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Sexual Behavioral Deficit and some Reproductive Abnormalities in Male Wistar Rats Exposed to Landfill Leachate

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ABSTRACT

Land filling is a common method of managing solid wastes around the world and this method of waste management causes nuisance to the environment through the release of chemical laden landfill gases and leachates that can affect multiple organ systems including the male reproductive system. This study was carried out to investigate the impact of 21 days exposure of Gwagwalada landfill leachate on sexual behaviour, reproductive hormonal imbalance, gonadal and extra-gonadal sperm reserve depletion and histopathology of the testis and epididymis of adult male Wistar rats. Twenty (20) adults male Wistar rats were used and randomly selected into two (2) groups. Group A were fed with normal rat chow and tap water as drinking water ad libitum while Group B were fed with normal rat chow and 10% of Gwagwalada landfill leachate as drinking water ad libitum. Exposure was for 21 days. Results showed that exposure to GLL induced decreased mount frequency and increased mount latency as well as decreased serum concentrations of LH, FSH and testosterone. Also, there was marked weight loss of the testis (especially the left) with different morpho-phenotypic pathologies of the testis and epididymis. The present study has demonstrated the detrimental effects of Gwagwalada landfill leachate to adult males.

Key words: Gwagwalada landfill leachate, Sexual behavioural deficits, Hormonal imbalance, Testis, Histopathology

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INTRODUCTION

Wastes are unavoidably produced by anthropogenic activities, and poor waste management has recently resulted in serious public health and environmental contamination hazards [1]. These issues increase together with the steady increasing human population and industrial activities [2-7]. Presently, land filling is the most usual and common process used in solid waste management at various places in the world and this process of waste management results to environmental hazards by the means of releasing heavy chemicals, landfill gases and leachates [1]. For more than four decades, Njoku et al., [8] have published extensive data relating solid waste chemicals to human and wildlife health issues.

Leachate is any liquid that extracts soluble or suspended solids in the course of passing through matter [9] and contains high concentrations of hazardous organic and inorganic chemicals, toxic heavy metals, particulate matter, radioactive substances and microorganisms [10-14]. Reports in Nigeria have focused on mixture of xenobiotics in leachates collected from Aba Eku and Olusosun landfills in Africa [15-16]. They [13, 15-16] have posited that exposure to leachate can cause severe alterations in daily body weight gain, erythrocyte morphology abnormalies and altered haematological indices as well as liver and kidney dysfunction [15-16] in rat experimental model. Also, Li et al. [17-18] posited that exposure to Xinguo, China leachate induced protein oxidation, lipid peroxidation as well as disturbances of antioxidant status in different organs including the brain, heart, liver, kidney and spleen.

Few information is available on the reproductive effects of leachate especially on male sexual parameters. Specifically, these reports are mainly on leachate induced alteration of the testicular tissue due to oxidative stress [1, 19-20]. The testes are paired

male gonads found in the scrotal sac which are responsible for the production of spermatozoa and some androgens such as testosterones [21]. Testes are susceptible to lipid peroxidation and oxidative injury resulting from exposure to xenobiotics [6, 19]. Herein, we evaluated the reproductive toxic effects of Gwagwalada landfill leachates (GLL) in adult male Wistar rats. We posit that exposure to GLL induced severe sexual behavioral, hormonal and structural alterations in testicular and epididymal tissues of male rats.

MATERIALS AND METHODS

Collection and preparation of 10% Leachate Raw leachate was collected from landfill holes (10 wells and about 3-10cm apart) in Phase 3, Gwagwalada Area Council; Federal Capital Territory, Abuja. Abuja is the capital of Nigeria having a population of above two million [2]. Abuja is located north of the confluence of River Niger and River Benue, and the centrality of the city in relation to other states makes it influential and important in various socioeconomic activities such as excavating and illegal gold mining [2, 22] resulting to increasing accumulation of solid waste. Ten liters of leachate was collected in July 2021, during the period of the rains. The leachate was collected in clean plastic containers and transported by road to the Veterinary Anatomy Department, University of Abuja Nigeria. Then, in order to remove debris, the leachate was therefore thoroughly mixed and then filtered. Using 10mls of the leachate, the physicochemical properties and metal analyses were determined (results not provided). After that, the leachate was kept refrigerated at 40°C until it was time to use it. At the time of usage, a 10% leachate was generated from a homogeneous mixture as amended by Adedara et al., [19] using tap water, according to a conventional process. To summarize, 100 mL of raw sample was mixed with 900 mL of tap water and mechanically agitated for 1 hour, and this was allowed to settle for 30 minutes before

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filtering (Whatman no. 42) in order to remove the suspended particles. Finally, at room temperature, the resulting filtrate was centrifuged at 3000 rpm for 15 minutes and the pH of the supernatant fluid was measured before storage at 4°C until use. The sample was labeled Gwagwalada landfill leachate (GLL).

Experimental Animals

The experiment utilized a total of 20 mature male Wistar rats weighing 158 $g \pm 0.68$ g. The male Wistar rats were obtained from the National Institute for Pharmaceutical Research and Development (NIPRD) in Idu-Karmo, Abuja, Nigeria, and transported to the Animal Core facility of the Department of Veterinary Anatomy, University of Abuja, Nigeria, in ventilated cages. The animals were acclimatized for two weeks before the experimental processes were commenced. A 12-hour/12-hour light/dark cycle was allowed and all animal used had unlimited access to food and water. The University of Abuja Ethics Committee for Animal Use (UAECAU/2020/006) approved the experimental protocol, which is in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) and the European Communities Council Directive of November 24, 1986 (86/609/EEC).

Experimental Design and Treatment Groups

Experimental animals were randomly divided to two experimental groups as follows:

Group A: Control group, had 10 male Wistar rats as members of the group. They were fed with normal chow rat and tap water as drinking water *ad libitum* throughout the period of the experiment.

Group B: Treated group, had 10 male Wistar rats as members of the group. They were given normal chow rat as feed and 10% of Gwagwalada landfill leachate as drinking water *ad libitum* throughout the period of the experiment.

All treatments were for twenty-one (21) days, within which animals were closely monitored for signs of toxicity by a veterinarian.

Determination of Sexual Behaviors

After 21 days of Gwagwalada landfill leachate treatment, the leachate exposure's effects on sexual behavior of these male Wister rats were evaluated following the modified method described by [23]. For the sexual behavioral studies, an open field arena of aluminum rat cage of 60 cm X 30 cm with 20 cm high, same as used for housing the animals during the period of experiment was used. The cages were emptied of bedding and moved to the sexual behavioral room. The video camcorder, Nikon coolpix L341 (Nikon Imaging, USA) used for video capturing of sexual behavior was positioned 90 cm above the centre of the open cage floor. The entire arena of the cage in the zone of the camera's view was captured from The video camcorder was this position. connected to a Samsung laptop, (Samsung Electronics, South Korea). The behavior observation tests started at 3.00 hour after the onset of darkness and were performed under red illumination and by pressing space bar key on the computer keyboard; the test starts and ends manually. In the next days, the animal's sexual behavior inside the cage arena was manually scored from the recorded videos by two investigators blinded to experimental groups with good concordance. Before the commencement of the actual sexual behavioral test, individual male Wistar rats were placed in the observation cage and were left for 10 minutes in order for them to get used to the new cage environment. Each individual's sexual behavioral test was initiated with the introduction of receptive female. Female Wistar rats introduced quietly from one side of the cage as teasers, and then observations were recorded for a period of 20 mins with a video camera. Each male had one female and no repetition was done in this study. In this sexual behavioral experiment, parameters studied were as described by Mos et al., [23] and

included the following:

Mount latency (ML): The interval between the introduction of a receptive female into the arena and the first mount – an indication of sexual desire/libido (in seconds). When a male rat mounts a female from behind and grasps her sides with his front feet, it is known as a mount.

Mount frequency (MF): The number of mounts to ejaculation within 20 minutes – an indicator of sexual drive/desire/libido.

The sexual behavioral testing was done on all of the Wistar rats in each group. At 8:00am of the next day, five millilitres (5 ml) venous blood was collected at room temperature through the retro-orbital sinus plexus of all rats in both groups into plain sterile tubes as described by Usende *et al.*, [6]. The collected blood samples were left standing in the tubes at room temperature for 45 minutes before they were centrifuged at 4000 rpm for 15 minutes to obtain a clear serum. The clear serum obtained was separated with Pasteur pipettes into another plain tube and stored at -20°C until the time of hormonal assays [6].

Some Reproductive Hormones Assay

For alterations in some male reproductive hormones evaluation, serum was taken and preserved from all Wistar rats (Gwagwalada landfill treated and control groups). Using a commercially available radioimmunoassay kit, serum testosterone, follicle stimulating hormone (FSH), and luteinizing hormone (LH) concentrations from each of the two groups were assessed in duplicate (Bristol Scientific Company, Sigma-Aldrich, Lagos Nigeria) following manufacturer's instruction as previously described by Usende et al., [6]. The sensitivity of the testosterone assay was 2.6 pg/ml (range 3.9-1000 pg/ml) and inter-assay and intra-assay coefficients of variation ranging from 3.26% to 9.45% and 5.58% to 9.78% respectively. Cross reactivity was grouped into four broad categories: Strong cross-reactivity (5% or greater), Weak CrossReactivity (0.5–0.49%), Very Weak Cross-Reactivity (0.05-0.49%), and Not Cross-Reactive (<0.05%). Percent cross—reactivity was defined as the ratio of observed "steroid" to the amount of test compound added, multiplied by 100 as previously described by Usende *et al.*, [6]. The sensitivity of LH was 0.04 ng at 80 %, whereas FSH sensitivity was 0.07 ng at 98 %. The intra-assay CV was 3.6 % for LH and 3.2 % for FSH. All the results generated were presented as UI/L (mean \pm standard error of mean (SEM).

Testicular Weight Measurement

Before the animals were sacrificed, a bench top sensitive scale was used to obtain the weight of each rat earlier before they were anaesthetized with lethal dose of Ketamine and Xylazine combination [24]. The left and right testis and its epididymis were carefully dissected out from 5 Wistar rats from each group. The weight of each testis (left and right) and the sum of the mean weight was obtained as previously described by Sakamoto et al., [25]. Thereafter, the testis and its epididymis were used for evaluation of gonadal and extragonadal sperm reserve. The testis and epididymis of the remaining five (5) Wistar rats were used for histopathological examination after transcardiac perfusion [24] followed by immersion fixation in Bouins fluid for 24 hours.

Determination of Gonadal and Extragonadal Sperm Reserve

With slight modifications, the gonadal and extragonadal sperm reserves were determined using the methods described by Otubanjo and Mosuro [26] and Mofio *et al.*, [27]. In summary, five male Wistar rats from the treated group and the control group had their left testis and caudal epididymis carefully dissected, adherent tissues were removed, and then the organs were weighed separately using a benchtop sensitive electronic balance (LP 502A, China) with a sensitivity range of 0.1 to 5 kg [28]. In a ceramic mortar and pestle, the testis



and caudal epididymis of the rats were each separately ground up, and the ground-up testis and caudal epididymis were then homogenized with 5 ml of normal saline. 0.5 ml of the filtrate was added to 4.5 ml of white blood cell (WBC) suspension diluting fluid (Turks Solution) and the suspension was carefully mixed and sieved into clean glass test tubes using a sterile double layer wire gauze. Sperm concentrations in the gonadal and epididymal regions were measured using hemocytometric counts. The number of sperm cells in the four major corner squares (each measuring 1 mm²) were counted, and a correction factor of 250,000 was used as a multiplication index to calculate the expected number of sperm cells per ml of gonadal and epididymal sperm reserve.

Animal Perfusion Fixation and Light Microscopy

The remaining five male Wistar rats from the control and treated groups were transcardially perfused first with normal saline to wash out the blood cells, then with freshly prepared 4% paraformaldehyde (PFA) in 0.1M phosphate buffer until well fixed, using muscle stiffness and liver pallor as indicators for good fixation [5, 24]. The testis and epididymis were dissected carefully after perfusion and immersed fixed in Bouins solution for additional 24 hours before being processed for light microscopy. Following the methodology outlined by Usende et al., [29], all materials were treated for paraffin sectioning and sections were stained with hematoxylin and eosin.

Statistical Analysis

Numerical data obtained were analyzed for statistical significance using Graph-pad prism

version 7.0 computer software. Means of the groups were expressed as mean \pm SEM and Student T-test was used to determine the significant difference between the treated group and the control group for all numeric data generated.

RESULTS

The present study evaluated the effects of 21 days exposure of 10% Gwagwalada landfill leachate on some reproductive parameters of adult male Wistar rats. This study explored sexual behaviour, hormonal changes, sperm parameters and histopathology of the testes and the epididymis.

Effects of Oral Exposure of Gwagwalada Land Fill Leachate on Sexual Behavioral Responses

All male Wistar rats of both control and Gwagwalada landfill leachate treated groups responded to females ano-genital sniffing which was followed by pursuit and mount. In this experiment, we focused on mount latency and frequency. In the control group, the mount behaviour was quick and in frequent succession. Exposure to Gwagwalada landfill leachate affected these parameters significantly. Specifically, the mount latency recorded from the leachate exposed group was significantly increased (P<0.01) compared to the control (Fig. 1a). Concerning the mount frequency, a statistically significant reduced frequency was recorded for the leachate exposed group (P<0.001) when compared to control (Fig. 1b).



Fig. 1: Bar graphs showing significant increased mount laten cy (A) and significant decreased mount frequency (B) in male Wistar rats exposed Gwagwalada landfill leachate compared to control. (*P<0.05; **P<0.01)

Effects of Oral Exposure of Gwagwalada Land Fill Leachate on Testicular Weight

With regards to testicular weight, exposure to Gwagwalada landfill leachate induced significant reduction (P<0.01) in the left testicular weight when compared to the control group (Fig. 2a). On the other hand, although the Gwagwalada landfill leachate exposed group had a lower right testicular weight compared to the control group, no statistically significant

difference (Fig 2b) was seen. More so, a further assessment was carried out to know if this reduction is unilateral or bilateral, we did a comparison on either the right or left testes singly as reported above, before taking the mean of the right and left testes and one parameter. The results showed a statistically significant decreased mean testicular weight in the leachate exposed group (P<0.01) relative to control (Fig 2c).



Fig. 2: Bar graphs showing significant decreased left testicular weight (A), a decreased ed that was not statistically significant in the right testicular weight (B) and significant decreased mean sum of left and right testicular weight in male Wistar rats exposed Gwagwalada landfill leachate compared to control. (*P<0.05; NS, not significant)

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Effects of Oral Exposure of Gwagwalada Land Fill Leachate on Gonadal and Extra Gonadal Sperm Reserve

The testicular and epididymal sperm cells reserve of male Wistar rats exposed to Gwagwalada landfill leachate together with the control group results are shown in Figure 3. Concerning the sperm count, both the gonadal (Fig 3a) and extra gonadal (Fig 3b) sperm reserve of Gwagwalada landfill leachate treated group were significantly reduced (P<0.001) compared to control match.



Fig. 3: Bar graphs showing significant decreased testicular (A) and Epidydimal (B) sperm count in male Wistar rats exposed Gwagwalada landfill leachate compared to control. (**P<0.01)

Effects of Oral Exposure of Gwagwalada Land Fill Leachate on Some Reproductive HormonalAssay

Result on the serum concentration of luteinizing hormone (LH), follicle stimulating hormone (FSH) and testosterone are presented in figure 4. The results showed significantly reduced concentrations of LH (P<0.01) (Fig 4a), FSH (P<0.01) (Fig 4b), and testosterone (P<0.001) (Fig 4c) in male Wistar rats exposed to Gwagwalada landfill leachate after 21 days in comparison to control.



Fig. 4: Bar graphs showing significant decreased serum concentration of luteinizing hormone (LH) (A), Follicle stimulating hormone (FSH) (B) and testosterone (C) of male Wistar rats exposed to Gwagwalada landfill leachate compared to control. (*P < 0.05; **P < 0.01)

Effects of Oral Exposure of Gwagwalada Land Fill-leachate Induced Histopathology of the Testes and Epididymis

On histopathological examination, significant lesions were seen in the testis and epididymis of Wistar rats exposed to Gwagwalada land fill leachate compared to the control group. Concerning the testis, the seminiferous tubules of control group appeared normal with proper organization of the spermatogenic cells. In the Gwagwalada landfill leachate treated group, the seminiferous tubules appeared distorted with disorganization of cellular arrangement and severe interstitial tubular oedema, destruction of interstitial connective tissues, as well as hypocellularity of the seminiferous tubule (Fig. 5). Also, a severe destruction of the basal cells, with pyknotic nuclei and scanty and deeply eosinophilic cytoplasm of these cells were seen in the seminiferous tubules of Wistar rats exposed to Gwagwalada landfill leachate (Fig. 5).



Fig. 5: A photomicrograph showing distorted and disorganization of cellular arrangement and severe interstitial tubular oedema, destruction of interstitial connective tissues (white asterisks), severe destruction of the basal cells, with pyknotic nuclei and scanty and deeply eosinophilic cytoplasm (red arrow) and clumps of these cells (white arrow head) in the seminiferous tubules of male Wistar rats exposed Gwagwalada landfill leachate (B) compared to control (A).

On the epididymis, there was a severe sloughing of the epididymal epithelium into the tubular lumen, with other forms of disrupted epithelial morpho-phenotypes and shrinkage of the epithelial lining in the Gwagwalada landfill leachate exposed group compared to control (Fig. 6). Also, results showed vacuolation of epidydimal epithelium together with shrinked clumps of the sperm cells (Fig. 6).



Fig. 6 A micrograph showing severe sloughing of the epididymal epithelium into the tubular lumen, with other forms of disrupted epithelial morpho-phenotypes (yellow and black arrows) with shrinked clumps of the sperm cells (white arrow) in the epidydimal tissue of Gwagwalada landfill leachate exposed male Wistar rat group (B) compared to control (A). H&E stain, scale bar: 50µm.

DISCUSSION

Report have shown that landfill leachate constitutes a complex cocktail of constituents such as xenobiotic organic compounds and dissolved organic matter, heavy metals and inorganic macro components [30]. At very close range to living organism, landfill leachate is very toxic [19]. Recent reports have shown that landfill leachate can instigate liver and kidney dysfunction, DNA, brain and importantly, testicular damages [1, 19]. Moreover, organs constituting the male reproductive tract (especially the testis) function with regards to formation of gamete (sperm) [6, 31] needed for fertilization of the female ova which as a result arise in progeny and continuity of life [6], and therefore of interest in the present study. Evidence have shown that any adverse effect in the testes is translated affecting further generations and if not controlled, can lead to extinction [6].

In this study, we evaluated the effects of 21 days oral exposure of 10% Gwagwalada landfill leachate on sexual behavioral and hormonal deficits, sperm reserve as well as abnormal morpho-phenotypes of the testis and epididymis. Humans as well as many wildlife living in highly polluted poor resource settling are constantly exposed to several environmental toxins through contamination of their food and major water source (such as wells, bore-holes and tap water) with leachate from dumpsite and may go into extinction [1-2, 5, 19]. We used *ad libitum* and oral exposure route because this is the pattern of exposure in nature. This is similar to the work of Adedara *et al.*, [19] who administered different concentrations of Olushosun landfill leachate to male Wistar rats for seven days and reported reproduction dysfunction in exposed animals. Recently, Ademola *et al.*, [1] exploited the intraperitoneal route of exposure using the Olushosun landfill leachate to male mice and reported similar results.

The present study is the first to evaluate the effects of exposure of leachate on sexual behaviour and revealed decline in sexual behaviour in the Gwagwalada landfill leachate exposed group compared to their control match. Specifically, we reported herein severe decline in mount latency and frequency in the exposed group compared to control. Sexual behavior is hormone-regulated and largely reflects the normal functioning of the hypothalamo-pituitary-gonadal axis, and it includes both sexual motivation (libido) and sexual performance [32], both of which were severely reduced in the Gwagwalada landfill leachate exposed group in the current study. Interestingly, the findings on decline of sexual

behaviour corroborate well with the significant depletion of serum concentrations of gonadotropins (LH and FSH) and testosterone reported herein. The hypothalamic-pituitarygonadal axis is well known for its regulation of development, reproduction, and aging in humans and animals [1] and gonadal steroids act on the hypothalamus to regulate gonadotropin-releasing hormone pulses and at the pituitary level to regulate gonadotropin (LH and FSH) secretion [1, 6]. FSH, in conjunction with LH, modulates spermatogenesis by reducing proapoptotic signals and, as a result, boosting spermatogenic cell survival [33]. During spermatogenesis, LH is the principal tropic hormone necessary to stimulate the Leydig cell to produce and secrete testosterone (which is dramatically reduced in this study) [1]. The significant decrease in serum concentrations of testosterone, LH, and FSH seen in the present investigation in Gwagwalada landfill exposed rats is similar to report of Ademola et al., [1] in Olushosun landfill leachate exposed rats.

In the present study, a significant reduction of spermatozoa was observed in the testis and epididymis of the Gwagwalada landfill leachate exposed group compared to control and this could be due possibly to disturbances of the process of spermatogenesis, a cyclical phenomenon of cell proliferation and or germ cell death [6]. Similar findings have been reported by Ademola et al., [1] and Adedara et al., [19]. The production of appropriate numbers of spermatozoa depends entirely on the stimulation of the testes by FSH and LH, and in response to LH, testosterone which is necessary for maintenance of spermatogenesis is produced [6, 31, 34]. The significant reduction in the serum concentrations of testosterone as well as FSH and LH in the 21 days Gwagwalada landfill exposed rats resulted to the quantitative low sperm count observed herein. FSH and testosterone are necessary for the beginning of spermatogenesis, and their considerable reduction explains why testosterone is unable to maintain spermatogenesis and stop the loss of germ cells [35-36]. In addition, acute testosterone reduction, as observed in the current study and also reported by Wang *et al.* [36], can cause changes that cause the spermatid to Sertoli cell cytoskeleton to dissociate, which can result in the depletion of testicular germ cells, particularly spermatocytes and spermatids [37] and induction of increased germ cell apoptosis [8]. It therefore implies that testosterone and FSH functions as cell survival factor, protecting germ cells from apoptosis [31, 34, 39]. This study has shown strong negative relationship in sexual behaviour, serum testosterone, LH and FSH levels and gonadal and extragonadal sperm reserve seen in Gwagwalada landfill leachate exposed rats.

Also, this study revealed that exposure to 10% Gwagwalada landfill leachate caused significant reduction of the mean sum of both right and left, and on the left testis but not the right testicular weight This is an interesting finding and the possible reason remains to be investigated. Moreso, it is interesting to note that while Ademola et al., [1] reported no significant testicular weight reduction after exposure of male rats to Olusosun landfill leachate and Adedara et al., [19] reported significant increase in testicular weight in mice exposed to same Olusosun landfill leachate and tributed it to oedema of the testicular tissue. The possible explanation for the difference in this present study and that of Adedara et al., [19] could be the route of exposure. While the present study explores the oral route and ad libitum exposure, Adedara et al., [19] exposed their mice intraperitoneally and at a single exposure per day. We hypothesis that the reduction in the mean sum of the left and right, and left testicular weights might be due to the possibly shrinkage of this organ. Reports have shown that the weight of male reproductive organs usually provides a useful reproductive risk assessment in experimental studies [40]. The observed significant reduction in the left and right, and left testicular weights in Gwagwalada landfill exposed group suggested the toxic effect of the components of this leachate on the testis [41].

Concerning the histopathological examination

of the testis and epididymis of male Wistar rats treated with Gwagwalada landfill leachate, the present study revealed that the seminiferous tubules of exposed rats appeared distorted with disorganization of cellular arrangement and severe interstitial tubular oedema, destruction of interstitial connective tissues, as well as hypocellularity of the seminiferous tubule, severe destruction of the basal cells, with pyknotic nuclei and scanty and deeply eosinophilic cytoplasm of these cells. On the epididymis, we reported herein, a severe sloughing of the epididymal epithelium into the tubular lumen, and other forms of disrupted epithelial morpho-phenotypes with shrinked clumps of the sperm cells. Similar findings have been reported by Adedara et al., [19] and Ademola et al., [1] on mice and rats testicular and epidydimal tissues respectively. Depletion of spermatogonia, spermatocytes, spermatids, alteration in size and number of seminiferous tubules, reduction in germinal epithelium height and lumen space and disorganization of the seminiferous tubules as reported herein in the Gwagwalada landfill exposed rats are lesions related to xenobiotic induced toxicity [6]. Adedara *et al.*, [19] and Ademola *et al.*, [1] had attributed these lesions seen in the testis and epididymis to the effects of heavy metals in landfill leachate. This is an indication that chemicals most importantly metals in landfill leachates can cross blood testes barrier to reach the testicular environment.

In conclusion, the present study demonstrated the detrimental effects of Gwagwalada landfill leachate to adult male Wistar rats, thus, continuous oral exposure to leachate suggests severe adverse effects on sexual behavior, reproductive hormones, depletion of gonadal and extragonadal sperm reserve and destructions of the testicular and epididymal tissues, which if prolonged could lead to total infertility.

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