Life With Geophagy: Biochemical Effects of "Calabash Chalk" on Swiss albino Rat"

A project Submitted
by
Feona Shermin Tumpa
ID: 13146033
Session: Spring 2013
to

The Department of Pharmacy
in partial fulfillment of the requirements for the degree
Bachelor of Pharmacy (Hons.)

Dhaka, Bangladesh
June, 2017

Dedicated to

my beloved family and respected teachers who supported and guided me through thick and thin and assisted me to achieve my goals.

Certification Statement

This is to certify that the project titled “Life with Geophagy: Biochemical effects of “Calabash Chalk” on Swiss Albino rat” 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. Sharmind Neelotpol, Assistant Professor, Department of Pharmacy, BRAC University and proper referencing have been made where the language, concept or writings of others are used.

Signed,

……

Countersigned by the supervisor,

……

Acknowledgement

My first and most heartily praise goes to the almighty Allah, who blessed me with the knowledge, power and ability to accomplish my final year project successfully. Without His endless grace I would be unable to reach my goals.

Foremost, I am obliged from the bottom of my heart to my honorable supervisor and teacher Dr Sharmind Neelotpol, Assistant Professor, Department of Pharmacy, BRAC University, for giving her valuable time to guide and support me. I honestly believe that without her guidance, criticize and direction it was not possible to finish this project. I am also thankful to my honorable Chairperson Dr. Eva Rahman Kabir and my other teachers for their constant superintendence.

Furthermore, I acknowledge my accountability to Professor Shahabuddin Kabir Choudhuri and Professor Masum Shahriar, Department of Pharmacy, Jahangirnagar University, who despite of their busy schedule spare time to help me. I would also like to give my appreciation to the support of the staffs and students of Department of Pharmacy, Jahangirnagar University.

Lastly, I thank to my family members and my friends who continuously boost up my confidence and motivate me throughout my project.

Feona Shermin Tumpa
May 2017

Abstract

"Geophagy", a common practice of deliberate consumption of non-food nutritive clay which is mostly consumed by the pregnant women and children in some specific region of the world. During pregnancy some women feel special craving for consumption of this special type of pica. In Bangladesh, same kind of clay has been found in Sylhet by the name of "Calabash Chalk". The purpose of this study is to determine the effect of this clay on haemato-biochemical profile, lipid profile of female Albino Wister rats. The study was conducted for 70 days. There were one control and three treated groups of 24 female albino Wistar rats weighing 129-158g. The doses were 0.5ml, 1ml, 1.5ml of the clay throughout the experiment. No significant changes were observed in body weight gain among the groups. No significant change was observed in haematology test results except neutrophils ($p = 0.0302$) and lymphocytes ($p = 0.008$) in treated group having dose of 0.5ml. that interpret, chances of microbial infection. In biochemical test, only creatinine in treated group having dose of 0.5ml, 1.5ml ($p = 0.008$) and alkaline phosphatase in treated group 0.5ml ($p = 0.005$), showed significant differences compared to the control group. However, all other parameters of biochemical test including cholesterol, triglycerides, HDL, LDL levels recorded showed no significant differences. All the rats of treated groups were pregnant earlier than the control group. Therefore, this clay might have effect on ovulation of the biological system.

Table of Contents

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ALT: Alanine aminotransferase

AST: Aspartate aminotransferase

ESR: Erythrocyte Sedimentation Rate

HCT: Hematocrit

MCH: Mean Corpuscular Hemoglobin

MCHC: Mean Corpuscular Hemoglobin Concentration

MCV: Mean Corpuscular Volume

RBC: Red blood cell

WBC: White blood cell

WHO: World Health Organization

FAO: Food and Agricultural Organization

HDL: High Density Lipoprotein

LDL: Low Density Lipoprotein

HgB: Haemoglobin

ALT: Alanine aminotransferase

AST: Aspartate aminotransferase

SGOT: Serum glutamic oxaloacetic transaminase

SGPT: Serum glutamic pyruvic transaminase

NEUT: Neutrophils

LYMP: Lymphocytes

MONO: Monocytes

EOSI: Eosinophils

RDW: Red cell distribution width

PLT: Platelet count

MPV: Mean platelet volume

ALP: Alkaline phosphatase

BUN: Blood urea nitrogen

Chapter 1

Background of the study

1.1 Introduction

“Geophagy” is a routine of expending non-sustenance or non-nutritive substances regularly referred as "pica" which is deliberately consumed by pregnant women and young ladies throughout different parts of the world. Numerous databases containing data on the name of “geophagy”, “geophagia”, “pica”, “clay eating” , “ chalk eating”, “cachexia Africana”,“dirt eating” ,“malacia” ,“citta,” “erdeessen” and “aardeeten”(Young et al. 2011). According to John M Hunter (1973), there is a confirmation of its different indications in the Mediterranean universe of Roman and later circumstances, in medieval Western Europe, and in present-day Iran, India, China, and, broadly in tropical worker universe of Indonesia, Oceania, and Africa.

Literature of human geophagy can be classified into five ways (Young et al.2011). These are:

1) Individual case reports—e.g. an African woman living in Africa ate chalk and clay every day;

2) A specific portion of people —e.g 59% of women of Namibia having anemia eat earth

3) Particular social gathering—e.g. Pregnant Otomacs (an extinct aboriginal people) regularly engage in clay eating

4) Soil analysis research—e.g, montmorillonite was a noteworthy segment of ch’aqu and

5)Writing surveys in which no new information were exhibited— e.g. as indicated by La Billardiere and affirmed by the reports of Hekmeyer dirt figures are grinded by ladies and kids.

Pica is the medieval Latin name for the bird called the magpie, who, it is claimed, has a penchant for eating almost anything. Specific form of pica is named differently. Young et al. (2008) , have portrayed various sorts of pica, for example, consumption of child powder, charcoal, calcium hydroxide (lime), slag, uncooked starch and ice.

Table 1.1 Greek words for some specific forms of Pica

Amylophagia	Starch
Cautopyrelophagia	Matches
Coniophagia	Dust from Ventian blind
Geomelophagia	Potatoes
Geophagia	Clay, dirt
Gooberphagia	Peanuts
Lectophagia	Lettuce
Lithophagia	Stones, pebbles. Rocks
Pagophagia	Ice
Stachtophagia	Ashes from cigarettes
Trichophagia	Hair
Xylophagia	Wood toothpicks

Source: Jakari, J. M. (2015)

1.2 History of geophagy: This propensity for eating soil was initially started in Africa and from there this practice moved and it expanded all around the world. Geophagy is an antiquated practice, that was initially recorded by Hippocrates (460-380bc) over 2000 years back (Young, 2011) . Which is still across the board in numerous piece of Asia, Africa, South America, North America and a portion of Europe (AL-Rmalli et al. 2010) .Gelfand (1945) , theorized that, the starting points of geophagy might be founded on the ripeness of the earth. According to Nyanza et al.(2014), the prevalence of geophagy changes between nations evaluated between 10-

75%.The earth soil ordinarily devoured by geophagic people contains a few mineral supplements including magnesium, zinc, copper, manganese, silicon, and iron, inclusive of some harmful mineral components, for example, lead and aluminum. (Ekosse et al, 2010). Geophagy among the

Negroes of the United States has been recorded by various twentieth-century scholars (Key et al.1982). It has been considered that, the practice is still widespread. However, it has diminished lately due to enhanced training, nourishment, developed education and altered culture (Key et al.1982). During some recent period of the twentieth century, geophagy has been reported a number of geological ranges from the tropics areas (Abrahams and Parsons, 1995).

1.3 Geophagy and religious view: According to Hunter (1973), Geophagy is a profoundly different marvel, reflecting religious conviction, social practice, psychiatric confusion, pathogenic response as well as psychological requirement. As per Jakari (2015), a confidence in the religious and magical power of soil may have been other early reasons why earth was expended. According to Hunter (1973), earth eating shapes, or has framed, as portion of numerous religious customs and services. He also mentioned some example like, Muslims used to eat gray colored, flat and oval shaped cake which is made up of dust collected from Prophet's tomb as a cure all diseases. Christians used to take whitish stones from Bethel helm grotto to nurse mothers. Furthermore, Hunter mentioned that, earth has been expended as mainstream pharmaceuticals to treat certain sickness.

1.4 Geophagy and cultural view: Most confirmation of earth-eating originates from Africa, where it is socially and culturally acknowledged among ladies, particularly at pregnancy, and among youngsters (Geissler, 2016). The practice of geophagy can be influenced by cultural and social motives connected with this propensity. Earth-eating will be portrayed as a socially appropriated rehearse, which is firmly identified with individuals' position in the family and group. Moreover writer mentioned that, as a social practice and a culture-particular talk, earth-eating goes into procedures of progress: on the individual level, the life course from adolescence to maturity, and on the societal level, chronicled changes identified with the provincial and post-frontier circumstance. All things considered it is of specific significance with respect to youngsters, who experience childhood in a complex and quickly evolving society (*ibid*).

1.5 Causes and consequences: Geophagy are involved with the craving and consumption of earth and remain enigmatic though it is not recognized as a practice having influence in well being of public health. Some researchers are advanced with the result of geophagy including nutrient

supplementation, detoxification , alleviation of gastrointestinal disorders such as diarrhea, craving and relief from morning sickness (Nyanza et al. 2014), part of cultural beliefs (Ekosse and Diko , 2014). According to Ekosse and Diko (2014), regardless of these gainful parts of geophagia, a few reviews have related the practice with hindering impacts, for example, iron deficiency anaemia, hypokalaemia , excessive tooth wear, enamel damage and erosion of the mucosal surface of the stomach, perforation of the colon , parasitic infections resulting from transmission of *Ascarislumbricoides*, and other highly toxigenic bacteria, causative agents of gas Gangrene, Tetanus and Botulism. Some minerals that are assayed in clays are said to have some nutritional properties. The earth soil ordinarily devoured by geophagic people contains a few mineral supplements including magnesium, zinc, copper, manganese, silicon, and iron, and in addition some harmful mineral components, for example, lead and aluminum. (Ekosse et al, 2010). Specific amount of sample has been collected and assayed. During experiment, some specific minerals including phosphorous, potassium, calcium, zinc, magnesium, iron, copper, manganese were found. Chromium been suspected as nutritional whereas, cadmium with harmful quantity. These minerals play various important roles in human health (Hunter, 1973).

1) Phosphorus is an important element that in combination with calcium and other elements helps for the formation of bone. Play important roles in cell division and protein synthesis. It also help to regulate transportation of nutrients and energy release. Deficiency og phosphorous therefore results in various pathological disorder of the skeletal system and in impaired growth.
2) Potassium another important element helps to regulate water balance inside body and incorporate with other ions in muscle contraction and in the nervous system. Moreover, it works as a catalyst for protein synthesis and energy release.
3) In conjunction with vitamin D and other catalysts Calcium elevate bone growth. Essential element in muscle contraction. Deficiency of calcium leads to rickets, osteomalacia, and osteoporosis.
4) Magnesium stimulated functions in protein synthesis, energy release, calcification, secretion of thyroxine, conduction of nerve impulses, and metabolic rate whereas

5) For hemoglobin formation, copper associates with iron which is essential in enzyme systems and deficiency of copper causes anemia, retarded growth, nervous disorders, deficiency of it impairs growth.and depigmentation.
6) Zinc is important for metabolism and a catalyst for enzyme systems which is linked with sexual development.
7) Manganese is another impetus for chemicals that help to store thiamine. Overabundance manganese decreases iron and copper in the body. Lack is related with poor regenerative capacity.
8) Iron an oxygen carrier which is essential for hemoglobin synthesis. An excess of iron causes siderosis or haemochromatosis; insufficiency causes anemia.

1.6 Some well-established benefits: For gastrointestinal diseases e.g. kaopectate, ingestion of clay is a well-accepted treatment. Moreover, in over the counter treatment for nausea, diarrhoea and vomiting, clay such as in kaolin (basic active ingredient) are used in a great extent (Jakari, 2015**).** According to Frankel (1997), numerous geophagists ascribe that, their pica works for the reduction of heartburns and nausea. Moreover, in some primates clay also shows some medicinal effects. According to Krishnamani and Mahaney (2002), monkeys that regularly eat soil have been noted to have lower parasite loads. Moreover, geophagic clay has some nutrient value such as, it prevent asthma. It also works as a n immunologic adjuvants that has capability to perform as vaccines (Jakari, J. M. 2015). There is an association of geophagy with exposure to toxins. For example, people in Northern Territory of Australia explained that they ate clay to "line the stomach" just before eating fish might be harmful (ibid). During pregnancy, Iron is a very important element. For the primitive society, geophagic clay was the most easy and available source of iron for pregnant women (Cooper, M. 1957).

1.7 Some bad impact on health of consuming clay: Consumption of geophagic clay does not only bring merits. However, it has also been reported to show some detriments. Some of the detrimental health conditions include heavy metal poisonings especially lead (Abraham et al., 2006) and alimentary canal damage (Ekosse et al., 2010). Consumption of geophagic clay increases the risk factor of the transmission of parasites- Nematodes *(Ascaris lumbricoides*), Roundworms (*Trichuris trichuria*), Whipworm (*Toxocaraspp).* Besides, it has been asserted that, clay can block

the intestines leading to a fetal condition where some soils by combining with minerals in diet may cause problems in absorption (Jakari, 2015). It is well established that, clay contains some most important metal elements of body for attending some biological function in such as -Na, K, Mg, and Ca(main group) , V, Cr, Mn, Fe, Co, Cu, Zn, Ni, Mo, and Cd(transitional metals) . Deficiency in some of them may causes diseases. However, presence at a large amount of these essential metals may also be toxic for health. (*ibid).*

1.8 Clay and retail market: Edible mud are separated, and are sent from makers through brokers to retailers. They achieve a wide purchaser opened through a system of occasional markets. Where this retail market of Africa is highly monopolized by Nigerian Yoruba ladies, they offer customary cures, charms, flavors and amulets including an assortment of land dirt from different geological formations (Hunter, 1973).

Source: (John M Hunter 1973)

Figure 1.1: Different types of edible clay in retail market

1.9 Physical properties of geophagic clay: Clays are sold in retail markets of Kumasi of Africa at a reasonable price. Clays are white or dark, once in a while with a fragile pink or purple tone that reflects geographical site conditions. Numerous clays are identified by normality of size, shape, shading, surface, and markings. Lacking enrolled trademarks and institutionalized pharmaceutical-sort readiness, the shapes, sizes, and other distinctive elements serve to recognize the mud items.

Source: (Al-Rmalli et al.2010)

Figure 1.2: Shape and color of clay

1.10 Forms of consumed clay: Generally women and children consume clay in various form such as broken piece of clay, made pottery, piece of earthen made wall, tablets earth that is found among dry beam (Young et al., 2010). According to, Geissler et al. (1997), the consumed soils were largely from termite mounds, clay found from roadsides, yellowish delicate stone found from the bottoms of dried streams, and even chalks used in classroom also weathered stones and dividers of cottages were the regularly consumed things by geophagists.

1.11 Geophagy and social view: Earth-eating is portrayed as a socially dispersed practice, which is firmly identified with individuals' position in the family, group, or in a social space and community. Etiology behind eating earth clay may be influenced by various reasons. It has been confirmed by the ethnocentric notion that, earth-eating is an 'aberration of appetite', might be stimulated by psychosocial push or mental unsettling influence (Geissler, 2016). In spite, of the widespread distribution of soil eating habit both today and past, the utilization of soil is still largely obscure, under reported, misjudged, or disregarded by the vast majority. In a study done by Geissler 2016, some questions were made to the general mass in African villagers regarding 'who can eat earth?'. Most of them replied, women and pregnant women at the time of reproductive period. They are supposed to give up eating after menopause as they will not be able to give birth then. If man eat earth, they consider it as a shameful matter. Through earth-eating they express their gender identity, where only women will eat earth because they symbolizes fertility and softness in nature (*ibid).*

1.12 Hypothesis of geophagy: There have been different theories progressed to clarify geophagic conduct. According to (young et al 2011), there are three hypothesis regarding geophagy.

The first one is hunger hypothesis. Under this hunger hypothesis, this speculation propose that there is no vantage of consumption of earth. Individuals consume earth because, either they have nothing to eat or those micronutrient insufficiencies have brought about neurological or sensory issues in brain. In secondary case, when people starving it affect appetite-regulating brain enzymes or changes taste sensitivity, which may be a reason of non-food substances become appealing for them. According to Young et al, (2011), hunger status have been reported in 72 cultural reports. Among these reports, geophagy was credited exclusively to hunger in just 16 (22%). Interestingly, yearning was unequivocally not connected with geophagy in 36 reports (50%). In the rest of the 20 reports (28%), earth was in some cases eaten out of yearning while different times for "joy," "custom," "desiring," or "propensity" (e.g., von Humboldt et al. 1821). It has been shown that, frequency of consuming clay is most in pregnant women and it is also supported by hunger theory. Pregnant women requires more calories during the period when embryo is growing rapidly inside than other age group of people (Young et al, 2011). In this way their demand for food is greater.

Under the second hypothesis (Young et al.2011) which is the protection hypothesis, according to that earth is consumed as medicine to reduce the short-term malaise and long-term effects of harmful chemicals, parasites and pathogens. Various human food and plants tend to produce venomous chemicals, such as tannins and glycoalkaloids that can guard themselves from biotic enemies (pathogens and herbivores) (Young et al. 2011). In the human diet, some more chemicals are enterotoxins secreted by food- and bacteria born in water such as, Escherichia coli, Staphylococcus aureus, Salmonella enterica, and Listeria monocytogenes (young et al 2011). Ingestion of these toxins can cause gastrointestinal distress, dizziness, and muscle pains. In sufficient quantities, they can be mutagenic, carcinogenic, or lethal. Furthermore, dangerous pathogens include food and waterborne bacteria, as well as, viruses and parasitic Nematodes (Young et al.2011). Besides, pregnant ladies are adaptively immune suppressed, therefore avoidance of parasites and pathogens is particularly imperative for the lady's own well health.

Moreover, under the second hypothesis, two mechanisms by which geophagic earth maybe protective, by lessening the permeability of the gut wall to poisons and pathogens and by binding directly to toxins and pathogens (Young 2010). The intestinal mucosal layer acts as a physical barrier between ingesta and the bloodstream by filtering out large molecules, as well as, a chemical barrier by maintaining a pH gradient. Geophagic earth, especially if it is clay-rich, can bind with gastrointestinal layer and thereby reinforce the protective mucosal layer and/or enhance mucosal secretion, thereby reducing permeability of the intestinal walls (ibid).

The second mechanism involves binding directly to toxins, parasites, and other pathogens.

This can either render them unobservable by the gut or inhibit their respiration (young et al, 2011) .A number of clays found in geophagic earths are capable of binding pathogens, including

Viruses, fungi, and bacteria, as well as toxins, including poisonous herbicides, pharmaceuticals, and plant secondary compounds. *(ibid*)

Under this hypothesis, it has been said that, because of micro nutrient inadequacies, individual eat non-food sustenance and try to build sufficiency by administration of iron, zinc or calcium (Wiley and Katz, 1998). From early 40 AD, a relation between iron deficiency anemia and geophagy has been confirmed repeatedly (Young et al, .2011). A statistical data have shown that, pregnant

women need more nutrient such as iron, calcium in late pregnancy because this nutrient are necessary for the development of various organ of fetus , however statistics shows that women have more craving for clay in their early pregnancy than in late period of pregnancy (Institute of medicine 2002).

The third hypothesis is about Non-adaptivity. This hypothesis proposes that there is no benefit to eating earth. Instead, people do so either because they have no food to eat or because micronutrient deficiencies has caused neurological or sensory problems (young2011). In the first case, earth is supposedly consumed to ease hunger pains when no other food is available. In the second case, cravings for earth are suggested to be epiphenomena of nutrient deficiencies that affect appetite-regulating brain enzymes or taste sensitivity causing non-food substances to become appealing (*ibid*).

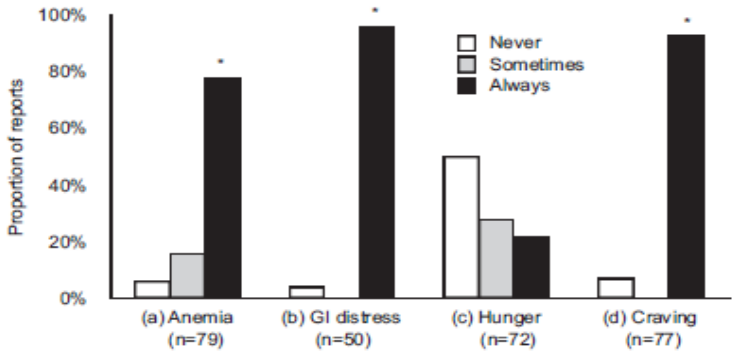

FIGURE 3. REPORTS OF THE FREQUENCY OF ASSOCIATION OF GEOPHAGY WITH (A) ANEMIA, (B) GI DISTRESS, (C) HUNGER, AND (D) CRAVING

Source :Young et,al. 2011

Figure 1.3: Reports of the frequency of association of geophagy

1.13 Geophagy and pregnancy: Pregnant slaves also continued the family practice of clay eating for cultural and possibly for nutritional reasons. (Abrahams and Parsons, 1995). Determined soil-eating brought about a stamped crumbling of the individual and substantial gatherings of Negroes

enjoyed intemperate soil-eating because of a firm conviction that after death they would return spiritual partner to their local home (Haller, 1972). The diseased associated with clay eating have been addressed as Cachexia Africana. Medical symptoms of Cachexia Africana found sluggishness, mental insensibility, profound muscular weakness and lassitude (Abrahams and parsons, 1995). Also iron deficiency and hypokalamia are most common symptom occurs from eating clay as have been demonstrated *(ibid*).

1.14 Geophagy in other primates: Consumption of geophagic clay has been reported in many primates. Primates consume various types of food items and selection of food items basically influenced by their nutrient and energy demand. They not only eat various kinds of fruits, green leaves, edible parts, insects but also been observed to consume carcoal, dead wool, soil. (Mahaney & Krishnamani , 1999). Writers also mentioned in writing that, Of the 185 extant species of primates only 39 (21.1%) are reported to ingest soil, either in the wild or in captivit Of these. 28.2% are obligate folivores, 20.5% are obligate, 7.7% are frugivores/insectivores, 17.9% are folivores/frugivores and 25.6% are frugivores/omnivores. Similarly, only three of the 12 apes (25%), seven of the 36 prosimians (19.4%), 10 of the 64 New World monkeys (15.6%) and 19 of the 73 Old World monkeys (26%) are known to ingest soil. According to Mahaney & Krishnamani(1999), in some species geophagy has been reported prominently such as African elephants(*Loxodonta Africana),* African buffaloes*(Synceros caffer)* , Mule deer*(Odocoileus hemionus),* Holstein cross cattle*(Bos primigentus),*Tapir*(Tapirus terrestris)* , Brocket deer *(Mazama sp.)* , Pacas (*Agouti paca)* as well as in some birds, reptiles, other mammals, in most of herbivores or omnivores. Moreover , they mentioned, behind consumption of geophagic clay there has been some medical value for primates such as , geophagy helps to reduce gastrointestinal disorders , works as antidiarrheal agent, source of mineral supplement and extra iron or generally as a supply of famine food.

1.15 Aim:

The aim of the study is to determine the biochemical changes in female Albino Wistar rats after the ingestion of "Calabash chalk"- a specific geophagic clay.

1.16 Objectives:

Various studies on soils consumed by humans could provide information about the relationship between health of geophagic individuals and physico-chemical, mineralogical and geochemical properties of soil to ascertain possible health implications on the geophagic individuals. On that basis, objectives of this study is divided in several folds. These are:

1. To determine the haemato-biochemical profile of Female Albino Wistar rats on consumption of clay.

2. To determine the effects of geophagic earth minerals in blood which included blood hemoglobin (Hb) concentration, mean values for Red blood cells (RBC), Haematocrit (HCT), Mean corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH), Mean corpuscular haemoglobin concentration MCHC) and White Blood Cells (WBC).

3. To determine kidney, liver function and lipid profile of female Albino Wister rats.

Chapter 2

Material and methods

2.1 Reagents:

The reagents used in this study were "Chalabash chalk", heparin, ketamine and formalin. Calabash chalk was collected from Sylhet. Heparine was purchased from drug store in the brand name of Heparine-rotex manufactured by Rotex and marketed by City overseas. Heparine was used as anticoagulant. Moreover, ketamine was bought in a brand name of G-Ketamine manufactured by Gonosashthaya pharmaceuticals ltd.

2.2 Experimental animal:

Twenty four female Albino Wistar rats were used in this experiment. Rats weighing 129-158g were kept in the pharmacology laboratory of Jahangirnagar University. Throughout the whole study rats were maintained in 22±1ºC temperature, and were allowed to drink water and foods for 80 days. Besides that, they were divided into four groups , six animals per group according to their body weight.

Among them five were female and one male. Each group rats were given specific doses of clay. Group 1 served as a control group and Groups 2, 3 and 4 were treated groups.

Controlled group weight – (145g-148g), male- 175g

Treated group 2 - (125g-135g), male-175g

Treated group 3 - (128g-130g), male- 180g

Treated group 4 - (130g- 137g), male -175g

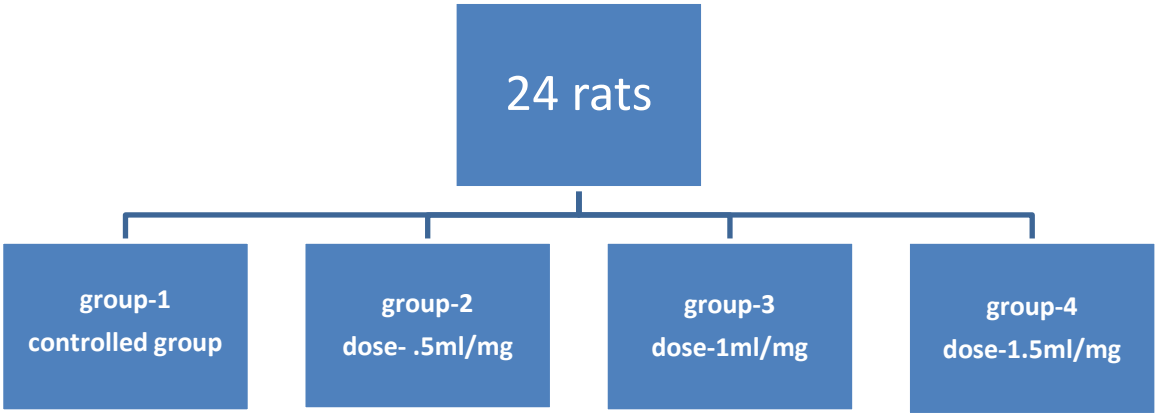

Figure 2.1: Grouping of experimental rats

2.3 Experimental procedure:

Collected geophagic clay sample were first sun dried for 3 days. Then the geophagic samples turned into powder using crucible mortar and pestle and sieved to obtain the desired powder. Powdered clay sample was prepared into stock solution. Here, 40g of powder dissolved in 1000ml of distilled water into suspension and was stirred continuously prior to administration. Each milliliter of suspension contained 40mg/1000ml. This prepared suspension was given to (4×6=24) rats orally in daily basis. Rats were weighed in order to keep records throughout the experiment. The clay was administered as a suspension in varied doses per body weights as follows; 0.5ml to Group 2 , 1ml to Group 3 and 1.5ml to Group 4 orally via gavage (using 1ml syringe) once daily to the rats. The Control Group 1 was fed only on distilled water .The doses were calculated based on the average weight of the rats. This was done for a period of 70 days.

Ketamine were administrated intra peritoneal to the rats before they were sacrificed. After that, blood samples were collected from post vena cava and then transferred into 10 ml heparinized Ethylene diamine tetra acetic acid (EDTA) tubes .The collected samples were taken to the laboratory, where these samples were tested for full blood count and biochemical analysis.

2.4 **Preparation of plasma:**

In order to separate the red blood cells and plasma, the collected bloods were centrifuged in a centrifugation machine at 4000 rpm for 10 minutes. The plasma samples were then collected using dry Pasteur pipette and stored for analysis in a refrigerator.

2.**5 Statistical analyses**:

Statistical analyses were carried out using the Portable IBM SPSS Statistics version 20. For continuous metric variables, mean and standard deviation, standard error was used as descriptive measures, while the independent t-test was used for comparisons when the data could be shown to be normally distributed.

Table 2.1: Average weight of control and three different doses treated Wistar rats from day 0 to 70:

Experiment Group	Average weight(g) On day 1	Average weight(g) 30 days	Average weight(g) 60 days	Average weight(g) 70 days
Control group 1	147.25g	162.75g	189g	179g
Treated group 2 (dose .5ml)	130.7g	176.25g	151g	163.75g
Treated group 3 (dose 1ml)	129.5g	177.75g	160.5g	167.75g
Treated group 4(dose 1.5ml)	134.25g	155.5g	179g	158.75g

Chapter 3

Result and discussion

3.1Body weight:

Absolute weights of rats before pregnancy period from Day 0 to 30 treated with varied doses of geophagic clay suspension has been shown in figure 3.1. From average weight of controlled and three treated groups we can observe that, absolute weight of all groups have increased at a positive scale with varied doses whereas, Jakari (2015),mentioned in his study that, on a 0 to 28 days treatment, all the controlled group gained marginal positive growth and treated group had negative growth. Moreover, he mentioned that, at a lower dose treated group gained highest weight far more than control group but when dose become increased weight of treated group decreased significantly ($P < 0.05$). In the same study he mentioned that, this negative weight could be an outcome for the presence of heavy metal such as lead and arsenic that exceeds the range of FAO/WHO of 0 .01ppm. In a similar study, Rafique et al., (2008) confirmed that animal having continuous exposure to heavy metals usually lose weight. However in our study , as we did not find any weight difference we can interpret that, presence of heavy metals in our clay was very negligible that it had no significant effect on the body weight of the experimental animals.

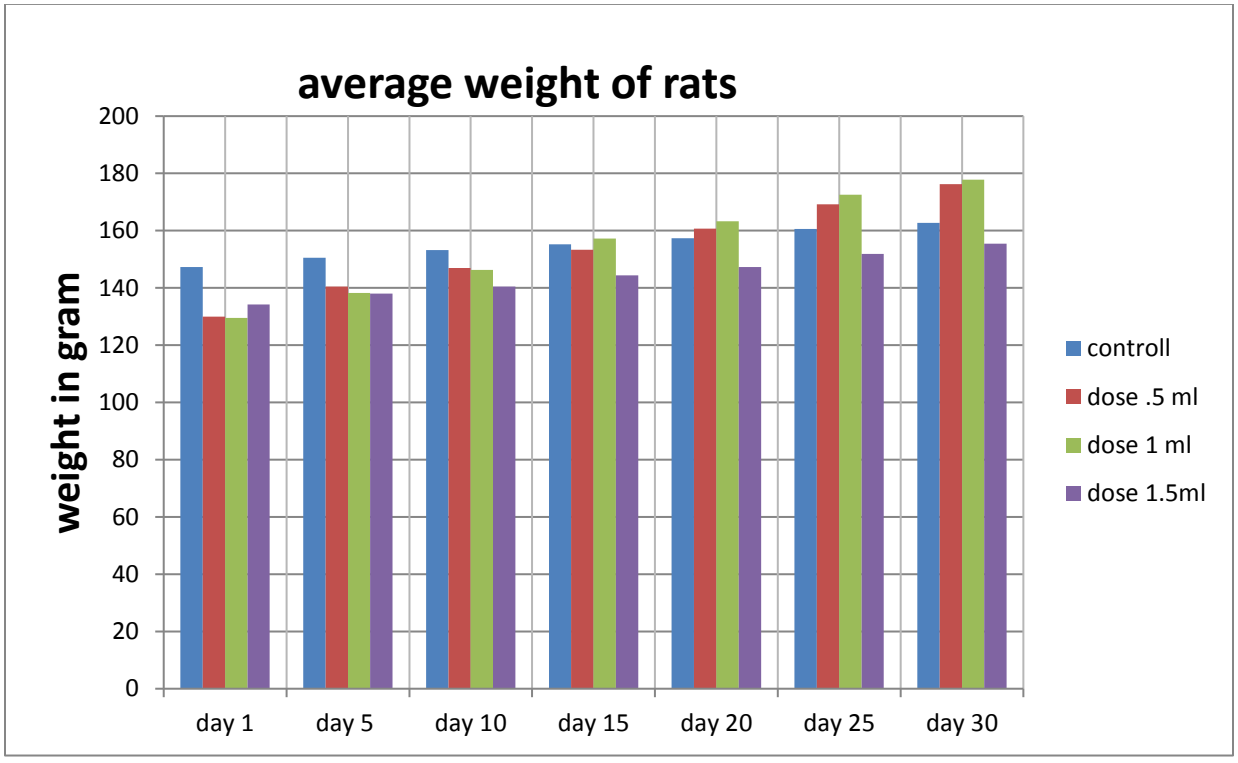

Figure 3.1-Comparison of body weight among control group and clay treated groups.

Table 3.1: Statistical output of body weight among control group and treated groups:

Day	Control group	Treated group .5 ml	Treated group 1 ml	Treated group 1.5ml
Mean±SE(F , P)				
Day 1	147.5±0.501	131.66±2.108 (2.667, 0.178)	129.33±0.421 (0.444,0.541)	133.66±1.20
Day 5	151.26± 2.79	141.66± 1.81	137.33 ±2.53 (1.195,0.451)	133.66 ±1.20 (2.865, 0.201)
Day 10	155.21± 3.75	148± 1.63	144.5± 4.36 (0.574, 0.615)	141.98 ±2.66
Day 15	157± 4.66	153.933 ±1.73	155.167± 6.67	146.33± 2.7
Day 20	160.5± 4.6	160.77 ±2.79 (1.084, 0.442)	161.1± 6.85 (0.376, 0.715)	149.93± 3.76
Day 25	162.5± 5.6	168.66± 3.35 (0.305, 0.757)	169.33± 9.18	154.83± 4.11
Day 30	164.66± 6.5	175.16± 4.6	174.66± 9.38	158.66± 4.66

From the collected blood samples, two types of tests were performed. One was hematological test and other test was biochemical test.

Haematology test: Haematology test are done to measure the elements in the body. To identify early physical illness this test can be proved very important. Different variations in the size, shape and number of blood cells can give early insight about general functioning of blood and the bone marrow where blood is made and some clinical factors that may affect it.

Under haematology test we did CBC (complete blood count) test. Complete blood count determines general health status. It is used as a screen for a variety of disorders, such as anemia and infection, inflammation nutritional status and exposure to toxic substances. Complete blood count test included- Red blood cell count (RBC), Red cell indices (MCV, MCH, MCHC, and RDW), White blood cell count (WBC), Neutrophils (NEUT), Lymphocytes (LYMP), Monocytes

(MONO), Eosinophils (EOSI), Basophils (BASO), Haemoglobin (HGB), Haematocrit (HCT), Platelet count (PLT),Mean platelet volume (MPV).

Table 3.2 shows the result of haematological examination RBC measures the number of red cells in the blood. A low count often accompanies anemia, excess body fluid and blood loss. A high count is commonly seen in dehydration and in a condition called polycythemia (Firkin&Gruchy,1990).Here the mean values for Red blood cells (RBC), from three treated group of dosage form 1ml, and 1.5ml showed no significant difference from the controlled group and treated group .5ml showed insignificant differences.

Platelet count (PLT) measures the number of platelets in blood. Platelets help the blood clot at the site of a wound. High platelet counts can be seen following strenuous activity in some infections and inflammatory conditions. Extremely low platelet counts can be associated with spontaneous bleeding. Lower platelet count in blood which is called thrombocytopenia. Circulating platelets are reduced by one or more of the following processes: trapping of platelets in the spleen, decreased platelet production or increased destruction of platelets (Firkin & Gruchy,1990). Here , Platelet count in treated group 1ml and 1.5ml did not show any significant differences and treated group .5ml here we can see showed statistically insignificant differences compared to control group

Haemoglobin (HgB) gives the red cells their color and carries oxygen from the lungs to the cells. This test is primarily used to determine the presence of anemia or its reverse, polycythemia (Firkin & Gruchy ,1990). Haemoglobin(HgB) and Erythrocyte sedimentation rate (ESR) component of RBC also did not show any significant difference from the controlled group.

.

3.2 Statistical output of Haematology test result and discussion:

Table 3.2: Effects of geophagic clay on the Haematology test of rats after 70 days of treatment

Parameters	Control group	Treated group .5ml	Treated group 1 ml	Treated group 1.5ml
Mean ±SE(F , P)				
WBC	7600±264.57	7433.33±352.76	7566±333(0.437,0.795)	8300±360.55
RBC	6733.33±120.7	5866±338(.379, 0.714)	6733.33±578.311	7333.3±233.33
PLT	661000±31320.9	529666±16666 (.267 , 0.875)	753333.3±37551.44	582333.33±36861.60
ESR	3±.575	5±1(.364 , 0.722)	2.66±.66(2.6 , 0.221)	4±1.52(.604 , 0.732)
HGB	12.33±.21	12.6±.288	12.7±.173	12..766±.145
HCT	43.3±1.42	45.16±.874	45.13±1.57 (7.82, 0.261)	43.70±1.80 (7.82 , 0.261)
MCV	53.9±1.77	53.6±1.25	58.66±1.97	59.80±.32
MCH	16.8±.416	17.266±.4702	16.9±.05 (.241 , 0.889)	18±.251 (3.241 , 0.392)
MCHC	28.23±.866	28.7±.702	28.03±.3756	30.5±.966(3.8 ,0.211)
NEUT	26.66±2.96	23.6±3.5(9,0.0302)*	27±2.5	24±1.15(3,0.102)
LYMPH	70.66±3.84	66.3±4.66 (.5 , 0.008)*	66.66±3.17	82±2 (4.250 , 0.347)
MONO	3.66±.330	4±.577 (0.06 ,0.973)	4±.577 (0.333 , 0.740)	2.3±.88(0.333, 0.740)
EOS	2.00 (±0.37)	1.80 (±0.20)	2.00 (±0.32)	0.012

Only SPSS given F and P values have been presented. No other F and P values have been generated from the rest of the data by SPSS analysis due to co-linearity

Haematocrit (HCT) measures the percentage of red blood cells in a standard volume of blood. It is used, in conjunction with the haemoglobin and red cell count, to determine the presence and type of anemia (Firkin & Gruchy 1990). In case of Haematocrit(HCT) treated group 1ml and 1.5ml showed insignificant differences compare to the control group.

There are three red blood cell indices, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). The MCV shows the size of the red blood cells. The MCH value is the amount of hemoglobin in an average red blood cell. The MCHC measures the concentration of hemoglobin in an average red blood cell. These numbers help in the diagnosis of different types of anemia. Red cell distribution width (RDW) can also be measured which shows if the cells are all the same or different sizes or shapes (Firkin & Gruchy 1990). Here, Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH), and Mean corpuscular hemoglobin concentration (MCHC) of the entire treated group showed no significant differences from Control group. Here from interpreting all the results we can say that, samples had no chance of having anemia.

White blood cell count (WBC) measures the number of white blood cells in blood. Types of white blood cell are neutrophils, lymphocytes, monocytes, eosinophils, and basophils. Immature neutrophils, called band neutrophils, are also part of this test. White cells protect against infection and allergies. High counts are seen during infection, after exercise and with stress. Low counts may be seen if there is suppression of the immune system (Firkin & Gruchy 1990). Here, the mean value of White blood cell (WBC) obtained from treated groups (0.5ml , 1ml, 1.5ml) did not show any significant differences compared to the control group.

Neutrophils (NEUT), Lymphocytes (LYMP), Monocytes (MONO), Eosinophils (EOSI), Basophils (BASO), they are the different types of white blood cell in the blood. Usually called a ‘differential’, in conjunction with the total white cell count, the levels give information about the immune system. The neutrophils and lymphocytes are the most important levels to measure. The other three types are less significant and are often measured together (MXD) (Firkin & Gruchy 1990). Here, the value of Neutrophils (NEUT) showed significant difference ($P<0.05$) for treated group 0.5ml but all other group showed no significant difference from the controlled group. Reason behind showing difference might be chance of viral infection for treated group dose .5ml.

Lymphocytes (LYMP) showed significant differences at level ($0.001<p<0.01$) for treated group dose 0.5ml but all other groups showed no significant difference from the controlled group. We all know that, Neutrophils and Lymphocytes are components of WBC that fight against the microbial invasion Therefore, reason behind showing difference might be chance bacterial/protozoal/viral infection. Moreover, a study done by Jakari, J. M. (2015) showed, Lymphocytes and Neutrophils were statistically significant at ($P <0.05$) when treatment groups were compared with Control.

Monocytes (MONO), Eosinophils (EOSI), Procalcitonin (PCT), Erythrocyte Sedimentation Rate (ESR) result obtained from three treated group 0.5ml, 1ml, 1.5ml showed no significant differences from the controlled group. The findings therefore, require further scientific enquiries.

Biochemical test:

Under biochemical test there are various test such as liver function test, Bilirubin (BILI) test, Alkaline phosphatase (ALP) test, Aspartate aminotransferase (AST) or SGOT test, Alanine transaminase (ALT) or SGPT test, blood urea nitrogen (BUN) test, Creatinine test.

In table 3.3, shows the result of biochemical examination. A blood urea nitrogen (BUN) test measures the amount of nitrogen in blood that comes from the waste product urea. When protein is broken down in body urea is produced. Urea is made in the liver and passed out of your body in the urine. BUN test is done to see how well kidneys are working. If kidneys are not able to remove urea from the blood normally. Heart failure, dehydration, or a diet high in protein can also make BUN level higher however liver disease or damage can lower BUN level (Guyton & Hall, 2006). From result we can see that, for Urea treated groups (0.5 ml, 1 ml, 1.5ml) did not show any significant differences compared to the controlled group.

Creatinine is a waste product made by the muscles. Creatinine passes into the bloodstream, and is usually passed out in urine. A high blood level of creatinine indicates that the kidneys may not be working properly. Creatinine is usually a more accurate marker of kidney function than urea.

(Guyton & Hall, 2006). Here, Creatinine level for the treated groups dose 0.5ml and 1.5ml showed significant differences compared to controlled group and treated group 1ml showed no significant change. Decreased cratinine level may indicate improved kidney function for both the treated group in comparison to the controlled group.

Bilirubin (BILI) is a pigment in bile broken down by the liver and excess will make sample look yellow. This is called jaundice. Mild increases are very common and are of no significance. Grossly elevated bilirubin may indicate poor liver function of sample (Guyton & Hall, 2006). Here, Bilirubin value for all three treated group did not show any significant difference from the control group. Bilirubin is a major product that results from the breakdown and destruction of old red blood cells that indicated jaundice. It is an important product with diagnostic values that is removed from the body by the liver (Jakari, J. M. 2015).

The plasma enzymes, Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) are the markers of hepatocellular damage. Aminotransferases are sensitive but relatively nonspecific indicators of liver cell injury. High level of Alanine transaminase (ALT) or SGPT indicated hepatocellular damage that means liver is not working properly (Guyton & Hall, 2006). Here we can see that, there was not any significant change in the value presence to indicate hepatocellular damage in body.

According to Mayne (1996), as body cell contains more AST than ALT, AST level are expected to be high in body. Usually, about 80% of AST is found in the mitochondria whereas ALT is purely cytosolic enzyme. Therefore, AST appears higher in concentration in a number of tissues (liver, kidney, heart and pancreas) and is released slowly in comparison to ALT (Jakari,2015). According to Al-Mamary et al., (2002), However, ALT enzymes are more sensitive marker for indicating hepatocellular damage than the AST enzyme. As there was not any significant difference in ALT result, so we can say that, there was no possible toxicity found in liver after ingesting clay.

3.3Statistical output of biochemical test result and discussion:

Table 3.3: Effects of geophagic clay on the biochemical test of rats after 70 days of treatment

Parameters	Control group	Treated group .5ml	Treated group 1ml	Treated group 1.5ml
Mean±SE(F,P)				
Urea	21.33±.553	26±.36(8.44 ,0.108)	24.6±.843(1.156 , 0.495)	22.3±.843(1.156, 0.495)
Creatinine	1.38±.389	.83±.01 (8806.417 , **0.008**)*	.786±.049 (5.25 , 0.315)	.783±.030* (8806.417 , **0.008**)
Bilirubin	.19(±.002)	.193±.011 (3.167 , 0.396)	.2133(±.01)	.233±.01 (0.604 , 0.732
ALT/SGPT.	57(±1.31)	62.33(±.91) (10.188 , 0.230)	57(±4.4)	56(±4.42)
AST/SGOT	86.66(±24.85)	146.33(±3.74)	177(±6.07)	176.33(±7.52)
Alkaline phosphatase	289(±5.51)	288±2.2* (52.620 , **0.005**)	294.33±7.8 (49.861 , 0.106)	276(±16.9)
Total Cholesterol	72.56(±.91)	74.66(±1.83)	73.66±1.72	72(±1.31)(1.132,0.460)
Triglyceride	42.66(±.91)	47.33(±.84) (10.417 , 0.228)	48.33(±3.80) (5.022, 0.171)	43(±.632) (2.494, 0.2)
HDL	48.33(±.76)	48.66(±1.87) (0.685 , 0.706)	51.33(±2.97) (0.685 , 0.706)	52.83(±1.42)
LDL	8.25(±2.71)	16±2.71	13.5±2.2	8±.577 (0.887 , 0.0600)
Total protein	5.3±.057	5.41±.079 (2.00 , 0.350)	5.4±.096	5.4±.103 (1.0 , 0.626)
Albumin	2.30±.07	2.33±.071 (.639 , 0.721)	2.51±.124	2.4±.141

Only SPSS given F and P values have been presented. No other F and P values have been generated from the rest of the data by SPSS analysis due to co-linearity.

Alkaline phosphatase (ALP) is an enzyme found in bones, bile ducts, and liver. An ALP test is typically done ordered in combination with several other tests. High levels of ALP may indicate liver damage, blockage of the bile ducts, or a bone disease of sample (Guyton & Hall, 2006). Here, high level of Alkaline Phosphatase (ALP), found only in treated group dose 0.5ml whereas all other treated group did not show any significant change compared to controlled group. As the AST and ALT result before in discussion confirmed that there is no chance of hepatocellular damage , so high level of ALP may indicate that , there is a problem for treated group .5ml related to bone. According to Jakari, J. M. (2015), significant elevation in Alanine aminophosphatase (ALP) level at 1.0g/kg could be an indication of bone disease but not liver since GGT and ALP are enzymes of the bile ducts and in situations where GGT is normal and ALP is elevated, it then means that there is possibility of disease relating to bones.

Result of Triglyceride level showed insignificant difference in all treated groups (0.5ml, 1ml, 1.5ml) compared to the controlled group. Which indicate no risk of artherosclerosis, hypothyroidism, liver disease, pancreatitis, myocardial infarction, metabolic disorders, toxemia, and nephrotic syndrome (Guyton & Hall, 2006) for all the treated group.

Result of HDL, LDL, Total protein and Albumin did not show any significant difference for all three treated group from the controlled group. According to Jakari, J. M. (2015), Mean values for VLDL concentrations increased in all the treated groups indicates values of the coronary risk.

Due to co-linearity SPSS did not give any outcome. In some cases it gave values where results were statistically not significant or insignificant.

3.4 Average rate of pregnancy between control and clay treated groups:

An interesting finding of this study we found here that, throughout the 70 days experiment over the samples after 30 days samples of different treated groups started to get pregnant. However, samples of control group did not get pregnant at the same time with the treated groups though their weight was more and environment was same. Samples of control group started to get pregnant from day 40-45 whereas treated group samples started to get pregnant after day 30. So here by interpreting results we can say that, as treated groups were ingested with sample geophagic clay it

stimulated the ovulation of treated group rats. On the other hand, as control group rats were not ingested the sample clay effect was not same for this group.

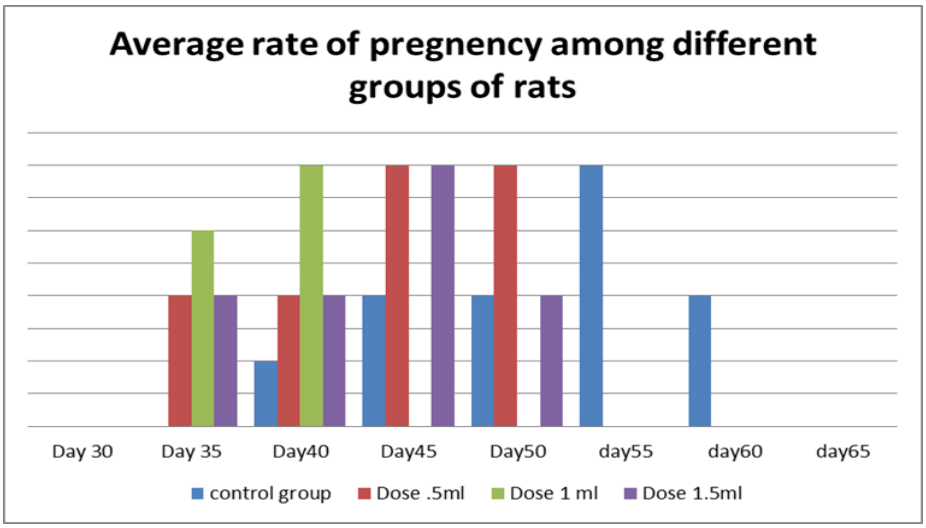

Figure 3.2: Average rate of pregnancy between control and different dose clay treated groups of rat throughout the experiment day 30-65.

Chapter 4

Conclusion

4.1 **Conclusion:**

Consumption of geophagic clay is a common practice in some region of earth especially in Nigeria and other sub-Saharan African countries, developed and some developing countries of South Asia (Ekanem, 2015). Though some study has showed that , geophagic clay may contain heavy metals especially led that might be toxic for human body if it is consumed at a high level (Abraham et al , 2006) yet , geophagic clay contains amount of Iron which is very much essential during period of pregnancy.

The study here revealed our geophagic clay sample "Calabash chalk" had no negative effect in terms of weight gain for rat samples. All the parameters of RBC were unaffected so therefore experimental rats had no chance of having anemia. Moreover, from the biochemistry test result we found improved kidney function among rats treated with sample clay. Besides, our study revealed interesting result about ovulation of treated group rats in comparison to the control group. However, our study showed some effect of microbial infection and possibilities of bone related diseases among the treated groups. Therefore, it requires some more study regarding these topic that may help us to provide a better conclusion.

4.2 Limitations of the study:

1. This study was performed for 70 days only. However, if the study could be carried out for at least three months, it might give better results (Jakari, J. M. 2015).

2. It would be better if the study could be conducted with few more groups having different doses treated with clay.

3. Because of budget limitations some tests were not done which might provide us better results.

CHAPTER 5

Reference

5.1 Reference

Abrahams, P. Follansbee, M. Hunt, A. Smith, B. and Wragg, J. (2006). Iron nutrition and possible lead toxicity: an appraisal of geophagy undertaken by pregnant women of UK Asian communities. *Appl Geochem* 21: 98 – 108.

Abraham, P. W. and Parson J. A. (1996). Geophagy in the Tropics: A literature review. The geographical journal,vol.162(1):63-72.

Almmary, M. , Al-Habori, M., Al-Aghabri, A.M., and Baker, M. M. (2002). Investigation into the toxicological effects of Catha edulis leaves: a short term study in animals. *Phytother.Resource* ,16: 127 -132.

Al-Rmalli1, S.W., Jenkins, R.O., Watts, M.J., and Haris, P.I. R. O. J.,(2010). Risk of human exposure to arsenic and other toxic elements from geophagy: trace element analysis of baked clay using inductively coupled plasma mass spectrometry. Environmental Health , 9(79).

Website: http://www.ehjournal.net/content/9/1/79

Cooper, M. (1957). Pica: Survey of the Historical Literature as Well as Reports from the Fields of Veterinary Medicine and Anthropology, the Present Study of Pica in Young Children, and a Discussion of Its Pediatric and Psychological Implications. Bannerstone House, Springfield,Illinois.

Nyanza1, E.C., Joseph, M., Premji, S. S., Thomas, D. SK., and Mannion, C.(2014). Geophagy practices and the content of chemical elements in the soil eaten by pregnant women in artisanal and small scale gold mining communities in Tanzania. *BMC Pregnancy and Childbirth*, 14(144).

Website:

https://bmcpregnancychildbirth.biomedcentral.com/articles/10.1186/1471-2393-14-144.

Ekosse, G. E., de Jager, L. and Ngole, V. (2010). Traditional mining and mineralogy ofgeophagic clays from Limpopo and Free State provinces, South Africa. *African Journal of Biotechnology*, 9(47): 8058-67. 38.

Firkin,F & De Gruchy, G.C (1990). De Gruchy's Clinical Haematology In Medical Practie. (5th ed) . New Delhi, ND: Oxford University Press.

Geissler, P. W. (2016). The Significance of Earth-Eating: Social and Cultural Aspects of Geophagy Among Luo Chitdren. *Africa: Journal of the International African Institute*, 70(4 (2000)): 653-682.

Gelfand, M. (1945). Geophagy and its relation to hookworm disease. East African Medical Journal, 22: 98–103.

Guyton, A.C. and Hall, J.E. (2006). Textbook of Medical Physiology. (11th ed). Philadelphia, PA, USA: Elsevier Saunders.

Hunter, J. M. (1973). Geophagy in Africa and in the United States: A Culture-Nutrition Hypothesis. *Geographical Review*, 63(2): 170-195.

Key, T. C., Horger, E.O. and Miller, J.M. (1982). Geophagia as a cause of maternal death. Obstet. *Gynecol* , 60(4): 525-526.

Krishnamani, R. and Mahaney, W.C.(2000). Geophagy among primates: adaptive significance and ecologicalconsequences. *Animal Behaviour* , 2000(59): 899–915.

Rafique, M. , Perveen, K., Khan, N. and Nigar , S. (2008). Lead intoxication causing loss of body weight and loss of absolute weight of testes in albino rats. *Hamdard Medical*, 51: 123 –128.

Sera L. Young, P. W. S., Julius B. Lucks and Gretel H. Pelto (2011). Why On Earth?: Evaluating Hypotheses About The Physiological Functions Of HumanGeophagy. *The Quarterly Review of Biology*, 86(2): 97-120.

Young, S. L., Jeffrey, Wilson, M., Hillier, H., Delbos, E., Ali, S. M. and Stoltzfus, J. R., (2010). Differences and commonalities in Physical, Chemical and Mineralogical Properties of Zanzibari Geophagic Soils. *J. Chem. Ecol* , 36: 129-140

Young, S. L., Wilson, M. J., Miller, D. and Hillier, S. (2008). Toward a comprehensive approach to the collection and analysis of pica substances, with emphasis on geophagic materials. *PLoS ONE* 3:e 3147.

Zakari, J. M. (2015, January). Health implications of some geophagic clays on growth performance, haemato-biochemical profile and histology of female sprague dawley rats.

Website : http://dspace.knust.edu.gh:8080/jspui/handle/123456789/7475.