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INVESTIGATION OF THE EFFECTS OF NIGERIAN CALCIUM BENTONITE ON ALBINO RAT PLASMA ALKALINE PHOSPHATASE AND LACTATE DEHYDROGENASE ACTIVITY AT 37oC, pH = 9.8.

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ABSTRACT

Nigerian calcium bentonite was analysed for its *in vivo* effects on rat plasma alkaline phosphatase and lactate dehydrogenase levels. The study showed that the bentonite increased the activity of the enzymes in a concentration dependent manner. The effects of the bentonite on the enzymes were also time dependent with the highest activation obtained at 28 days duration while the least increase occurred at 7 days duration. ALP showed the highest increase of 233.25 ±19.363 vs control 60.00±12.000 and the least increase of 171.25 ±13.048 vs control 60.00 ±12.000) p>0.05. LDH showed the highest increase of 325.25 ±19.363 vs control 57.00 ±18.000 and the least increase of 259.50 ±09.147 vs control 57.00 ±18.000. The result indicate that liver function tests are required for anyone using bentonite in large quantities and for long periods of time.

KEYWORDS: Alkaline phosphatase, Bentonite, Enzymes, Lactate Dehydrogenase

INTRODUCTION

For ages bentonite has been regarded as the clay with a thousand uses, its importance is evident in various fields such as medicine, drilling, agriculture, cosmetics etc. While Bentonite has several beneficial effects, very little research has been carried out on its adverse effect on health. Bentonite is aluminum phyllosilicate impure clay consisting mostly of montmorillonite. The most important non clay mineral in bentonite is silica polymorphs (Silica; Quartz and Opals), Feldspars, Zeolites, Carbonates, Sulfites, Sulfate, Oxide and Hydroxide (Bates and Jackson, 1987; Moore *et al.*, 1997, Bentonite presents a strong colloidal properties and an increasing volume when in contact with water, it therefore creates a gelatinous and viscous fluid (Virta, 2001). Mikolaichik and Morozova (2009) reported that diets for cows supplemented with bentonite can increase milk production, digestibility and improve milk quality.

Varadyova (2003), stipulated that adding bentonite in high concentrate diets of ruminants can control rumen pH and improve the rumen environment.

Aflatoxin is a type of mold-like compound produced by organisms that thrive on a variety of different food crops. These toxic substances are detectable in peanut butter and some cooking oils, and research shows that they can interfere with the functioning of the immune system. Aflatoxins may also contribute to possible liver damage, including liver cancer. Animal models have shown that bentonite clay may reduce health damage associated with the ingestion of dietary aflatoxin. Bentonite clay is typically used for cleansing the body of toxic metals, compounds that may degrade the health of the body over time. The most damaging toxic metals are mercury, cadmium, lead, and benzene, compounds contained in some processed foods (especially those with high fructose corn syrup), drinking water, building materials, and the environment. One study has revealed that bentonite clay may be helpful for reducing these harmful metals. (Patterson, 1983; Odom, 1984; Virta, 2001; Varadyova 2003; Mikolaichik and Morozova, 2009).

Phosphatases are enzymes capable of hydrolyzing organic esters of phosphoric acid to organic compounds and free inorganic phosphate. Alkaline phosphatase (ALP) has optimum pH of about of about 9.8 present in the blood plasma, bone, kidney, intestine, lungs. In adults the normal serum level of ALP is 98 – 279 IU/L. Most of the serum ALP is derived from the liver (Okoye *et al.*, 2012). Lactate dehydrogenase is an enzyme which catalyzes the reversible conversion of lactic acid to pyruvic acid. Values in the upper range are generally seen in children. Strenuous exercise would slightly increase the value. LDH level is 100 times more inside the red blood cell than the plasma.

Therefore minor amount of hemolysis would result in a false positive test. LDH activity is increased during a heart attack. Increase in total LDH level is also seen in hemolytic anemia, hepatocellular damage, muscular dystrophy

carcinomas, leukemia's and any condition which causes necrosis of body cells. 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 to ascertain its effects on the organs of the body. Therefore the aim of this research work is to investigate the effect of bentonite on the liver using the liver makers ALP and LDH of wistar albino rats.

MATERIALS AND METHODS

Calcium bentonite clay was obtained from bentonite deposit at Anambra state in Nigeria. Reagent kits were bought from Randox Laboratories Ltd. Ardmore, Diamond Road, Crumlin, Co. Antrim, United Kingdom BT29 4QY. 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. 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 in to sub groups (A, B, C and D).

Bentonite clay was administered orally at various grams (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 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 anaesthesia (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.

Alkaline Phosphatase analysis:
Reagent kit contained 1: Buffer Diethanolamine buffer (1mol/l,pH 9.8), $MgCl_2$ 0.5 mmol/l. 2: Substrate p-nitrophenylphosphate (10 mmol).

ALP levels were determined by colorimetric method. 0.05ml of sample was mixed with 3.00ml of reagent in a 1cm light path cuvette. The absorbance of the mixture was read against air at 37 ^{o}C at 405nm using spectronic - 20 spectrophotometer. A timer was started simultaneously and the absorbance of the mixture was read again after 1, 2 and 3 minutes.

Calculation:
The ALP activity was calculated using the following formulae
IU/L = 3300 x ΔA405nm/min

Lactate dehydrogenase analysis:
Reagent kit contained: R1a: Buffer/substrate: Phosphate buffer (50mmol/l, pH 9.8), Pyruvate (0.6mmol/l), R1b: NADH (0.18mmol/l).

LDH levels were determined by UV kinetic method. 0.05ml of sample was mixed with 3.00ml of reagent in a 1cm light path cuvette. The absorbance of the mixture was read against air at 37 ^{o}C at 340nm using spectronic - 20 spectrophotometer. A timer was started simultaneously and the absorbance of the mixture was read again after 1, 2 and 3 minutes

STATISTICAL ANALYSIS

All data were expressed as mean ± standard deviation. Statistical analysis was performed using SPSS version 16.0. The data were analyzed using one-way analysis of variance (ANOVA) where p values <0.05 were considered as significant.

RESULTS AND DISCUSSION

The results shown in Table 1 and 2.

Table 1: *In vivo* effects of bentonite on rat plasma alkaline phosphatase activity at 37°C at pH = 9.8 expressed in (IU/L)

Bentonite g/100g body wt	7 days (IU/L)	21 days (IU/L)	28 days (IU/L)
CONTROL (0.00)	60.25±11.500	60.00±11.195	60.00±12.000
0.02	171.25±13.048	174.50±7.550	176.25±8.770
0.04	178.50±14.617	190.50±95.434	201.50±97.753
0.05	205.25±8.770	210.25±8.382	218.00±101.469
0.07	216.00±10.551	220.75±112.191	233.25±19.363

No significant difference was observed at $p<0.05$ for ALP at different concentrations. Values are shown as Mean ± Standard deviation

Table 2: *In vivo* effects of bentonite on rat plasma lactate dehydrogenase activity at 37°C at pH = 9.8 expressed in (IU/L)

Bentonite g/100g body wt	7 days (IU/L)	21 days (IU/L)	28 days (IU/L)
CONTROL (0.00)	57.25^{a}±11.500	57.00^{b}±09.695	57.00^{a}±18.000
0.02	259.50^{a}±09.147	282.00^{b}±9.487	292.75^{b}±12.148
0.04	287.25^{a}±16.581	291.50^{a}±181.911	300.75^{a}±152.555
0.05	311.00^{a}±16.533	315.25^{a}±8.770	318.00^{a}±177.403
0.07	319.25^{a}±04.349	321.50^{a}±173.146	325.25^{a}±19.363

Values are means ± Standard Deviation of measurements. Means in the same row with the same alphabets are not significantly different at $p<0.05$.

Tables 1 and 2 showed the mean results ± SD of the *in vivo* effects of Nigerian calcium bentonite on rat plasma ALP and LDH. The study showed that the bentonite activated the activities of the enzymes as compared to the controls in a concentration dependent manner. The effects of the bentonite on the enzymes were also time dependent with the highest activity obtained at 28 days duration. While the least of the activation occurred at 7 days duration. ALP showed the highest increase of 233.25 ±19.363 vs control 60.00±12.000 and the least increase of 171.25 ±13.048 vs control 60.00 ±12.000) ($p>0.05$. LDH showed the highest increase of 325.25 ±19.363 vs control 57.00 ±18.000 and the least increase of 259.50 ±09.147 vs control 57.00 ±18.000.

The liver plays a major role in metabolism with numerous functions in the human body including regulation of glycogen storage, decomposition of red blood cells, hormone production and detoxification. High ALP levels can occur if the bile duct is obstructed. (Lange *et al.*, 1982). LDH is a protein that normally appears throughout the body in small amounts. Many cancers raise LDH levels. So LDH may be used as tumor marker but at the same time, it is not useful in identifying a specific kind of cancer. Non-cancerous conditions that raise LDH levels include heart failure.
Bentonite clay has strong internal cleansing properties and has become a popular ingredient in some detoxification programs. Many people have used bentonite clay to address symptoms of constipation like bloating and gas. Ma and Guo (2008), reported that the addition of bentonite to chicken feed can significantly improve the growth and performance of chicken at the age of 320 days. This study agrees with the work of Ma and Gou (2008) who reported that bentonite feed improved the activities of intestinal maltase, amino peptidase N and alkaline Phosphatase.
From the results of this work, it has been observed that bentonite used in small amounts for over a short period of time has no significant effect on ALP and LDH of wistar rat. However using large amounts of bentonite for a long period of time showed significant effect on ALP and LDH. Therefore it is important that liver function tests are undertaken while using bentonite. Those who already have elevated levels of ALP and LDH should avoid or limit the amount of bentonite used.

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REFERENCES

Bates R.E. and Jackson J.A. (1987). Glossary of geology, 3rd ed. Alexandria, Virginia, American Geological Institute. Pp 65 - 432.

Lange P.H., Millan, J.L., Stigbrand, T., Vessella, R.L., Ruoslahti E. and Fishman, W.H. (1982). Placental alkaline phosphatase as a tumor marker for seminoma. *Cancer Res*. 42 (8): 32 – 44.

Ma Y.L. and Guo T. (2008). Intestinal morphology, brush border and digestive activities of broiler fed on a diet containing Cu^{2+} loaded montmorillonite. *Br. Poult. Sci.* 49: 65-73

Mikhail R.S., Guindy N.M., & Hanafi S.J. (1979). Colloid *Interf. Sci*. 70: 282-292

Moore D.M. and Raynolds R.C. (1997). Xray diffraction and the identification of analysis of clay minerals, 2nd ed., Oxford University Press, New York. Pp 1-50

Mikolaichik I. N and Morozova L.A. (2009). Biological basis of using bentonite-based mineral-vitamin premix when increasing the milk yield of cows. *Russian Agric. Sci*., 35: 199-201.

Odom I.E. (1984). Smectite clay minerals: Properties and uses. Philosophical transactions of the engineering sciences. Pp 311- 391.

Okoye N. F., Uwakwe A. A., Nwachoko N. and Ayakeme T. (2012). Effects of Microgynon and primolut-N on Albino Rat plasma and erythrocyte alkaline phosphatise ALP (EC 3.1.3.1) activity at 37oC, pH=9.8. *Int. J. Pharmaceut. Sci. Healthcare.* 2: (4) 39-46.

Patterson S.H. and Murray H.H. (1983). Industrial minerals and rocks, 5th ed., New York. American Institute of mining, metallurgical and Petroleum Engineers. Pp 585-651.

Virta R.L. (2001). US. Geological Survey mineral industry surveys. *Clay and shale*. 1- 5

Varadyova Z. M., Baran P., Siroka, I. and Styriakova. (2003). Effect of silicate minerals (Zeolite, bentonite, kaolin, granite) on in vitro fermentation of amorphous cellulose, meadow hay, wheat straw and barley. *Berl. Munch*.116: 317-321.

