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EFFECTS OF BENTONITE ON PLASMA UREA AND CREATININE OF WISTAR ALBINO RATS.

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ABSTRACT

The in vivo effect of Nigerian calcium bentonite clay on wistar albino rat plasma urea and creatinine levels were investigated. The rats were fed for a period of four weeks with varying concentrations of the bentonite clay, and the urea and creatinine levels determined using spectrophotometric methods. Test results showed that the bentonite clay had a lowering effect on both the plasma urea and creatinine levels in a concentration and time dependent manner. The highest decrease of urea (0.63 ± 0.16mmol/l) was obtained at the 4 weeks 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 the plasma urea and creatinine levels at 95.0% confidence level (P<0.05). From the study, it was clear that the bentonite clay decreased the wistar albino rat plasma urea and creatinine levels. The significant decrease in the values of urea levels could suggest that the entire renal function may not have been compromised following the administration of bentonite substance.

Key words: Bentonite, Calcium, Creatinine, Plasma, Urea.

INTRODUCTION

Bentonite Clayis an absorbental uminum phyllosilicate, impure clay consisting mostly of montmorillonite. The absorbent clay was given the name bentonite by Wilbur C. Knight in 1898, after the Cretaceous Benton Shale near Rock River, Wyoming in America

There are different types of bentonite, each named after the respective dominant element that it contains, such as potassium (K), sodium (Na), calcium (Ca), and aluminum (Al). Bentonite usually forms from weathering of volcanic ash, most often in the presence of water. For industrial purposes, two main classes of bentonite exist: sodium and calcium bentonite(Hosterman and Patterson, 1992).

Bentonite is a mixture from the smectite group with high ion exchange capacity which binds with different cations. Bentonite has been utilized as a useful material in both high-roughage and high-concentrate based diets of ruminants due to its ability to absorb toxic products from digestion and lowering the accumulation of toxic substances in tissues (Huntington *etal*.,1977; Fenn and Leng, 1989; Varadyova*et al*., 2003).

The bentoniteclay has been prescribed as a bulk laxative, and it is also used as a base for many dermatologic formular (Hosterman

and Patterson 1992).Bentonite clay has a strong negative ionic charge. This negative ionic charge is the reason that bentoniteclay are so helpful in detoxifying the body (Danica, 2011).

A negative charge allows the clay to attract only substances that have a positive charge, such as toxins, harmful bacteria, pesticides, heavy metals, and pathogens-without leaching away any beneficial elements. This clumping action prevents toxic molecules from passing through the walls of the intestines and entering the blood stream and together with the clay; the toxins are eliminated harmlessly out of the body through the kidneys (Danica, 2011).

It's no wonder that bentonite is a key ingredient found in many colon cleansing and detox products. Experts believe montmorillonite is the mineral that gives bentonite its beneficial qualities. But it also contains magnesium and 67 other trace minerals. The properties of these particles, as well as their placement within the bentonite molecule, give the clay its healing effects-including its all-important negative charge (Danica, 2011).

Urea is synthesized in the body of many organisms as part of the urea cycle, either from the oxidation of amino acids or from ammonia. Amino acids from ingested food that are not used for the synthesis of proteins and other biological substances are oxidized by the body, yielding urea and carbon dioxide, as an alternative source of energy generation (Sakami, *etal*., 1963). The oxidation pathway starts with the removal of the amino group by a transaminase; the amino group is then fed into the urea cycle. Creatinine is a protein breakdown product and its level is a reflection of the bodies muscle mass. Creatinine is a metabolite of creatine. Creatinineproduction is endogenous and is determined by muscle mass. Unlike urea, creatinine concentration is almost independent of diet. It increases in renal failure (Wootton and Freeman, 1982).

There has been some work done on how bentonite clay are used in the feed industry (Mikolaichik and Morozova, 2009), but there is little or no work done on the effects of this bentonite on biochemical parameters such as urea and creatinineand hence the objective of this work.

MATERIALS AND METHOD

Calcium bentonite clay was obtained from bentonite deposit at Anambra state in Nigeria. Urea and Creatinine kits were obtained from Randox Laboratories, Limited United Kingdom. Other reagents used were of analytical grade and were prepared with distilled water.

A total of forty five male and female wistarabino rats (Ratusrattus) 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 daily at 12 hours interval for 28 days.

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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.

Urea determination

Urea levels were determined by enzymatic colorimetric endpoint method. The principle of this method is that urea is hydrolysed by urease into ammonia and carbon dioxide. The ammonia generated reacts with alkaline hypochlorite and sodium salicylate in the presence of sodium nitroprusside as coupling agent to yield a blue cromophore. The intensity of the colour formed is proportional to the concentration of urea in the sample (Young, 1995; Tietz, 1995).

The reagent kit contained reagent 1: (urease >500U/ml), stabilizers. Reagent 2/; (buffered chromogen), phosphate buffer (20mmol/l pH 6.9), EDTA (2 mmol/l), sodium salycilate (60 mmol/l), sodium nitroprusside (3.4mmo/l). Reagent 3: Alkaline hypochlorite, sodium hypochlorite (10 mmol/l), NaOH (150mmol/l), urea standard, urea (8.3 mmol/l). The working reagent was prepared by mixing 1ml of reagent 1 with 24 ml of reagent 2.

1.00ml of the working reagent was mixed with 10µl of the sample. The standard tube contained 1.00ml of the working reagent and 10µl of the standard. The blank tube had 1.00ml of working reagent. The mixture was incubated for 5 minutes at 37^{o}C and absorbance of sample read against the reagent blank at 600nm with Spectronic-20 spectrophotometer.

Calculations:

Concentration of Sample $= \frac{\Delta A_{sample}}{\Delta A_{standard}} \times C_{standard}$ = mg/dL urea x 0.1665 = mmol/l

Normal values: 2.5 – 6.6mmol/l.

Creatinine determination

Creatinine levels were determined by colorimetric method (with deproteinization)

The principle of this method is that creatinine in alkaline solution reacts with picrate to form a coloured complex (Henry, 1974; Traymor, et al, 2006).

The Reagent kit contained solution 1: Standard (177umol/l), solution 2: Picric acid (35 mmol/l), solution 3: Sodium hydroxide (1.6 mol/l), TA 651 Trichloroacetic acid (TCA) (1.2mol/l). The Working reagent was prepared by mixing 10ml of solution 2 and 10ml of solution 3.

The sample was first deproteinized by mixing 1.0ml of Trichloroacetic acid (TCA) and 1.0ml of sample. The mixture was vigorously stirred with a glass rod to evenly disperse the precipitate. The mixture was then centrifuged at 2500 rpm for 10 minutes, the supernatant was then separated and used for the assay as listed below:

1.00ml of the working reagent was mixed with 1.00ml of the supernatant. The standard tube contained 1.00ml of the working reagent, 0.5ml of TCA and 0.5 ml of solution 1. The blank tube had 1.00ml of working reagent, 0.5ml of TCA and 0.5ml of distilled water. The mixture was let to stand for 20 minutes at 25^{o}C and the absorbance of the sample and standard were read against the blank at 520nm with Spectronic-20 spectrophotometer.

Calculations:

$\frac{\Delta A_{sample}}{\Delta A_{standard}}$ x 177 = µmol/l

Normal values : 44 – 80 µmol/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: *In vivo* effect of bentonite on rat plasma urea expressed in mmol/l

Bentonite dose	Urea (mmol/l)		
g/100g body wt.	Week 1	Week 3	Week 4
0.00 (Control)	0.98 ± 0.00	0.92 ± 0.15	0.99 ± 0.00
0.02	0.88 ± 0.18[a]	0.72 ± 0.48[b]	0.71 ± 0.18[c]
0.04	0.85 ± 0.18[a]	0.71 ± 0.10[b]	0.65 ± 0.08[c]
0.05	0.84 ± 0.15[a]	0.70 ± 0.16[b]	0.64 ± 0.08[c]
0.07	0.81 ± 0.20[a]	0.66 ± 0.08[b]	0.63 ± 0.16[c]

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

Table 2: *In vivo* effect of bentonite on rat plasma creatinine expressed in µmol/l

Bentonite dose	Creatinine (µmol/l)		
g/100g body wt.	Week 1	Week 3	Week 4
0.00 (Control)	105± 0.00	105 ± 0.01	105 ± 0.00
0.02	104 ± 6.83[a]	93 ± 3.12[b]	85 ± 2.95[c]
0.04	100 ± 4.50[a]	91 ± 1.58[b]	82 ± 1.63[c]
0.05	98 ± 2.73[a]	89 ± 2.54[b]	84 ± 3.26[c]
0.07	97 ± 1.93[a]	88 ± 3.26[b]	79 ± 1.87[c]

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

DISCUSSION

The mean results ± SD of urea and creatinine determinations are presented in Tables 1 and 2 respectively. Test results showed that the urea and creatinine levels when compared with the control decreased significantly in a concentration and time dependent manner throughout the experimental period. For plasma urea, the highest decrease of 0.63 ± 0.16 vs control 0.99 ± 0.00 mmol/l was obtained after the 4 weeks duration at the highest concentration of bentonite (0.07g/100g body weight). For plasma creatinine, the highest decrease of 79 ± 1.87 vs control 115 ± 0.00 µmol/l was obtained after the 4 weeks period at the highest concentration of bentonite (0.07g/100g body weight). The differences in weight and weeks were statistically significant on the effect of the bentonite on the plasma urea and creatinine levels at 95.0% confidence level (P<005).

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The biochemical indices evaluated in this study are useful parameters to indicate impairment in functional capacities of the renal organs. Blood Urea Nitrogen is a waste product derived from protein breakdown in the liver. Elevated levels of plasma urea can be caused by excessive protein intake, certain drugs and low fluid intake and kidney damage. The clinical importance of the urea level in plasma is normally determined in conjugation with the plasma creatinine level (Ivan, 1992; Okoye*etal*. 2011). Creatinine is measured primarily to assess kidney function and has certain advantages over the measurement of urea. The plasma levels of creatinine is relatively independent of protein ingestion, water intake, rate of urine production and exercise. Since its rate of production is constant, elevation of plasma creatinine is indicative of under excretion, suggesting kidney impairment. Very low levels of plasma creatinine are rare and not clinically significant. (Zhao, *etal*., 2010 and Okoye*etal*. 2012).

The significant decrease in the values of plasma urea and creatinine levels in table 1 and 2, point to the fact that the entire renal functionis not compromised following the administration of Bentonite substance. This study therefore supports the fact that bentonite is widely utilized in the feed industry. From this study, plasma urea and creatinine levels were not raised after the ingestion of bentonite, this is good for the feed industry because bentonite has a strong adsorption capacity and cation exchange capacity therefore, in the feed industry, bentonite acts as a carrier of the premix, an anti-caking agent that absorbs particles such as feed mycotoxins. Also diets for cows supplemented with bentonite has been reported to aid digestibility, and increase milk production and milk quality (Mikolaichik and Morozova 2009). Varadyova*etal*., (2003) stated that adding bentonite to high concentrate diets of ruminants can control rumen pH.Berthianzine*etal*., (2007) and Ambula, *etal.*, (2003) studies showed that adding bentonite to silage can increase the amount of animal feed, average daily gain and utilization of silage. Khadem*et al*., (2007) and Aquilera-soto*etal*., (2008) found that adding 2 to 4% bentonite to the diet can improve the feed intake of sheep. Ma and Guo (2008) observed that the addition of bentonite can significantly improve the growth performance of chicken at the age of 320 days and improve activities of intestinal maltase, aminopeptidase N and alkaline phosphatase. These results are in good agreement with the results reported in this study.

From this study, it was clear that Bentonite clay decreased plasma urea and creatinine levels of the wistar rat. The significant decrease in the values of plasma urea and creatinine levels, is suggestive of non-compromise of renal function following the administration of Bentonitesubstance, thus encouraging the opinion that Bentonite clay has no deleterious effects upon consumption.

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