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Effect of Petroleum Products Inhalation on Oxidative Stress Parameters of Male Wistar Albino Rats

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ABSTRACT

Background: The widespread use of petroleum products in modern industries has raised concerns about their potential toxic effects on biological systems. The study explored the effect of petroleum product inhalation on the oxidative stress parameters of the male Wistar albino rats.

Method: Forty-five rats, aged three 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, with lungs and hippocampal tissue homogenate samples collected at day 0, 14, and 28 to measure hydrogen peroxide (H_2O_2) scavenging activity, nitric oxide (NO) activity, reduced glutathione (GSH) level, superoxide dismutase (SOD) activity, and malondialdehyde (MDA) level.

Results: The results showed that H_2O_2 scavenging activity, NO activity and MDA level were significantly increased ($p>0.05$) (24.34 ± 1.19, 4.90 ± 0.54, and 1.20 ± 0.14 mg/dl) when compared to control (31,04 ± 1.11, 4.15 ± 0.59 and 1.46 ± 0.20 mg/dl) while GSH level and SOD activity were significantly decreased ($p<0.05$) (1.93 ± 0.31 and 11.12 ± 0.48 mg/L) when compared with the control (3.28 ± 0.30 and 12.87 ± 0.93 μ/mg).

Conclusion: The results inferred the detrimental effects of inhaling petroleum products on critical organ systems, particularly the brain and lungs.

Keywords: SOD, H_2O_2, Nitric oxide, MDA, GSH, Petroleum products

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INTRODUCTION

Petroleum products are widely used today and serve important functions in sectors like home operations, energy production, and transportation[1]. However, their extensive use raises serious concerns about possible health dangers, especially when exposure happens through inhalation[2].

Inhaling petroleum products has been linked to several toxicological effects, including oxidative stress, tissue damage, and abnormal behaviour[3]. Oxidative stress, which is characterised by an imbalance between the production of reactive oxygen species (ROS) and the capacity of the antioxidant defence system, is one of the primary reasons behind the toxicity of these chemicals[4]. ROS can potentially harm DNA, proteins, and lipids, among other components of cells. They are created either during metabolism or due to exposure to outside pollutants. According to studies, inhaling petroleum products increases oxidative stress by lowering the activity of antioxidant enzymes such as glutathione (GSH) and superoxide dismutase (SOD) and raising levels of the lipid peroxidation marker malondialdehyde (MDA)[5].

Despite remarkable advancements, there are still a number of gaps in our knowledge of the harmful impacts of petroleum products. Without examining the possible connections between these variables, a significant amount of the current research has concentrated on specific endpoints, such as behavioural outcomes or oxidative stress markers[6].

The usage of petroleum products, which are known to discharge harmful substances into the environment, has increased due to increased industrial activity. These substances pose serious health concerns when inhaled, especially to the respiratory and central neurological systems[7]. One important part of the brain that controls memory and learning, the hippocampus, is particularly vulnerable to oxidative stress, which can be worsened by exposure to harmful chemicals like those in petroleum products[8]. In a similar vein, inhaling these toxicants can cause structural and functional abnormalities in the lungs, which are the main route of exposure[9].

Although the broad health impacts of inhaling petroleum products have been studied in the past, more focused research on the oxidative stress parameters is required. Because of its physiological and anatomical similarities to humans, the male Wistar albino rat, a popular model for human health studies, provides a pertinent system to examine these impacts[10]. The effects of inhaling petroleum products on these particular indices may help identify possible health hazards to people and aid in the management strategies.

This research attempts to give a thorough grasp of how inhaling petroleum products affects the lungs and hippocampal regions by investigating oxidative stress indicators like MDA and antioxidant enzymes. The results will add to the expanding body of research on petroleum toxicity and could influence occupational safety regulations and public health initiatives that try to lower exposure hazards. Given that petroleum products have an impact on oxidative stress, this study would help understand the systems underlying their harmful effects while opening up the possibilities to the creation of focused therapies.

METHOD

Experimental design

Forty-five (45) adult male Wistar rats aged three (3) months, weighing between 180 – 220 grams, were procured from the Department of Human Physiology, University of Nigeria, Enugu campus, Enugu State. The experiment was laid out in a complete randomized design. The rats were housed in a wire-gauze ventilated cages at Power-Tech Analytical and Scientific Research Laboratory, Independence Layout, Enugu, Enugu State. They were fed on standard rat chow and clean water *ad-libitum* and kept under normal room temperature of 25 ± 2^0C with humidity of 45 ± 5%. The rats were allowed to acclimatize for 2 weeks before the start of the experiment. The procedure involving animals and their care was performed in accordance with the National Institute of Health (NIH) guidelines for the care and use of animals. The rats were grouped into five (5) cages labeled A-E, which comprised three (3) rats each; this was replicated thrice. The groups were grouped as follows: Group A (Control Group) comprised of nine rats that were fed with normal rats' chow and clean water without exposure to any petroleum products; Group B comprised of nine rats that were exposed to 500 ml of Premium Motor Spirit (PMS); Group C comprised of nine rats that were exposed to 500 ml of dual purpose kerosene (DPK); Group D comprised of nine rats that was exposed to 500 ml of diesel; and Group E, comprised of nine rats that was exposed to 500 ml of a combined mixture of PMS, DPK, and diesel. The experiment lasted for 28 days post-petroleum products exposure. Lungs and hippocampal tissue homogenate were taken from three rats each from the experimental and control groups for oxidative stress parameters on

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week 0, week 2, and week 4 post-exposure. The sample was taken to the Power–Tech Analytical and Scientific Research Laboratory for oxidative test analysis.

Animal Model Selection: Forty-five (45) adult male Wistar rats aged three (3) months, weighing between 180 – 220 grams, were obtained from the Department of Human Physiology, University of Nigeria, Enugu campus, Enugu State. They were housed in well-ventilated cages. They were fed on standard rat chow and tap water *ad-libitum*. The male Wistar albino rat is a commonly used animal model for toxicological studies due to its physiological similarity to humans.

Petroleum-Products Inhalation Protocol: The method of exposure employed in this study was inhalation. The animal was placed in a sealed, ventilated exposure chamber. Petroleum product (PMS, DPK, diesel) was gotten from 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 was placed in the exposure chamber and the animals was allowed to inhale the fumes evaporating from the cans. The exposure period lasted for 5 hours daily. The experiment lasted for twenty-eight (28) days. The time of exposure was between 9.00 am to 2.00 pm, after which the animals were transferred to the fume-free section of the experimental animal house.

Collection and preparation of respiratory tissue homogenate: The lungs of the sacrificed rat were harvested using the dissection method, and they were weighed and homogenized with a Potter-Elvehjem tissue homogenizer in a potassium phosphate buffer, 10Mm and pH 7.4. The respiratory tissue homogenate was centrifuged at 10,000 revolutions per minute for fifteen (15) minutes in a cold centrifuge, and the resultant supernatant was used for different estimations of oxidative stress markers.

Collection and preparation of hippocampal tissue homogenate: The rats were sacrificed using cervical dislocation, and the hippocampus of the sacrificed rat was harvested using a dissection method. The hippocampi were weighed and homogenized with a Potter-Elvehjem tissue homogenizer in a potassium phosphate buffer, 10Mm and pH 7.4. The tissue homogenate was centrifuged at 10,000 revolutions per minute for fifteen (15) minutes in a cold centrifuge, and the resultant supernatant was used for different estimations of oxidative stress markers.

Oxidative Stress Analysis

Hydrogen peroxide (H_2O_2) scavenging assay: Hydrogen peroxide (H_2O_2) was determined by the method of Ruch *et al.*[11]. A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (50 mM, pH 7.4). The concentration of hydrogen peroxide (H_2O_2) was determined by absorption at 230 ηM using a spectrophotometer. One milliliter of serum sample in two milliliters of physiological saline was added to hydrogen peroxide, and absorbance at 230 ηm was determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging was calculated as follows:

% scavenged (H_2O_2) = $[(A_i-A_t)/A_i] \times 100$. Where A_i is the absorbance of the control, and A_t is the absorbance of the test.

***Nitric oxide scavenging activity*:** The method illustrated by Marcocci *et al.*[12] was used. Two (2.0) mL of 10mM sodium nitroprusside dissolved in 0.5 mL. Phosphate buffer saline (pH 7.4) was mixed with 0.5 mL of the sample at various concentrations (0.2 – 0.8 mg/mL). The mixture was then incubated at 25 ^{0}C. After 150 min of incubation, 0.5 mL of the incubated solution was withdrawn and mixed with 0.5 mL of Griess reagent [(1.0 mL sulfanilic acid reagent (0.33 % in 20% glacial acetic acid at room temperature for 5min with 1.0 mL of naphthyl ethylenediamine dichloride (0.1% w/v)].

The mixture was incubated at room temperature for 30min, and its absorbance pouring into a cuvette was measured at 546 ηm. The amount of nitric oxide radical inhibition was calculated following this equation: % inhibition of NO radical = $[A_0-A_t]/A_0 \times 100$ Sample (μM TE/g).

Reduced glutathione (GSH) estimation: The method illustrated by Ellman[13] was used for the determination of reduced glutathione. The tissue homogenate (in 0.1 M phosphate buffer, pH 7.4) was taken and added with an equal volume of 20% trichloroacetic acid (TCA) containing 1.0 mM EDTA to precipitate the tissue proteins. The mixture was allowed to stand for 5 min before centrifugation for 10 min at 2000rpm. The supernatant (200 μL) was then transferred to a new set of test tubes and added with 1.8 mL of Eliman's reagent (5,5-dithiobis-2-nitrobenzoic acid (0.1 mM) prepared in 0.3 M phosphate buffer with 1% of sodium citrate solution). Then all the test tubes were made up to the volume of 2.0mL. After completion of the total reaction, the solution was measured at 412 ηm

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against the blank. Absorbance values were compared with a standard curve generated from known GSH.

Determination of superoxide dismutase (SOD): The method described by McCord and Fridovich[14] was applied to the determination of superoxide dismutase. It was estimated in the erythrocyte lysate prepared from the 5% RBC suspension. 50µL of the lysate, 7 Mm of Tris-HCl buffer (pH 8.2), 30 mM EDTA, and 2mM of pyrogallol added. An increase in absorbance was recorded at 420 nm for 3 min by spectrophotometer. One unit of enzyme activity were 50% inhibition of the rate of autooxidation of pyrogallol as determined by change in absorbance/min at 402ηm. The activity of SOD was expressed as units/mg protein.

Malondialdehyde: The levels of MDA were measured using thiobarbituric acid reactive substances (TBARS) as described by Donnan[15]. In all these methods, MDA reacts with TBARS in acidic medium at 100°C to generate a pink/red-coloured product which can be extracted with butanol and measured using a spectrophotometer at an absorbance of 520-535 nm or by a fluorimeter at excitation = 515 nm and emission = 555 nm. The TBARS method is rapid and easy; however, aldehydes other than MDA may also react with TBARS, producing derivatives that absorb light in the same wavelength range.

Statistical Analysis: All the statistical analyses were processed by using the Statistical Package of Social Science (SPSS) for Windows (version 16). The value of the measured parameters was expressed as mean ± SEM. Two-Way Analysis of Variance (2-way ANOVA) was used to determine the effect of different inhalants and days of post-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 a 0.05 probability level.

RESULTS

Oxidative Stress Parameters

Hydrogen peroxide scavenging activity of male Wistar albino rats exposed to petroleum products: The effect of petroleum product inhalation on H_2O_2 scavenging activity in male Wistar albino rats was presented. The baseline result (Day 0) showed no significant difference ($p>0.05$) between the test group and the control. There was also no difference in the mean value of H_2O_2 (mg/L) among the test groups. The H_2O_2 scavenging activity on the 14th day post-exposure was significantly lower between the test groups (28.46 ± 1.47, 25.36 ± 0.82, 29.06 ± 1.29, and 29.44 ± 1.97 mg/L) when compared with the control (31.16 ± 1.63 mg/L). There was a significant difference ($p < 0.05$) between groups B and C. The mean value of H_2O_2 was found to be significantly lower in the kerosene group compared to the other test groups. On day 28 of exposure, H_2O_2 scavenging activity maintained the same trend as on day 14, when compared with the control. Moreso, there were significant differences ($p<0.05$) among all test groups. All the petroleum products exhibited increased H_2O_2 scavenging activity on day 28, except in group E, which showed a further significant decrease ($p < 0.05$). On days of exposure, there was no significant difference ($p>0.05$) for the control, PMS, and diesel, except for day 28 of diesel, which showed a significant difference ($p<0.05$). However, kerosene and mixed petroleum products presented a significant difference ($p<0.05$) across exposure days (Table 1).

Nitric oxide (NO) activity of male Wistar albino rats exposed to petroleum products: The effect of petroleum product inhalation on nitric oxide activity in male Wistar albino rats was presented. The baseline result (Day 0) showed no significant difference ($p>0.05$) between the test group and the control. There was also no significant difference ($p>0.05$) in the mean value of nitric oxide activity (µ/mg) among the test groups. Nitric oxide activity significantly increased on the 14th day post-exposure between the test groups (4.73 ± 0.38, 5.22 ± 0.44, and 4.98 ± 0.06 µ/mg) except for group E, which was significantly lower (4.09 ± 0.16 µ/mg) when compared with the control (4.15 ± 0.59 µ/mg) ($p<0.05$). There was no significant difference between A and E, and no significant difference ($p>0.05$) between groups B, C, and D. The nitric oxide activity was found to be significantly higher in rats exposed to kerosene when compared to other test groups. On the 28th day of exposure, nitric oxide activity maintained the same trend as on day 14, when compared with the control. However, there were no significant differences ($p > 0.05$) in mixed petroleum product exposure when compared to the control. There was no significant difference ($p>0.05$) across the days of exposure for the control, kerosene, PMS, diesel, and mixed petroleum products except for day 14 of diesel, PMS, and day 0 of mixed petroleum products, which were significantly different ($p<0.05$) (Table 2).

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Table 1: Effect of petroleum product inhalation on hydrogen peroxide (mg/L) scavenging activity of male Wistar albino rats

GROUPS	DAY 0	DAY 14	DAY 28
A (Control)	31.16 ± 1.63^{a1}	31.04 ± 1.11^{c1}	31.04 ± 1.11^{b1}
B (PMS Exposure)	32.98 ± 4.45^{a1}	28.46 ± 1.47^{b1}	35.08 ± 1.24^{c1}
C (Kerosene Exposure)	31.10 ± 2.57^{a2}	25.36 ± 0.82^{a1}	41.86 ± 2.39^{d3}
D (Diesel Exposure)	31.78 ± 1.70^{a1}	29.06 ± 1.29^{b1}	34.72 ± 0.90^{c2}
E (Mixed PP)	33.10 ± 1.23^{a3}	29.44 ± 1.97^{b2}	24.34 ± 1.19^{a1}

The values are expressed as (mean ± SEM)

Mean values with different letters of the alphabet down the column are significantly different ($p<0.05$) while mean values with the same figures or number as superscript are not significantly different ($p>0.05$).

Table 2: Effect of petroleum product inhalation on Nitric Oxide (µ/mg) activity of male Wistar albino rats

GROUPS	DAY 0	DAY 14	DAY 28
A (Control)	5.13 ± 0.39^{a1}	4.15 ± 0.59^{a1}	4.15 ± 0.59^{a1}
B (PMS Exposure)	7.07 ± 1.04^{a2}	4.73 ± 0.38^{b1}	6.11 ± 0.31^{b2}
C (Kerosene Exposure)	6.07 ± 1.12^{a1}	5.22 ± 0.44^{b1}	7.25 ± 1.02^{c1}
D (Diesel Exposure)	6.25 ± 1.17^{a2}	4.98 ± 0.06^{b1}	7.82 ± 0.38^{c2}
E (Mixed PP)	6.49 ± 0.51^{a2}	4.09 ± 0.16^{a1}	4.90 ± 0.54^{a1}

The values are expressed as (mean ± SEM)

Mean values with different letters of the alphabet down the column are significantly different ($p<0.05$) while mean values with same figures or number as superscript are not significantly different ($p>0.05$).

Reduced glutathione level of male Wistar albino rats exposed to petroleum products

The effect of petroleum product inhalation on reduced glutathione levels in male Wistar albino rats was presented. The baseline result (Day 0) showed no significant difference ($p>0.05$) between the test group and the control. There was also no significant difference ($p>0.05$) in the mean value of reduced glutathione level (mg/dl) among other test groups. GSH level was significantly reduced on the 14th day post-exposure, between the test groups (2.69 ± 0.63, 1.92 ± 0.16, 2.44 ± 0.48, and 2.32 ± 0.55mg/dl) when compared with the control (3.28 ± 0.30mg/dl) ($p<0.05$). Group C showed no significant difference ($p>0.05$) with group D and E. GSH level was further reduced on day 28 of exposure. However, there was no significant difference ($p>0.05$) between kerosene and diesel, and no significant differences ($p>0.05$) between PMS and mixed petroleum products. There was no significant difference ($p>0.05$) across the days of exposure for all test groups and control except for day 14 of diesel and mixed petroleum products, which presented a significant difference ($p<0.05$) (Table 3).

Table 3: Effect of petroleum product inhalation on reduced glutathione (mg/dl) level of male Wistar albino rats

GROUPS	DAY 0	DAY 14	DAY 28
A (Control)	2.81 ± 0.49^{a1}	3.28 ± 0.30^{c1}	3.28 ± 0.30^{c1}
B (PMS Exposure)	2.14 ± 0.71^{a1}	2.69 ± 0.63^{b1}	1.76 ± 0.12^{b1}
C (Kerosene Exposure)	1.32 ± 0.12^{a1}	1.92 ± 0.16^{a1}	1.39 ± 0.27^{a1}
D (Diesel Exposure)	1.54 ± 0.42^{a1}	2.44 ± 0.48^{a2}	1.38 ± 0.16^{a1}
E (Mixed PP)	1.42 ± 0.26^{a1}	2.32 ± 0.55^{a2}	1.93 ± 0.31^{b1}

The values are expressed as (mean ± SEM)

Mean values with different letters of the alphabet down the column are significantly different ($p<0.05$) while mean values with the same figures or numbers as superscript are not significantly different ($p>0.05$).

Superoxide dismutase activity of male Wistar albino rats exposed to petroleum products

The effect of petroleum product inhalation on superoxide dismutase activity in male Wistar albino rats was presented. The baseline result (Day 0) showed no significant difference ($p > 0.05$) between the test groups and the control. There was

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also no significant difference ($p>0.05$) in the mean value of superoxide dismutase activity (μ/dl) among other test groups. SOD activity was reduced on the 14th day post-exposure in the test groups (11.57 ± 0.28, 11.13 ± 0.74, 11.41 ± 0.45, and 11.75 ± 1.39 μ/dl) compared to the control (12.87 ± 0.93 μ/dl). The superoxide dismutase activity among test groups showed no significant difference ($p > 0.05$) among groups B, C, D, and E. On day 28 of exposure, the mean value of superoxide dismutase activity maintained the same trend as on day 14, when compared to the control; the mean value of superoxide dismutase activity in the kerosene group was significantly lower ($p < 0.05$) compared to the other test groups. However, there was no significant difference ($p > 0.05$) among groups B, D, and E. On days of exposure, there was no significant difference ($p > 0.05$) across the days of exposure for control, PMS, kerosene, diesel, and mixed petroleum products except for day 28 of kerosene, which was significantly different ($p<0.05$) (Table 4).

Table 4: Effect of petroleum product inhalation on superoxide dismutase (μ/dl) activity of male Wistar albino rats

GROUPS	DAY 0	DAY 14	DAY 28
A (Control)	11.44 ± 0.82^{a1}	12.87 ± 0.93^{b1}	12.87 ± 0.93^{c1}
B (PMS Exposure)	10.56 ± 0.45^{a1}	11.57 ± 0.28^{a1}	10.21 ± 0.83^{b1}
C (Kerosene Exposure)	10.22 ± 0.76^{a2}	11.13 ± 0.74^{a2}	8.96 ± 0.93^{a1}
D (Diesel Exposure)	9.89 ± 1.26^{a1}	11.41 ± 0.45^{a1}	10.23 ± 0.30^{b1}
E (Mixed PP)	9.53 ± 0.33^{a1}	11.75 ± 1.39^{a1}	11.12 ± 0.48^{b1}

The values are expressed as (mean ± SEM)

Mean values with different letters of the alphabet down the column are significantly different ($p<0.05$) while mean values with the same figures or numbers as superscript are not significantly different ($p>0.05$).

Malondialdehyde (MDA) level of male Wistar albino rats exposed to petroleum products

The effect of petroleum product inhalation on malondialdehyde in male Wistar albino rats was presented. The baseline result (Day 0) showed no significant difference ($p > 0.05$) between the test groups and the control. There was also no significant difference ($p > 0.05$) in the mean value of MDA level (mg/dl) among other test groups. MDA level on the 14th day post-exposure was increased between the test groups (2.15 ± 0.31, 2.53 ± 0.35, 2.43 ± 0.55, and 2.02 ± 0.06mg/dl) when compared with the control (1.46 ± 0.20mg/dl) ($p < 0.05$). There was no significant difference ($p>0.05$) among groups B, D, and E. On the 28th day of exposure mean value of MDA maintained the same trend as day 14, except for group E; however, groups D and E showed no significant difference ($p > 0.05$) when compared to the control. On days of exposure, there was no significant difference ($p > 0.05$) across groups: control, PMS, kerosene, and diesel, except for the day 28 of group kerosene group, which was significantly different ($p < 0.05$). However, the mixed petroleum products group was significantly different ($p < 0.05$) when compared to day 0 (Table 5).

Table 5: Effect of petroleum product inhalation on malondialdehyde (mg/dl) level of male Wistar albino rats

GROUPS	DAY 0	DAY 14	DAY 28
A (Control)	2.60 ± 0.34^{a1}	1.46 ± 0.20^{a1}	1.46 ± 0.20^{a1}
B (PMS Exposure)	2.67 ± 0.32^{a1}	2.15 ± 0.31^{b1}	2.95 ± 0.22^{b1}
C (Kerosene Exposure)	3.74 ± 0.57^{a1}	2.53 ± 0.35^{c1}	4.50 ± 1.07^{c2}
D (Diesel Exposure)	3.11 ± 0.41^{a1}	2.43 ± 0.55^{b1}	1.60 ± 0.16^{a1}
E (Mixed PP)	4.59 ± 0.32^{a2}	2.02 ± 0.06^{b1}	1.20 ± 0.14^{a1}

The values are expressed as (mean ± SEM)

Mean values with different letters of the alphabet down the column are significantly different ($p<0.05$) while mean values with the same figures or numbers as superscript are not significantly different ($p>0.05$).

DISCUSSION

Oxidative stress parameters of male Wistar albino rats exposed to petroleum products

The current research demonstrated that the inhalation of petroleum products significantly increased H_2O_2 activity in a time-dependent manner, indicating that petroleum

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products induced high antioxidant activity. These results are consistent with the work of Moronkeji *et al.*[16], who found that exposure to gasoline generator fumes caused a rise in H_2O_2 levels in male Wistar rats, depending on the duration of exposure.

The present study showed that petroleum product inhalation significantly increased nitric oxide activity in a time-dependent manner. The results are in agreement with Moronkeji *et al.*[16], who reported increased NO levels in male Wistar rats exposed to gasoline generator emissions. Their study highlighted the role of air pollutants in promoting intracellular oxidative stress, with the increase in NO attributed to prolonged exposure and accumulation of toxic byproducts. However, the decline in NO levels observed by Oni *et al.*[17] in mice exposed to polluted groundwater suggests that prolonged or indirect exposure routes may impair NO synthesis or increase its breakdown due to persistent oxidative damage. The conflicting outcomes may stem from variations in experimental conditions.

The study revealed that petroleum product inhalation significantly decreased GSH levels, indicating poorer health and increased susceptibility to oxidative stress. This finding agreed with Khan *et al.*[18], who reported a decline in intracellular GSH levels following exposure to crude oil paint, demonstrating the harmful effects of hydrocarbon-based substances on antioxidant systems.

The present study showed that petroleum product inhalation significantly decreased superoxide dismutase activity. This outcome supports the work of Kemabonta *et al.*[5] and Azeez *et al.*[19] who documented a notable decrease in SOD activity in animals exposed to petroleum vapours, highlighting the vulnerability of antioxidant enzymes to hydrocarbon pollutants.

The present study demonstrated that petroleum product inhalation significantly increased the malondialdehyde level. This is consistent with the work of Owagboriaye *et al.*[20]; Fang *et al.*[21]; Moronkeji *et al.*[16] who demonstrated that increased MDA in lung tissue corresponds with heightened lipid peroxidation following inhalation of harmful airborne chemicals.

Strengths and limitations of the study

The study's examination of the impact of petroleum product inhalation on oxidative stress measures in male Wistar albino rats exhibits a number of strengths. The adoption of a controlled experimental design with a well-defined control group, which enables efficient comparison between exposed and non-exposed animals, is one of its main advantages. The scientific validity of the results is further improved by the use of Wistar albino rats, a widely used model in toxicological research. The study also assesses several biomarkers of oxidative stress, such as nitric oxide, reduced glutathione, superoxide dismutase, hydrogen peroxide scavenging activity, and malondialdehyde.

Studying multiple biochemical markers leads to greater insight of oxidative damage and antioxidant defense systems. Another strength is the comparison of several petroleum compounds, such as petrol (PMS), kerosene, diesel, and their mixtures, which increases the data' relevance to real-world environmental exposures. Despite these strengths, the research has several limitations. The very small sample size of forty-five rats may restrict the statistical power and generalizability of the findings. Furthermore, the exposure parameters, such as the exact vapor concentration and duration of daily treatment, were not properly stated, potentially affecting repeatability. The study also used only male rats and lasted only 28 days, limiting insight into long-term or sex-related consequences.

Implications of the findings

The study underscores the need for strict environmental regulations and public health interventions to minimize exposure to petroleum products in order to protect vulnerable populations. Further studies are therefore recommended to evaluate the long-term impacts of low doses of petroleum products. Determining the exact concentration inhaled by the animals helps in the establishment of a clearer dose-response relationship and comparison with exposure levels often observed in occupational settings such as gasoline stations and refineries.

Extending the length beyond the twenty-eight days used in this study might provide more information about the long-term and cumulative effects of petroleum products inhalation on significant organs. Increasing the sample size to include both male and female animals will strengthen the statistical strength of the findings and allow for the examination of any sex-related differences in susceptibility to petroleum toxicity. Further research should broaden the scope of biochemical indicators studied and combine these findings with a thorough histological evaluation of the lungs and hippocampus. Incorporating behavioral and neurological tests may also

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assist identify whether oxidative changes in the hippocampus are linked to functional deficits.

CONCLUSION

The findings inferred that exposure to petroleum products induces oxidative stress, as evidenced by increased levels of reactive oxygen species and alterations in antioxidant enzyme activity.

Abbreviation and Nomenclature

SOD: Superoxide Dismutase
H_2O_2: Hydrogen Peroxide
MDA: Malondialdehyde
GSH: Reduced Glutathione
NO: Nitric Oxide
ROS: Reactive Oxygen Species
DNA: Deoxyribonucleic Acid
PMS: Premium Motor Spirit
DPK: Dual Purpose Kerosene
NIH: National Institute of Health
NNPC: Nigerian National Petroleum Corporation
EDTA: Ethylenediaminetetraacetic acid
TBARS: Thiobarbituric Acid Reactive Substances
SPSS: Statistical Package of Social Science
ANOVA: Analysis of Variance

Declarations

Conflict of Interest Declaration: The authors declare that there is no conflict of interest.

Ethical Conformity Statement: All experimental procedures involving animals were conducted in accordance with the National Institute of Health (NIH) guidelines for the care and use of laboratory animals. Ethical approval was obtained from the appropriate Institutional Animal Ethics Committee of Enugu State University of Science and Technology (ESUT), Enugu, Nigeria.

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