Effects of Sub Chronic Exposure to Diesel Combustion Fumes on Hematological Parameters of Wister Rats

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ABSTRACT

Introduction: Several studies on the health effects of diesel exhaust from conventional road and non road sources have been documented but no record of any such work done on combustion fumes from a diesel lamp.

Aim: The present study was undertaken to evaluate the effects of exposure to diesel combustion fumes from an open lamp on the haematological parameters of Wister rats.

Method: Fifteen rats were weighed and divided into 3 groups, n = 5; control rats were unexposed to diesel combustion fumes, group A and group B rats had daily (4hrs) exposure to diesel combustion fumes in an enclosed chamber with open vents for 2 and 3 weeks respectively. At the end of which they were weighed again before their hematological parameters were assessed using standard laboratory methods.

Result: The results showed a statistically significant difference in mean blood level of bicarbonate ions of 20.0±1.47 mmol/L in the control group compared to 25.25± 0.85 mmol/L in rats exposed to fumes for 3 weeks. It also showed decreases in the mean packed cell volume, blood hemoglobin concentration, total white blood cell count (relative lymphocytosis). Increased erythrocyte sedimentation rate accompanied by increasing neutrophil counts in rats in groups A and B compared with their matched controls. These changes were however not statistically significant (all P> 0.05).

Conclusion: There was no significant impact of diesel exhaust fume from the lamp on most of the hematological parameters assessed in this study.

Key: Words: Effects, Sub-chronic exposure, Diesel, Combustion fume, Hematological parameters, Rats

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INTRODUCTION

The exploration and use of petroleum by human population has been associated with environmental (land, water and air) pollution. Gaseous fumes released from the industrial and domestic combustion of petroleum products have been reported to cause inimical health effects in human.

In the ambient environment, human exposure to diesel exhaust (DE) has been reported to come from both non-road and road engine exhaust. On road diesel engines

include vehicles while non road diesel engines include locomotives, marine vessels and heavy duty equipments[1]. Although no longer as common, diesel also serves as fuel in lamps that provide domestic lighting in certain poor regions of the world plagued with either no or unreliable electrical power supply. Diesel exhaust is a complex mixture of hundreds of constituents in either a gas or particle form. Gaseous components of DE have been reported to include carbon dioxide, oxygen, nitrogen, water vapor, carbon monoxide, nitrogen compounds, sulfur compounds and numerous low molecular weight hydrocarbons[1]

The particles present in DE (i.e diesel particulate matter [DPM]) are composed of a central core of elemental carbon and adsorbed organic compounds as well as small amounts of sulfate, nitrate, metals and other trace elements [1].

Available evidence suggests that human health hazards are associated with exposure to diesel exhaust. Hazards to health have been broadly subdivided into acute exposure related symptoms, chronic exposure non cancer effects and lung cancers. Acute irritation of eyes ,throat ,and bronchial tree ; neuro-physiological symptoms such as lightheadedness, nausea; respiratory symptoms like cough ,phlegm[2] and immunological effects like exacerbation of allergenic responses to known allergens and asthma like symptoms [3,4] have been reported as the health effects of short term exposure to diesel combustion fumes. Also results from extensive animal studies on diesel exhaust has been judged to constitute a chronic respiratory hazard to humans with dose dependent inflammation and histo-pathological changes in the lungs reported in several animal species including rats, mice, hamster, and monkeys[5-7] A considerable body of evidence also supports the association between DE exposure and increased cancer risk among workers in varied occupations where diesel engines historically have been used[8] as well as diesel particulate matter (DPM) carcinogenicity and associated DPM organic compounds extracts in rats and mice by non-inhalation routes of exposure [9-12].

While the properties and health effects of the conventional non-road and road diesel exhaust have been studied the effect of the combustion fumes from the use of diesel as fuel in domestic lamps have not been studied. Also there is relative paucity of information on the effects of diesel combustion fume on the hematological parameters of either humans or laboratory animals [9,10,13-16] and none of these considered the effects of the fumes from an open diesel lamp. In one of the above studies no changes in the heart mass or hematology was reported at any exhaust level in either rats or guinea pigs after 78weeks exposure[14].This study assesses the effects of diesel combustion fume from a lamp on the hematological parameters of Wistar rats.

METHODOLOGY

Animal Procurement and Husbandry: Fifteen male Wistar rats weighing between 180-230g were procured from the breeding colony of the College of Health Sciences, Delta State University, Abraka and housed in plastic cages in the animal facility of the Faculty of Basic Medical Sciences, Delta State University Abraka . They were acclimatized for 2 weeks before the experiment commenced. Throughout the experiment they were allowed free access to clean drinking water and standard rat feed (Vital Feeds Nigeria)

Animal Experiment: The fifteen rats were divided into three groups consisting of five per group. Group A (Control subject) had no exposure to diesel combustion fumes during

the study period. Groups B and C rats were exposed to (diesel combustion fume polluted air) in closed chambers for 4 hours everyday for two and three weeks respectively. The rats were weighed before and at the end of the experiment before they were sacrificed.

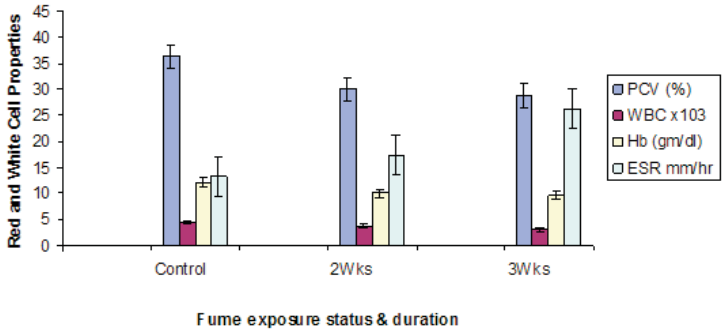
Exposure to diesel combustion fume: To expose the group B and C rats to the diesel combustion fume, they were transferred into two separate wooden boxes connected by rubber tubing to another metal container within which a burning lamp containing diesel was lit with flame. The fume from the diesel combustion was conveyed by the tube into the enclosed boxes where the rats were kept for 4hours everyday. Few holes were bored at the upper part of the enclosed box to allow some atmospheric oxygen. Group A rats also spent 4 hours everyday in the enclosed box but without a burning diesel lamp that emitted fumes into their inspired air. The rest part of each day of control and experimental rats were spent in their plastic cages.

Blood Collection: At the end of the exposure to diesel combustion fume the rats in each group were anaesthetized with chloroform, and 5 ml of blood was collected by cardiac puncture. 2.5ml of the blood collected was stored in lithium heparin bottles while the other half was stored in labeled EDTA bottles that were used for haematological studies. The animals were humanely killed by decapitation at the end of the procedure.

Specimen analyses: The blood samples stored in EDTA bottles were used to undertake the following studies: The packed cell volume (PCV) was determined by Hawksley micro-capillary centrifugation at 5.000 r.p.m for 10 minutes[17]; haemoglobin(Hb) concentration was determined by Sahli-haemoglobinometer method[17]; Erythrocyte sedimentation rate (ESR) was by Westergreen method[17]; WBC total count and platelets were done in neubeur counting chamber [17]; white blood cell differentials was carried out by Leishman Staining techniques[17]. The blood levels of the different electrolytes were done by spectrophotometeric methods using test kits (Cromatest, Spain) as specified in the Linear Chemical Manual of Cromatest reagent company .

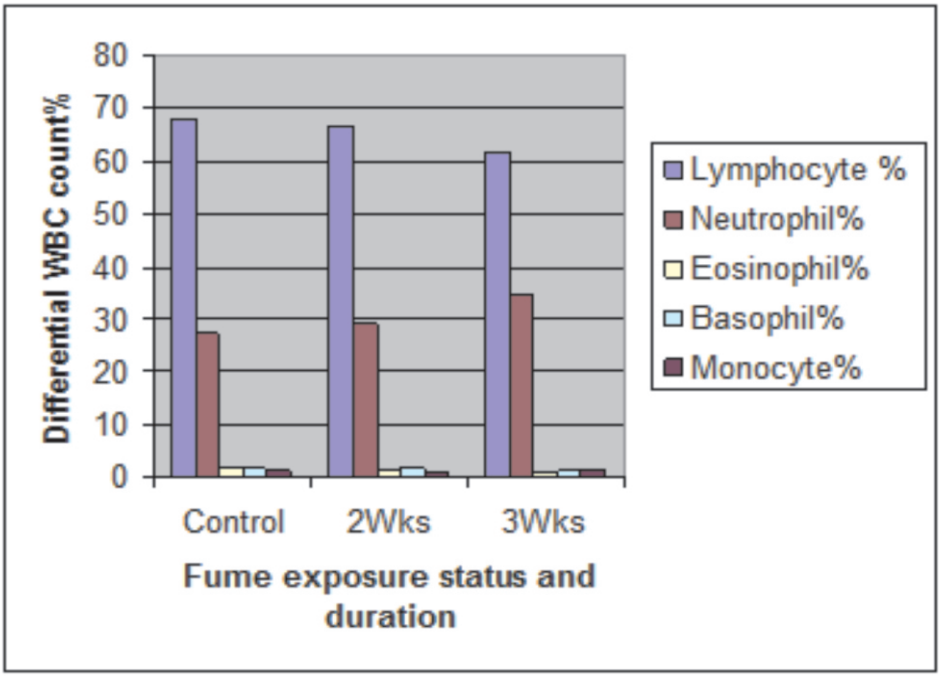
Statistical Analysis: The results expressed as Mean ± SEM. The Means of experimental and control groups were compared with each other using computerized software – Microsoft Excel 2003 by the Students t- Test assuming unequal variance and the single factor ANOVA test. P values less than 0.05 were considered to be statistically significant.

RESULTS



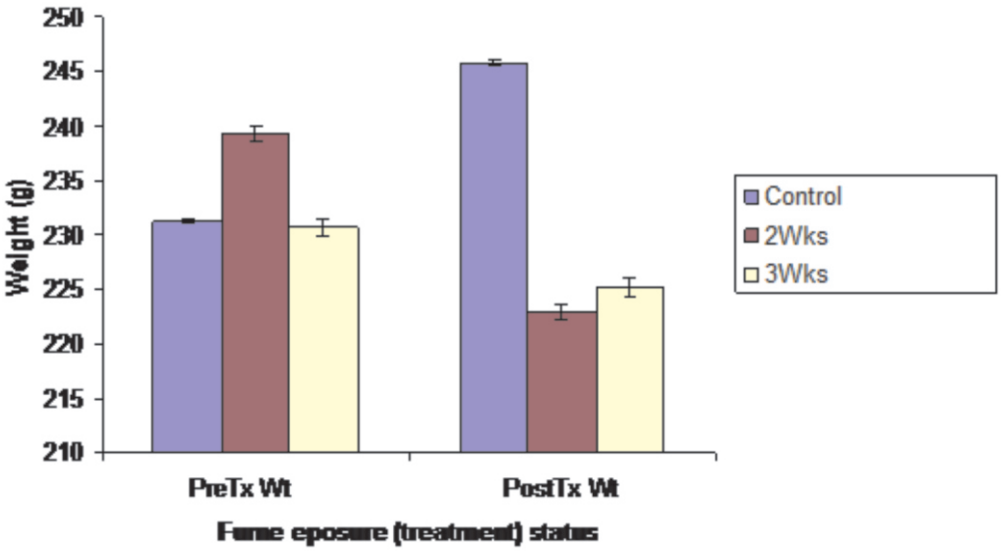
Total White Blood Cell Count (WBC)/ mm3, Blood Hemoglobin Concentration (Hb) and Erythrocyte Sedimentation Rate(ESR) of Wistar Rats. Mean ± SEM, n= 5, Control no fume exposure,2wks (daily fume exposure for 2weeks);3wks(daily fume exposure for 3weeks).

Figure 1: Effects of Diesel Combustion on the Packed Cell Volume (PCV)



Mean ±SEM, n=5,Control no fume exposure,2wks (daily fume exposure for 2weeks);3wks(daily fume exposure for 3weeks)

Figure 2: The Effects of Diesel Combustion on Differential White Blood Cell Count of Wister Rats



Mean ± SEM, n=5, Control no fume exposure, 2wks (daily fume exposure for 2weeks); 3wks(daily fume exposure for 3weeks)

Figure 3: The Effects of Diesel Combustion on the Body Weights of Wister Rats

Table 1: The Effects of Diesel Combustion Fumes on Blood Electrolytes,Urea and Creatinine of Rats

Parameters	Unexposed to fumes Control	Exposed to fumes daily 2Weeks	3Weeks
$[Na^+]$(mmol/L)	134 ±2.04	129.8± 1.93	137.8± 3.42
$[K^+]$(mmol/L)	3.80± 0.4	4.25± 0.11	4.05± 0.17
$[Cl^-]$(mmol/L)	116.25± 9.53	98.25± 1.70	101.5± 2.54
HCO_3 (mmol/L)	*20.0 ±1.47	*20.75± 1.43	*[a]25.25± 0.85
Urea (mg/dl)	42.3± 5.40	37.48± 0.03	37.5± 6.02
Creatinine(mg/dl)	3.17± 0.34	2.83± 0.40	2.67± 0.24

Mean ± SEM, n=5, Control no fume exposure,2wks (daily fume exposure for 2weeks);3wks(daily fume exposure for 3weeks)
Na+-Sodium ion,K+-Potassium ion,Cl-Chloride ion,HCO3-Bicarbonate.
*P< 0.05 (Single Factor Anova significance test:Control vs 2wks exposure vs 3wks exposure)
aP< 0.05 (Student t-Test Exposed vs Control).

DISCUSSION

The results of this study showed that sub-chronic exposure of Wister rats to diesel combustion fumes from a lamp caused a significant ($P < 0.05/0.01$) increase in blood level of bicarbonate,(Table1) compared to that of unexposed rats. This finding differs from the observation of reduced arterial blood bicarbonate level reported in diesel exhaust exposed mice and hamster[5] and unaffected arterial blood gases and standard bicarbonate reported in rats exposed to irradiated diesel exhaust [18]. The bicarbonate concentration was 20mmol/L, 20.75mmol/L and 25.25 for control, 2 weeks and 3 weeks diesel fume exposed rats respectively. ln other words, a steady increase in bicarbonate blood concentration was observed in rats exposed daily to diesel combustion fumes for 2 weeks and 3 weeks compared with control rats that were unexposed and this was statistically significant. A ready explanation for this observation may not be available. The method of blood sampling using the open cardiac puncture under anaesthesia could result in admixture of venous and arterial blood unless caution was strictly exercised to withdraw from the left ventricles[19] . Venous blood is known to contain high levels of carbon IV oxide produced by the tissues. Most of these are conveyed in blood bound to red cell hemoglobin while a smaller portion is transported dissolved in plasma to form carbonic acid which dissociates into hydrogen and bicarbonate ions. An unrecognized admixture of venous and arterial blood complicating collection of blood sample through cardiac puncture could have resulted in the observed increase in the blood bicarbonate concentration. Secondly, this may be as a result of the rats having been exposed to excessive carbon (IV) oxide, a by product of diesel combustion in the air that they inspired. Although the immediate effect of excessive carbon dioxide inhalation should be a respiratory acidosis, it is possible that excessive compensatory mechanisms such as renal leading to increased tubular re-absorption of bicarbonate[20] after a prolong exposure to diesel combustion fumes resulted in the higher blood levels of bicarbonate observed in the fume exposed rats in this study.

There were also observed decreases in other

haematological parameters including packed cell volume (PCV), blood heamoglobin concentration (Hb), total white blood cell count (WBC) while the erythrocyte sedimentation rates (ESR) increased in the rats exposed to diesel combustion fume compared to their respective control (Figure 1). However there were no statistically significant differences between the observations for these parameters in the fume exposed rats from those of their respective control groups (all P values > 0.05).This observation was in line with previously reported lower erythrocyte and leucocyte counts in Hamsters exposed to diesel exhaust for 78 weeks[15] but contradicted reported increases in red blood cell count, Hb, Hct (hematocrit) and total WBC counts in rats that were exposed to diesel exhaust for even a longer time period [9,10].

Figure 2 showed that there was a decrease in the mean differential lymphocyte count from 67.8 3.47 in the control rat to 61.5 5.36 in rats that had 3 weeks fume exposure, which was also not significant statistically. The progressive decrease in the percentage lymphocyte count was observed to be accompanied by a progressive increase in the neutrophil fraction of the total white blood cells. Increases in banded neutrophils have also been reported in rats exposed to diesel exhaust in other studies [13,16]. This might be related to the fact that inhaled combustion fumes could result in reduced immunity and increased susceptibility to respiratory infections as previously reported in mice after exposure to dilute exhaust from light duty diesel engines [21].

The observation in this study that the fume exposed rats had lower mean post treatment body weights compared with their pre-treatment weights while the control rats gained weight before they were sacrificed (Figure 3) was in line with results in previous studies conducted on rats and mice exposed to either DPM or diluted diesel exhaust which had separately reported significant weight loss at high concentration of DPM ;but no effect at low concentration[22] and insignificant loss of body weight [23]. The post-treatment weights of the diesel exposed rats in this study were statistically significantly different (P< 0.05) from their pre treatment weights; hence the weight loss induced by such exposure was significant.

CONCLUSION

Although this study suggest that sub-chronic exposure of laboratory rats to diesel combustion fumes from open lamps may have some effects on their hematological parameters, other than the effect on the blood level of bicarbonate ion, no statistically significant effect was observed in the parameters that were assessed. This suggests that subchronic exposure to limited amount of diesel combustion fumes from lamps sometimes used as a domestic light source may not have far reaching hematological effects. How this observation may be affected following prolonged exposure to limited dose or short term exposure to high dose need to be evaluated in subsequent studies.

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