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Research Article

Alterations in Reproductive Indices in Mice Exposed to Contaminated Urban Groundwater From an Automobile Spare Parts Market in Ibadan, South-Western Nigeria

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Abstract
Contamination of underground water with pollutants such as spent engine oil from automobile scrap sales and repair activities has health implications on biota and public health. This study investigated the effects of contaminated groundwater within the Araromi spare-parts market, Ibadan on some reproductive indices in male Swiss albino mice. Samples from two wells (CWW1 and CWW7) known to be contaminated with high levels of heavy metals and PAHs concentrations were purposively selected for experimental studies. Four groups of 10 mice each were exposed to graded concentrations (25%, 50%, 75%, and 100%) of the well-water samples, while a control group of 10 mice received distilled water. Semen characteristics, reproductive hormonal levels (LH and FSH) were analysed at days 21, 42 and 84 post-exposure for changes induced by the contaminated well-water. Data were analysed using one-way ANOVA. Sperm count and percentage sperm motility were significantly lower ($p<0.05$) in exposed mice compared to the control and lower concentration groups (25%) throughout the experiment. Various types of morphological abnormalities including long pin and pin head were observed at day 84 in 75% CWW1 and 100% CWW7 respectively. Significantly elevated ($p<0.05$) levels of LH and FSH occurred in 100% CWW1 at day 84, while FSH levels were only significantly higher in 100% CWW7 exposed group at day 84. Our findings indicate that the contaminated well-water induced reproductive toxicity in exposed mice and may have negative health implications in orally exposed human populations with prolonged domestic usage of the contaminated water.

Key Words: *Reproductive indices, Groundwater, Spent engine oil, Toxicity*

INTRODUCTION

Water is an important resource required by all living organisms. However, it also serves as a medium of exposure to hazardous contaminants (WHO, 2014). Groundwater is at risk from either point source pollution, which are activities that can be controlled by adherence to the relevant laws and regulations, or non-point source pollution which is very difficult to manage due to the multiplicity of contaminant sources (EPA, 1991). The Araromi Automobile spare-parts market represents a point source of pollution for groundwater within its vicinity. It is a major market for the sale and repair of automobile parts in Ibadan, South-Western Nigeria. Various automobile parts are displayed for sale at the market and these parts are covered with lubricants such as waste oils especially spent/used engine oil, which may be leached into underground water sources during precipitation. Used engine oil is a hazardous environmental contaminant which is enriched with toxic substances such as heavy metals particularly arsenic, lead, cadmium, nickel and hydrocarbons such as polycyclic aromatic hydrocarbons (PAHs) amongst other contaminants. During precipitation, contaminants in the spent oil may percolate into underground water which serves for drinking and other domestic purposes, thereby resulting in contamination / pollution of this vital resource with adverse health effects in the resident population (CEPA, 1994; Arise *et al.,* 2012). The rising cases of infertility in the society today calls for concern. The appreciation for the potential impact of environmental contaminants on male reproductive function was awakened in the 70s as a result of fertility problems observed in spouses of men occupationally exposed to a hazardous chemical fungicide, dibromochloropropane (DBCP) (Moline *et al*, 2000). Since then, the effects of various hazardous contaminants on male reproductive function has been assessed by various authors. The contamination of groundwater with spent engine oil is a serious environmental problem that may have negative consequences on human reproductive health (Ochiogu *et al*, 2009).

Several reports have indicated that organisms exposed to contaminated groundwater have shown severe reproductive defects such as deformed spermatozoal middle piece, reduced sperm motility and infertility among other effects (EPA, 1991; CEPA, 2002; Liu *et al.,* 2003; Ochiogu *et al.,* 2009). The impact of contaminated groundwater may be observed in the male reproductive system of mice using biomarkers such as Gonadotrophin releasing hormones (GnRH) secreted by the

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hypothalamus, which in turn stimulates the pituitary gland to release two hormones: Luteinizing Hormone (LH) and Follicle Stimulating Hormone (FSH). Luteinizing hormone binds to receptors on the surface of the Leydig cells found in the testicles, ultimately resulting in the production of testosterone which is important for spermatogenesis (Oh, 2014). The production of testosterone and its product estradiol, feedback to the hypothalamus and pituitary to suppress LH and its subsequent production of testosterone. In response to the reduction of testosterone, GnRH and LH are again produced. FSH acts independently and in concert with testosterone to stimulate the proliferation of the Sertoli cells and to produce signaling molecules and nutrients which support spermatid maturation (Oduwole *et al.,* 2018). It also stimulates the Sertoli cells to produce androgen-binding protein (ABP) and inhibin. ABP keeps testicular testosterone concentrations at levels necessary for spermatogenesis, while inhibin feeds back to the anterior pituitary to cease production of FSH (Pardue *et al.,* 2017). Exposure to contaminated groundwater can however lead to elevated levels of GnRH (and subsequently FSH and LH) production, acrosomal dysgenesis, nuclear malformation and decline in semen/sperm quality (Jihen *et al*., 2008). Other effects include reduced ejaculation, abnormal spermatozoa, reduced mating desire, and decreased testosterone production resulting in infertility or sterility (Moss *et al.,* 1979; Ochiogu *et al.,* 2009). Diminished secretion of LH and FSH can result in failure of gonadal function (hypogonadism) which manifests in males as reduced production of normal number of sperm, while excessive secretion also results in gonadal failure or pituitary tumors (Auyeung *et al.,* 2010). Excessive secretion also suggests a failure of the negative feedback mechanism on the hypothalamus and pituitary to suppress the secretion of LH and FSH. Semen quality is a measure of the ability of the spermatozoa to fertilize an egg. It is the measure of fertility in males and semen quality involves both sperm quality and quantity as decreased spermatozoa is a major factor in male infertility (Auyeung *et al.*, 2010). Normal sperm morphology (shape) and motility (ability to swim forward) determine a typical ejaculate of a healthy physically matured sperm with no fertility related problems (Liu *et al.*, 2003). Healthy human semen contains between 300-500 million normal spermatozoa and the quality can be determined via sperm motility, sperm agglutination test, indirect immunofluorescence test and FSH, LH and Testosterone enzyme-linked immunosorbent assay. Sperm livability is an important test for fertility as it helps to determine the percentage of living and non-living sperm cells, damage to DNA and substrate presence of anti-sperm antibodies (Van der Merwe *et al.*, 2005). The reproductive organ is one of the major repository sites for many contaminants and can cause hormonal imbalances and reproductive defects (Ogbuewu *et al.*, 2009). In this study, we sought to investigate the likely effects of exposure to contaminated groundwater from the Araromi automobile spare parts market, Ibadan on male reproductive parameters in Swiss albino mice; as indicators of reproductive toxicity and potential source of antifertility in exposed human populations.

MATERIALS AND METHODS

Study Area: The study area was the Araromi automobile spare parts market located within Ibadan North East Local Government Area of Oyo State, Nigeria. Geographically, it lies between 3^{O} 55.35 North and 7^{O} 23.49 North and longitudes 3^{O} 55.80 East to 3^{O} 55.23 East (Fig. 1).

Water Sampling: Water samples from two (out of seven) selected wells (W1 and W7; Fig. 1) with high levels of metals and PAHs above regulatory limits were used for the assessment of the reproductive toxicity. Details on the water sampling, analysis for heavy metals, PAHs and the results of the analysis are discussed in a separate paper. However, a few highlights are presented in the discussion section.

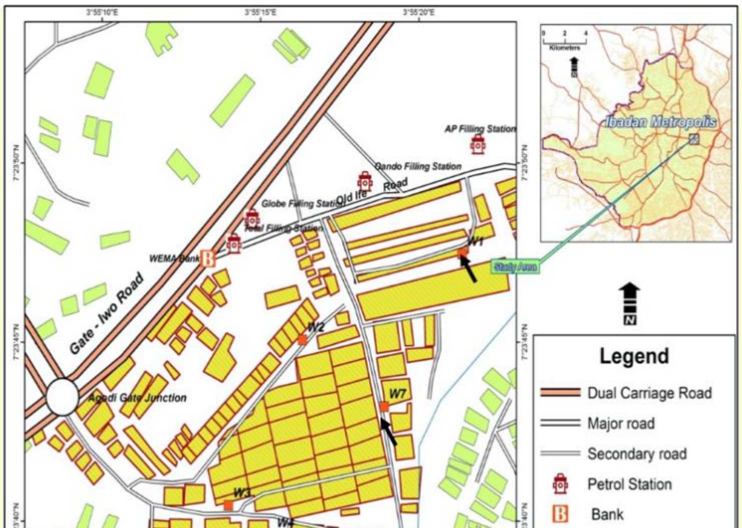


Plate 1
Map of Ibadan metropolis showing the Araromi automobile spare-parts Market and the selected sample wells.

Experimental animals: Ninety male Swiss albino mice were obtained from the Animal House Units of the Department of Physiology, and Department of Zoology, University of Ibadan, Ibadan, Nigeria respectively. They were kept in clean and well ventilated cages in the experimental Animal House of the Department of Zoology and fed with standard pelletized feed obtained from Ladokun feed® mill, Ibadan. The mice were allowed access to good quality drinking water (distilled water) *ad libitum* and acclimatized under laboratory conditions, feeding and handling procedures for a period of eight weeks, before the commencement of the experiment. Wood shavings were used for beddings and the beddings changed and cages cleaned every 48 hours throughout the acclimatization and experimental period.

Experimental design: Experimental design: Ninety male animals were randomly selected into eight (8) treatment groups and untreated control comprising of ten animals each. Animals in each concentration group were however housed five per cage in order to minimize stress associated with overcrowding. The animals in the treatment groups were administered with normal feed and various concentrations (25%,-low dose, 50%-medium dose, 75% and 100%-high dose) of the contaminated groundwater (designated CWW 1 and CWW 7) respectively, while the control group was administered distilled water (DW) only.

The contaminated water and distilled water control were administered as drinking water sources for the animals and the various concentrations and control were replaced every 48 hours. A 200ml measuring cylinder was used to determine the volumes of contaminated groundwater which was then mixed with the respective volumes of distilled water to make up the desired percentage concentrations of 25%, 50%, 75% and 100% respectively. Animals were anesthetized with chloroform and sacrificed via cervical dislocation on days 21, 42 and 84 of the exposure period respectively. Regulations on good laboratory practice (WHO, 1998) and principles of laboratory animal care (NIH, 1985) were adhered to during acclimatization and experimental design.

Immuno-Assay for LH and FSH: Prior to sacrifice, blood samples were collected from the mice via ocular puncture using plain capillary tubes and transferred into plain sample bottles. The clotted blood samples were centrifuged at 2500rpm for 10 minutes using a Biofuge pico D37520 centrifuge. The sera were separated from the clotted blood into well labeled Eppendorf tubes for biochemical estimation of the fertility hormones: Follicle stimulating hormone (FSH) and Luteinizing hormone (LH) respectively. The sera were assayed using the Enzyme Linked Immuno-Sorbent Assay (ELISA) test kit (Bio-inteco®, UK). LH and FSH were determined according to the protocol described by Abraham, (1981) and Uotila *et al.,* (1981).

Body and Reproductive organ weight: After blood sample collection, the mice were placed in a desiccator and anaesthetized using chloroform after which they were sacrificed via cervical dislocation. Three mice from each test group and control were sacrificed after exposure on days 21, 42 and 84. The animals were weighed using a weighing balance. The reproductive organs (testis and epididymis) were excised after dissection and also weighed using a weighing balance to determine the absolute weight of the organs. The organosomatic indices of the organs were also determined as described by Radoslav et al., (2016).

The percentage weight increase (PWI) of the animals was determined using the formula**:**

$$PW1 = W_u - W_v / W_v \times 100;$$ where,

W_u represents final mean total body weight; and

W_v represents Initial mean total body weight.

Semen collection and analysis: Semen samples were collected from the mice on each sacrifice day. The anaesthetized mice were sacrificed after a mid-caudo-ventral abdominal incision was made with a pair of sterilized scissors to access the testis. The epididymis was separated and trimmed from each testis and semen sample was then collected from the tail of the epididymis via an incision made with a scalpel blade. Each semen sample was evaluated for sperm vitality as described by Bjorndarhl *et al.,* (2003).

Sperm Morphology Assay and Testicular Histopathology: After sacrificing the mice, the testes were removed and the cauda-epididymis were separated from the testes. Semen suspensions were prepared and assessed for morphological changes as described by Takeda, *et al.,* (2016). For the histopathology, the testes was fixed in 10% formalin for 72h, thoroughly washed under running water and dehydrated in ascending series of ethyl alcohol, cleared in xylene and embedded into soft paraffin wax, after which 5μm slices were cut with a microtome. They were then stained using hematoxylin-eosin dye and the slides examined under a light microscope at x 400 magnification.

Statistical Analysis

Data obtained were analyzed using one way ANOVA. Differences between the means and Standard Deviation (SD) of the control and experimental groups were considered significant at $p<0.05$.

RESULTS

LH and FSH Reproductive Hormones of Mice Exposed to Contaminated Well Water: Tables 1 and 2 show the results of the effects on LH and FSH levels respectively, in mice orally exposed to ground water contaminated with leachate from scrap automobile spare parts in the Araromi automobile spare parts market in Ibadan Nigeria. On day 84 post exposure, mean LH and FSH levels were significantly elevated ($p<0.05$) in three of the four 100% concentrations of CWW 1 and CWW 7, when compared to the control (DW) and lower dilutions of 25-75% of both contaminated well-water samples respectively. LH in mice given 100% CWW 1 was significantly elevated at 0.97±0.45mlU/mL, while LH in the 100% CWW 7 mice group (1.21±1.18 mlU/mL) was elevated but not significant at $p<0.05$. The mean FSH levels in mice given 100% water from both contaminated wells (CWW 1 and CWW 7) were significantly elevated when compared to the control and lower concentration groups.

Body weight: The results of the body and organ weights of mice orally exposed to the contaminated ground water are presented in Table 3, Figs. 2 and 3 respectively. By day 42, the control mice showed a significant weight gain when compared to other test concentrations (for both CWW 1 and CWW 7 ($p<0.05$). On the other hand, mice exposed to the contaminated water, 100% CWW 1 gained more weight by day 21 compared to the control. Mice exposed to varying concentrations of

CWW 7 also showed significantly increased weight gain in the 25% concentration by day 21; while mice in the 50-75% concentration group experienced significantly increased weight gain compared to all other test concentrations and the control by day 84. All the above differences were significant at $p<0.05$ (Table 3).

Organosomatic indices

Testes: Mice exposed to varying concentrations of CWW 1 showed no significant differences ($p<0.05$) in testes weight and testicular organosmatic index between all concentrations and the control over the study period. In addition, the weights observed did not follow a dose dependent pattern (Figs.2-3). For mice exposed to CWW 7, again, no dose dependent effects were observed in testes weight in all concentrations and control. However, a significant increase ($p<0.05$) was observed in the testicular organosomatic index of mice in the 75% CWW 7 concentration on both days 21 and 84, when compared to the control; while mice in the 25% concentration group showed a significant reduction in testicular organosomatic index when compared to the control by day 84.

Table 1:
LH Hormone of Mice Exposed to Distilled Water (DW), Contaminated Well Water l and 7 (CWW 1 and 7)

	CWW1			CWW 7		
Groups/ Days of Exposure	**Day 21**	**Day 42**	**Day 84**	**Day 21**	**Day 42**	**Day 84**
Control (DW)	0.06 ± 0.06^{a}	0.06 ± 0.00^{a}	0.06 ± 0.06^{a}	0.06 ± 0.06^{a}	0.06 ± 0.00^{a}	0.06 ± 0.06^{a}
25%	0.54 ± 0.42^{a}	0.04 ± 0.04^{a}	0.07 ± 0.05^{a}	0.11 ± 0.11^{a}	0.03 ± 0.03^{a}	0.50 ± 0.41^{a}
50%	0.16 ± 0.02^{a}	0.04 ± 0.04^{a}	0.04 ± 0.01^{a}	0.21 ± 0.21^{a}	0.10 ± 0.10^{a}	0.09 ± 0.02^{a}
75%	0.16 ± 0.06^{a}	0.03 ± 0.03^{a}	0.01 ± 0.01^{a}	0.14 ± 0.14^{a}	0.04 ± 0.04^{a}	0.05 ± 0.02^{a}
100%	0.11 ± 0.03^{a}	0.04 ± 0.04^{a}	0.97 ± 0.45^{b}	0.57 ± 0.42^{a}	0.04 ± 0.04^{a}	**1.21 ± 1.18^{a}**

Values are expressed as Mean ± SD. Means with the same letters are not significantly different. LH- Luteinising Hormone.

Table 2:
FSH Hormone of mice exposed to distilled water (DW) and contaminated well water l and 7 (CWW 1 and 7)

	CWW 1			CWW 7		
Groups/ Days of Exposure	**Day 21**	**Day 42**	**Day 84**	**Day 21**	**Day 42**	**Day 84**
Control (DW)	0.05 ± 0.04^{a}	0.03 ± 0.03^{a}	0.05 ± 0.04^{a}	0.05 ± 0.04^{a}	0.05 ± 0.05^{a}	0.05 ± 0.05^{a}
25%	0.08 ± 0.00^{a}	0.03 ± 0.03^{a}	0.07 ± 0.00^{a}	0.05 ± 0.05^{a}	0.03 ± 0.03^{a}	0.65 ±0.57^{a}
50%	0.19 ± 0.12^{a}	0.03 ± 0.03^{a}	0.06 ± 0.00^{a}	0.03 ± 0.03^{a}	0.04 ± 0.04^{a}	0.10 ± 0.03^{a}
75%	0.09 ± 0.00^{a}	0.03 ± 0.03^{a}	0.03 ± 0.00^{a}	0.20 ± 0.20^{a}	0.03 ± 0.03^{a}	0.06 ± 0.01^{a}
100%	0.08 ± 0.00^{a}	0.03 ± 0.03^{a}	**1.45 ± 0.78^{b}**	0.70 ± 0.54^{a}	0.04 ± 0.04^{a}	**1.11 ± 1.02^{c}**

Values are expressed as Mean ± SD. Means with the same letters are not significantly different. FSH- Follicle stimulating Hormone, LH- Luteinising Hormone

Table 3:
Body Weights of Mice Exposed to CWW 1, CWW 7 and Distilled Water at Day 21, 42 and 84

	DAY 21			DAY 42			DAY 84		
GROUPS/ CONC. (%)	**IBW (g)**	**FBW (g)**	**PWC (%)**	**IBW (g)**	**FBW (g)**	**PWC (%)**	**IBW (g)**	**FBW (g)**	**PWC (%)**
Cont. (DW)	25.9±0.3^{c}	33.6±4.1^{c}	29.7±10.7^{a}	11.9±3.0^{a}	30.7±2.7^{bcd}	158.6±59.2$_{b}$	25.9±0.3^{c}	33.6±4.1^{c}	29.7±10.7^{a}
25% CWW1	17.8±2.0^{a}	19.2±4.1^{a}	7.9±0.4^{ab}	18.0±0.4^{ab}	26.5±2.1^{a}	47.1±2.3^{a}	19.2±0.6^{b}	27.0±1.1^{abc}	40.7±29.2^{a}
50% CWW1	19.9±2.1^{ab}	26.0±5.2^{abc}	30.7±8.7^{ab}	19.±1.8^{c}	27.0±2.6^{ab}	37.1±5.7^{a}	16.6±0.6^{ab}	26.0±5.2^{ab}	56.8±9.4^{a}
75% CWW1	22.1±1.0^{b}	29.5±0.1^{bc}	33.5±7.5^{ab}	18.1±0.5^{c}	31.9±3.7^{d}	76.0±10.8^{a}	16.4±1.8^{ab}	27.4±2.9^{abc}	66.8±23.8^{a}
100%CWW1	19.9±0.3^{ab}	30.5±2.0^{bc}	53.3±18.3$_{b}$	17.6±2.3^{bc}	31.2±1.3^{cd}	76.8±16.6^{a}	13.8±2.6^{a}	24.9±0.8^{a}	80.1±14.5^{a}
25% CWW7	18.0±1.0^{bc}	27.4±4.4^{bc}	52.2±24.2^{b}	17.4±0.7^{bc}	28.4±1.8^{abcd}	63.3±15.2^{a}	18.2±1.8^{b}	30.8±1.8^{abc}	69.4±5.9^{a}
50% CWW7	22.0±2.7^{b}	25.9±0.9^{abc}	18.0±4.0^{a}	14.7±1.1^{ab}	26.4±0.8^{a}	79.0±6.3^{a}	14.7±1.1^{a}	29.9±0.3^{abc}	111.3±28.1$_{b}$
75% CWW7	23.3±0.7^{b}	23.0±1.8^{ab}	-1.3±0.3^{a}	19.5±1.6^{c}	27.7±1.5^{abc}	42.3±3.5^{a}	19.5±1.6^{b}	29.2±4.2^{abc}	139.2±5.5$_{b}$
100%CWW7	22.1±2.1^{b}	28.6±4.1^{bc}	29.4±2.4^{ab}	17.4±2.3^{bc}	27.6±1.7^{abc}	58.9±4.6^{a}	17.4±2.3^{a}	32.8±3.1^{bc}	58.0±15.8^{a}

Values are expressed as Mean ± SD. Means with the same letters are not significantly different.
IBW- Initial Body weight, FBW-Final Body Weight, PWI (Percentage weight increase)= (Wf-Wi)/(Wi) × 100

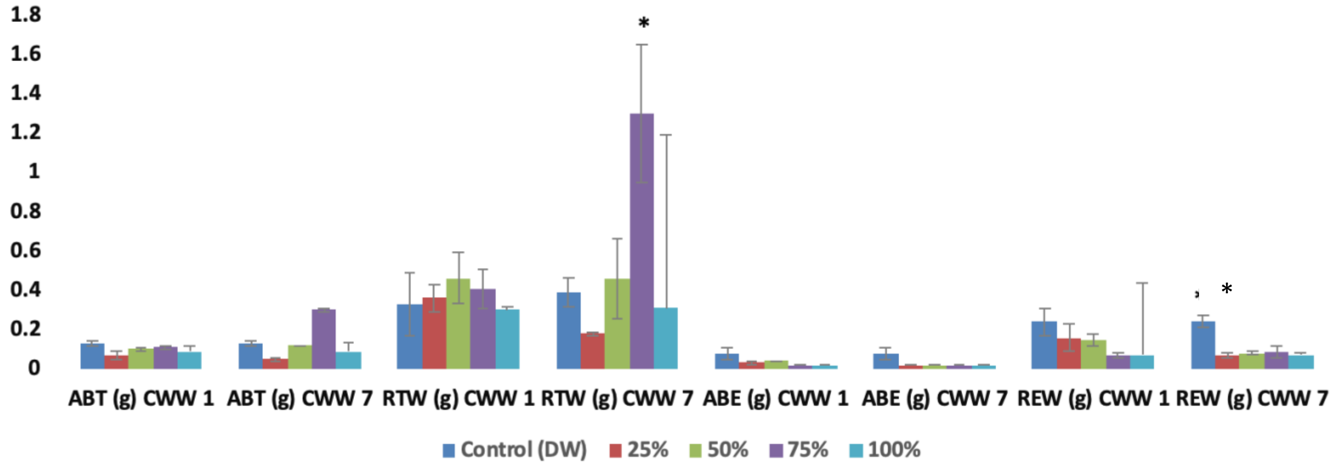


Figure 1
Absolute and Relative Organ Weight of mice exposed to the Contaminated Water and Distilled Water Control at day 21

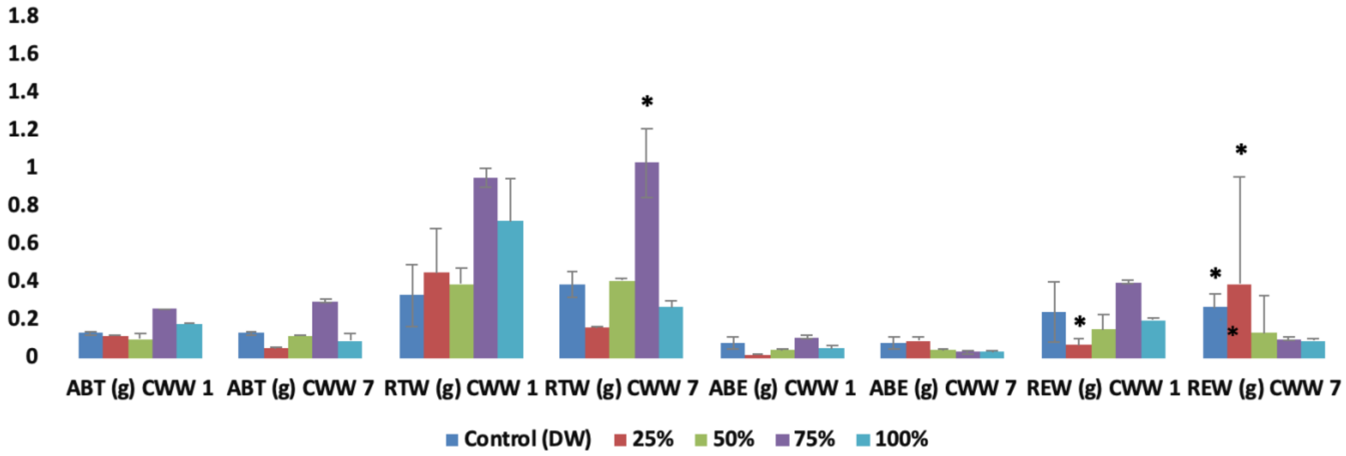


Figure 2:
*Values are expressed as Means ± SD. *Significant at p<0.05; ATW- Absolute Testis Weight, TOI- Testicular Organosomatic Index, AEW- Absolute Epididymis Weight,*
EOI- Epididymal Organosomatic Index.

On the other hand, the testicular organosomatic index of mice in the other two test concentrations (50% CWW 7 and 100% CWW 7) did not differ significantly from those of the control (Figs. 2-3).

Epididymis: Mice exposed to contaminated well water (CWW 1) showed a non-significant but dose-dependent decrease in the epididymal organosomatic index compared to the control by day 21 (Fig. 2), while by day 84, the 25% concentration group showed significant reduction in weight compared to the control and other test concentrations (Fig. 3). On day 21, the epididymal organosomatic index of mice exposed to contaminated well water 7 (CWW 7) were significantly reduced in all test concentrations when compared to the control (Fig. 3). Similarly by day 84, higher concentrations of 50 – 100% CWW 7 showed a significant reduction in their epididymal organosomatic index when compared to the control and the low-dose (i.e. 25% concentration CWW 7) (Fig. 3)

Sperm and Semen Characteristics: By day 21, mice exposed to the contaminated water CWW 1 showed significant ($p<0.05$) dose-dependent reduction in sperm count and percentage motility when compared to control (DW); with the lowest values for sperm count and percentage motility observed in mice exposed to the highest concentration (Table 4). Mice exposed to the contaminated water, CWW 7 also showed a significant ($p<0.05$) reduction in sperm count and motility (except the 50% concentration group), although this reduction was not dose-dependent. Sperm livability showed a dose-dependent reduction in CWW 1 exposed mice compared to the control, although these differences were not significant at $p<0.05$. Except for the 75% concentration group, the CWW 7 exposed mice showed a marginal reduction in livability compared to the control, although this difference was not significant at $p<0.05$ and did not follow a dose-dependent pattern (Table 4). Sperm volume however remained constant in control and treated mice (Table 4). By day 84, the CWW 1 and CWW 7 treated groups showed a significant reduction in sperm motility when compared to the control. However, livability and sperm volume in contaminated water treated groups did not differ significantly from the control at $p<0.05$ (Table 5). Sperm count in the 50-100% concentration groups were significantly ($p<0.05$) reduced compared to the control and the 25% concentration group for CWW 1; while the CWW 7 treated groups showed a non-significant reduction in sperm count compared to the control except for the 25% treated group where this reduction was significant ($p<0.05$) (Table 5).

Table 4:
Mean values for sperm and semen characteristics of Swiss albino Mice test groups at day 21.

Parameters			CWW 1				CWW 7		
	Control (DW)	**25%**	**50%**	**75%**	**100%**	**25%**	**50%**	**75%**	**100%**
Motility (%)	92.50±3.54^{b}	75.00±7.07^{a}	70.00±0.00^{a}	70.00±0.00^{a}	65.00±7.07^{a}	70.00±0.00^{a}	80.00±0.00^{a}	70.00±0.00^{a}	75.00±7.07^{a}
Livability (%)	98.00±0.00^{a}	95.00±0.00^{a}	95.00±0.00^{a}	95.00±0.00^{a}	91.50±9.19^{a}	95.00±0.00^{a}	96.50±2.12^{a}	98.00±0.00^{a}	96.5±2.12^{a}
Volume (m/s)	5.10±0.00^{a}	5.10±0.00^{a}	5.10±0.00^{a}	5.10±0.00^{a}	5.10±0.00^{a}	5.10±0.00^{a}	5.08±0.04^{a}	5.10±0.00^{a}	5.08±0.04^{a}
Sperm Count (x10^{6}sperm/ml)	126.50±6.36^{c}	119.50±0.00^{c}	101±0.00^{b}	105.00±0.00^{b}	83.00±7.07^{a}	89.00±0.00^{a}	117.50±3.54$_{c}$	98.00±0.00$^{a}{}_{b}$	108.00±14.14$^{a}{}_{b}$

Means with similar letters are not significantly different at $p<0.05$

Table 5:
Mean values for sperm and semen characteristics of Swiss albino Mice in test groups at day 84.

Parameters			CWW 1				CWW 7		
	Control (DW)	**25%**	**50%**	**75%**	**100%**	**25%**	**50%**	**75%**	**100%**
Motility (%)	90.00±3.54^{b}	70.00±0.00^{a}	70.00±0.00^{a}	70.00±0.00^{a}	75.00±7.07^{a}	70.00±0.00$_{a}$	70.00±0.00^{a}	70.00±0.00^{a}	75.00±7.07^{a}
Livability (%)	98.00±0.00^{a}	98.00±0.00^{a}	95.00±0.00^{a}	98.00±0.00^{a}	96.50±2.12^{a}	96.50±2.12^{a}	96.50±2.12^{a}	96.50±2.12^{a}	96.50±2.12^{a}
Volume (m/s)	5.10±0.00^{a}	5.10±0.00^{a}	5.10±0.00^{a}	5.08±0.04^{a}	5.10±0.00^{a}	5.08±0.04^{a}	5.08±0.04^{a}	5.08±0.04^{ab}	5.10±0.00^{a}
Sperm Count (x10^{6}sperm/ml)	126.50±6.36^{c}	121.50±0.71$_{c}$	101.00±0.0^{a}	106.5±2.12$_{b}$	99.50±2.12$_{b}$	98.00±0.00$_{b}$	103.0±7.07$_{bc}$	111.50±9.19$_{c}$	97.00±11.31$_{bc}$

Table 6:
Mean Values of Spermatozoa Morphology Of Swiss Albino Mice In Different Treatment Groups For Contaminated Well Water (CWW 1 And 7) At Day 21.

Parameters		CWW 1	CWW 7	Parameters		CWW 1	CWW 7	Parameters	
	Control (DW)	**25%**	**50%**		**Control (DW)**	**25%**	**50%**		**Control (DW)**
No Hook	1.20±0.45^{a}	2.80±2.05^{a}	1.80±0.84^{a}	1.60±0.89^{a}	5.60±2.41^{b}	1.50±0.58^{a}	2.20±2.68^{a}	1.80±0.84^{a}	5.60±2.41^{b}
Folded	0.00±0.00^{a}	1.33±0.58^{b}	0.00±0.00^{a}	0.00±0.00^{a}	1.00±0.00^{b}	1.00±0.00^{b}	0.00±0.00^{a}	0.00±0.00^{a}	1.00±0.00^{b}
Amorphous head	1.00±0.00^{a}	4.00±2.92^{a}	2.00±1.73^{a}	2.25±1.89^{a}	2.40±2.07^{a}	1.25±0.50^{a}	1.33±0.58^{a}	2.20±0.84^{a}	2.40±2.07^{a}
Short Hook	0.00±0.00^{a}	1.20±0.45^{b}	1.00±0.00^{ab}	1.75±0.96^{b}	1.40±0.55^{b}	1.33±0.58^{b}	0.00±0.00^{a}	2.00±1.00^{b}	1.40±0.55^{b}
Bent Knob Hook	1.33±0.58^{ab}	1.60±0.89^{ab}	1.00±0.00^{a}	2.33±0.58^{b}	1.60±0.89^{ab}	1.00±0.00^{a}	1.00±0.00^{a}	1.40±0.00^{a}	1.60±0.89^{a}
Banana Head	0.00±0.00^{a}	1.33±0.58^{b}	0.00±0.00^{a}	1.50±0.71^{b}	1.00±0.00^{b}	2.20±1.10^{ab}	3.67±3.79^{b}	0.00±0.00^{a}	1.00±0.00^{a}

TABLE 7:
Mean Values Of Spermatozoa Morphology Of Swiss Albino Mice In Different Treatment Groups For Contaminated Well Water (CWW 1 AND 7) AT DAY 84.

Parameters	Control (DW)	CWW 1				CWW 7			
		25%	50%	75%	100%	25%	50%	75%	100%
No Hook	1.20±0.45^{a}	10.50±7.05^{b}	5.00±4.30^{ab}	7.80±5.22^{ab}	11.00±6.16^{b}	7.75±8.92^{a}	7.75±6.80^{a}	8.40±11.3^{a}	6.60±7.89^{a}
Folded	1.00±0.00^{a}	2.50±0.71^{a}	2.00±0.00^{a}	2.00±1.41^{a}	1.50±0.71^{a}	1.67±0.58^{a}	4.50±4.95^{a}	1.33±0.58^{a}	2.00±0.00^{a}
Amorphous head	2.50±0.71^{a}	7.40±5.68^{a}	4.60±4.22^{a}	6.40±3.65^{a}	10.60±6.10^{a}	9.00±11.27^{a}	5.00±3.65^{a}	6.75±7.59^{a}	7.20±7.60^{a}
Short Hook	1.00±0.00^{a}	2.00±1.41^{a}	2.25±1.26^{a}	6.20±3.77^{a}	4.00±4.44^{a}	5.25±7.85^{a}	3.75±1.50^{a}	8.67±6.66^{a}	8.50±4.93^{a}
Bent Knob Hook	1.00±0.00^{a}	7.00±7.68^{a}	4.00±4.08^{a}	7.80±5.50^{a}	4.00±5.10^{a}	2.50±1.29^{a}	4.20±2.68^{a}	3.75±3.77^{a}	6.75±5.56^{a}
Banana Head	0.00±0.00^{a}	2.00±1.00^{a}	2.00±1.41^{a}	2.00±1.41^{a}	4.20±4.44^{a}	2.50±1.91^{a}	2.50±1.73^{a}	1.33±0.58^{a}	2.50±1.29^{a}
Thin Mid Piece	1.50±0.71^{ab}	0.00±0.00^{a}	0.00±0.00^{a}	1.00±0.00^{ab}	2.50±2.12^{b}	0.00±0.00^{a}	1.00±0.00^{b}	1.00±0.00^{b}	1.00±0.00^{b}
Rough Surface Mid Piece	0.00±0.00^{a}	2.50±2.12^{b}	2.00±1.41^{ab}	1.33±0.58^{ab}	1.33±0.58^{ab}	1.33±0.58^{ab}	1.00±0.00^{ab}	2.50±2.12^{b}	3.00±0.00^{ab}
Short Tail	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	1.00±0.00^{ab}	2.00±1.41^{b}	2.00±0.00^{ab}	2.00±1.41^{b}	1.50±0.71^{ab}	1.50±0.71^{ab}
Wrong Tail Attachment	0.00±0.00^{a}	1.00±0.00^{ab}	1.00±0.00^{ab}	1.50±0.71^{ab}	1.67±1.15^{b}	7.50±7.78**b**	0.00±0.00**a**	1.00±0.00**ab**	1.67±1.15**b**
Pin Head	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	6.40±4.62^{b}	3.40±1.82^{ab}	0.00±0.00^{a}	0.00±0.00^{a}	3.60±1.95^{ab}	5.00±3.39^{b}
Long Pin Head	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	6.40±4.62^{b}	3.40±1.82^{ab}	0.00±0.00^{a}	0.00±0.00^{a}	2.00±0.82^{a}	9.20±5.26^{b}

Means with similar letters are not significantly different at p<0.05

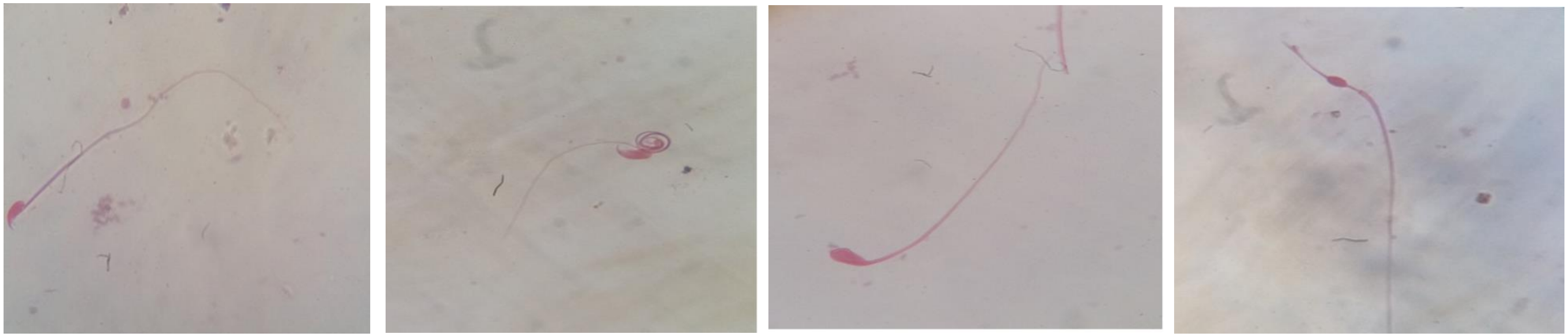
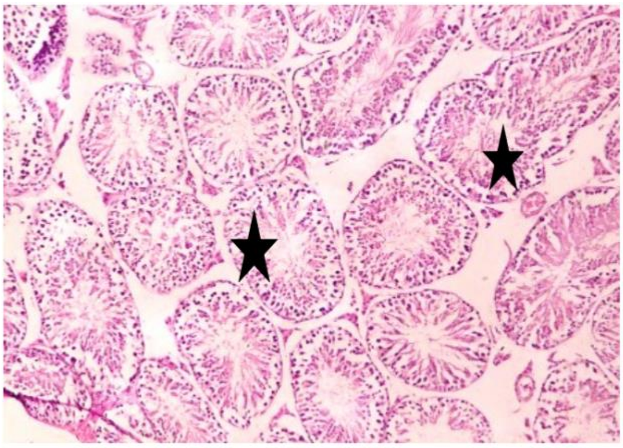
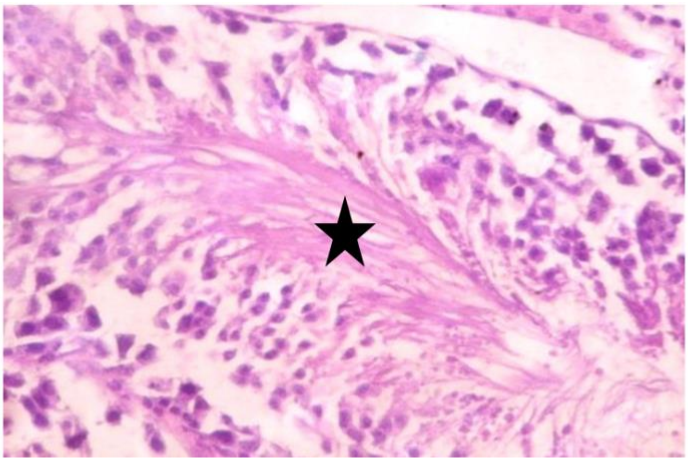


Plate 2: a: Normal Sperm b: Folded sperm with folded mid piece (×100 obj.**).** C: Amorphous head d: Long Pin Head (×100 obj.)

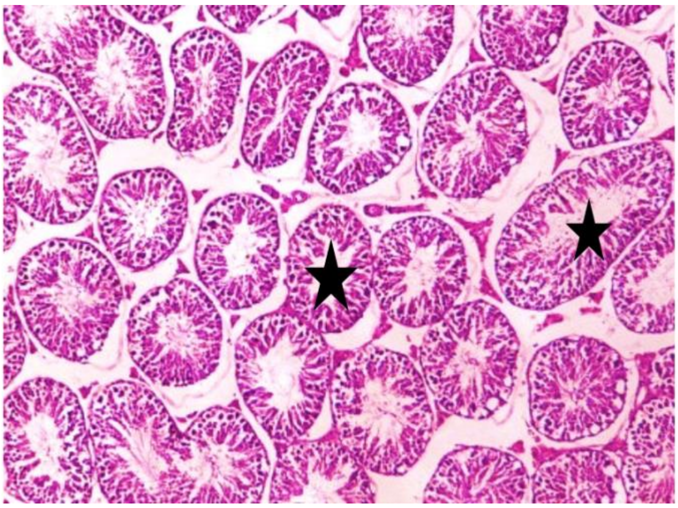
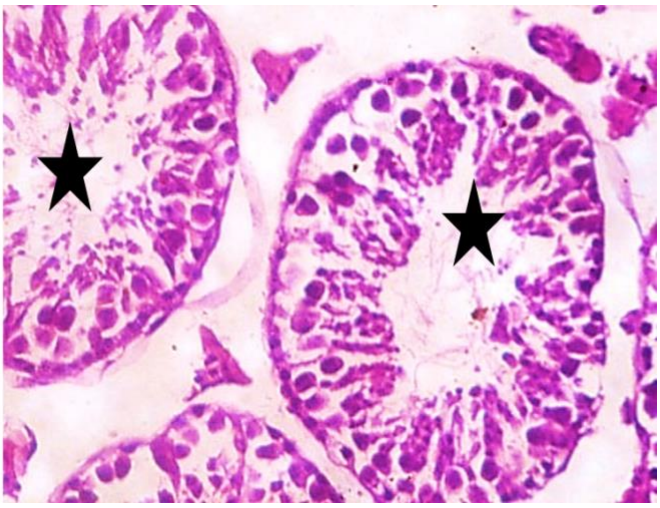
Sperm Morphology: A normal spermatozoon of Swiss albino mice is shown in Plate 2a. The sperm head has a distinct hook attached to the acrosome and a tail which is attached to the head at a right angle (Plate 2a). Tables' 6-7 show the morphology of spermatozoa exposed to the contaminated groundwater and the distilled water control over the exposure period. It is interesting to note that the different types of abnormal spermatozoa observed increased over the 84-day exposure period from six types of abnormal spermatozoa in exposed mice by day 21 to twelve by day 84 respectively (Tables 6-7). Other morphologic abnormalities observed by day 84 in exposed mice were thin mid piece, rough surface mid piece, short tail, abnormal tail attachment as well as sperms with pin head and long pin head respectively (Table 7). On the contrary, abnormalities observed in control mice ranged from three by day 21, to six by day 84 (Tables 6-7) with the numbers of these significantly lower ($p<0.05$) in control mice compared with exposed mice. Exposed mice showed increased numbers of abnormal sperm compared to control, with these differences significant at $p<0.05$ for no hook, folded sperm, short hook (100% CWW 1 and 7 respectively at day 21) and banana head (100% CWW 1 at day 21). There was a significant progressive increase in abnormalities such as no hook from 5.60 ± 2.41 in the 100% CWW 1 concentration by day 21 (Table 6) to peak as high as 11.00 ± 6.16 in the same concentration group by day 84 (Table 7). Amorphous head occurrence also increased from 2.40 ± 2.07 in the same concentration group by day 21 (Table 6) to 10.60 ± 6.10 by day 84 (Table 7). Short tail, abnormal tail attachment, thin mid piece, pin head and long pin head were abnormalities observed in exposed mice at significantly higher ($p<0.05$) concentrations of 75% and/or 100% CWW 1, as well as at the 25% and 100% concentrations for CWW 7 on day 84 (Table 7)

Testicular Histology: The photomicrographs of sections of the testes of control and exposed mice are presented in Plates 3 – 5 a and b respectively. The testicular tissue of control mice showed normal features with the seminiferous tubules (Plates 3a & 3b) and maturing spermatids in their lumen (3b). Histology of testes of mice exposed to 50% and 100% of CWW 1 and 7 indicated the presence of few spermatids in the lumen of the seminiferous tubules (Plate 4a), while the testicular tissue of mice in the CWW 7 50% concentration group showed epithelial degeneration (Plate 4b).

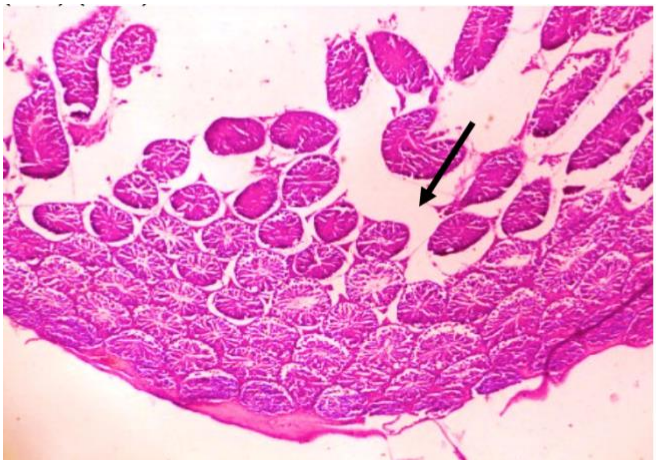
The testicular tissue of exposed mice in the 100% concentration groups (CWW 1 100%; day 21) and (CWW 7 100%; day 84) showed abnormalities such as mild fluid exudation into the interstitial space and degeneration of the germ cells (Plates 5a & 5b)

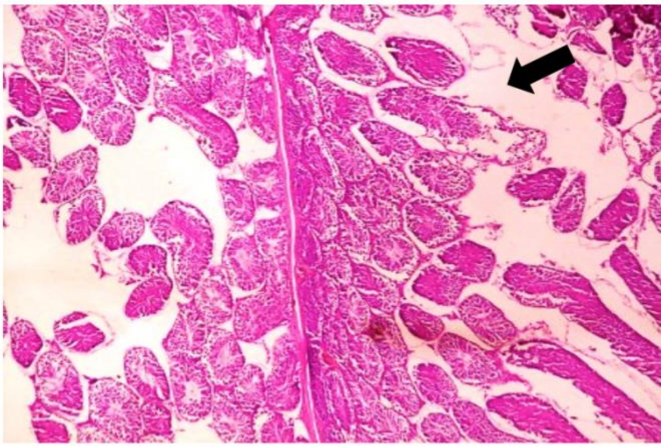



Plates 3a & 3b:
Testicular tissue of control mice showing the seminiferous tubules (L) (x200) with maturing spermatids (star) in the lumen (R) (x400).

Plates 4a & 4b:
Testicular tissue of exposed mice (L) CWW1 50% day 84 showing seminiferous tubules with few spermatids in their lumen (x200) and (R) CWW 7 50% day 21 showing tubular epithelial degeneration (star) (x400).





Plates 5a & 5b:
Testicular tissue of exposed mice (L) CWW 1 100% day 21 and (R) CWW 7 100% day 84 showing mild fluid exudation into the interstitial space (arrow) and degeneration of the germ cells (Mag: x100).

DISCUSSION

Water is a major means of exposure to hazardous chemicals. The concentrations of iron, lead, cadmium and arsenic present in the groundwater used in this study have been shown to exceed the permissible limits of WHO (2017). Details are presented in a separate paper (Oni *et al.,* 2020; in preparation). Exposure to lead is associated with a wide range of effects including impaired fertility and adverse pregnancy outcomes (WHO, 2017). Food and drinking water including beverages made from contaminated drinking water constitute the most important sources of exposure to metals such as arsenic. Arsenic concentrations of 10µg/L (0.01 mg/L) in drinking water as observed in the groundwater indicates a source of human and animal intake of arsenic (WHO, 2017). There is overwhelming evidence that the consumption of elevated levels of arsenic via drinking water is causally related to the development of cancer at several sites (WHO, 2017). Benzo (a) pyrene, anthracene and benzo(a) anthracene were detected in well 1; while benzo (b) fluoranthene, anthracene, benzo 1, 2-anthracene and benzo (a) anthracene were detected in well 7 with levels exceeding the EPA regulatory limits (EPA, 2002) thus confirming the polluted status of the well water. Furthermore, four of eight PAHs identified in the water are priority pollutants categorized as carcinogens according to the International Agency for Research on Cancer (IARC) and USEPA. These include benzo (a) pyrene, which is classed as a group I carcinogen; and benzo (a) anthracene, benzo (b) fluoranthene and chrysene which are listed as group II carcinogens (Abdel-Shafy and Mansour, 2016). The soils of the study area are darkened due to the presence of spent engine oil, crankcase oil, brake fluid, grease, fuel and other volatile compounds arising from the sales, service or repair activities of the engines and other automobile parts. The heavy metals and PAHs may have been leached into the underground water from these toxic wastes. Intake of the contaminated water can lead to exposure to the toxic effects of the leachates and the associated toxicities as observed in this study.

Reece (1997) reported that exposure to metals and hydrocarbons could alter sex hormonal levels which in turn could affect spermatogenesis or cause abnormalities in seminal fluid resulting in functional or structural impairment of sperm. The alterations in sex hormonal levels due to exposure to the polluted groundwater suggest that some of the chemical pollutants in the water may alter the function of the endocrine system consequently causing adverse reproductive effects. The observed increase in FSH and LH levels in all the 100% concentrations of CWW 1 and CWW 7 by day 84, probably indicate some degree of interference with spermatogenesis. LH stimulates testosterone synthesis by the Leydig cells, which in turn acts on the Sertoli cells to initiate spermatogenesis. FSH is responsible for spermatogenesis by binding with receptors on the Sertoli cells to complete the development of the spermatozoa from the spermatids (young sperm cells). FSH is thus responsible for sperm cell production (Ajayi *et al*, 2011), while LH triggers the process with the secretion of testosterone. Increased levels of FSH have been associated with a decrease in spermatogenesis, causing reduced sperm motility, alterations in morphology, sperm concentration and viability (Broer *et al*, 2014).

It is noteworthy that the 100% contaminated water concentrations group showed the highest levels of FSH and this was associated with the lowest sperm motility and the highest number of certain morphologic abnormalities such as sperms with no hook or short hook. This suggests that the increase in FSH (and LH) is associated with decreased and abnormal spermatogenesis and could therefore likely lead to reduced fertility. Previous report has shown that men with azoospermia (no sperm) and severe oligozoospermia (few numbers of sperm) have elevated levels of FSH which were indicative of damaged seminiferous tubules (Lanes *et al*, 2010). Sperm cell development begins in the Sertoli cells, located in the lower parts of the seminiferous tubules. As the sperm cells mature, they move along the seminiferous tubules and are stored in the upper part of the tubules (Oyeyemi and Ubiogoro, 2005). Although testosterone levels were not determined in the mice in this study, decreased spermatogenesis as indicated by the above results suggest that testosterone levels could probably have been reduced. Normally, increased levels of testosterone should result in a decrease in the secretion of Gonadotropin releasing hormones (GnRH) from the hypothalamus thus causing a negative feedback and a resultant decline in the levels of LH and FSH (Tabatabaei *et al.*, 2009). The fact that LH and FSH levels remained significantly high at the highest contaminated water concentrations of 100% for both CWW 1 and CWW 7 by the end of the exposure period suggests a failure in the secretion of the GnRH and the negative feedback mechanism probably due to reduced testosterone production. The significant reduction in sperm motility, sperm count and increased number of certain sperm abnormalities observed in this study is highly suggestive of reduced levels of testosterone which ultimately resulted in the significantly elevated levels of LH and FSH observed.

Mice that received the contaminated water, CWW 1 and CWW 7 showed decreased body weight compared to the control by day 42. The reduction in body weight in exposed mice may be due to the additional energy requirement involved in resisting the pollutants associated with the well-water, through avoidance, exclusion, removal and complexing. This extra energy requirement may likely have decreased the "scope for growth" ultimately resulting in reduced growth (Spurgeon and Hopkin, 1996). While it is unclear why some of the exposed mice showed increased growth rate compared to the control, Jeng and Bocca, (2013) in their studies on the influence of exposure to Benzo (a) Pyrene (BaP) on mice testicular germ cells during spermatogenesis similarly observed a marginal increase in the body weight of the mice at 1 and 10 mg/kg/day BaP exposure when compared to the control. It is possible that other organic pollutants in the water served as some form of nutrient sources for enhanced growth in the mice. However, more studies will be required to elucidate this assertion.

The significant increase in testicular organosomatic index in mice in the 75% CWW 7 concentration group and the significant reduction in the epididymal organosomatic index observed when compared to the control shows the effects of the pollutants in the water on spermatogenesis. Wirth and Mijal, (2010) reported that lead accumulates preferentially in the epididymis and other accessory organs and causes changes in the weight of accessory reproductive organs. Other adverse effects of lead include disruption of the hypothalamic-testicular-pituitary axis with resultant alterations in semen quality (Wirth and Mijal, 2010), as observed in this study. Aromatic hydrocarbons such as Benzo(a) Pyrene have also been associated with significant dose-dependent reduction in the weights of epididymis and testis in studies involving prenatal exposure of mice to BaP suggesting the possible contributory role of PAHs as well (Nakamura *et al*, 2012). Similarly, Jeng and Bocca, (2013) observed a significant decrease in epididymis and testis weights in mice exposed to BaP. However, studies by Jeng and Yu, (2008) on alterations in sperm quality and hormone levels by PAHs did not show any significant reduction in body, testes and epididymis weights. The significant reduction in sperm motility and sperm count observed in the treatment groups compared to the control is suggestive of the antifertility potential of the pollutants in the water over time. According to Nwaigwe *et al.* (2012), the efficiency of spermatogenesis is assessed according to the amount of spermatozoa produced per gram of testicular parenchyma and it is not influenced by the differences in testicular size among animals. The significant reduction in spermatozoa count (oligozoospermia) and the increase in sperm abnormalities (teratozoospermia) observed in the cauda epididymis sperm reserves of the exposed mice suggests an interference with testicular spermatogenesis. Most causes of male infertility are related to sperm health quality such as abnormal sperm morphology and poor motility. Other causes include sperm that cannot attach its head to the egg, or sperm that cannot penetrate the egg (Egwurugwu *et al*, 2013). The lack of a hook on the sperm head as observed with increased exposure in this study may compromise the sperm's ability to attach and penetrate the egg. Olukole and Obayemi, (2010) observed that abnormally shaped spermatozoa such as those with round, pin, very large or double heads and absent tails may reduce its ability to fertilize the egg. The higher numbers of abnormal sperm observed over time in this study may also contribute to progressive reduction in male fertility. Crude or spent oil exposure have previously been observed to cause developmental defects, structural abnormalities of the spermatozoa and testicular changes such as lack of sperm, reduced sperm count and sterility (Igwebuike *et al*. 2007). The occurrence of sperm head abnormalities may be attributed to chromosomal aberrations which occur during the packaging of genetic material in the sperm head. It could also be due to the occurrence of point source mutation in testicular DNA or errors during the differentiation of spermatozoon during spermatogenesis (Bakare *et al.*, 2005).

Heavy metals may accumulate in testicular tissues due to disruption of the Blood-Testis Barrier (BTB) (Chandra *et al*, 2007). This in turn may lead to the disappearance of Sertoli cells and loss of gametes in the lumen of the seminiferous tubules. Animal studies have also demonstrated that PAHs such as benzo[a]pyrene can cause histologic changes in the testis of adult male animals. Histological sections of testicular tissue in this study showed reduced presence of spermatids associated with degeneration of germ cells. The interstitial tissues surrounding the tubules contain the Leydig cells, which are responsible for the synthesis of testosterone following stimulation by the LH. The mild interstitial pathology may lead to loss of these cells, which may in turn result in reduced secretion of testosterone and may perhaps explain the reduction in spermatogenesis as indicated by the significantly reduced numbers of spermatozoa in exposed mice when compared to the control. Ultimately, other processes of spermatogenesis may be affected and this may also explain the scanty presence of spermatids which may suggest an interference with the maturation processes of the spermatids.

In conclusion, Swiss albino mice as mammals share a high degree of homology to humans. The implications of the findings from this study highlights the potential male antifertility effects of oral exposure to contaminated well-water from the Araromi automobile spare-parts market, Ibadan, South-Western Nigeria on prolonged domestic consumption of the water. This calls for urgent remedial action by the relevant authorities and the need to take necessary steps to protect exposed populations in order to safeguard human and animal health.

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