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FULL BLOOD COUNT VARIABILITY IN FEMALE ALBINO WISTAR RATS EXPOSED TO ORAL CONSUMPTION OF CALABASH CHALK COMPOSED OF SPECIFIC PHYSICOCHEMICALS

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ABSTRACT

Background: Calabash Chalk consumpuion is a common habit among a lot of persons especially women in African part of the world. Regional differences have been reported in the concentration and Physicochemical constituents of calabash chalk, this study therefore, aimed at evaluating the variability of Haematologic parameters of female albino Wistar rats fed orally with calabash chalk containing high quantity specific physicochemicals, such as hexamethyl- Arsenous acid and quinoline Acridine. **Materials and Methods:** After the physicochemical, analsyis of calabash chalk purchsed from mile 3 market in port Harcourt, 56 rats which weighed between 75-200g, purchased from Department of Animal Science in Rivers State University, Port Harcourt were used for the experiment. The rats were domicile in the animal house of Department of Animal and Environmental Biology of same University. Six rats grouped into two having 3 each were used for pilot study to determine the median lethal dose (LD50) of the calabash chalk. None of the rats died or showed any sign of toxicity, the LD50 from the pilot study was used to estimate the various grade doses used for the study. The remaining 50 rats were divided into groups of 10 each according to their body weights and kept in well-ventilated cages tagged (1-5). The rats were allowed to acclimatize for 1 week and they were fed with standard feeds and clean water. The first group (control) was fed water and food only, while the test groups were also fed 200, 300, 400 and 500mg/kg body weight of calabash chalk respectively. After 30 days treatment, the animals were sacrificed, samples were taken in Ethylene-diamine Tetra Acetic (EDTA) bottles for evaluation of Haematologic paramters in the laboratory. Results obtained in this study were statistically analyzed using the Statistical Analysis Software (SAS), version 9.4. The results were expressed as mean ± standard error of mean (mean±SEM). One way Analysis of Variance (ANOVA), was used for comparison of mean in the various groups. The level of significance was set at $P<0.05$. **Results:** Significant increase in the WBC and Lymphocytes was observed for the test groups as against the control group at $p<0.05$. There were decrease in the PCV, HB and Platelet values of the test group compared to control groups at $p<0.05$. **Conclusion:** It was concluded that oral consumption of calabash chalk had adverse effect, on the complete blood count of the Wister albino rats rats resulting to low haemoglobin content, low haematocrit, leucocytosis and thrombocytopenia. Consumption of this substance should therefore, be discouraged.

KEYWORDS: female albino Wistar rats, Calabash Chalk, Haematologic parameters, oral consumption.

INTRODUCTION

Calabash chalk popularly known in some Nigerian languages as nzu (Igbo) Edom (Effik/Ibibio) and Eko in Bini/Edo is an edible clay found and consumed mostly in Nigeria and other West African countries, even outside the countries of West Africa due to migration. It can also be found in ethnic stores in countries such as UK, Canada and United States.[1] Consumption of this naturally or artificially prepared clay is dominated by gravid women whose claims for consumig the substance is associated to alleviation of nausea, vomiting and stomach problems. The rest of its consumers, eat it for pleasure and pica syndrome (geophagia in particular) cravings caused by deficency of zinc, calcium and iron in the body. In medical practice, full blood count is often recommended and carried out as part of a medical evaluation. Its usefulness is tied to health monitoring and disease diagnosis. It is an anlysis that gives needed information about the cell constituents of the blood present in a person. The blood cell constituents include; the red blood cells, white blood cells and the platelets. The red blood cells and its indicies give information on

the average size, and haemoglobin content of the red cells, the patelets play a centeral role in maintaing haemostasis, while white blood cells and its population found throughout the body, blood and the lymphatics are cells of the immune system which also has a unique duty of protecting the body against infectious diseases.[2] Though, there are previous studies on how consumption of calabash chalk affect the biology of human health, there is limited information on the specific physicochemical constituents of the particular calabash chalk consumed by persons living in Port Harcourt, which have the propensity to cause alterations in the full blood count of its consumers. This study therefore carried out a research to evaluate the variability of haematological parameters of Albino Wister Rats fed with calabash chalk in Port Harcourt containing high quantity specific Physicochemical, such as hexamethyl-Arsenous acid and quinoline Acridine among other chemicals.

MATERIALS AND METHODS

Quantitative Analysis of the Chemical Composition of Calabash Chalk (Nzu)eaten in Port Harcourt

The quantitative analysis of the chemical composition of calabash chalk is shown in Table 3.1. From the table, the extract contains a variety of organic and inorganic compounds and their quantities.

A quantitative chemical analysis was done to ascertain the compounds present in calabash chalk.

Table 3.1: Quantitative Physicochemical Analysis of Sample of Calabash Chalk (Nzu) eaten in Port Harcourt.

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- 4,6-trimethyl-benzenesulfonamide m-Cresol, TMS derivative	9.220
4	Trisiloxane, 1,1,3,3,5,5-hexamethyl- 6-Aminoindazole Benzoic acid, 2-amino-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-2-carboxylic 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 1-Decanol, 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)] undec-8-en-1 1-one, 3-[(2-methoxyethoxy)methoxy]-2-methyl- Decanoic acid, 2-hydroxy-	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-1-en -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, 2-hexyl-	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 2-propyl ester Oxalic acid, allyl octadecyl ester	2.037

Experimental Animals

A total of 56 (fifty-Six) female albino Wistar rats that weighed between 75-200g was used for this study. The rats were purchased from Department of Animal Science of Rivers State University, Port Harcourt. A total of 6 rats were used for the pilot study to determine the median lethal dose (LD50) of the calabash chalk. The remaining 50 were used for the main study. The rats were domicile in the animal house of Department of Animal and Environmental Biology of Rivers State University, Port Harcourt. For the pilot study, the 6 rats were divided into two groups of 3 each (High dose set and low dose set). For the main study, the 50 rats were divided into groups of 10 each. The rats were kept in well-ventilated cages tagged (1-5) according to their groups. The rats were allowed to acclimatize for 1 week and were fed with standard feeds and were given clean water. The animals were treated according to Guidelines for the Care and Use of Laboratory Animals.[3]

Experimental Design

This study was an experimental study. The pilot study was firstly done by weighing 500g of Calabash chalk and grinding it into powder with a ceramic mortar and pestle. It was then sieved, to give 350gm powder of calabash chalk. Distilled water was added and stirred. The suspension was given to the rats immediately using the formula below as dosage calculation.

$$\text{Calculation for low dose in mg (200mg/kg)} = \frac{1000}{\text{Body wt of animals x dose (mg)}}$$

The Experimental rats were weighed and in each cage, 10 rats of similar body weights were kept and tagged (1-5) according to their groups. The first cage had rats of varying weights; this cage housed the control groups. They were served just water and feed. The second to the fift cage housed the experimental rats, they were fed calabash chalk for one month (30 days) by gastric lavage. The different groups of the rats were fed of calabash chalk solution, water and feed according to their average body weight.

Sample Collection

At the end of each phase, the rats were anaesthetized with chloroform and blood samples were collected through cardiac puncture. using standard phlebotomy techniques.[3]

Estimation of the Full Blood Count

using the Fully Automated Haematology Analyzer MSLAB21 (Guamgzhou Medsinglong Medical Equipment Co., Ltd China). The full blood count parameters determined using this equipment are WBC, RBC, PLT, HB, HCT, LYMPH, MID, MCV, MCH, MCHC, RDW-CV, RDW-SD, MPV, PDW, PCT and P-LCR. The count principle of the instrument is based on the measurement of change in electrical resistance produced by a particle passing through a ruby aperture sensor.

Statistical Analysis

The result obtained in this study were statistically analyzed using the Statistical Analysis Software (SAS), version 9.4. The results were expressed as a mean ± standard error of mean (mean±SEM). One way Analysis of Variance (ANOVA), was used for comparison of mean in the various groups. The level of significance was set at $P<0.05$. Test of relationship was performed using Pairwise correlation, and the results were presented graphically to show nature of relationship.

RESULTS

Comparing the Effect of consumption of Calabash chalk on Haematological Parameters

The Mean ±SEM of the haematological parameters of the control group (group 1) and the different 4 groups (group 2, 3, 4, 5)fed with calabash chalk and water are as follows WBC; (7.92±0.53, 12.77±0.77, 10.42±0.35,10.29±0.35, 11.29±0.65), Lymph (59.71±1.46, 12.77±0.77, 10.42±0.35, 10.29±0.35, 11.29±0.65), MID(8.08±0.35, 7.62±0.44, 5.66±0.34, 8.39±0.93, 5.49±0.49), Gran(32.21±1.38, 12.33±1.22, 10.31±1.49, 12.99±0.68, 10.96±2.05), RBC(5.81±0.08, 4.23±0.09, 3.98±0.18, 4.37±0.13, 4.42±0.09), Hb(13.38±0.38, 8.24±0.36, 8.12±0.53, 8.34±0.47,9.49±0.25), HCT(39.44±0.49, 24.62±1.08, 23.83±1.76, 25.02±1.40, 28.52±0.73), MCV(68.65±0.52, 58.02±1.84, 59.90±2.28, 57.12±2.15, 64.79±0.79), MCH(22.83±0.18, 19.42±0.61, 20.64±0.33, 19.08±0.73, 21.55±0.27), MCHC(33.23±0.06, 33.48±0.10, 33.30±0.05, 33.31±0.05, 33.24±0.03), RDW-CV(1.07±0.35, 0.83±0.25, 1.33±0.41, 1.08±0.31, 1.19±0.36), RDW-SD(30.55±1.27, 33.32±1.76, 32.39±1.08, 29.97±1.54, 30.29±0.36), PLT(525.80±22.98, 355.40±14.42, 380.20±12.40, 391.80±16.72, 377.30±13.37), MPV(7.25±0.28, 7.46±0.22, 7.25±0.15, 7.24±0.24, 7.01±0.27), PDW(14.36±0.36, 14.51±0.17, 14.14±0.12, 14.52±0.31, 14.28±0.46) PCT(3.10±0.26, 3.03±0.27, 3.09±0.22, 2.92±0.24, 2.89±0.23), P-LCR(11.10±0.64, 11.48±0.72, 10.01±0.35, 12.10±0.50, 11.77±0.59).

Table 3.2a: Mean ±SEM of Haematological Parameters of Female Albino Wistar Rats fed Calabash.

Characteristic Groups	n	WBC (10^9/L)	LYMPH (%)	MID (%)	GRAN (%)	RBC (10^{12}/L)	Hb (g/dl)	HCT (%)
Group 1 (Control)	10	7.92±0.53^{a}	59.71±1.46^{a}	8.08±0.35^{a}	32.21±1.38	5.81±0.08^{a}	13.38±0.38^{a}	39.44±0.49^{a}
Group 2	10	12.77±0.77^{b}	79.93±1.17^{b}	7.62±0.44^{ab}	12.33±1.22	4.23±0.09^{b}	8.24±0.36^{b}	24.62±1.08^{b}
Group 3	10	10.42±0.35^{c}	84.03±1.20^{b}	5.66±0.34^{b}	10.31±1.49	3.98±0.18^{b}	8.12±0.53^{b}	23.83±1.76^{b}
Group 4	10	10.29±0.35^{c}	78.65±1.07^{b}	8.39±0.93^{a}	12.99±0.68	4.37±0.13^{b}	8.34±0.47^{b}	25.02±1.40^{b}
Group 5	10	11.29±0.65^{bc}	83.16±1.83^{b}	5.49±0.49^{b}	10.96±2.05	4.42±0.09^{b}	9.49±0.25^{b}	28.52±0.73^{b}
Test Statistics	*F-Ratio*	10.1498	52.6211	6.1208	41.7570	35.0445	29.7459	30.1314
	p-value	<0.0001****	<0.0001****	0.0005***	<0.0001****	<0.0001****	<0.0001****	<0.0001****

Abbreviations: SEM: Standard Error of Mean; WBC: White Blood Cell; LYMPH: Lymphocytes; Hb: Hemoglobin; HCT: Hematocrit; RBC: Red Blood Cell; GRAN: Granulocytes, MID: MID cells and percentage.
Within each parameter, mean±SEM with different superscripts a,b,c are significantly different at P<0.05
*Significance Level: *=p<0.05; **=p<0.01; ***=p<0.001; ****=p<0.0001; ns=not significant (p>0.05).*

Table 3.2b Mean ±SEM of Haematological Parameters of Female Albino Wistar Rats fed Calabash Chalk.

Characteristic Groups	N	MCV (fL)	MCH (pg)	MCHC (g/dL)	RDW-CV	RDW-SD (fL)	PLT (10^9/L)	MPV (fL)
Group1 (Control)	10	68.65±0.52^{a}	22.83±0.18^{a}	33.23±0.06^{b}	1.07±0.35^{a}	30.55±1.27^{a}	525.80±22.98^{a}	7.25±0.28^{a}
Group 2	10	58.02±1.84^{c}	19.42±0.61^{c}	33.48±0.10^{a}	0.83±0.25^{b}	33.32±1.76^{b}	355.40±14.42^{b}	7.46±0.22^{b}
Group 3	10	59.90±2.28^{bc}	20.64±0.33^{bc}	33.30±0.05^{ab}	1.33±0.41^{a}	32.39±1.08^{b}	380.20±12.40^{b}	7.25±0.15^{a}
Group 4	10	57.12±2.15^{c}	19.08±0.73^{c}	33.31±0.05^{ab}	1.08±0.31^{a}	29.97±1.54^{ab}	391.80±16.72^{b}	7.24±0.24^{a}
Group 5	10	64.79±0.79^{ab}	21.55±0.27^{ab}	33.24±0.03^{ab}	1.19±0.36^{a}	30.29±0.73^{a}	377.30±13.37^{b}	7.01±0.27^{a}
Test Statistics	*F-Ratio*	8.4474	10.7159	2.7451	0.2996	1.2222	17.2362	0.4602
	p-value	<0.0001****	<0.0001****	0.0398*	0.8767^{ns}	0.3148^{ns}	<0.0001****	0.7645^{ns}

Abbreviations: SEM: Standard Error of Mean; MCV: Mean Corpuscular Volume; MCH: Mean Corpuscular Hemoglobin; MCHC: Mean Corpuscular Hemoglobin Concentration; RDW-CV: Red Blood Cell Distribution Width Coefficient of Variation; RDW-SD: Red Blood Cell Distribution Width Standard Deviation; PLT: Platelet; MPV: Mean Platelet Volume.
Within each parameter, mean±SEM with different superscripts a,b,c are significantly different at P<0.05
*Significance Level: *=p<0.05; **=p<0.01; ***=p<0.001; ****=p<0.0001; ns=not significant (p>0.05).*

Table 3.2c Mean ±SEM of Haematological Parameters of Female Albino Wistar Rats fed Calabash Chalk.

Characteristic Groups	n	PDW	PCT (mL/L)	P-LCR (%)
Group 1 (Control)	10	14.36±0.36	3.10±.26	11.10±0.64
Group 2	10	14.51±0.17	3.03±0.27	11.48±0.72
Group 3	10	14.14±0.12	3.09±0.22	10.01±0.35
Group 4	10	14.52±0.31	2.92±0.24	12.10±0.50
Group 5	10	14.28±0.46	2.89±0.23	11.77±0.59
Test Statistics	*F-Ratio*	0.2698	0.1509	1.9587
	p-value	0.8959^{ns}	0.9616^{ns}	0.1171^{ns}

Abbreviations: SEM: Standard Error of Mean; PDW: Platelet Distribution Width; PCT: Procalcitonin; P-LCR: Platelet-Large Cell Ratio.
Within each parameter, mean±SEM with different superscripts a,b,c are significantly different at P<0.05
*Significance Level: *=p<0.05; **=p<0.01; ***=p<0.001;****=p<0.0001; ns=not significant (p>0.05).*

DISCUSSION

After the investigation, the Haematologic Parameters which includes; white blood cells, Lymphocytes, Red cell indices and platelets were observed in Wistar rats fed with calabash chalk. Haemoglobin and Haematocrit had significant decrease variations amomg the various test groups as against the control group at $p<0.001$, and MID also had significant decreased alterations amoung the test groups against the control group at $p<0.05$ but the platelets were significantly reduced ($p<0.05$).

An increase in White blood cells is usually due to the presence of an infection, inflammatory condition or distorted immune regulation. From the quantitative analysis carried out on the chemical composition of the calabash chalk consumed in Port Harcourt, The calabash chalk contained high quantity of hexamethyl- Arsenous acid, which could have caused the spike in the white blood cells and lymphocytes. The body might have responded to the calabash chalk as a foreign, or an irritating substance thereby mounting an immune response against it. The increase in WBC could also be due to the body's defense system in response to an inflammation and immunotoxicity linked to arsenic exposure.[4] This explains why there is a significant increase in the lymphocyte population. Since calabash chalk contains high quantity of some toxic chemicals such as Arsenic acid, the increase in WBC could be the body's method of neutralizing these toxic substances. This assumption supports the study carried out by[5], which also stated that calabash chalk contains toxic substances harmful for consumption. It also agrees with the report of[6], on the effect of Arsenic poisoning on the haematological parameters of Wistar rats, from that study, rats poisoned with Arsenic had a higher WBC than the control groups. This study disagrees with the study of[7], in which rats fed with aqueous solution of calabash chalk had a reduced WBC, and lymphocytes count, but however agrees with the findings in this study on the significant decrease in granulocytes and MID of rats fed aqueous solution of calabash chalk. Our finding however, does not also agree with the study of[8] in which there was no significant difference between the WBC of rats fed with calabash chalk and the control groups.

A marked decrease in the red blood cells and Haematocrit, Hb and RBC was also observed with rats fed with calabash chalk as against the control group. Parasite infection found in calabash chalk and can cause iron loss which further reduces the iron available for erythropoiesis.[9] Also, this reduction could be as a result some toxic substances contained in calabash chalk. This finding is in line with the findings of[7], in which oral administration of aqueous mixture of calabash chalk in rats caused a significant reduction in the value for RBC, Hb and PCV. In research carried out by[10] he discovered that Oxalic acid causes anaemia by binding with minerals, especially iron and calcium in the gut, which forms insoluble crystals which the body cannot absorb. When this happens, excessive oxalates can make it difficult for the body to absorb iron-rich food even when it is consumed by an individual, potentially leading to iron-deficiency anaemia. This study also agrees with[11], from the study, it was discovered that arsenic can decrease heme metabolism and could bind to haemoglobin, resulting in lower haemoglobin concentrations. Also, the research carried out by[6], agrees that rats poisioned with Arsenic had reduced Haematocrit, RBC, and Hb. Studies carried out by[12] who worked on mechanism of erythrocyte death in human population exposed to arsenic through drinking water, his analysis has shown that arsenic can also alter erythrocyte morphology and induce erythrocyte death which also agrees with this study. However, this study does not agree with the study carried out by[8], from his analysis, there was no significant difference in the HCT, Hb and RBC when compared with the control groups.

There was a significant decrease in the red cell indices with MCV and MCH having a higher level of significance at $p<0.05$ when compared to MCHC at $p<0.05$. The significant decrease in the Red Cell Indices can be traced to the decrease in the Haemoglobin, Haematocrit and RBC. However, this does not agree with the study of[6], in his findings, there was no significant difference in the red cell indices (MCV, MCH, MCHC) of rats poisoned with Arsenic.

There was no significant difference between RDW-CV, RDW-SD, and MPV as against the control groups ($P<0.05$). This is in line with the research of[6], whose research showed no significant difference amongst these parameters.[8] also recorded no significant difference for the MPV values between rats fed with calabash chalk and the control group.

The platelet count was significantly reduced as against the control group at $p<0.05$. This reduction can be linked to the arsenic acid present in calabash chalk. Arsenic acid has been implicated to cause apoptosis in human platelets through mitochondrial pathways. This mechanism is said to alter platelet function as well as cause a reduction in circulating platelets, thereby leading to thrombocytopenia[13] Research carried out by[14] shows that veratric acid which is present in calabash chalk can indirectly have an effect on the function of platelets by causing a reduction in the platelet activation factors. Also, the research of[7], agrees that rats fed with calabash chalk has reduced platelet count compared to the control group. However, the research of[8], disagrees with the findings from this study as his findings recorded a significant increase in the platelet values. In his study, there was no significant different in the platelet levels between rats fed with calabash chalk, and the control group. There was no significant difference between PDW, PCT, P-LCR respectively and the control group. The values gotten from the test group were not statistically significantly different from those value gotten for the control group at $P<0.05$. However, the

findings from[7], disagrees, as a reduction in the value of PCT was observed.

CONLUSION

Consumption of calabash chalk admnistered to Female Albino Wister rats had adverse effect capable of inducing anaemia, leucocytosis and thrombocytopenia. Since rat's physiology resembles that of humans, consumption of this substance by humans should be discouraged.

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