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INVESTIGATION OF THE *IN VIVO* EFFECTS OF NIGERIAN NATURAL BENTONITE CLAY ON PLASMA CALCIUM AND PHOSPHORUS IN WISTAR ALBINO RATS

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ABSTRACT

Bentonite is a widely used clay for so many purposes, especially as a body detoxifier. In this research, the investigation of the effect of bentonite on calcium and phosphorus levels in Wistar rat plasma was examined. A total of 45 female and male adult Wistar albino rats were used. The rats were put into 5 groups of 9 rats each. Group 1, 2, 3 and 4 were given varying concentrations of bentonite (0.02g, 0.04g, 0.05g, 0.07g respectively) while group 5 was kept as control and was fed with basal diet. The experiment lasted for 28 days. The results showed that there was a slight increase in the calcium levels ($P \leq 0.05$). In addition, there was also a significant increase (3.60mmol/l) in phosphorus levels observed for 28 days duration of the experiment. This indicates that long-term exposure to increasing concentration of bentonite has a significant effect on the electrolyte levels in rat plasma and by extrapolation, the human body. As a result, care should be taken while ingesting bentonite for other health benefits to avoid hypercalcemia and hyperphosphatemia. It is also recommended that blood test is undertaken at regular intervals for heavy users of the bentonite product.

Keywords: Bentonite, Calcium, Electrolyte, Phosphorus

INTRODUCTION

Bentonite is defined as a naturally occurring material that is composed predominantly of the clay mineral smectite. Most bentonites are formed by the alteration of volcanic ash in marine environments and occur as layers sandwiched between other types of rocks [1].

Many types of skin conditions have been treated by the application of medicinal clay. Montmorillonite has shown its effectiveness in this area. It has also been used as a base ingredient for tissue engineering. Clay is used in many dermatological over-the-counter remedies, such as in acne treatments. One of the many medical uses for bentonite clay has been to treat diarrhoea, and modern science is discovering its benefits for patients suffering from irritable bowel syndrome (IBS) [2]. Bentonite clay benefits include a multitude of valuable effects apart from attracting positively charged toxins from the internal systems. When it is taken orally, it has an outstanding cleaning capacity. It pulls away built-up refuse and plague that block the absorption of essential nutrients from food and supplements. This action alone can raise the energy level in as little as 1 to 3 days. There are three main reasons bentonite clay benefits are great for boosting energy; It revitalizes, it absorbs, and acts as a catalyst for the transformation of food into usable energy [3].

Bentonite clay has a strong negative ionic charge. This negative ionic charge is the reason that bentonite clay benefits are so helpful in detoxifying the body. The negative charge allows the clay to attract only substances that have a positive charge, such as toxins, pesticides, heavy metal, harmful bacteria and pathogens without leaching away beneficial element [4]. This clumping action prevents toxic molecules from passing through the walls of the intestines and entering the bloodstream and together with the clay; the toxins are eliminated harmlessly out of the body through the kidneys. It is for this reason that bentonite is a key ingredient found in many colon cleansing and detox products. Experts believe montmorillonite is the mineral that

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gives bentonite its beneficial qualities. But it also contains magnesium and 67 other trace minerals. Bentonites are used for decolorizing various vegetable, minerals, and animal oils. They are also used in clarifying wines, ciders, liquors, beer, vinegar [2]. Bentonite has been prescribed as a bulk laxative, and it is also used as a base for many dermatologic formulas [5, 6].

Bentonite's inclusion in liquid cosmetics and skin cares products help prevent formulas from clumps. Foundations that contain trace amounts of bentonite are less likely to appear cakey when applied to the skin. Bentonite can also be added to skin care products to increase their thickness or viscosity and to prevent them from separating. This inclusion of bentonite in formulas also reduces the transparency of creams, gels and lotions, making products more usually appealing. Apart from skin dryness, bentonite is not known to cause side effects and is not a common allergen. Due to its natural origin, bentonite is a common ingredient in natural skin care lines, including organic and vegan products [2, 7, 8]. Bentonite is used as filler in pharmaceuticals and due to its absorption/adsorption functions, it allows paste formation and such applications include industrial protective creams, calamine lotion, antioxidants and wet compresses for eczema. 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 [9]. Laundry detergents and liquid hand cleansers/soaps rely on the inclusion of bentonite, in order to remove the impurities in solvents and to soften the fabrics [10].

Electrolytes are minerals in the blood and other body fluids that carry an electric charge. Electrolytes affect the amount of water in the body, muscle function, the acidity of the blood and other important processes. Calcium is the most abundant electrolyte in the body. Total calcium in the human body is about 1 to 1.5kg, 99% of which is seen in bone and teeth where it promotes their formation, 1% in extracellular fluid nerve cells, body tissues, blood and other body fluids. Phosphorus is a mineral that makes up about 1% of a person's total body weight, 80% of which is seen in bone and teeth and 10% in muscle. Phosphorus is mainly an intracellular ion and is seen in all cells [11, 12, 13].

Bentonite has been known to have a lot of uses and benefits to both man and plants 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 calcium and phosphorus of Wistar rat and by extrapolation, the human body.

MATERIALS AND METHODS

Calcium bentonite clay was obtained from bentonite deposit at Anambra state in Nigeria. Reagent kits were obtained from Randox Laboratories, Limited United Kingdom. A total of forty-five male and female Wistar albino rats (*Ratus 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 12hours 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 the following doses 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 at days 7, 21, and 28 days. This was done by cardiac puncture with the animal under anaesthesia (chloroform) in a desiccator. The blood collection was done immediately and was stored in lithium heparin sample containers.

The electrolyte testing method used for this experiment were the colorimetric method for calcium analysis and the ultraviolet-visible spectroscopy for phosphorus analysis. Instructions were followed as directed in the reagent kit.

Determination of Calcium: Calcium ions form a violet complex with O-Cresolphthalein complexone in an alkaline medium. The reagent composition is shown in Table 1.

Calculation:

Conc. (mmol/l) = A_{sample} / A_{sample} x Standard Conc. (mmol/l)

Table 1: Reagent composition for determination of calcium

Contents	Initial Concentration of Solutions
CAL. Standard	
Calcium	5.5ml
R1. Buffer	
2-amino-2-methylpropan-1-ol	3.5 mol/l, pH 10.7
R2. Chromogen	
O-Cresolphthalein complexone	0.16mmol/l
8-Hydroxyquinoline	6.89mmol/l
Hydrochloric acid	60mmol/l
R3 EDTA	150mmol/l

Phosphate Inorganic:

Inorganic phosphorus reacts with ammonium molybdate in the presence of sulphuric acid to form a phosphomolybdate complex which is measured at 340nm. The reagent composition is shown in Table 2.

Table 2: Reagent composition for determination of Inorganic phosphorus

Contents	Initial Concentration of Solutions
R1a. Blank Reagent	
Sulphuric acid	0.36mol/l
Sodium Chloride Detergent	154mmol/l
R1b. Molybdate Reagent	
Ammonium Molybdate	3.5mmol/l
Sulphuric Acid	0.36mol/l
Sodium Chloride	154mmol/l
CAL. Standard	
Potassium Phosphate	1.65mmol/l

Statistical analysis:

Data analysis was performed using the Statistical package for the Social Scien ces 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

Results in Table 3 showed that at 7 days duration, the value of 2.17mmol/l was obtained for calcium level at 0.02g/100gbody weight concentration of bentonite. However, at 0.07g/100g body weight concentration of bentonite, 2.35mmol/l was the value obtained. When compared with the control which was 2.10mmol/l, the *in vivo* effect of bentonite on the rat plasma level increased. At 21 days, at 0.02g/100g body weight concentration of bentonite, 2.20mmol/l was obtained, while at 0.07g/100g body weight concentration of calcium bentonite, 2.30mmol/l was observed. When compared with the control which is 2.10mmol/l, the *in vivo* effect of bentonite on the rat plasma level increased. At 0.02g/100g body weight concentration of bentonite at 28 days duration, the value was 2.15mmol/l, at 0.07g/100g body weight concentration of calcium bentonite, the value was 2.37mmol/l. When compared with the control which was 2.00mmol/l, the *in vivo* effect of bentonite on the rat plasma level increased. This implies that the *in vivo* effect of calcium bentonite in *wistar albino rats* leads to increase in levels. This increase is expected as the bentonite analysed is calcium bentonite.

Results in Table 4 indicated that at 7 days duration, at 0.07g/100g body weight concentration of calcium bentonite, the value obtained for phosphorus level was 3.60mmol/l. When compared with the control which was 2.90mmol/l, the *in vivo* effect of calcium bentonite on the rat plasma level decreased. At 0.02g/100g body weight concentration of calcium bentonite at 21 days, at 0.07g/100g body weight concentration of calcium bentonite, 3.50mmol/l was obtained. When compared with the control which was 2.77mmol/l, the *in vivo* effect of bentonite on the rat plasma level decreased. At 0.02g/100g body weight concentration of bentonite at 28 days duration, the value was 3.20mmol/l, at 0.07g/100g body weight concentration of bentonite, the value obtained was 3.60mmol/l. When compared with the control which was 3.00mmol/l, the *in vivo* effect of bentonite on the rat plasma level decreased. This implies that the presence of bentonite decreases phosphorus but increases calcium in the rat plasma based on the results. When there is too much calcium, a bone is broken down and calcium is released into the blood stream. This condition is called hypercalcemia and is usually the result of a tumor in parathyroid gland. Some symptoms of hypercalcemia include: thirst,

Table 3: In vivo effect of calcium bentonite on rat plasma level of calcium in Wistar albino rats

Conc. (g/100g body weight)	Calcium (mmol/l)		
	7 days	21 days	28 days
Control (0.00)	2.10 ± 0.00[(a)]	2.10 ± 0.07[(a)]	2.00 ± 0.00[(a)]
0.02	2.17 ± 0.13[(a)]	2.20 ± 0.05[(b)]	2.15 ± 0.05[(b)]
0.04	2.20 ± 0.16[(b)]	2.25 ± 0.05[(b)]	2.20 ± 0.08[(c)]
0.05	2.27 ± 0.13[(b)]	2.30 ± 0.12[(c)]	2.30 ± 0.08[(d)]
0.07	2.35 ± 0.11[(c)]	2.30 ± 0.08[(c)]	2.37 ± 0.15[(d)]

Mean ± Standard error of mean. Values with different superscripts show a mean difference that is significant at $P \leq 0.05$.

Table 4 In vivo effects of calcium bentonite on rat plasma level of phosphorus in Wistar albino rats.

Conc. (g/100g body weight)	Phosphorus (mmol/l)		
	7 days	21 days	28 days
Control (0.00)	2.90 ± 0.00[(a)]	2.77 ± 0.08[(a)]	3.00 ± 0.00[(a)]
0.02	3.30 ± 0.10[(b)]	3.15 ± 0.05[(b)]	3.20 ± 0.07[(b)]
0.04	3.45 ± 0.05[(c)]	3.35 ± 0.05[(c)]	3.36 ± 0.05[(c)]
0.05	3.50 ± 0.20[(d)]	3.12 ± 0.02[(b)]	3.40 ± 0.08[(d)]
0.07	3.60 ± 0.07[(e)]	3.50 ± 0.08[(d)]	3.60 ± 0.07[(e)]

Mean ± standard error of mean. Values with different superscripts show a mean difference that is significant at $P \leq 0.05$.

frequent urination, confusion, stomach upset, vomiting, loss of appetite, nausea, risk of coma and death. Hypocalcemia occurs due to wide spread blood infection, as well as infection in other tissues and some symptoms of hypocalcemia includes: numbness in the hands and or feet, confusion and se izures Hyperphosphatemia which is an electrolyte disturbance in which there is abnormally elevated levels of phosphate in the blood. Some symptoms include: bone and joint pain, sleep disturbances, fatigue, shortness of breath anorexia. Hypophosphatemia is an electrolyte disturbance in which there is an abnormally low levels of phosphate in the blood and the symptoms include: encephalopathy, seizures, neuromuscular disturbances may occur, osteomalacia, anorexia, and muscle weakness, mental status changes [11].

In conclusion, the risks of oral administration of b entonite increase with heavy use. This puts prolonged consumers of bentonite at risk of constipation, as it absorbs too much water from the intestine.

This study has been able to ascertain that small amounts of calcium bentonite has no significant effect on calcium and phosphorus plasma levels of rat and by extrapolation, the human body. This implies that calcium bentonite used in many products (such as toothpaste, antacids, and cosmetics), would have no significant effect on the electrolyte parameters of the consumer.

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