



Research Journal of
**Environmental
Toxicology**

ISSN 1819-3420



Academic
Journals Inc.

www.academicjournals.com

Examination of Cardiovascular Toxicity and Trace Elements Status in Albino Rats Treated with Okposi and Uburu Salt Lakes (Nigeria)

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ABSTRACT

This study was aimed at investigating the effect of water and salt samples from Okposi and Uburu salt lakes on some serum trace elements and the cardiovascular system. Serum levels of copper (Cu), cadmium (Cd), cobalt (Co) manganese (Mn), cardiac troponin I (cTnI) and lipid profile were measured in albino rats after treating them with different doses of salt and water from the lakes for seven consecutive days. Average body weight, physical activities and food and intake decreased in all the treated groups compared with the control. The levels of Cu, Cd, Co and Mn in test groups were significantly higher ($p < 0.05$) than in the control. There was a significant increase ($p < 0.05$) in the concentrations of cardiac troponin I, cholesterol, triacylglycerol and low density lipoproteins, in the treated groups relative to the control while high density lipoproteins in the control were significantly higher ($p < 0.05$) than the treated groups. The observations were also statistically more pronounced ($p < 0.05$) in the groups given water samples than those administered salt samples. The differences in the levels of these parameters between groups given Okposi samples and those treated with Uburu samples were not significant ($p > 0.05$). The effects of the samples were found to be dose- dependent. These results indicate that the samples may be toxic to the cardiovascular system and this toxicity may be reduced by the methods of processing the salt. The toxicity of the samples may be due to the chemical contents of the lakes.

Key words: Serum, deionized water, trace elements, cardiac troponin I, lipid profile

INTRODUCTION

Lakes are products of volcanic, glacial, tectonic and river activities which leave depressions and cavities on land surface (Charles *et al.*, 1999). 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 g salt per litre). Salt lakes have been shown 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). Indeed, a salt lake is a complex solution of mineral salts and decayed biological matter that results from life in the lake. The chemical constituents of salt lakes originated from either natural processes (erosions and weathering of coastal materials) or anthropogenic sources (domestic, industrial and agricultural practices) (Charles *et al.*, 1999).

Okposi and Uburu salt lakes are located in Ohaozara local government area of Ebonyi state, Nigeria. The lakes serve as salt (obtained after heating lake water to dryness) and water sources for most domestic purposes of the inhabitants of the communities who are mainly farmers.

Akubugwo *et al.* (2007) have reported the presence of metallic and non-metallic ions in the lakes. Hepatotoxicity of the salt lakes has been demonstrated by Akubugwo and Agbafor (2007).

Copper (Cu) toxicity may lead to liver impairment, gastrointestinal tract disturbance, loss of appetite, anemia, nervous system damage, renal failure, cardiovascular collapse, tachycardia and so on (Sarava *et al.*, 2007). Exposure to cobalt results to a wide spectrum of toxicities in mammals. Cobalt toxicity results to polycythemia, hypertriglyceridemia, hypercholesterolemia, cardiomyopathy, etc. (Taylor and Marks, 1978). The adverse health effects caused by ingestion or inhalation of cadmium (Cd) include renal tubular dysfunction from high urinary excretion of Cd, lung damage, lung cancer and hypotension (Yu, 2001). Exposure to manganese (Mn) may lead to neurological disorders similar to those of Parkinson's disease. Manganese interferes with absorption of dietary iron, resulting to iron deficiency. It also impairs the activity of Cu metalloenzymes (Blaurock, 1997).

The cardiovascular system refers to the heart and the blood vessels. The heart is a muscular organ responsible for moving blood through the vessels to all parts of the body (Burtis and Ashwood, 2003). Cardiac troponins I are cardiac markers with extraordinary high specificity for myocardial cell injury. They can be used in a variety of clinical situations, including differentiation of skeletal from cardiac muscle injury; detection of minor myocardial cell damage, detection of preoperative myocardial infarction and estimation of infarct size (Coudry, 1998). Acute myocardial injury can be sensitively and accurately identified by measurement of plasma or serum concentration of cardiac troponin I (cTnI), one of the contractile proteins of the myocardium. A rise in the concentration of TnI in the circulation indicates various degrees of myocardial cell damage (Cardinale *et al.*, 2002). Lipid profile constitutes total cholesterol, High Density Lipoproteins (HDL), Low Density Lipoproteins (LDL), Very Low Density Lipoproteins (VLDL) and triacylglycerols. Examination of lipid profile is useful in assessment of the risk of cardiovascular disease (Robert, 2006). Okposi and Uburu salt lakes have been reported to be toxic to hepatic and renal systems. In this communication, the present study investigated the effect of the lakes on the cardiovascular system.

MATERIALS AND METHODS

Collection of samples: Each of the lakes was divided into transects of North, South, East and West from a reference epicenter. The study was performed in the month of March, 2010. Five water samples were randomly collected from each of the transects and pooled to get a unity sample. In the same vein, five salt samples of each lake were obtained from the local people and pooled in each case to also get a homogenous unity sample. The 0.1 g mL⁻¹ salt solution was prepared with deionized water.

Animals and handling: Forty-five adult male albino rats, weighing 110-122 g were brought from the animal house of Biochemistry Department, University of Nigeria, Nsukka. They were placed in nine groups (A-I) of five rats in each group and kept in animals house of Biochemistry Department, Ebonyi State university Abakaliki for seven days to acclimatize. All the rats were allowed free access to feed (rat chaw) and water before and throughout the experiment.

Animal groups and treatments:

Group A : 50 mg kg⁻¹ body weight salt solution from Okposi lake

Group B : 100 mg kg⁻¹ body weight salt solution from Okposi lake

- Group C :** 1 mL kg⁻¹ body weight salt water from Okposi lake
- Group D :** 2 mL kg⁻¹ body weight salt water from Okposi lake
- Group E :** 50 mg kg⁻¹ body weight salt solution from Uburu lake
- Group F :** 100 mg kg⁻¹ body weight salt solution from Uburu lake
- Group G :** 1 mL kg⁻¹ body weight water from Uburu lake
- Group H :** 2 mL kg⁻¹ body weight water from Uburu lake
- Group I :** Deionized water (used to dissolve the salts)

The treatment was done orally for seven consecutive days.

Collection of blood for analysis: Blood samples were collected from the animals following an overnight fast through cardiac puncture under mild anaesthesia using diethylether. The samples were put into specimen bottles without anticoagulant.

Analysis of trace elements: Serum levels of Cu, Cd, Co and Mn were measured with flame Atomic Absorption Spectrophotometer (AAS) using a direct method described by Kaneko (1999).

Measurement of serum cardiac troponin I levels lipid profile: The method employed for cardiac troponin I (cTnI) determination was an immunoenzymatic fluorescent assay of Bodor *et al.* (1992). The serum concentrations of the individual component of lipid profile were determined according to the methods used by Akpanabiatu *et al.* (2005).

Data analysis: Statistical analysis was done using Analysis Of Variance (ANOVA). Means were compared for significance using Duncan's Multiple Range test ($p < 0.05$) (Sokal and Rohlf, 1969).

RESULTS AND DISCUSSION

There were significant decreases in physical activities, food and water intake in the treated groups (data not shown) while the control group thrived. These observations may be attributed to the chemical constituents of the salt lakes (Yu, 2001). The presence of Pb, Mn, Cr, Cu, Fe, Cd etc, in concentrations higher than WHO'S permissible limit have been reported in water sources, including the lakes and biological fluids of residents of Okposi and Uburu communities (Akubugwo *et al.*, 2007; Akubugwo and Agbafor, 1997). The manifestations of Pb poisoning among other disorders include muscle aches, pains and loss of appetite (Yu, 2001). These may have contributed to the observed decrease in physical activities, food and water intake. Further, distortion of metabolism by other constituents of the lakes may not be ruled out. Some surface water in Nigeria are known to be polluted such that their constituents elicit adverse effects (Agbafor *et al.*, 2010).

The result of changes in body weight of the animals during the period of treatment is shown in Table 1. There was a significant decrease ($p < 0.05$) in weight of the test groups compared with the control (which gained weight). This may be attributed to the reported reduction in food and water intake. However, direct effect of the constituents of the lakes water in the rats which will cause metabolic changes consequent upon their ingestion certainly contributed to observed changes. For example, symptoms of cadmium (Cd) toxicity include sensory disturbances and weight loss (Yu, 2001). These observations are inline with those reported by Akubugwo and Agbafor (2007).

Table 1 also presents the effect of the samples on trace elements (Cu, Cd, Co and Mn) of the animals after administration. There was a significant increase ($p < 0.05$) in the levels of the trace

Table 1: Change in body weight and serum levels of Cu, Cd, Co and Mn of the rats after seven days of treatment

Group	Change in wt g ⁻¹	Mn (µg dL ⁻¹)	Cu (µg dL ⁻¹)	Cd (µg L ⁻¹)	Co (µg L ⁻¹)
A	-4.95±1.11 ^a	80.51±6.30 ^a	144.04±3.55 ^a	86.30±2.11 ^a	0.64±0.13 ^a
B	-10.13±1.06 ^b	159.73±4.50 ^b	160.35±2.81 ^a	133.52±3.09 ^a	0.89±0.04 ^a
C	-13.20±1.23 ^b	176.81±5.31 ^b	189.77±5.60 ^a	165.35±2.22 ^b	1.66±0.15 ^b
D	-17.88±1.60 ^b	208.20±5.72 ^c	226.23±5.14 ^b	198.72±3.55 ^b	1.90±0.11 ^b
E	-6.31±0.94 ^a	69.62±4.40 ^a	130.45±4.60 ^a	80.13±2.50 ^a	0.49±0.08 ^a
F	-8.66±0.70 ^a	146.30±5.55 ^b	151.80±3.62 ^a	121.70±3.16 ^a	0.53±0.03 ^a
G	-12.02±1.33 ^b	166.54±6.11 ^b	170.88±4.33 ^a	160.51±2.75 ^b	1.25±0.21 ^b
H	-16.53±1.04 ^b	191.72±5.80 ^c	213.15±4.70 ^b	185.62±2.30 ^b	1.53±0.13 ^b
I	5.83±0.76 ^c	46.33±3.60 ^d	83.41±3.62 ^c	47.54±2.55 ^c	0.17±0.02 ^c

Values are Mean±SD. Values bearing different superscripts differ significantly (p<0.05). n = 5. Table 1 shows changes in weight and trace element levels of the rats during and after treatment respectively. Weight changes ranged from 4.95±1.11 to 17.88±1.60 g while in trace elements, Cu produced highest value of 226.23±5.14 µg dL⁻¹ and Co least with a value of 0.17±0.02 µg L⁻¹

elements in treated groups relative to the control. The increase in the levels of these trace elements may be related to their reported high levels in the salt lakes (Akubugwo *et al.*, 2007; Akubugwo and Agbafor, 1997).

The effects of the water and salt samples on serum levels of cardiac troponin I and lipid profile are presented in Table 2. The concentrations of cardiac troponin I in the treated groups were significantly higher (p<0.05) than in the control group. Although the biochemical basis of this increase is currently obscure, it may be attributed to damage of heart muscle cells (myocytes). Measurement of plasma or serum concentration of cardiac troponin I, one of the contractile proteins of the myocardium, can be used to sensitively and accurately identify acute myocardial injury. Elevation of circulatory level of this protein indicates various degrees of myocardial cell damage (Cardinale *et al.*, 2002). The chemical constituents of the lakes may be responsible for this possible myocardial cell injury. For example, animal studies indicate that manganese is capable of quickly accumulating in the heart tissue, resulting in acute or subacute cardiovascular disorders, such as acute cardiodepression and hypotension. These toxic outcomes appear to be associated with Mn-induced mitochondrial damage and interactions with the calcium channel in the cardiovascular system (Jiang and Zheng, 2005). Copper toxicity has been linked with cardiovascular collapse, hypotension and tachycardia. Severe methemoglobinemia, caused by Cu, can result in cardiac dysrhythmia and hypoxia which could contribute significantly to cardiovascular collapse. Other factors are direct effect of Cu on vascular and cardiac cells and sepsis due to transmucosal invasion (Sarava *et al.*, 2007). Further, according to Liu *et al.* (2010), microscopic observation showed that high-dose cobalt chloride caused significant hyperemia, swelling of the heart and spotty necrosis. Cobalt ions are present in the body as radical ions which can cause lipid peroxidation, leading to decreased fluidity, increased fragility and altered permeability of cell membranes (Liu *et al.*, 2010).

The results of lipid profile shown in Table 2 indicates the levels of cholesterol, triacylglycerols and low density lipoproteins in the test groups increased significantly (p<0.05) relative to the control while high density lipoprotein in the control was significantly higher (p<0.05) than in the treated groups. The actual mechanism involved in these observations is currently being studied. However, the effects of the constituents of the samples administered to the animals may have contributed. For instance, one effect of cobalt administration on blood is an increase in triacylglycerols, cholesterol and free fatty acids (Taylor and Marks, 1978). This may be due to

Table 2: Serum cardiac troponin I levels and lipid profile of the rats after seven days of treatment

Group	cTnI (mg mL ⁻¹)	TC (mg dL ⁻¹)	TG (mg dL ⁻¹)	HDL (mg dL ⁻¹)	LDL(mg dL ⁻¹)
A	1.04±0.16 ^a	261.45±3.66 ^a	218.60±5.17 ^a	28.53±1.40 ^a	197.20±4.12 ^a
B	2.35±0.08 ^b	344.60±2.79 ^a	275.18±4.10 ^a	16.35±2.01 ^a	273.21±3.55 ^a
C	2.77±0.13 ^b	335.16±4.11 ^a	288.62±4.53 ^a	11.61±1.22 ^a	265.83±3.17 ^a
D	3.50±0.12 ^b	428.77±3.50 ^b	402.17±3.23 ^b	4.65±0.63 ^b	343.69±2.62 ^b
E	1.07±0.05 ^a	253.13±6.21 ^a	223.52±5.33 ^a	22.40±2.31 ^a	186.03±2.86 ^a
F	2.23±0.15 ^b	306.72±4.33 ^a	280.43±3.60 ^a	20.05±1.32 ^a	230.58±4.40 ^a
G	2.65±0.21 ^b	340.35±3.73 ^a	311.76±4.12 ^a	15.34±1.05 ^a	262.66±2.63 ^a
H	2.94±0.15 ^b	386.48±5.16 ^b	360.55±4.34 ^b	8.20±1.12 ^b	59.14±2.13 ^b
I	0.42±0.02 ^c	134.33 ±2.66 ^c	98.15±3.50 ^c	39.51±2.10 ^c	46.36±3.44 ^c

Values are Mean±SD. Values bearing different superscripts differ significantly (p<0.05). n = 5. In Table 2, serum cTnI ranged from 0.42±0.02 to 3.50±0.12 mg mL⁻¹. TC, TG, HDL and LDL were 134.33±2.66 to 428.77±3.50, 98.15±3.50 to 402.17±3.23, 4.65±0.63 to 39.51±2.10, 46.36±3.44 to 343.69±2.62, respectively, LDL: Low density lipoprotein, TC: Total cholesterol, cTnI: Cardiac troponin I, TG: Triacylglycerol, HDL: High density lipoprotein

inhibition of tissue lipoprotein lipase, resulting in the failure to clear very low lipoproteins (Taylor and Marks, 1978) and perhaps by stimulation of lipoprotein synthesis by the liver (Eaton, 1972).

The chief role of cholesterol, triacylglycerols and lipoproteins (except high density lipoproteins) in pathological processes is as factors in the genesis of atherosclerosis of vital arteries, causing cerebrovascular, coronary and peripheral vascular disease (Murry *et al.*, 2003). For example, low density lipoproteins pose a risk for cardiovascular disease when it invades the endothelium and becomes oxidized, since the oxidized form is more easily retained by proteoglycans (Cromwell and Otvos, 2004). This oxidation is chiefly stimulated by free radicals in the endothelium (Cromwell and Otvos, 2004).

Comparing the groups treated with salt solution with those given lake water, the later consistently showed higher potency. This points to the fact that the procedures involved in the processing of the salt water into salt affect the chemical composition of the salt lakes. The difference between the results from the groups treated with samples from Okposi and those from Uburu was not significant (p>0.05). The effects of all the treatments were found to be dose-dependent. These observations have been reported by Akubugwo and Agbafor (2007).

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. The methods of salt production used by the indigenes contributed to reduction of this toxicity. The results of this study suggest that continuous consumption of water or salt samples from the lakes may elicit various disorders, including loss of appetite and cardiovascular system disorders. However, proper methods of processing are required to reduce the toxicity of the lakes water.

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