American-Eurasian Journal of Toxicological Sciences 7 (2): 55-62, 2015
ISSN 2079-2050

DOI: 10.5829/idosi.aejts.2015.7.2.93125

Changes in Reproductive Functions of Adult Male Rats Administered Water and Salt Samples from Okposi and Uburu Nigerian Salt Lakes

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Abstract: Polycyclic aromatic hydrocarbon compound and heavy metal composition of Okposi and Uburu salt lakeand the possible changes to the testicular content of Albino rats administered water and salt samples from the same Lakes were determined. The polycyclic aromatic hydrocarbon compound composition was determined using gas chromatography coupled with mass spectroscopic equipment while heavy metals were analyzed using atomic absorption spectrophotometer.170 Albino Rats were grouped into eighteen groups of A to Q. Group D received 0.5ml, 1.0ml, 2.0mls and 4.0mls of water from Uburu salt Lake. Groups E to H received 50mg/kg, 100mg/kg, 200mg/kg and 400mg/kg of salt from Uburu salt Lake. Group I to L received 0.5ml, 1.0ml, 2.0mls and 4.0mls of water from Okposi salt Lake and group M to P received 50mg/kg, 100mg/kg, 200mg/kg and 400mg/kg of salt from Okposi salt Lake. Group while group Q received deionised water to serve as the control. Administration lasted for ninety days. At the end of ninety days, the animals were sacrificed. Reproductive tissues such as the testes, epididymis and prostate gland were immediately dissected out, cleared from the adhering tissues, blotted dried and weighed individually. Result revealed a significant reduction in the testicular weight, epididymal weight and prostate weight. The testicular contents such as cholesterol, glycogens, sialic acids and total proteins also decreased significantly. Chemical analysis of the Lakes showed a significant level of eight out of the sixteen world health organization priority polyclic aromatic hydrocarbon analyzed. The heavy metals found to be present in milligram litre were Cd (0.003), Cr (0.06), Pb (0.027), Ni (0.03), Cu (0.015), Mn (1.421), Zn (0.038), Hg (0.002), Fe (.026) Mg (15.200), Al (0.063), Ca (483.860) and F (1.87). 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: Reproduction · Heavy Metals · Polycyclic Aromatic Hydrocarbon and Testicular Contents

INTRODUCTION

River activity such as volcanic, glacial and tectonic eruptions leaves depression and cavities on land surfaces. The major products of these activities are formation of lakes [1]. A salt lake is simply a land that is locked by water that has salt concentration (Mostly sodium chloride) and many other minerals/elements in significant quantities higher than that of other lakes (Mostly referred to as 3g per salt per litre). Most salt lakes have been proved to contain both metallic and non-metallic ions including calcium, cadmium, lead, magnesium, mercury, fluorine, sulfate, manganese etc, as well as sodium chloride [2]. Physicochemical properties like organic compound content, nitrogen, phosphate and carbonate compositions, temperature, salinity and pH of which values are higher than that of the World Health Organization's standard for normal drinking water has been elucidated [3]. Indeed, complex solution of minerals including decayed organic matters that result from life in the lake is simply the term salt lake [4].

Long range transport of persistent toxic substances or persistent organic pollutants (POPs) by oceanic water currents and air from agricultural product and anthropogenic activities have lead to very high level of these pollutants in top predators in our ecosystem [5].

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Most of these persistent organic pollutants are lipophilic and are very resistant to metabolic breakdown and excretion, they tend to build up in lipid reach tissues and are thereforebimagnified with increasing trophic level in food chain [6]. The constituents of lakes such as chemical constituents either originate from natural processes such as erosion, weathering of coastal materials or from (Man-made) anthropogenic sources like domestic, industrial and agricultural practices [7]. The fact that most surface water and lakes are used for domestic purposes and as sources of drinking water has generated growing concern by the general public about their contamination.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*. [2] reported the presence of metallic and non-metallic ions in the lakes. Cardiovascular toxicity has also been reported [8]. These toxic effects have been attributed to the chemical constituents of the lakes [3-5].

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 [9]. Approximately 30% of cases of infertilities are as a result of a male factor. Several phenomenons can interrupt spermatogenesis and lower sperm quality, efficiency and production. Infertility have become a major clinical issues over the years, affecting 15% of all reproductive-aged married people, male factors, such as decreased semen quality, low sperm count as well as abnormal sperm morphology are responsible for 25% of these cases across the globe. 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 [9,10]. 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 male: female sex ratio of offspring, inhibition of spermatogenesis and ovogenesis, destruction of seminiferous epithelium and hydroceles resulting to decrease in fertility [10]. 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 [11].

MATERIALS 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*. [3], Agbafor *et al*. [4] and Agbafor *et al*. [5]. 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.

Animals Samples and Treatment: A total of 170 male bred Albino 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: A total of 170 Albino Rats were grouped into eighteen groups of A to Q. Group A to D received 0.5ml, 1.0ml, 2.0mls and 4.0mls of water from Uburu salt Lake. Group E to H received 50mg/kg, 100mg/kg, 200mg/kg and 400mg/kg of salt from Uburu salt Lake. Group I to L received 0.5ml, 1.0ml, 2.0mls and 4.0mls of water from Okposi salt Lake and group M to P received 50mg/kg, 100mg/kg, 200mg/kg and 400mg/kg of salt from Okposi salt Lake. Group while group Q received deionised water to serve as the control. Administration lasted for ninety days.

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. 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.

Collection of Epididymal Sperm: The epididymides were detached carefully from the right testes. The epididymis was apportioned into 3 parts; the tail, the body and the head. A pair of scissors was used to trim the tail of the epididymis and were dipped in 1.0ml of 0.1M phosphate buffer of pH 7.4. It was shaken vigorously to achieve a homogenous mixture, release and dispersal of sperm cells. Semen samples were analyzed for number, motility and gross morphology according the method of Mehran *et al*. [11].

Chemical Analysis: The water samples from both lakes were filtrated through glass fiber membrane (0.45µm, Fisher brand, USA) filters, two different 1ml water samples were obtained by extraction by the use of two solid phase extraction (SPE) cartridge which contained octadecysilane (Envi-C18, 1g, 6ml, Supko, USA), respectively. One C18 column was eluted with 10ml of high purity hexane to exclude PCBs and OCPs while the column was eluted with 10ml of 20% dichloromethane in hexane for PAHs. The targeted analytes includes 16 USA EPA priority PAHs, 20 PCBs congeners and 16 OCPs. The determination of the concentrations of PAHs were performed by the use of an HP 6890 GC/5973 MSD (Agilent US) and HP-5MS capillary columns (30m x 0.25mm id x 0.25µm film thickness). Helium and nitrogen were used as the carrier gases for MSD and µECD respectively. Program temperature for PAHs were set at 40°C for 4minutes before raising it to 320°C at 8°C/min and kept steady for 5min. The GC injector and µECD were maintained at 250°C and 280°C respectively. Injector and interface temperature for GC/MSD were kept at 270°C and 250°C accordingly. The blank of the method contained no detectable amount of target analytes. The limit of detection was fixed at 0.01mg/l for PAHs.

Determination of Testicular Glycogen: Testicles (25-75mg) were crushed with 5.0mls of deproteinizing solution in a centrifuge tube. The mark was made on the fluid level in the centrifuge tube and the tube was closed using a glass cap and was placed inside boiling water bath for a period of 15mins. it was removed after 5 mins and then centrifuged at 3000 rev/min for 5min. 1ml of the supernatant fluid was added to 3ml of 0.125N H_2SO_4 in a test tube and vigorous shaken to mix it together. The mixture was made hot using a boiling water bath for exactly 6min and later cooled in running tap water. The degree of pink color generated were measured with the aid of spectrophotometer, the concentration of glycogen produced were read from a standard curve in relation to its glucose equivalent. Glycogen gives exactly the same color intensity as an equivalent amount of glucose will do Mendel *et al*. [11].

Determination of Testicular Cholesterol: Total cholesterol assay kits (Cell Technology Inc, CA94043 USA) were used.

The principle of the assay used was based on an enzyme-coupled reaction that detects and quantify both free cholesterol and cholesterol esters. Cholesterol esters is broken down by cholesterol esterase to cholesterol, this is then oxidized by cholesterol oxidase to hydrogen peroxide and cholest-4-en-3-one (Ketone). The reaction between hydrogen peroxide and cholesterol probe (Detection reagents) in a 1:1 stoichiometry produces stable fluorescent product and this absorbance can be read at 570nm according to the kit protocol using spectrophotometer.

Determination of Testicular Sialic Acid: Sialic acid was assayed by the method of Ames *et al*. [6] as modified by Saradha and Mathur [12]. 40µl of the sample homogenate was added to 250µl of periodate reagent (25mM periodic acid in 0.125N H_2SO_4, pH 1.2) in polypropylene test tubes, incubated at 37°C in a warm water bath for period of 30mins. Sodium arsenite (2% solution of sodium arsenite in 0.5M HCl) was employedfor an excess of periodate. As soon as the yellow color of liberated iodine began to fade away after 1-2min, 2ml of thiobarbituric acid (0.1M solution of 2-thiobarbituric acid with pH adjusted to 9.0 with NaOH) was incorporated and mixed together. The test tubes were heated up for 7min in boiling water, cooled in ice blocks and shaked with hands with 5ml of an acid butanol mixture. After thorough vortexing and quick centrifugation, the intensity of the colour in the butanol phase was measured on a spectrophotometer at 549nm. 10µl of the 100mM sialic acid standard was serially diluted with 990µl dH_2O to get 1mM standard sialic acid. We added 0, 2, 4, 6, 8 and 10µl of the diluted sialic acid in a 96-well plate to obtain 0, 2, 4, 6, 8 and 10nmol/well

standard and this was used to raise a standard curve. The levels of sialic acid were calculated from the standard curve and expressed as mg/g proteins.

Estimation of Testicula Total Proteins: The testicular total protein levels were assayed respectively by the methods described by Habeebu *et al*. [13].

RESULTS AND DISCUSSION

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 [3-6] and on hepatotoxicity as well as cardiovascular and renal function impairment [13 - 17]. 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.

Only very few PAHs of the sixteen World Health Organization priority PAHs were observed at quantifiable concentrations. No PCB waspresent; this might be that PCBs are not present in this lake or that they occur at concentration that is below the detectable limit which is 0.01mg/L. PAHs with 3 and 4 rings such as naphthalene, phenanthrene and fluroanthene were detected at greater concentrations than that of PAHs containing 5 to 6 rings. The concentrations of most of the detected organochlorinated compounds were below the water quality limit set by the WHO and 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 [18]. 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.

Sources of PAHs can be assessed by use of ratios of concentrations of individual PAHs [18 - 22]. The ratios of Phe/Ant within the two ring group of PAHs and Fl/Pyr within the four rings group of PAHs were used to differentiate among sources. A Phe/Ant ratio >15 suggest petrogenic sources and Phe/Ant ratios of <10 are suggestive of pyrogenic sources. The Fl/Pyr ratio of 0.6 which is < 10 observed in this study indicates that PAHs originated from pyrogenic sources. Petrogenic PAHs generally originate from the leakage of crude oil and the refined products such as gasoline [23-27]. Pyrogenic PAHs originate primarily from combustion especially of fossil fuels. Concentration of polycyclic aromatic hydrocarbons were lower compared to those report from urbanized and highly industralized countries [28-32].

Effect of Both Lakes on Body Weight and Organ Weight: In the *in vivo* studies, epididymal cell and testicular cells of adult male rats were chosen to assess the reproductive toxicity of salt and water from these lakes which have been proven to contain some organic compounds based on this research and metal including non - metals and heavy metals [2]. The testes and epididymis contain Sertoli cells, spermatogenic cells and Leydig cells. Sertoli cells are one of the most important somatic cells in the reproductive organs [21]. Spermatogenic cells are precursor cells, sperm cells [25] and Leydig cells play a role of nutrition, support and mediate transfer [20].

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. Other persistent organic pollutants found present at a reasonable concentration such as polycylic aromatic hydrocarbons also have antiestrogenic activities.

Testes-body weight ratios and indices of secretory function of the testes: proteins, glycogen, sialic acid, cholesterol, testosterone, follicle stimulating hormone, GGT can be used to assess testicular function and the degree of spermatogenesis in mammals. Activities of the testes are closely controlled by androgens [7]. Because androgens, especially testosterone are produced in the seminiferous tubules of the testes, the assessment of such biomolecules as well as sperm analysis of the testes may give useful information about organ damage and rogenicity and indirect assessment of fertility in male rats. An elevation in the organ body weight ratio may either indicate inflammation or an increase in the secretory ability of the organ while reduction in the value of organ-body weight ratio may be as a result of cellular atrophy. Measurement of organ body weight ratios therefore can be used to indicate organ swelling, atrophy or even hypertrophy. In the present study, testes-body

Table 1: 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*
Cholesterol (mg/L proteins)	16.9±0.4	6.9±1.2*	5.4±1.1*	4.9±0.9*	3.6±0.18*
Glycogen (mg/L proteins)	2.69±0.22	2.49±0.20*	2.24±0.21*	2.02±0.20*	1.31±0.07*
Sialic acid (mg/L)	5.16±0.05	5.0±0.04*	4.14±0.06*	3.02±0.03*	1.71±0.02*
Epididymal TP (mg/L proteins)	220.0±15.2	129.1±7.9*	124.2±5.9*	115.2±4.7*	109.2±2.9*
Testicular TP (mg/L proteins)	209.2±14.3	203.02±12.2*	170.9±11.0*	190.0±7.8*	105.2±10.1*

Table 2: 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*
Cholesterol (mg/L proteins)	16.59±0.48	6.4±1.30*	5.0±0.90*	4.2±0.70*	3.2±0.14*
Glycogen (mg/L proteins)	2.29±0.22	2.08±0.11	1.09±0.10*	1.34±0.30*	1.0±0.20*
Sialic acid (mg/L)	5.16±0.05	4.09±0.04*	4.0±0.03*	2.09±0.02*	1.42±0.01*
Epididymal TP (mg/L proteins)	220.0±15.12	128.2±7.6*	120.4±5.2*	112.1±3.8*	100.5±1.8*
Testicular TP (mg/L proteins)	209.2±14.3	200.01±10.1	192.1±9.8*	185.9±9.4*	113.8±10.2*

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*
Testicular sialic acid (mg/L)	5.7±0.05	4.4±0.04	4.9±0.03*	4.0±0.03*	2.8±0.02*
Epididymalsialic acid(mg/L)	3.7±0.09	1.9±0.07	1.8±0.05*	1.8±0.03*	1.7±0.01*

Table 4: Physicochemical Properties of Okposi and Uburu Salt Lake Water (mg/L)

Metals	Okposi salt lake water	Uburu salt lake water	NAFDACPermissible limit
Al	0.063	0.061	0.2
Cd	0.003	0.005	0.003
Cr	0.06	0.07	0.05
Fe	0.26	0.25	0.3
Cu	0.015	0.028	1.0
Mn	1.421	1.341	0.2
Pb	0.027	0.026	0.01
Zn	0.038	0.062	3.0
Mg	15.200	17.200	0.2
Ca	485.860	479.950	-
Na	21.700	20.93	200
Hg	0.002	0.002	0.001
Ni	0.03	0.04	0.02
F	1.87	2.12	1.5

Results are mean of three replicates

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

Table 5: Concentration of Polycyclic Aromatic Hydrocarbons (PAHs) in Salt Okposi and Uburu salt Lakes Salts (mg/L)

PAHs	Sample B Okposi salt lake	Sample B1 Uburu salt lake	WHO/NAFDAC permissible limit
Phthalates	Nd	nd	0.07
Phenanthrene	0.03	0.04	0.07
Fluoranthene	0.07	0.10	0.07
Benzo(a)pyrene	Nd	0.01	0.07
Coronene	Nd	nd	0.07
Benzo(b)fluoroanthene	Nd	nd	0.07
Benzo(ghI)perylene	Nd	nd	0.07
Pyrene	0.02	0.03	0.07
Benzpo(e)pyrene	0.11	0.14	0.07
Chrysene	Nd	nd	0.07
Fluorene	0.02	0.01	0.07
Dibenzo(a,h)anthracene	Nd	nd	0.07
Benzo(I)fluroanthene	Nd	nd	0.07
Naphthalene	0.66	0.03	0.07
Anthracene	0.04	0.32	0.07
Fluoranthane	0.02	nd	0.07

nd = not detected. WHO maximum permissible limit=0.007mg/L

of the animals given salt and water from Okposi and Uburu salt lakes is in agreement with work of Waalkes *et al*. [30] who observed reduction in the weight of the accessory sex organs of animal given $NaAsO_2$. Yu *et al.* [32] made a similar observation in rats given NaF, Cd as well as those given chlorpyrifos and profenofos pesticides [8]. Impairment of capacity of the organ to synthesize or secrete some biomolecules such as proteins, as seen in the present study may account for the reduction in the testes: body weight ratios, final body weight and the reduction in the organ weights. It is also an indication of impairment at testicular, pituitary or hypothalamic level and may adversely affect the normal functioning of the testes [32].

Effects on Biochemical Parameters: Testicular protein which is dependent on testosterone action plays a role in the maturation of spermatozoa [16]. Furthermore, a study has shown that the administration of anti- androgens could reduce among others, protein content in both the testes and epididymis of animals. The testicular fluid also contains both stimulatory factors as well as inhibitory factors that selectively alter the protein secretions [22]. Therefore, the significant (p<0.05) decrease in testicular protein caused by the administration of salt samples and water samples from both Okposi and Uburu salt lakes may be associated with anti-androgenic activity, more so, when the level of testosterone was also significantly reduced by the salt and water samples in the present study. The glycogen content in the cell indicates energy storage. Sertoli cells and spermatogonia often contain glycogen where it serves to provide reserve of carbohydrates for seminiferous tubular cells [22]. The glycogen levels in laboratory animals have been found to be directly proportional to the steroid hormones. The significant decrease in glycogen content of the testes after administration of salt and water samples from Okposi and Uburu salt lakes for twelve weeks may be due to the inhibition of glycogen phosphorylase activation which is actively in glycogenolysis or depletion of certain other enzymes which could block androgen synthesis. Such decreased testicular glycogen content may reduce energy which needs to be made available for spermatogenic activity and this might result in spermatogenic arrest. The substance that act as a lubricant facilitating the the movement of sperm from the epididymis down to the vagina and also aid in reduction of friction among spermatozoa is the sialic acid [23]. Reduction of this in our research might also contribute in reduction of spermatogenesis. The reduction in glycogen level could affect protein synthesis because protein synthesis in spermatogenic cells is dependent on glucose [15-16]. Sialic acid known also as acetylated muramic acid has its synthesis dependent on androgen. There is also a direct relationship between the levels of androgen and the concentration of sialic acid in the testes of animals. Sialic acid play a role of lubrication to facilitate movement of sperm cells by lowering friction among spermatozoa thereby facilitating their upward movement within the lumen of the testes as well as during their transit through the epididymis and vagina. Acetylated muramic acid also has a role in the maturation of spermatozoa and in the maintenance and retention of structural integrity of the membranes of spermatozoa [29]. Therefore, the significant

reduction in testicular sialic acid following the administration of water and salt samples from Uburu and Okposi salt lakes may adversely affect structural integrity of the acrosomal membranes which ultimately affects the metabolism, motility and fertilizing capacity of the spermatozoa. Thus, it is possible that the constituents of salt and water from Okposi and Uburusalt lakes might have negatively interfered with the synthesizing phases and machinery of the testes that is responsible for producing and secretion of sialic acid. The chemical consituents of Okposi and Uburu salt lakes may also promote the catabolism of sialic acids in the testes of the animals higher than its biosynthesis thereby reducing its concentration in testes. This is agree with the work of Gyan *et al*. [20] who reported that Vanadylsuphate reduced testicular sialic acid in male rats.

Cholesterol is one of the precursors responsible for the anabolic effect of testosterone in the males and a constant supply of the lipid is required for the synthesis of steroid hormone [14]. The synthesis of testosterone via steroidogenesis is dependent on concentration testicular cholesterol. Therefore, the significant reduction in the testicular cholesterol of rats treated with salt and water samples from Okposi and Uburu salt lakes as compared with the control may not only account for the corresponding decrease in testosterone obtained in this same study, but also suggest impaired steroidogenesis. There is therefore a need for the proper processing of water and salt from these lakes before consumption.

REFERENCES

1. Charles, C.D., M.G. David and H.C. Draire, 1999. Salt Lakes: Physical Geology. Eight Edition, WCB/ Mchraw-Hill Company, United States of America, pp: 142-160.
2. Akubugwo, I.E., C.J. Ofoegbu and C.U. Ukwuoma, 2007. Physicochemical studies on Uburu salt Lake, Ebonyi State-Nigeria. Pakistan Journal of Biological science, 10(8): 3170-3174.
3. Agbafor, K.N., P.M. Ajah, C.E. Offor, I.O. Igwenyi and U.A. Ibiam, 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, 5: 229-234.
4. Agbafor, K.N., N. Nwachukwu and M.E. Ogbanshi, 2010. Effect of Okposi and Uburu (Nigeria) Lakes on trace elements levels and hematological system in rats. Journal of Science Technology, 16: 52-58.
5. Agbafor, K.N., U.A. Ibiam and N.O. Omaka, 2008. Analysis of Hg, Zn, Fe, Pb, Cu, Cd, Cr, Na and Cl^- in Uburu and Okposi salt lakes, Journal of Applied and Natural Sciences, 3: 20-23.
6. Ames, B.N., M.K. Shingenga and T.M. Hagen, 1993. Oxidants, antioxidants and degenerative diseases of aging. National Academic Society, 90: 7915-7922.
7. Agrawal, A., S Chauhan and R. Mathur, 1986. Antifertility effects of embolin in male rats. Andrologia, 18(2): 125-131.
8. Afaf, A., G. El-kashoury, A. Hanan and Tag El-din, 2010. Chlorpyrifos (from different sources): Effects on testicular Biochemistry of male Albino rats. Journal of American Science, 6(7): 252-26.
9. WHO, 1992. Environmental Health Criteria, 134, Cadmium. Geneva: World Health Organisation.
10. Budzinski, H., L. Janes, J. Belloaq, C. Pier and P. Carrigues, 1997. Evaluation of sediment contamination by polyclic aromatic hydrocarbons in the cironde estuary. Marine Chemistry, 58: 85-97.
11. Mendel, B., A. Kemp and D.K. Myers, 1954. Method in biochemistry glycogen measurement. Biochemical Journal, 56: 639-644.
12. Saradha, B. and P.P. Mathur, 2006. Induction of oxidative stress by lindane in epididymis of adult male rats. Environ Toxicol. Pharmacol., 22: 90-96.
13. Habeebu, S.S., J. Liu and C.D. Klaassen, 1998. Cd induced apoptosis in mouseliver. Toxicol. Appl. Pharmacol., 149: 203-209.
14. Carreaus, 1996. Paracrine control of human Leydig cells and Sertoli cells functions. Folia Histochem and lytol., 34: 111-119.
15. Das, K.K. and S. Dasgupta, 2002. Effect of Nickel sulphate on testicular steroidogenesis in rats during protein restriction. Environ and Health Persp, 110: 923-926.
16. Dixit, V.P., C.L. Gupta, S.K. Bhargava and J.S. Sandhu, 1972. Long-term effects of metopiron (SU-4885CIBA) on the testicular functions of dogs. Folia Biol., 27: 277-285.
17. Gupta, R.S., J.B. Kachhawe and R. Chaudhary, 2004. Antifertility effects of methanolic pod extract of *Albizzia lebbeck*. Asian J. Andro., l6: 155-159.
18. Gupta, R.S., P. Kumar, V.P. Dixit and J.S. Dobhad, 2000. Antifertility studies of the root extract of *Barleria Prionitis* Linn in male albino rats with special reference to testicular cell population dynamics. J. Ethnopharm, 70: 111-117.
19. WHO, 1984. Guideline for drinking water quality. Health Criteria, 2: 335.

20. Gyan Chand Jain, HemantPareek, Sameer Sharma, Mamta Bhardwaj and Balvant S. Khajja, 2007. Reproductive Toxicity of VandylSulphate in male Rats. Journal of Health Science, 53(1): 137-141.
21. Hotchkiss, A.K., C.V. Ride, C.R. Blystane, V.S. Wilson, P.C. Hartig and G.T. Ankley, 2008. Environmental endocrine disrupters and human and wildlife health. Toxicol Sci., 105: 235-259.
22. Karl, J. and M. Carpel, 1998. Sertoli cells of the mouse testes originate from thecoeloric epithelium. Developmental Biology, 203: 323-333.
23. Morakinyo, A.O., P.U. Achema and O.A. Adegoke, 2010. Effect of *Zingiber officinale* (Ginger) on sodium arsenite induced reproductive toxicity in male rats. African Journal of Biomedical Research, 13: 39-45.
24. Pathak, S. and A.O. Praskash, 1989. Post-coital contraceptive effect of fluorine. Phytotherm Res., 3: 61-68.
25. Quill, A.T. and L.O. Garblers, 2002. "Sperm Motility Activation and Chemo attraction". In M. Daniel, Hardy Fertilization. 6th Edition. California: Academic Press., pp: 29-38.
26. Roel, H.A., P. Hoet and D. Lison, 1999. Usefulness of biomarkers of exposure to inorganic mercury, lead or cadmium in controlling occupational and environmental risks of nephrotoxicity. Ren. Fail, 21: 251-262.
27. Saravu, K., J. Jose, M.N. Bhat and B.A. Shastry, 2007. Acute ingestion of copper sulphate. A review on its clinical manifestations and management. Industrial Journal of Critical Care Medicine, 11: 74-80.
28. Sarkar, M., R.G. Chaudhuri, A. Chattopadhyay and N.M. Biswas, 2003. Effect ofsodiumArsenite on spermatogenesis, plasma gonadotropin and testosterone in rats. Asian Journal of Andrology, 5: 27-31.
29. Virginia, A., L. Victoria, B. Montserrat, A. Maria, J. Juan, L. Jose and J.S. Domenec, 2009. Sulfasalazine induced oxidative stress: A possible mechanism of male infertility. Reproductive Toxicology, 27: 35-40.
30. Waalkes, M.P., J.M. Ward, J. Liu and B.A. Diwan, 2003. Transplacental carcinogenicity of inorganic arsenic in the drinking water: induction of hepatic, ovarian, pulmonary and adrenal tumors in mice. Toxicology and Applied Pharmacology, 186: 7-17.
31. Yunker, M.B., R.W. MacDonald, F. Vingarzen, R.H. Mitchell, D. Goyette and S. Sylvester, 2002. Polycyclic aromatic hydrocarbons (PAHs) in the presser river basins: A critical appraisal of PAHs ratios as indicator of PAHs source and composition. Organic Geochemistry, 33: 489-515.
32. Yu, H.N., S.R. Shen and J.J. Yin, 2007. Effects of metal ions, catechins and theirinteractions on prostate cancer. Critical Review of Food Science and Nutrition, 47(8): 711-719.