

Biomarker response of albino rats (*Rattus norvegicus*) to generator fumes

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Abstract

This study was carried out to determine the effect of generator fumes on albino rats using the blood and lung histology as biomarkers. Rats were exposed to generator fumes in a five-week exposure. Blood samples were collected from the control and exposed animals weekly. The following blood parameters were tested: pH, Neutrophil, Lymphocytes, Eosinophil, Monocytes, red blood cells, and carbon monoxide. The result obtained indicate pH in week 1 was acidic (5) but the pH in weeks 5 and 4 were alkaline 7.5 and 8 respectively. Neutrophil was seen to be low in all groups from week 1 to week 5. Lymphocytes were high indicating an inflammatory response. Eosinophil in week 3, group 2, and week 5 group 3 were 0. Monocytes were normal in all groups from week 1 to week 5 except for group 4 in week 5 which was high with 10 as its value indicating the possible inception of infections. RBC in all the weeks ranged from 3.9 -5.8. Carbon monoxide was low in week 1 and high in weeks 3 and 5. The lung histology indicates hemorrhage, capillary congestion and edema. The results indicate that exposure to generator fumes predisposes one to lung damage, pulmonary edema and chronic obstructive pulmonary diseases, hence a safe distance should be kept when around an operating generator to avoid inhaling the fumes.

Keywords: Generator Fumes; Carbon monoxide; Lung Histology; Haematology; Albino rats

1. Introduction

Generators have been implicated by many authors and researchers in the emission of large amounts of gaseous and particulate pollutants especially in the developing countries [1], with many reported adverse effects due to the exposure [2,3 & 4]. Exhaust fumes contain many known or suspected carcinogens and they pose a cancer risk that is 7.5 times higher than the combined risk from all other air toxins [1]. Many hazardous pollutants such as carbon monoxide, nitrogen oxides, particulate matter, and polycyclic aromatic hydrocarbons are produced in the process of gasoline and diesel combustions [5], but of all these pollutants associated with petroleum related exhaust fumes, polycyclic aromatic hydrocarbons (PAHs) and carbon monoxide (CO) are of great significance due to their carcinogenic and acute CO intoxication (tissue hypoxia) respectively [1]. Polycyclic aromatic hydrocarbons (PAHs) are considered to be the most acutely toxic components of crude oil, with its toxicity being due to its ability to interfering with membrane fluidity [6]. Polycyclic aromatic hydrocarbons (PAH's) are highly lipophilic compounds and are easily taken up by marine organisms thus increasing the carcinogenic potential [7]. They are also readily absorbed from the gastrointestinal tract of mammals. They are rapidly distributed in a wide variety of tissues with a marked tendency for localization in body fat [8]. Cancer is the most significant endpoint of PAHs toxicity. Although PAHs generally have a low degree of acute toxicity to humans, polycyclic aromatic hydrocarbons (PAHs) are one of the most widespread organic environmental pollutants that pose a potential risk to human health [9]. Many factors affect the toxicity of PAHs such as the species, route of exposure, and molecular structure of the PAH [10,11, 12 & 13]. Carbon monoxide (CO) is a gas that has no odor and

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color, it is a very dangerous gas that can cause sudden illness and death. It is found in combustion fumes, such as those made by cars, trucks, small engines (generator sets), fireplaces etc. Among the most concerning pathophysiological effects of CO is its propensity for causing severe neurological symptoms, brain damage, or even death after exposure to high CO concentrations [14]. Carbon monoxide poisoning is not a 'pure' pathological process because injuries may be precipitated by a combination of cardiovascular effects linked to hypoperfusion or frank ischemia, COHb-mediated hypoxic stress, and intracellular effects, including free radical production and oxidative stress. For example, CO poisoning cause elevations of glutamate and dopamine in experimental models and human fatalities [15 & 16]. Carbon monoxide can inhibit a number of hemoproteins found in cells, such as myoglobin found in heart and skeletal muscle, neuroglobin found in the brain, as well as cytochrome c oxidase, cytochrome P450, dopamine b hydroxylase, and tryptophan oxygenase. Inhibition of these enzymes could have adverse effects on cell function [17]. Laboratory animal studies indicate that nitrogen and oxygen-based free radicals are generated in vivo during CO exposures [18]. Exposure to CO at concentrations of 20 ppm or more for 1 hr will cause platelets to become a source of the nitric oxide free radical (NO) in the systemic circulation of rats [19]. Due to the challenges facing electricity in developing countries, the use of generators has increased significantly and so has Carbon monoxide levels especially in homes and offices, this study is therefore aimed at determining the effects of generator fumes which contains carbon monoxide on haematology and lung histology of albino rats in line with relating it to possible effects on man considering the similar physiology.

2. Material and methods

2.1. Experimental design

A total of Sixteen (16) healthy albino adult males weighing 190grams to 250grams were used in this study, and were allowed to acclimatize to laboratory condition (24-26°C) for 7 days before the commencement of the study. They were housed in a cage during the duration of study. Complete Randomized design was used and the animals were divided into 4 groups; Group 1(control,), Group 2 (1 hour), Group 3 (2 hours) and Group 4 (4 hours). The generator fumes were produced by placing the generator in an isolated environment with ventilation, the generator is switched on and the smoke channelled to a ventilated room where the 3 cages labelled one hour, two hours and four hours were kept for exposure, a stop watch was used for timing the duration of exposure of the rats in each cage from when the generator is switched on. The period of study was 5 weeks.

2.2. Biochemical Analysis

Standard procedures were ensured during the collection of the blood and the parameters were obtained using CELL-DYN 3700 fully automatic haematological analyzer. The lung histology was carried out according [20].

3. Results

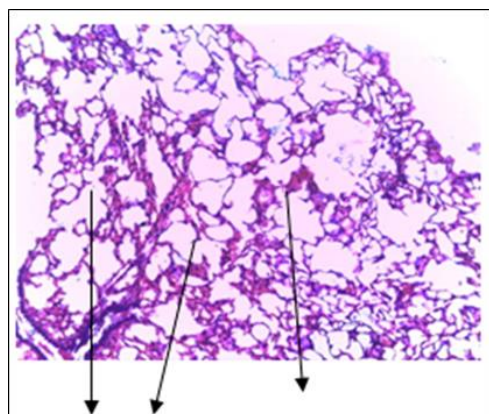
Table 1 Effects of generator fumes on Haematological parameters

WEEK	GROUP	pH	N	L	E	M	RBC	CO
Week 1	Group 1	5	21	75	3	1	4.7	6.83
	Group 2	5	8	90	2	0	5.5	24.59
	Group 3	5	25	69	3	3	4.8	6.95
	Group 4	5	8	85	4	5	3.9	2.75
Week 3	Group 1	7	14	83	3	0	5.6	171.75
	Group 2	7.5	14	84	0	2	4	114.5
	Group 3	7.5	15	80	1	4	4.3	125.95
	Group 4	8	25	71	2	2	3.9	148.85
Week 5	Group 1	7	20	65	4	5	4.7	137.17
	Group 2	7.5	30	57	8	5	5.8	475.79
	Group 3	7.5	17	66	0	6	5.4	164.22
	Group 4	8	30	55	5	10	4.3	23.58

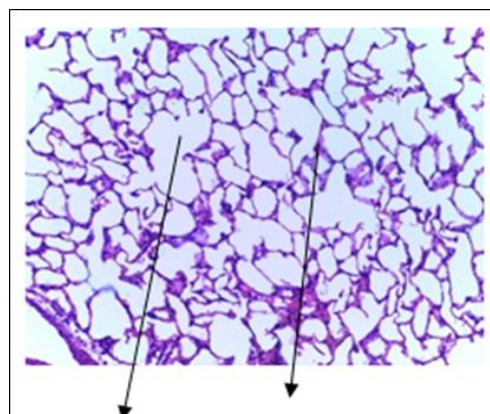
Key: N= Neutrophil, L= Lymphocyte, E= Eosinophil, M= Monocytes CO= Carbon monoxide estimate/bicarbonate

3.1. Histology of the Rat's Lungs

The lungs of the rats used were subjected to histological examination and the result presented below:

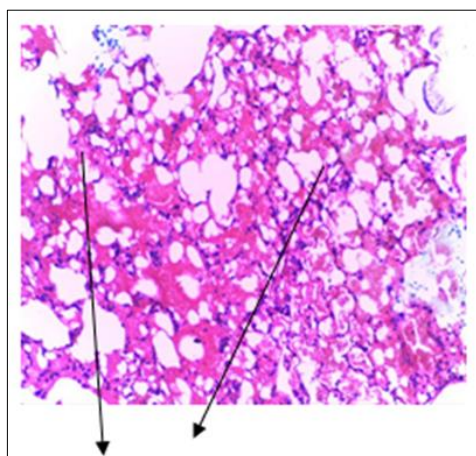


A Alveolar sacs pulmonary capillaries

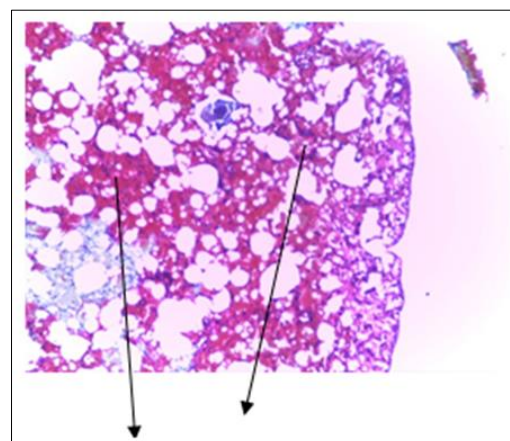


B Alveolar sac pulmonary capillaries

Figure 1 Histology of the Lungs of Rats not exposed to generator fume (A= week 1, B= week 5)

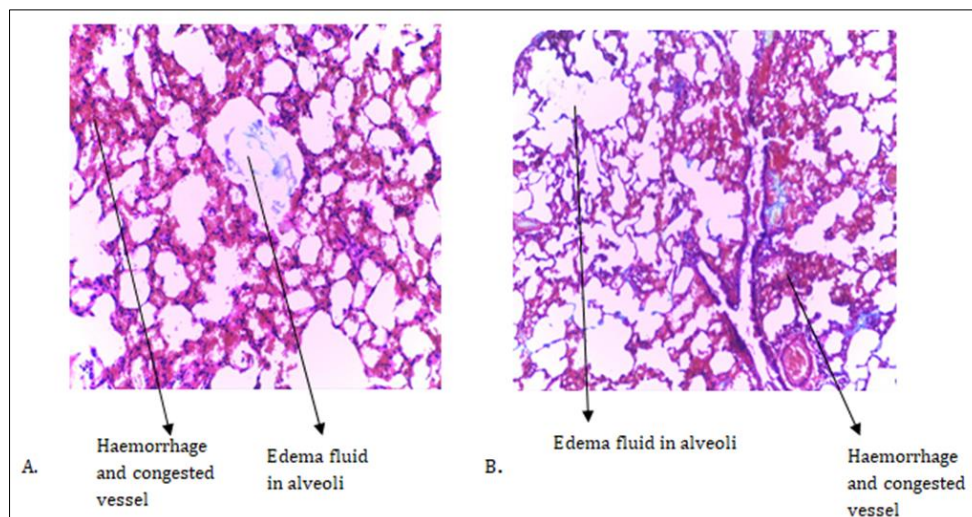


A. Congested cappilaries and hemorrhage



B. Congested cappilaries and hemorrhage

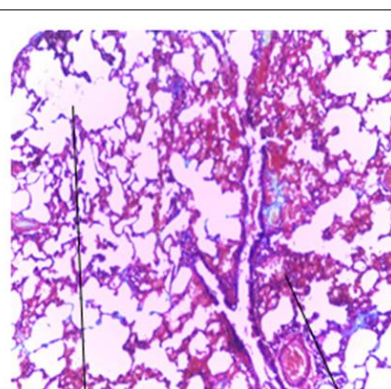
Figure 2 Histology of the lungs of Rats Exposed to generator fume for 1hr (A= week 1, B= week 5)



A.

Haemorrhage
and congested
vessel

Edema fluid
in alveoli



B.

Edema fluid in alveoli

Haemorrhage
and congested
vessel

Figure 3 Histology of the lungs of rats Exposed to generator fume for 2hrs (A= week 1, B= week 5)

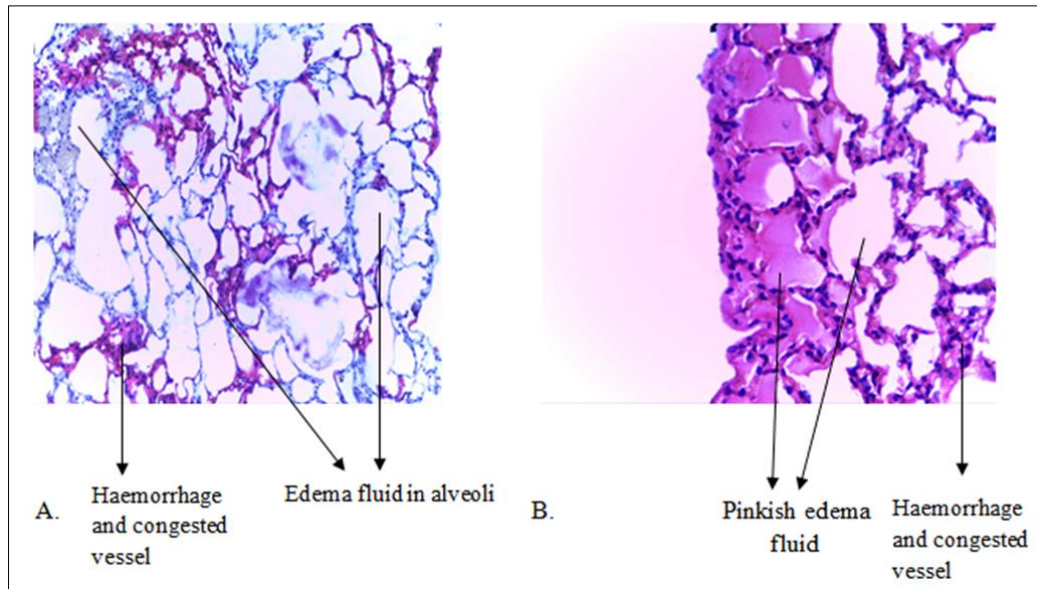


Figure 4 Histology of the lungs of Rat Exposed to generator fume for 4hrs (A= week 1, B= week 5)

4. Discussion

The results for blood pH for week 1 showed no change in the value when compared to the control, while in week 3, the pH value increased gradually from group 2 to 4 showing that an increase in the duration of exposure gradually increases the pH of the blood. Week 5 had similar result as week 3. Acute change in blood pH induces powerful regulatory effect at the level of the cell, organ and organism [21]. In [22] gammaglobulin receptors on the erythrocyte membrane were damaged due to changes in pH value. The clear effect is oxygen displacement and generation of carboxyhemoglobin, which is virtually unable to deliver oxygen to the tissue and is thereby responsible for various degree of hypoxia [23]. The Neutrophil was recorded to be low in the treated group compared to the control; group 2 and 4 had a lower value compared to the control while group 3 had a higher value compared to the control in week 1, in week 2 the value of neutrophil increased compared to the control, Neutrophils are the most abundant type of white blood cell in mammals and form an essential part of the innate immune system. They are formed from stem cells in the bone marrow. They are short-lived and motile [24]. Week 3 also recorded a higher neutrophil when compared to the control with the exception of group 3, the high value recorded might be due the damages done to the alveoli as seen in the lung histology on Fig 4 considering the fact that neutrophils are also types of white blood cells that, chronic bacterial infection may also add to inflammation of neutrophil granulocyte and macrophages as well as injuries to the lungs [25]. The lymphocyte was also observed to be higher in the treated group compared to the control group in week 1 while in the subsequent weeks 3, the level was slightly lower in group 3 and 4. Week 3 recorded a very low count for lymphocyte in group 4 that had the exposure for 4 hours, this low value might be due to the loss of blood fluid which contains lymphocytes through the blood vessels. The eosinophil was observed to be higher in the last week in the treated groups when compared to the preceding weeks 1 and 3, this might be because chronic exposure must have triggered an increase in the blood eosinophil level, since eosinophils are known to consume foreign substances [26]. Therefore, after prolonged exposure there might be an accumulation of particulate matter or other pollutant in the lungs that will necessitate the release of more eosinophil into the blood. The high monocyte levels seen in to be generally higher in the treated groups compared to the control groups for all the weeks, the reason for this might be due to the damages done to the lungs and body generally by the generator fumes. The red blood cells was observed to increase during the first week of treatment except for group 4, this increase in the amount of red blood cells might be to enable the body get more oxygen in the oxygen poor environment, the increased red blood cells is also linked to COPD [27], the decrease in group 4 might be due to possible damages to the kidney incurred by the prolonged exposure to the generator fumes considering the fact that the level never rose above the control group even in week 5. In week 5, group 2 and 3 were still higher red blood cells count than the control group showing that the exposure levels in group 2 and 3 wasn't severe to cause any damage to the kidney but was enough to lead to COPD. The high level of CO/biocarbonate recorded in the group 2 and 3 when compared to the control group is linked to certain diseases of the lungs such as chronic obstructive pulmonary disease COPD, while low levels is attributed to kidney diseases among other factors [28], and this values recorded indicates prolonged exposure to generator fumes predisposes one to COPD. This result corroborates the report of [27] that fumes can increase the chances of COPD. Fig 1 above shows the histology of the lungs of rats not exposed to generator fume all through the project. From the plate above we can see that the rats not exposed to generator fume had a normal histology,

but in Fig 2, it can be observed that the rats sustained injury in their lungs which was primarily congested capillary and Haemorrhage. Haemorrhage is the cut or opening on a tissue which leads to loss of blood. Congested capillary is the blocking of the blood vessel carrying oxygenated blood and nutrients essential to the different tissues of the body. The result shows a severe influence of the generator fume on the lungs of rat in group 2 (1 hour). Fig 3 above shows the histology of the lungs of rat exposed to generator fume for 2hrs and from the picture, it can be observed that the level of lung damage was more severe than that of rats exposed to 1hr (fig 2 above). There were cases of edema in all their lungs in addition to haemorrhage and congestion in the capillaries as shown in Fig 3 above. The fluid accumulation or pulmonary edema is known to be caused by damage of the lungs due to the inhalation of smokes and inhaling certain toxins, this causes the alveoli to be filled with fluid instead of air. In line with this, we can rightly say that generator fumes damage the lungs which leads to pulmonary edema, but the damage was only observed in the group 3 and group 4 showing that exposures for up to 2 hours and more can cause pulmonary edema. This result shows an acute damage of the lungs of rat in group 3. From fig 4 above, rats exposed to generator fume for 4hrs showed severe lung damage as can be seen from the level of lung congestion observed from them, also pinkish edema fluid were also observed in them. This result shows that the effect of the generator fume is acute to the lungs of rat in group 4. The histological results show that exposure to generator fumes possess serious danger to the lung and can increase the risk of lung cancer, this agrees with a study carried out by [29] on traffic related air pollution and lung cancer and also [1] on the use of generators. Another author suggested that exposure to engine exhaust fumes may increase the risk of lung cancer and neurological conditions in rats [30]. Some reported adverse effect of exhaust pollutant includes increased infant mortality [31], chronic deficit in lung development of children aged 10-18 years [3] and other ovarian cancer.

5. Conclusion

Generator fume according to the study damages the lungs and adversely affects the body especially the respiratory system with increased risk of lung cancer and chronic obstructive pulmonary disease, hence when operating a generator, a safe distance from the house and office should be ensured to prevent inhaling the fumes.

Compliance with ethical standards

Acknowledgments

All individuals who have contributed to this work have been listed as authors.

Disclosure of conflict of interest

No potential conflict of interest reported by the authors.

Statement of ethical approval

University standard written ethical permission was sought for, granted and has been preserved by the author(s).

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