**Effect of Calabash Chalk on Some Haemathological Parameters in Pregnant Wistar Rat**

**ABSTRACT**

**Introduction**

Calabash chalk, a type of edible clay, is commonly consumed by various individuals, particularly pregnant women, despite containing substances that may cause haematological abnormalities such as anaemia and bleeding disorder.

**Aim:** This experimental study investigated the effects of calabash chalk consumption on hematological parameters in pregnant Wistar rats during pregnancy.

**Material and Methods**: A total of 40 Wistar rats comprising of 20 pregnant and 20 non-pregnant rats were selected and assigned to four groups (A-D) each comprising five pregnant and five non pregnant rats. Group A served as control and was fed with standard animal feed and distilled water at a dose of 10 mg/kg body weight daily for 21 days. Groups B, C and D received calabash chalk suspension at doses of 200 mg/kg, 400 mg/kg and 600 mg/kg body weight respectively, along with feed and distilled water for 21days. At the end of the experiment, the rats were anaesthetized, and blood samples were collected from the orbital sinus into ethylenediaminetetraacetic acid (EDTA) sample containers for analysis. The samples were analyzed for hematological parameters (full blood count). Data from pregnant and non-pregnant rats were statistically analysed using the statistical analysis system (SAS) version 9.4. Results were expressed as mean ± standard error of mean (SEM). Group comparisons were made using One-way Analysis of Variance (ANOVA) . Results were considered statistically significant at a 95% confidence interval (P< 0.05).

 Result: Statistical analysis with a significant level set at P< 0.05 showed a significant increase in white blood cell (WBC) and neutrophil counts (P=0.001 and P= 0.002, respectively) compared to control group. Significant decreases were also observed in haematocrit (HCT), haemoglobin (HB), and mean corpuscular volume (MCV), with P- values of 0.001, 0.032, and 0.012, respectively. Platelet counts showed significant difference (P=0.001).

**Conclusion:** This study revealed that while calabash chalk may be used for different reasons during pregnancy, it can significantly alter some haematological parameters like haemoglobin concentration, haematocrit, white blood cell counts and platelet count, potentially posing health risks due to its constituents.

**Keywords: haematological parameters; pregnant; non pregnant; calabash chalk**

**INTRODUCTION**

Calabash Chalk is a type of consumable clay, commonly ingested by different populations, especially pregnant women, for perceived health or cultural benefits. It is widely consumed in parts of West Africa including Nigeria, Ghana and South Africa (Ngole *et al*.,2010; Kortei *et al*.,2020), and report suggests its usage may extend to regions in India (Traugott *et al*., 2019). The widespread availability and distribution are partly facilitated by regional trade and migration.

Geophagia, the practice of eating clay, stems from religious beliefs, medicinal purposes, or pleasure. However, calabash chalk poses safety concerns, despite pregnant women believing it alleviates nausea, morning sickness, vomiting and excessive salivation. This belief lacks scientific substantiation (Abraham *et al*.,2013).

Both humans and animals engage in geophagia. In humans, it is classified as pica, characterized by craving non-food items (Young,2008).

Studies show geophagia transcends regional and gender boundaries (Waywodt *et al*.,2002)., prevalent among all socioeconomic classes, particularly as a means to suppress hunger in low-income groups. In some part of Nigeria, calabash chalk is called Nzu while in French it is called Agile or La Craie. It can be gotten from the soil and can be seen as dull whitish substance or ash colour like substance which is solid and can dissolve in water. Sometimes it can be processed by adding some other substances like wood ash, salt or it can be gotten in its natural state in places where they are sold in the form of pellets or moulded as small blocks (Aprioku *et al*.,2018).Calabash chalk is believed to have different uses such as antacid (Olatunji *et al*.,2014)., contraceptive, antidiarrheal, nutritional supplement, for wound healing, skin care and sociocultural activities. (Okeke *et al*.,2014).

 Calabash chalk reduces morning sickness and suppress appetite which is experienced during pregnancy (Wiley, 2008). Since calabash chalk is gotten from the soil it is very possible that the substance is contaminated with microorganisms. Research conducted by the Food and Drug Administration in 2019, showed that calabash chalk contains heavy metals, and its composition varies depending on the region and location where it is gotten from.

 La *et al*,2014., discovered that calabash chalk contains aluminum as one of its main components and other chemicals like lead, arsenic acid and chromium. Ekong *et al*.,(2015). carried out research and discovered that calabash chalk contains metals, metalloids and organic pollutants. Recently the Texas Department of State Health Services and the Food and Drug Administration has raised concerns of potential health risk which could arise from the consumption of calabash chalk mainly in pregnant women and lactating mothers since calabash chalk is contaminated with some quantity of lead and arsenic. This research becomes very important since a lot of physiological changes occur during the pregnancy period and the process could be complicated by the presence of chemicals like lead contained in calabash chalk. Vofo *et al*., (2019).,.

**2 MATERIAL AND METHODS**

**2.1 Experimental Animals**

This study was an experimental study designed to evaluate the effect of calabash chalk consumption on the hematological parameters of the pregnant Wistar rats.

The study involved of two categories of Wistar rat.

The non- pregnant Wistar rats and the pregnant Wistar rats.

A total of 40 healthy Wistar rats weighing between 125-178 g were used, consisting of 20 pregnant and 20 non-pregnant Wistar rats. These animals were obtained from the animal farm of the Department of Physiology, University of Port Harcourt, Nigeria. The rats were housed in clean plastic cages, fed standard animal pellets (Ultima Plus Finisher Mash produced by Olam Ultima Feeds in Kaduna, Nigeria), and provided with clean distilled water. They were maintained under natural light-dark conditions. The animal handling followed the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals. (CPCCSEA).

Female rats in the estrous phase were paired with male rats at a ratio of 2:1 (i.e., two males to four females per cage). They cohabited for one week and were monitored daily for signs of pregnancy, including the presence of a copulatory plug. The estrous phase was confirmed by microscopic examination of vaginal smears, identifying cornified epithelial cells, in line with the description by Marcondes *et al*., (2002).

Twenty (20) confirmed Pregnant rats were selected and randomly divided into four groups of five rats each. Similarly, twenty (20) non-pregnant rats were sorted and assigned to four groups (five rats per group).

#  **2.2 Calabash Chalk**

The study was conducted between March and May 2024. Salted calabash chalk was purchased from Oil Mill Market, Eleme Junction, in Port Harcourt, Rivers State, Nigeria.

# **2.3 Preparation of Aqueous Calabash Chalk**

 A total of 500g of salted calabash chalk was grounded into powder form. The powder was sieved, and 300 g was collected for suspension preparation. To this 1000 ml of distilled water was added. The suspension was used to prepare a 30g stock solution that was used for the experiment.

Converting 30 g to mg = 30 g × 1000 mg/g = 30,000 mg

To calculate the concentration in mg/ml = amount of calabash chalk in mg/ total vol of solution in ml, therefore 30,000 mg/1000 ml = 30 mg/ml. Therefor the concentration of the stock solution was 30 mg/ml. This stock solution was used to prepare test doses of 200 mg/kg, 400 mg/kg and 600 mg/kg body weight which were administered daily for 21days.

**2.4 The Physicochemical Analysis of calabash chalk**

The physicochemical analysis of calabash chalk was conducted in the chemistry laboratory in Rivers State University Port-Harcourt, Rivers state.

**2.5 Pilot Study**

Determination of LD50 for the calabash chalk

The acute toxicity of the aqueous extract of calabash chalk was assessed in Wistar rats following the Organization for Economic Co-operation and Development (OECD) Guideline 423.

Four groups, each consisting of three female rats were used. Three groups were orally administered calabash chalk extract doses of 1000 mg/kg ,1500 mg/kg and 2000 mg/kg body weight respectively. The control group received 10 ml/kg body weight distilled water.

These animals were closely monitored for signs of toxicity. Observations were made every 20 minutes during the first 6 hours post administration, periodically over the next 48 hours, and daily for 7 days thereafter.

 They were monitored for signs of toxicity like lethargy, behavioral changes, altered appetite, changes in skin or fur, respiratory distress, tremor or convulsion. No signs of toxicity or mortality were observed within the first 24 hours window or during the 7- day observation period. It was therefore concluded that the LD50 of the aqueous calabash chalk suspension was greater than 2000 mg/kg.

The dosage of the substance (calabash chalk) used was calculated according to body weights using the formula according to Oghenesuvwe *et al*., (2014).

# **2.6 Experimental Design**

This experimental study was carried out by separating the Wistar rats into groups and the rats in each group was weighed after which the average weight of the rats in each group was noted.

**2.6.1 Animal Grouping and Administration of Calabash Chalk**

The animals were separated into two sets comprising of:

Twenty pregnant Wistar rats (20)

Twenty non-pregnant Wistar rats **(20)**

# **The First Set**

The first set consists of the twenty pregnant Wistar albino rat which were randomly separated into four different groups of five rat each and were kept in a plastic cage.

**Control Group A**

The cage labeled Group A contained five pregnant rats with an average body weight of 159 g and they were fed animal feed + distilled water daily 10 ml/kg body weight for 21 days.

# **Group B**

The cage labeled Group B contained five pregnant Wistar rats with an average body weight of 155 g. They were fed with distilled water + animal feed + 200 mg/kg body weight of the calabash chalk suspension for 21 days.

**Group C**

 The cage labeled Group C also contained five pregnant Wistar rats with average body weight of 157 g. They were fed with distilled water + animal feed ＋ 400 mg/kg body weight of the calabash chalk suspension for 21days.

**Group D**

The cage labeled Group D contained five pregnant Wistar rats with average body weight of 161g and were fed with distilled water + animal feed + 600 mg/kg of body weight of the calabash chalk suspension for 21days.

**Second Set**

The Second set comprise of Twenty female non- pregnant Wistar rats that were also separated into four different groups of five rats each.

These rats in the different groups A, B, C, D were also arranged like the rats in the first set comprising of:

 Group A – Control 10 ml/kg distilled H2O + feed

 Group B -- Animal feed + H2O + 200 mg/kg suspension

 Group C -- Animal feed + H2O + 400 mg/kg suspension

 Group D – Animal feed + H2O + 600 mg/kg suspension

The rats in the two sets (pregnant and non- pregnant) containing Wistar rats were fed in this manner using oral cannular for 21 days.

**2.7 Sample collection and Preparation**

On day 3 after delivery, the rats were taken to the laboratory, anaesthetized, and blood sample were collected from the orbital sinus of those that gave birth using a capillary tube while the non-pregnant rats were sacrificed and blood sample collected and dispensed into EDTA bottle (approximately 3ml) for hematological analysis.

**2.7.1 Labelling of Sample Bottles**

 The bottles were properly labeled according to the samples that were collected from the different groups.

**2.7.2 Serum Preparation**

Blood samples were processed immediately in the Laboratory. Full blood count tests were performed immediately using an automated hematology analyzer.

**2.7.3 Sample Analysis**

Full Blood Count (FBC) samples were analyzed using the 5-part automated hematology analyzer (Mind ray BC - 5150) within 2 hours of sample collection.

**2.8 Statistical Analysis.**

Data was analyzed using Statistical Analysis Software (SAS) version 9.4. Results were expressed as mean ± standard error of mean (SEM). One-way analysis of variance (ANOVA) was used to compare mean across the groups, with a significance level set at P ≤ 0.05.

**3.Result**

**Result of pilot study**

The LD50 (lethal dose for 50% of experimental animals) of the calabash chalk used was determined through preliminary dosing. Rats were administered 1000 mg/kg, 1500 mg/kg and 2000 mg/kg body weight doses of the calabash chalk. At all these dose level no sign of toxicity was observed among the Wistar rats. Indicators such as lethargy, respiratory distress, convulsion, confusion, loss of appetite and body weight were absent.

The body weight of Wistar rats used in the study ranged from 150-179 g.

**3.1 Quantitative Analysis of the Chemical Composition of Calabash Chalk**

Table 1 shows the quantitative analysis of the chemical constituents of the calabash chalk; the substance contains different organic and inorganic compounds, and their quantity is shown in this table after a quantitative chemical analysis was carried out on the substance. This table shows the different compounds and their percentage value in the calabash chalk.

**Table 1 Showing the Chemical Composition of Calabash Chalk (Nzu) and the Various Percentages of Each Composition.**

|  |  |  |
| --- | --- | --- |
| **S/N**  | **Chemical composition**  | **% value**  |
| 1  | Cyclotrisiloxane, hexamethyl-Cyclotrisiloxane, hexamethyl- Arsenous acid, tris (trimethylsilyl) ester  | 15.640  |
| 2  | Phenanthridine Benzo [g] quinoline Acridine  | 14.922  |
| 3  | 3-Methoxyphenyl isothiocyanate N-[2-(Adamantan-1-yloxy)-ethyl] 2- 4,6trimethyl-benzenesulfonamide m-Cresol, TMS derivative  | 9.220  |
| 4  | Trisiloxane, 1,1,3,3,5,5-hexamethyl- 6-Aminoindazole Benzoic acid, 2amino-4-methyl-  | 9.867  |
| 5  | 4H-Furo [3,2-b] pyrrole-5-carboxylic acid, methyl ester Veratric acid, TMS derivative Adamantane-1-carbothioamide  | 1.820  |
| 6  | Dotriacontyl isobutyl ether 1-Decanol, 2-octyl-Oxalic acid, allyl octadecyl ester  | 1.923  |
| 7  | Cyclotetrasiloxane, octamethyl- Cyclotetrasiloxane, octamethyl- Cyclotetrasiloxane, octamethyl-  | 7.541  |
| 8  | 5-Bromo-1-methylindole-2-carboxylic acid 5-Bromo-1-methylindole-2carboxylic acid Benz (a) anthracene-7-carbonitrile  | 6.092  |
| 9  | Phthalazine-1,4(2H,3H)-dione, 2-(2 -methyl-5-nitrophenyl)- Benzo [h] quinoline, 2,4-dimethyl- Cyclotrisiloxane, hexamethyl-  | 4.919  |
| 10  | Tetrapentacontane, 1,54-dibromo-Oxalic acid, cyclobutyl heptadecyl ester 1Decanol, 2-hexyl-  | 1.971  |
| 11  | trans-4-(2-(5-Nitro-2-furyl) vinyl) -2-quinolinamine Cyclotetrasiloxane, octamethyl- Benzene, 1-phenyl-4-(2-cyano-2-phenylethenyl)  | 2.286  |
| 12  | 1-(1-Propen-1-yl)-2-(2-thiopent -3-yl) disulfide Tricyclo [5.2.2.0(2,6)] undec8-en-1 1-one, 3- [ (2-methoxyethoxy) methoxy ]-2-methyl- Decanoic acid, 2hydroxy-  | 2.228  |
| 13  | Propenone, 3-(2-benzoxazolylthio)- 1-phenyl- Benzophenone-4,4'-dicarboxylic acid dimethyl ester m-Phenylenediamine, N, N, N'-trimethyl-N'[2-(N-methylanilino) ethyl]-  | 3.025  |
| 14  | Carbonic acid, octadecyl prop-1-en -2-yl ester Dodecyl nonyl ether Carbonic acid, decyl hexadecyl ester  | 3.612  |
| 15  | 2-Trimethylsiloxy-6-hexadecenoic acid, methyl ester 1H-Isoindole-1,3(2H)dione, 2-hydroxy- Trimethylsilyl 2-(trimethylsilyloxy) propaneperoxoate  | 2.258  |
| 16  | 1-Bromoeicosane Nonahexacontanoic acid Carbonic acid, octadecyl prop-1en -2-yl ester  | 2.963  |
| 17  | 8-Heptadecene 8-Heptadece Cyclopentane, (4-octyldodecyl)-  | 2.827  |
| 18  | Methoxyacetic acid, heptadecyl ester 1-Decanol, 2-octyl- 1-Dodecanol, 2hexyl-  | 2.176  |
| 19  | Carbonic acid, eicosyl vinyl ester Oxalic acid, cyclobutyl pentadecyl ester Undecane  | 2.673  |
| 20  | Cyclopentane, 1-methyl-3-(2-methylpropyl)- Sulfurous acid, octadecyl 2propyl ester Oxalic acid, allyl octadecyl ester  | 2.037  |

**3.2 Hematological Parameters of Pregnant and Non-Pregnant Wistar rats Fed Graded Levels of Calabash Chalk (Pregnancy Status + Treatment)**

The hematological parameters of the Wistar rats were statistically analyzed after separating them into different category. Each category (pregnant and non-pregnant) was further subdivided into control and experimental group receiving graded doses of aqueous calabash chalk suspension. Hence, there were 5 rats in each category

**Table 2 White Blood Cell and Its Subset**

Table 2 presents the WBC count and its subsets, and the RBC count in pregnant rats that were treated with graded doses of aqueous calabash chalk suspension with a control group and non-pregnant Wistar rats that were treated with graded doses of aqueous calabash chalk suspension with a control group. WBC count in the pregnant rats fed with 200 mg/kg body weight dose was 8.44±1.69, those fed with the 400mg/kg body weight was 8.74±1.63 while those that were fed the 600mg/kg body weight was 11.34±1.63. The non pregnant rats fed with the 200mg/kg dose recorded 6.47±1.11, the group fed with the 400mg/kg recorded 6.09±0.61 while the group fed 600mg/kg recorded 7.32±0.79 when these values were statistically analyzed using P= 0.05 as the level of significance there was significant difference (P =0.001). Neutrophil count in the pregnant rats fed with 200 mg/kg body weight dose was 5.97±1.98, those fed with the 400mg/kg body weight was 5.89±1.61 while those that were fed the 600mg/kg body weight was 9.23±1.78. The non pregnant rats fed with the 200mg/kg dose recorded 2.97±0.57, the group fed with the 400mg/kg recorded 3.57±0.38 while the group fed 600mg/kg recorded 3.84±0.80 the neutrophil count showed significant difference (P = 0.002). Monocytes count in the pregnant rats fed with 200 mg/kg body weight dose was 0.26±0.07, those fed with the 400mg/kg body weight was 0.41±0.09 while those that were fed the 600mg/kg body weight was 0.44±0.09. The non pregnant rats fed with the 200mg/kg dose recorded 0.36±0.07, the group fed with the 400mg/kg recorded 0.35±0.06 while the group fed 600mg/kg recorded 0.34±0.03. Eosinophil count in the pregnant rats fed with 200 mg/kg body weight dose was 0.11±0.05, those fed with the 400mg/kg body weight was 0.09±0.04 while those that were fed the 600mg/kg body weight was 0.01±0.01. The non pregnant rats fed with the 200mg/kg dose recorded 0.16±0.05, the group fed with the 400mg/kg recorded 0.08±0.05 while the group fed 600mg/kg recorded 0.09±0.04. The monocytes and eosinophil also recorded significant difference (P= 0.009 and 0.012 respectively). Lymphocyte count in the pregnant rats fed with 200 mg/kg body weight dose was 2.07±0.40, those fed with the 400mg/kg body weight was 2.35±0.63 while those that were fed the 600mg/kg body weight was 1.59±0.31. The non pregnant rats fed with the 200mg/kg dose recorded 2.99±0.82, the group fed with the 400mg/kg recorded 2.10±0.25 while the group fed 600mg/kg recorded 3.04±0.22. The lymphocytes recorded no significant difference (P = 0.8227).

The red blood cells count (RBC) showed that as the dosage was increased there was a slight decrease in the RBC values. RBC count in the pregnant rats fed with 200 mg/kg body weight dose was 4.60±0.22, those fed with the 400mg/kg body weight was 4.10±0.12 while those that were fed the 600mg/kg body weight was 4.08±0.31. The non pregnant rats fed with the 200mg/kg dose recorded 4.88±0.31, the group fed with the 400mg/kg recorded 4.19±0.20 while the group fed 600mg/kg recorded 4.23±0.16. RBC recorded no significant difference (p=0.5300).

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Status | WBC (x109/L)  | NEUT(x109/L) | LYM(x109/L) | MONO(x109/L) | EOS(x109/L) | BASO(x109/L) | RBC(x109/L)  |
| P (A) |  8.82±1.40a | 6.14±1.19a | 2.23±0.32 | 0.35±0.12a | 0.04±0.03a | 0.00±0.00 | 5.94±0.91  |
| P (B) | 8.44±1.69a | 5.97±1.98b | 2.07±0.40 | 0.26±0.07b | 0.11±0.05b | 0.00±0.00 | 4.60±0.22  |
| P (C) | 8.74±1.63a | 5.89±1.61b | 2.35±0.63 | 0.41±0.09c | 0.09±0.04b | 0.00±0.00 | 4.10±0.12  |
| P (D) | 11.34±1.63b | 9.23±1.78c | 1.59±0.31 | 0.44±0.09c | 0.01±0.01c | 0.00±0.00 | 4.08±0.37  |
| NP (A) | 5.74±0.56c | 2.94±0.24d | 2.33±0.37 | 0.35±0.13a | 0.10±0.05b | 0.00±0.00 | 5.17±0.18  |
| NP (B) | 6.47±1.11d | 2.97±0.57d | 2.99±0.82 | 0.36±0.07a | 0.16±0.05b | 0.00±0.00 | 4.88±0.31  |
| NP (C) | 6.09±0.61d | 3.57±0.38e | 2.10±0.25 | 0.35±0.06a | 0.08±0.03b | 0.00±0.00 | 4.19±0.20  |
| NP (D) | 7.32±0.79e | 3.84±0.80e | 3.04±0.22 | 0.34±0.03a | 0.09±0.04b | 0.00±0.00 | 4.23±0.16  |
| F-ratio | 22.344 | 8.988 | 0.3035 | 18.033 | 7.182 |   | 0.7508  |
| p-value | <0.001 | 0.002 | 0.8227 | 0.009 | 0.012 |   | 0.5300  |
| Remarks | S | S | NS | S | S |   | NS |

**Table 2. Haematological Parameters of Pregnant and Non-Pregnant Wistar rats Fed Graded Levels of Calabash Chalk** (MEAN±SEM) (Pregnancy status ＋ Treatment)

 Abbreviations: WBC= White Blood Cell; NEU=Neutrophils; LYM=Lymphocytes; Mono=Monocytes; Eosin= Eosinophils; Baso=Basophils; RBC=Red Blood Cell. S= significant P <.0.05 ns = non-significant, P > 0.05.

**Table 3. Red Blood Cell Parameters**

Comparison of red blood cell parameters of pregnant and non-pregnant Wistar rats fed with graded levels of calabash chalk. Haemoglobin concentration in the pregnant rats fed with 200 mg/kg body weight dose was 12.52±0.26, those fed with the 400mg/kg body weight was 11.52±0.56 while those that were fed the 600mg/kg body weight was 10.52±0.69. The non pregnant rats fed with the 200mg/kg dose recorded 12.98±0.66, the group fed with the 400mg/kg recorded 11.36±1.01 while the group fed 600mg/kg recorded 11.50±0.38 . Haematocrit level in the pregnant rats fed with 200 mg/kg body weight dose was 35.32±1.31, those fed with the 400mg/kg body weight was 30.16±1.40 while those that were fed the 600mg/kg body weight was 29.28±1.50. The non pregnant rats fed with the 200mg/kg dose recorded 37.72±1.68, the group fed with the 400mg/kg recorded 33.50±2.65 while the group fed 600mg/kg recorded 34.40±0.81. There was a statistical decrease in hemoglobin and the hematocrit level as the dosage was increased across the different groups (P = 0.032 and 0.001 respectively). The MCV and RDW-SD also showed significant difference (P = 0.012and 0.033 respectively).

 **Table 3: Hematological Parameters of Pregnant and Non-Pregnant Wistar rats Fed Graded Levels of Calabash Chalk**

**(MEAN±SEM) CONTD (Pregnancy status ＋ Treatment)**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Status/ Group** | **Hb (g/dl)** | **HCT (%)** | **MCV (fl)** | **MCH (pg)** | **MCHC (g/dl)** | **RDW-CV** | **RDW-SD (fl)** |
| P (A) | 13.84±0.67a | 40.12±1.95a | 78.20±5.71a | 26.58±2.49 | 33.76±1.03 | 14.31±1.24 | 41.84±1.76a |
| P (B) | 12.52±0.26a | 35.32±1.31b | 80.80±2.52b | 27.52±1.12 | 34.04±0.53 | 14.28±0.65 | 44.68±2.32b  |
| P (C) | 11.52±0.56b | 30.16±1.40c | 79.74±5.68a | 27.06±2.16 | 33.90±0.35 | 14.34±0.96 | 43.02±2.15b |
| P (D) | 10.52±0.69b | 29.28±1.50c | 75.46±4.16c | 25.20±1.52 | 33.36±0.25 | 18.62±1.96 | 52.52±6.73c |
| NP (A) | 14.64±0.88c | 42.92±1.62a | 83.16±2.34b | 28.44±1.47 | 34.14±0.80 | 14.90±1.43 | 47.30±5.11d |
| NP (B) | 12.98±0.66a | 37.72±1.68d | 77.94±3.57a | 26.68±1.25 | 34.26±0.31 | 13.58±0.62 | 41.32±1.90a |
| NP (C) | 11.36±1.01a | 33.50±2.65e | 79.92±5.00b | 27.06±1.91 | 33.82±0.41 | 14.20±0.93 | 43.28±0.67b  |
| NP (D) | 11.50±0.38a | 34.40±0.81b | 82.02±4.64a | 27.46±1.68 | 33.44±0.43 | 13.38±0.32 | 42.84±2.57a |
| F-ratio | 31.322 | 22.722 | 18.622 | 0.3560 | 0.0590 | 2.7268 | 33.442  |
| p-value | 0.032 | <0.001 | 0.012 | 07851 | 0.9809 | 0.0603 | 0.033  |
| Remarks | S | S | S | NS | NS | NS | S |

Values in the same column but having different superscripts are significantly different from each other (p≤0.05)

Hb-Haemoglobin, HCT-Haematocrit, MCV-Mean Cell Volume, MCH-Mean Cell Haemoglobin, MCHC-Mean Cell Haemoglobin Concentration, RDW-CV-, RDW-SD

**Table 4. Platelet Parameters**

Comparison of platelet parameters of pregnant and non-pregnant Wistar rats fed with graded levels of calabash chalk. The platelet value was observed to increase as the dosage was increased.

Platelet level in the pregnant rats fed with 200 mg/kg body weight dose was 249.29±29.12, those fed with the 400mg/kg body weight was 274.20±36.11 while those that were fed the 600mg/kg body weight was 279.20±33.06. The non pregnant rats fed with the 200mg/kg dose recorded 229.80±26.50, the group fed with the 400mg/kg recorded 242±26.77 while the group fed 600mg/kg recorded 250±17.29. This showed significant difference across the different groups (P =0. 001). The P-LCC and P-LCR also recorded statistical difference (p=0.011 and 0.001 respectively).

**Table 4: Platelet Parameters**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Status/ Group | PLT (109/L) | MPV (fl) | PDW | PCT | PLCC (109/L) | PLCR (%) |
| P (A) | 327.80±50.90a  | 9.50±0.64  | 15.70±0.28 | 0.30±0.04 | 84.80±11.47a | 0.24±0.04a  |
| P (B) | 249.20±29.12b  | 9.62±0.40  | 15.78±0.17 | 0.24±0.02 | 58.20±2.78b | 0.25±0.03a  |
| P (C) | 274.20±36.11c  | 10.46±16.64  | 15.62±0.17 | 0.26±0.03 | 65.40±7.31c | 0.25±0.03a  |
| P (D) | 279.20±33.06c | 9.40±0.24  | 15.62±0.14 | 0.26±0.03 | 67.60±8.66c | 0.24±0.01a  |
| NP (A) | 230.00±30.89d | 10.40±0.53  | 15.80±0.25 | 0.24±0.03 | 68.40±8.10c | 0.31±0.04b  |
| NP (B) | 229.80±26.50d | 11.96±19.62  | 15.80±0.13 | 0.22±0.02 | 55.00±6.44b | 0.25±0.03a  |
| NP (C) | 242.40±26.77b | 10.56±0.41  | 16.06±0.14 | 0.25±0.02 | 72.00±3.70d | 0.31±0.03b  |
| NP (D) | 250.00±17.27b | 9.68±0.31 | 15.50±0.09 | 0.24±0.02 | 61.60±7.55c | 0.25±0.02a  |
| F-ratio | 45.355 | 2.458 | 0.672 | 0.411 | 19.356 | 36.891  |
| p-value | <0.001 | 0.808 | 0.575 | 0.746 | 0.011 | <0.001  |
| Remarks | S | NS | NS | NS | S | S |

S= significant P <.0.05 ns = non-significant, P > 0.05.

Abbreviations: PLT= Platelets, MPV= Mean Platelet Volume, PDW= Platelet Distribution Width, PCT= Procalcitonin, P-LCC=, P-LCR=Platelet-Large Cell Ratio.

# **4.1 Discussion**

A pilot study was conducted to determine an LD50 and establish a safe dose of the aqueous calabash chalk suspension to be administrated to Wistar rats for a 21-day duration. Wistar rats which weighed less than 125g were excluded from this study. Chemical composition of calabash chalk (Table 1) revealed that the calabash chalk used for this experiment contained Cyclotrisiloxane, hexamethyl-Cyclotrisiloxane, hexamethyl- Arsenous acid, tris (trimethylsilyl) ester with the latter being the most abundant component (15.6％). The table shows that Phenanthridine Benzo quinoline Acridine is the second most abundant constituent of the calabash chalk used for this study with percentage value of 14.992％, followed by 3-Methoxyphenyl isothiocyanate N-[2-(Adamantan-1-yloxy)ethyl] 2- 4,6-trimethyl-benzenesulfonamide m-Cresol, TMS derivative (9.220%) and Trisiloxane, 1,1,3,3,5,5-hexamethyl- 6-Aminoindazole Benzoic acid, 2-a***.*** mino-4methyl-made up 9.867% .Other chemical constituents were present in smallerquantity-

Table 2 which summarizes some of the white blood cell count (WBC) and differentials of pregnant and non-pregnant Wistar rats that were treated with graded level of aqueous calabash chalk suspension revealed that there was a significant increase in WBC count in pregnant rats and non-pregnant rats with increasing doses (P<0.001). Although the white blood cell value was still within the reference range of white blood cell during pregnancy. This increase in WBC is in line with study carried out by Kortei *et al* (2020) which stated that calabash chalk contains certain toxic substances which could be harmful to the body and this increase in WBC count could be as a result of immunological response to these toxins and microbial contaminants present in the calabash chalk. Alternatively, the increase in the white blood cell of the experimental rats could reflect the normal immunological response or possible inflammatory changes associated with pregnancy which is in line with Bonet *et al., (*2020). who reported that pregnancy is characterized by immune modulation and increased leukocytes levels due to inflammatory processes. This study also agrees with study carried out by Campbell in 2014 which revealed that rats administered with arsenic acid had an increased WBC count when compared to those that were not administered the arsenic acid However, this contradicts findings by Chinko *et al., (*2020) that revealed a significant decrease in mean values of WBC and neutrophil and also Amabe *et al., (*2010). who reported no significant changes in WBC count following calabash chalk administration

 A significant increase in neutrophil count (P=0.002) was also noted as the doses were increased (just like the white blood cell count) across the different groups. This pattern is consistent with physiological stress and immune adaptations in pregnancy, as noted by Chandra *et al., (*2012). who emphasized the predominance of neutrophil during gestation. The specific constituents or origin of calabash chalk may also have contributed to this outcome. However, there were no significant difference in the lymphocytes (P=0.8227), of both the pregnant and non-pregnant population as the doses were increased suggesting that calabash chalk may not exert a direct effect on lymphocyte level.

Moreso there was a decrease in red blood cell (RBC) count among rats treated with increasing doses of calabash chalk, although not statistically significant this decrease suggest a potential suppression effect of calabash chalk on erythropoiesis or red cell survival. This may be due to the presence of kaolin and other substances in calabash chalk that can reduce iron bioavailability Awad *et al., (*2017); Mongogoa *et al., (*2011) since impaired iron absorption can negatively impact red blood cell production. Mongogoa *et al., (*2011) also observed that kaolin consumption may increase parasitic infections leading to iron loss.

 Table 3 which described additional red blood cell parameters revealed a significant reduction in haemoglobin concentration and haematocrit level with increase in the doses of the aqueous calabash chalk suspension (P=0.032 and P=0.001respectively). This finding may indicate anaemia suggesting that calabash chalk and its constituents exerts a suppressive effect on the red blood cell production and oxygen carrying capacity of the cells of the experimental rats as this finding corroborate with the study by Ogbuagu *et al., (*2017) which reported that chemicals in calabash chalk can cause alteration in the haematocrit and haemoglobin concentration of Wistar rats exposed to calabash chalk by chelating iron thereby reducing it bioavalability in the system. Additionally, the haemodilution effect of pregnancy caused by plasma volume expansion due to activation of the renin- angiotensin -aldosterone system could also contribute to the decrease in HB and HCT levels, Chandra *et al., (*2012) and Parvod *et al., (*2010) also noted that HB levels often decrease during pregnancy due to expanded plasma volume a condition referred to as physiological anaemia of pregnancy.

The mean cell volume (MCV) also showed a significant decrease suggesting a potential microcytic anaemia. This supports the hypothesis that calabash chalk may affect red cell morphology and HB content potentially producing a detrimental effect in the experimental animals.

 A statistically significant difference (P=0.001) also occurred in the platelet concentration of the pregnant and non-pregnant Wistar rats, with the platelet concentration being decreased in the pregnant group as compared to the control. This is in line with the study by Wu *et al., (*2014), which reported decreased platelet count following arsenic acid exposure a known component of calabash chalk. Platelet elevation in pregnancy could reflect physiological adaptation to the hypercoagulable state of pregnancy as suggested by Clemeston *,(*2012). However, studies on platelet trends in pregnancy remain inconsistent, with some showing decline, others stability, and a few showings increases Reese *et al., (*2017). Ekong *et al., (*2015), reported altered platelet count and morphology in rats exposed to calabash chalk. Similarly, this study observed changes in platelet large cell ratio (P-LCR) and platelet large cell concentration (P-LCC) a treatment and pregnancy related effect. The variation and effect may depend on the source and composition of the calabash chalk which differ by geographical location.

**5.Conclusion**

The study has shown that although calabash chalk has a broad safety margin (LD50 > 2000mg/kg) this particular doses which were administered that is 200mg\kg body weight, 400mg\kg body weight, 600mg\kg body weight had a statistically significant negative effect on some of the haematological parameters of these experimental animals which could lead to some haematological abnormalities like anaemia and as such pregnant women should be discouraged from eating calabash chalk.

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