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Original Article

Exposure to Petroleum Hydrocarbon: Implications in Lung Lipid Peroxidation and Antioxidant Defense System in Rat

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ABSTRACT

Objective: Various studies have implicated automobile exhausts as risk factors in cardiovascular and pulmonary diseases; however, there is little or no documentation on the role of the main source of the exhausts, petroleum hydrocarbons, on cardiopulmonary pathologies. Thus, we investigated the effect of petroleum hydrocarbons, using various petroleum products, on histomorphology of the lung and the role of lipid peroxidation in it. **Materials and Methods:** Control rats were not exposed to any of the petroleum products, whereas petrol-exposed, diesel-exposed, and kerosene-exposed rats were exposed to petrol, diesel, and kerosene by inhalation, respectively. **Results:** Exposure to petroleum hydrocarbons significantly induced lipid peroxidation with a consequent rise in malondialdehyde (MDA), and a decrease in superoxide dismutase (SOD) and catalase (CAT) activities and glutathione (GSH) level. Exposure to petroleum hydrocarbons also caused an alteration in the histomorphology of lung tissues. **Conclusion:** Our findings imply that exposure to petroleum hydrocarbons by inhalation is a risk factor in the pathophysiology of pulmonary dysfunction. This is associated with oxidative stress.

Key words: Lipid peroxidation, lung, oxidant, oxidative stress, petroleum hydrocarbons

INTRODUCTION

Exposure to a high level of air pollution has been implicated in the increased morbidity from cardiovascular diseases and respiratory diseases.[1] Crisis in air quality resulting from automobile exhaust has become a growing problem,[2] posing health challenges in urban regions. This has been associated with the rise in allergic diseases in industrialized countries.[3,4] Automobile emissions consist of oxides of nitrogen, carbon monoxide, particulate matter, and others, and have been reported to enhance airway response to inhaled allergens in susceptible subjects and cause injury to the terminal bronchioles and a decrease in the pulmonary compliance and vital capacity.[4,5]

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According to the report of Gupta,[2] among the motor vehicle-generated air pollutants, diesel exhaust particles account for a highly significant percentage of the particles emitted in many towns and cities. Irritation of eyes and nose, changes in lung function, headache, fatigue, and nausea are common acute effects of exposure to diesel exhaust. Chronic exposure is associated with cough, production of sputum, and decrements in lung function.[6]

Although the constituents of petroleum products are quite different from those of automobile exhaust, they are the source of the emission. They are hydrocarbons,

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a heterogeneous group of substances primarily made up of hydrogen and carbon molecules.

Despite large data on the effect of automobile exhaust on lung function, there is still a dearth of knowledge on the implication of petroleum hydrocarbons, the source of exhaust fumes, on lung pathology. This study bridges this gap and thus documents the effect of the inhalation of petroleum products (the seemingly commonest form of exposure) on lung oxidative status and histomorphology.

MATERIALS AND METHODS

Animal treatment

Sprague Dawley rats of comparable weights were used for the study. They were housed in well-ventilated cages maintained at 25 ± 2°C, on a 12-hour light-dark cycle. The rats were fed on standard rat chow and tap water without restriction. They were acclimatized for two weeks before the experimental period.[7,8] Procedures involving animals and their care were performed in accordance with the guidelines of the Institution Animal Ethics Committee and the National Institutes of Health (NIH) for the care and use of animals.

The rats were assigned randomly to one of the four experimental groups as follows with five rats per group:

Control: Rats not exposed to any form of petroleum hydrocarbon
Petrol inhalation (Pi): Rats exposed to petrol inhalation
Diesel inhalation (Di): Rats exposed to diesel inhalation
Kerosene inhalation (Ki): Rats exposed to kerosene inhalation

The rats were kept in the exposure chambers saturated with the appropriate petroleum-fractionated product vapors. All treatment sessions lasted for eight weeks.

Exposure to petrol, diesel, and kerosene vapors

A modified nose-inhalation exposure method was used as previously described.[9-11] Briefly, the cages housing the animals were placed in respective exposure chambers with calibrated beakers of 1000 cm^3 containing 500 cm^3 of kerosene, petrol, and diesel, respectively. The petroleum-fractionated products were allowed to evaporate freely within the respective exposure chambers at ambient humidity and temperature, and the animals were exposed to vapors (0.5 ± 0.08 cm^3/min/kg/m^3/day) generated from direct evaporation of the petroleum products. The animals were exposed for five minutes daily. At the end of the exposure, the animals were transferred to a petroleum-free section of the animal house. The initial and final volumes of petroleum products in the beaker before and after exposure were respectively recorded. The differences in volume per day were used as the estimate relative concentrations of the vapors used.

Lipid peroxidation and anti-oxidant assay

Blood sample was collected via cardiac puncture. Lung tissue malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH) were assayed using standard laboratory methods using kidney homogenate.[12-14]

Histomorphological study

After the experimental period, the animals were sacrificed through cervical dislocation and opened up. The lungs were collected and preserved in 10% formalin. Tissue processing was carried out by autotechnique; 5µ thick sections were prepared and mounted on slides and stained with hematoxylin and eosin (H and E). The stained sections were morphologically evaluated and microphotographs were taken.

Statistical analysis

All data were expressed as mean ± standard error of mean (SEM). The results were analyzed by one-way analysis of variance (ANOVA), followed by pairwise comparison between test and control groups using Student's *t*-test. Duncan's multiple range test was used as post hoc. Differences between groups were considered significant at $P < 0.05$.

RESULTS

Petroleum hydrocarbons led to a significant rise in MDA, an index of lipid peroxidation. Though the rise in MDA was similar in the petrol- and kerosene-exposed rats, it was more in the diesel-exposed rats compared to other groups [Figure 1].

Figures 2-4 show the effect of petroleum hydrocarbons on some enzymatic antioxidants. All forms of petroleum products used in the study caused a significant decrease in GSH and in the activities of SOD and CAT.

Histomorphologic studies also showed that petroleum hydrocarbons caused edema and hemorrhagic necrosis of the lung alveoli and parenchyma [Figure 5].

DISCUSSION

Petroleum products remain necessary environmental hazards. Their uses cannot be overemphasized; however, exposure to their hydrocarbon constituents poses significant threats to health. The present study documents the effect

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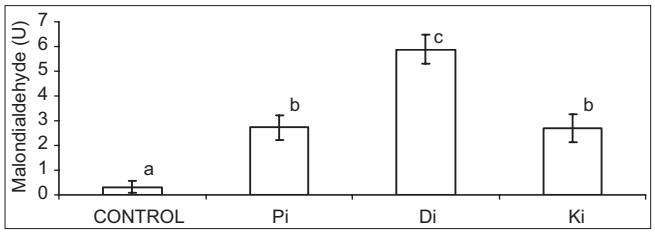


Figure 1: Effect of Petroleum Hydrocarbon Inhalation on Lung Tissue Malondialdehyde Bars carrying different letters are significantly different at $P < 0.05$

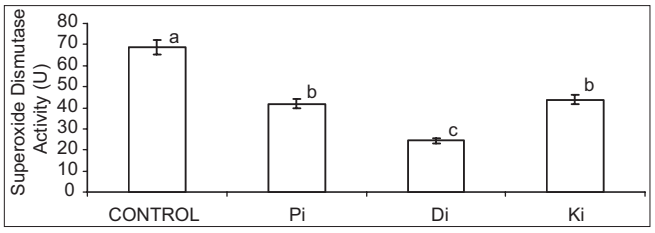


Figure 2: Effect of Petroleum Hydrocarbon Inhalation on Lung Tissue Superoxide Dismutase Bars carrying different letters are significantly different at $P < 0.05$

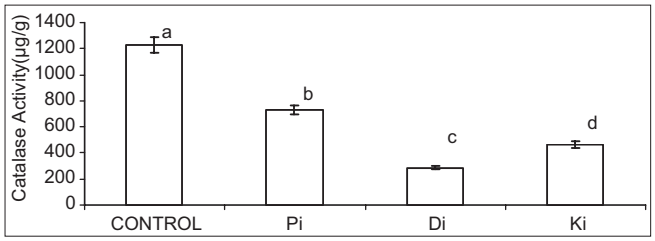


Figure 3: Effect of Petroleum Hydrocarbon Inhalation on Lung Tissue Catalase Bars carrying different letters are significantly different at $P < 0.05$

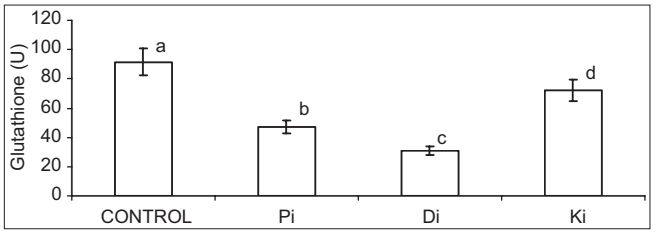


Figure 4: Effect of Petroleum Hydrocarbon Inhalation on Lung Tissue Reduced Glutathione Bars carrying different letters are significantly different at $P < 0.05$

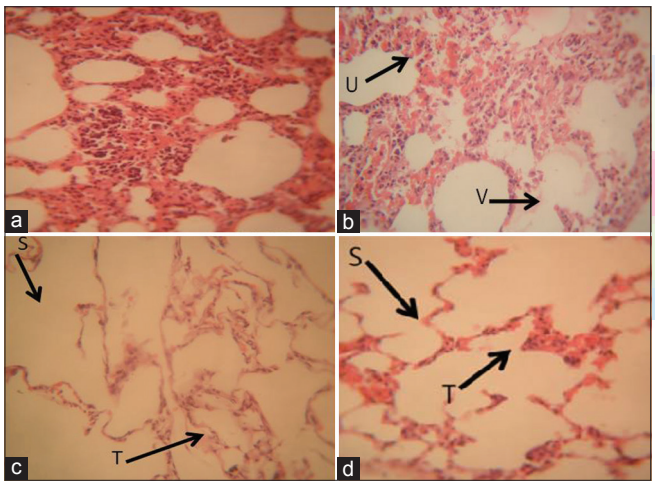


Figure 5: Photomicrograph of lung in rats exposed to various petroleum products, (a) Control showing normal parenchyma and alveoli (hematoxylin and eosin stain, ×400), (b) Petrol inhalation showing hemorrhage (U) and edema of the alveoli (V), (c) Diesel inhalation showing widespread necrosis of the lung alveoli (S) and parenchyma (T) (hematoxylin and eosin stain, ×400), (d) Kerosene inhalation showing widespread necrosis of the lung alveoli (S) and parenchyma (T) (hematoxylin and eosin stain, ×400)

and possible mechanism of petroleum hydrocarbons on lung tissue. Exposure of experimental animals to various petroleum hydrocarbons triggered oxidative stress. Oxidative stress is a threat to well-being.[15] The antioxidant enzymes SOD, CAT, and glutathione peroxidase (GPx) serve as a primary line of defense in destroying the free radicals[16] produced by oxidative stress. SOD reduces the radical superoxide (O_2-) to form hydrogen peroxide (H_2O_2) and oxygen (O_2); then, CAT and GPx work simultaneously with the protein glutathione to reduce H_2O_2 and ultimately produce water (H_2O). The increased MDA level and decreased SOD, CAT, and GSH suggest that exposure to petroleum hydrocarbons caused oxidative stress by consuming the protective free radical scavengers.

The antioxidant defense system via dismutation, oxidation, and hydrolysis also protects the biological integrity of cells and tissues against the harmful effects of superoxide free radicals and are thus natural endogenous protection against the generation of reactive oxygen species.[17,18] This implies that consumption of the antioxidant defense system results in loss of cell and tissue integrity. This could explain the edema and hemorrhagic necrosis of lung tissue seen in this study following exposure to petroleum hydrocarbons. This may be due to the leakage of fluid into the extravascular space with resultant hemorrhagic necrosis. Studies have reported that exposure to petroleum hydrocarbons impairs type II pneumocytes resulting in a decreased production of surfactant and consequent alveolar collapse, ventilation-perfusion mismatch, and hypoxemia. This ultimately leads to hemorrhagic alveolitis, interstitial inflammation, intra-alveolar hemorrhage and edema, hyperemia, bronchial necrosis, and vascular necrosis.[19,20]

Findings in this study are similar to the case report by Prasad *et al.*[19] which documented that aspiration of kerosene led to bilateral hemorrhagic pleural effusion. In their study, management of the patient with corticosteroids to reduce the inflammatory process, oxygen supplement to prevent hypoxic cell death, and antibiotics to possibly prevent or control superimposing bacterial infection improved the status of the patient without any pulmonary sequel.

Though there are insufficient data in scientific literature

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that document the effect of petroleum hydrocarbons by inhalation of various petroleum products on pulmonary function, findings from the present study reveal that inhalation of petroleum hydrocarbons is a risk factor in pulmonary pathologies.

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