PUCPR

HEPATOTOXIC EVALUATION OF WATER AND SALT FROM OKPOSI AND UBURU SALT LAKES, NIGERIA

Avaliação da hepatotoxicidade da água e sal obtidos nos lagos salgados Okposi e Uburu, Nigéria

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Abstract

Evaluation of possible health implications of chronic consumption of salt and water from Okposi and Uburu salt lakes was carried out using adult male albino rats. The rats were placed in groups and orally administered I ml/kg of the raw lake water, semi-processed water or solution of 10% (W/V) of processed salt from the lakes for seven consecutive days. Physical activity, body mass changes, total serum protein, and transaminase activities were monitored and compared with those of control animals given equivalent volume of distilled water. Results showed that the raw (un-processed) lake water samples from both Okposi and Uburu salt lakes caused the greatest observed reduction in physical activities but elicited the highest serum transaminase activities, while the processed salt solution elicited the lower effects, when compared to control. The raw lake waters also caused the greatest reduction in the total serum protein levels ($p<0.05$) relative to the control. However, while the transaminase activities followed the order: raw lake water > semi-processed > processed salt solution, the order in the obtained reduction of weight and total serum protein is; raw lake water > processed salt solution > semi-processed lake water. These results suggest that the raw lake water is toxic and caused the greatest liver damage. Yet, the processing seems to improve the safety of the salt lake water. Processing methods aimed at reducing the possible adverse health effects should be employed.

Keywords*: Okposi lake; Uburu lake; Hepatotoxicity; Water processing.*

Resumo

A avaliação de possíveis implicações na saúde decorrentes do consumo crônico de sal e água provenientes dos lagos salgados Okposi e Uburu foi levada a termo empregando ratos albinos machos adultos. Os ratos foram alocados em grupos que passaram a receber, por via oral, doses de 1 ml/kg de água lacustre in natura, água semiprocessada ou soluções de sal lacustre, processado 10%, por sete dias consecutivos. A atividade física, as erações na massa corpórea, as concentrações de proteínas séricas totais e a atividade de transaminases foram monitoradas e comparadas com aquelas dos animais-controle que receberam volumes equivalentes de água destilada. Os resultados revelaram que as águas dos dois lagos in natura promoveram a maior redução de atividade física, mas também responderem pelas maiores atividades e transaminases séricas, ao passo que as soluções de sal processado parecem ter causado os menores efeitos, quando comparados com os do grupo controle. As águas in natura também causaram as maiores reduções nos níveis de proteínas séricas totais ($p<0.05$) em relação ao controle. Contudo, enquanto as atividades de transaminases seguiram a seguinte ordem: águas in natura > água semiprocessada > soluções de sal processado, a ordem de redução de massa corpórea e de proteínas séricas totais foi: água in natura > soluções de sal processado > água semiprocessada. Estes resultados sugerem que a água dos lagos salgados Okposi e Uburu consumida in natura é tóxica e causou os maiores danos hepáticos. Ainda, o processamento parece melhorar a segurança das águas dos lagos em questão. Métodos de processamento que visem a redução de possíveis efeitos adversos à saúde devem ser empregados.

Palavras-chave: Lago Okposi; Lago Uburu; Hepatotoxicidade; Processamento de água.

INTRODUCTION

Salt lakes are products of volcanic, glacial, tectonic and river activities which leave depressions and cavities (1). A salt lake is a land locked body of water which has a concentration of salts (mostly sodium chloride) and other minerals significantly higher than most lakes (often defined as 3,000 miligram salt per litre). Salt lakes have been shown to contain metals and non-metallic ions such as cadminum, calcium, cobolt, lead, manganese, mercury, nickel, iron, biocarbonate, sulphate, bromine, fluorine, in addition to sodium and chlorine (2).

In deed, high heavy metals presences have been reported in Wadi EL Natrun saline lakes of Egypt (3) and even the Uburu salt lake in Ebonyi state, Nigeria (4).

Heavy metals are released into water bodies such as streams, lakes, rivers and ground water from either natural processes or anthropogenic sources (5, 6, 7). Major sources of heavy metals arising from anthropogenic activities are domestic, industrial waste waters and solid wastes, as well as fertilizers for agricultural practices. Natural sources of heavy metals may include natural erosion and weathering of crustal materials (8). In Ohaozara local Government Area where Okposi and Uburu salt lakes are situated, there are active lead mining operations. These contributed to increased lead levels in water and biological fluids of residents (9).

As a follow up to our study on the physicochemical properties of Uburu salt lake, the present study has been designed to evaluate possible health implications of consumption of the salt water and or salt produced from Okposi and Uburu salt lakes. The liver was chosen because it is an organ central in biochemical homeostasis (10). To assess the functional state of the liver, the transaminases were chosen. They are enzymes which catalyse the transfer of amino groups from alpha-amino to alpha-keto acids. Aspartate amino transferase (AST) and Alanine amino transferase (ALT) are important in diagnosis of liver damage caused by chemical toxicity or infection. These enzymes are located intracellularly, but leak out into the blood stream when the tissue is damaged. AST rises up to 100 times the upper limit in severe tissue injury such as acute hepatitis and liver necrosis. Concomitant rise in ALT levels occur with AST. Since ALT is found in both cytoplasm as well as mitochondria (AST is not present in mitochondria), greater release of it suggest gross damage that have penetrated beyond the cytoplasm as occur in severe and / or chronic damage (11, 12).

Though consumption of water from Utah's Great Salt Lake in USA have been reported to produce amazing health benefits (because of its trace elements content) (13) our earlier studies on the physicochemical properties of Uburu salt lake did not seem to suggest that its consumption will be beneficial to health (4).

The present study is therefore designed to give a clear picture of possible health implications of consumption of water and salt produced by evaporation of water from the salt lakes.

MATERIALS AND METHODS

Sampling site, sample collection and treatment

Okposi and Uburu salt lakes are situate in Ohaozara Local government area of Ebonyi State, Nigeria. The community settlers are predominantly farmers. Lead mining activities are also active in the local government area.

The samples used for this study were collected in the month of March directly from the sources. The raw (un-processed) salt lake water samples were collected with sterilised plastic bottles directly from the lakes. Each of the two lakes was divided into transects of North, South, East and West about an epicentre. Five water samples were collected randomly from each transect, pooled together to obtain a unity sample that was used for the study. The semi-processed (filtrate) samples were also collected with sterilised bottles from the local people. Semi-processed sample are lake water samples that have been allowed to stand in the sun for sometime in earthen pots containing mixtures of salty dry clay soil and ash and filtered. The pots have tiny opening at the bottom through which the brine being filtered drips into another earthen pot placed beneath it. This is the first stage of salt production process employed by the local people. The processed salt samples are obtained by evaporating the filtrate obtained in stage one above to dryness using fire wood. Five different samples of each sample type (either semi-processed or processed) were now randomly collected and pooled to obtain unity sample used for the study. The salt solution from the processed salt was prepared by dissolving 1g of the salt in 10cm of distilled water. ($10\%^{W}/_{V}$).

Animals and handling

35 adult male albino rats were purchased from the animal house of the University of Nigeria Teaching Hospital, Enugu. They were transported to the animal house, Department of Biochemistry and Biotechnology, Ebonyi State University, Abakaliki in plastic cages with raised metal mesh. Here, they were acclimatised for one week, weighed and placed in seven groups (A-G) of five animals each. The animals were allowed free access to water and feed (rat chaw) before and through out the period of the experiment.

Animal groups and treatment

Animals were placed in seven groups, and administered a daily dose of 1ml/kg of test salt solutions for seven consecutive days as follows

Group A: Unprocessed Okposi salt lake water
Group B: Semi-processed Okposi salt lake water
Group C: 10% ($^{w}/_{v}$) solution of processed salt from Okposi salt lake
Group D: Un-processed Uburu salt lake water
Group E: Semi-processed Uburu salt lake water
Group F: $10\%(^{W}/_{V})$ solution of processed salt from Uburu salt lake
Group G: distilled water (used to dissolve salt in group C and F).

Collection of blood for Analysis

Blood samples were collected from the rats following an overnight fast through cardiac puncture under mild anaesthesia using diethylether. The blood samples were allowed to clot and then centrifuged at 3000xg for 10 minutes to obtain a clear serum used for the determinations.

Determination of transaminases activity

ALT and AST activity were measured according to (14) as described by (15) 0.5ml of substrate (2mM oxoglutarate +0.2M alanine +1M NaOH + phosphate buffer pH 7.4 for ALT; (2mM oxogluterate + 0.2M aspartate + 1M NaOH + phosphate buffer pH 7.4 for AST) was incubated at 37^{0}C for 5 min before 0.1ml of serum was added. The mixture was incubated at 37^{0}C for 60 min

(AST) and 30 min (ALT) 0.5 ml of 2,4-dinitrophenylhydrazine was added and the entire mixture kept at room temperature for 20 min before the addition of 5 ml of NaOH. The absorbances were read at 540 nm against a blank. The amount of keto acid formed which represent enzyme activity was obtained from keto acid standard curve.

Determination of total protein

Protein levels in the serum were determined by the method of Lowry (16), using bovine serum albumin as standard. 0.4ml of serum was mixed with 0.2ml of 2x Lowry concentrate and incubated at 37^0C for 10 min before adding 0.2 ml of 0.2 N Folin reagent. The mixture was incubated further at 37^oC for 30 min. Absorbance was read at 75 0nm against a blank. 2x Lowry concentrate – 30ml of copper reagent + 10ml 1% SDS + 10ml 0.04M NaOH. Copper reagent – 20g Na_2CO_3 in 260ml of H_2O + 0.4g $CuSO_4$. $5H_20$ in 260 ml. of water + 0.2g NaK tartarate in 20 ml H_20.

Data analysis

Statistical analysis was done using Analysis of variance (ANOVA). Means were compared for significance using Duncan's multiple Range test ($P<0.05$) (17).

RESULTS AND DISCUSSION

Decreases in physical activities, food and water intake were observed in the test animals (data not shown) while the animals in the control group thrived. These observations may be linked to the chemical constituents of the salt lakes (18, 19). The presence of Pb in concentrations higher than WHO'S permissible limit have been reported in many potable water sources and biological fluids of residents of Okposi and Uburu communities (10). It has also been observed that Mn and Fe occurred at levels higher than WHO's permissible limits in Uburu salt lake (4). These heavy metals are known to be toxic to organism at high concentration. The manifestations of Pb poisoning among other disorders include muscle aches and pains as well as loss of appetite (20). Therefore, lead toxicity may account in part for the observed decrease in physical activity in the exposed animals.

The body weight of the animals in the test groups decreased (Table 1), while the control animals gained weight. These weight loses were significant ($p<0.05$) relative to the control. The observed loss of appetite may account in part for the weight loss. Apart from this, direct effect of the constituents of the lake water in the animals which will cause metabolic changes consequent upon their ingestion certainly contributed to the observed changes.

TABLE 1 - Weight changes, serum total protein and transaminases activities in rats after seven days administration of water samples From Okposi and Uburu salt lakes – Animal Test

Group	Protein conc.Mg/dl	Transaminase activity (1U/L) ALT	AST	Change in weight (g) (final – Initial)
A	56.00 ± 3.00^b	35.92 ± 1.43^b	38.18 ± 0.96^b	-17.79 ± 2.87^a
B	58.00 ± 3.00^b	44.99 ± 3.10^c	48.38 ± 1.35^c	-15.05 ± 3.11^a
C	37.00 ± 1.00^a	75.68 ± 2.52^e	80.16 ± 2.11	-12.71 ± 0.68^a
D	53.00 ± 2.00^b	38.20 ± 1.94^b	47.02 ± 2.36^c	-5.91 ± 2.90^b
E	55.00 ± 1.00^b	42.15 ± 2.30^c	50.95 ± 1.20^c	-11.53 ± 0.98^a
F	42.00 ± 1.00^a	66.52 ± 2.53^d	74.36 ± 1.44^d	-13.81 ± 0.87^a
G	61.00 ± 2.00^b	11.12 ± 1.23^a	12.75 ± 0.86^a	12.14 ± 1.72^c

Figures are mean ± SD, Values bearing different superscripts differ significantly ($P<0.05$); N = 5

Legend:
Group
A = Animals given 1ml/kg, raw Opkosi salt lake water
B = semi-processed
C = 10% (w/v) salt solution of processed salt from Okposi salt lake water
D = raw Uburu salt lake water
E = semi-processed uburu salt lake water
F = 10% (w/v) salt solution of processed salt from Uburu salt lake water
G = of distilled water (control)

Total serum levels decreased in the test animals compared to the control (table 1). The observed decreases were only significant ($P<0.05$) in the animals given the raw lake water from both Okposi and Uburu salt lakes. Processing of lake water reduced their toxicity. A closer look at the serum protein level indicated that the processed samples were more toxic compared to the semi-processed samples. This seeming paradox may be explained by the lake water constituents and the possible effects of processing methods. Processing may have either resulted in the concentration of some factors which inhibit protein synthesis or converted some factors to their more toxic forms.

When a toxic substance enters an organism, binding occurs to various ligands in its cells or tissues. If the number of molecules of the toxic substance is small, there is usually no detectable influence on the function of the cell. When larger numbers of the molecules are bound the degree of effect depends on the function of the binding ligand (21). Heavy metals like lead and cadmium accumulate in the body during exposure and exert their effects once the critical concentration is attained in the critical organ (22). This will account for most of the observed changes in the test animals. It is known for example that one of the ways heavy metals exert their toxic effects is by binding to the sulfhydryl groups in proteins leading to inhibition of activity or disruption of structures or by displacing an essential element resulting in deficiency effects.

The effect of administration of the lake waters on serum transaminase activities is shown in table 1. The result indicates that raw lake water from each of the two sources (Okposi and Uburu) caused significant ($p<0.05$) elevation of transaminase activity in each treatment relative to the control .This suggests high hepatotoxic effect of the test samples. Indeed, heavy metals such as Fe and Mn which have been reported in high concentration in the lake water are hepatotoxic (23).

CONCLUSION

Salt and water from Okposi and Uburu salt lakes are hepatotoxic in rats. Their toxicity may be related to their constituents which include lead. Obviously, the method of production of salt employed by the local people contributed to reduction in the toxicity of the lake water. The results of this study strongly suggest that continuous consumption of salt or water samples from the lakes may cause various disorders including liver damage. However, proper processing methods are necessary to detoxify the salt water and make it safe for consumption.

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