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EFFECT OF CALCIUM BENTONITE ON LIPID PARAMETERS IN WISTAR ALBINO RAT

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ABSTRACT

The in vivo effect of Nigerian calcium bentonite clay on rat plasma cholesterol and triglyceride levels of Wistar albino rats was investigated. The rats were fed for a period of four weeks with varying concentrations of the bentonite clay, and the cholesterol and triglyceride levels determined using spectrophotometric methods. Test results showed that the bentonite clay had an increasing effect on both the plasma cholesterol and triglyceride levels in a concentration and time dependent manner. The highest increase of cholesterol (3.38± 0.10mmol/l) and triglyceride (0.54 ± 0.01mmol/l) were obtained at the 4th week with the highest concentration of bentonite (0.07g/100g body wt) (P<0.05). From the study, it was clear that the bentonite clay increased the plasma cholesterol and triglyceride levels. The slight increases in the values of cholesterol and triglyceride levels point to the fact that the bentonite substance did not significantly affect the lipid profile following the administration of calcium bentonite.

Key words: Calcium bentonite, Plasma cholesterol and triglyceride.

INTRODUCTION

Bentonite is an absorbent aluminium phyllosilicate impure clay consisting mostly of montmorillonite. Bentonite has been prescribed as a bulk laxative, and it is also used as a base for many dermatologic formulas.Granular bentonite is being studied for use in battlefield wound dressings. Bentoquatam is a bentonate-based topical medication intended to act as a shield against exposure to urushiol, the oil found in plants such as poison ivy or poison oak (Hosterman and Patterson, 1992).

Bentonite is effective in killing bacteria *in vivo* and *in vitro* and it has been reported that many types of skin conditions have been treated by the application of the bentonite medicinal clay. Montmorillonite has shown its effectiveness in this area. It has also been used as a base ingredient for tissue engineering. Bentonite clay is used in many dermatological over-the-counter remedies, such as in acne treatments(Wang *et al*., 2007).

Cholesterol is a sterol or modified steroid, a lipid molecule and is biosynthesized by all animal cells because it is an essential structural component of animal cell membranes that is required to maintain both membrane integrity and fluidity. In addition to its importance within cells, cholesterol also serves as a precursor for the biosynthesis of steroid hormones, bile acids, and vitamin D

(Hanukoglu, 1992). Cholesterol is the principal sterol synthesized by animals. In vertebrates, the hepatic cells typically produce greater amounts of cholesterol than other cells. It is almost completely absent among prokaryotes, although there are some exceptions such as mycoplasma, which require cholesterol for growth. (Razin and Tully, 1970).

Triglyceride is an esterderived from glycerol and three fatty acids. As a blood lipid, it helps enable the bidirectional transfer of adipose fat and blood glucose from the liver. There are many triglycerides: depending on the oil source, some are highly unsaturated, some less so (Hanukoglu, 1992).Bentonite has been known for years to be of medicinal importance to man yet, there is no information available in literature regarding the effect of bentonite on lipid parameters. Hence this is what informed this research.

MATERIALS AND METHOD

Calcium bentonite clay was obtained from bentonite deposit in Anambra State of Nigeria. The cholesterol reagent kit was obtained from Randox Laboratories Limited, United Kingdom. The triglyceride reagent kit was from Human Gesellschaft fur Biochemica und DiagnosticambH, Max Planck-Ring 21D -65205 Wiesbaden, Germany.

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. They were housed in 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 sub groups (A, B, C and D) made up of 9 rats per sub group.

Various amounts of bentonite clay (0.02g, 0.04g, 0.05g, and 0.07g in 2 ml of distilled water), were administered orallyusing a syringe 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 experiment.

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

Cholesterol determination

The plasma cholesterol levels were determined by enzymatic endpoint method. The principle of this method is that cholesterol is determined after enzymatic hydrolysis and oxidation. The indicator quinoeimine is formed from hydrogen peroxide and 4-aminoantipyrine in the presence of phenol and peroxidase. (Trinder, 1969; Wootton, 1982; Young, 1995). Reagent kit contained 4-aminoantipyrine (0.30mmol/l), phenol (6mmol/l), peroxidase (≥0.5 μ/ml) cholesterol esterase (≥0.15 μ/ml), cholesterol oxidase (≥0.1 μ/ml), pipes buffer

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(80mmol/l;pH 6.8) and standard 5.17mmol/l (200mg/dl).
One millilitre of reagent was mixed with 10µl of the sample. The standard tube contained 1.00ml of reagent and 0.01ml of reagent and 10µl of distilled water. The mixture was incubated for 10 minutes at 37^{o}C. The absorbance was read against the reagent blank within 60 minutes at 546nm with spectrophotometer (spectronic 20).

Calculation

$$\text{Cholesterol in sample} = \frac{\text{Change in absorbance of sample}}{\text{Change in absorbance of Standard}} \times \text{Conc. of Standard}$$

Normal values is less than 5.17mmol/l.

Triglycerides levels were determined by enzymatic colorimetric test with lipid clearing factor (LCF). The principle of this method was that the triglycerides were determined after enzymatic hydrolysis with lipases. Indicator wasquinoneimine formed from hydrogen peroxide, 4 – aminoantipyrine and 4-chlorophenol under the catalytic action of peroxidase (Schettler&Nussel, 1975; Jacobs and VanDemark,1960). Reagent kit contained buffer (pH 7.5) (50mmol/l), 4-chlorophenol (5mmol/l), 4 – aminoantipyrine (0.25mmol/l), magnesium ions (4.5 mmol/l), ATP (2mmol/l), lipases (≥1.5 µ/ml), glycerol – 3- phosphate oxidase (≥1.5 µ/ml) and 3 ml standard (200 mmol/l).
To 10 µl of the sample was added 1000 µl of the reagent and mixed. The standard tube contained 1000µl of reagent and 10µl of the standard. The blank tube had 1000µl of reagent. The mixtures were incubated for 5 minutes at 37 ^{o}C. The absorbance of the samples were read against the reagent blank within 60 minutes at 546nm with spectronic 20 spectrophotometer.

Calculations

$$\text{Triglyceride in sample} = \frac{\text{Change in absorbance of sample}}{\text{Change in absorbance of Standard}} \times 2.28\text{mol/l}$$

Normal values is less than 1.71 mmol/l.

Statistical Analysis

Data analysis was performed using the Statistical package for the Social Sciences software (SPSS, version 11.0). 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 was $P<0.05$.

RESULTS

The initial body weights of the rats were obtained before they were fed with the calcium bentonite. The average weight was observed to be 100g.

The results below show the effect of various concentrations of bentonite on the cholesterol and triglyceride levels in Wistarrats (Tables 1 and 2). The results show that the calcium bentonite increased the plasma cholesterol and triglyceride levels in a concentration and time dependent manner. The highest level of cholesterol (3.38 ± 0.10 vs control 2.25 ± 0.13) was obtained at the fourth week with the highest concentration of bentonite(0.07g/100g body weight of rat). However, the lowest level of 2.30 ± 0.08 was obtained at the first week with the lowest concentration of 0.02g/100g body weight of rat. Also the highest level of triglyceride (0.54 ± 0.01 vs control 0.45 ± 0.11) was obtained at the fourth week with the highest concentration of bentonite (0.07g/100g body weight of rat). However, the lowest level of 0.47 ± 0.06 was obtained at the first week with the lowest concentration of 0.02g/100g body weight of rat.

Table 1: *In vivo* cholesterol levels (mmol/l) of rats fed with bentonite.

Amount of bentonite (g/100g body weight of rat)	Concentration of Cholesterol (mmol/l) Week 1	Week 3	Week 4
Control	2.22 ± 0.13^{b}	2.15 ± 0.14^{a}	2.25 ± 0.13^{a}
0.02	2.30 ± 0.08^{a}	2.40 ± 0.08^{a}	2.43 ± 0.05^{a}
0.04	2.40 ± 0.08^{a}	2.48 ± 0.05^{a}	2.70 ± 0.08^{b}
0.05	2.68 ± 0.10^{a}	2.83 ± 0.10^{a}	3.00 ± 0.13^{b}
0.07	2.90 ± 0.08^{a}	3.18 ± 0.13^{b}	3.38 ± 0.10^{a}

The results are expressed as mean ± standard deviation. Values with superscript (b) show a mean difference that issignificant at $P \geq 0.05$.

Table 2: *In vivo*triglyceride level (mmol/l) of rats fed with bentonite.

Concentration (g/100g body weight of rat)	Concentration of triglyceride (mmol/l) Week 1	Week 3	Week 4
Control	0.45 ± 0.10	0.45 ± 0.11	0.45 ± 0.11
0.02	0.47 ± 0.06	0.48 ± 0.06	0.49 ± 0.04
0.04	0.48 ± 0.01^{a}	0.49 ± 0.01^{a}	0.50 ± 0.01^{a}
0.05	0.49 ± 0.03	0.50 ± 0.04	0.51 ± 0.04
0.07	0.52 ± 0.01^{a}	0.53 ± 0.01^{a}	0.54 ± 0.01^{a}

The results are expressed as mean ± standard deviation. Values with superscript (a) show a mean difference that is not significant at $P \geq 0.05$.

DISCUSSION

The prevalence of cardiovascular disease (CVD) related death in the world is on the increase and high blood pressure is one of the major contributors of these diseases. The clinical consequences of these conditions are severe and exert major research efforts to improve knowledge of its pathogenesis and thereby provide a more rational approach to its prophylaxis and therapy

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(Lewington*et al* 2007). The results for the effects of various concentrations of bentonite on total cholesterol level showed slight increase in the amount of total cholesterol in the Wistar rats at the first, thirdand fourth week (Table 1). This suggests that the bentonite did not adversely affect cholesterol biosynthesis which resulted instability within normal range in the level of cholesterol in the blood. The values for the test groups at the various weeks were not significantly ($p>0.05$) different and were within the range for those of the control. These results are in agreement with the work of (Dwyer *et al*., 1997; Ledoux*et al*., 1999; Miles & Henry, 2007; Uwakwe*et al*, 2012), who carried out work on the effect of eggplant juice on plasma lipid levels. The possible mechanism of action of this bentonite on cholesterol metabolism has not been clarified.

The results of triglyceride level showed slight increase after 3 weeks of feeding with bentonite but they were not significant($p>0.05$) as shown in Table 2. A recent study by Galuh*et al.*, (2015), showed that calcium bentonite reduced blood glucose levels in Wistar albino rat, however, nothing has been reported concerning the effect of bentonite on lipid parameters.

This study has been able to ascertain that low doses of calcium bentonite over a short period of time results in no significant effect on the serum cholesterol and triglyceride levels on adult Wistar albino rats.

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