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Administration of Water and Salt Samples from Okposi and Uburu Nigerian Salt Lakes Induce Oxidative Stress in the Reproductive Parameters of Adult Male Sprague-Dawley Rats

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Abstract: Organochlorinated pesticidespolychlorinated biphenyls composition of Okposi and Uburu salt lakes and the possible effect of its consumption to some reproductive parameters were determined. The organochlorinated pesticides compound and polychlorinated biphenyls composition were determined using gas chromatography coupled with mass spectroscopic equipments. 200 adult male Sprague -Dawley rats were grouped into eighteen groups of A to Q. Group A to D were given 0.5ml, 1.0ml, 2.0mls and 4.0mls of water from Uburu salt Lake. Group E to H were administered 50, 100, 200 and 400mg/kg of salt respectively, fromUburu salt Lake. Group I to L were given 0.5, 1.0, 2.0 and 4.0mls of water from Okposi salt Lake and group M to P were administered 50, 100, 200 and 400mg/kg of salt from Okposi salt Lake while group Q received deionised water to serve as the control. The experiment lasted for 90 days. At the end of 90 days, the animals were sacrificed. Testes, epididymis and prostate gland were immediately removed and dissected out, cleared from the adhering tissues, blotted dried and weighed individually.Tissues homogenateswere prepared and were used to measure the extent of lipid peroxidation in the reproductive tissues. There was a significant decrease in the testicular weight, epididymal weight and prostate weight in all the treated groups. Rats treated with Okposi and Uburu salt lake water and salt sample showed significant elevation in the level of formation of thiobarbituric acid reactive substances concentration. Reduced glutathione concentration and the activities of catalase and superoxide dismutase in the testes were all significantly reduced in the Okposi and Uburu salt lake sample treated male albino rats in this study. Chemical analysis of the Lakes showed a significant level of some of the organochlorinated pesticides. No polychlorinated biphenyls were found to be present in both lakes.Result shows that consumption of Okposi and Uburu salt lake unprocessed water and salt samples could be dangerous to health and might lead to reproductive function impairment.

Key words: Reproductive Toxicity · Persistent Organic Pollutants · Heavy Metals · Antioxidants · Salt Lakes and Fertility

INTRODUCTION

OkposiandUburu salt lakes are found in Ohaozara Local Government Area inEbonyi State, Nigeria.The lakes serve as salt (obtained after heating lake water to dryness) and water sources for most domestic purposes for the rural inhabitants of these communities who are mainly farmers. Akubugwo *et al.*[1] have reported the presence of metallic and non-metallic ions in these lakes. Cardiovascular toxicity has also been reported [2]. These toxic effects have been attributed to the chemical constituents of the lakes [3].

The World Health Organization defines infertility as the inability of a couple to achieve conception or bring a pregnancy to term after 1 year or more of regular, unprotected sexual intercourse [4]. Presently, the etiology of suboptimal semen quality is not clearly understood and data on it is very limited, many physiological, environmental, genetic factors, as well as oxidative stress have been associated to infertility [5]. Exposure to insecticides, pesticides, heavy metals and organochlorinated compounds has been documented (in animals and humans) with occurrence of spontaneous abortion, low birth weight, birth defects and change in

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male: female sex ratio of offspring, inhibition of spermatogenesis and ovogenesis, destruction of seminiferous epithelium and hydroceles resulting to decrease in fertility [6].

Okposi and Uburu salt lakes located in Ohaozara Local Government Area of Ebonyi State Nigeria is two of the most important lakes used as source of water for domestic purposes and as cooking salt obtained after heating lakes water to dryness. There are a large number of farms and cities that are potential sources of contamination to these lakes. Carbon tetrachloroethane is a carcinogen that might also cause acute effects to liver, kidneys, cardiovascular systems as well as reproductive impairment [7]. Most of the sturdies by researchers including workers exposed to these toxicants and other populations with high body burdens of polychlorinated biphenyl revealed association between polychlorinated biphenyl and hepatic indices involving microsomal enzymes of the liver and lipids [8]. Human populations that were orally exposed to mixtures of polychlorinated biphenyl and a host of other persistent organic pollutants showed high alteration and weakening of the immune status in infants as well as in adult humans [9]. The children of mothers who consumed Lake Michigan or Sheboygan River fish was found to be infected by some infectious diseases which was later found to be associated with polychlorinated biphenyls. IgA and IgM antibody in the serum decreased drastically in Yusho and Yu-Cheng population and even in the Inuit children [10].

Lake Michigan fishes have a high content of polychlorinated biphenyls and women who eat fishes from this lake are highly exposed to these contaminants. Newborns from these women who eat a lot of fishes from this lakes were found to have greater numbers of abnormal reflexes and more motor immaturity when compared to newborns from mothers who eat less fishes from this lake (decreased polychlorinated biphenyl exposure) suggesting that persistent organic pollutants could be associated with neurological effects [11-14]. Close findings were observed in a North Carolina research with children from women with reduced polychlorinated biphenyl exposure levels. Also, in an Oswego, New York examination of children from women with very high consumption of polychlorinated biphenyls from fish obtained from Lake Ontario revealed similar result [15- 18]. Prenatal exposure to polychlorinated biphenyls was noted to be associated with memory and intelligent quotient score deficits when measured by polychlorinated biphenyls in umbilical cord blood. The children that were most highly exposed were discovered to have three times as likely to have low average intelligent quotients scores and possibly twice as likely to be up to one year backward in comprehension during reading [19].

On a general note, a study of reproductive end points in humans is limited. However, markers such as weight of existing humans and data on laboratory animals showed that persistent organic pollutants such as polychlorinated biphenyls, tertiary methyl butyl ether and organochlorinated pesticides metabolites is possibly a potential reproductive hazard to man[20]. Other studies which looked at the reproductive endpoints in mothers with diets containing fishes from Great Lakes found that consumption of the fish might be linked with a little shorter length menstrual cycle and decreased fecund ability within couples trying to bring pregnancy to term. However, not with elevated risks of conception difficulty and delay. Menstruation variation (intervals altered, flow and duration) have all been noticed in women who were exposed to high doses of polychlorinated biphenyls during the *Yusho* poisoning incident [21- 24].

MATERIAL AND METHODS

Collection of Samples: Samples were collected in the month of March 2011 during dry season. The bottles for sample collection were washed with deionized water. The lakes were aportioned into transact of North, South, East and West. Four samples were collected differently from each transact and mixed to get a homogenous sample which were used for the study according to the method of Agbafor *et al*. [2].

Salt Sample Collection and Preparation: Five salt samples were bought from the local people and ground together to get a homogenous unity sample. A stock solution of 400mg/ml was prepared by dissolving 40g of salt in 100mls of deionized water.

Determination of Organochlorinated Pesticides and Polychlorinated Biphenyls: The determination of the concentration of OCPs and PCBs in the samples were performed using an Agilent 6890 gas chromatography coupled with mass spectroscopy (GC/MS) that is equipped with a micro cell electron capture detector (μECD) and DB-5 capillary columns (35m x 0.32mm id x 0.25μm film thickness). The temperature of the oven for OCPs were set at 50°C and was held for 5min before increasing it to 220°C at 35°C/min and then finally kept steady for 20min. For PCBs, it was set at 50°C and then held for 5min before raising it to 280°C at 4°C/min and

held for 10min. The recoveredmatrix spikes varied from 76.6% to 126% for polychlorinated biphenyls and thatorganochlorinatedpesiticdes varied from 59.6% to 87.4% for. The limits of detection were 0.01mg/l for PCBs and 0.01mg/l for OCPs

Animals Samples and Treatment: 200 male bred Sprague-Dawley Rats weighing 170-200g obtained from Manchester school of veterinary institute were used in the study. The animals were housed in a centralized animal care facility maintained at 22 to 25°C with a relative humidity of 76 ± 5%. Standard pelleted food and deionized water were provided for the animals' *ad libitium*.

Administration of Samples: Salt samples from Okposi and Uburu salt lakes were dissolved in deionized water and labeled sample A and A_1 respectively. The raw water samples from Okposi and Uburu salt lakes were designated sample B and B_1 respectively. The animals were grouped into 20 groups with each group containing five rats. 50, 100, 200 and 400mg/kg body weight of sample A were administered to group C, D, E and F respectively while group G were given deionized water only to serve as the control group. 50, 100, 200 and 400mg/kg body weight of sample A_1 were administered to group H, I, J and K respectively. 0.5, 1.0, 2.0 and 4.0ml/kg body weight of sample B were administered to group L, M, N and O respectively. 0.5, 1.0, 2.0 and 4.0ml/kg body weight of sample B_1 were administered to group P, Q, R and S respectively. All the administrations were done orally. The experiment was performed in three batches and in each batch, administration lasted for eight weeks

Collection of Samples from the Animals: At the end of eight weeks of administration, the animals were sacrificed. Reproductive tissues such as the testes, seminal vesicles, epididymis and prostate gland were immediately dissected out, cleared from the adhering tissues, blotted dried and weighed individually. Semen samples were obtained from the epididymis and biochemical analysis was carried out in each batch.

Preparation of Tissue Homogenate: A part of the right testicles and epididymis of each rats were collected for biochemical analysis and homogenates were prepared. Sample were perfused in 0.9% saline, testes and epididymis were crushed in 0.2M sodium phosphates buffer with pH 6.25 (1:20, w/v) in an Elvehjem Potter homogenizer coupled with a Teflon pestle. The homogenates were centrifuged at 10,000g for a period of 1hr and the supernatants obtained were preserved at -20°C and utilized for biochemical analysis within one week.

Evaluation of Testicular Superoxide Dismutase SOD Activity: Superoxide dismutase activity was assayed using the methods of Mari *et al.*[13]. Cu, Zn-SOD from bovine erythrocytes, Sigma chemical Co.USA was used to prepare a standard solution of SOD. 100µl part of the sample mixture was added in 2.7ml of 50mM Tris-HCl buffer that contained 1mMEDTA at pH 8.2 and 200µl of 0.4Mm pyrogallol in a test tube. Immediately, mixture was measured on a spectrophotometer at 325nm as the increase in absorbance against a blank at 5 seconds for 1min. SOD activity was expressed as U/mg proteins.

Determination of Testicular Catalase CAT Activity: Catalase activity was assayed by using the method of Alvarez, (1989). 90µL aliquot of the sample mixture was added to 1.9ml of phosphate buffer with 0.05, pH 7.0, 1ml of 30mM hydrogen peroxide and 10µl of Triton X-100 (1%), mixed thoroughly and immediately measured at 240nm spectrophotometer. The reduction in absorbance against blank was recorded at 15s interval for 1min. Catalase activity was expressed as U/mg proteins.

Determination of Testicular Lipid Peroxidation: The method described by Meistrich and VanBeek [14] was used to determine the extent of MDA formation, the breakdown of lipid peroxidation, with a thiobarbituric acid reactive substances (TBA) assay. The procedure involved addition of 0.2ml of sample mixture to 3ml of 1% H_3PO_4 in Pyrex test tubes.Tetraethoxyropane (TEP) standard solutions were prepared in increasing concentration of (0.825, 1.65, 3.30 and 6.60mM/0.2ml) with stock TEP (8.26mM). A 0.2ml aliquot of each prepared standard solution was added in the Pyrex test tubes and TEP was exchanged with the exactly same volume of ethanol in the test tube containing the blank. 0.8ml of KCl and 1ml of TBA solution (42mM) was added to each test tube. The mixtures were vortexed to mix thoroughly, heated up for 45min in boiling water bath and then allowed to cool in running tap water. The mixture were made up by addition of 4ml of butanol, vortexed very hard for 20s and centrifuged at 1000g for 20min. The absorbance of the supernatant was measured on a spectrophotometer at 532nm. Level of MDA was obtained by comparison with the absorbance of standard solutions.

RESULTS AND DISCUSSIONS

Literature reports on physicochemical properties and toxicity of consumption of samples from Okposi and Uburu salt lakes have been focused on metal and non-metal composition [2,3] and on hepatotoxicity as well as cardiovascular and renal function impairment [3]. Not much has been reported on persistent organic pollutant composition and possible reproductive effect of Okposi and Uburu salt lakes. This work was set at determining the composition of persistent organic pollutants present in these lakes and their possible effect on the reproductive functions when consumed over a long period of time.

No PCB was present; this might be that PCBs are not present in this lake or that they occur at concentrations that is below the detectable limit which is 0.01mg/L. The concentrations of most of the detected organochlorinated compounds were below the water quality limit set by the WHO and NAFDAC which is 0.07mg/L. The concentrations of OCPs were higher than the toxic limit for these compounds in drinking water which is 0.01mg/L [22]. The concentrations of these organic contaminants were significantly lower in salt samples. The reduction of these persistent organic pollutants might be as a result of heat since the salt is obtained after heating lake water to dryness. This might have lead to evaporation of these compounds since most organic compounds are volatile especially when heated up to high temperatures.

The organochlorinated pesticides present in these lakes might be from agricultural practices since rural dwellers are mostly farmers who apply pesticides and herbicides to kill pests and weeds most of which leaks into these lakes. Atmospheric deposition might also be considered as one of the possible source of most of these organochlorine in these lakes.Under aerobic conditions, DDTs are biodegraded to DDE and DDD [6]. In our study, DDT metabolites weredetected at lower concentration (DDD and DDE) though DDT was detected in all the samples from both lakes. Heavy metals and trace elements were also found to be present in both lakes of which most of them were significantly higher in concentration than the NAFDAC and WHO permissible limits in drinking water.

The result shows that treatment of the rats with Okposi and Uburu salt lake water and salt samples resulted in a certain degrees of reproductive toxicity. Testes weight depends on the extent of sperm cells that undergoes differentiation. The testes weight decrease observed in this research might be due to reduced tubular size,lowered number of germ cells elongation of spermatid phase. The reduction in the weight of accessory sex organs here might be due to decreased bio-availability of estrogenic and anti-androgenic activities caused by heavy metal, organochlorinated pesticides such as DDT and its metabolites like DDD and DDE. Other persistent organic pollutants found present at a reasonable concentration such as polycylic aromatic hydrocarbons also have antiestrogenic activities.

Table 1: Concentration of Organochlorinated Pesticides in Water from Okposi and Uburu Salt Lakes Water (mg/L).

OCPs	Sample B	Sample B1	
Okposi salt lakes	Uburu salt lake	permissible limit	NAFDAC/WHO
DDT	2.04	1.05	0.01
PCP	1.03	0.02	0.01
DDD	0.07	0.08	0.01
Dieldrin	0.09	0.06	0.01
Mirexndnd	0.01		
Clordanendnd	0.01		
TCDD	1.20	1.00	0.01

nd = not detected. NAFDAC and WHO maximum permissible limit=0.01mg/L.

Table 2: Concentration of Organochlorinated Pesticides in the Salt from Okposi and Uburu Salt Lakes Salts (mg/L).

OCPs	Sample A	Sample A1	
Okposi salt lakes	Uburu salt lake	Permissible limit	WHO/NAFDAC
DDT	1.04	0.75	0.01
PCP	0.63	0.01	0.01
DDD	0.04	0.02	0.01
Dieldrin	0.04	0.03	0.01
Mirexndnd	0.01		
Clordanendnd	0.01		
TCDD	0.20	0.03	0.01

<nd = not detected. (NAFDAC and WHO maximum permissible limit=0.01mg/L

Table 3: Changes in biochemical parameters of adult Sprague- Dawley rats administered water from Okposi salt lake

Parameter	Control	0.5ml/kg	1.0ml/kg	2.0ml/kg	4.0ml/kg
Initial b.wt (g)	198.2±5.8	197.0±4.2	197.8±5.2	196.6±4.9*	197.0±5.8*
Final b.wt(g)	197.2±7.1	194.0±4.4	193.1±5.6	189.5±7.2*	189.04±5.1*
Body weight ratio	0.0079±0.002	0.0049±0.003*	0.0028±0.001*	0.0026±0.0012*	0.0021±0.0010*
SOD (U/mg/L proteins)	7.56±0.47	6.43±0.29	5.32±0.41*	3.32±0.28*	3.00±0.22*
CAT (U/L proteins)	7.06±0.46	6.95±0.38	4.80±0.36*	4.32±0.29*	3.83±0.21*
TBARS (nmol/mg tissues	2.23±54	2.46±0.49*	3.96±0.47*	4.23±0.35*	6.43±0.42*

Values are means ± SD. Values bearing the superscripts * are significantly different from control. N=5

Table 4: Changes in biochemical parameters of adult Sprague- Dawley rats administered with water from Uburu salt lake

Parameters	Control	0.5ml/kg	1.0ml/kg	2.0ml/kg	4.0ml/kg
Initial b.wt (g)	198.8±4.2	197.0±3.9	195.2±3.3	194.0±3.1	192.0±2.9
Final b.wt (g)	198.8±3.4	189.6±3.6*	190.0±3.0*	184.2±2.9*	180.0±3.1*
Testis wt (mg/100g b.wt)	1321±18.9	1027±31.0*	1237±40.25*	974.0±30.21*	940.0±20.11*
Prostate (mg/100g b.wt)	297.4±12.32	209.0±11.8*	205.2±10.6*	203.0±9.8*	200.2±9.0*
Epididymis (mg/100g.b.wt)	399.7±20.0	260.3±7.8*	261.32±6.8*	258.3±4.8*	250.2±4.6*
SOD (U/L proteins)	915.9±101.3	908.4±102.2	900.0±90.2	891.4±85.1*	872.2±70.2*
CAT (U/L proteins)	0.892±0.34	0.724±0.3	0.632±0.27*	0.512±0.21*	0.467±0.16*
TBARS (nmol/mg protein)	0.140±0.026	0.154±0.012*	0.203±0.044*	0.324±0.023*	0.389±0.055*
GSH (nmol/mg proteins)	65.03±17.72	59.02±7.56*	52.86±6.26*	42.88±7.12*	33.80±9.23*

Data are means ± SD. Value bearing * are significantly different from the control. N=5

Table 5: Changes in biochemical parameters of adult Sprague- Dawley rats administered with salt from Uburu salt lake

Parameters	Control	50mg/kg	100mg/kg	200mg/kg	400mg/kg
Testis wt(mg/g b.wt)	1410.4±19.3	1331.4±15.1*	1228.3±14.22*	1223.0±13.22*	1200.0±10.1*
Epididymalwt (mg/g b.wt)	402.8±24	392.7±22	360.6±21*	286.6±25*	254.3±33*
Prostate wt (mg/g b.wt)	302.3±13.11	300.2±12.0	200.4±11.02*	234.1±10.0*	216.7±8.2*
TBARS (nmol/mg proteins)	0.16±0.030	0.22±0.027*	0.287±0.065*	0.298±0.064*	0.360±0.072
CAT (U/L proteins)	1.13±0.443	0.827±0.114*	0.800±0.112*	0.734±0.163*	0.62±0.114*
SOD (U/L proteins)	930.6±120.4	920.0±112.3	900.0±105.2	880.0±97.6*	800.0±82.7*
GSH (nmol/mg proteins)	65.02±17.72	61.08±15.33	56.9±16.4*	50.23±15.44*	44.42±14.3*

Data are means ± SD. Value bearing * are significantly different from the control. N=5

In the present study testes-body weight ratios decreased significantly in the groups administered water and salt from both Okposi and Uburu salt lakes. There was also significant decrease in body weights and organ weights of the treated rats compared to controls. Therefore, the reduction in the body weights, organ weights and reduction in testes body weight ratios by the salt and water samples from Okposi and Uburu salt lakes in this study may suggest atrophy, reduced tubule size, spermatogenic arrest or inhibition of steroid biosynthesis in the Leydig cells. This decrease in weight of the animals given salt and water from Okposi and Uburu salt lakes is in agreement with work of Saravu *et al*.andSarkar *et al*. [16, 17] who observed reduction in the weight of the accessory sex organs of animal given $NaAsO_2$. Yu *et al*. [23] made a similar observation in rats given NaF, Cd as well as those given chlorpyrifos and profenofos pesticides [3]. In this work, it does appear that the DDT, DDD, TCDD and PCP found in Okposi and Uburu salt lakes have contributed to toxic effects of these lakes to the reproductive organs of the given rats.

The spermatozoa, in common with all cell types have developed an elaborate antioxidant defense system consisting of enzymes such as catalase, superoxide dismutase and protein such as reduced glutathione that are involved in scavenging and suppressing the formation of reactive oxygen species like singlet oxygen, peroxynitrile and hydrogen peroxide [18]. Estimation of end product of lipid peroxidation as thiobarbituric reactive species is an index of the extent of oxidative damage to cellular structure [21]. In this study, rats treated with Okposi and Uburu salt lake water and salt sample showed significant elevation in the level of formation of thiobarbituric acid reactive substances concentration. This might be a consequence of decreased production of antioxidants in the treated rat tissues. The shift in the delicate balance in favor of reactive oxygen species ultimately will lead to a plethora of pathologic damage to

sperm cells and concomitant loss of functions. Reduced glutathione concentration and the activities of catalase and superoxide dismutase in the testes were all significantly reduced in the Okposi and Uburu salt lake sample treated male albino rats in this study. This result is in agreement with previous study which revealed that exposure to polychlorinated biphenyls and polycyclic aromatic hydrocarbons during early development can disrupt adult reproductive function by mediated depletion of antioxidants [9] and elevation of lipid peroxidation by Cd intoxication [11]. It has also been suggested that organochlorinated pesticides generate free radicals [13]. These free radicals interfere with the antioxidant defense system in the testes and results in the tissue injury. Studies have also revealed that levels of reactive oxygen species correlate with motility of spermatozoa [11]. Reactive oxygen species appears to play a role in apoptosis of spermatozoa. Therefore, overproduction of free radicals and hence oxidative stress induced by organochlorinated pesticides and other possible contaminants present n these lakes may account at least in part for the testicular toxicity associated with the Okposi and Uburu salt lakes.

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