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A STUDY OF THE EFFECT OF NATURAL BENTONITE ON TOTAL PROTEIN CONTENT OF WISTER ALBINO RATS

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ABSTRACT
The *in vivo* effect of Nigerian calcium bentonite clay on wistar albino rat plasma total protein contents were investigated. The rats were fed for a period of four weeks with varying concentrations of the bentonite clay, and the total protein levels determined using spectrophotometric methods. Test results showed that the bentonite clay had an increasing effect on whole total protein levels in a concentration and time dependent manner. The highest increase of (44.00±1.83 g/l) was obtained at the 28 days duration with the highest concentration of bentonite (0.07g/100g body wt). The differences in weight and weeks were statistically significant on the effect of the bentonite on total protein levels at 95.0% confidence level (P<0.05). From the study, it was clear that the bentonite clay increased the wistar albino rat plasma total protein. The slight increase in the values of the total protein could suggest that body functions may not have been affected negatively following the administration of bentonite substance. The results from this study further support bentonite clay as a healing clay. However, blood tests have to be undertaken in the event that bentonite is to be taken in large quantities for long periods of time.

KEYWORDS: Bentonite, Calcium, Natural, Plasma, Total protein.

INTRODUCTION
The term bentonite was first used for clay found in about 1890, in upper cretaceous tuff near Fort Benton, Wyoming. The main constituent, which is the determinant factor in the clay's properties, is the mineral montmorillonite. Furthermore, Calcium bentonite is a useful adsorbent of ions in solution (Odom 1984; Robertson, 1986; Hosterman and Patterson 1992). The presence of these minerals can impact the industrial value of a deposit, reducing or increasing its value depending on the application. Bentonite presents strong colloidal properties and its volume increases several times when coming into contact with water, creating a gelatinous and viscous fluid. The special properties of bentonite (hydration, swelling, water absorption and viscosity) make it a valuable material for a wide range of uses and applications (Ivan *et al*., 1992; Guyonnet *et al*.; 2005, Khadem 2007). In medicine, bentonite is used as an antidote in heavy metal poisoning. Personal care products such as mud packs, sunburn paint, baby and face powders, and face creams may all contain bentonite (Fen and Leng 1989; Noble *et al*., 2001). Bentonite clay is also used for farming although costs are higher, but, due to more production and quality of the crops, clay farmers could afford to invest and grow more and better food, compared to non-clay-using farmers (Suzuki, *et al*.; 2007, Aquilera –Soto *et al*. 2008; Saleth *et al*., 2009). Proteins perform a vast array of functions within living organisms, including catalysing metabolic reactions, replicating DNA, responding to stimuli, and transporting molecules from one location to another. Plasma total protein is a biochemical test for measuring the total amount of protein in blood (Henry 1974). Bentonite is known to have a lot of uses and benefits to mankind yet not much research work has been done in this area to ascertain its effects on the body. Therefore the aim of this study is to investigate the effect of bentonite on the total proteins of wistar rat and by extension, the human body.

MATERIALS AND METHOD
Calcium bentonite clay was obtained from bentonite deposit at Anambra state in Nigeria. Reagents kit for total protein was from Human Gesellschaft fur Biochemica and Diagnostica mbH, Max-Planck-Ring, 21 – DD-65205 Wiesbaden – Germany. A total of forty five male and female wistar abino rats (Ratus rattus) were obtained from the small animal holding unit of the department of Biochemistry, University of Port-Harcourt, Choba Nigeria. The average weight of the rats is 100g. 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 12hours interval for 28 days. The rats in Group 2 (36 rats) of average weight of 100g, were further divided into sub groups (A, B, C and D). Bentonite clay was administered orally at various concentrations (0.02g, 0.04g, 0.05g, and 0.07g) per 100 gram body weight of rat twice

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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 experiment. The animals in the two groups were sacrificed in days 7, 21, and 28 days. This was done by cardiac puncture with the animal under anesthesia (chloroform) in a desiccator. The blood collection was done immediately and were stored in a lithium heparin sample containers. The blood was centrifuged at 3000 rotations per minute for 3 minutes and the blood plasma were separated and used for analysis.

Blood total proteins

Total protein levels were determined by Buiret method (colorimetric test). The Principle of this method is that cupric ions react with protein in alkaline solution to form a purple complex. The absorbance of this complex is proportional to the protein concentration in the sample (Weichselbaum 1946; Henry 1974).

Reagent kit contained sodium hydroxide (200 mmol/l), potassium sodium tartrate (32 mmol/l),copper sulphate (12 mmol/l), potassium iodide (30 mmol/l). Standard contained Protein (80g/l), sodium azide (0.095%). Soudium tetrephenyl boron (2.1 mmol/l), potassium standard (4 mEq/L).

One milliliter of reagent was mixed with 0.02 ml of the sample. The standard tube contained 1.00 ml of reagent and 0.02 ml of the standard. The mixtures were incubated for 10 minutes. The absorbance of the sample and standard were read against the reagent blank within 30 minutes at 546nm with spectronic – 20 spectrophotometer.

Calculations

$$\text{Protein concentration (C)} = 80 \times \frac{\Delta A_{sample}(g/l)}{\Delta A_{STD}}$$

Normal values: (62 – 80g/l).

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

Table 1: Effect of Nigerian Calcium Bentonite on Wistar Albino Rat Plasma Albumin

g/100g body weight	7 days(g/l)	21 days(g/l)	28 days(g/l)
CONTROL (0.00)	35.75 ± 17.50	35.25 ± 1.708	35.50 ± 19.00*
0.02	36.50 ± 1.29	36.70 ± 1.29	37.50 ± 1.29*
0.04	37.00 ± 0.82	37.25 ± 21.08	38.25 ± 19.57*
0.05	39.00 ± 0.81	39.50 ±2.08	39.90 ± 21.06*
0.07	40.50 ± 1.29	41.75 ± 21.20*	44.00 ± 1.83

Results are means of three determinations ± standard deviation.
* Different letters in a given row denote significant difference, P<0.05.

Table 2: Effect of Nigerian Calcium Bentonite on Wistar Albino Rat Plasma Globulin

g/100g body weight	7 days (g/l)	21 days (g/l)	28 days (g/l)
CONTROL (0.00)	34.25 ± 14.50	34.25 ± 3.31	34.50 ± 17.00*
0.02	34.50 ± 2.65	31.75 ± 3.94*	35.00 ± 0.00
0.04	35.75 ± 2.87	36.50 ± 21.42*	36.75 ± 17.63
0.05	36.75 ± 2.36	36.85 ± 1.50	37.25 ± 17.63*
0.07	37.25 ± 2.50	37.75 ± 17.91*	37.90 ± 3.32

Results are means of three determinations ± standard deviation.
* Different letters in a given row denote significant difference, P<0.05.

Table 3: Effect of Nigerian Calcium Bentonite on Wistar Albino Rat Plasma Total Protein

g/100g body weight	7 days (g/l)	21 days (g/l)	28 days (g/l)
CONTROL	70.00 ± 32.00	69.50 ± 1.71	70.00 ± 36.00*
0.02	71.00 ± 2.58	68.45 ± 3.10*	72.50 ± 1.29
0.04	72.75 ± 2.99	73.75 ± 42.45*	75.50±37.04
0.05	75.75 ± 1.71	76.35 ± 1.71	77.15 ± 38.51*
0.07	77.75 ± 1.71	79.50 ± 39.01	81.90 ± 4.44*

Results are means of three determinations ± standard deviation.
* Different letters in a given row denote significant difference, $P<0.05$.

DISCUSSION

The mean test results ± SD of plasma total protein determination are shown in tables 1-3. The bentonite had an increasing effect on the albumin, globulin and blood total protein. Plasma total protein is a measure of the total amount of protein in the blood. A low or high total protein does not indicate a specific disease, but it does indicate that some additional tests may be required to determine if there is a problem (Briggs *et al*., 1976; Okoye *et al*., 2012). Total protein is an important component of the serum as a marker for many physiological states of the body. An anomaly in the concentration of total protein is useful in detecting or estimating the imbalance in organ systems. Thus the study of the effect of bentonite, on the total protein content is of great importance. As was seen in the results of this study, bentonite at concentrations 0.02g/l, 0.04g/l 0.05g/l and 0.07g/l showed gradual increases for the total protein with the highest increase of 81.90 ± 4.44 vs control 70.00 ± 36.00 obtained at 0.07g/l concentration at the 28 days duration. The result indicates that bentonite had a slight increasing effect in the plasma total protein content, and as such, the proteins can still perform their vast array of functions within living organisms including catabolizing metabolic reactions, replicating DNA, responding to stimuli and transporting molecules from one location to the other.

However, if bentonite is to be taken in large quantities for a prolonged time, then the albumin, globulin and total protein should be monitored at given intervals. Although taken bentonite for a short period of time would seem not to pose much problems, as seen from the results because they fall within the reference range. From the findings, it follows, then, to say that the beneficial applications of bentonite in medicine, pharmaceuticals, agriculture, drilling and the host of them, need not be set aside seeing that there is minimal risk of intoxication from bentonite.

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