ISSN: 2714-4674 (Online)
ISSN: 2714-4666 (Print)

Annals of Clinical and Experimental Medicine

(ACEMedicine)

This Journal Is A Publication of
ASSOCIATION OF SPECIALIST MEDICAL DOCTORS IN ACADEMICS SOKOTO STATE CHAPTER

Volume 3, No. 1, January - June 2022

In this issue

www.acemedicine.asmeda.org

Prophylactic and Therapeutic Effect of *Moringa oleifera* Leaf Extract on Petrol-Induced Hematological, Serum Biochemical, and Histological Changes in Wistar Rats

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ACCESS TO THIS ARTICLE ONLINE

DOI: 10.47838/acem.26011977.127132022.asmeda.3.6

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Abstract

Background: Premium motor spirit (Petrol or gasoline) is a complex mixture of volatile hydrocarbons derived through distillation from crude petroleum, it's a mixture of complex compounds (Aliphatic and aromatics) such as benzene, xylene, toluene, hundreds of saturated and unsaturated hydrocarbons and their by-products contribute to air pollution and global warming. High-energy-demanding equipment, machines and erratic electricity supply leads to the use of generators, and predispose households, their environment to emitted hydrocarbons and causes environmental pollution. Cardiac failure and sudden death are on the increase, the causes of which are neither often diagnosed at the hospital nor at postmortem. Moringa oleifera has medicinal uses which include antioxidant, anti-inflammatory and wound healing properties. Zeatin, quercetin and alkaloids present in Moringa oleifera have been found to have some ameliorative effects against the petroleum hydrocarbons-linked health hazards.

Materials and Methods: An experimental study was conducted, a total of 25 adult Wistar rats randomly divided into five groups: group A (negative control); group B (Exposed to petrol fumes only); group C (treated with Moringa extract later exposed to petrol fumes); group D (exposed to petrol fumes later treated with Moringa); and group E (treated with Moringa only). 40mg/kg/rat of Moringa extract and 0.008 cm3/min/rat of petrol were used for this study.

Results: The results showed polycythemia, neutrophilia, and lymphocytosis. Serum biochemistry analysis revealed hyponatremia, hyperkalemia, and hyperchloremia while histology slides show no tissues damage and treatment with Moringa oleifera showed a positive response.

Conclusion: Petrol caused changes in cardiovascular parameters, and prophylactic and treatment with Moringa oleifera leaf extract showed a promising result.

Keywords: opioids, acute urinary retention

Introduction

Petrol, often known as gasoline, is a complex mixture of volatile hydrocarbons that is made from crude petroleum through distillation (1). The burning of petrol is a significant source of ambient air pollution and global warming (2). In addition, petrol is a mixture of hundreds of saturated and unsaturated hydrocarbons, including aliphatic aromatics like benzene and toluene (3). However, exposure to petroleum hydrocarbons (benzene and xylene) has been linked to cardiovascular illness in both clinical and experimental trials (4). Workers are at danger of developing various diseases that affect various bodily systems, including the cardiovascular system, due to ongoing exposure to the vapors of this mixture and working circumstances at occupational settings (5). Exposure to petroleum products causes cardiotoxicity, baroreflex disability, and an increase in blood pressure and heart rate (6,7). It was approved that the toxicity of inhaled vapors, including benzene, is due to its biotransformation into reactive oxygen species. The liver converts benzene to phenol, which is then subjected to hydroxylation to produce hydroquinone and 1,4-benzenetriol by bone marrow peroxidase or by autoxidation (8). The toxic effects of benzene and its metabolites on the hematological system result in bone marrow suppression (9). The main harmful effect of benzene on personnel exposed to it continually is increased sensitivity to injuries and infections due to leucopoiesis suppression (10). Because it includes protein, vitamins, and a variety of phenolic compounds, *Moringa oleifera* has a high nutritional value (11,12). *Moringa oleifera*'s zeatin, quercetin, and alkaloids have been discovered to have some ameliorative effects against health risks associated with petroleum hydrocarbons (7,13,14).

Hematological assessments are thought to be a crucial technique for assessing an animal's health without killing it (15). Animals exposed to toxicants and other situations have blood that serves as a pathological mirror of their health (15). Studies have shown that Wistar rats exposed to petrol vapors (inhalation) have higher white blood cell (WBC) counts and lower packed cell volume (PCV), hemoglobin (Hb), and red blood cell (RBC) counts (16). The distribution of electrolytes in extracellular and intracellular fluid determines how the heart beats electrically, so any change in the concentration of any one of the electrolytes will affect the electrical activity of the heart. In the physiology of the cardiovascular system, calcium is crucial. Aberrations from a normal serum calcium level are known to be

Early Proof

associated with several cardiovascular diseases, and they are known to play a crucial role in the pathophysiology of many diseases (17).

Poor electricity supply leads to the use of generators, which predisposes household owners to hydrocarbons. High-energy-demanding equipment and machines also use petroleum products, which emit hydrocarbons into the environment and cause environmental pollution that has resulted in various health challenges (heart problems) in both animals and humans. This study will provide updated data on the leverage of *Moringa oleifera* in preventing the damaging effects of petroleum product fumes on the cardiovascular system. The data will also evaluate the prospective and promise of *Moringa oleifera* as a clinical remedy for prophylactic use against petroleum-borne intoxications. The aim of this study is to evaluate the prophylactic effects of *Moringa oleifera* leaf extract on petrol-induced cardiovascular dysfunctions in the Wistar Albino rats' model.

Materials and Methods

Study Area: The study was conducted in the laboratory of the Department of Veterinary Physiology and Biochemistry, Faculty of Veterinary Medicine, Usmanu Danfodiyo University, Sokoto, Sokoto State. Sokoto State is in the extreme northwest of Nigeria, with a land area of 28,232.37 square kilometers. The State is located between longitudes 11° 30` to 13° 50` East and latitudes 4° to 6° North. It is bordered in the North by the Niger Republic, Zamfara State to the east, and Kebbi State to the South and West (18)

Experimental Animals and Acclimatization: A total of 25 male and female adult Wister rats weighing between 130 and 250g were purchased from the animal house at the Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University Sokoto, Sokoto. They were housed in a well-ventilated cage. The rats were on standard rat chow and tab water *ad' libitum*. They acclimatized for two weeks before the experimental period (4) under a control temperature of (25 ± 2°C) room on a normal 12-h light-dark cycle.

Study Design: An experimental study was conducted between February and April 2021. A random sampling technique was employed. A total of 25 male and female adult Wister rats aged 8 – 10 weeks, weighing between 130 – 250 g, were randomly divided into five groups, comprising five rats in each group: Group A (negative control); Group B: exposed to petrol fume 5 minutes daily for 8 weeks, served as treatment group; Group C: treated with *Moringa oleifera* for 2-3 hours and later exposed to petrol fume 5 minutes daily for eight weeks, served as prophylactic group; Group D: exposed to petrol fume 5 minutes daily for 8 weeks and later treated with *Moringa oleifera* extract daily for additional 4 weeks, served as treatment group; and Group E: treatment with *Moringa* extract daily for eight weeks, served as positive control II. Forty (40) mg/kg/rat of 80% methanolic leaf extract of *Moringa oleifera* was used as the dosage throughout the study period; this was obtained after a toxicity study.

Plant Materials/Preparation of Plant Extract: *Moringa oleifera* leaves were obtained from Danchadi village, Bodinga LGA, Sokoto State as seen in Plate 1. The 80% methanol cold extraction method was used (19). The plant was identified at the herbarium unit of the Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto (PCG/UDU/SOR1/0001).

Plate 1: *Moringa oleifera* leaves.

Exposure to Petrol: A modified human nebulizer nose inhalation exposure method was used as described by (4) as seen in Plate 2, 3, and 4. Group A was not given anything (negative control), group B was exposed to petrol fume only for 8 weeks, group C was treated with *Moringa oleifera* leaf extract using an oral cannula for 2-3 hours before they were placed in the fume chamber of petrol for five minutes, group D was exposed to petrol vapor for 8 weeks after being treated with *Moringa* extract, and group E was treated with *Moringa* only. The average exposure dosage was 0.5 ± 0.008 $cm^3/min/kg/m^3/day$ (4). The fume chamber is a 10-liter bucket with a very tight lid. During the exposure period, rats from each group were placed in the chamber, the nebulizer cup was filled with petrol and the liquid petrol turned to vapor. They were allowed to stay in the fume chamber for 5 minutes (0.5 ± 0.08 $cm^3/min/kg/m^3/day$) and removed back to their cages in the vapor free section of the experimental room. This was done for all the exposed groups daily for eight weeks.

Evaluations of Hematological and Biochemical Parameters: Blood samples were collected after anesthetized with chloroform as approved by FAREC (see below; ethical approval), via the retro-orbital sinus into the EDTA bottles for a complete hemogram and into the plain sample bottles, centrifuged at 3000 rpm for 15 minutes, and serum was obtained for serum biochemistry analysis for sodium, calcium, chloride, and potassium.

Gross and Histological examination: The rats were later sacrificed using chloroform as approved by FAREC (see below; ethical approval) ; the heart was removed, dropped into formalin-saline, and prepared for histology. Using H & E staining technique. The technique was done in the Department of Veterinary Anatomy and Histology, Faculty of Veterinary Medicine, Usmanu Danfodiyo University Sokoto. Nigeria.

Ethical approval: Ethical approval was sought from the Faculty Animal Research and Ethics Committee (FAREC) of the Faculty of Veterinary Medicine, Usmanu Danfodiyo University, Sokoto (UDUS/FAREC/AUP-R16/2019). Procedures involving animals and their care were performed in accordance with the National Institute of Health (NHI) guidelines for the care and use of animals.

Statistical Analysis: Data are expressed as means ± standard error of means (SEM); statistical analysis was done using a one-way ANOVA and Tukey-Kramer Multiple Comparisons Test. If the value of q is greater than 4.232 then the P value is less than 0.05. P <0.05 was considered significant. All analysis was done using SPSS software. In case of abnormal distribution Kruskal-Wallis Test (Nonparametric ANOVA) was used.

Plate 2: Human Nebulizer

Plate 3: Inhalation bucket

Plate 4: Inhalation chamber

Results

The results of the study on the effect of petrol on the hematological parameters of Wistar rats are presented in Table 1. There was a statistically significant increase (P <0.05) in pack cell volume count (P < 0.0017), hemoglobin (P < 0.04), red blood cells count (P < 0.0015), mean corpuscular volume (P < 0.0070), and mean corpuscular hemoglobin (P < 0.0015) in the treatment group. However, there were no statistically significant (P > 0.05) differences in white blood cells count (P > 0.4648) and mean corpuscular hemoglobin concentrations (P > 0.5648).

There was a statistically significant (P <0.05) increase in neutrophils (P < 0.0596) in the petrol-treated group (Group B) compared to the negative control (Group A), Group C, Group D and Group E. There was a significant (P <0.05) decrease in lymphocytes in the petrol-treated group (Group B) compared to the negative control (Group A), Group C, Group D and Group E. However, there were no statistically significant (P <0.05) differences in monocytes (P > 0.6242), eosinophils, or basophils in all the groups.

The effect of petrol on serum biochemical parameters in Wistar rats is presented in Table 2. There was a significant (P < 0.05) decrease in Sodium (P < 0.0039) in the petrol-treated group (Group B), compared to Group C, Group D and Group E. There was a statistically significant (P < 0.05) decrease in Potassium (P < 0.0003) in petrol-treated group (Group B) compared to Group C, Group D and Group E. There was also a statistically significant (P <0.05) decrease in chloride (P < 0.0255) in petrol treated (Group B) compared to Group C, Group D and Group E. There was a statistically significant (P < 0.05) decrease in Calcium (P < 0.0210) in petrol treated (Group B) compared to Group C, Group D and Group E.

Histological examination of the longitudinal section of ventricular myocardium of the Wistar rats (Group A: Negative control) showed normal structure as showed in figure 1.

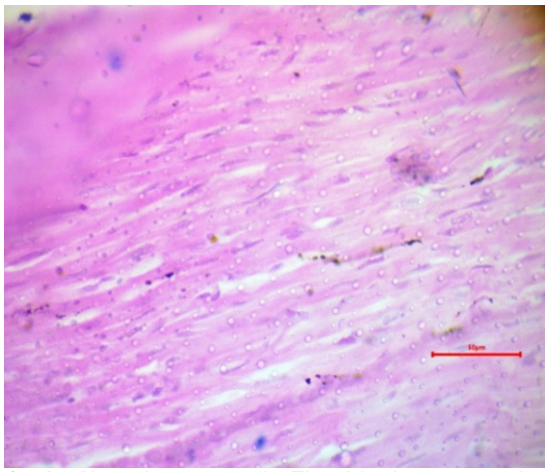

Fig1: A: Negative control. The black arrow: showing photomicrographs of the longitudinal section of ventricular myocardium of the rats at 400 magnification using H & E staining technique.

Table 1: Means and standard deviation of PCV, Hb, RBC, MCV, MCH, MCHC and differential leucocytes count of rats exposed to petrol and administered *Moringa oleifera* leaf extract (N = 5)

Parameters	A	B	C	D	E
PCV (%)	38.40 ± 0.87 ^{b}	41.20 ± 1.07 ^{ab}	40.60 ± 0.75 ^{ab}	32.20 ± 3.07 ^{bc}	42.40 ± 0.93 ^{a}
HB (g/dL)	11.84 ± 0.28 ^{b}	12.73 ± 0.36 ^{ab}	12.57 ± 0.27 ^{ab}	12.34 ± 1.22 ^{ab}	13.09 ± 0.32 ^{a}
RBC x 10^{6}/mm^{3}	4.69 ± 0.41 ^{c}	7.08 ± 0.19 ^{a}	5.89 ± 0.20 ^{b}	4.66 ± 0.72 ^{bc}	5.33 ± 0.06 ^{bc}
MCV (fl)	8.19 ± 0.46	5.82 ± 0.88	6.90 ± 0.55	6.0 ± 2.35	7.95 ± 0.61
MCH (pg/cells)	2.52 ± 0.13	1.80 ± 0.17	2.13 ± 0.07	2.65 ± 0.50	2.46 ± 0.26
MCHC (g/dl)	0.31 ± 0.02	0.31 ± 0.02	0.31 ± 0.02	0.38 ± 0.02	0.31 ± 0.02
WBC x 10^{3}/mm^{3}	5.28 ± 1.39	5.65 ± 0.43	5.87 ± 1.27	3.93 ± 0.45	3.90 ± 0.94
Neutrophils %	16.80 ± 1.24 ^{b}	26.20 ± 1.77 ^{a}	18.80 ± 2.03 ^{b}	18.60 ± 3.27 ^{ab}	18.60 ± 1.96 ^{b}
Lymphocytes %	81.80 ± 0.92 ^{a}	72.80 ± 2.35 ^{b}	80.60 ± 2.11 ^{a}	80.20 ± 3.12 ^{ab}	79.40 ± 1.63 ^{a}
Monocytes %	1.40 ± 0.60 ^{ab}	1.40 ± 0.68 ^{ab}	0.60 ± 0.24 ^{b}	1.20 ± 0.37 ^{ab}	1.80 ± 0.37 ^{a}
Eosinophils %	0.00 ± 0.00	0.40 ± 0.24	0.00 ± 0.00	0.00 ± 0.00	0.20 ± 0.20
Basophils %	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

KEY: A: Negative Control; B: Exposed to petrol fume at 0.008 cm^{3}/min/rat only; C: Treated with 40 mg/kg *Moringa oleifera* leaf extract then Exposed to petrol fume at 0.008 cm^{3}/min/rat; D: Exposed to petrol fume at 0.008 cm^{3}/min/rat later treated with 40 mg/kg *Moringa oleifera* leaf extract and E: treated with 40 mg/kg *Moringa oleifera* leaf extract only; PCV: Packed cell volume; Hb: Hemoglobin; RBC: Red blood cells; MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration; WBC: White blood cells. ^{abc}Means with different superscript is considered statistically significant (P <0.05). Using one-way ANOVA and Tukey-Kramer Multiple Comparisons Test.

Table 2: Means and standard deviation of Na^{+}, K^{+}, Cl^{-}, and Ca^{2+} of rats exposed to petrol and administered *Moringa oleifera* leaf extract (N = 5)

Parameters	A	B	C	D	E
Na^{+} (mmol/l)	142.80 ± 0.97 ^{b}	127.00 ± 4.44 ^{c}	131.60 ± 0.74 ^{c}	147.00 ± 0.71 ^{a}	135.60 ± 6.23 ^{abc}
K^{+} (mmol/l)	4.76 ± 0.17 ^{b}	27.24 ± 3.70 ^{a}	21.06 ± 0.96 ^{a}	5.5 ± 0.45 ^{b}	15.18 ± 6.36 ^{ab}
Cl^{-}(mmol/l)	106.60 ± 1.33 ^{b}	112.40 ± 2.73 ^{a}	109.80 ± 0.37 ^{ab}	105.80 ± 0.58 ^{b}	106.40 ± 1.29 ^{ab}
Ca^{2+} (mmol/l)	2.54 ± 0.08 ^{a}	2.05 ± 0.18 ^{ab}	1.98 ± 0.13 ^{b}	2.67 ± 0.13 ^{a}	1.93 ± 0.30 ^{ab}

KEY: A: Negative Control; B: Exposed to petrol fume at 0.008 cm^{3}/min/rat only; C: Treated with 40 mg/kg *Moringa oleifera* leaf extract then Exposed to petrol fume at 0.008 cm^{3}/min/rat; D: Exposed to petrol fume at 0.008 cm^{3}/min/rat later treated with 40 mg/kg *Moringa oleifera* leaf extract and E: treated with 40 mg/kg *Moringa oleifera* leaf extract only; Na^{+}: Sodium; K^{+}: Potassium; Cl^{-}: Chloride; and Ca^{2+}: Calcium. ^{abc}Means in a row with a different superscript differ significantly (P < 0.05). The data was analyzed using one-way ANOVA and Tukey-Kramer Multiple Comparisons Test.

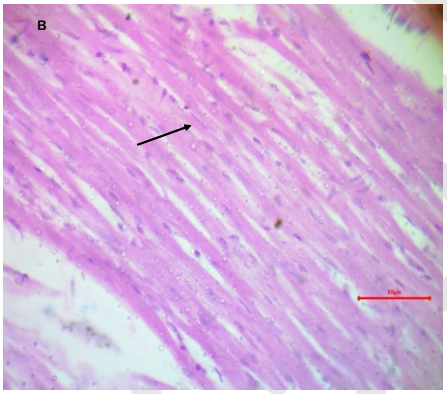

Fig2: B: Exposed to petrol fume only. The black arrow: showing photomicrographs of the longitudinal section of ventricular myocardium of the rats at 400 magnification using H & E staining technique.

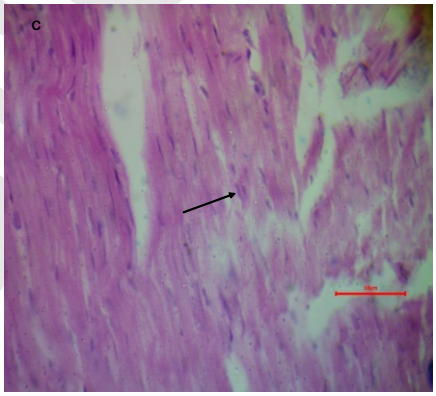

Fig3: C: Given *Moringa oleifera* later exposed to petrol fume; The black arrow: showing photomicrographs of the longitudinal section of ventricular myocardium of the rats at 400 magnification using H & E staining technique.

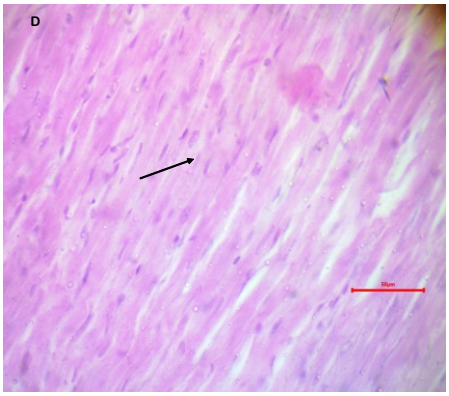

Fig4: D: Exposed to petrol fume for 8 weeks and later treated with *Moringa oleifera* leaf extract: The black arrow: showing photomicrographs of the longitudinal section of ventricular myocardium of the rats at 400 magnification using H & E staining technique.

Histological examination of the longitudinal section of ventricular myocardium of the rats exposed to petrol only (Group B) showed normal structure, similar to the structural morphology of myocardium observed in rats from the control group as showed in figure 2.

Histological examination of the longitudinal section of ventricular myocardium of the rats given *Moringa oleifera* for 2-3 hours and later exposed to petrol fume (Group C) showed normal structure, similar to the structural morphology of myocardium observed in rats from the control group as showed in figure 3.

Histological examination of the longitudinal section of ventricular myocardium of the rats exposed to petrol fume for 8 weeks and later treated with *Moringa oleifera* (Group D) showed normal structure, similar to the structural morphology of myocardium observed in rats from the control group as showed in figure 4.

Histological examination of the longitudinal section of ventricular myocardium of the rats given *Moringa oleifera* only (Group E) showed normal structure, similar to the structural morphology of myocardium observed in rats from the control group as showed in figure 5.

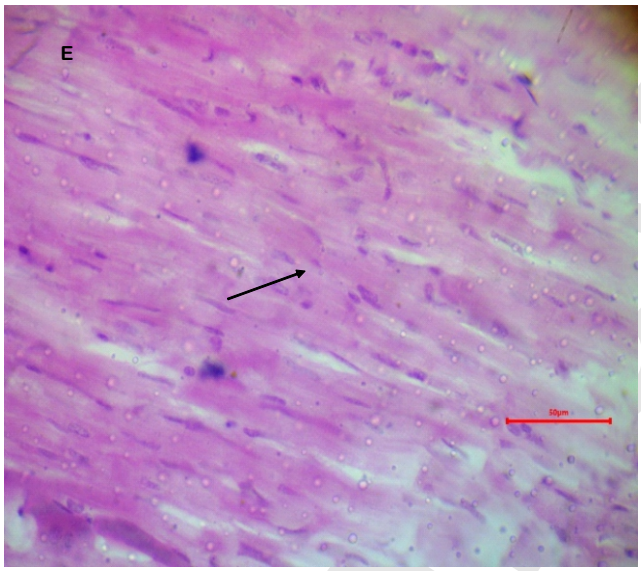

Fig5: E: Given *Moringa* oleifera only: The black arrow: showing photomicrographs of the longitudinal section of ventricular myocardium of the rats at 400 magnification using H & E staining technique.

Discussions

Inhalation exposure to petrol fumes for 5 minutes daily for eight weeks in rats causes an increase in packed cell volume, hemoglobin, and red blood cell count, which leads to polycythemia. This is contrary to the findings of (20, 16), who reported a decrease in PCV, hemoglobin and RBC after exposure to petrol fumes. The polycythemia might be secondary absolute polycythemia, which is caused by a physiologically appropriate release of erythropoietin resulting from chronic hypoxemia because of pulmonary and cardiac compromise, an anomaly with right-to-left shunting, or hemoglobinopathy. *Moringa oleifera* seemed to have protected the membrane integrity of the erythrocytes, thereby stabilized the cells, and made them osmotically resistant to the redox effect of petrol, as seen in group C (treated with *Moringa* extract and later exposed to petrol), which is consistent with the work of (20). Neutrophilia and lymphocytosis were seen in this study. This is consistent with the work of (21). The neutrophilia and lymphocytosis were due to epinephrine release, which is usually caused by fear, excitement, and vigorous exercise, which in turn causes a transient mature neutrophilia by shifting neutrophils from the marginal neutrophil pool to the circulating neutrophil pool, which likewise causes increased blood flow and washes marginated lymphocytes back into circulation (21). The decrease in sodium level (hyponatremia) observed in this study is similar to the finding observed by (22, 23), who reported low levels of serum sodium in acute myocardial infarction. This could also be due to the hydrocarbon components of the petroleum products that interfere with the membrane sodium pump mechanism that maintains a low level of sodium ion concentration. The increased potassium level (hyperkalemia) observed in this study after exposure to petrol fumes in rats is similar to the findings of (24) who report smoking shows a strong association with serum potassium level increase but is contrary to the findings of (22) who reported a low level of serum potassium level in acute myocardial infarction. The increase in serum potassium level might be due to a decrease in cardiac output or increase in cardiac pressure, which caused decreased renal perfusion and subsequently reduced renal filtration and hence hyperkalemia. Likewise, decreased renal perfusion activates the Renin-Aldosterone-Angiotensin System, which in turn increases Angiotensin II and Aldosterone concentration, hence hyperkalemia. The increased chloride (hyperchloremia) serum level observed in this study after exposure to petrol is similar to the findings of (22). The hyperchloremia might be due to decrease in renal perfusion and respiratory alkalosis which occurs due to excessive breathing of the petrol vapor. Similarly, *Moringa oleifera* has been able to prevent the effects of petrol for both sodium, potassium, and chloride (seen in group B); this is similar to the findings of (4, 7 and 20). The decrease in calcium (hypocalcemia) level observed is contrary to the findings (25) who reported that the primary causes of acute kidney injury include ischemia, hypoxia, or nephrotoxicity. The hypocalcemia might be due to disruption of calcium regulation when the heart muscle is damaged, when the heart pumping ability is reduced due to damage it causes reduced cardiac output, and hence affects the blood flow to the kidneys, leading to altered calcium handling by the kidneys, which can contribute to hypocalcemia. This study showed no significant reduction in body weight when compared between the negative control and exposed groups, and this is contrary to the findings of (26, 27 and 20), who reported exposure to petrol vapor for 10 minutes daily for eight weeks caused an incredibly significant reduction in body

weight. In this study, the histological findings show no tissue damage. This is contrary to the work of (20), who reported a range of degrees of cellular degeneration in rats after exposure to gasoline, diesel, and kerosene for 10 minutes daily for eight weeks. This is because the rats in this study were exposed to petrol 5 minutes daily for 8 weeks, and probably the damage is still in progress.

Conclusion

Increased PCV, Hb, and RBC, as well as neutrophilia and lymphocytosis, are suspected to be of cardiac origin, as found in this research. The decrease in sodium and increased potassium and chloride concentrations in the blood found in this research are also indicative of acute myocardial injury. Histological findings in this research showed no evidence of architectural damage to the heart tissues. In conclusion, petrol caused cardiovascular parameters changes, and prophylactic and therapeutic administration of *Moringa oleifera* leaf extract showed promising results.

Acknowledgment

My sincere and profound appreciation goes to my lovely mother and late father for their sacrifice. I want to acknowledge the efforts of all staff of the Department of Physiology and Biochemistry, Faculty of Veterinary Medicine, Usmanu Danfodiyo University Sokoto, Nigeria.

Conflict of Interest

There is no conflict of interest.

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