

## INVESTIGATION OF THE EFFECT OF NATURAL NIGERIAN CALCIUM BENTONITE ON HAEMATOLOGICAL PARAMETERS OF WISTAR ALBINO RATS

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### ABSTRACT

Bentonite clay is widely distributed all over the world and it has been consistently used for its healing properties. In this study, the *in vivo* effect of natural Nigerian calcium bentonite clay on haematological parameters of wistar albino rat were investigated. The rats were fed for a total period of 28 days with varying concentrations of the bentonite clay. The haemoglobin count, packed cell volume, total white blood cell count, red blood cell count, platelet count, neutrophil and lymphocyte count were assayed. Test results showed that the bentonite had a lowering effect on haematological parameters studied in a concentration and time dependent manner with differences in concentration and time at 95 % confidence levels ( $P < 0.05$ ). The highest decrease of  $12.60 \pm 0.36$  vs control  $14.35 \pm 0.00$  (g/dl) was obtained for Hb at the highest concentration of 0.07g at 28 days duration. PCV, WBC, RBC, platelet, neutrophil and lymphocyte analysis also showed highest decrease at 28 days duration at 0.07g/100g body weight. The results showed that using bentonite in small amounts for a short period of time had little effect. However, it is imperative that anyone intending to take large amounts of bentonite for long periods of time to undergo blood tests from time to time.

**Keywords:** Bentonite, Blood, Calcium, Haematological, Natural, Parameters.

### INTRODUCTION

Bentonite is a widely distributed clay used as bonding agents in animal feed, clarifying agent in wine, and as medicine. Therefore, there is need to ascertain the effect of bentonite on blood variables since it is being consumed by human and mammals in general. In both value and amount of annual production, bentonite is one of the leading minerals worldwide.<sup>1</sup> Bentonite is used in a large number of different cosmetic products, such as paste masks, skin care and cleansing preparations, eyeliners, foundations, and others.<sup>2</sup> Inclusion of bentonite in the diet of cattle reduced the transfer of radio-caesium into milk. Furthermore, there is an extensive literature documenting the ability of bentonite to adsorb toxins. Bentonite is used as an adsorbent for oil, grease, and animal waste and as a carrier for pesticides and fertilizers. It is also used in

filtering, clarifying, decolorizing and serving as filler in paints, adhesives, and pharmaceuticals.<sup>3</sup>

Bentonite often contains quartz, and exposure to quartz is causally related to silicosis and lung cancer. Statistically significant increases in the incidence of or mortality from chronic bronchitis and pulmonary emphysema have been reported after exposure to quartz.<sup>1,3,4</sup> Haematology refers to the study of the numbers and morphology of the cellular elements of the blood – the red cells (erythrocytes), white cells (leucocytes), and the platelets (thrombocytes) and the use of these result in the diagnosis and monitoring of diseases.<sup>5,6</sup> Haematological studies are of ecological and physiological interest in helping to understand the relationship of blood characteristics to the environment.<sup>7,8</sup> Haematological parameters are good indicators of the physiological status of animals.<sup>9,10,11,12</sup> The major functions of the white blood cell and its differentials are to fight infections, defend the body by phagocytosis against invasion by foreign organisms and to produce or at least transport and distribute antibodies in immune response.<sup>13</sup> Thus, animals with low white blood cells are exposed to high risk of disease infection, while those with high counts are

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**Table 1: In vivo effects of Nigerian calcium bentonite on haematological parameters of wistar albino rats after 7 days of feeding**

Parameter	Control (0.00) g/100g body weight	0.02 g/100g body weight	0.04 g/100g body weight	0.05 g/100g body weight	0.07 g/100g body weight
Haemoglobin (g/dl)	13.07 ± 0.25 <sup>a</sup>	12.87 ± 1.91 <sup>a</sup>	12.33 ± 0.35 <sup>a</sup>	12.87 ± 0.98 <sup>a</sup>	11.57 ± 1.50
PCV (%)	41.00 ± 0.00 <sup>a</sup>	38.67 ± 5.69 <sup>a</sup>	37.00 ± 1.00 <sup>a</sup>	35.67 ± 2.89 <sup>a</sup>	34.67 ± 4.62 <sup>a</sup>
WBC (x10 <sup>9</sup> /l)	8.00 ± 0.00 <sup>a</sup>	5.87 ± 0.93	5.73 ± 0.64 <sup>a</sup>	5.67 ± 0.76	4.23 ± 1.54 <sup>b</sup>
RBC (x10 <sup>12</sup> /l)	5.30 ± 0.10	4.90 ± 0.53 <sup>ab</sup>	4.30 ± 0.30 <sup>b</sup>	4.60 ± 0.35	4.07 ± 0.46
Platelets (x10 <sup>9</sup> /l)	250.00 ± 0.00	240.00 ± 79.37 <sup>a</sup>	216.67 ± 15.28 <sup>c</sup>	176.67 ± 5.77 <sup>b</sup>	163.33 ± 7.40 <sup>b</sup>
Neutrophils (cells/μl)	33.33 ± 1.53 <sup>a</sup>	32.33 ± 5.13 <sup>a</sup>	34.33 ± 5.13 <sup>a</sup>	35.00 ± 8.69 <sup>a</sup>	30.33 ± 1.53 <sup>a</sup>
Lymphocytes (cells/μl)	68.33 ± 0.58 <sup>a</sup>	67.67 ± 5.14 <sup>b</sup>	65.67 ± 5.13 <sup>a</sup>	65.00 ± 8.66 <sup>a</sup>	64.67 ± 4.04 <sup>a</sup>

Results are means of three determinations ± SD. The alphabets are statistically significant at 95% confidence level. (P < 0.05).

capable of generating antibodies in the process of phagocytosis and have high degree of resistance to diseases.<sup>11,14</sup>

## MATERIALS AND METHODS

Calcium bentonite clay was obtained from bentonite deposit at Anambra state in Nigeria. A total of forty-five male and female Wistar albino rats (*Rattus rattus*) were obtained from the small animal holding unit of the department of Biochemistry, University of Port-Harcourt, Choba Nigeria. They were housed in clean metabolic cages which were cleaned of wastes twice daily at 12 hours each of day and night at room temperature.

The rats were maintained on normal rat diet and water and they were allowed to acclimatize for seven days after which they were randomly divided into two groups. Rats in group 1 (9 Rats) served as the control and were given their normal feed and distilled water twice daily at 12 hours interval for 28 days. The rats in Group 2 (36 rats) were further divided into subgroups (A, B, C and D).

Bentonite clay was administered orally in dose of 0.02g, 0.04g, 0.05g, and 0.07g, twice daily at 12 hours interval for 28 days. The bentonite clay and distilled water were administered at the same time daily throughout the duration of the experiment.

The animals in the two groups were sacrificed on days 7, 21, and 28 days by cardiac puncture with the animal under anaesthesia (chloroform) in a desiccator. The blood collection was done immediately and was stored in an EDTA sample containers.

### HAEMOGLOBIN

Blood (0.20ml) was measured and dispensed into 4ml Drabkins solution. The mixture was allowed to stay at room temperature for 4 – 5 minutes, absorbance were measured using spectrophotometer and read at 540nm. The haemoglobin values were read using the calibrate graph.

### PACKED CELL VOLUME

The capillary tube was filled with ¾ well mixed EDTA blood. The unfilled end was sealed with a sealant and place in a microhaematocrit centrifuge for 5 minutes. After centrifuging, the PCV was read using a microhaematocrit reader.

### TOTAL WHITE BLOOD CELLS:

Measured 0.38ml of diluting fluid was dispensed into a small container and 0.20ml of well mixed EDTA blood was added and mixed and remixed in the counting chamber using Pasteur pipette. The chamber was left undisturbed for 20 minutes to

**Table 2: In vivo effects of Nigerian calcium bentonite on haematological parameters of wistar albino rats after 21 days of feeding**

Parameter	Control (0.00) g/100g body wt	0.02 g/100g body wt	0.04 g/100g body wt	0.05 g/100g body wt	0.07 g/100g body wt
Haemoglobin (g/dl)	14.00 ± 2.00 <sup>a</sup>	13.23 ± 1.69 <sup>b</sup>	13.00 ± 2.00 <sup>b</sup>	12.23 ± 1.66 <sup>a</sup>	12.90 ± 0.88 <sup>c</sup>
PCV (%)	42.00 ± 6.00 <sup>a</sup>	36.33 ± 5.13 <sup>b</sup>	36.00 ± 6.00 <sup>b</sup>	35.67 ± 3.06 <sup>a</sup>	34.67 ± 2.51
WBC (x10 <sup>9</sup> /l)	7.00 ± 2.00 <sup>a</sup>	6.70 ± 1.26 <sup>a</sup>	6.50 ± 1.50 <sup>b</sup>	6.33 ± 1.53	6.23 ± 1.53 <sup>a</sup>
RBC (x10 <sup>12</sup> /l)	5.67 ± 0.85 <sup>a</sup>	4.87 ± 0.87 <sup>b</sup>	4.80 ± 0.80	4.60 ± 0.72 <sup>a</sup>	4.63 ± 0.15 <sup>c</sup>
Platelets (x10 <sup>9</sup> /l)	433.33 ± 15.26 <sup>a</sup>	276.67 ± 25.17 <sup>d</sup>	260.00 ± 10.00 <sup>b</sup>	248.33 ± 27.08 <sup>b</sup>	166.67 ± 15.28 <sup>d</sup>
Neutrophils (cells/μl)	26.33 ± 1.53 <sup>a</sup>	31.33 ± 1.15 <sup>b</sup>	32.33 ± 2.52 <sup>b</sup>	27.67 ± 2.53 <sup>a</sup>	35.00 ± 3.00 <sup>b</sup>
Lymphocytes (cells/μl)	73.33 ± 1.54 <sup>a</sup>	67.33 ± 4.62 <sup>b</sup>	67.33 ± 2.52 <sup>b</sup>	65.32 ± 1.53 <sup>a</sup>	64.00 ± 3.00 <sup>b</sup>

Results are means of three determinations ± SD. The alphabets are statistically significant at 95% confidence level. (P < 0.05).

allow time for the white blood cells to settle. They were then counted.

$$\text{WBC count (per/liter)} = \frac{N \times DF \times 10}{A \times D}$$

Where N = no of cell counted  
DF = dilution factor  
A = Area counted  
0.1 = depth of chamber

#### PLATELET COUNT

Measured 0.38ml of diluting fluid was dispensed into a small container and 0.20ml of well mixed EDTA blood was added and mixed and remixed in the counting chamber. The chamber was left undisturbed for 20 minutes to allow time for the white blood cells to settle. They were then counted.

$$\text{Platelet count} = \frac{N \times 20 \times 10}{0.2 \times 0.1}$$

#### RED BLOOD CELL COUNT

Measured 4.00ml of formal citrate fluid was dispensed into a small container and 0.20ml of well mixed EDTA blood was added and mixed and remixed in the counting chamber using Pasteur pipette. The chamber was left undisturbed for 20 minutes to allow time for the white blood cells to settle. They were then counted.

$$\text{RBC count (per liter)} = \frac{N \times DF \times 10^9}{A \times D}$$

Where N = no of cell counted

DF = dilution factor

A = Area counted

0.1 = depth of chamber

#### NEUTROPHIL AND LYMPHOCYTE

A drop of blood was placed on the end of a clean, dry slide. A clean, smooth edge of the spreader was used to spread the blood to make a film of about 40-50mm in length. The film was air dried and fixed in absolute methanol. The slide was covered with undiluted Leishman stain and allowed to stand for 2 minutes. Buffered water of pH 6.8 was used to mix the stain, and it was allowed to stand for 8 minutes. It was washed with tap water and allowed to dry. It was examined using oil immersion objective lens.

#### STATISTICAL ANALYSIS

Data analysis was performed using the Statistical package for the Social Sciences software (SPSS, version 11.0). Data is displayed in mean + SD. The statistical method of one way analysis of variance (ANOVA) was used to compare the mean values obtained among different groups. Differences were considered significant whenever the p-value is P<0.05.

#### RESULTS AND DISCUSSION

The mean results ± SD of the *in vivo* effects of Nigerian calcium bentonite on haematological

**Table 3: In vivo effects of Nigerian calcium bentonite on haematological parameters of wist.ar albino rats after 28 days of feeding**

Parameter	Control (0.00) g/100g body wt	0.02 g/100g body wt	0.04 g/100g body wt	0.05 g/100g body wt	0.07 g/100g body wt
Haemoglobin (g/dl)	14.35 ± 0.00 <sup>a</sup>	14.10 ± 0.17 <sup>a</sup>	13.60 ± 0.36 <sup>b</sup>	12.80 ± 3.00 <sup>c</sup>	12.60 ± 0.36 <sup>b</sup>
PCV (%)	43.00 ± 1.00 <sup>a</sup>	41.67 ± 0.58 <sup>a</sup>	40.67 ± 2.08 <sup>a</sup>	38.33 ± 3.06 <sup>a</sup>	38.00 ± 1.00 <sup>a</sup>
WBC (x10 <sup>9</sup> /l)	8.07 ± 0.12	7.53 ± 0.25 <sup>a</sup>	7.47 ± 0.58 <sup>a</sup>	7.33 ± 0.29 <sup>a</sup>	7.13 ± 0.12 <sup>a</sup>
RBC (x10 <sup>12</sup> /l)	5.13 ± 0.12 <sup>a</sup>	4.64 ± 0.15 <sup>b</sup>	4.60 ± 1.17 <sup>b</sup>	4.50 ± 0.30 <sup>a</sup>	4.29 ± 0.12 <sup>b</sup>
Platelets (x10 <sup>9</sup> /l)	293.67 ± 28.87 <sup>a</sup>	283.33 ± 11.55 <sup>a</sup>	263.33 ± 40.41 <sup>a</sup>	256.67 ± 5.77 <sup>b</sup>	243.33 ± 15.28 <sup>b</sup>
Neutrophils (cells/μl)	35.33 ± 4.62 <sup>a</sup>	34.67 ± 2.89 <sup>b</sup>	33.67 ± 1.53 <sup>b</sup>	31.33 ± 1.15 <sup>a</sup>	30.00 ± 1.70 <sup>a</sup>
Lymphocytes (cells/μl)	72.33 ± 2.52 <sup>a</sup>	71.00 ± 3.61 <sup>a</sup>	70.00 ± 1.00 <sup>a</sup>	69.33 ± 1.12 <sup>b</sup>	66.00 ± 1.73 <sup>b</sup>

Results are means of three determinations ± SD. The alphabets are statistically significant at 95% confidence level. (P < 0.05).

parameters are shown in Tables 1 to 3. The highest decrease of 12.60 ± 0.36 vs control 14.35 ± 0.00 (g/dl) was obtained for Hb at the highest concentration of 0.07g at 28 days duration. PCV showed the highest decrease of 38.00 ± 1.00 vs control 43.00 ± 1.00 (%). The highest decrease of the total WBC count was 7.13 ± 0.12 vs control 8.07 ± 0.12 (x10<sup>9</sup>/l). The highest decrease of RBC count was 4.29 ± 0.12 vs control 5.13 ± 0.12 (x10<sup>12</sup>/l). Platelet, neutrophil and lymphocytes counts showed the highest decreases of 243.33 ± 15.28 vs control 293.67 ± 28.87 (x10<sup>9</sup>/l), 30.00 ± 1.70, vs control 35.33 ± 4.62 (cells/μl) and 66.00 ± 1.73 vs control 72.33 ± 2.52 (cells/μl) respectively.

Haematological studies investigate the numbers and morphology of the cellular elements of the blood. The results showed that the bentonite in comparison with the controls decreased the blood variables studied. The bentonite decreased the haematological parameters in a concentration and time dependent manner with the highest decrease obtained at the 28 days duration at the highest concentration of 0.07g with differences in concentration and time at 95 % confidence levels (P < 0.05). Haematological studies are useful in the diagnosis of many diseases as well as investigation of the extent of damage to blood.<sup>15,16</sup> In addition, blood also act as a pathological reflector of the status of

exposed animals to toxicants and other conditions.<sup>17</sup> As reported by Aderemi (2004) animals with good blood composition are likely to show good performance.<sup>12</sup>

There have been wide a wide range of studies on the medicinal uses of bentonite ranging from its use in battlefield wound dressing and its use as a medication intended to detoxify the body from different toxins. This study is in agreement with the work of Kececi et al (1998), which stipulated that when compared to control, the addition of polyvinyl polypyrrolidone (PVPP), synthetic zeolite (SZ), bentonite (BNT) and their combinations in the diet without aflatoxin have not significantly altered the serum biochemical and haematological parameters of growing broilers and also that the adsorbents in the study were inert and nontoxic.<sup>18</sup> Long-term occupational exposures to bentonite dust may cause structural and functional damage to the lungs. However, available data are inadequate to conclusively establish a dose–response relationship or even a cause-and-effect relationship due to limited information on period and intensity of exposure and to other factors, such as exposure to silica and tobacco smoke.<sup>1,3</sup>

The results from this study showed that using bentonite in small amounts for a short period of time had little effect on the haematological

parameters. It is recommended that bentonite and other healing clays should not be used by those with clinically diagnosed iron intolerance without going for blood sample monitoring and secondly, obtaining a laboratory analysis of the clay used. However, it is imperative that anyone intending to take large amounts of bentonite for long periods of time to undergo blood tests from time to time to rule out anaemia and other associated problems.

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