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Research Article

Comparative Studies on Noise Effects on Reproductive Hormones of Wistar Albino Rats and Assessment of Bioaccumulated Heavy Metals from Fumes of a Running Generator

Nwuke Chinedu Paulinus[1], Alaebo Prince Ogochukwu[1]*, Achi Ngozi Kalu[1], Onyeabo Chimaraoke[1] and Ezeigwe Obiajulu Christian[2]

[1]Department of Biochemistry, College of Natural Sciences, Michael Okpara University of Agriculture, Umuahia, Abia State, Nigeria

[2]Department of Applied Biochemistry, Faculty of Biosciences, Nnamdi Azikiwe University Awka, Anambra State, Nigeria

***Corresponding Author:** Alaebo Prince Ogochukwu, Department of Biochemistry, College of Natural Sciences, Michael Okpara University of Agriculture, Umuahia, Abia State, Nigeria.

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Abstract

The study comparatively evaluates the effects of high sound level from a generator set on testosterone and progesterone levels using albino rats of the Wistar strain. Fifty (50) Wistar albino rats of known weight were acclimatized for 14 days and divided into 3 main groups, which includes a control group, group 1 and group 2. Group 1 (for high sound level of 85db -105db) and group 2 (for low sound level of 40db - 55db) were sub divided into subgroups of five albino rats each and exposed to way off fumes from electrical generator sounds at different sound level for 8 hours each day for 28days. There were significant ($p < 0.05$) decreases in the testosterone and progesterone levels of rats in group one when compared with rats in the control group (group 0). However, the Testosterone and progesterone levels in group 2 did not decrease significantly when compared to the control group. This shows that there are significant adverse effects from that sound level of group 1 on the body's testosterone and progesterone levels. Heavy Metal Bioaccumulation in the Wistar rats showed the presence of lead, zinc, arsenic, chromium at significant ($p < 0.05$) levels. This shows that high noise pollution from the generator set can cause adverse effects to the body immune defense system.

Keywords: Noise; Reproductive Hormones; Wistar Rats; Heavy Metals; Generator

Introduction

Noise is a common physical nonspecific stressor and its physiological response as a stressor is the same as any other nonspecific physical stressor [21], noise affects the normal functioning of the cardiovascular, endocrine [2], and immune systems in the body as they manage to balance with the environmental or perceived demands of the individual [4]. This results to an imbalance between the demand and the individual's resources to adapt which determines the individual's ability to deal with noise-induced stress [26]. Therefore, the body's inability to handle any overstimulation can result to hazardous stress implications which can affect the immunity and hence causing disease state [3]. Noise stress may lead to noise-induced hearing loss by causing changes in the cell-mediated immune response [5]. This noise stress can also result to sleep deprivation because of its effect on the immune function and also this immune suppression from the stress load can increase the risk of acquiring diseases [6].

Many people are exposed to hazardous noise levels from work environment, urban traffic, or household appliances, etc., which affects their general health and life style [8]. In a particular industrious layout in Aba-north local government area of Abia state, where the noise level is high between 65 decibels to 95 decibels and above [9], the population which includes traders, transporters, club houses [24], tailors and many mini production workshops depend on their noisy generators to run their industrial machines and other electrical appliances [11]. These independent workshops run generator which is their main source of power for over 8 hours on the average every day in order to power their heavy machines and electrical appliances [16], the generators are not sound proof and also emits poisonous fumes from its combustion of gasoline [12].

Also, the generators are kept very close to their work table and machine as they have very limited space which exposes them to the hazardous health challenges from the noisy generator [26] and its poisonous fumes [17].

Exposure to this environmental condition has an adverse effect on the said reproductive hormones of the Lap Rats and in humans as a whole.

The environment is densely populated during the day due to activities of hundreds of production workshops, thousands of traders in that area and also noisy activities of motorist passing by [13]. This noise stress can reduce their testosterone and progesterone levels which can affect their sexual and reproductive life [20]. This is in agreement to a work done by Smaila and Odusote [28] on the serum testosterone level on workers in work place in which she discovered that noise exposure was associated with significantly lower total and free testosterone in active workers between 16years to 85years [24], however more severe in workers aged ≥ 37 years [27]. This decrease in testosterone in male animal models is due to the effects of the high noise level on the hypothalamic-pituitary-testicular axis [23]. Also, the chemical toxicants that are inhaled from generator set also contribute to so many health challenges as they are very poisonous for the health [21].

Materials and Methods

List of chemicals/reagents used

Reagents/Chemicals	Manufacturer
Buffer	Randox Kit, UK
Phosphate buffer	Randox Kit, UK
α – oxoglutarate	Randox Kit, UK
2, 4-dintrophenyl hydrazine	Randox Kit, UK
Carbonate buffer Ph	Randox Kit, UK
Xanthine oxide 0.3µ/ml	Randox Kit, UK
Sodium hydroxide solution	Randox Kit, UK
2-Amino,2-methly-1-propanol pH 11(7.9 M)	Randox Kit, UK
Na_2PO_4 (80 nM)	Randox Kit, UK
ethylene diamine tetra acetate (EDTA)	Drug House (BDH) Ltd
Tris-HCl	Drug House (BDH) Ltd

Table 1: List of chemicals and reagents and their manufacturers.

All the chemicals used in this study were of analytical grades and products.

Experimental animals

Fifty (50) albino rats of the Wistar strain aged 10-12 weeks and weighing 70 -100g bred in the animal house of the Department of Zoology and Environmental Biology (ZEB), University of Nigeria, Nsukka Enugu State were used for this study. The animals were transported in aluminum cages to Michael Okpara University of Agriculture Umudike. All animals were housed at controlled room temperature of about 27-30°C with a photoperiod of 12-hour light and 12-hour dark per day. The animals were fed Vital Growers Mash and water ad libitum and allowed to acclimatize to their environment for 7 days before experimentation.

Source of the albino rats

The animals were bought from Dr. Samuel of Veterinary Medicine Department of the University of Nigeria Nsukka and were transported to Michael Okpara University of Agriculture, Umudike in aluminum cages where the experiment was carried out.

Toxicological study design

The fifty (50) albino rats of the Wistar strain aged 10 - 12 weeks and weighing 70 -100 g bred were all fed with Vital growers' mash and water, then were randomly grouped into 3main groups, which consist of a control group, group 1 and group 2. Group 1 (for high sound level of 85db - 105db) and group 2 (low sound level of 40db - 55db) were sub divided into subgroups with five albino rats and exposed to way off fume from electrical generator sounds at different noise levels for 8 hours each day for 28days as follows.

Control group: kept away from generator set at noise level less than 30 decibels

- Group 1a: Exposed to noise level of 85 decibels
- Group 1b: Exposed to noise level of 95 decibels
- Group 1c: Exposed to noise level of 105 decibels
- Group 1d: Exposed to noise level of above 105 decibels
- Group 2a: Exposed to noise level of 55 decibels
- Group 2b: Exposed to noise level of 50 decibels
- Group 2c: Exposed to noise level of 45 decibels
- Group 2d: Exposed to noise level of 40 decibels
- Group 2e: Exposed to noise level of below 40 decibels

They were anaesthetized with chloroform, sacrificed by cervical dislocation and blood samples collected through cardiac puncture

using 2ml syringes. Blood samples for biochemical assays were collected in plain tubes and allowed to clot before centrifugation and the sera were separated thereafter and used for the assays.

Determination of serum progesterone concentration

This measurement procedure describes the measurement of total progesterone (free and protein bound progesterone) in human serum using isotope dilution high performance liquid chromatography tandem mass spectrometry (ID-HPLC/MS/MS).

- **Principle:** The ISO/IUPAC definition of the quantity measured with this method is 'total progesterone', the measurement is 'serum total progesterone; amount of substance concentration equal to x nmol/L'. To facilitate the clinical use of these measurements, results are converted into ng/dL. The three principal steps in this measurement procedure are: Dissociation of the analyte from binding proteins, extraction of the analyte form the sample matrix and quantitation of the analyte by isotope dilution high performance liquid chromatography tandem mass spectrometry (ID-HPLC/MS/MS) using stable isotope labeled internal standards and calibrators.
- **Procedure:** Isolation of the analyte is achieved using liquid-liquid extraction. ID-HPLC/MS/MS is performed with a triple quadrupole mass spectrometer using electrospray ionization in positive ion mode. Progesterone is identified based on the agreement to a work done by Smaila and Odusote [28] on the serum testosterone level on workers in work place in which she discovered that noise exposure was associated with significantly lower total and free testosterone in active workers between 16years to 85years, however more severe in workers aged ≥ 37 years [28]. This decrease in testosterone in male animal models is due to the effects of the high noise level on the hypothalamic-pituitary-testicular axis. retention time and on specific mass to charge ratio transitions using selected reaction monitoring (SRM). A 13C isotope-labeled progesterone is used as an internal standard.

The analysis of total progesterone is done in the following steps;

- Preparation of samples solution.
- Dissociation of progesterone from binding proteins
- Isolation of lipids fraction from samples using liquid-liquid extraction
- Removal of phospholipids and other polar lipids from lipid fraction using liquid- liquid extraction
- Analysis of total progesterone by ID-HPLC/MS/MS
- Data processing and result calculations

Determination of the concentration of serum testosterone

This measurement procedure describes the measurement of total testosterone (free and protein bound testosterone) in human serum using isotope dilution high performance liquid chromatography tandem mass spectrometry (ID-HPLC/MS/MS).

- **Principle:** The ISO/IUPAC definition of the quantity measured with this method is 'total testosterone', the measurement is 'serum total testosterone; amount of substance concentration equal to x nmol/L'. To facilitate the clinical use of these measurements, results are converted into ng/dL. The three principle steps in this measurement procedure are: Dissociation of the analyte from binding proteins, extraction of the analyte form the sample matrix and quantitation of the analyte by isotope dilution high performance liquid chromatography tandem mass spectrometry (ID-HPLC/MS/MS) using stable isotope labeled internal standards and calibrators.
- **Procedure:** Isolation of the analyte is achieved using liquid-liquid extraction. ID-HPLC/MS/MS is performed with a triple quadrupole mass spectrometer using electrospray ionization in positive ion mode. Testosterone is identified based on chromatographic retention time and on specific mass to charge ratio transitions using selected reaction monitoring (SRM). A 13C isotope-labeled testosterone is used as an internal standard.

The analysis of total testosterone is done in the following steps

- Preparation of samples solution.
- Dissociation of testosterone from binding proteins
- Isolation of lipids fraction from samples using liquid-liquid extraction
- Removal of phospholipids and other polar lipids from lipid fraction using liquid- liquid extraction
- Analysis of total testosterone by ID-HPLC/MS/MS
- Data processing and result calculations.

Determination of heavy metal bioaccumulation

Bioaccumulation of heavy metals in the albino wister rats exposed to fumes from the electrical generator sets was determined as described by Oparaji., *et al*. 2017 using mass spectrometry.

- **Principle:** This method directly measures the Cd, Mn, Hg, Pb, and Se content of whole blood specimens using mass spectrometry after a simple dilution sample preparation step. During the sample dilution step, a small volume of whole blood is extracted from a larger whole blood patient specimen after the entire specimen is mixed to create a uniform distribution of cellular components. This mixing step is important because some metals (e.g., Pb) are known to be associated mostly with the red blood cells in the specimen and a uniform distribution of this cellular material must be produced before a small volume extracted from the larger specimen will accurately reflect the average metal concentration of all fractions of the larger specimen. Clotted samples are not analyzed by this method due to the inhomogeneity factor.
- **Procedure:** Dilution of the blood in the sample preparation step prior to analysis is a simple dilution of 1 part sample + 1 part water + 48 parts diluent. The effects of the chemicals in the diluent are to release metals bound to red blood cells making them available for ionization, reduce ionization suppression by the biological matrix, prevent clogging of the sample introduction system pathways by undissolved biological solids, and allow introduction of internal standards to be utilized in the analysis step. Tetramethyl ammonium hydroxide (TMAH, 0.4% v/v) and Triton X-100 (0.05%) in the sample diluent solubilizes blood components. Triton X-100 also helps prevent biological deposits on internal surfaces of the instrument's sample introduction system and reduce collection of air bubbles in sample transport tubing. Ammonium pyrrolidine dithiocarbamate (APDC) in the sample diluent (0.01%) aids in solubilizing metals released from the biological matrix. Ethyl alcohol in the sample diluent (1%) aids solubility of blood components and aids in aerosol generation by reduction of the surface tension of the solution. Mass spectrometric analysis is done to determine the various metal constituents.

Data analysis and statistical procedures

Statistical analysis of the data was carried out with SPSS version 22.0 using One Way Analysis of Variance (ANOVA). The statistically analyzed data were reported as Mean ± SD. Significant difference was accepted at 95% confidence level of probability i.e. if $P < 0.05$.

Results

Effect of Induced Noise Stress on Progesterone Concentration (pg/ml) of Wistar Rats

The result in figure indicates a significant ($p < 0.05$) increase in the progesterone concentration of group 2 exposed to low noise level (between 55 db – 40 db) when compared with the control group and group 1, while group1 exposed to high noise level (between 85 db - 105 db) showed a significant ($p < 0.05$) decrease when compared with the control group and group 2.

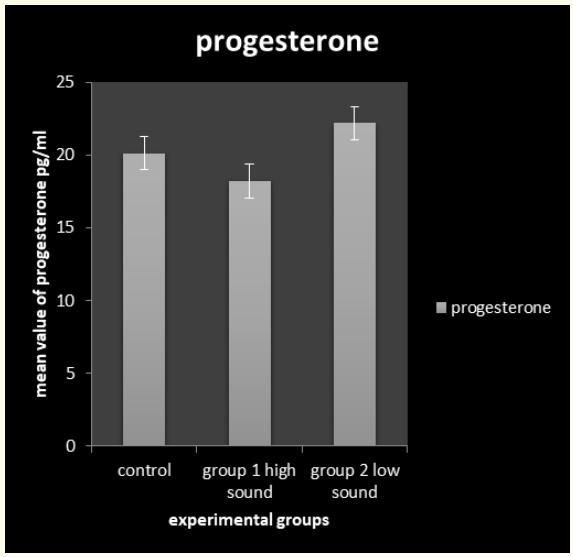

Figure 1: Group mean value of progesterone concentration for animal group 1 and group 2.
Control = 20.13
Group 1 high = 18.21
Group 2 low = 22.19.

Effect of induced noise stress on testosterone concentration (ng/ml) of Wistar rats

The result in figure indicates a significant ($p < 0.05$) decrease in the testosterone concentration of group 2 exposed to low noise level (between 55 db – 40 db) and group 1 exposed to high noise level (between 85 db - 105 db) when compared with the control group.

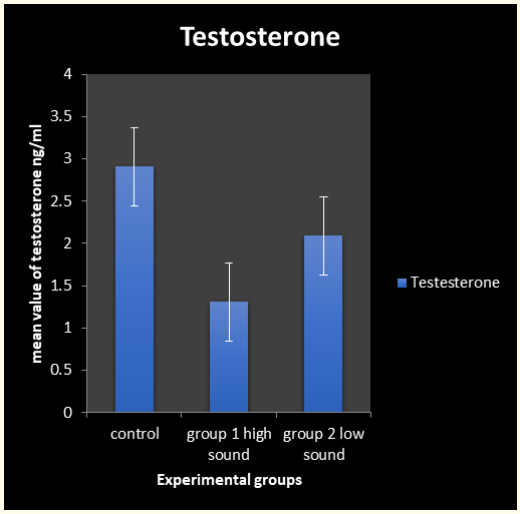

Figure 2: Group mean value of testosterone concentration for animal group 1 and group 2.
Control = 2.91
Group 1 high = 1.31
Group 2 low = 2.09.

	Progesterone	Testosterone
CONTROL	20.12 ± 0.18^{c}	0.82 ± 0.78^{a}
Group1a	22.6 ± 0.62^{cd}	0.30 ± 0.014^{a}
Group1b	20.9 ± 0.05^{b}	0.31 ± 0.035^{a}
Group1c	18.7 ± 0.45^{a}	0.63 ± 0.064^{a}
Group1d	20.9 ± 0.03^{bc}	0.82 ± 0.014^{a}
Group2a	23.4 ± 0.01^{e}	3.38 ± 0.021^{b}
Group2b	23.4 ± 0.14^{e}	3.57 ± 0.156^{b}
Group2c	22.2 ± 0.02^{c}	1.54 ± 1.760^{a}
Group2d	23.1 ± 0.11^{d}	0.98 ± 0.064^{a}
Group2e	21.4 ± 0.29	0.78 ± 0.064

Table 1: Effect of noise induced stress on testosterone, progesterone and interleukin concentration of Wistar rats at week 4.

Groups with different subscripts are significant ($p < 0.05$) when compared with the control

Group 0: Control

Group 1a: Noise exposure to wistar rat at 85 decibels

Group 1b: Noise exposure to wistar rat at 95 decibels

Group 1c: Noise exposure to wistar rat at 105 decibels

Group 1d: Noise exposure to wistar rat at above 105 decibels

Group 2a: Noise exposure to wistar rat at 55 decibels

Group 2b: Noise exposure to wistar rat at 50 decibels

Group 2c: Noise exposure to wistar rat at 45 decibels

Group 2d: Noise exposure to wistar rat at 40 decibels

Group 2e: Noise exposure to wistar rat at below 40 decibels.

Discussion

In this study the effects of generator noise pollution from a running noisy generator set on testosterone and progesterone levels were evaluated using wistar albino rats kept at different positions which is dependent on the measured sound level. The effects of noise pollution at these different sound pressure levels were considered and compared to the control group. This was achieved in two different groups of the animal exposure, in order to know the effects of our parameters at this distinguished sound pressure levels in group one and group two. Noise exposure higher than 90 dB is considered a source of stress.

There were significant ($p < 0.05$) decreases in the testosterone and progesterone levels of rats in group one which include the wistar rats exposed to high noise level (between 85 db -105 db) when compared with rats in the control group (group 0). This is in agreement to a work done by Smaila and Odusote [28] on the serum testosterone level on workers in work place in which she discovered that noise exposure was associated with significantly lower total and free testosterone in active workers between 16years to 85years, however more severe in workers aged ≥ 37 years. This decrease in testosterone in male animal models is due to the effects of the high noise level on the hypothalamic-pituitary-testicular axis. The hormones of the rats in group two which include the Wistar rats exposed to low noise level (between 55 db – 40 db) did not decrease as much as those in group one. However, their levels were

significantly ($p < 0.05$) lesser compared to those of the control group rats. This also shows that noise level at that sound pressure is not very strong to affect the normal serum testosterone or progesterone level.

Heavy metals cause severe health abnormalities such as nephrotoxicity, neurotoxicity, cancer [26]. Heavy metals have been proven to be toxic at a very low concentration upon ingestion with obvious deficiency symptoms. This is because it cannot be broken down [14,18,25]. The result of the exposure of the Wistar rats to the fumes from a running generator set shows the presence of so many heavy metals which include both the nutritionally important heavy metals like iron, zinc and copper which were very significant, and non-nutritional heavy metal which are toxic to the body system such as lead, mercury, chromium, cadmium and arsenic. The concentration of mercury detected was below detectable limit while the other poisonous heavy metals were very significant. It is good to note that heavy metals are very poisonous even at a very low concentration [27,30]. The study reveals that the bioaccumulation of heavy metals also adds to the immune dysfunction and damages caused by high noise pollution seen in work place.

Conclusion

This current study suggested that chronic noise exposure from a running generator set could adversely affect the testosterone and progesterone levels in the Wistar rats. Chronic noise exposure can reduce the testosterone and progesterone levels. Also, bioaccumulation of heavy metals can be toxic at even low concentration since they are not broken down, and can cause alterations in many important biosynthetic pathways by substituting important elements in the pathway. Therefore, individuals who are closely exposed to noisy generator set during their work period may be victims of this health hazards. The findings of this study have proved the importance of sensitizing the general public on the adverse health implications of chronic exposure to noise, especially, to people who run generator sets very close to their working desk or living rooms, which endangers them more to this health hazards. Owing to these findings, health policy makers should sensitize the general public through health awareness programmed on the health consequences of their exposure to noisy generator set and also make recommendations on safety measures they should adopt to protect themselves from these exposures during work and in their living houses.

Further studies are required to assess the health consequences of individuals exposed to other sources of stress during work which will give a general guide to the stressors that cumulatively cause the health hazards.

Key Findings

After the experiment was carefully carried out, about 80-92% of the Lab Rats responded negatively to the condition they were subjected to. The noise from the fuming generator had a negative impact to the reproductive hormones (Testosterone and Progesterone) of both the male and female albino rats as they were all reduced.

Toxicity was observed even at low concentration as a result of the unscathed bioaccumulated heavy metals.

In general, being exposed to chronic noise or an overly noisy environment can have severe effect of the sexual and reproductive hormones of individuals.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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