Prolonged Exposure to Environmental Black Soot Impacted Negatively on the Reproductive Functions of Male Wistar Rats

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Abstract

Residents of the oil rich city of Port Harcourt and its environs have been inundated since November 2016 by a fresh wave of pollution – plumes of thick black soot in the air capable of impacting health and wellbeing of the people. The current study investigated the relationship between exposure to environmental black soot and some reproductive and testicular function parameters in male Wistar rats. The study consisted of four groups of rats that were exposed to drinking water alone, while the remaining three groups were exposed to soot and sacrificed after four weeks, eight weeks and twelve weeks. Additionally, the control was also sacrificed at the end of weeks four, eight and twelve respectively. Results obtained indicated a statistically significant decrease (p < 0.05) in some sperm parameters and male sex hormones (follicle stimulating hormone, luteinizing hormone and testosterone) especially between the control and the group exposed to black soot for up to twelve weeks. Taken together, environmental exposure to black soot may alter hormonal profile and subsequently lead to infertility in exposed male rats.

Keywords: Black Soot; Testicular Function; Sperm Parameters

Introduction

Across Nigeria’s oil producing Niger Delta Region, environmental pollution has long been a part of daily lives. While residents have become used to multiple oil spills which have damaged livelihoods and farmlands, the residents of the oil industry hub of Port Harcourt face a new kind of dangerous soot particles in the air–black soot.

Residents of Port Harcourt in Rivers State, Nigeria, and its environs have since the last quarter of 2016 been experiencing adverse environmental impacts of particle (soot) pollution. This “double air pollution burden”-the unresolved prevailing widespread air pollution and the “added” emergence of particle pollution considered an environmental health threat, led to protests against government inaction in some parts of the state. In February 2017, several months following the onset of the pollution, the government declared an Emergency, and set up a Task Force to investigate and find a solution to the problem. Global research suggests that particle pollution correlates positively with a range of morbidities and an increased risk of mortality among exposed populations [1].

According to the World Health Organization air pollution constitutes the largest among all of the environmental risks: 3 million deaths a year are linked to exposure to outdoor air pollution. Indoor air pollution can be just as deadly. In 2012, an estimated 6.5 million deaths...
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(11.6% of all global deaths) were associated with indoor and outdoor air pollution together. Nearly 90% of air-pollution-related deaths occur in low- and middle-income countries, with nearly 2 out of 3 occurring in WHO’s South-East Asia and Western Pacific regions.

Ninety-four per cent are due to non-communicable diseases – notably cardiovascular diseases, stroke, chronic obstructive pulmonary disease and lung cancer. Air pollution also increases the risks for acute respiratory infections [2].

The ubiquitous presence of the soot constitutes hazards of air pollution and thus have appreciable effects on air quality, visibility and ultimately exacerbates the changing climate impacts in the Port Harcourt metropolis and the surrounding communities in the region. Most coastal communities contiguous to the artisanal refineries whose activities generate the most part of this air pollution dilemma is constantly 'fumigated'; experiencing continuous showers of soot deposits, enveloping every sphere their blackened daily existence [1].

The quality of air in the Port Harcourt metropolis and surrounding towns, as well as other parts of the Niger Delta have been studied by various scholars, who rank the region's air quality amongst the top 10 most polluted regions in the world [3-8]. Results from the investigations into the composition of the black soot in Port Harcourt revealed that lead (Pb), Nickel (Ni), Cobalt (Co) and Polycyclic Aromatic Hydrocarbons all exceed the WHO/EPA allowable limits [9,10].

Infertility is a disease of reproductive system defined by failure to achieve the clinical pregnancy after 12 months or more of regular unprotected sexual intercourse. It can also be defined as failure of couple to conceive after 12 months of regular intercourse without the use of contraception in women < 35 years; and after 6 months of regular intercourse without the use of contraception in women ≥ 35 years [11]. There has however, been a marked increase in cases of infertility in Rivers State. A recent study conducted in port-Harcourt, the Rivers state capital reported that 87.4% of men had varying degrees of anatomical abnormality of their sperm cells (teratozoospermia) [12]. The current work investigated an association between an extended exposure to black soot and infertility in male Wistar rats.

Materials and Method

Chemicals

Follicle stimulating hormones (FSH) (RPN 2560, Amersham, United Kingdom), luteinizing hormone (LH) (RPN 2562, Amersham, United Kingdom) and testosterone (EIA - 5179, DRG Diagnostic GmbH, Marburg, Germany). Other reagents bought from British Drug House (Poole, Dorset, UK) were of high analytical grade.

Animal model

Forty adult male Rats weighing 150 - 170g were bought from the Central Animal House, Faculty of Science, Rivers State University, Rivers State. The animals were caged in plastic cages sited in a well-ventilated vivarium and subjected to natural photoperiod of 12 h light:12 h dark photocycle throughout the period of the experiment. They were fed with rat pellets and given drinking water ad libitum for two weeks before the commencement of the experiment. Animal care and experimental protocols were carried out in agreement with the approved guidelines set by the Rivers State University Ethical Committee and the Guide for the Care and Use of Laboratory Animals' prepared by the National Academy of Science (NAS) as recommended by the National Institute of Health.

Experimental design

Subsequent to acclimatization, the rats were randomly allocated to four groups of ten rats each. The animals in each group were exposed to soot for four (4) weeks, eight (8) weeks and twelve (12) weeks consecutively in this manner:

- **Control group**: Rats received normal drinking water alone.
- **4 weeks group**: Rats were exposed to soot for 4 weeks consecutively.

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- **8 weeks group**: Rats were exposed to soot for 8 weeks consecutively.
- **12 weeks group**: Rats were exposed to soot for 12 weeks consecutively.

**Tissues sampling**

Twenty-four hours after the last treatment, the final body weight of the experimental rats was taken and were thereafter sacrificed at the end of the different exposure week. Five testes were fixed in 4% paraformaldehyde solution for histology and the remaining five testes were processed for sperm analysis (such as sperm appearance, volume, viscosity, morphology, motility and total sperm count) as well hormone profile (FSH, LH and testosterone).

**Measurement of pituitary and testicular hormones**

The circulatory levels of the pituitary and testicular hormones were assayed using available commercial enzymes immunoassay kits that is specific for rats such as FSH, LH and testosterone according to the instructions of the manufacturer. The analysis was done on the same day to avoid inter-assay variation. The sensitivity of the hormones was as follows: FSH was 0.07 ng at 94%, LH was 0.06 ng at 90% and testosterone was 0.08 ng/ml with a cross-reactivity with other androgen derivatives such as methyl testosterone, androstenedione and 5α-dihydrotestosterone that was negligible. The intra-coefficient of FSH, LH and testosterone were 3.9%, 3.7% and 3.4% respectively.

**Histological analysis**

Testicular tissues collected were dehydrated in ascending grades of alcohol, cleared in xylene and embedded in paraffin wax. Serial sections of five microns thickness were obtained using a rotary microtone. The sections were then stained Haematoxylin and Eosin (H and E) and viewed under a light microscope.

**Statistical analysis**

Data was presented as mean ± standard error of mean (mean ± SEM). They were analyzed using ANOVA and Fisher’s post hoc test as appropriate on SPSS (version 20) software. Only p values less than 0.05 (p < 0.05) were considered statistically significant.

**Results**

**The effect of soot exposure on sperm functional parameters in male rats**

The effects of Mn and EtOH co-treatment on sperm functional parameters namely daily sperm production, testicular sperm number, epididymal sperm count, viability, motility and abnormalities in male rats as shown on table 1. Exposure to soot caused a significant (p ≤ 0.05) decreases in sperm daily sperm production, testicular sperm number, epididymal sperm count and motility whereas it resulted in significant increase in sperm morphological abnormalities without causing any significant effect on sperm viability in the treated rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>A (Control)</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vol(ml)</td>
<td>0.22 ± 0.03a</td>
<td>0.15 ± 0.02a</td>
<td>0.16 ± 0.03a</td>
<td>0.13 ± 0.17a</td>
</tr>
<tr>
<td>FSH</td>
<td>7.60 ± 0.00a</td>
<td>8.00 ± 0.00b</td>
<td>8.00 ± 0.00b</td>
<td>8.00 ± 0.00b</td>
</tr>
<tr>
<td>Nm(%)</td>
<td>81.30 ± 1.23a</td>
<td>73.50 ± 1.49c</td>
<td>56.50 ± 1.44b</td>
<td>38.00 ± 2.38a</td>
</tr>
<tr>
<td>Am(%)</td>
<td>18.60 ± 1.19a</td>
<td>26.50 ± 1.49b</td>
<td>43.50 ± 1.44b</td>
<td>62.00 ± 2.38b</td>
</tr>
<tr>
<td>Active(%)</td>
<td>79.40 ± 1.23a</td>
<td>65.60 ± 2.50c</td>
<td>55.60 ± 1.27b</td>
<td>40.50 ± 1.48a</td>
</tr>
<tr>
<td>Sluggish(%)</td>
<td>15.90 ± 1.44a</td>
<td>25.10 ± 2.44b</td>
<td>23.80 ± 1.88b</td>
<td>21.90 ± 2.51b</td>
</tr>
<tr>
<td>Dead(%)</td>
<td>4.70 ± 1.31a</td>
<td>7.10 ± 1.25c</td>
<td>20.60 ± 0.99b</td>
<td>37.60 ± 2.49c</td>
</tr>
<tr>
<td>Sperm count</td>
<td>73.80 ± 1.31c</td>
<td>64.10 ± 2.67b</td>
<td>49.50 ± 4.13b</td>
<td>35.90 ± 2.39c</td>
</tr>
</tbody>
</table>

**Table 1**: Comparative study of the level of significance among mean values of sperm parameters among the 4 groups of selected test specimen.

***Means having the same superscript are not significantly different.

nm = normal morphology; am = abnormal morphology.

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Soot induced decrease circulatory hormones concentration in rats

The effects of soot exposure on circulatory concentrations of FSH, LH and testosterone in rats are shown in figure 1 to 4. The levels of LH, FSH and testosterone in rats exposed to soot showed marked decrease in the levels of FSH, LH and testosterone.

Figure 1: The effect of soot exposure on follicle stimulating hormones (FSH) after 4 weeks, 8 weeks and 12 weeks. a, b and c: Values differ significantly from control and exposed groups (p ≤ 0.05).

Figure 2: The effect of soot exposure on follicle luteinizing hormone (LH) after 4 weeks, 8 weeks and 12 weeks. a, b and c: Values differ significantly from control and exposed groups (p ≤ 0.05).
Figure 3: The effect of soot exposure on follicle luteinizing hormone testosterone after 4 weeks, 8 weeks and 12 weeks. a, b and c: Values differ significantly from control and exposed groups (p ≤ 0.05).

Figure 4: Representative histopathological sections of the testes exposed to soot. The rats in the control group showed normal architecture of the testes. There were histopathological alterations in soot exposed group which included decreased germinal epithelium and vacuolization (arrow) in the testes. There was also focal area of oedema in the interstitial spaces and the tubules appear bloated with sloughing of the germ cells (arrows) (8 and 12 weeks).
Soot exposure Induces histological changes in testes of male rats

Figure 1 shows the representative histological sections of the testes of the experimental rats. Light microscopic examination indicated disruption of the histology sections of the testes exposed to soot. In detail, the rats in the control group showed normal architecture of the testes. There were histopathological alterations in soot exposed group which included decreased germinal epithelium and vacuolization (arrow) in the testes. There was also focal area of oedema in the interstitial spaces and the tubules appear bloated with sloughing of the germ cells (arrows) (8 and 12 weeks).

Discussion

This study investigated the effects of exposure to environmental black soot on the reproductive functions of male Wistar rats. Residents of Port Harcourt, the Rivers state capital have been inundated the plumes of black soot which permeates all parts of the environment. However, neuroendocrine regulation of physiological events leading to the production of mature sperm must occur to ensure the fertilization of mature oocytes, the development of normal embryos and delivery of viable offspring. The disruption of any part of this precise sequence of events leads to infertility or abnormal fetal growth if a sperm with a genetic defect fertilizes normal mature eggs.

The result showed no difference statistically in both the sperm volume and pH across the groups. However, there was a significant decrease in the normal morphology of sperm cells with the control group being significantly higher than the groups exposed to the black soot. The result also indicates that the longer the duration of exposure, the greater the effect on the sperm morphology.

This could be as a result of DNA fragmentation caused either by the heavy metals such as lead or other toxicants present in the black soot [13]. Lead represents a significant ecological and public health concern due to its toxicity and its ability to accumulate in living organisms. Earlier studies have demonstrated that lead can pass through the blood testes barrier, accumulate in the testis and/or epididymis and seriously affect the spermatogonia, primary spermatocytes, spermatids or spermatozoa (germinal cells different levels of differentiation) [14].

The result also shows a statistically significant decrease in the percentage of actively motile sperm cells between the control group that was not exposed and the other groups. The longer the duration of exposure, the more inactive and sluggish the sperm cells became. This result is in keeping with what Aboul-Ela reported. He detected a significant increase of structural chromosomal aberrations in bone marrow cells and primary spermatocytes of albino mice treated with lead [15]. Another major constituent of the black soot is polycyclic aromatic hydrocarbons (PAHs). Selevan., et al. [16] reported an association between air pollution episodes of elevated PAHs and increased asthenospermia, abnormal morphology and abnormal chromatin in human sperm [16].

Another study found that the presence of PAH–deoxyribonucleic acid (DNA) adducts in sperm is associated with abnormal morphology. The presence of the toxic heavy metals and PAHs may have increased the concentration of reactive oxygen species (ROS). In this context, in sperm, reactive oxygen species (ROS) can cause potential damage to plasma membranes and DNA integrity, motility and overall semen quality.

The total sperm count was not statistically different between the control group and the group exposed for four weeks, but was significantly different from the groups exposed for eight and twelve weeks respectively. The result also showed that the testicular hormones (FSH, testosterone and LH) significantly reduced with increased duration of exposure. This suggests the black soot may have affected the hypothalamo-pituitary –testicular neuroendocrine axis. As seen in the graph, there is an initial increase in the level of FSH seen in the group of animals exposed for four weeks. The up-regulation of FSH level is believed to be associated with reduced negative feedback regulation triggered by decreased testosterone levels in blood circulation. The synergetic action of luteinizing hormone/testosterone and FSH is necessary for the initiation, maintenance and reinitiating of normal spermatogenesis. The current results also demonstrated a positive correlation coefficient between testosterone and total motility and progressive motility of sperm.
Conclusion

This present study shows that environmental exposure to black soot may alter hormonal profile and subsequently lead to infertility in exposed male rats.

Bibliography