



Original Research Article

Acid Phosphatase Activity of Albino Rats Administered with Salt and Water Samples from Okposi and Uburu Salt Lakes

Agbafor, K. N.*

Biochemistry Department, Ebonyi State University, Abakaliki, Nigeria

*Corresponding author

ABSTRACT

Water and salt samples from Okposi and Uburu salt lakes have been reported to elicit some toxic properties. This research was set up to study the effect of water and salt samples from Okposi and Uburu salt lakes on the prostate in albino rats. Forty five albino rats, placed in nine groups (A-I) of five rats in each, were used for this research. The animals in groups A and B were treated with 1.5 and 3.0ml/kg body weight respectively of water from Uburulake, while C and D were given 1.5 and 3.0ml/kg body weight respectively of water from Okposi lake. Groups E and F received 100 and 200mg/kg body weight respectively of salt solution from Okposi lake, and G and H were given 100 and 200mg/kg body weight respectively of salt solution from Uburu lake, while group I was the control. Administration was done orally for seven consecutive days. Average body weight, physical activities, feed and water intake decreased in all the treated groups relative to the control. Total protein concentrations of the groups administered with the samples were found to be significantly lower ($P < 0.05$) than the values obtained in the control animals. The acid phosphatase activity in the test groups was significantly higher ($P < 0.05$) than in the control. The difference in the levels of these parameters between groups given Okposi sample and Uburu samples was not significant ($P > 0.05$). The difference between the results from the groups treated with water samples and those given salt samples was significant ($P > 0.05$). The effects of all the treatments were found to be dose-dependent. These results suggest that water and salt samples from Okposi and Uburulake maybe toxic, and this toxicity may be reduced by the method of processing the lakes water into salt.

Keywords

Acid phosphatase, Salt lakes, Serum, Total protein, Toxicity

Introduction

A salt lake is a land locked body of water which has a concentration of salts (mostly sodium chloride) and other dissolved minerals significantly higher than most lakes (often defined as at least three grams of salt per liter). Salt lakes are formed from volcanic, glacial, tectonic and river activities

which leave depressions and cavities on land surface. Salt lakes have been reported to contain metallic and non-metallic ions such as calcium, cadmium, lead, mercury, manganese, bicarbonate, sulphate, bromine, fluorine, etc. in addition to sodium and chlorine (Agbafor et al., 2010). The

chemical constituents of salt lakes originated from either natural process (erosions and weathering of coastal materials) or anthropogenic sources, such as domestic, industrial and agricultural practices (Charles et al., 1999). These heavy metals, when higher than the maximum contaminant limit (MCL), results to toxicity, which is detrimental to human health (Matloob, 2003).

Okposi and Uburu salt lakes are located in Ohaozara Local Government Area of Ebonyi state, Nigeria. The origin of these lakes can be traced to as early as life. The lakes serves as salt (obtained after heating lake water to dryness) and water sources for many domestic purposes of the inhabitants of the surrounding communities. The presence of Pb, Mn, Cr, Cu, Fe, Cd, etc., in concentrations higher than WHO's permissible limit have been reported in the lakes (Akubugwo et al., 2007).

Under the name acid phosphatase (ACP) are included all phosphatases with optimal activity below a pH of 7.0. However, ACP of greatest clinical importance is the one derived from the prostate that has a pH optimum of 5-6. Acid phosphatase test is done to diagnose prostate cancer and to know if it has spread to other parts of the body (metastasized), especially bones, and to check the effectiveness of treatment. The test has been largely supplanted by the prostate specific antigen test (PSA). The prostate contains high concentration of acid phosphatase and elevations in serum have been used as biomarkers of damage to the prostate gland (Burtis and Ashwood, 2003; Roobol et al., 2005).

Studies have revealed that water and salt samples form Okposi and Uburu lakes may be toxic to liver and other organs (Agbafor et al., 2007; Akubugwo and Agbafor, 2007;

Agbafor et al., 2010; Agbafor et al., 2011; Ogbanshi et al., 2015). Hence, the present research investigated the effect of the samples on serum acid phosphatase activity in albino rats.

Materials and Methods

Collection of materials

Water samples from Okposi and Uburu salt lakes were collected using sterile containers in the month of March, 2015, during the peak of dry season, while already processed salt samples were collected from the residents in Uburu and Okposi communities into sterile containers. They were transferred to Biochemistry department's laboratory of Ebonyi State University.

Animal handling and treatment

Ethical approval for use of animals in research was given by Ebonyi State University Research and Ethics Committee.

Fourty-five (45) adult male albino rats, weighing 145 – 205g, were randomly distributed into nine (9) groups A, B, C, D, E, F, G, H, and I, each group contained five rats. The animals were fed *ad libitum* with grower's marsh and water throughout the experiment.

Administration of water and salt solution to the animals

After seven-day acclimatization, water and salt solutions were administered to the animals orally for seven consecutive days. Animals in groups A and B were treated with 1.5 and 3.0ml/kg body weight respectively of water from Uburulake. Groups C and D were given 1.5 and 3.0ml/kg body weight of water from Okposi lake. Groups E and F received 100 and

200mg/kg body weight of salt solution from Okposi lake, G and H were given 100 and 200mg/kg body weight of salt solution from Uburu lake, while group I was administered deionized water.

Collection of blood from the animals

Blood samples were collected from animals, following an overnight fasting, through cardiac puncture under mild anesthesia (using chloroform). The samples were put into specimen bottles without anticoagulant and allowed to clot before serum was obtained.

Measurement of parameters

Prostatic acid phosphatase activity in serum was determined by the method described by Albesten et al (2005), while the method of Lowry (1951) was adopted for measurement of total protein.

Statistical analysis

Data generated were expressed as mean \pm SD. Statistical significance of difference was determined using the program SPSS 12 (SPSS, USA) by performing one-way ANOVA with post-hoc comparisons between the control group and each of the treated groups by Duncan's multiple comparison test. A p-value less than 0.05 was considered statistical significant.

Results and Discussion

Administration of salt and water samples from Okposi and Uburu salt lakes to the test animals (A-H) resulted to a significant decrease in physical activities, feed and water intake relative to control (data not shown). The reason for this observation is yet not fully understood. However, it could be attributed to the changes in metabolic activities of the treated animals elicited by

constituents of the salt lakes. Similar observation has been reported by Agbafor and Akubugwo (2007), after treating albino rats with samples from both lakes.

Salt lakes have been shown to contain metallic and non-metallic ions such as calcium, cadmium, lead, mercury, manganese, bicarbonate, sulphate, bromine, fluorine, in addition to sodium and chlorine (Agbafor et al., 2010). The manifestations of lead poisoning, among other disorders, include muscle aches, pains and loss of appetite (Yu, 2001). These may have contributed to the decrease in physical activities, feed and water intake. Further, distortion of metabolism by other constituents of the lakes may not be ruled out.

The average body weight of groups given the samples decreased throughout the period of administration, while that of the control increased (tables 1 and 2). This decrease in body weight may be attributed to the observed decrease in feed and water intake. Although the actual basis to support this result is still obscure, it is consistent with the findings of Akubugwo et al., (2007). Some surface water in Nigeria is known to be polluted such that their constituents elicit some adverse effects (Akubugwo and Agbafor, 2007).

The total protein concentrations obtained in serum of test animals were significantly lower ($P < 0.05$) than those in the control group (tables 3 and 4). Occurrence of aquatic pollutants (such as heavy metals) has been reported to alter immune system and the incidence of infectious diseases. Even very low sub lethal doses of certain heavy metals can have profound effects upon the structure and / or functions of the immune system that could be almost as harmful as direct toxic doses (Saxene et al., 2008). It is known that metals act as

mutagenic/genotoxic compounds, interfere with xenbiotic metabolic pathways and may also affect glycolysis, the Krebs cycle, oxidative phosphorylation, metabolism of protein and amino acid as well as carbohydrate and lipid metabolism (De la Torre et al., 2000; Drastichora et al., 2005). Habib and Samah (2013) recorded a

decrease in protein synthesis in catfish exposed to heavy metals. This effect on serum total protein may be due to the hepatotoxicity of water and salt samples from the lakes reported by Akubugwo and Agbafor (2007), liver being the major site of protein synthesis.

Table.1 Changes in average weight (g) of animals administered the lakes water for seven consecutive days

NO OF DAYS	GROUP A	GROUP B	GROUP C	GROUP D	GROUP I
1	205.15±4.73	170.21±5.05	165.63±4.95	200.34±10.01	146.34±2.11
2	200.34±6.84	139.19±4.25	125.05±4.42	198.19±8.24	150.02±4.48
3	200.05±8.73	131.55±4.12	125.05±4.42	195.23±8.11	150.02±2.14
4	190.40±10.11	128.06±3.18	125.07±4.75	181.21±6.56	155.10±5.11
5	188.10±8.37	125.21±2.15	119.03±2.34	171.21±7.02	156.27±2.40
6	176.04±7.71	123.20±2.25	119.05±2.22	154.34±5.25	159.11±5.65
7	160.23±6.12	113.22±2.09	100.33±2.10	135.55±5.34	167.44±6.02

All values are expressed as mean ± standard deviation. N=5

Key: Group A = 1.5ml/kg body weight Uburu lake water.

Group B = 3.0ml/kg body weight Uburu lake water.

Group C = 1.5ml/kg body weight Okposi lake water.

Group D = 3.0ml/kg body weight Okposi lake water.

Group I = 0.2ml deionized water.

Table.2 Changes in average weight (g) of animals administered the lakes' salt solution for seven consecutive days

NO OF DAYS	GROUP E	GROUP F	GROUP G	GROUP H	GROUP I
1	178.41±10.89	180.21±9.11	150.21±7.25	219.55±9.97	146.34±2.11
2	178.41±10.76	150.54±7.05	144.05±7.04	200.02±9.85	150.02±4.48
3	177.22±9.23	145.72±5.58	144.05±7.05	204.05±9.80	150.02±2.14
4	173.05±9.05	140.66±4.45	136.88±6.32	195.72±7.33	155.10±5.11
5	163.50±7.78	140.56±4.50	128.23±6.30	190.81±7.21	156.27±2.40
6	157.11±7.25	138.42±4.28	125.17±6.05	188.51±7.05	159.11±5.65
7	147.15±5.62	125.32±4.02	122.08±5.49	180.33±7.02	167.44±6.02

All values are expressed as mean ± standard deviation. N=5

Key: Group E = 100mg/kg body weight Okposi lake salt.

Group F = 200mg/kg body weight Okposi lake salt.

Group G = 100mg/kg body weight Uburu lake salt.

Group H = 200mg/kg body weight Uburu lake salt.

Group I = 0.2ml deionized water.

Table.3 Acid phosphatase activity and total protein in albino rats treated with water samples from the lakes for seven consecutive days

NO of Groups	Enzyme Activity (U/l)	Total Protein (mg/ml)
A	8.69±0.51 ^a	0.42±0.05 ^a
B	15.30±1.67 ^b	0.28±0.06 ^b
C	7.44±0.47 ^a	0.38±0.07 ^a
D	17.38±0.48 ^b	0.21±0.11 ^b
I	3.25±0.26 ^c	0.86±0.07 ^c

All values are expressed as mean ± standard deviation N=5. The values in same column with different superscript differ significantly (P < 0.05).

Key: Group A = 1.5ml/kg body weight Uburu lake water.

Group B = 3.0ml/kg body weight Uburu lake water.

Group C = 1.5ml/kg body weight Okposi lake water.

Group D = 3.0ml/kg body weight Okposi lake water.

Group I = 0.2ml deionized water.

Table.4 Acid phosphatase activity and total protein in albino rats treated with sal samples from the lakes for seven consecutive days

NO of Groups	Enzyme Activity (U/l)	Total Protein (mg/ml)
E	7.20±0.99 ^a	0.46±0.08 ^a
F	13.16±0.75 ^b	0.30±0.06 ^b
G	6.82±0.41 ^a	0.47±0.07 ^a
H	12.12±0.71 ^b	0.28±0.08 ^b
I	3.25±0.26 ^c	0.86±0.07 ^c

All values are expressed as mean ± standard deviation. N=5. The values in same column with different superscript differ significantly (P < 0.05).

Key: Group E = 100mg/kg body weight Okposi lake salt.

Group F = 200mg/kg body weight Okposi lake salt.

Group G = 100mg/kg body weight Uburu lake salt.

Group H = 200mg/kg body weight Uburu lake salt.

Group I = 0.2ml deionized water.

The activity of prostatic acid phosphatase obtained in the treated groups was significantly higher (P<0.05) than in the control tables 3 and 4). This may be as a result damage of the prostate gland by toxicants in the samples. Human acid phosphatases are normally found at low concentrations. However, pronounced changes in their synthesis occur in particular diseases, where unusually high or low enzyme expression is seen as part of the pathophysiological process. This observation suggests that acid phosphatases

could be diagnostically useful as serological and histological markers of disease, and could also be of use in the investigation of the pathophysiology of the associated disease. Prostate acid phosphatase (PAP) has been used extensively as a serum marker for cancer of the prostate. It is released into the serum from the prostate gland in increasing amounts as malignant tissue proliferates. In recent years, the enzyme has lost its clinical interest to prostate specific antigen (PSA), which is claimed to be a more sensitive marker for early stage disease (Akimoto et

al., 1997). However, PAP is now receiving renewed interest as a prognostic indicator (Allen, 1995).

These effects of the samples were linearly dose-dependent, and did not differ significantly ($P>0.05$) between groups given Okposi lake samples and those treated with Uburu lake samples. However, the effects produced by lake water were significantly higher ($P<0.05$) than those of salt solutions, suggesting reduction in concentration of toxicants by methods of processing the water into salt. These observations are consistent with previous studies (Agbafor et al., 2007; Akubugwo and Agbafor, 2007; Akubugwo et al., 2007; Agbafor et al., 2010; Agbafor et al., 2011).

In conclusion, Water and salt from Okposi and Uburu salt lakes are toxic. Their toxicity may be due to their constituents which include high levels of trace elements reported in the samples. The methods of salt production used by the indigenes contributed to reduction of this toxicity. The results of this study show that continuous consumption of water or salt samples from the lakes may elicit various disorders, including prostate-related ones. However, efforts are in progress in our laboratory to use more specific markers to confirm this toxicity to prostate, and identify the toxicants responsible. Proper methods of processing are required to reduce the toxicity of the lakes water.

Reference

Agbafor K. N, Akubugwo E. I. 2007. Hypocholesterolaemic effect of ethanolic extract of fresh leaves of *Cymbopogon citratus*, *African Journal of Biotechnology* 6 (5): 596 – 598.

Agbafor, K. N, Akubugwo, I. E, Ugbogu, A.

E. 2007. Toxicological evaluation of Okposi and Uburu salt lakes, *Int. J. Biotechnol. and allied sci* 2 (2): 186 - 189.

- Agbafor, K. N., Nwachukwu, N., Ogbashi, M. E. 2010. Effect of Okposi and Uburu (Nigeria) lakes on trace elements levels and hematological system in rats. *Journal of Science and Technology*, 16: 52-58.
- Agbafor, K. N., Ajah, P. M., Offor, C. E., Igwenyi, I. O, Ibiam, U. A. 2011. Examination of cardiovascular toxicity and trace elements status in albino rats treated with Okposi and Uburu salt lakes (Nigeria). *Research Journal of Environmental Toxicology*. 12: 1-8.
- Akimoto S, Furuya Y, Akakura K, .1997. Relationship between prostate-specific antigen, clinical stage, and degree of bone metastasis in patients with prostate cancer: comparison with prostatic acid phosphatase and alkaline phosphatase. *Int J Urol* 6:572–5.
- Akubugwo, I. E., Agbafor, K. N. 2007. Hepatotoxic evaluation of water and salt from Uburu and Okposi salt lakes, *Estud. Biol.* 29 (66): 99 - 104.
- Akubugwo, I.E., Ofoegbu, C.S., Ukwoma, C.U. 2007. Physicochemical studies on Uburu salt lake, Ebonyi State. *Journal Environmental Toxicology* 1:26-29.
- Albertsen, R.P, Killh, M.S, Yeboah, E.O. 2005. Prostate specific antigen and prostatic phosphatase for monitoring therapy of carcinoma of the prostatic, *Journal of urology*, 156 (10):1026-1010.
- Allen, S. M. 1995. An enzyme linked immunosorbent assay (ELISA) for detection of seminal fluid using a monoclonal antibody to prostatic acid phosphatase. *J*

- Immunoassay*16:297–308.
- Burtis, C. A, Ashwood, E. R. 2003. Tietz Fundamentals of Clinical Chemistry, 5th ed. Elsevier Pub. India
- Charles, C., and David, M.C.G., Diarie H.C. 1999. Physical geology, 8th edition, McGraw Hill companies, New York, 142-160.
- De la Torre, F.R., Saliian, A., Ferrari, L. 2000. Biomarker assessments in Juvenile *Cyprinus carpio* exposed to water born cadmium. *Environ. Pollut.*, 109: 277-282.
- Drastichova, J.E., Svestkova, V. L., Svobodova, Z. 2005. Cytochemical study of carp neutrophil granulocytes after acute exposure to cadmium. *J. Applied Ichthyol.*, 21: 215-219.
- Habib S, Samah, A. 2013. "Effect of Heavy Metals Pollution on Protein Biosynthesis in Catfish," *Journal of Water Resource and Protection*, 5(5): 555-562.
- Lowry, O. H. 1951. Protein measurement with the Folin phenol reagent. *J Biol Chem.* 193(1):265-75.
- Matloob, M.H. 2003. Simultaneous determination of Cd, Pb, Cu, and Zn concentration of heavy Khatchewers using anodic stripping voltammetry, *Journal of the Saudi chemical society*, 7(2): 1-8.
- Ogbanshi, M. E., Agbafor, K. N., Ominyi, C.M., Ugwu, O. P. C., Nwali, B. U., Ali, F. U. 2015. Changes in Reproductive Functions of Adult Male Rats Administered Water and Salt Samples of Okposi and Uburu Nigerian Salt Lakes. *American-Eurasian Journal of Toxicological Sciences*, 7(2): 55-62.
- Roobol, B.M., Anthony, F., Charles P. 2005. Advance prostate cancer symptoms, *Journal of medicine*, 23(7): 142-149.
- Saxena, M., Saxena, H., Sangha, G, Kaur, K. 2008. Effect of heavy metal pollution of water on total plasma proteins and serum protein profiles of Common Carp fish (*Cyprinus carpio*). *The Internet Journal of Veterinary Medicine*, 5(2): 1-2.
- Yu, M. 2001. Environmental toxicants on living system. Lewis publishers, London, 152-205.