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HAEMATOLOGICAL STUDIES AND HEAVY METAL CONCENTRATION OF THE HEART, LUNGS AND LIVER OF ALBINO RATS (*SPRAGUE DAWLEY*) EXPOSED TO VEHICULAR EXHAUST FUMES AND DUST ON MAJOR ROADS OF ABEOKUTA, NIGERIA

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ABSTRACT

The rapid increase of shops and small scale businesses along busy roads in Abeokuta metropolis is becoming alarming. This study was therefore aimed at investigating the effect of vehicular exhaust fumes on the blood biochemistry and heavy metal concentration in the lungs, heart and liver of Albino rats (*Sprague dawley*). Forty-five albino rats were randomly divided into three groups of 15 rats (three replicates each). The first two groups were placed along two major roads of Abeokuta (Oke-Ilewo and Ijemo) while the third (control) was placed at the Forest Nursery of the Federal University of Agriculture, Abeokuta for 12 weeks respectively. Haematological studies and blood biochemistry was conducted post-exposure using standard methods while the concentrations of some heavy metals in the organs of the rats was done using Atomic Absorption Spectrophotometer. Results revealed significantly higher ($P<0.05$) concentration of heavy metals in the lungs, heart and liver of Albino rats placed along the two major roads than those of the control. The concentrations of Zn, Pb and Co in the organs of the rats followed the trend Lungs > Heart > Liver. Also, heavy metal concentration in the heart and lungs of the rats followed the trend: Zn>Pb>Mn>Co. White Blood Cell and Haemoglobin were also observed to be significantly higher in the rats placed along the roads than those of the control. Continuous and prolonged exposure to vehicular exhaust fumes could therefore posse threat to the health and wellbeing of humans.

Keywords: Pollution, vehicular exhaust fumes, physiology, blood biochemistry, heavy metals

INTRODUCTION

In recent time, there has been increased number of people living in urban areas. According to United Nations (1998) human societies are rapidly becoming more urbanized. More than a third of the world population live in cities (Chirenje *et al*., 2003). With this increase in population coupled with urbanization, there is also the need for increase in transportation network. This trend has led to a substantial increase in air pollution caused by vehicular exhaust emissions due to addition of more and more vehicles on roads to meet the transportation demand of people in the cities (Na *et al*., 2002).

On the global sense, transportation is the major culprit of air pollution accounting for over 80% of total air pollutants (Abam and Unachukwu, 2009) which comes in the form of gaseous, solid and liquid emissions that arose from using fossil fuels as source of energy to power these engines (LAQM, 2005). Exposure to traffic emissions has been shown to cause a variety of acute and chronic health impact (Brook *et al*., 2004). For instance, numerous studies presented a link between respiratory problems in children and residents with proximity to vehicle traffic (Kim *et al*., 2004; Peel *et al*., 2005; Lin *et al*., 2009). Chronic exposure to traffic exhaust was associated with leukemia and other childhood cancers (Pearson *et al*., 2000). There is also an association between exposure to vehicular traffic and onset of a myocardial infraction within the subsequent one to three hour period (Peters *et al*., 2004). According to Pope et al (2002) vehicles contribute significantly to population exposure to particulate matter (PM), which has been associated with lung cancer and premature mortality.

In Nigeria, much attention is given on general industrial pollution and pollution in oil industries, with little reference on damage of pollution caused by mobile transportation sources of air pollution (Faboye, 1997). Over the years, there has been an increasing growth in commercial activities along the roads of major cities of Abeokuta which is the largest city and the state capital of Ogun State, Nigeria. Most of the business owners erect small shops and umbrellas beside the roads while others hawk their goods along these busy roads. To determine the effect of vehicular exhaust fumes inhaled by these people including the traffic control officers on daily basis, an experiment was setup along two major roads in Abeokuta using Albino rats (*Sprague dawley*) as model.

MATERIALS AND METHODS

Experimental area: Abeokuta is the largest city and the state capital of Ogun State, South-western Nigeria. It is situated on the east bank of Ogun River. From the 2006 National Census of the National Population Commission, the population of Abeokuta was estimated as 449,088 people.

Three sites were used for this experiment namely Ijemo road in front of Ogun State Fire Service Station (latitude $7^0$16'N and Longitude $3^0$35'E) with high traffic flow, Oke-Ilewo road in front of Ogun State Water Corporation (Latitude $7^0$14'N and Longitude $3^0$34'E) with low traffic flow and a control site at the Forest Nursery of the Federal University of Agriculture, Abeokuta (latitude $7^0$22'N and longitude $3^0$44'E) with no vehicular activities.

Experimental Animals: Forty five Albino rats of wistar strain (*Sprague dawley*) weighing between (100-120 g) were purchased from the College of Veterinary Medicine of the Federal University of Agriculture, Abeokuta. The animals were acclimatized for a week in the Forest Nursery of the University of Agriculture, Abeokuta where they had free access to food and clean water. All experiments were undertaken with the consent of the animal ethics in accordance with the guidelines set out by the committee of the University of Agriculture, Abeokuta Nigeria.

Experimental Design: Forty five albino rats were randomly divided into three groups each group contains 15 rats. Two groups were placed in close proximity to roads at Oke-Ilewo and Ijemo roads respectively in Abeokuta, Ogun State while the third group (Control) was kept in Forest Nursery of the University of Agriculture, Abeokuta far away from traffic movement

for 12 weeks respectively. Each of the rat groups was further divided into three replicates of five (5) rats each. These were placed at different points in each of the locations.

Dissection and Blood Collection: At the end of the experiment, the rats were sacrificed and blood samples were collected from each rat by cardiac puncture method. The lung, heart, liver and kidney of each rat were also collected.

Heavy metal analysis of the organs: Samples of the lungs, hearts and livers were digested with a mixture of concentrated hydrochloric acid and nitric acid (1:3 v/v) and cooled to room temperature. The concentrations of chromium, cobalt, iron, magnesium, lead and zinc were determined using an Atomic Absorption Spectrophotometer (AAS).

Haematological analysis: This was done according to the methods described by Adeyi et al (2012). The PCV was determined by using the microhaematocrit centrifuge. The total haemoglobin concentration (Hb) of the blood samples was estimated using the cyanomethaemoglobin method. The white blood cell count (WBC), Red blood cell count (RBC), Mean cell haemoglobin (MCH), mean cell volume (MCV), and the mean cell haemoglobin concentration (MCHC) were also determined.

Blood biochemical composition: The biochemical composition of the blood of the experimental rats was also determined according to the methods described by Adeyi *et al.* (2012). Flame photometry method was used to determine the concentration of sodium and potassium in the plasma of each rat while colorimeter method was used to determine the concentration of calcium in the blood of the animals. Blood glucose was determined using the strip of digital ACCU-CHEK advantage glucose meter (Roche diagnostic, Mannheim Germany). A drop of blood was obtained from tip of conscious rats and placed on the strip. The reading on the meter was noted and recorded as the blood glucose concentration. Concentration of total protein in the blood of each animal was determined by using the biuret method while total cholesterol and triglyceride were determined using the enzymatic (cholesterol oxidase) and (colorimeter) methods, respectively.

Statistical Analysis: Data obtained were subjected to Analysis of Variance (ANOVA) using the Statistical Package for Social Sciences (SPSS version 16.0). Means were separated using the Duncan's Multiple Range Test (DMRT). The results were presented as Mean ± Standard deviation. $P<0.05$ was regarded as statistically significant.

RESULTS AND DISCUSSION

Heavy metal concentration in the organs: Variation in the concentration of heavy metals in the lungs of Albino rats at different locations are presented in Table 1. From this study, Pb was not detected in the organs of the control rats. Similarly, the highest concentration of heavy metals in the lungs of Albino rats was found in rat at Ijemo road followed by Oke-Ilewo road and least at Forest Nursery of the University of Agriculture, Abeokuta. Similar trend were observed in the heart and liver of Albino rats. Ijemo road is a major road which linked the bulk part of Abeokuta with Sapon, a major commercial area of Abeokuta. Hence, more traffic flows through Ijemo road on daily basis than the Oke-Ilewo road. Elsom (1992) reported that a large percentage of pollutants from exhaust fumes are deposited by the road side and are widely dispersed by wind. Abulude *et al.* (2006) also reported higher concentration of heavy metals in the blood of cows which were close to busy roads. Therefore, higher concentration of heavy metals recorded in the organs of the rats placed along the roads could have resulted from exhaust fumes and dust released into the air from vehicles transiting the roads.

The concentrations of Zn, Pb and Co in the organs of the rats followed the trend Lungs>Heart>Liver (Table 1). The lung is an internal sac-shaped respiratory organ. During inhalation process, large volume of air pumped into the lungs gets to the alveoli where gaseous exchange takes place. Oxygen is picked up from the air through the pulmonary capillaries into the lungs (Miller and Harley, 1996). As reported by Babalola *et al*. (2005) the alimentary and respiratory tracts have been reported as the main portals of entry for heavy metals into the body. Therefore, higher concentrations of these metals as recorded in the lung and heart in this study could have resulted from the deposition of metal contaminated particles in the lung tissues during inhalation of vehicular exhaust fumes as well as deposition of the fumes on the animal feed.

Also, heavy metal concentration in the heart and lungs of the rats followed Zn > Pb > Mn > Co (Table 1). The observed higher concentrations of Zinc (Zn) and Lead (Pb) in the heart and lungs of the rats placed along the roads could be as a result of the use of leaded gasoline and high vehicular movement on the roads. Idrees (2009) reported higher concentrations of zinc and lead in the surface soils of busy roads than those of industrial and residential roads of Amman, Jordan. These metals are particularly dangerous because of their ability to bio-accumulate in the body tissues and organs (Luckey *et al*., 1975; Babalola *et al*., 2005). It is therefore expedient to empower the emission control agents as well as educating people whose business activities are proximate to major roads on the use of nose guards.

Haematological composition: The haematological parameters of Albino rats at different locations are presented in Table 2. The levels of White Blood Cell (WBC) and Haemoglobin (Hb) were observed to be significantly higher ($P < 0.05$) in the rats exposed to vehicular exhaust fumes from the roads than those of the control rats. These values were higher in rats placed along Ijemo road than those placed along Oke-Ilewo. Olson *et al*. (2000) earlier reported that changes in haematological system have higher predictive value for human toxicity when the data are translated from animal studies. The white blood cell (WBC) or the white corpuscles is responsible for the defence action of the blood. Etim *et al*. (2014) also identified that the white blood cell and its differentials are to fight infections, defend the body by phagocytosis against invasion by foreign organisms and to produce or at least transport and distribute antibodies in immune response. The significant increase ($P < 0.05$) observed in the white blood cell (WBC) count in the rats exposed to busy road than those of the control could have resulted from an altered physiological process by the vehicular exhaust fumes. This could be an indication that the immune system was stimulated to protect the rats against the effect of the vehicular exhaust on the physiology of the rats. However, no significant difference ($P > 0.05$) in the PCV and MCHC of the rats exposed to vehicular exhaust fumes along the roads and those of the control rats (Table 2). MCV and MCH were significantly lower in the rats

Table 1. Concentrations of heavy metals (mg/kg) in the lungs, heart and liver of Albino rats among the three locations in Abeokuta, Nigeria

	Heart				Lungs				Liver			
	Zn	Pb	Co	Mn	Zn	Pb	Co	Mn	**Zn**	**Pb**	**Co**	**Mn**
Control	125.20^{c}	Nil	8.23^{c}	24.03^{c}	172.90^{c}	Nil	18.03^{c}	25.40^{c}	87.87^{c}	Nil	4.37^{c}	5.77^{b}
Oke Ilewo	155.80^{b}	Nil	11.10^{b}	27.43^{b}	210.23^{b}	29.87^{b}	34.50^{b}	32.97^{b}	102.70^{b}	Nil	5.97^{b}	11.90^{b}
Ijemo	225.80^{a}	50.07^{a}	18.07^{a}	29.05^{a}	312.97^{a}	71.00^{a}	45.17^{a}	150.00^{a}	112.13^{a}	4.07^{a}	8.60^{a}	30.55^{a}

^{abc}Mean values in the same column having the same superscript are not significantly different ($p > 0.05$). Control = UNAAB Forest Nursery

Table 2: Haematological parameters of Albino rats at different locations in Abeokuta, Nigeria

Parameter	UNAAB Forestry	Oke-Ilewo Road	Ijemo Road
PCV (%)	28.67 ± 1.6^{a}	30.67 ± 1.0^{a}	32.67 ± 1.8^{a}
RBC x 10^{6}/mm^{3}	5.2 ± 0.2^{a}	4.6 ± 0.1^{b}	5.4 ± 0.1^{a}
WBC cm^{-3}	4533.3±389.6^{c}	9200.0± 1182.0^{b}	9816.0±1488.9^{a}
Hb x 10^{12}g/dl	4.58 ± 0.2^{c}	5.35 ± 0.1^{b}	11.02 ± 0.6^{a}
MCH g/dl	21.1 ± 0.2^{a}	19.5 ± 0.6^{b}	21.1 ± 0.3^{a}
MCHC pg	0.34 ± 0.0^{a}	0.34 ± 0.0^{a}	0.34 ± 0.0^{a}
MCV fl	6.27 ± 0.1^{a}	5.74 ± 0.2^{b}	6.26 ± 0.2^{a}

^{abc}Mean (± Standard deviation) values in the same column having the same superscript are not significantly different ($p > 0.05$). PCV - Packed Cell Volume, RBC - Red Blood Cell, WBC -White Blood Cell, Hb – Heamoglobin, MCH - Mean Cell Heamoglobin, MCHC - Mean Cell Heamoglobin Concentration, MCV - Mean Cell Volume

Table 3: Blood chemical parameters of Albino rats placed at the locations in Abeokuta, Nigeria

Locations	Na^{+} (mmol/L)	K^{+} (mmol/L)	Cl^{-} (mmol/L)	Ca^{2+} (mmol/L)	HCO_3^{-} (mmol/L)	Total Protein (g/L)	Glucose (mg/dl)	Total Cholesterol (mg/dl)	Triglyceride (mg/dl)
Oke Ilewo Road	149.0±2.7^{a}	6.3±0.7^{a}	104.0±5.1^{a}	9.9±0.1^{a}	23.3±1.8^{a}	63.6±2.8^{a}	72.5±6.7^{a}	135.9±16.7^{a}	95.3±14.8^{a}
Ijemo Road	145.3±2.4^{a}	4.2±0.7^{a}	89.1±7.7^{a}	9.2±0.3^{b}	25.0±1.7^{a}	53.0±3.8^{a}	78.1±8.9^{a}	111.2±14.8^{a}	67.2±18.8^{a}
Control	69.1±7.7^{b}	4.3±1.1^{a}	99.3±2.6^{a}	9.1±0.2^{b}	24.7±1.8^{a}	53.6±3.9^{a}	69.1±7.7^{a}	97.9±3.2^{a}	67.1±14.1^{a}

^{abc}Mean (± Standard deviation) values in the same column having the same superscript are not significantly different ($p > 0.05$).

exposed to Oke-Ilewo road while no significant difference exists in the MCV and MCH of rats placed along Ijemo road and the control. Red blood count (RBC) was significantly lower in rats placed along Oke-Ilewo roads while no significant difference exist between RBC values of rats placed along Ijemo road and the control.

Blood biochemical composition: Variation in the blood chemistry of Albino rats at different locations are presented in Table 3. There was no significant difference in concentration of K^{+}, Cl^{-}, HCO_3^{-}, Total Protein, Glucose, Total Cholesterol and Triglyceride of all the experimental rats at the different locations. However, Na^{+} concentration was observed to be significantly higher in the rats exposed to vehicular exhaust at Oke-Ilewo and Ijemo roads than the control. Also, Ca^{2+} was significantly higher in the blood of rats placed along Oke-Ilewo road but no significant difference exist between Ca^{2+} concentration in the rats placed along Ijemo road and the control.

CONCLUSION

This study has revealed that a frequent and prolonged exposure to vehicular exhaust fumes has the potential to damage vital organs such as the heart, lungs and liver and the health of human whose daily activities predispose them to vehicular exhaust fumes. Hence, there is the need to enforce the emission control awareness as well as educating road users and people who have their daily businesses along the road on how to prevent the inhalation of vehicular exhaust fumes, especially in Abeokuta, Ogun State, Nigeria.

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