Although in different articles it is found that mainly pregnant women have the tendency of consumption of clay there is no specific and proved reasons behind this. This phenomenon is quite highlighted in Bangladesh now-a-days. In an interview, a gynecologist of Bangladesh mentioned that most of the pregnant women consume this clay in quite a large quantity (Al-Ramalliet al.2010).

In modern societies consumption of clay is found to be shameful. People basically do not want to come forward and talk about this type of habits. They think if they talk about this type of tendency the others may find them different from them and that will be very shameful for them. This stereotypical thoughts result in the negligence of researchers interested about the research of geophagy. Even after that, the research field of geophagy is quite wide in some countries like-Africa compared to Bangladesh. Next, if we see for the statistical data of western Kenya there is difference in mothers attending antenatal clinics and the in community survey.

The consumption of calabash chalk among the Bangladeshi population is of particular concern as because contaminated ground water and rice make them exposed to high level of Arsenic (As) (Al-Rmalli et al.2010). Because these contaminated substances contain a pretty good amount of heavy metals in them which along with calabash chalk can raise the level to toxic. For Bangladeshi people the consumption level of this is approximately 50 -60g per day (Al-Rmalliet al.2010). As a quite good number of populations are exposed to it, therefore this study is aiming to find out the histopathological changes of unusual ingestion of soil containing different heavy metals on rat. The solubilized substances from the soil can have different types of effects on different organs of the body.

1.2. Aim:

The aim of this study is to evaluate the histological changes of different organ of *Swiss albino* mice after ingestion of calabash chalk.

1.3. Objectives:

The objectives of the study are:

- to evaluate the changes in brain.
- to evaluate the changes in different organs of the body such as: lung, heart, uterus, stomach, kidney, and liver .
- to evaluate the changes in reproductive organ.

Chapter 2
Materials and
Method

2.1. Materials:

Samples of dry clay lumps were obtained from sylhet. The clay were dried in the sun for seven days to remove the moisture.

2.2. Reagents:

The reagents used in this study were chloroform and ketamine. Chloroform was used as to preserve the organs and to prevent them from deterioration. Ketamine was used as anesthesia for those rats.

2.3. Experiment animal:

Female albino Wistar rats (20) weighing 135-185g were kept in the pharmacology laboratory of Jahangirnagar University. Throughout the whole study the rats were maintained in almost 22 degree Celsius temperature and allowed drinking water and food for 70 days. Beside this, they were divided into 4 groups, with five animals per group.

1. Only deionized water was given to the controlled group.
2. One of the groups was treated with 0.5ml dose of calabash chalk
3. The second group was treated with 1.0ml dose of calabash chalk
4. Another group received 1.5ml dose of calabash chalk.

The solution of calabash chalk was given orally every day for 10 weeks

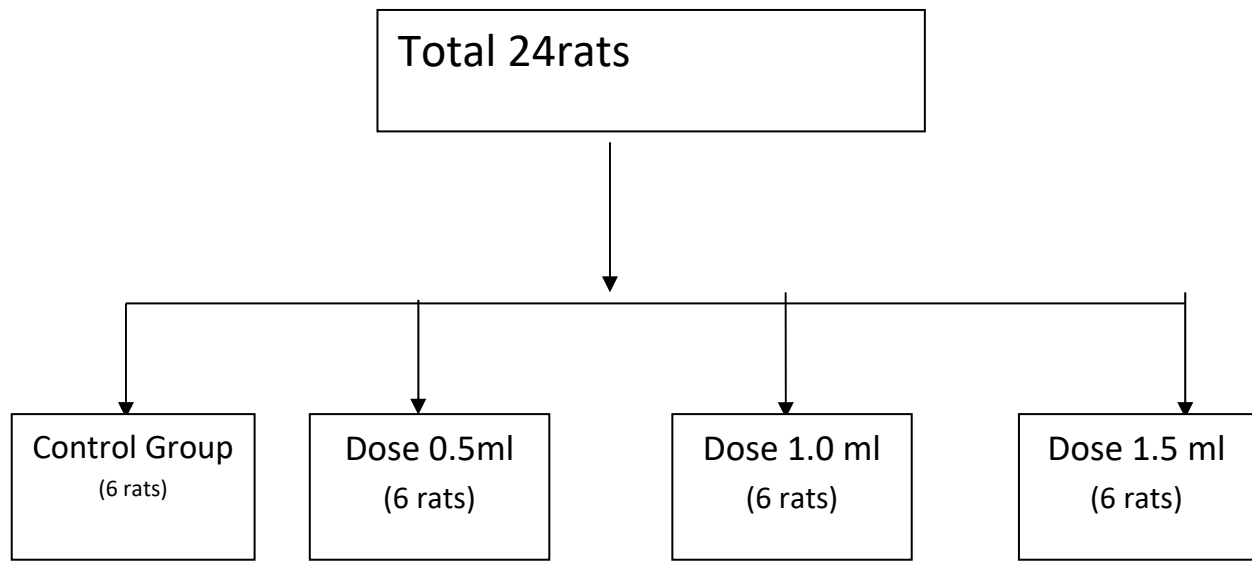


Figure 2.1: Grouping of experimental rats

2.4. Experimental Procedure:

Firstly, the samples were dried in the sun in order to ensure that moisture and extraneous contamination is reduced. This was the only pretreatment done for this experiment. The dried chalk was kept in sterile polythene bags. The sealed bag was carefully taken to the laboratory where they were crushed into fine powder to be dissolved in water. For, the purpose of crushing crucible mortar and pestle was used. The solution of the clay was made by dissolving 40g of the fine powder in 1000ml of water and stirred continuously before administration. It has already been said that the experimental rats were grouped into four small groups with $4 \times 6 = 24$ rats. These different groups of rats was provided with different doses as per body weight and the doses were 0.5ml, 1.0ml, 1.5ml. These rats were receiving the treatments orally on a daily basis for 10 weeks and within these 10 weeks the rats were weighed in order to keep the records. After 10 weeks, these rats were sacrificed. Before sacrificing, ketamine was administered intra-peritoneally to the rats. After this, requiring organs from the rats were collected and were kept in formalin. Next to this, histopathological tests were done.

2.5. Histopathology:

At first, the kidneys, brains, lungs, livers of five rats from group were collected. After that the fat of these organs were removed and kept in container containing formalin. The organs were cleared in xylene and embedded in paraffin. After staining with hematoxylin and eosin dye, the organ tissues were sectioned and examined under the light microscope

Chapter 3

Result

3.1. Result:

The liver, kidney, brain, lung of the rats were collected in order to conduct the histopathological test.

3.1.1. Histopathological output of liver:

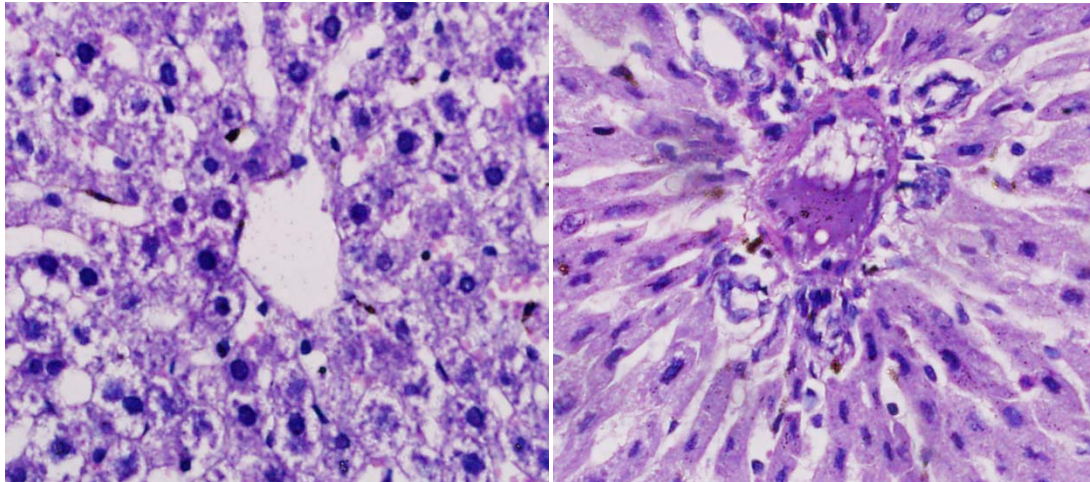


Figure 3.1: Histopathological study of rat from control group

Figure 3.2: Histopathological study of rat treated with 0.5ml

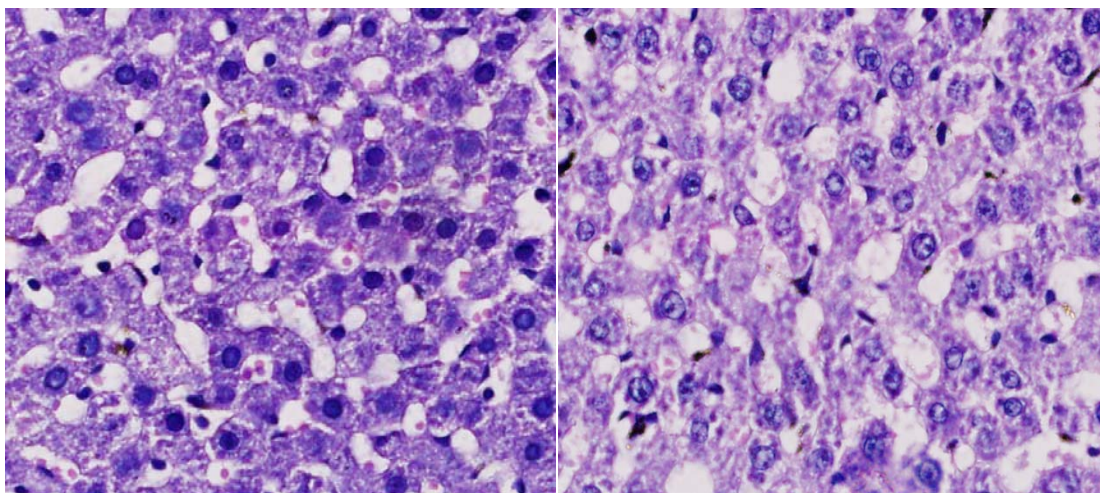


Figure 3.3: Histopathological study of rat treated with 1.0ml dose

Figure 3.4: Histopathological study of rat treated with 1.5ml dose. Result: No visual changes have seen in case of liver.

3.1.2. Histopathological output of kidney:

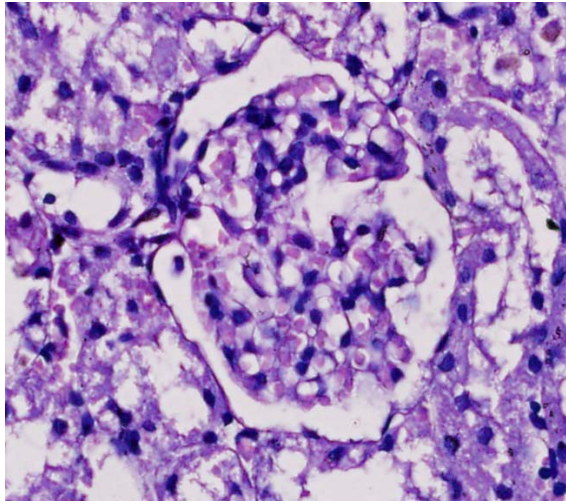


Figure 3.5: Histopathological study of rat from control group

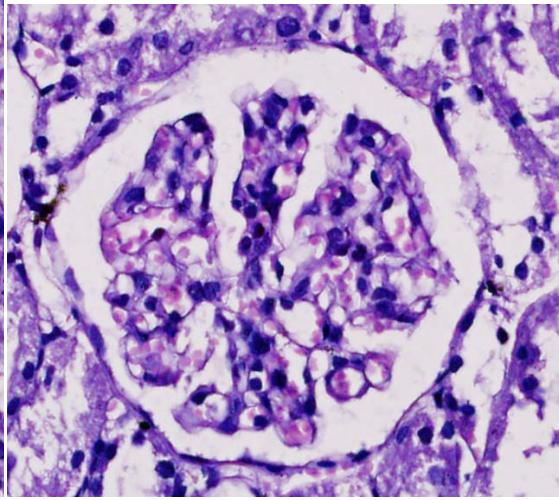


Figure 3.6: Histopathological study of rat treated with 0.5ml dose

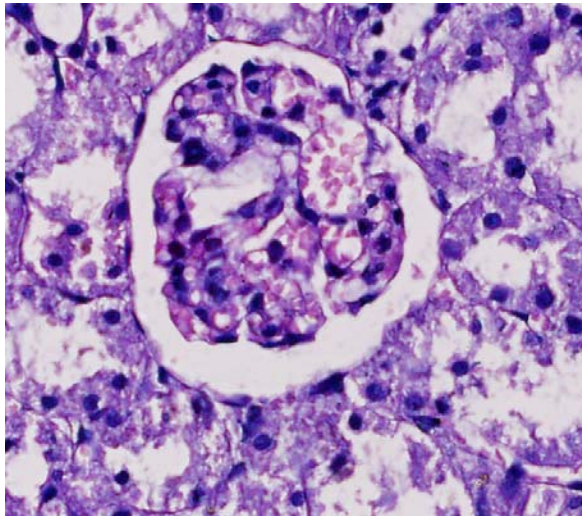


Figure 3.7: Histopathological study of rat treated with 1.0ml dose

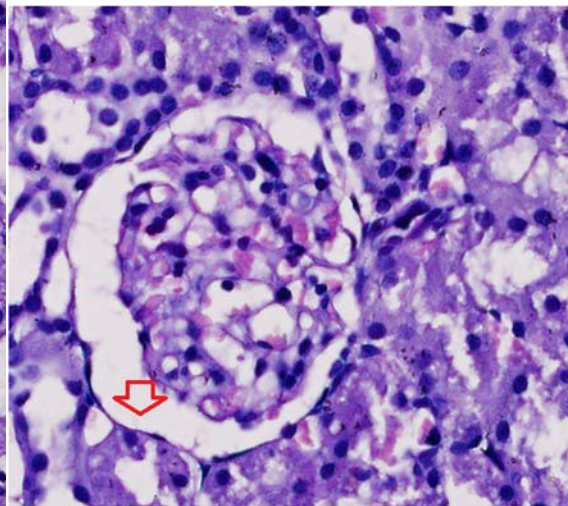


Figure 3.8: Histopathological study of rat treated with 1.5ml dose

Result: Little expansion of the kidney.

3.1.3. Histopathological output of brain:

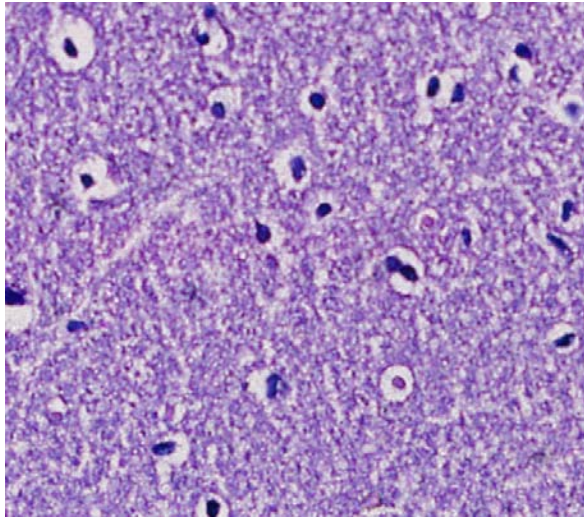


Figure 3.9: Histopathological study of rat from control group

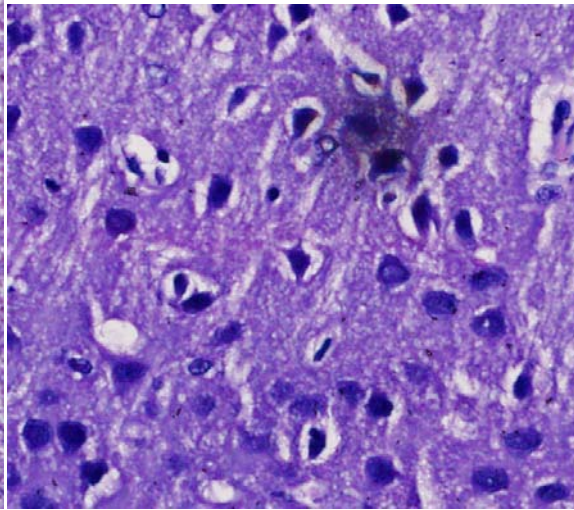


Figure 3.10: Histopathological study of rat treated with 0.5ml dose

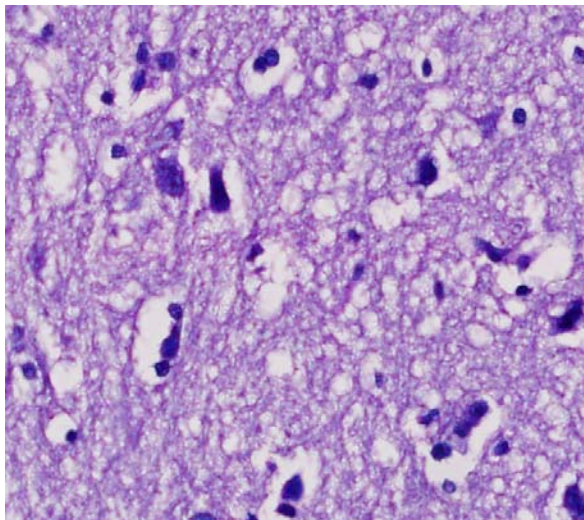


Figure 3.11: Histopathological study of rat treated with 1.0ml dose

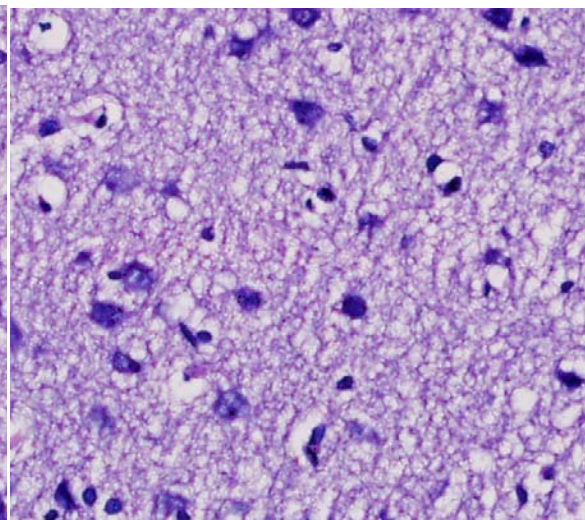


Figure 3.12: Histopathological study of rat treated with 1.5ml dose

Result: No visual change was found.

3.1.4. Histopathological output of lung:

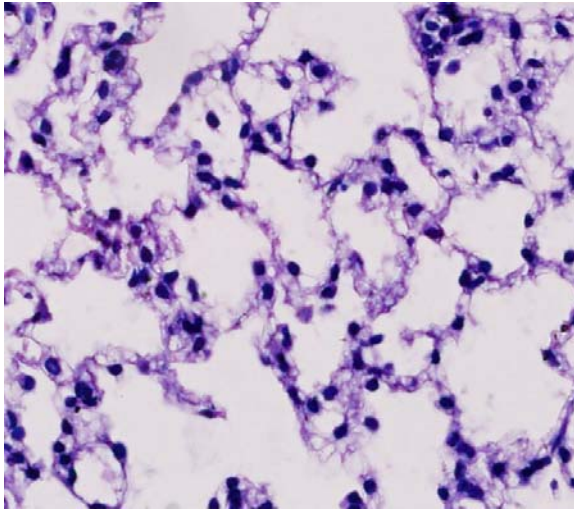


Figure 3.13: Histopathological study of rat from control group

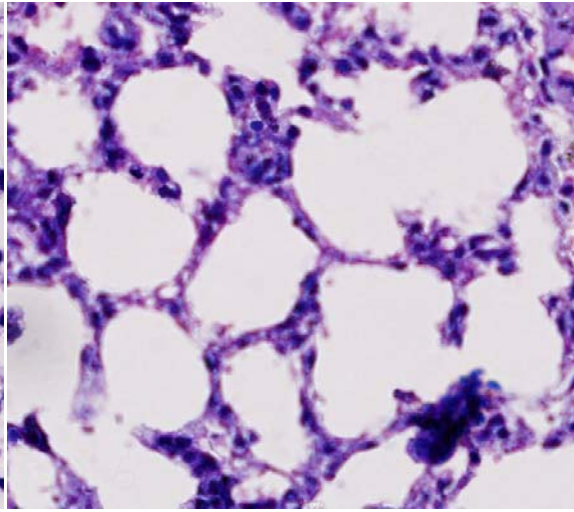


Figure 3.14: Histopathological study of rat treated with 0.5ml dose

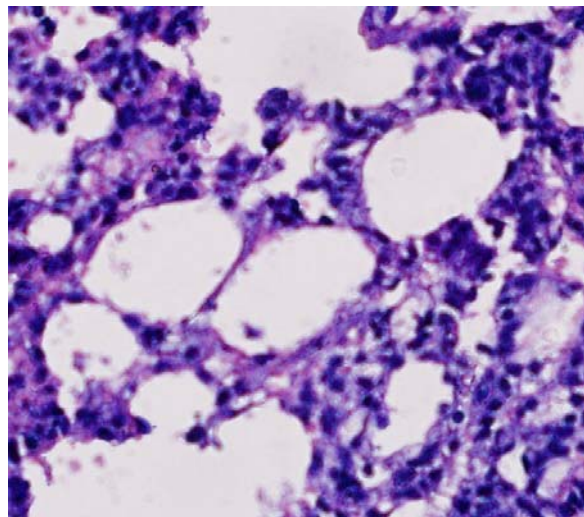


Figure 3.15: Histopathological study of rat treated with 1.0ml dose

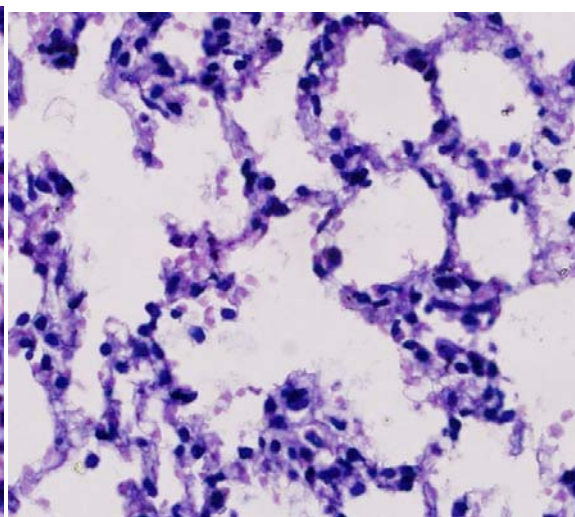


Figure 3.16: Histopathological study of rat treated with 1.5ml dose

Result: No visual change was found.

3.1.5. Histopathological output of uterus:

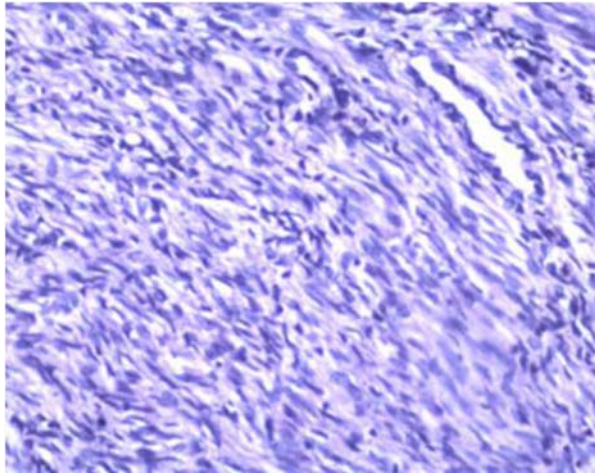


Figure 3.17: Histopathological study of rat from control group



Figure 3.18: Histopathological study of rat treated with 0.5ml

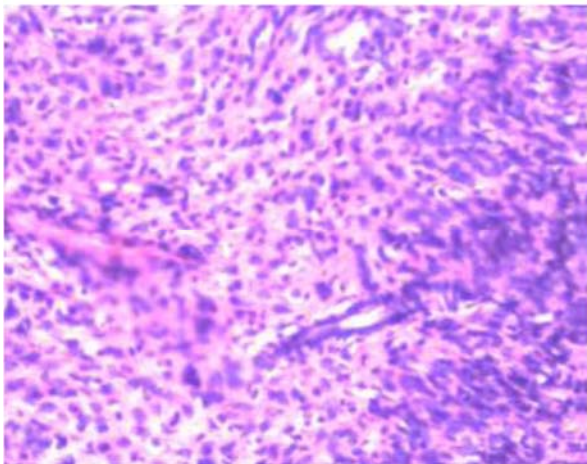


Figure 3.19: Histopathological study of rat treated with 1.0ml dose

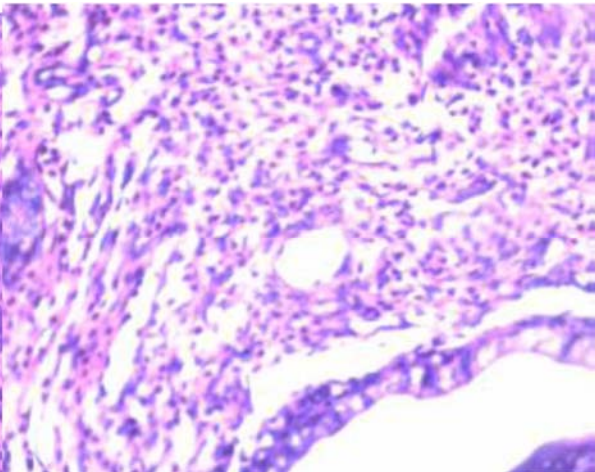


Figure 3.20: Histopathological study of rat treated with 1.5ml dose

Result: No visual change was found.

Chapter 4

Discussion

4.1. Discussion:

It is difficult to determine the exact amount of the composing substances in calabash chalk. We know that whether a substance will act as a supplement or a toxic it depends mostly on the composition and the amount of the materials in it. There is a term known as the percentage of permitted maximum tolerable daily intake (PMTDI) for different elements. An article of Al-Rmalli , shows that in case of sikor the PMTDI is 126, 60, 12000, 210 microgram per day for As, Cd, Mn, Pb respectively. But sikor contains a large amount of Pb like 13 to 32mg per kg (Al-Rmalli et al. 2010). Because of lead toxicity there can be behavioral functioning, diminish hearing acuity, difficulty learning and decreased stature. (Mitra et al. 2009). Pica can also have its effect on metabolism, intestinal obstruction or perforation which can even lead to medical or surgical emergency (Bhatia et al. 2014). Therefore, the material we are having for our nutritional value may become a reason for our sickness. For this reason, we need to be very careful about consumption of any substance. Since it is known that this practice is common among the pregnant women many articles have published about the effects on the babies of those mothers having the craving for clay. It is said that the mothers who used to consume clay are thought to give birth to babies with low birth weight, having prematurity or some sort of physical abnormalities and sometimes even leads to death (Bhatia et al. 2009).

Liver is an important organ in case of production of many important substances. Albumin which works as a former component of blood is found to be produced by the liver. So, whenever we consume any substance it is very important to have a clear idea about the effect of that substance on liver. The main markers of liver are known to be alanine aminotransferase (ALT), aspartate aminotransferase (AST) and glutamyltransferase (GGT) which show change in their amount whenever there is any kind of damage (Zakari et al 2015). The study that is done by Zakari has not shown any significant change in liver function. This study also has not found any effect of this clay on the function of liver. The tissues of the dissected rats were normal and no inflammation was seen. The change in dose and the time period the study can increase the possibility of liver damage.

Kidney is another important organ of human body which is responsible for excretion. The assessment of the amount of creatinine and uric acid is usually used to measure the function of

kidney (Sunmonu and Oloyeda et al. 2006). In case of kidney functioning there found a dose dependent reduction of creatinine than the control group (Zakari et al. 2015). This variation in the marker of kidney function indicates that there can be some problems in the functioning of kidney. This study somehow merges the interpretation of the study previously mentioned. There is seen expansion of the kidney. This can be because of the heavy metals present in the clay, which somehow interfered the production of creatinine and ultimately hampers the normal working capacity of kidney. As a result, the clearance of kidney will also be affected. There can be about 50 to 70% decrease in Glomerular Filtration Rate (GFR) which is quite a high amount (Zakari et al. 2015). This finding is very different from the toxicological result of Kaneko (1989). That study shows relatively positive effect of the clay on kidney function. The findings of the study explain that the rats that fed on nature generally much healing capacity and in other study good clearance level of the unnecessary metabolites from body was found (Oduola et al. 2007). This study shows little expansion of kidney which can be a result of the presence of heavy metals in calabash chalk. But this is only for a single dose which is 1.5ml and cannot be exactly said that it is the effect of calabash chalk. There is possibility that the rat had some kidney problem before the ingestion of calabash chalk. So, ensure this is the result of calabash chalk more doses, variation in time and more experimental animals in to be included in the experiment.

Lung is an important organ for gaseous exchange. Due to damage of lungs there might arise different types of diseases related with respiration. The study that was done by Zakari shows that there is a reduction in the weight of lungs of the rats. The histopathological results of the tissue of lung have seen to remain normal after consumption of the clay. There is no sign of any kind of damage or inflammation in the tissues of lung. Both the treated and the control group was found to be normal and properly working in case of lung functioning.

Brain plays the most crucial part in human life. It is mainly responsible for our thinking capability, reasoning and about learning things properly (Colom et al. 2010). Whenever we consume any kind of substance there is possibility of having either positive or negative effect on our brain. Sometimes the substance can prove to be neutral and having no effect upon it. This happened in the case of this study. This study shows a normal state of the brain. Although there is no sign of damage or any kind of negative effect of calabash chalk on brain, the variation in dose and the time period may show some results which can be different from this study.

After these comes the most important organ in case of pregnancy. As this study is mainly related with the tendency of the pregnant women who consume clay at the time of the pregnancy period. Proper functioning of uterus is required for aiding the transport of the embryo to the implantation site, remodeling the tissue walls to host placenta, protecting the fetus during gestation, contracting forcefully for a safe parturition and post partum, remodeling back to its non pregnant condition (Myers et al.2017). Sections from both the control and the treated group showed infiltration by acute inflammatory cells and indicates stromal oedema and heamorrhage as there was presence of neutrophils (Zakari et al.2015). The study also interpreted the result as it is the result of cyclic changes of rats and not an effect of the clay. The result of this study about the changes of uterus is somehow similar to the study done by Zakari. In this study nothing significant about the functioning of uterus was found rather than the presence of neutrophils. Changes in dose may give a different result from this.

Chapter 5
Conclusion

5.1. Limitations of the study:

- It would be better if the study could be carried out with few more groups having different doses of calabash chalk. That would make the result even better.
- If the time period varied the results could be different.

5.2. Recommendations:

- A regular deworming program can be there to reduce the parasite burden as well, as the risk of geophagy(Njiru et al. 2011).
- For women who consume calabash chalk as an iron source there can be a shifting from calabash chalk to iron supplement.
- Different awareness programs can be organized to make people aware about the harmful effects of geophagic tendency.
- Dopaminergic activity may prove to be beneficial in this case. Olanzapine is thought to be an effective treatment of geophagic tendency (Bhatia et al. 2014).
- As pica can have some effect on cognitive behavior, cognitive behavioral therapy can be given.
- More study should be performed in order to know the effect of calabash chalk.

5.3. Conclusion:

As there is a gradual increase in the use of metals in our houses, industries, agriculture and technological field, the chance of mixing of these substances with soil is increasing. So, calabash chalk can contain high level of toxic substances even after not containing those substances in toxic amount before mixing with the metals. Besides, this soil can be a source of microbes which can be pathogenic (Hunter et al. 1973). So, these excess metals and the pathogens can do much harm to different parts of our body. Though there is still lack of data about the specific histopathological tests some of those shows some adverse effect of this clay on human organs. The consequences of geophagy can be malnutrition, nervous system damage, lead poisoning (Hackley and Katz-Jacobson et al.2003, Mills et al. 2007). As a result, of lead poisoning there can be maternal and fetal kidney damage. The result of this study also shows some difference in kidney structure. This study is also associated with the risk of diseases related with uterus. All though there is a huge advancement in health sector, complain about the lacking of initiatives and specifications of toxicity are seen on a common basis. In order to dispel this, more study need to be performed to find out the exact effect of consumption of calabash chalk and ways to reduce these problems. From this study we can have an idea that calabash chalk has some effect on kidney and may have effect on ovary of as well which can adversely affect different organ structure nad their function. By doing extensive research on this we can find out the mechanism of those organ destruction and can find out some ways to prevent those.

5.4. Future Research Plan:

A toxicological study can be done to specify the toxic level of this special type of clay which is known as calabash chalk.

Chapter 6

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