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ORGANOCHLORINATED COMPOUND AND PHYSICOCHEMICAL COMPOSITION OF OKPOSI SALT LAKE AND ITS POSSIBLE EFFECTS ON MALE REPRODUCTIVE FUNCTIONS

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Abstract:

Water and salt samples from Okposi salt lakes have been reported to elicit various degrees of toxicity. Reproductive toxicity of samples from the lakes were studied in the present research. The quantity of selected persistent organic pollutants in these lakes includes polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and organochlorinated pesticides (OCPs) were assessed using gas chromatographic and mass spectroscopic equipment. Heavy metals were measured using atomic absorption spectrophotometer. 85 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%. The levels of Cd, Cr, Mn, Pb, Mg and Ni found in the lakes samples were significantly ($p<0.05$) higher in concentration than the WHO permissible limits for drinking water. Ten of the WHO priorities PAHs were detected. The concentration of individual PAHs ranged from 0.02-0.24mg/L. Five OCPs was detected and no PCBs were detected. Concentrations of the detected OCPs and PAHs were higher than the WHO toxic limits for drinking water. The concentration of most of these contaminants were significantly lower ($p<0.05$) in the salt samples relative to the lakes water. These lakes samples lower significantly ($P<0.05$) the testicular body weights ratio, body weight and organ weights in administered rats. Testicular levels of sialic acid and testosterone were all reduced significantly ($p<0.05$). The levels of antioxidant markers:- catalase, superoxide dismutase and reduced glutathione were lowered significantly ($p<0.05$) in the treated group while the testicular lipid peroxidation marker (thiobarbituric acid reactive substances) was significantly increased ($p>0.05$). Photomicrograph of testes of the treated rats showed severe degenerative changes on the spermatogenic cells and atrophy of the testes unlike the control. These effects were found to be dose- dependent to the dose administered. The results are indicative of the toxicity of these lakes to the reproductive systems with raw water samples being more toxic than the l ocally processed salt samples.

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INTRODUCTION:

Okposi salt lakes are found in Ohaozara Local Government Area in Ebonyi 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. [1] have reported the presence of metallic and non-metallic ions in the lakes. The hepatotoxicity of these lakes was demonstrated by [2]. Cardiovascular toxicity has also been reported [3]. These toxic effects have been attributed to the chemical constituents of the lakes [4].

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 (WHO, 1995). Approximately 30 % of cases 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 (WHO, 1995). 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 [5].

Okposi salt lakes located in Ohaozara Local Government Area of Ebonyi State Nigeria is one 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 this lake. Carbon tetrachloroethane is a carcinogen that might also cause acute effects to liver, kidneys, cardiovascular systems as well as reproductive impairment [6]. Most of the studies 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 [7]. 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 [8]. 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 (AMAP, 2004).

METHODOLGY:

Collection of Samples:

Samples were collected in the month of March 2018 during dry season. The bottles for sample collection were washed with deionized water. The lakes were apportioned 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 [9]; [10].

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.

Animals Samples and Treatment:

85 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^{o}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 salt lakes was dissolved in deionized water and labeled appropriately. The raw water samples from Okposi salt lakes were also labeled accordingly. The animals were grouped into 17 groups with each group containing five rats. 50, 100, 200 and 400mg/kg body weight of water sample from Okposi salt lake were administered to group F, G, H and I respectively. 0.5, 1.0, 2.0 and 4.0ml/kg body weight of salt sample from Okposi salt lake were administered to group N, O, P and Q respectively. All the administration were done orally it 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

Preparation of Tissue Homogenate:
A part of the right testicles and epididymis of each rats were collected for biochemical analysis and homogenates were prepared using the method of [11]. 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^{o}C and utilized for biochemical analysis within one week.

Tissue Processing:
Tissue processing involved were dehydration, de-alcoholization (clearing), impregnation (infiltration), embedding, microtomy (sectioning) and staining of the testicular tissues in accordance with the method of [12].

Evaluation of Testicular Superoxide Dismutase SOD Activity:
Superoxide dismutase activity was assayed using the methods of [13], modified by [14]. 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 1mM EDTA 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 [15]. 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 [16] 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.

Determination of Testicular Sialic Acid:
Sialic acid was assayed by the method of [17] as modified by [18]. 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^{o}C in a warm water bath for period of 30mins. Sodium arsenite (2% solution of sodium arsenite in 0.5M HCl) was employed for 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.

Determination of Testicular Reduced Glutathione (GSH):
The reduced glutathione (GSH) content of the testes homogenates were determined using the method described by [19]. The GSH determination method is dependent on the reaction between Ellman's reagent 5'5'dithiobis and the thiol group of GSH at pH 8.0 to

release 5-thiol-2-nitrobenzoate which is yellow at 412nm. Malondialdehyde (MDA) is the highest abundant individual aldehyde from lipid peroxidation in biological systems and it has been utilized as an indirect index of lipid peroxidation. We have employed the method used by U.S EPA, (1996) in this work for the determination of MDA that is based on its interactions with Thiobarbituric acid (TBA) to form a pink complex with absorption maximum at 535nm.

Determination of Testicular Testosterone:
Testosterone concentration were measured using ELISA kit obtained from Diagnostic Freiburg, Germany. The method stated in the manufacturer's protocol version (2001) was used in the assay. The assay procedure consist of three major different steps of antibody reaction with testicular mixture of testosterone and kit testosterone label, magnetic solid phase separation step and color development step which were all based on a direct measurement of competitive type followed by the general reaction between antibody and antigen as based on the enzyme-linked immunosorbent assay (ELISA) using serozyme 1 serone (Diagnostic Freiburg, Germany). The testosterone concentration in the testicular sample mixture were obtained by comparing the absorbance of the testicular sample at 550nm with the corresponding absorbance on the standard curve.

Determination of Testicular LH and FSH:
Solid phase enzyme-linked immunoabsorbent assay method described by [20] was employed in the determination of LH and FSH concentration. Briefly, the assay principle involved a mouse monoclonal anti-LH antibody in the solid stage/phase (microliter wells) immobilization and another monoclonal anti-LH antibody in the antibody enzyme (horsradish peroxidase)-conjugated solution. Fifty microliter of the standard, test and control were pipetted into suitable distinguished wells after which 100µl of enzyme conjugated reagent was put to each of the wells and mixed together. The mixture was incubated for 45mins. Hundred microliter of TMB reagent was put into each well by pipetting and incubated in the dark for 20min. The reaction was terminated by puting 100µl of the stop solution to all of the wells and kept for color development from blue to yellow. The absorbance at 450nm was interpreted with microtitre plate reader in 15mins. The absorbance was compared with that of the standard curve to get the amount of the LH in the testicular homogenates. For FSH the same procedure was used and FSH standard was used instead of LH standard.

RESULTS:

Table 1: Concentration of Polycyclic Aromatic Hydrocarbons (PAHs) in Water from Okposi Salt Lakes (mg/L)

PAHs	Sample B Okposi salt lake	WHO/NAFDAC permissible limit
Phthalates	Nd	0.07
Phenanthrene	0.05	0.07
Fluoranthene	0.09	0.07
Benzo(a)pyrene	Nd	0.07
Benzo(b)fluoroanthene	Nd	0.07
Benzo(ghI)perylene	Nd	0.07
Pyrene	0.04	0.07
Benzpo(e)pyrene	0.17	0.07
Fluorene	0.03	0.07
Dibenzo(a,h)anthracene	Nd	0.07
Benzo(I)fluroanthene	Nd	0.07
Naphthalene	0.76	0.07
Anthracene	0.08	0.07
Fluoranthane	0.03	0.07

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

Table 2: Concentration of Polycyclic Aromatic Hydrocarbons (PAHs) in Salt from Okpos salt Lakes Salts (mg/L)

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

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

Table 3: Concentration of Organochlorinated Pesticides in Water from Okposi Salt Lakes Water (mg/L).

OCPs	Sample B Okposi salt lakes	NAFDAC/WHO permissible limit
DDT	2.04	0.01
PCP	1.03	0.01
DDD	0.07	0.01
Dieldrin	0.09	0.01
Mirex	nd	0.01
Clordane	nd	0.01
TCDD	1.20	0.01

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

Table 4: Concentration of Organochlorinated Pesticides in the Salt from Okposi Salt Lakes Salts (mg/L).

OCPs	Sample A Okposi salt lakes	WHO/NAFDAC Permissible limit
DDT	1.04	0.01
PCP	0.63	0.01
DDD	0.04	0.01
Dieldrin	0.04	0.01
Mirex	nd	0.01
Clordane	nd	0.01
TCDD	0.20	0.01

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

Table 5: Physicochemical Properties of Okposi Salt Lake Water (mg/L)

Metals	Okposi salt lake water	NAFDAC Permissible limit
Al	0.063	0.2
Cd	0.003	0.003
Cr	0.06	0.05
Fe	0.26	0.3
Cu	0.015	1.0
Mn	1.421	0.2
Pb	0.027	0.01
Zn	0.038	3.0
Mg	15.200	0.2
Ca	485.860	-
Na	21.700	200
Hg	0.002	0.001
Ni	0.03	0.02
F	1.87	1.5

(Table 6): 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*
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*
GSH (nmol/mg proteins)	70.48±8.4	68.48±8.2	58.42±7.2*	54.48±6.2*	50.56±5.4*

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

(Table 7): Changes in biochemical parameters of adult Sprague- Dawley rats administered salt 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)	5.80±0.04	4.6±0.06	5.0±0.05*	4.4±0.04*	2.9±0.03*
SOD (U/mg/L proteins)	7.65±0.53	6.33±0.38	4.31±0.31*	3.43±0.37*	3.02±0.33*
CAT (U/L proteins)	8.07±0.52	7.75±0.42	5.70±0.28*	5.41±0.28*	4.93±0.11*
TBARS (nmol/mg tissues	2.43±53	2.57±0.48*	4.76±0.49*	5.13±0.44*	7.44±0.43*
GSH (nmol/mg proteins)	75.48±7.4	70.48±5.1	60.42±4.1*	58.48±5.2*	53.56±4.3*

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

Plate 1: Micro-photograph of rat testis of control group showing the normal histological structure of mature seminiferous tubules with complete spermatogenic series i.e. normal morphology of seminiferous tubules. Lumen is filled with sperm. (H and E X40.)

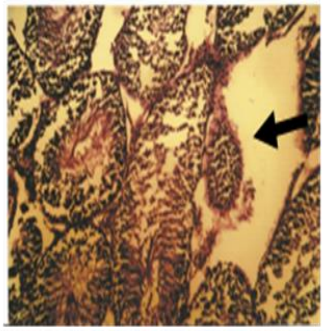

Plate II. Photomicrograph testis of male Sprague-Dawley rats administered with 400mg/kg body weights of salt from Uburu salt lake (H and E X40). Arrow indicates decreased number of spermatogenic cells. Lumen is filled with sperm debris. Seminiferous tubules degenerated with distorted basement membrane.

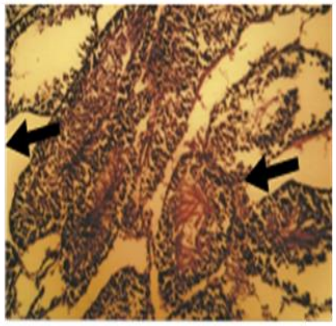

Plate III: Photomicrograph testis of male Sprague-Dawley rats administered with 400mg/kg body weights of salt from Okposi salt lake (H and E X40). Arrow shows too many spaces between the tubules, damaged seminiferous tubules containing degenerated spermatogenic cells.

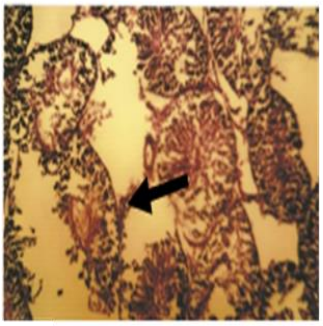

Plate IV. Photomicrograph testis of male Sprague-Dawley rats administered with 4.0ml/kg body weights of water from Okposi salt lake (H and E X40). Arrow shows shrunken seminiferous tubules with degenerated spermatozoa. Seminiferous tubular epithelium is irregular and increased intertubular spaces with less connective tissues and decreased sperm cells and reduction in seminiferous tubule diameter

Plate v:Photomicrograph testis of male Sprague-Dawley rats administered with 4.0ml/kg body weights of water from Uburu salt lake (H and E X40). Arrow indicates increased intertubular spaces. Shrunken tubules with their walls greatly thickened and reduction in seminiferous tubule diameter.

DISCUSSION:

Persistent Organochlorinated Pesticides Present in Okposi Salt Lake:

Literature reports on physicochemical properties and toxicity of consumption of samples from Okposi salt lakes have been focused on metal and non-metal composition [21]; [22] and on hepatotoxicity as well as cardiovascular and renal function impairment [23]. Not much has been reported on persistent organic pollutant composition and possible reproductive effect of Okposi salt lakes. This work was set at determining the composition of persistent organic pollutants present in this lake 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 was present; this might be that PCBs are not present in this lake or that they occur at concentrations that are 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. The concentrations of OCPs were higher than the toxic limit for these compounds in drinking water which is 0.01mg/L [24]. 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 [25]; [26]. 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 [27]. Pyrogenic PAHs originate primarily from combustion especially of fossil fuels [28]. Concentration of polycyclic aromatic hydrocarbons were lower compared to those report from urbanized and highly industralized countries .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 theses organochlorine in these lakes. Under aerobic conditions, DDTs are biodegraded to DDE and DDD is the resultant product of DDT degradation in anaerobic condition (HELCOM (2002)). In our study, DDT metabolites were detected at lower concentration (i.e 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 WHO permissible limits in drinking water.

Effects on Antioxidant Levels:

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 is involved in scavenging and suppressing the formation of reactive oxygen species like singlet oxygen, peroxynitrile and hydrogen peroxide [29]. Estimation of end product of lipid peroxidation as thiobarbituric reactive species is an index of the extent of oxidative damage to cellular structure [30]. In this study, rats treated with Okposi 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 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 [23] and elevation of lipid peroxidation by Cd intoxication [11]. It has also been suggested that organochlorinated pesticides generate free radicals [3]. 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 [7]. Reactive oxygen species appears to play a role in apoptosis of spermatozoa. Therefore, overproduction of free radicals and hence oxidative

stress induced by polycyclic aromatic hydrocarbons, organochlorinated pesticides and heavy metals present in these lakes may account at least in part for the testicular toxicity associated with the Okposi salt lakes.

Effect on androgen levels:

Testosterone as the main male gonadal hormone produced by the interstitial cells of the leydig cells in the testes however, reduced significantly. The significant reduction of the testicular testosterone as observed in this study is an indication of anti-androgenic potentials of the salt and water from Okposi salt lakes probably due to the DDD, DDT, PCP and some heavy metals present in these lakes. Such reduction in the testosterone content of the testes might have resulted from the decreased cholesterol and lueteinizing hormones concentrations observed in the study. Cholesterol is the starting material for androgen biosynthesis and lueteinzing hormones stimulates testosterone biosynthesis in leydig cells [17]. A crucial level of androgen in the blood is required for the maintenance of normal sexual desires, nocturnal penile tumescence and non - erotic penile erections in most men. A certain concentration of androgen is also needed for instigation and maintenance of spermatogenesis and for stimulation of growth and function of the prostate and seminal vesicles [9]. Testosterone also helps in maintaining body shape and increasing muscle mass and strength. Therefore, the reduction in the testosterone as observed here will negatively affect androgen dependent parameters like mating behavior, blood count, bone density and maintenance of spermatogenesis. Hence, the anti-androgenic potential of the water and salt from Okposi salt lakes is due to interference with steroidogenesis at the testicular level. Similar observation was recorded by [5]; [6] who reported that animal given DDT had a drastic decrease in testicular testosterone level without alteration in serum LH and FSH. However, Okposi salt lake water and salt administration reduced lueteinizing hormone and follicle stimulating hormone probably because of other contaminants like heavy metals and PAH.

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