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Effects of Petroleum-based Particulate Air Pollutants on Lipid Profiles of Adult Wister Rats in PortHarcourt Metropolis, Nigeria

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Abstract
This work centred on the toxicological impact of particulate emissions from the combustion of crude oil on vital organs as lungs, heart, kidneys and liver of male Wister rats with a view to establishing the possible pathological effects of inhalation of petroleum-based particulates. This work assessed lipid profiles as bases for establishing the effects on the rats. In this study, TC was found to have bio-concentrated more than TG, HDLs and LDLs into vital organs. High level of TC in this study is an indication of a heart predisposed to cardiac issues like ischemic heart attack, stroke and other cardiovascular diseases. Elevated values shown in this study over prolonged exposure for TC was evident of ill health or disease condition, too. Lower levels of HDLs and a dramatic increase in LDLs as noticed on the twenty eight day of exposure also meant predisposition to diseased condition or a deficiency. This study has therefore demonstrated that prolonged exposure to petroleum-based particulate pollutants in the study area could predispose rats to increased chances of stroke and heart attack, most especially, diabetics.

Key Words: Effects, Toxicological Assessment, Petroleum-based Particulates, Air Pollutants.

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I. Introduction

Particulate matter is a composite combination of suspended particles of movable origin, composition, dimension, form and density with diverse health and environmental significance to living systems. Particulate matter is an air pollutant comprising a complex range of chemically and physically diverse substances that exist in any local atmosphere in a combination of solid and liquid particles suspended in the air (WHO, 2006). These suspended particles differ in size, configuration and source (Ndubuisi, 2017). Particles are often categorized by their aerodynamic properties for the reason that: (a) these properties determine the conveyance, transformation and removal of particles from the ambient air; (b) they also determine their admission within the respiratory system and (c) they are associated with the chemical composition and sources of particles.

According to DEQO (2006), natural forms of particulate matter include pollen, sea salt, wind-blown dust from arid areas, volcanic dust, and products of combustion from wildfires. Anthropogenic sources, on the other hand, include but not limited to motor vehicles; utility boilers; industrial boilers; dust from paved and unpaved roads; agricultural, construction, and mining activities; prescribed fires and other forms of open burning; as well as fugitive emissions from industry. Particulate matter is directly emitted into the air by processes such as combustion, incineration, construction, mining, metal smelting, metal processing, and grinding (Atiku *et al.*, 2016). Particles formed in the atmosphere by condensation or the transformation of emitted gases such as SO_2 and VOCs are also considered particulate matters (Merkisz & Pielecha, 2015).

According to Tawari and Abowei (2012), the Niger Delta is Nigeria's most endowed region in terms of oil mineral reserves and one of the most industrialized, after Lagos. The consequence is that despite enormous potential for economic development and sustainable growth, Niger Delta is fueled with environmental pollution on land, water and air (Ukpeh, 2010). The activities of these industries, especially the upstream and downstream petroleum sectors as well as other related happenings emits substantial amounts of substances like volatile organics, oxides of carbon, nitrogen, sulphur, particulate matter, heavy metals and other toxic substances at levels that most times exceed both the national and international guidelines. Ede and Edokpa (2015) have noted that the Niger Delta area is engulfed with pollutant concentrations in the atmosphere due to the increasing human activities which is directly or indirectly connected to oil and gas exploration. Within the Niger Delta communities, there are diverse sources of air pollution. A closer look at the Niger delta area shows that common air pollution sources are usually biomass combustion, bush burning, automobile emissions, generator emissions,

pipeline explosions, industrial emissions, gas flaring, and the recent soot suspected to be a by-product of artisanal refineries is not left out in this classification.

Biomass burning is a significant emission source of particulate matter with an aerodynamic diameter less than 2.5µm (Zhang *et al*. 2013). According to Tawari and Abowei (2012), this pattern of combustion is related to the usage of firewood, coal, bamboo etc. These are of course, common sources for cooking in Niger Delta's rural areas. Enormous amounts of automobiles traverse the Niger Delta roads and each one is a potential source of particulate emission.Major events linked with oil and gas exploration include gas flaring, pipeline explosions, fugitive emissions, soot emissions etc. All these emission sources contribute to the concentrations of pollutants especially particulates in the lower atmosphere (Ede & Edokpa, 2015). The dumping of waste has been a foremost non-eco-friendly issue in the Niger Delta area particularly in the urban areas. According to Tawari and Abowei (2012), the combination of waste from several sources, when burnt, intensifies the likelihoods of accumulating particulates into the habited atmospheric environment. Other sources of particulate matter in the Niger Delta are use of gasoline generator (Stanley *et al.*, 2010) and bush fires (Wardoyoa *et al.*, 2007). Since the first oil well was struck in 1956 in Oloibiri community, present day Bayelsa State, oil exploratory activities have always had its attendant environmental impacts. These had presented itself in major ways as land, water and air pollution, respectively (Amukali, 2019). According to Onwuka (2006), this has of course redefined the chemistry of the atmosphere.

Exposure to fine particulates has been known to be the cause of increase in deaths and some disease conditions (Mohapatra & Biswal, (2014); NCAA, 2011). For instance, The World Health Organization (WHO) made a declaration in 2012 that 1 out of every 8 persons dies as a result of air pollution related diseases. Recently, a study, named, Global Burden of Disease (GBD) ranked $PM_{2.5}$ exposure to be the ninth leading cause of death in Korea. In the study, 17,224 deaths out of the total deaths within the research period of 2013 was attributed to $PM_{2.5}$. The causes of deaths included; ischemic stroke, hemorrhagic stroke, tracheal, bronchial, and lung cancer; and ischemic heart disease, all of which were caused by ambient particulate air pollution, and ischemic stroke was the highest cause of death (Jong *et al.*, 2018). Similar data have not been obtained for Nigeria, even though there are a few researches on disease patterns in Port Harcourt of particulate concentrations in some busy road junctions as well as spatial and seasonal concentrations of particulates and their health implications (Ede & Pere, 2013; Ede & Edopka, 2017; Happy *et al*., 2018). More studies still needs to be done because sequel to the aforementioned, the exact extent of the damages this soot could cause have not been quantified, thus the need for a study of this kind. This study was therefore designed to assess the toxicological effects of inhalation exposure of petroleum-based particulates on the lipid profiles of various functional organs of Wister rats (specifically the lungs, kidneys, heart, liver, pancreas and blood) and by extension humans.

II. Study Area

In the city of Port Harcourt, particulate air pollution has assuredly taken another dimension with the presence of soot in the ambient environment for some time now. Houses, cars, clothing, e.t.c. are now coated with fine layers of particulate, perhaps of petroleum origin (Ede & Edopka, 2017). In addition, Ede and Pere (2013) earlier stated that the level of particulate air pollution in the city of PortHarcourt was way beyond both WHO and local standards. According to them, Total Suspended Particulate (TSP) values of about 360ug/m^3 was recorded as against the then annual standard of between 150 - 230ug/m^3 and 60 - 90ug/m^3 24 hour standards. This becomes worrisome considering the fact that particulate air pollution is associated with premature deaths in people with pre-existing heart or lung diseases, heart attacks, irregular heartbeat (cardiac arrhythmia), decreased lung function and respiratory problems such as, emphysema, asthma etc.

III. Materials and Methods

3.1. Research Design

True experimental design employed in this experiment was complete randomized method since there was absolute control over the treatment group. The adult Wister rats were used for this study because the genetic, biological and behavioral characteristics of rats closely resemble humans and many ailments of humans can be induced in rats for experimental purposes (Allexandro, 2011). In addition adult Wister rats were used as opposed to younger ones since adult rats are fully developed and any change observed in the course of the experiment could correctly be attributable to the treatment rather than a developmental change (Chan *et al*., 2011).

3.2 LC_{50} Determination

LC_{50} refers to the lethal concentration of material in air, which causes the death of 50% of a group of animals in an experiment. Determination of LC_{50} is a sure way of ascertaining the poisoning potential (acute toxicity) of a substance being considered in an experimental procedure (CCOHS, 1997).In this study, a pilot

study was conducted for ascertaining the safe dose that was introduced into the air which didn't kill the experimental animals. To achieve this, the research entailed using minimum number of animals (Festing & Altman 2002) at the onset. Thus, Six (6) rats were initially used. A hand held particulate monitor was used to measure the particulate concentration in the environment that the rats were housed. The crude was burnt for a period of Six (6) hours. Measurements of the temperature, humidity, particulate concentrations of PM_{10} and $PM_{2.5}$ were taken on an hourly basis for the Six (6) hours. This was done from 12.08 mid-day to 6.08pm each time and these were accurately documented.

3.3 Laboratory Protocol: Excise of Internal Organs

Detailed study of the internal organs was done following the method adopted by Allexandro (2011). Before surgery, animals were made to undergo general anesthesia induced by inhalation of chloroform which is applied to a piece of cotton wool and put in a closed desiccator containing the rat to be used. After reaching plane of anesthesia with no response to pain stimulus applied on the adipose pad of animals paw and absence of corneal reflex, the rats were positioned horizontally in dorsal decubitus with all paws held in place with the aid of board pins. The animals were then shaved on the anterior walls of their abdomen with new blades and the exposed areas were swapped with antiseptic of a solution of alcohol and 2% iodine, to prevent compromising the integrity of specimens (Kelly & Fussell, 2012).

With the aid of a scalpel, an incision of about 4cm was made using a Metzembaum scissors, and laparotomy was then considered completed. Next, an Adisonutostatic retractor was placed to expose the peritoneal cavity and inventory was then carried out on all the organs. The organs of interest were carefully removed and individual vital organs such as the heart, lungs, liver, kidneys and pancrease were kept aside for further analysis (Allexandro, 2011). The organs so excised were kept separately to undergo fixation in 10% formaldehyde solution for 24hrs and the rest of the parts not needed were placed in plastic bags and properly disposed-off, following the method described by Ibid (2011). After fixation, the specimens were rinsed in water and completely immersed in a 70% alcohol solution.

In this experiment, adult Wister rats were used, and the mode of administration of the petroleum-based particulate pollutants was by inhalation. This required care and thorough planning, to ensure that the treatment was administered in a manner that it got to its target destination and the dose administered is such that it did not unduly cause adverse experimental effects to the Wister rats (Allexandro, 2011).

The Adult Wister rats were placed in two groups; treatment group and control group. All rats in both groups were kept under similar meteorological conditions i.e. temperature, pressure, humidity, sunlight etc. Water and food were provided for the rats during the day only, so as to avoid contamination with the petroleum particulate and also to direct the treatment only to the target organs which are the lungs, kidneys, heart and liver. All procedures of the experiment were done abiding by the Rules and Guidelines of the Ethics Committee of the Rivers State University with a view to ensuring good standard of experiment and compliance with international best practices. Two way Analysis of Variance (ANOVA) was employed to test for significance for data owing to data generated.

IV. Results and Discussions

Figure 1 below showed the somewhat complicated nature of relationship between time and PM10, PM2.5, humidity and temperature respectively. It was clearly seen that with passage of time, exposure of the rats to petroleum-based particulate pollutants did not show much significant difference between values of PM10 and PM2.5, even as both showed a parabolic relationship with time passage (Figure 2). Passage of time upon exposure of the rats to the petroleum-based particulate pollutants did not also significantly affect humidity and temperature (Figure 3).

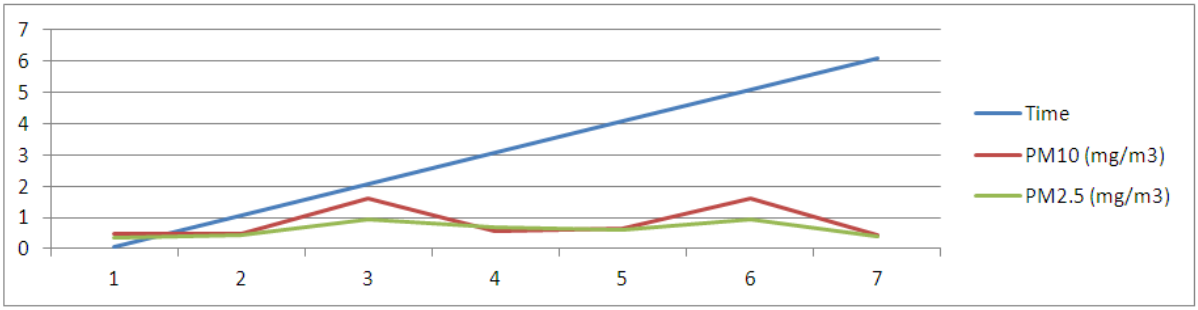

Figure 1: Concentrations of $PM_{2.5}$ PM_{10} and Metrological Parameters of the Rat.
Source: Stephen (2021)

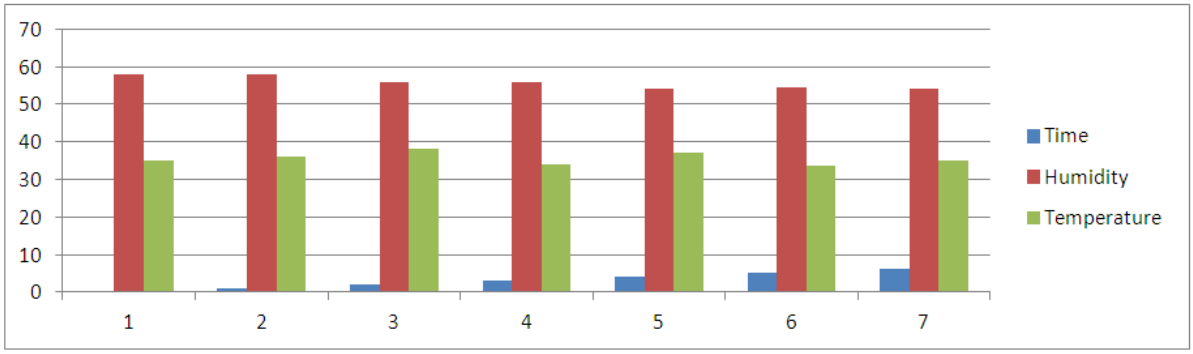

Figure 2: Time versus PM10 and PM2.5
Source: Stephen (2021)

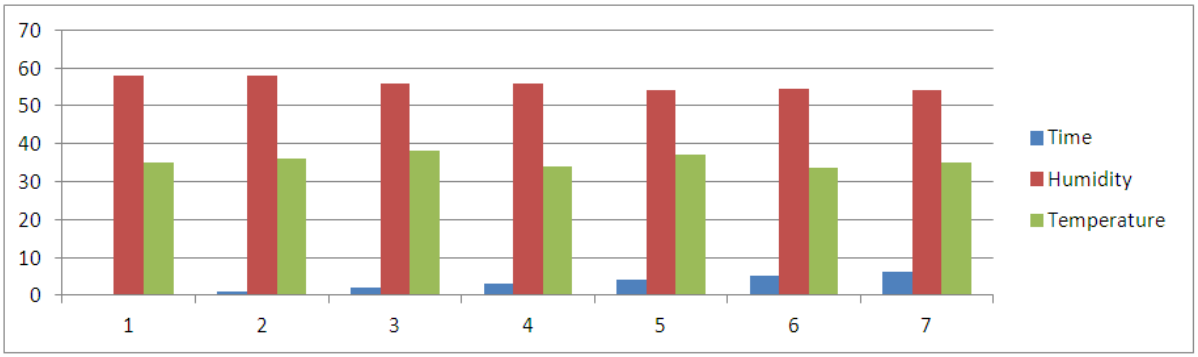

Figure 3: Time versus Humidity and Temperature.
Source: Stephen (2021)

The principal toxicologicalparameter that was used to determine the possible effects of exposure to petroleum-based particulate pollutants generated from crude oil in this very study was lipid profile data generated from vital organs as liver, kidneys, gall blader and pancrease, respectively. Parameters as Total glycerol (TG), Total cholesterol (TC), High density lipids (HDL) and low density lipids (LDL) were specifically evaluated in this study. From Figure 4, the concentrations of the lipid profile between the experimental and the control group over 28 days exposure to petroleum-based particulate pollutants was shown below. From Figure 4.1 below, it was observed that exposure of the rats to petroleum-based particulate pollutants did affect the lipid content values in vital organs of the rats. For instance, Total Cholesterol was found to be 2.44mmol/L for the control group (on the first day), but reduced to 2.128mmol/L by the Seventh day of exposure. From there, TC consistently increased from 2.164mmol/L on the Fourteenth day of exposure, 2.332mmol/L on the Twenty First day of exposure and 2.92mmol/L on the Twenty Eight day of exposure respectively. Among all the lipid parameters, TC was observed to have had bio-concentrated more into vital organs than TG, HDL and LDL respectively. High level of total cholesterol is an indication of a heart predisposed to cardiac issues like ischemic heart attack, stroke and other cardiovascular diseases (Mahendran *et al*., 2013).

Values with different superscript are statistical significant at ($p< 0.05$). Superscript (a,b) compares 7 days treatment, 14 days treatment, 21 days treatment and 28 days treatment to control across the group

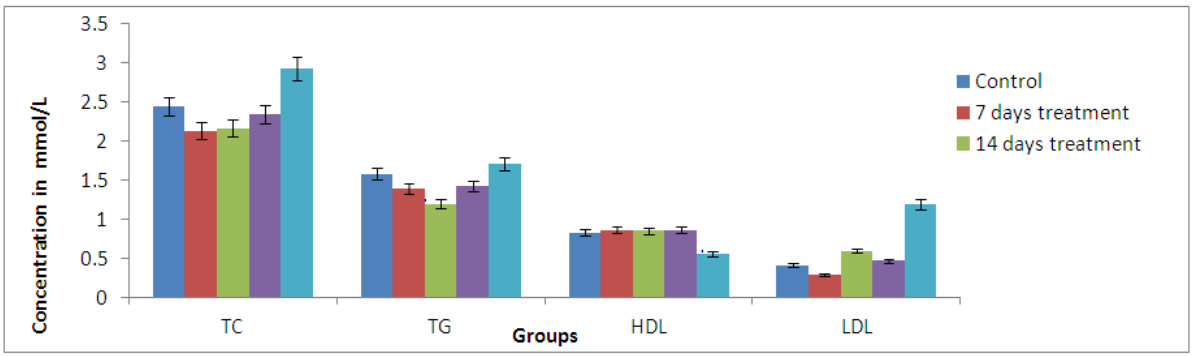

Figure 4: Showing concentrations of Lipid Profile (TC, TG, HDL, and LDL against Period of Exposure

For Total Glycerol (TG), it was observed that highest value occurred by the 28th day of exposure as compared to the least value which occurred on the 14th day of exposure. Among all of these, the fourteen days treatment appears to have the smallest concentrations. This phenomenal behavior could be owing to an initial negative impact of the petroleum-based particulate pollutants on the rats but after the 14th day, the rats lost characters that enabled them cope with the environmental hazard created by the particulate pollutants. Total glycerol has been established as a biological marker in the prediction of hyperglyceamia and type II diabetes (Mahendran *et al*., 2013) and so the elevated values shown here is evident of ill health or disease condition in the rats exposed for twenty eight days (28). It could also be stated that fewer days of exposure to petroleum-based particulate pollutants, like 0 to 14 days, showed a suppressive effect on disease conditions like hyperglycaemia and Type II diabetics

4.1 High Density Lipids

For the control group, the mean value of the concentration of HDLs was 0.8263mmol/L, the seventh day exposure was0.858 mmol/L, fourteenth day exposure was 0.858mmol/L while the twenty eight day exposure had a mean concentration of 0.555mmol/L. High density lipids was found to be lowest on the twenty eight day and this meant predisposition to diseased condition or a deficiency, since high density lipid is actually beneficial to the body. The concentrations of high density lipids for the various days did not vary much.

4.2 Low Density Lipids

The mean value of the concentration of low density lipids for the control group, seventh, fourteenth and twenty first days of exposure were all observed to be less than 1.000mmol/L while the twenty eight day had a mean value of 1.19 mmol/L after the exposure. The dramatic increase on the twenty eight day was predictably expected, since that is the highest period of exposure. Low density lipids are actually called bad cholesterol, and are known for collecting in the walls of the blood vessels, increasing the chances of heart condition like stroke or heart attack especially in diabetic patients (Barter, 2011). Thus, prolonged exposure to petroleum-based particulate pollutants in the area could predispose rats to increased chances of stroke and heart attack, especially in diabetics.

V. Conclusion

This work explored the toxicological impact of particulate emissions from the combustion of crude oil on the lungs, heart, kidneys and liver of male Wister rats with the aim of developing an understanding on the possible pathological impacts as a consequence of inhalation of such petroleum-based particulates by higher-order animals like man.

Among all the lipid parameters, Total Cholesterol was observed to have had bio-concentrated more into vital organs than TG, HDLs and LDLs respectively. High level of TC is an indication of a heart predisposed to cardiac issues like ischemic heart attack, stroke and other cardiovascular diseases, hence high values in thus case showed serious health concerns. Total Glycerol was observed to be highest by the 28th day of exposure as compared to the 14th day which recorded the least value. This phenomenal behavior could imply an initial negative impact of the petroleum-based pollutants on the rats but after the 14th day, the rats lost characters that enabled them cope with the environmental hazard created by the particulate pollutants. Thus, elevated values shown in this study was evident of ill health or disease condition in the rats exposed for 28 days and fewer days of exposure to petroleum-based particulate pollutants showed a suppressive effect on disease conditions like hyperglycaemia and Type II diabetics

High density lipids was observed to be lowest on the twenty eight day and this meant predisposition to diseased condition or a deficiency, since HDLs are actually beneficial to the body. This implied that relatively lower levels of HDLs on prolonged exposure to petroleum-based particulates meant suppression of beneficial HDLs, thus increased predisposition to diseases. A dramatic increase in LDLs was observed on the twenty eight day of prolonged exposure. These LDLs, called bad cholesterol, are known for collecting in the walls of the blood vessels and complicating chances of development of heart condition like stroke or heart attack especially in diabetic patients (Barter, 2011). This implied that prolonged exposure to petroleum-based particulate pollutants in the study area could predispose rats to increased chances of stroke and heart attack, most especially, diabetics.

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