• Original Article

Reproductive toxicity assessment of Olususon municipal landfill leachate in *Mus musculus* using abnormal sperm morphology and dominant lethal mutation assays

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Mixture of xenobiotics in Olususon landfill leachates (OSL) induced somatic and germ cell genotoxicity in eukaryotic cells. However, whether the DNA-damaged germ cells can increase reproductive failure, embryotoxicity and/or teratogenicity during fertilization remained unclear. The study herein investigated reproductive toxicity and embryotoxic effects of OSL in mice using abnormal sperm morphology and dominant lethal mutation assays. Six mice per group were exposed to 0.5 mL of 5, 10, 25, and 50% OSL (v/v, leachate/distilled water) for 5 consecutive days. Similar treatment was giving to distilled water and cyclophosphamide (20 mg/kg body weight), as negative and positive control groups, respectively. At 35 day post-treatment, the mice were sacrificed and examined for cauda epididymal abnormal sperm morphology, testicular weight change and histopathological lesions. Male mice were mated to untreated nulliparous females (1 treated male per 2 untreated females) until vaginal plugs were observed. Pregnant females were sacrificed on 14th day post-mating and uterine content examined for number of implants per female, resorptions, and live and dead fetuses. OSL significantly increased teratozoospermia by 31.02% with amorphous sperm head having the highest frequency and sperm with two heads, the least. Histology of the testes revealed congestion of the interstitial blood vessels, spermatid retention and disorganization of the germinal epithelium from the basal compartment. OSL insignificantly increased absolute and relative testes weights but reduced body weight of exposed mice. There was insignificant induction of dominant lethal mutation, although low fertility was observed in the treated mice. The analyzed physico-chemical parameters and heavy metals/ metalloids in OSL were higher than permissible limits. The metals, physico-chemical parameters and other unanalyzed substances in OSL induced abnormal sperm morphology, altered testes pathology, but did not significantly induce dominant lethal mutation in mice. Constituents of OSL are toxic to the male reproductive organs in mice and suggests harmful impact on public health. Indiscriminate disposal of solid wastes in the environment should be prevented.

Keywords: abnormal sperm morphology, dominant lethal mutation, histopathology, Olususon landfill leachate, Mus musculus

Introduction

Anthropogenic activities generate wastes, but inappropriate management of these wastes causes major public health and environmental contamination issues. These issues increase with human population and industrial activities [1,2]. Landfilling, the most common method of managing solid wastes around the world, causes nuisance to the environment via the release of chemical laden landfill gases and leachates. Njoku et al. reported enormous evidence linking exposure to solid waste chemicals with human and wildlife health challenges for over four decades [3].

Olususon landfill, an unsanitary and the largest dumpsite in Africa [4], receives over 1.0 million tons of solid wastes per annum. These wastes are generated from various human activities in Lagos State, Nigeria [5]. The dumpsite, which is located in the public domain and surrounded by residential quarters, generates and discharges landfill gases and leachates into the environment [6,7]. Leachates generated from Olususon landfill solid wastes contain microorganisms, hazardous organic and inorganic chemicals including heavy metals and radiations [8-12]. These xenobiotics can contaminate surrounding underground and surface water, soil and air, posing threat to human

health. Previous studies showed that multiple xenobiotics in Olususun landfill leachate (OSL) increased chromosome aberration and micronucleus formation in somatic cells of vertebrates inhabiting various ecological habitats and induced DNA damage and cytotoxicity in three human cell lines [6,11,12]. Similarly, Bakare et al. showed that landfill leachate increased abnormal sperm morphology in cauda epididymis of exposed mice [13]. The reports on somatic and germ cell genotoxicity induced by complex mixture of xenobiotics in landfill leachates suggest increased mutation frequency that may be associated with increased outcome of reproductive disorder and fitness instability [14]. Whether the abnormal sperm cells formed during exposure to landfill leachate are associated with reproductive failure, embryotoxicity and/or teratogenic effects in human and animal models is yet to be studied.

The outcome of studies investigating reproductive failure in rodents exposed to landfill leachates may be useful in understanding how humans residing and working within 2–3 km radius of landfill facilities expressed high incidence of low birth weight, different congenital malformations, adverse pregnancy outcomes and other reproductive or developmental anomalies [3,15,16]. In the study herein, a dominant lethal test, an assay that detects mutagens capable of increasing male germ cell mutation during spermatogenesis, embryonic viability and/or present risk of transmissible genetic damage [17], was used to evaluate embryonic viability in female mice mated with leachate exposed male mice. The study utilizes the sperm head abnormality and dominant lethal mutation test as biomarkers to assess the potentials of OSL to increase reproductive abnormalities and reduce embryonic viability in mice.

Materials and methods

Sampling site, leachate collection and processing

The sampling site, Olususun landfill, Lagos State, Nigeria (6°34′ N 3°24′ E) is the largest landfill in Nigeria covering 42 hectares of land with a life span of 35 years from inception in 1999 [18]. Raw leachate was collected from 20 different leachate wells within the landfill and thoroughly mixed to obtain a homogenous mixture [7]. This sample was transferred to the laboratory, in a pre-cleaned 10 L transparent plastic container for the subsequent experiment. The leachate sample was aliquoted for analysis of the physico-chemical parameters and heavy metal composition. The other aliquot was used for the animal experiment. The aliquot for the animal experiment was filtered with a glass wool and Whatmann® No. 42 filter paper to remove suspended particles [7,19]. The resultant filtrate was centrifuged at 600 g for 15 minutes and the supernatant

used as stock solution (100% OSL). 5, 10, 25, and 50% solutions were prepared from the stock solution based on previous studies [7,20] using distilled water (v/v, leachate/distilled water) as diluent and kept at 4°C.

Physico-chemical and heavy metal analyses of the leachate

The physico-chemical parameters; nitrate, ammonia, chloride, phosphate, sulphate, total hardness, total alkalinity, biochemical oxygen demand (BOD), chemical oxygen demand (COD), total solids (TS) and concentration of heavy metals: iron (Fe), lead (Pb), copper (Cu), manganese (Mn), arsenic (As), cadmium (Cd), and mercury (Hg), present in OSL were analyzed in accordance with standard analytical methods [21,22].

Animal acclimatization and leachate exposure regimen

Male and female mice between 10- to 12-week-old with body weight range of 18.6–25.1 g, obtained from the Animal facility of the Department of Zoology, University of Ibadan, were used. They were acclimatized to laboratory conditions of 12 hours light/dark cycle for two weeks and allowed access to food and water *ad libitum*. 0.5 mL of the various OSL concentrations; 5, 10, 25, and 50% were intraperitoneally injected into each mouse in a group of 6 male mice for 5 consecutive days. Negative (distilled water) and positive (cyclophosphamide, 20 mg/kg body weight) control groups received same treatment [23]. Cyclophosphamide, an alkylating agent, was selected as the positive control due to its ability to significantly induce abnormal sperm cells and subsequently increase dominant lethal mutations in mammalian systems [55,56]. The treated and control mice were sacrificed at 5 weeks of post-treatment, this is to allow for a complete spermatogenic cycle of 34.5 days in mice [24]. The body weight of the mice was recorded before and at the termination of the experiment using Acculab® USA, Model-vic-303 electronic analytical weighing balance (Sycamore, IL, USA). Percentage body weight gain was determined using equation (1).

$$\frac{Final\ body\ weight - Initial\ body\ weight}{Initial\ body\ weight} \times 100 \qquad (1)$$

International standard guide for care and use of laboratory animals in accordance with the US National Institute of Health (NIH Publication No. 85–23, revised in 1996) was strictly adhered during the experiment.

Sperm head abnormality assay and testis histopathological analysis

At post-exposure, the control and OSL treated mice were sacrificed and their testes surgically excised, rinsed in phosphate buffered saline (pH 7.4), blotted in Whatmann® No. 42

filter paper and absolute weight measured. Relative testes weight was determined from the absolute weight using the formula:

$$Relative\ testes\ weight = \frac{Absolute\ testes\ weight}{Final\ body\ weight} \quad (2)$$

The right testis from each mouse was fixed in Bouin's fluid for histopathological analysis. The cauda epididymis was surgically removed for abnormal sperm morphology test, which was carried out in accordance with standard procedure [24]. The cauda epididymis was minced in physiological saline using fine needle to produce sperm suspension. The suspension was stained using 1% eosin Y (9:1, physiological saline/eosin Y) for 45 minutes and 6 slides prepared per mouse, air-dried and blind coded for microscopic analysis. A thousand of sperm cells per mouse were assessed for abnormal sperm head morphology in accordance with Wyrobek et al. [24].

Bouin's fluid fixed testes were processed and slides prepared for Haematoxylin-Eosin (H-E) staining. The tissues were dehydrated in ascending order of ethyl alcohol-water concentrations, cleared in xylene and sequentially embedded in paraffin wax blocks using rotary microtome. Tissue sections of 3-5 μm thick were cut and prepared on clean slides for H-E staining and mounted in neutral DPX, mounting medium for pathological assessment, using light microscope at 400×and 100× magnification.

Dominant lethal assay

The male mice from OSL-treated and control groups were used for the dominant lethal test in accordance with the protocol by the Organization of Economic Co-operation and Development (OECD) [23]. At the end of 35-day exposure, a treated male was mated to 2 untreated nulliparous (virgin) females un-

til vaginal plug (indication of the congealed contents of the seminal fluid) was observed in the females (Figure 1A), usually within 2–3 days. The mated females were then isolated into separate cages and labeled according to the treatment groups. At pregnancy day 14, the females were sacrificed and the uterus contents were examined for the number and status of implantation site (Figure 1B and 1C). The total number of implants, number of live implants, number of early resorptions or moles and late death were recorded at the time of dissection of each mouse [23] (Figure 1B and 1C). The frequency of induced dominant lethal mutations was determined and expressed as percentage of the dominant lethality using the formula below:

$$(1 - \frac{live\ implants\ per\ female\ in\ the\ treatment\ group}{live\ implants\ per\ female\ in\ the\ negative\ control\ group}) \times 100 \quad (3)$$

Statistical analysis

Data were analyzed using Graphpad prism 5.0® computer software. One-way analysis of variance (ANOVA) was used to determine the significant difference among the various groups for the body weight gain and the absolute and relative testis weight, and abnormal sperm morphology. Dunnett multiple posthoc-test (DMPT) was used to compare the level of significance ($p<0.05$) of each treated group with the negative control. Difference between the negative control and each treatment group for the dominant lethality was analyzed using the Student's t-test at $p < 0.05$.

Results

Physico-chemical properties of Olususun landfill leachates sample

The analysis of physico-chemical parameters and concentra-
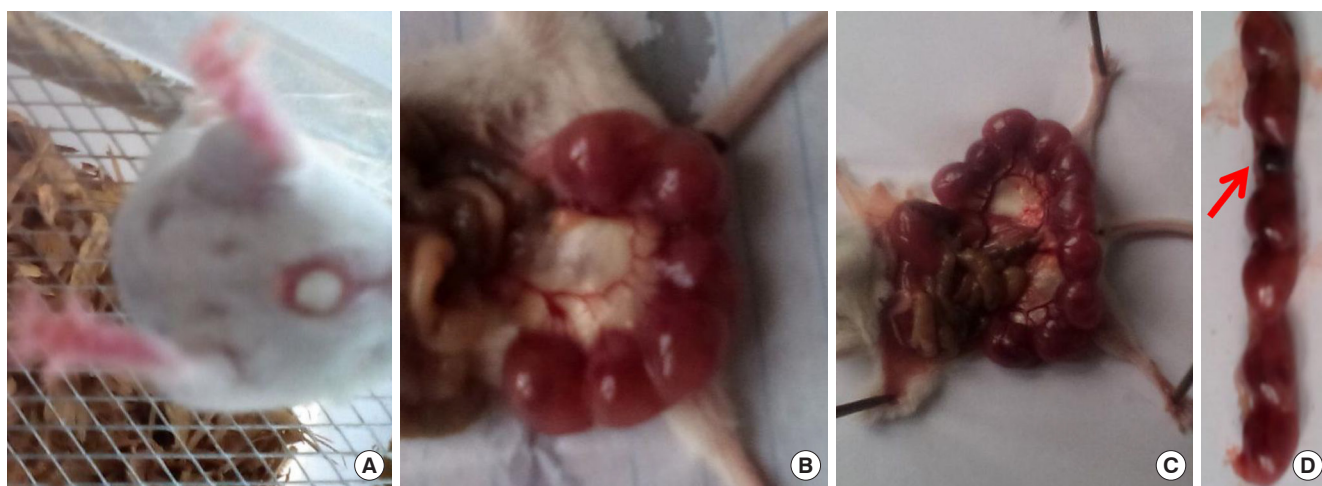
Figure 1. (A) A female mouse showing vaginal plug which is an indication of pregnancy. (B, C) The total number of implants and number of live implants. (d) Red arrow points at resorbed foetus in Olususun landfill leachate (OSL)-exposed mouse.

tion of heavy metals in OSL are presented on Table 1. OSL which was foul smelling and in brown colour was slightly alkaline (pH 7.5). The analyzed parameters - BOD, COD, TDS and concentration of chlorides, phosphate, Fe, Cu, Pb, Hg, Mn, As, and Cd - were higher than standard permissible limits from National Environmental Standards and Regulations Enforcement Agency (NESREA) in Nigeria and United States Environmental Protection Agency (USEPA). However, alkalinity and concentration of sulphate and nitrate were below the limits set

by these standards, hardness was within the range of the limits while ammonia was higher than permissible limit of USEPA, but below NESREA permissible limit.

Percentage body weight gain and testes weight of mice treated with OSL and the controls

Change in body weight showed that body weight of mice treated with OSL decreased compared to the negative control. However, this was not significant (p=0.6913; r = 0.4516) (Table 2). Also, the absolute and relative testes weight increased in the exposed mice, howbeit these were not statistically significant compared with the negative control; absolute (p=0.1182, r=0.6941) and relative (p=0.1633, r=0.6673) (Figure 2).

Abnormal sperm morphology analyzed in the Olususun landfill leachates treated and control mice

OSL significantly (p>0.05) increased the frequency of abnormal spermatozoa in the treated mice compared to the negative control (Figure 3). Abnormal spermatozoa increased 2.53-,

Table 1. Physico-chemical parameters and concentration of heavy metals analyzed in Olususun landfill leachate (OSL)

Parameters[a]	OSL	NESREA[e]	USEPA[f]
pH	7.5	6.0–9.0	6.5–8.5
Colour	Brown	-	-
Alkalinity	2.2	150	20
Hardness	75.7	-	0–75
BOD[b]	1,315	50	-
COD[c]	2,650	90	-
TDS[d]	920	-	-
Chlorides	2,450	250	250
Sulphates	4.2	250	250
Nitrates	6.5	10	10
Ammonia	1.3	10	0.02
Copper (Cu)	11.3	0.5	1.3
Iron (Fd)	2.43	-	0.3
Lead (Pb)	0.4	0.05	0.015
Cadmium (Cd)	0.19	0.2	0.05
Manganese (Mn)	0.82	0.2	0.05
Arsenic (As)	0.3	-	0.01
Mercury (Hg)	0.2	-	-

[a]All values are in mg/L except pH; [b]BOD: Biochemical oxygen demand; [c]COD: Chemical oxygen demand; [d]TDS: Total dissolved solid; [e]Maximum permissible limits for wastewater set by National Environmental Standards and Regulation Enforcement Agency (NESREA) (2011); [f]United States Environmental Protection Agency (USEPA) (2006).

Table 2. Change in body weight of mice exposed to Olususun landfill leachate (OSL)

| OSL Concentration (%)	Initial body weight (g)	Final body weight (g)	Body weight gain (%)
Control	18.53 ± 1.42	26.47 ± 3.25	42.85
5	21.53 ± 0.83	28.47 ± 2.43	32.23
10	23.33 ± 1.88	29.17 ± 1.71	24.99
25	19.87 ± 1.09	27.67 ± 1.46	39.26
50	20.27 ± 0.54	26.90 ± 0.96	32.71
Cyp[a]	21.57 ± 2.22	28.87 ± 0.40	33.84

[a]Cyp: Cyclophosphamide (20 mg/kg).
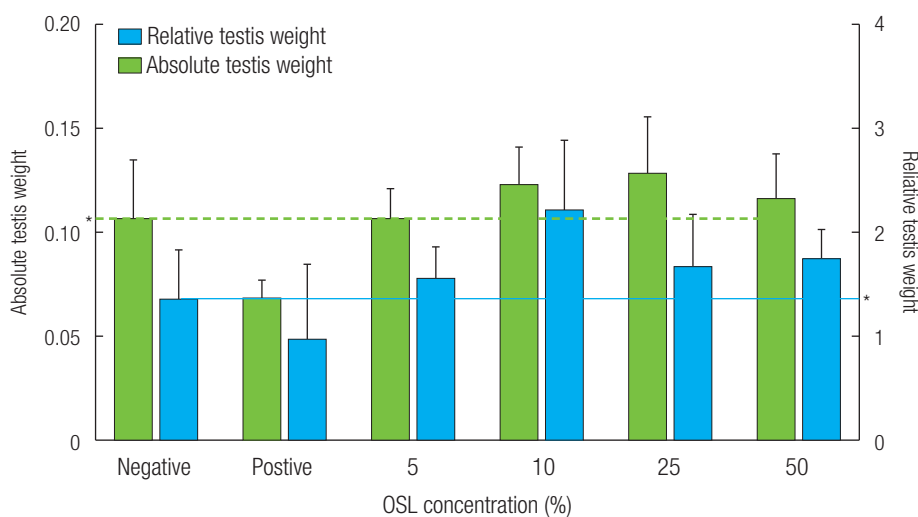
Figure 2. Absolute (p=0.1182) and relative (p=0.1633) testes weight measured in mice exposed to Olususun landfill leachate (OSL) compared with the negative control. Negative (Distilled water) and positive (cyclophosphamide; 20 mg/kg) controls.
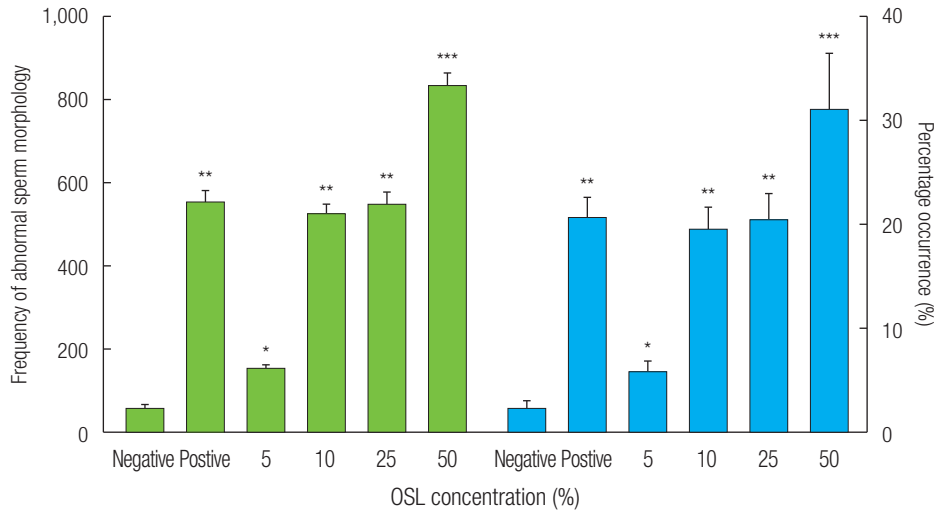
Figure 3. Frequency of sperms with abnormal morphology and the percentage occurrence of abnormal spermatozoa induced in Olusosun landfill leachate (OSL)-exposed mice. *p<0.05, **p<0.01, ***p<0.001 compared to negative control.
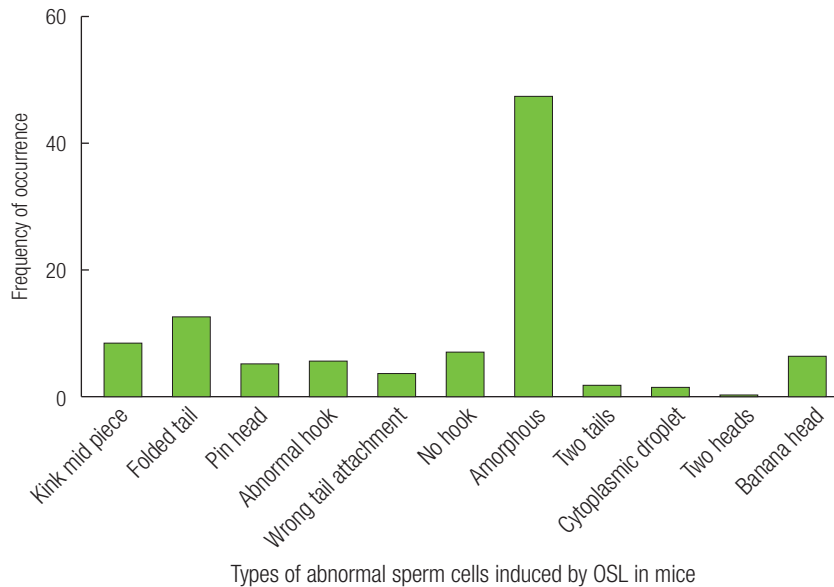
Figure 4. Frequency of occurrence of the various abnormal spermatozoa observed in Olusosun landfill leachate (OSL)-treated mice.

8.50-, 8.88- and 13.43-fold in 5, 10, 25, and 50% of OSL treated mice, respectively, which revealed concentration dependence. Frequency of the type of abnormal spermatozoa induced by OSL in the treated mice showed that amorphous head spermatozoon with 47.68% was the highest while spermatozoa with two heads scoring 0.34% are the least induced type of sperm abnormalities (Figure 4). Other abnormal sperm morphology induced in OSL-treated mice include cytoplasmic droplet, sperm without hook, two tail sperm cell, wrong tail attachment on the head, folded, banana and pin head (Figure 5). Histological structure of the testis from the negative control mice presents numerous uniformly sized seminiferous tubules with regular architectures. Also, there were moderate quantity

of spermatogenic cells with widened luminal spaces, predominant spermatocytes with scanty numbers of elongated (late stage) spermatids and small quantity of interstitial cells (Figure 6A). However, testicular architecture of OSL-treated mice presents numerous interstitial cells, congestion of the interstitial blood vessels (vascular congestion), spermatid retention, disorganization of the germinal epithelium from the basal compartment into the lumen of the seminiferous tubules and variations in height and width of the seminiferous tubule lumen (Figure 6B–6D).

Dominant lethal analysis

Frequency of dominant lethality in female mice mated with
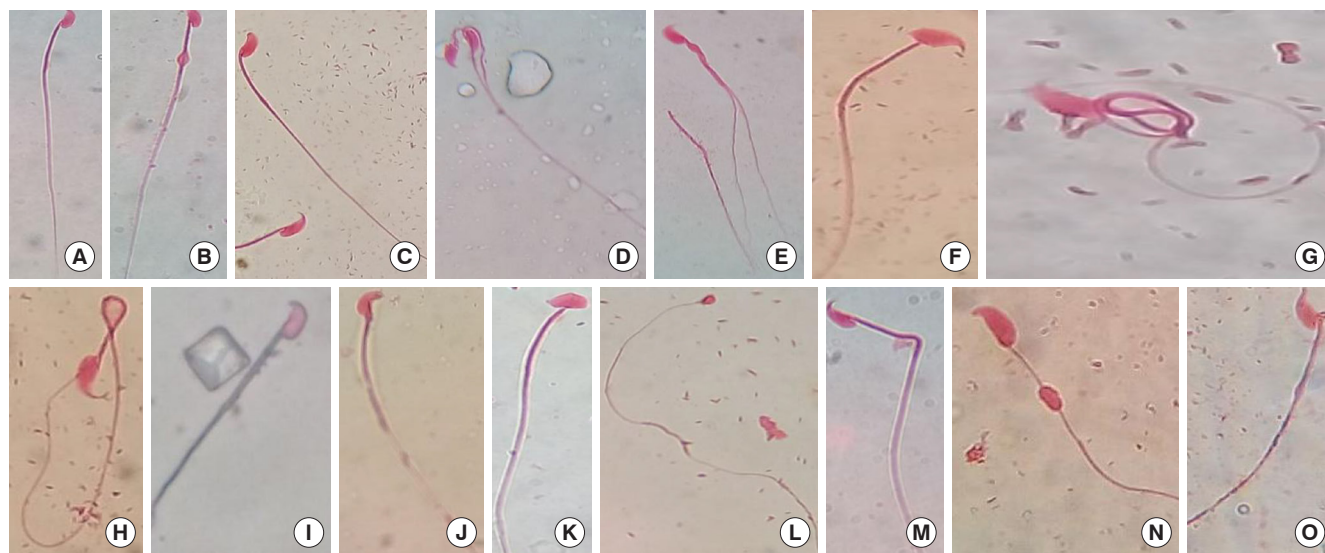
Figure 5. Abnormal spermatozoa induced in mice exposed to Olususun landfill leachate (OSL). (A) Normal spermatozoon. (B) Spermatozoon with cytoplasmic droplet at the mid piece. (C) Spermatozoon without hook. (D) Spermatozoon with two heads. (E) Spermatozoon with two tails. (F). spermatozoon with wrong tail attachment to the head. (G) Folded spermatozoon. (H) Spermatozoon with abnormal hook. (I) Spermatozoon with short hook. (J) Spermatozoon with banana head and without hook. (K) Spermatozoon with amorphous head. (L) Spermatozoon with pin head. (M) Spermatozoon with kink or bent tail. (N) Spermatozoon with short hook and cytoplasmic droplet at the mid piece. (O) Spermatozoon with wrong tail attachment and without hook. Mag.×1,000.
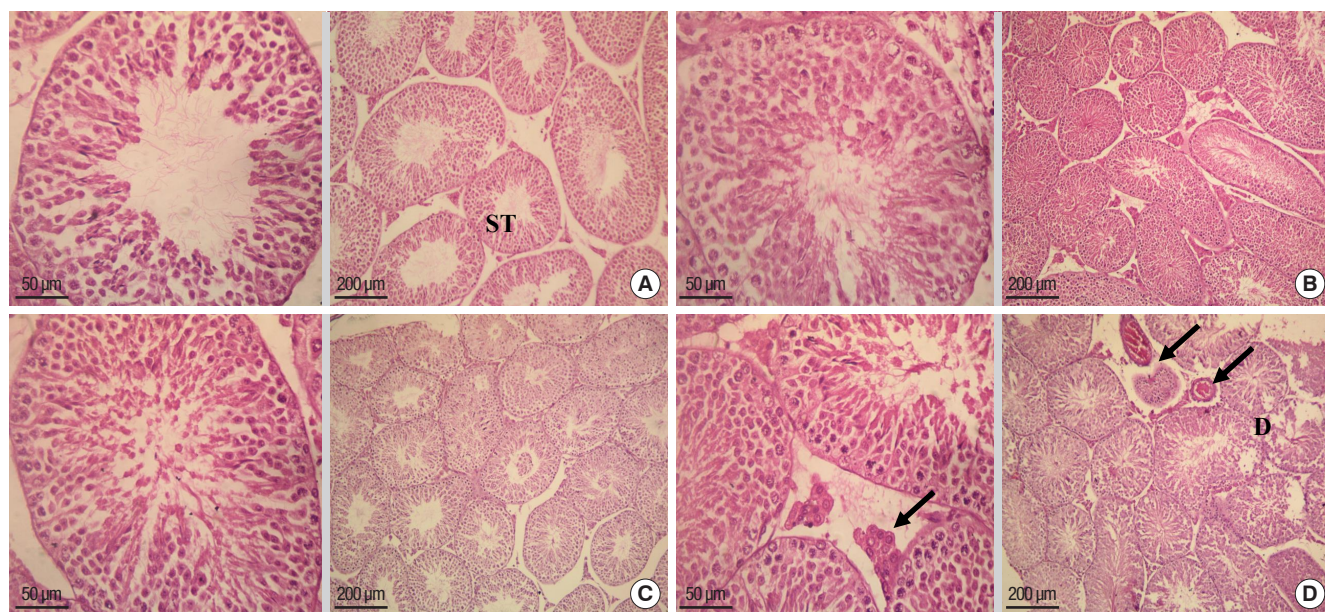
Figure 6. (A) Section of testis from the negative control mouse showing seminiferous tubule (ST) with an apparently normal luminal space and regular outlines. There are moderate amounts of spermatogenic cells, predominant spermatocytes and scanty number of elongate (late-stage) spermatids. Also there are little amounts of interstitial cells. Mag.×200 µm and 50 µm. (B) Section from 5% OSL treated mouse showing numerous uniformly-sized STs with regular outlines. There are numerous spermatogenic cells (increased height of spermatogenic epithelium), which almost obscure the luminal space in some STs. There are sufficient numbers of spermatocytes as well as early and late-stage spermatids. Mag.×200 µm and 50 µm. (C) Section from 10% OSL treated mouse showing numerous, large and regular STs. STs contain moderate amounts of spermatogenic cells with mild increased height of germinal epithelium. Numerous mitoses observed at the basal compartment of the STs. There are predominant spermatocytes and late-stage spermatids with numerous interstitial cells. Mag.×200 µm and 50 µm. (D) There are numerous, closely-packed, large STs with regular outlines which contain densely packed numerous spermatogenic cells (increased height of spermatogenic epithelium) which almost obscure the luminal space in some STs. Spermatocytes predominated with frequent late-stage spermatids. There are moderate congestion of interstitial blood vessels (black arrow). Also there was disorganisation of the ST epithelium (D). Mag.×200 µm and 50 µm.

Table 3. Summary of the results of the dominant lethal test on male mice treated with Olusosun landfill leachate (OSL)

OSL Concentration (%)	Pregnant female mice		Total implants		Live implants		Dead implants[b]		Dead implants per female (%)	Dominant lethal (%)
	Number	%	Number	Per female	Number	Per female	Number	Per female		
0	5	83.3	39	7.8	39	7.8	0	0	0	0
5	4	66.7	32	8	32	8	0	0	0	2.56
10	5	83.3	43	8.6	43	8.6	0	0	0	10.26
25	4	66.7	33	8.25	32	8	1	0.25	3.03	2.56
50	3	50	19	6.33	19	6.33	0	0	0	18.85
CYP[a]	4	66.7	32	8	31	7.75	1	0.25	3.13	0.64

[a]CYP: cyclophosphamide (20 mg/kg); [b]Dead implants: number of early resorptions or moles.

OSL-treated male mice is presented in Table 3. The mating schedule was selected to represent OSL exposure covering the entire cycle of pre- and post-meiotic stages of spermatogenesis. All female mice from both control and OSL treated groups were considered in calculating percentage (%) fertile mating considering that the frequency of infertility, notwithstanding OSL treatment, is expected to be the same. All females that had implants were regarded as "pregnant" and the number of implants, live embryos and dead implants per female (Figure 1B–1D) were determined based on pregnant females (Table 3).

There were no dead implants (0.0%) among females of the negative control mice and 5, 10, and 50% OSL treatment, while 3.03 and 3.13% dead implants were observed among females mated using males exposed to 25% OSL and cyclophosphamide respectively. Using t-test there was no significant difference between the frequencies of pregnancy in female mice mated with the negative control and treated male mice (Table 3). Similarly, OSL did not induce significant pre- and post-implantation dominant lethal effects in female mice, only 25% OSL and cyclophosphamide treated male mice produced resorptions in the mated female and this was not statistically significant.

Discussion

Mixture of xenobiotics in OSL increased numerical and structural chromosomal aberrations in somatic and germ cells of eukaryotic organisms [6,11–13,27–29]. Chromosome aberrations have been implicated with mild to severe genetic and developmental disorders both in humans and wildlife [30]. Exposure to contaminants emitted from landfills has been described a risk factor in congenital anomalies, including Down syndrome, chromosomal congenital anomalies, fetal and neonatal deaths and developmental anomalies [2,31–33] among human population residing within 2–3 km circumference of landfill facilities. In this study, we hypothesized that OSL will increase abnormal sperm production in mice. Also, the out-

come of mating between abnormal sperm morphology laden mice and normal nulliparous female mice will increase foetal/neonatal death.

The murine sperm abnormality assay, capable of screening xenobiotics (mutagens, teratogens, and carcinogens) that can cause differentiation errors in spermatogenic stem cells [24,34], was used to screen OSL for germ cell genotoxicity. Significant increase in the frequency of abnormal sperm morphology induced in OSL treated mice signifies germ cell genotoxic effects of xenobiotics in the leachate [13,29]. It implies that the toxic metals and metalloids and other substances in the leachate (Table 1) interfered with the integrity of spermatogonia chromosomes and or expression of genes that stabilize the fidelity of DNA during spermatogenesis [15,35]. For instance, Cu and Mn are observed in the leachate, though they are essential elements which readily enhance normal functioning of enzymes and biological molecules but at high concentrations they can increase DNA damage by directly binding to sensitive molecules and or via free radical formation [50,51]. Also, Pb and Cd are metals that lack any biological relevance at threshold concentrations in the mammalian body, they can induce germ cell mutation and reproductive toxicity [52,53]. High concentrations of TDS, BOD, and COD in the leachate usually signal high organic pollutants in the leachate. This may corroborate our previous report wherein 23 different polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) were analyzed in OSL [11]. The presence of these organic pollutants correlated with the induction of DNA damage and cytotoxicity in OSL-exposed human cell lines. The analyzed metals along with physico-chemical parameters and unanalyzed organic compounds in OSL individually and or interactively induced chromosome aberration and or DNA damage which occurred during mitotic and meiotic stages of spermatogenesis, and invariably resulted in the formation of abnormal sperm morphology [34,36,54]. Xenobiotics, including heavy metals, in OSL had similarly being reported to interfere with DNA integrity in spermatogonia nuclei of exposed

boar [37] and peripheral erythrocytes and bone marrow polychromatic cells of fish, Japanese quail and rat [6,12]. These reports are in tandem with the observed abnormal sperms in the OSL-treated mice. Furthermore, that 47.68% of the total abnormal sperm cells in OSL-treated mice were with amorphous head (Figure 4) corroborated the report that human exposed to environmental toxicants including metals had higher teratozoospermia, high frequency of sperm cells with abnormal head morphology [38]. The observed impaired spermatogenesis in OSL-treated mice and humans exposed to environment toxicants were associated with free radical induced oxidative stress [37,38].

Increase in the absolute and relative testes weight of OSL exposed mice suggests eodema [39]. While decrease in body weight of treated mice has been linked to OSL induced systemic toxicity [7,19,20]. Histopathology of the testes further revealed an evidence supporting OSL toxicity on the testes. For instance, spermatid retention observed in OSL treated mice corroborates the observed high abnormal sperm morphology [40,41]. Adequate blood supply to the testes is a requirement for active spermatogenesis and hormonal secretion [42]. Congestion of the interstitial blood vessel (Figure 6D) suggests that chemicals in OSL interrupted blood flow hence impairing spermatogenesis [40,41]. Depletion of spermatogonia, spermatocytes and spermatids, alterations in size and number of seminiferous tubules, reduction in germinal epithelium height and lumen space, and disorganization of the seminiferous tubules are lesions related to xenobiotic induced toxicity [39]. This is an indication that chemicals, most importantly metals, in landfill leachates can cross blood barrier to reach the testicular environment. Metals analyzed in OSL (Table 1) readily accumulate in testes and directly interfered with the tissue arrangement to produce pathological lesions [39]. Also, metals can indirectly inhibit growth and differentiation of spermatogonia via increase oxidative stress and lipid peroxidation. This will result in abnormal sperm morphology and chromosomal abnormalities in the primary spermatocytes [43,44].

Teratozoospermia along with the other types of abnormal sperm shapes, are determinants in genetic heritability [24]. A human retrospective cohort study showed that a case of severe teratozoospermic condition caused low implantation and spontaneous term pregnancy rate and higher miscarriage rate when compared with patients with normal sperm morphology in an in vitro fertilization study [45]. The dominant lethal assay is a cytogenetic test used for regulatory testing of chemicals suspected to be mutagenic to germ cells. It detects alterations in genetic composition of germ cells via zygotic death produced before, during, or post-implantation [23]. This assay

did not present significant difference between OSL-treated mice and the negative control mice in the percentage of post-implantation loss and increase mutagenic index. Although, there was reduced pregnancy outcome compared to the negative control, but this was not statistically significant. Considering that the highest selected concentration (50%) of OSL induced 31.02% teratozoospermia in the treated male mice (Figure 3), it is possible that the remaining 69.98% of the normally produced spermatozoa fertilized the oocytes in the female mice. Since fertilization involves a random fusion of a sperm cell from among numerous ejaculated sperm cells with an oocyte, it is possible that the outcome of the dominant lethal test was due to the inability of any among the 31.02% teratozoospermia to fertilize the oocytes. This assertion lends credence to the report that in patients with 100% teratozoospermia and amorphous sperm head, there was complete failure during fertilization [45].

Although, the concentrations of OSL selected in this study significantly increased numerical and structural chromosome aberrations in somatic and germ cells of eukaryotic systems [6,12,13,27,29]. However, it seems that the abnormal sperm cells produced were not selectively favoured during fertilization in the study herein. This is because sperms with teratozoospermia and amorphous heads increase congenital anomalies, embryonic death, developmental disorders and / or other birth outcomes if fertilized viable oocytes [30]. Finding from the dominant lethal mutation test herein does not suggest a high risk of transmissible genetic damage from OSL-treated male mice. Therefore, the huge evidence of congenital anomalies, fetal and neonatal deaths and other developmental defects reported among infants born to women living near hazardous waste sites [31,34,46] may not be totally linked to the exposure of men to landfill chemicals. The reports that Cd, Hg and Pb increased chromosome aberration in bone marrow cells and abnormal sperm cells but did not significantly increase dominant lethality in rodents are in concert with our observation [43,44,47,48]. Considering that both male and female contribute equally to percentage infertility [49], we suggest that further studies on landfill leachate induced infertility, embryotoxicity and teratogenicity be carried out by mating non-treated males with leachate treated females and vice versa. This will enhance proper understanding of the role of sex in landfill induced infertility, embryotoxicity and teratogenicity. Furthermore, it is possible that selection of leachate concentrations that will increase the abnormal sperm morphology beyond 50% may results in significant dominant lethality.

In conclusion, the findings herein showed that OSL significantly increased abnormal sperm morphology and induced

histopathological lesions in the testes of exposed mice. Also, OSL insignificantly increased absolute and relative testes weight and decreased body weight gain in mice. Sperm cells from leachate-treated male mice that fertilized oocytes from non-treated nulliparous females did not significantly increase spontaneous abortion, resorptions and post-implantation deaths. The findings suggest that the selected OSL concentrations and exposure duration, did not significantly induce dominant lethal mutations. It is therefore imperative to confirm whether congenital and developmental anomalies reported among residents in close proximity to landfill sites occur mainly due to defects during spermatogenesis, oogenesis and/or post fertilization events.

CRidiT authors statement

OJA: Experimental Investigation, Data analysis, Writing-Original draft preparation; CGA: Conceptualization / Visualization of the study, Supervision, Data analysis, Writing- Original draft preparation, Reviewing and Editing; AAB: Supervision, Writing- Reviewing and Editing.

Conflict of interest

Authors declare no form of conflict of interest exist.

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