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Sub-chronic exposure to EOMABRS leachate induces germinal epithelial cell lesions, sperm abnormalities and oxidative damage in rats

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ABSTRACT

Objective: To explore the possible link between reproductive abnormalities among men and exposure of toxic chemicals in the environment. **Methods:** The study investigated the sperm functions and the antioxidant defence system of rats exposed to leachate obtained from Elewi Odo municipal battery recycling site (EOMABRSL) via oral route. **Results:** EOMABRSL had significant effects on both absolute and relative testicular weight. Formation of sperm abnormalities was observed following EOMABRSL exposure. Antioxidant enzymes including superoxide dismutase and catalase were significantly altered in the testes resulting into increased lipid peroxidation. Reduced glutathione (GSH) levels were found to be significantly ($P<0.05$) depleted relative to the control group. Considerable necrosis of leydig cells and loss of germ cells in the seminiferous tubules with the clumping of interstitial space were seen in EOMABRSL-treated rats. The mechanism of toxicity is linked to individual, synergistic, antagonistic, competitive or collective interaction of the metals with normal testicular biochemical processes. **Conclusion:** The study concluded that possible mechanisms by which EOMABRSL at the investigated doses elicits spermatotoxicity could be linked to the testicular oxidative stress and damage to germinal epithelial cells by mixed-metal exposure. However, this may suggest possible reproductive health hazards in subjects with environmental or industrial exposure.

1. Introduction

Male reproduction and its development can be affected by exposure to a wide variety of agents including dioxins, poly-chlorinated biphenyls (PCBs), phyto-estrogens such as iso-flavones, heavy metals, chlorination disinfection by-products in water, organic solvents, poly-aromatic hydrocarbons, particulate air pollution, and caffeine. These toxic chemicals had been suggested to disturb the pro-oxidant/antioxidant balance leading to excessive generation of free radicals[1]. Also, it has been observed that the oxidative damage to testicular cells induced by various pro-oxidants or ROS can cause testicular dysfunctions. And as such cause male infertility[2]. Cellular oxidative damage becomes apparent when oxidants overwhelm the antioxidant defence system in cells. The excess oxidants take part in specific and non-specific reactions with nearby cellular components such as unsaturated lipids, proteins and DNA, and as such impairing normal cellular processes[3, 4]. It can also arise from a high turnover of oxidants by cells or due to the low levels of enzymatic and non-enzymatic antioxidant defence molecules[5, 6].

It is a common practice throughout the world to collect, transport, recover and recycle waste auto-batteries on account of their value as a major source of lead units. This has led to a collection method whereby auto-battery wastes are gathered from their generational sources, sorted, and delivered to the secondary smelter for recovery of lead and other materials[7-10]. However, the search for the lead as raw material has increased its recycling rates in the societies.

Previous studies in our laboratory have implicated leachate sources with cadmium (Cd), cobalt (Co), copper (Cu), iron (Fe), manganese (Mn), nickel (Ni), lead (Pb) and zinc (Zn) [11, 12],

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and these metals have also been reported to exceed the maximum permissible concentration for drinking water[13, 14]. Moreover, high levels of Cd, Cr, Fe, Mn, Pb and Ni in leachate have been reported to cause in vitro oxidative damage in livers, hearts and kidneys; also as evidence of environmental contamination[12]. The possible sub-chronic deleterious effects of this effluent/leachate in exposed rats on prostate, steroidogenesis and sex hormones have also been reported [15, 16]. Likewise, the leachate from small scale industry in Nigeria has been reported to activate key enzymes linked to Non-insulin dependent diabetes mellitus (NIDDM) [17].

In Nigeria today, the recycling rate for batteries containing Co, Ni, Cr and Cd is generally low. Even, the few common methods of waste recovery currently being practiced contradict the approved standard recommended by the regulatory agencies[18]. Elewi odo municipal battery recycling site, located in ancient city of Ibadan, Ibadan North Local Government Area (INLGA) of Oyo State, Nigeria. The site is largely set aside for recycling of auto-battery wastes. It is sited at the back of a stream of the residential area. It covers about 2 acres of land which generates a liquid material. The liquid material is known as leachate; discharged from heap of auto-battery recycling wastes into the drinking water body, thereby polluting the entire environment. Information regarding the effects of sub-chronic exposure to leachate (mixture of chemicals) from Elewi Odo municipal auto-battery recycling site (EOMABRSL) on relevant antioxidant enzymes in the testes is scanty[19]. Also its resultant effects on sperm parameters are not fully elucidated. This poses a great concern to Nigeria regulatory agencies and the entire communities. This study was aimed to evaluate the toxic effect of EOMABRS-leachate on both enzymatic and non-enzymatic antioxidants and to fully know its resultant effects on sperm index using male rats.

2. Materials and methods

2.1. Sampling site and leachate preparation

The leachate was obtained from Elewi Odo municipal battery recycling site, located at Ibadan North LGA of Oyo State, Nigeria (latitude$7^0$25.08'N and $7^0$25.11'N and longitudes 3^0 56.45'E and 3^0 56.42'E). A randomized sampling technique was employed to collect the first horizon solid soils (0-15 cm deep) from different points on the municipal auto-battery recycling site. At least five randomly collected samples from each site were pooled to make a single representative sample. The sample was air-dried, finely ground with a mortar and pestle, and sifted through a 63-μm (pore size) sieve to obtain a homogenous mixture.

Leachate (100%) was prepared from homogenous mixture according to the procedure of Ferrari *et al*[20] with little modification by adding 100 g of sample to 100 mL of distilled water (w/v) and shaken for 48 h at 32 ℃. Thereafter, the sample was left to sediment for 30 min, and the supernatant was filtered with a 2.5-μm filter paper; the filtrate was stored at 4 ℃ for further use. The leachate from Elewi Odo municipal auto-battery recycling site was designated as EOMABRSL. Sample waters were collected from well around the site. They were designated as WELL-A and WELL-B. The stream water collected near the site was regarded as STREAM; the drinking water sample (8 km far from recycling site) was collected and used as reference control (designated as CDW).

2.2. Heavy metal analysis

Nine metals viz copper (Cu), lead (Pb), cadmium (Cd), cobalt (Co), chromium (Cr), zinc (Zn), iron (Fe), nickel (Ni) and manganese (Mn) were analyzed in the EOMABRSL, well and control water sample. Briefly, 100 mL each of EOMABRSL and water sample was digested by heating with concentrated HNO3 and the volume was reduced to 2-3 mL. This volume was made up to 10 mL with 0.1 N HNO3 and the concentrations of the metals were estimated using atomic absorption spectrophotometer (AAS) [21]. The levels of these metals were assessed because of their reported occurrences in both solid and liquid wastes in Nigeria[11].

2.3. Chemicals and reagents

Epinephrine, Reduced GSH, 5, 5-dithio-bis-2-nitrobenzoic acid, hydrogen peroxide and thiobarbituric acid (TBA) were purchased from Sigma (St Louis, MO, USA). Except stated otherwise, all other chemicals and reagents were of analytical grades and were obtained from the British Drug Houses (Poole, Dorset, UK) and the water used was glass distilled.

2.4. Experimental protocol

Thirty adult male wistar rats weighing approximately (128±19.24) g obtained from the Department of Physiology, University of Ibadan, Nigeria were randomly assigned into 6 groups of 5 animals per group. They were housed in a plastic suspended cage placed in a well ventilated rat house, provided rat pellets and water ad libitum, and subjected to a natural photoperiod of 12 h light and 12 h dark cycle. All the animals received humane care according to the criteria outlined in the 'Guide for the Care and Use of Laboratory Animals' prepared by the National Academy of Science and published by the National Institute of Health. Ethic regulations have been followed in accordance with National and institutional guidelines for the protection of animal welfare during experiments[22].

The rats in group 1 served as control and were administered 1 ml of distilled water by gastric intubation. Animals in groups 2–6 received 1 ml each of 20%, 40%, 60%, 80%, and 100% of EOMABRL, respectively, by gastric intubation. The experiment lasted for 60 days (sub-chronic exposure). The animals were fasted overnight, weighed and sacrificed by decapitation 24 h after the last treatment, testes and epididymes were removed and cleared of adhering tissues, washed in ice-cold 1.15% potassium chloride and dried with blotting paper.

The absolute weights of the rats and relative weight of testes were recorded in gram (g).

2.5. Biochemical assay

The testes were homogenized in 50 mM Tris–HCl buffer (pH 7.4) containing 1.15% KCl and the homogenate was centrifuged at 10 000 g for 15 min at 4 ℃. The supernatant was collected for the estimation of catalase (CAT) activity using hydrogen peroxide (H_2O_2) as substrate according to the method of Clairborne[23]. Also, H_2O_2 level was estimated using the method described by Clairborne[23]. Superoxide dismutase (SOD) activity was determined by measuring the inhibition of autoxidation of epinephrine at pH 10.2 at (30±1) ℃ according to Misra and Fridovich[24]. Protein concentration was determined by the method of Lowry, *et al* [25].

2.5.1. Reduced glutathione (GSH) assay

Reduced glutathione (GSH) was determined at 412 nm using the method described by Jollow, *et al* [26].

2.5.2. Lipid peroxidation assay

Lipid peroxidation was quantified as malondialdehyde (MDA) according to the method described by Ohkawa *et al*, [27] and expressed as μmol/mg tissue.

2.5.3. Sperm analysis

A small quantity of semen was collected from the caudal epididymis using needle and dropped onto the slide for 2 hrs to liquefy. 0.02 mL liquefied semen was diluted in 0.38 mL of 5% formol saline solution to make 0.4 mL solution (1 in 20 dilutions). Sperm motility was assessed by the method described by Zemjanis[28]. Epididymal sperm count or number was obtained as described by Pant and Srivastava[29]. Dead sperms and total sperm deformity were microscopically determined according to the method described by Wells and Awa[30]. Furthermore, daily sperm production was estimated according to method explained by Joyce, *et al* [31].The results were expressed as percentage.

2.6. Histopathological examination

The testes were fixed in Bouin's fluid for 24 hours, before they were cut longitudinally into 2 equal halves and again post-fixed in fresh Bouin's fluid for next 24 hours. The tissues were dehydrated in the ascending strengths of alcohol, cleared in xylene. Infiltrated and embedded in paraffin wax, the tissue blocks were made, cut into 5 μm thick sections using rotatory microtome. The sections were mounted on albumenized glass slides and stained with eosin and hematoxylin. Morphological study of testes was done with the help of ocular micrometer scale under light microscope.

2.7. Statistical analysis

The results of the replicates were pooled and expressed as mean ± standard deviation. A one way analysis of variance (ANOVA) was used to analyze the results and Duncan multiple test was used for the post hoc[32]. Statistical package for Social Science (SPSS) 17.0 for windows was used for the analysis and the least significance difference (LSD) was accepted at $P<0.05$.

3. Results

3.1. Heavy metal concentration in the leachate and water samples [15,16]

The results of nine heavy metals such as copper (Cu), zinc (Zn), Lead (Pb), cadmium (Cd), manganese (Mn), Cobalt (Co), chromium (Cr) Iron (Fe) and nickel (Ni), obtained from EOMABRSL, STREAM, WELL-A, WELL-B and CDW (control) are presented in Table 1. The heavy metal contents of the EOMABRSL ranged from 0.006 mg/L (Cadmium) to 7.842 mg/L (Manganese). The heavy metal contents of the STREAM around the recycling site ranged

Table 1

Concentration of heavy metals detected in EOMABRSL, STREAM, WELL-A, WELL-B and CDW[16].

Parameter	EOMABRSL	STREAM	WELL-A	WELL-B	CDW	WHOLimits	NESREA Limits
Cadmium	0.006	0.002	0.002	0.003	BDL	0.003	0.01
Cobalt	0.049	0.004	0.003	0.002	BDL	0.05	-
Chromium	0.068	0.011	0.015	0.014	BDL	0.05	0.05
Copper	0.341	0.012	0.010	0.010	BDL	2.00	-
Iron	2.667	1.076	0.011	0.030	0.050	0.30	0.3
Manganese	7.842	0.223	0.239	0.239	BDL	0.40	-
Nickel	0.050	0.048	0.044	0.049	0.027	0.02	0.05
Lead	0.015	1.548	0.068	0.306	BDL	0.01	0.01
Zinc	0.010	0.126	0.053	0.011	0.010	3.00	-

EOMABRSL: Elewi Odo municipal battery recycling site leachate, CDW: Drinking water sample was used as control. All values are in mg/L.The contents of heavy metals detected in EOMABRSL, STREAM and WELLS around the site were higher than the drinking water sample (CDW) [14]. BDL- Below detection level [15], Least Observable Effective Concentration (LOEC) set by World Health Organisation[58, 62]; Permissible limits set by National Environmental Standard and Regulatory Enforcement Agency[59]. Values in the brackets: increase than the permissible limits in drinking water.

from 0.002 mg/L (Cadmium) to 1.548 mg/L (Lead). The heavy metal contents of the WELL-A and WELL-B ranged from 0.002 mg/L (Cadmium) to 0.239 mg/L (Manganese) and 0.002 mg/L (Cobalt) to 0.239 mg/L (Manganese) respectively. And, the heavy metal contents of the control drinking water (CDW) ranged from below detection level (Chromium, Copper and Manganese) to 0.027 mg/L (Nickel). In addition, EOMABRSL: Cd (0.006 mg/L), Cr (0.068 mg/ L), Fe (2.667 mg/L), Ni (0.05 mg/L), Pb (0.015 mg/L) and Mn (7.842 mg/L) were higher by 2.00, 1.36, 8.89, 2.50, 1.50 and 19.61-folds respectively (Table 1) when compared with the acceptable limits set by World Health Organisation (WHO). Also, similar trend occurred when compared with regulatory limits given by National Environmental Standard and Regulatory Enforcement Agency (NESREA). STREAM: Fe (1.076 mg/ L), Ni (0.048 mg/L) and Pb (1.548 mg/L) were higher than WHO permissible limits by 3.59, 2.40 and 154.8-folds respectively (Table 1). WELL A: Ni (0.044 mg/L) and Pb (0.068 mg/ L) exceeded the WHO permitted limits in the drinking water by 2.20 and 6.80-folds respectively. A similar trend was observed in WELL B as Ni (0.049 mg/L) and Pb (0.306 mg/L) exceeded WHO permissible limits in drinking water by 2.45 and 30.60-folds respectively. So, there was a considerable increase in the heavy metal contents of WELL A, WELL B and STREAM when compared with National Environmental Standard and Regulatory Enforcement Agency (NESREA). In contrast, Co, Cu and Zn were lower in all the samples when compared with the Least Observable Effective Concentration (LOEC) set by WHO and NESREA as shown in Table 1.

3.2. Absolute and relative testicular weight gain

The absolute weights of testes are presented in Table 2. The group treated with EOMABRSL depicted significant (P<0.05) decrease in the absolute weights of testes in a non-dose dependent manner when compared with the control group. Similarly, the relative weights (organ-to-body weight ratio) of testis are presented in Table 2. The group treated with EOMABRSL showed a significant (P<0.05) decrease in the relative weights of testis when compared with the control group.

Table 2

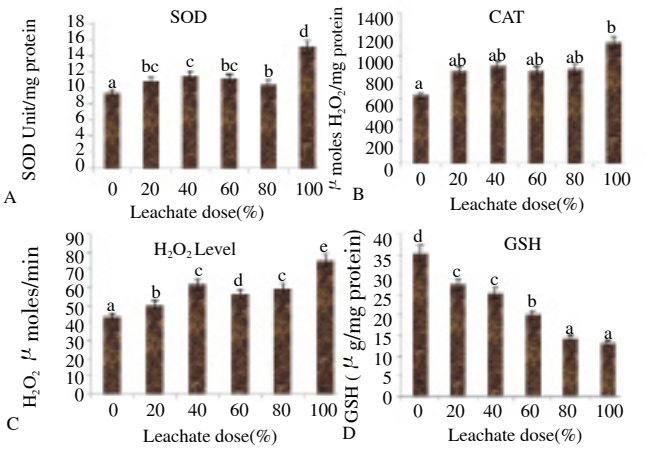
Effect of EOMABRSL on absolute and relative testicular weight gain of treated rats[15].

Groups	Dose %	Body weight gain (g)	Testes weight gain (g)	Relative weight gain (g/b.wt)
Control	0	206.000 ±19.500^a	1.980±0.210^a	0. 010 ± 0.001
Group 1	20	194.000 ±32.100^b	1.870±0.400^b	0.008± 0.002^b
Group 2	40	180.000 ±16.300^c	1.880±0.040^b	0.009 ±0.001^a
Group 3	60	179.000 ±20.200^c	1.710±0.060^c	0.008±0.000^b
Group 4	80	190.000 ±5.300^b	1.880±0.230^b	1.880±0.230^b
Group 5	100	174.000 ±36.300^c	1.660±0.130^d	0.007±0.001^c

EOMABRSL significantly (P<0.05) decreased body weight, testicular and relative weight of treated rats. n=5; Values with different superscript (P<0.05) are significantly different.

3.3. Antioxidant status in the testes

The antioxidant levels, testicular marker enzymes and markers of oxidative stress were evaluated. The activity of SOD in the post-mitochondrial fraction of rat testes increased significantly (P<0.05) by 20.6%, 24.4%, 23.0%, 19.8% and 63.6% respectively compared with the control group (Figure 1A). Similarly, CAT activity was significantly (P<0.05) elevated by 18.6%, 27.5%, 22.4%, 27.9% and 83.1% respectively when compared with the corresponding control group (Figure 1B). Administration of EOMABRSL caused a significant increase (P<0.05) in the production of hydrogen peroxide (H_2O_2), a testicular marker for reactive oxygen species (Figure 1C). Conversely, the reduced glutathione (GSH) levels were significantly (P<0.05) depleted in a dose-dependent manner by 21.8%, 27.6%, 42.8%, 56.1% and 62.6% respectively relative to the control group (Figure 1D). The level of testicular total protein was significantly (P<0.05) decreased following exposure to EOMABRL (Figure 2A)

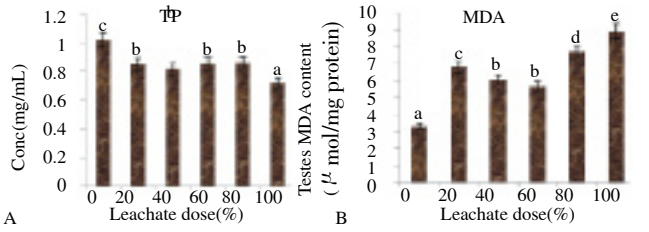


Figures 1. (A-D): Effect of EOMABRSL on the activity of superoxide dismutase (SOD), catalase (CAT) activity, hydrogen peroxide (H_2O_2) level and reduced glutathione (GSH) level in treated rats.

n=5; values with different superscript (P<0.05) are significantly different.

3.4. Marker of oxidative damage

The levels of MDA (malondialdehyde), maker of lipid peroxidation, in testes increased significantly (P<0.05) in rats exposed to EOMABRSL by 105%, 82%, 71%, 132% and 168% respectively compared to the control group (Figure 2B).



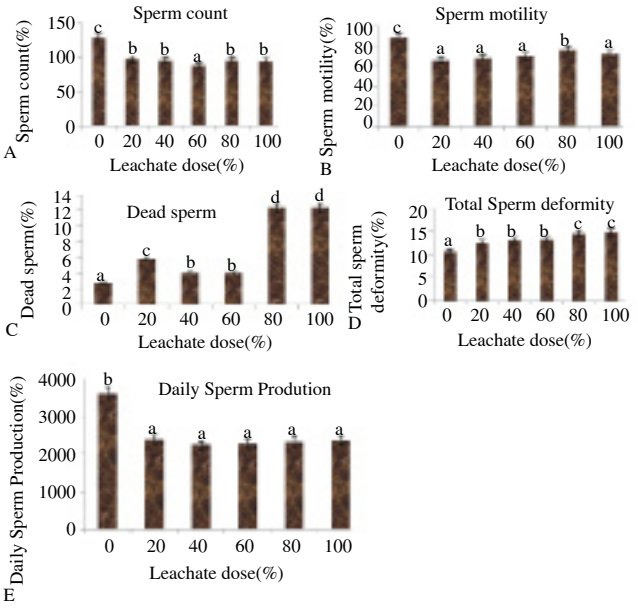
Figures 2. (A-B): Effect of EOMABRSL on level of total protein (TP) and testicular lipid peroxidation (MDA) in treated rats.

n=5; Values with different superscript (P<0.05) are significantly different.

3.5. Sperm function

Data on the sperm count, sperm motility and live/dead count

are presented in Figures 3 (A-C), Administration of EOMABRSL significantly (P<0.05) decreased sperm count (Figure 3A) and sperm motility (Figure 3B) by 25%, 26%, 32%, 26%, 27% and 25%, 23%, 21%, 14%, 18% respectively, when compared with the corresponding control group. Conversely, percentage dead spermatozoa was significantly (P<0.05) elevated following exposure to EOMABRSL by 111%, 46%, 46%, 349% and 349% respectively, when compared with the corresponding control group (Figure 3C). Similarly, there was significant (P<0.05) increase in the total spermatozoa abnormalities/deformities in the EOMABRSL-treated rats (Figure 3D) by 18.14%, 23.99%, 24.50%, 37.61% and 41.31% respectively when relative to the control group. Conversely, the treatment of EOMABRSL significantly (P<0.05) induced low daily sperm production as presented in Figure 3E by 34%, 38%, 37%, 35% and 34% respectively when compared with the control group. Additionally, animals that were exposed to EOMABRSL caused considerable significant abnormalities in sperm functions as depicted in table 3. The major abnormalities consisted of headless tails, bent tails, folded tail, curved mid-piece, amorphous head and bent mid-piece (Table 3). Amorphous head and headless tails occurred less frequently in the treated animals, while bent tails, folded tail, curved mid piece and bent mid piece constituted the major abnormalities in the treated animal (Table 3).



Figures 3. (A-E): Effect of EOMABRSL on epididymal sperm count, sperm motility, dead sperm, total sperm deformity and daily sperm production in rat treated.

n=5; Values with different superscript (P<0.05) are significantly different.

Table 3

Some morphology characteristics of spermatozoa in rats exposed to EOMABRSL (%).

Dose (%)	AmorphousHead	Headless tail	Headless tail	Folded tail	Curvedmid piece	Bent mid piece
0	0.74±0.01^{a}	0.98±0.03^{a}	1.72±0.03^{a}	1.93±0.30^{a}	1.80±0.14^{a}	1.84±0.13^{a}
20	1.33±0.13^{b}	1.24±0.02^{b}	2.36±0.13^{b}	2.57±0.13^{b}	2.48±0.27^{b}	2.42±0.14^{b}
40	1.49±0.01^{c}	1.34±0.17^{c}	2.30±0.24^{b}	2.47±0.03^{c}	2.68±0.17^{c}	2.45±0.02^{b}
60	1.47±0.02^{c}	1.34±0.15^{c}	2.42±0.24^{c}	2.57±0.13^{b}	2.55±0.17^{b}	2.42±0.16^{b}
80	1.48±0.03^{c}	1.38±0.18^{c}	2.49±0.02^{d}	2.89±0.13^{d}	2.73±0.22^{c}	2.89±0.18^{c}
100	1.38±0.18^{b}	1.38±0.18^{c}	3.03±0.34^{e}	2.80±0.18^{d}	2.60±0.27^{b}	3.15±0.34^{d}

Values represent Mean ± Standard deviation; n=5; Values with different superscript (P<0.05) are significantly different.

3.6. Germinal epithelial cells and sperm damage

In the control group, there was normal arrangement of tubules with intact interstitium (Figure 4 A). Also, large number of seminiferous tubules with regular and intact basement membrane was observed in control animals. The germ cells were arranged regularly. The line of cells was present from spermatogonia to spermatid. The spermatids attached normally to the sertoli cells. And the lumen contained the spermatozoa without slough. In the testes of animals treated with 20% EOMABRSL (Figure 4 B), testes showed shrinkage of seminiferous tubules. The interstitial space was degenerated coupled with necrosis. Basement membranes of the tubules were ruptured. Arrangement of germinal epithelium was distorted. The lumen contained slough with dead spermatozoa. For animals exposed to 40% EOMABRSL (Figure 4 C), there was cellular lesions and degeneration of spermatids with dark ''ring-like''. Also, nuclear chromatins clumping were seen in the epithelium and present free in the luminal of the tubules. Furthermore, severe damage was done to multinucleated giant cells of the seminiferous tubules (as shown in figure 4 D). Also, there was considerable loss of germ cells. Additionally, the damaged spermatids were inter-mixed with matured spermatozoa. Following 80% EOMABRSL exposure (as shown in figure 4 E), severe lesions had occurred which include loss of spermatids. Spermatocytes and Spermatogonia were brutally injured. Lastly, massive loss of germ cells (necrospermia) in 100% exposed animals (Figure 4 F). Seminiferous tubules and sertoli cells were devoid of germ cells.

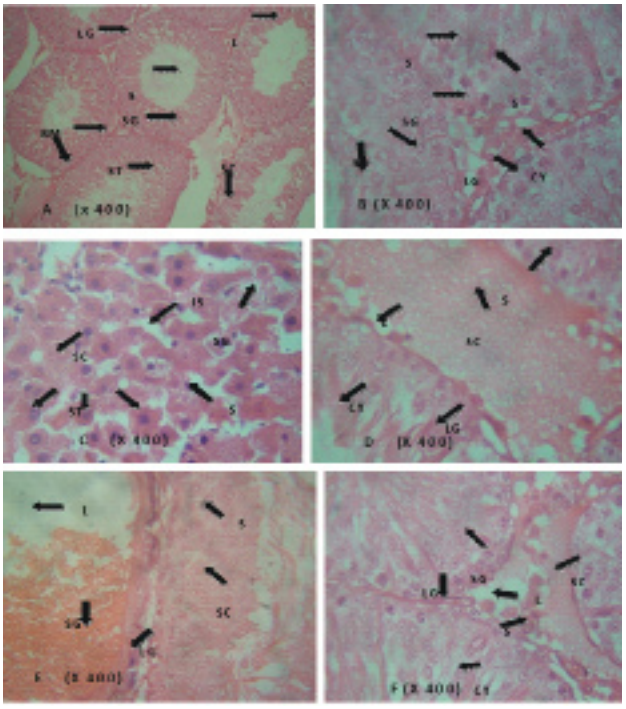


Figure 4. (A–F): Microscopic findings of eosin and hematoxylin 5 μm thick stained section of rat testes.
(L= lumen, ST= Spermatids, SC= Spermatocyte, SG= Spermatogonia, S= Sperm. LG= Leydig cell, CY=Cytoplasm, IS= Interstitial space, BM=Basement membrane).

4. Discussion

The exposed animals showed psychomotor behavioural symptoms in the course of the experiment. This includes body weakness, loss of body hair, pus or discharge from the eyes and light weight. This suggests that EOMABRSL exposure could have some lethal effects in mammals. Also, the loss in absolute and relative testicular weight further validates its toxicity. Similarly, the considerable drop of relative testicular weight in experimental rat suggests testis toxicity [33]. This may consequently results to low sperm count[34]. It has been established that the weight of testes is largely dependent on the mass of the differentiated spermatogonia[19, 35]. However, the reduction in the relative weight of the testes may be traced to the depleted number of germ cells. This may be linked to inhibition of spermatogenesis and steroidogenic enzyme activity[36, 15]. Also, the observed weight loss of relative testicular organ may be linked to drastic reduction of sex/serum hormones in circulations[16]. This observation is in line with the finding of Schrade[37]. It also supported the reports of Gupta *et al*, [38] which stated that administration of pro-oxidants to wistar rats by oral route caused significant testicular weight loss. Hence, the significant weight loss in testes of EOMABRSL-treated rats speculates its reproductive toxicity.

SOD generally dismutases the superoxide anion radicals into H_2O_2. This is degraded by CAT and GSH peroxidase using reduced GSH. Antioxidant enzymes CAT, peroxidases and SOD protect against tissue damage by inhibiting superoxide anions[39]. Reactive oxygen metabolites had been considered cytotoxic because of their ability to induce lipid peroxidation in membrane tissues[40]. As observed in the present study, EOMABRSL significantly overwhelmed the defence capacity of the testicular antioxidant enzymes of the treated rats. This further suggests that EOMABRSL could cause impairment to male testes through induction of oxidative stress after sub-chronic exposure. The increased activity of SOD may be linked to the high level of superoxide anions (02-) induced by EOMABRSL. Similarly, high activity of CAT suggests the precipitation of reacting oxygen species, H_2O_2 in the testicular tissue. This may leads to production of hydroxyl radical (OH•), causing damages to proteins; bio-membrane and DNA molecule of the testis. In the same vein, increase in the activity of CAT could also be linked to its induction to counter the effect of oxidative stress. The present investigation is consistent with earlier report of Guangke *et al* [41] and Farombi *et al* [11].

The GSH plays a crucial role in protecting the cells from oxidative damage[42]. In our study, testicular GSH content was considerably depleted after EOMABRSL administration. This decline in GSH content under the present experimental model suggests unbalanced glutathione system. This is by causing oxidative stress under the influence of ROS generated from EOMABRSL in the testes. Consequently brings about the diminution of sperm counts[43].

Lipid peroxidation (LPO) has been associated with testicular toxicity and carcinogenicity[15]. Increased lipid peroxidation as observed in the present finding potentiates H_20_2 and 02- to produce OH- in the presence of transition metals. However, OH- diffuses freely across biological membranes to initiate lipid damage[44]. The observation corroborates the earlier work which reported that elevated lipid peroxidation directly results from free radical-mediated toxicity[45–47]. Therefore, the mechanism by which EOMABRSL exerts its oxidative stress in male germinal cells may be related to its ability to increase lipid peroxidation. More so, the decrease in total protein content may be attributed to the direct inhibitory effect of EOMABRSL on protein synthesis[48].

The abnormalities of sperm parameters observed in the study may be linked to the division and differentiation of immature spermatogonia into mature elongated spermatid within the testis. This supports the earlier work that a decrease in epididymal sperm count and increase in sperm deformities have been associated with immature differentiation of spermatocytes[49, 50]. Also, low daily sperm production in the sub-chronic exposed rats may not be unconnected to the sertoli cells that have been harmfully distorted [51]. In addition,

the observed infertile semen might be linked to its contamination with leukocytes; mainly neutrophils and macrophages. However, EOMABRSL is suspected as apoptotic and mutagenic agent to spermatozoa because Neutrophil-derived HOCl was recently reported to induce apoptosis in human spermatozoa[52]. Besides, necrotic damage of leydig and sertoli cells of the interstitial cells as revealed from the study may again lead to deformed spermatids and folded sperms. This could lead to motionless spermatozoa without flagella (amorphous head) in the lumen.

Hypothalamic hypophysis axis responsible for the development of spermatogenesis had been considered to be highly susceptible to lead and cadmium intoxication[53]. However, the direct injury on germ cells could be linked to the stable metabolites and other organic pollutants/species in the EOMABRS leachate. Cd had been implicated in prostate cancer[54, 55]. Pb, Fe and Mn were known to reduce sperm count and sperm motility[56]. Cr and Ni were identified as agents of enlarged intracellular spaces and dramatic loss of gametes in treated rats[57]. The spermatotoxic effect of this study may be attributed to high doses of Cd, Cr, Pb, Mn, Fe and Ni in EOMABRS leachate when compared with No Observable Effective Limit (NOEL) set by World Health Organisation[58] and National Environmental Standard and Regulatory Enforcement Agency[59]. The levels of inorganic elements in EOMABRSL were higher than STREAM, WELL-A, WELL-B and CDW. Their high levels may be because soil can easily form ligands with metals or it has high capacity to retain heavy metals than inorganic solvents[14, 16]. The bio-metal effects could be linked to individual, synergistic, antagonistic, competitive or collective interaction of the metals with normal body biochemical processes. When these metals are ingested, they are converted into their stable oxidation states (Fe^{2+}, Pb^{2+}, Cd^{2+} etc). They consequently combine with the body's bio-molecules of the testes to form strong and stable chemical bonds, thus, mutilating their structures, hampering their functions to induce sperm toxicity [11, 12, 16]. Additionally, low doses of Co, Zn and Cu relative to WHO and NASREA limits as observed from the study could cause depletion of testicular antioxidant protein; GSH. The deficiencies of these trace elements had been implicated in decreased sex hormones and low testicular glutathione[60]. Therefore, interactions of heavy metals viz Cd, Cr, Mn, Pb, Fe, and Ni with other organic environmental pollutants in leachate from a small scale industry (battery recycling site) might suggest some certain health risk to testes and other related environmental problems. Also, this suggests that EOMABRSL would increase sperm abnormalities. This work supported the previous investigation which stated that increase in lipid peroxidation products had been associated with abnormalities in sperm morphology[61].

Humans and animals are exposed to combined toxicants from the environment. Also, quest for industrial development of recycling products to boost economy in Nigeria is raising a serious burden of heavy metals and several organic pollutants to the environment. This might pose some threats to the reproductive health. However, administration of EOMABRSL had significant effects on both absolute and relative testicular weight. Formation of sperm abnormalities was increased. Antioxidant enzymes including superoxide dismutase and catalase were significantly altered in the testes resulting into increased lipid peroxidation. A reduced response on glutathione level in the testes was also observed. The mechanism of toxicity is not unlinked to individual, synergistic, antagonistic, competitive or collective interaction of the metals with normal testicular biochemical processes. Taken together, we conclude that the possible mechanism by which interactions of heavy metals with other organic environmental pollutants in leachate at the investigated doses elicits sperm damage in rats could be through induction of oxidative stress and damage to germinal epithelial cells.

Conflict of interest statement

We declare that we have no conflict of interest.

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