

EFFECT OF PETROLEUM PRODUCT INHALATION ON THE BEHAVIORAL INDICES OF MALE WISTAR RATS

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Abstract

Petroleum products are ubiquitous in modern society, and inhalation exposure represents a significant route of potential neurotoxicity. This study analyzed the effect of petroleum product inhalation on the behavioral indices of male Wistar albino rats. Forty-five rats aged 3 months and weighing 180–220 g were divided into five groups: Group A (Control), Group B (PMS exposure - 500 ml), Group C (Kerosene exposure - 500 ml), Group D (Diesel exposure - 500 ml), and Group E (Mixed petroleum products - 500 ml). The exposure lasted for 28 days. Anxiety and cognitive tests were performed. The results of the behavioral indices showed a slight cognitive decline in rats exposed to diesel after 14 days, and petroleum products caused anxiety-like behavior in rats. These results emphasize the harmful effects of inhaling petroleum products on critical organ systems, particularly the brain and lungs.

Introduction

Petroleum products are essential components of modern industrial and domestic activities. However, their widespread use presents a significant risk of human exposure, particularly through inhalation (Ahmed *et al.*, 2018). Occupational exposure in refineries, gas stations, and transportation industries, as well as accidental spills and improper storage, can lead to substantial inhalation of volatile petroleum hydrocarbons (Olasehinde and Olaniran, 2022).

Previous studies have indicated that exposure to petroleum products can induce a range of neurological effects in humans and animals. For example, reports have documented cognitive deficits, including memory impairment and attention deficits, among workers chronically exposed to petroleum vapors (Malloul *et al.*, 2018).

Given the potential for widespread human exposure and growing concern about the neurotoxic effects of petroleum products, a systematic investigation of the behavioral impacts of petroleum product inhalation is warranted. This study aims to contribute to a better understanding of the potential neurobehavioral risks associated with petroleum product inhalation, providing valuable insights for public health and occupational safety.

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Justification of the study

The increasing use of petroleum products in modern life has raised concerns about the release of toxic compounds into the environment (Robinson *et al.*, 2017). Petroleum hydrocarbons are neurotoxic, and they can cross the blood-brain barrier and affect the central nervous system (Olasehinde and Olaniran, 2022). The nervous system is particularly vulnerable to the effects of petroleum products (Antonelli *et al.*, 2020). The effects of inhaling petroleum product fumes on neurological function are not fully understood. Studies on rats can provide insights into the risks to humans in high-exposure environments. This study aimed to address existing knowledge gaps by evaluating the effects of petroleum fume inhalation on the behavioral indices of male Wistar albino rats.

Aim of the study

The aim of this study was to investigate the effect of petroleum product inhalation on the behavioral indices of male Wistar albino rats.

Objectives of the study

The specific objectives of the study were as follows:

- Determine the effect of petroleum product inhalation on the cognitive function of male wistar albino rats.
- Determine the effect of petroleum product inhalation on the anxiety of male wistar albino rats.

Method

Experimental design

Forty-five (45) adult male Wistar rats aged 3 months, weighing between 180 and 220 g were procured from the Department of Human Physiology, University of Nigeria, Enugu campus, Enugu State. They were housed in wire-gauze ventilated cages at Power-Tech Analytical and Scientific Research Laboratory, Independence Layout, Enugu, Enugu State. The rats were fed standard rat chow and clean water *ad-libitum* and were kept under a normal room temperature of $25 \pm 2^{\circ}\text{C}$ with humidity of $45 \pm 5\%$. The rats were allowed to acclimatize for 2 weeks before the start of the experiment. Procedures involving animals and their care were performed in accordance with the National Institutes of Health (NIH) guidelines for animal care and use. The rats were grouped into five (5) cages labeled A-E, which consisted of three (3) rats each, each replicated three times. The groups were grouped as follows: Group A (Control Group) comprised nine rats that were fed with normal rats' chow and clean water without exposure to any petroleum products; Group B comprised nine rats that were exposed to 500 ml of Premium Motor Spirit (PMS); Group C comprised nine rats that were exposed to 500 ml of dual purpose kerosene (DPK); Group D comprised nine rats that were exposed to 500 ml of diesel; and Group E, comprised nine rats that were exposed to 500 ml of a combined mixture of PMS, DPK, and diesel. The experiment lasted for 28 days after exposure to the petroleum products. Rats were taken to the Y-maze and elevated plus maze test on weeks 0, 2, and 4 post-exposure. The samples were taken to the Power-Tech Analytical and Scientific Research Laboratory for biochemical (oxidative test) analysis, behavioral indices, and histological analysis.

Animal Model Selection (male Wistar rats)

Forty-five (45) adult male Wistar rats aged 3 months, weighing between 180 and 220 g were obtained from the Department of Human Physiology, University of Nigeria, Enugu campus, Enugu State. They were housed in well-ventilated cages. The rats were fed standard rat chow and tap water *ad-libitum*.

The male Wistar albino rat is a commonly used animal model for toxicological studies because of its physiological similarity to humans. These rats have been widely employed to assess the toxic effects of environmental pollutants, making them suitable subjects for investigating the respiratory and hippocampal consequences of petroleum product inhalation.

Petroleum-Product Inhalation Protocol

The method of exposure employed in this study was inhalation. The animal was placed in an exposure chamber that was sealed and ventilated. Petroleum product (PMS, DPK, diesel) was obtained from the Nigerian National

Petroleum Corporation (NNPC) Mega Filling Station Enugu, Nigeria. Four highly perforated 1000 ml cans containing 500 ml of diesel, dual purpose kerosene (DPK), premium motor spirit (PMS), and mixed doses of the three were placed in the exposure chamber, and the animals were allowed to inhale the fumes evaporating from the cans. The exposure period lasted for 5 h. The experiment lasted for 28 days. The exposure time was between 9.00 am and 2.00 pm, after which the animals were transferred to the fume-free section of the experimental animal house.

Behavioral Indices

Cognitive test

Cognitive tests were performed using the Y-maze. The test was performed according to the instructions provided by Djeuzong *et al.* (2019). The Y-maze test, which recorded random rearrangements, was used to examine the animals' working memory. A wooden maze with three independent arms (40 cm long, 35 cm tall, and 12 cm wide) was used, each staggered by a 120° inclination. To distinguish them, the walls of each arm were adorned with various designs, which were named X, Y, and Z. Rats were individually placed at the ends of maze branches for free exploration. The sum of visits to each arm was counted throughout the 5-min period. To mitigate the odor, the device was cleaned with 10% ethanol after every exercise. Three sequential entries in three separate arms, such as XYZ, ZXY, and YZX, were defined as random alternations.

Anxiety test

Anxiety-like behavior was assessed using the elevated plus maze test (EPM) developed by Pellow *et al.* (1985). A brown Plywood apparatus (Coulbourn Instruments, Allentown, PA, USA) formed of a central sheath raised 50 cm above the surface, with two opposing closed arms (49 × 10 cm) and two opposing open arms, was used for each animal testing. The rats were centrally positioned within the maze, oriented to the same open arm, and allowed to explore the area for 5 min. The observer recorded the following parameters: time spent in the open and closed arms, and the entries made on the open and closed arms (Fraser *et al.*, 2010). Before testing each rat, the maze was thoroughly cleaned with cotton and 10% ethanol solution and completely dried with paper towels.

Statistical Analysis

All the statistical analysis were processed by using the Statistical Package of Social Science (SPSS) for the window (version 16). The values of the measured parameters are expressed as mean \pm SEM. Two-way analysis of variance (2-way ANOVA) was used to determine the effect of different inhalants and number of days after exposure on the parameters that were studied, and the differences between means were separated using Duncan's multiple range test. Test for significance was considered at 0.05 probability level.

Results

Cognitive Test/Spatial Memory Test

The range on the control chart is high on day zero, indicating that the exposure is normal and has no significant impact on the rats; on day 14, the exposure has no significant impact on the rats; and on day 28, the exposure has no significant impact on the rats.

Regarding PMS, there is a slight negative effect on the rats exposed to it on day zero when compared to the control; on day fourteen when compared to the control, there is a slight negative effect on the rats exposed to PMS; and on day twenty-eight when compared to the control, there is a slight positive effect on the rats exposed to PMS on day twenty-eight.

For Kerosene, there is a slight negative effect on the rats exposed to it on day zero when compared to the control; on day fourteen when compared to the control, there is a slight positive effect on the rats exposed to Kerosene on day fourteen; and on day twenty-eight, when compared to the control, there is a slight positive effect on the rats exposed to Kerosene on day twenty-eight.

When it comes to Diesel, the rats exposed to it on day zero experience a slight negative effect; on day 14, the rats exposed to it on day 14 experience a slight negative effect; and on day 28, the rats exposed to it on day 28 experience a slight positive effect. These differences in response to Diesel are reflected in the fuel reduction in the chart range and its increase compared to the control.

Rats exposed to the mixture of petroleum products on day zero showed a slight unfavorable effect because the amount of Mixture is lowered on the chart range compared to the control. Mixture of petroleum products: on day 14, it is lower on the chart range compared to the control, indicating a slightly negative effect on the rats exposed to it; on day 28, it is higher on the chart range compared to the control, indicating a positive effect on the rats exposed to mixture of petroleum products (Fig. 1).

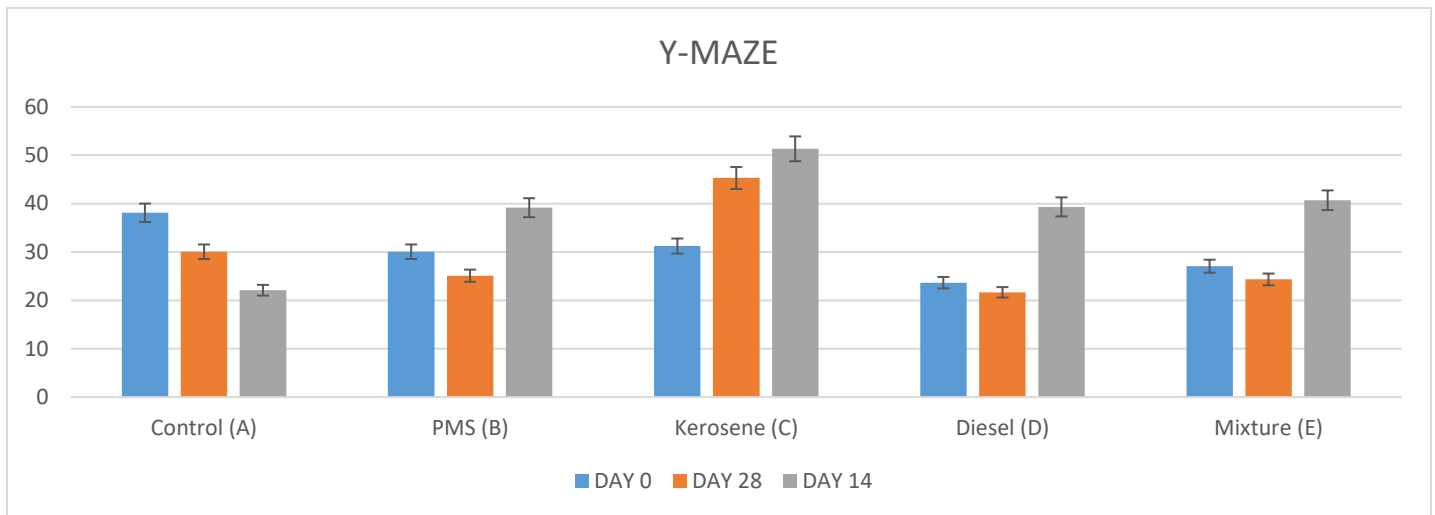


Fig. 1: Image showing the Y-Maze Cognitive test result.

Anxiety Test

The range on the chart for anxiety test on day 0, higher number of animals entered the closed arm and open arm for the control when compared to the test groups; for PMS, the number of animals entry in the closed arm entry were high and there is much avoidance for open arm entry, indicating high level of fear; kerosene diesel and mixed petroleum products maintained the same trend as PMS on day 0. There was no entry recorded for open arm entry on 14 for control, PMS, kerosene, and mixed petroleum products; however diesel recorded a very low entry for open arm entry. On day 28, there was high avoidance of open arm entry for all test groups except for the control (Fig 2).

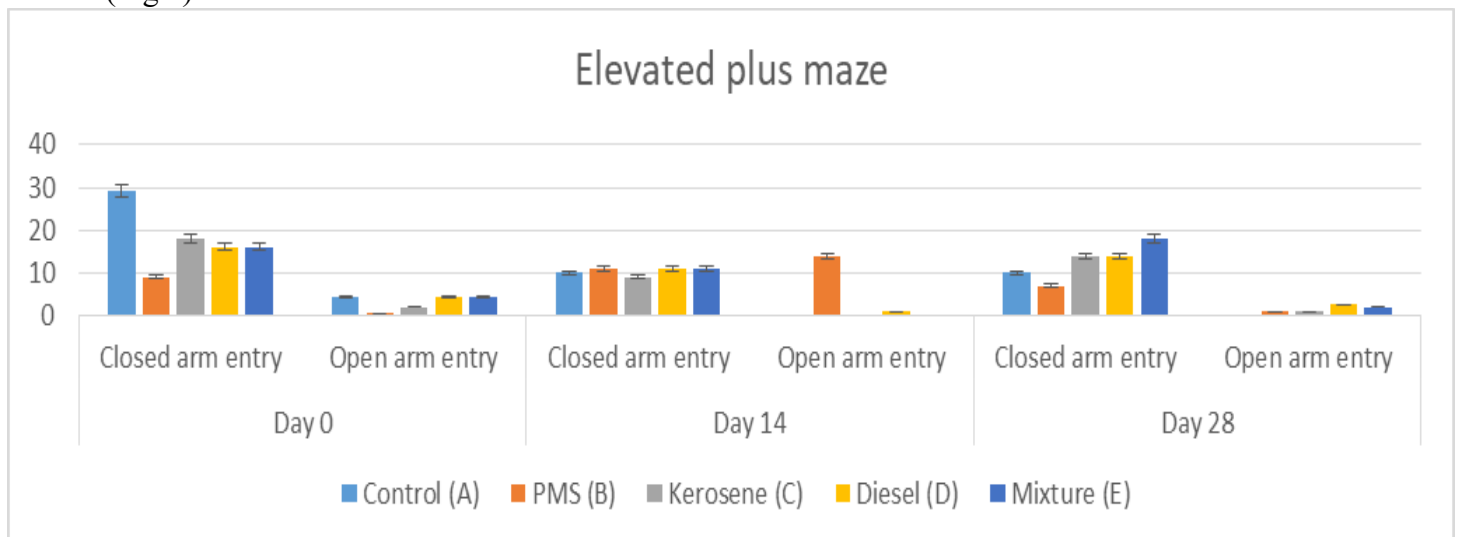


Fig. 2: Elevated plus maze (Anxiety test result).

Discussion, Conclusion, and Recommendations

Discussion

Behavioral Indices of male wistar rats exposed to petroleum products

Cognitive test

The current study showed slight cognitive decline in rats exposed to diesel after 14 days, which is in agreement with the study of Enogieru and Williams (2024), who investigated the cognitive and memory-boosting effects of cinnamon (*Cinnamomum zeylanicum*) aqueous extract in rats exposed to lead acetate. Researchers discovered that rats exposed to lead exhibited cognitive, learning, and memory problems, according to a comparison of neurobehavioral activities.

Anxiety test

The research showed that rats exposed to petroleum products caused anxiety-like behavior in rats, which was expressed by a significant lessening time spent by the rats in the open arm stemming from control to all test groups and across all days of test. This research is in agreement with Sadeghi *et al.* (2023), who investigated how exposure to crude oil vapor causes oxidative stress and changes in histopathological markers in the rat hippocampal region, which in turn causes behavioral impairments. They found that apoptosis is induced and antioxidant enzymes such as CAT, TAC, SOD, GPx, and MDA are disrupted. This ultimately causes rats to exhibit more anxiety-like behavior and experience trouble with spatial memory.

The present experiment agreed with the study by Branch and Hamedan (2016), who examined the Comparison between Gasoline and Diesel Oil Vapor Inhalation on Anxiety in Male Rats and discovered that enhanced anxiety following exposure to gasoline vapor may come from physiological changes occurring in the nervous system.

Conclusion

The findings illustrated that exposure to petroleum products on behavioral assessments revealed notable changes in cognitive function and anxiety level, suggesting potential neurotoxicity. These results emphasize the harmful effects of inhaling petroleum products on critical organ systems, particularly the brain.

Recommendations

This study encourages environmental regulations and public health interventions to minimize exposure to petroleum products and protect vulnerable populations. Further studies are therefore recommended to evaluate the long-term impacts of exposure to low-dose petroleum products. The use of a greater range of behavioral tests (such as the forced swim test, social interaction tests and novelty suppression tests) in addition to anxiety-related behavior would provide a better understanding of the effects on mood, learning, memory, and social behavior.

Declaration

We declare that this manuscript titled "Effect of Petroleum Product Inhalation on Histological Indices of Hippocampal Tissue of Male Wistar Albino Rats" is original and has not been published nor submitted elsewhere for publication. All data were collected and analyzed following ethical guidelines for animal research. There are no conflicts of interest to declare, and all authors have approved the final version of the manuscript for submission.

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Conflict of Interest: This study focuses on eco-toxicological effect of petroleum product on human health. The authors declare no conflicts of interest regarding the manuscript titled “*Effect of Petroleum Product Inhalation on Histological Indices of Hippocampal Tissue in Male Wistar Albino Rats.*”

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