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Ameliorative effects of *Moringa oleifera* on haematology, cardiac-troponin-I, electrolytes and histology of Wistar rats exposed to diesel fumes

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Abstract

Diesel fuel is a complex mixture of hydrocarbons obtained and used in various applications, such as motorboats, heavy equipment, generators, trucks, buses, and trains. However, the emissions from diesel engines contribute to air pollution and compromise air quality. This experimental study was carried out to evaluate the prophylactic and therapeutic effects of *Moringa oleifera* on diesel-induced changes on cardiovascular parameters. A total of 25 adult Wistar rats were randomly divided into five groups: group A (negative control); group B (exposed to diesel fumes only); group C (treated with *Moringa* extract and later exposed to diesel fumes); group D (exposed to diesel fumes and later treated with *Moringa* extract); and group E (treated with *Moringa* extract only). Forty (40) mg/kg/rat of 80% methanolic leaf extract of *Moringa oleifera* and 0.008 cm^3/min/rat of fumes from liquid diesel were used as dosage of extract and volume of fumes respectively. The haematology showed polycythemia in the exposed group. Serum biochemical analysis revealed hyponatremia, hyperkalemia, hyperchloremia, and hypercalcemia in the exposed groups. The result also revealed an increase in cardiac troponin-I concentration. In conclusion, exposure to diesel fumes caused changes in cardiovascular parameters, likewise prophylactic and therapeutic administration of *Moringa oleifera* leaf extract showed a promising result.

Keywords: Cardiac-troponin-I, Diesel, Electrolytes, Haematology, Histology, *Moringa Oleifera*, Wistar Rat

Introduction

Diesel fuel, a complex mixture of hydrocarbons is obtained from crude oil distillation and enhanced with specific additives to improve performance. It exists as a liquid with a distinct odour, and is used in various applications, such as motorboats, heavy equipment, generators, trucks, buses, and trains (Chilcott, 2006). However, the emissions from diesel engines contribute to air pollution and compromise air quality (Chilcott, 2006). Many veterans working in the oil and gas industry have reported exposure to diesel fuel and oil fires (Stuart *et al.,* 2002). Long-term inhalation of diesel exhaust particles (DEPs) has been linked to respiratory tract inflammation (Kafoury & Madden, 2005), wheezing, bronchitis, and asthma (Koike *et al*., 2004). Additionally, exposure to diesel through ingestion, inhalation, or skin contact can lead to various toxic effects, including headaches, dizziness, respiratory distress, and skin irritation (Chilcott, 2006). Diesel possesses specific physicochemical properties, such as low volatility, flammability, and the potential to release toxic fumes during combustion (Schwarze *et al*., 2013).

The primary function of the cardiovascular system is to convey blood to tissues, which provides the cells with necessary nutrients for digestion and protects the cells from waste products (Klein, 2020). Diesel causes numerous dysfunctions of the heart after exposure (Sun *et al*., 2020). DEPs have been shown to impair endothelial function, which is crucial for regulating blood vessel tone and maintaining vascular health (Sun *et al*., 2020). Exposure to diesel can promote the development of atherosclerotic lesions by triggering inflammatory responses and promoting the accumulation of lipid deposits in blood vessels (Bai *et al*., 2011). Chronic exposure to diesel exhaust has been associated with adverse changes in cardiac structure and function which are characterized by hypertrophy of the heart muscle and fibrosis, which can impair cardiac contractility and contribute to heart dysfunction (Bradley *et al*., 2013). Diesel exhaust exposure has been shown to disrupt autonomic nervous system balance, specifically by increasing sympathetic activity and reducing parasympathetic activity. This can result in abnormal heart rate variability, blood pressure dysregulation, and an increased risk of cardiac arrhythmias (Rankin *et al*., 2021).

The cardiac regulatory proteins that control the calcium mediated interaction between actin and myosin are called cardiac troponin T (cTnT) and troponin I (cTnI) (Bertrand *et al*., 2000). Cardiac troponins are the biomarkers of choice currently available for the diagnosis of myocardial injury because they are the most sensitive and cardiac-specific laboratory measures of myocardial injury (Thygesen *et al*., 2010; Agewall *et al*., 2011). The sensitivity of cTnI/T for myocardial injury is well established, but claiming specificity for any disease is not feasible (Thygesen *et al*., 2012). The sarcomere, the basic contractile unit of the heart, consists of thick and thin filaments composed of myosin and actin, respectively.

Moringa is widely distributed and still used as an herbal plant is still trustworthy and widely used as one of the alternatives in the medicinal field (Abalaka *et al.,* 2009). *Moringa oleifera* has high nutritional value because it contains protein, vitamins, and various phenolic compounds (Anwar *et al*., 2007; Munguro & Lemma, 2007). Zeatin, quercetin, and alkaloids present in *Moringa oleifera* leaf have been found to have some ameliorative effects against hydrocarbon-linked health hazards (Azeez *et al*., 2015). *Moringa oleifera* is rich in antioxidants (Chumark *et al*., 2008; Oliveira *et al.*, 1999). Arti Verma & Chandana (2009), and Sreelatha & Padma (2009) reported that the antioxidant compounds found in the leaf of *Moringa oleifera* have therapeutic potential for the prevention of cardiovascular diseases.

Poor electricity supply leads to the use of generators, which exposes household owners to hydrocarbons. High-energy-demanding equipment and machines also use diesel products, which emit hydrocarbons into the environment and cause environmental pollution that results in various health challenges in both animals and humans. The aim of this study was to evaluate the therapeutic effects of *Moringa oleifera* leaf extract on diesel-induced changes on cardiovascular parameters in Wistar rats' model. The study will provide updated data on the effects of inhalation of diesel on cardiovascular parameters such as haematological parameters, serum biochemical and histological alterations in Wistar rat.

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^{o} 30` to 13$^{o}$ 50` East and latitudes 4^{o} to 6^{o} North. It is bordered in the North by the Niger Republic, Zamfara State to the east, and Kebbi State to the South and West. (ICT Directorate, Sokoto State Government, 2023)

Ethical approval

Ethical approval was sought for and granted by the Faculty Animal Research and Ethics Committee (FAREC) of the Faculty of Veterinary Medicine, Usmanu Danfodiyo University, Sokoto (UDUS/FAREC/AUP-R14/2019).

Experimental animals and acclimatization

A total of 25 male and female adult Wistar 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. They were housed in a well-ventilated cage. The rats were on standard rat chow and tap water *ad libitum*. They acclimatized for two weeks before the experimental period. Procedures involving animals and their care were performed in accordance with the National Institute of Health (NIH)

guidelines for the care and use of animals (NRC, 2011).

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 Wistar rats aged 8–10 weeks, were randomly divided into five groups, comprising five rats in each group: Group A (negative control); Group B (exposed to fumes from liquid diesel); Group C (treated with 40mg/kg/rat of *Moringa oleifera* for 2-3 hours, later exposed to diesel fume, served as prophylactic group); Group D (exposed to diesel fume for 8 weeks, later treated with *moringa* extract, served as treatment group); and Group E (treatment with *Moringa* only, served as positive control). Forty (40) mg/kg/rat of 80% methanolic leaf extract of *Moringa oleifera* and 0.008 cm3/min/rat of fumes from liquid diesel were used as dosage of extract and volume of fumes respectively.

Plant materials/preparation of plant extract

Moringa oleifera leaves were obtained from Danchadi village, Bodinga LGA, Sokoto State. The plant was identified at the herbarium unit of the Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto (PCG/UDU/SOR1/0001). The 80% methanol cold extraction method was used (Abdulazeez *et al*., 2020). Forty (40) mg/kg/rat was used as the dosage throughout the study period; this was obtained after a toxicity study.

Exposure to diesel

A modified human nebuliser nose inhalation exposure method was used as described by Azeez *et al*. (2012) as seen in Plate 1. Group A was not given anything (negative control), group B was exposed to diesel fume only five minutes for eight weeks, group C was treated with *Moringa oleifera* leaf extract using an oral cannula for 2-3 hours daily for eight weeks before they were placed in the fume chamber for five minutes, group D was exposed to diesel vapor five minutes after it was treated with *Moringa* extract for eight weeks, and group E was treated with *Moringa* only. The average exposure dosage was 0.5 ± 0.008 cm^3/min/rat/kg/day.

Evaluation of haematological and biochemical parameters

Blood samples were collected via the retro-orbital sinus into EDTA bottles for a complete haemogram and into the plain sample bottles, centrifuged at 3000 revolutions per minute for 15 minutes, and serum was obtained within 5 minutes of collection for analysis of serum calcium, chloride, and potassium.

Plate I: Inhalation chamber; A: Human nebulizer and B: Improvised inhalation bucket containing the rats

Determination of cardiac troponin I

The cardiac troponin I (cTn-I) assays were determined using non-competitive enzyme-linked immunosorbent assays (ELISA) based on the sandwich principle, utilizing the high specificity and affinity of antibodies (Melanson *et al*., 2007).

Gross and histological examination

The rats were later sacrificed; the hearts were removed, dropped into formalin-saline, and prepared for histology.

Statistical analysis

Data were expressed as means ± standard error of means (SEM); statistical analysis was done using a one-way ANOVA (Tukey-Kramer Multiple Comparisons Test). P <0.05 was considered statistically significant. All analyses were done using InvivoStat software (Version 4.2.0).

Results

The result of the study on the effect of diesel on the haematological parameters of Wistar rats is presented in Table 1. There was statistically significant decrease ($P < 0.05$) in packed cell volume (PCV) (p value = 0.0040) and Hb (p value = 0.0058) in the treated groups. However, there were no statistically significant ($P > 0.05$) differences in red blood cells (RBC) (p-value = 0.2684), white blood cells

(WBC) (p value = 0.6761), neutrophils (p value = 0.7971), monocytes (p value = 0.5910), lymphocytes (p value = 0.6406), mean corpuscular volume (MCV) (p value = 0.1875), and mean corpuscular haemoglobin (MCH) (p value = 0.7753), while eosinophils, basophils, and MCHC were not processed because standard deviations are all zero. ANOVA requires at least one column's standard deviation to be nonzero in all the groups.

PCV Results: the result is shown in Table 2. The P value is 0.0040, considered statistically significant. The variation among column means is significantly greater than expected by chance. Tukey-Kramer Multiple Comparisons Test If the value of q is greater than 4.232, then the P value is less than 0.05.

Haemoglobin Results: are shown in table 3. The P value is 0.0058, considered statistically significant. Variation among column means is significantly

Table 1: Means and standard error of means of PCV, Hb, RBC, MCV, MCH, MCHC and differential leucocyte counts of rats exposed to diesel and administered *Moringa oleifera* leaf extract. (N = 25)

Parameters	**A**	**B**	**C**	**D**	**E**
PCV (%)	38.40 ± 0.87^{c}	39.80 ± 1.02^{abc}	43.20 ± 0.86^{ab}	38.20 ± 1.20^{c}	42.40 ± 0.93^{b}
Hb (g/dL)	11.84 ± 0.28^{d}	12.33 ± 0.35^{cd}	13.35 ± 0.28^{abc}	14.14 ± 0.64^{a}	13.09 ± 0.32^{abc}
RBC x 10^6/mm^3	4.69 ± 0.41	4.82 ± 0.42	5.76 ± 0.47	6.06 ± 0.82	5.33 ± 0.06
MCV (fl)	8.19 ± 0.46	8.26 ± 0.60	7.50 ± 0.39	6.30 ± 0.38	7.95 ± 0.61
MCH (pg/cells)	2.52 ± 0.13	2.56 ± 0.07	2.32 ± 0.19	2.33 ± 0.18	2.46 ± 0.26
MCHC (g/dl)	0.31 ± 0.02	0.31 ± 0.02	0.31 ± 0.02	0.37 ± 0.02	0.31 ± 0.02
WBC x 10^3/mm^3	5.28 ± 1.39	5.13 ± 0.93	4.12 ± 0.58	5.73 ± 1.12	3.90 ± 0.94
N (%)	16.80 ± 1.24	19.20 ± 2.85	20.20 ± 1.80	19.60 ± 2.58	18.60 ± 1.96
L (%)	81.80 ± 0.92	79.60 ± 2.70	79.00 ± 1.76	79.2 ± 2.75	79.40 ± 1.63
M (%)	1.40 ± 0.60	1.20 ± 0.37	0.80 ± 0.37	1.20 ± 0.37	1.80 ± 0.37
E %)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.20 ± 0.20
B (%)	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 diesel fume at 0.008 cm^3/min/rat only; C: Treated with 40 mg/kg *Moringa oleifera* leaf extract then Exposed to diesel fume at 0.008 cm^3/min/rat; D: Exposed to diesel 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: haemoglobin; RBC: Red blood cells; MCV: Mean corpuscular volume; MCH: Mean corpuscular haemoglobin; MCHC: Mean corpuscular haemoglobin concentration; WBC: White blood cells; N: Neutrophils; L: Lymphocytes; M: Monocytes; E: Eosinophils and B: Basophils. ^{abc}Means with different superscripts is considered statistically significant (P <0.05). Using one-way ANOVA and Tukey-Kramer Multiple Comparisons Test

Table 2: Effect of Diesel Fumes on Packed Cell Volume in Wistar rats and its Mitigation by Moringa oleifera Leaf Extract. (N = 25)

Comparison	Mean Difference	Q	P value
B vs D	1.600	1.626 ns	P>0.05
B vs A	1.400	1.423 ns	P>0.05
B vs E	-2.600	0.643 ns	P>0.05
B vs C	-3.400	3.456 ns	P>0.05
D vs A	-0.2000	0.2033 ns	P>0.05
D vs E	-4.200	4.269*	P<0.05
D vs C	-5.000	5.082*	P<0.05
A vs E	-4.000	4.066 ns	P>0.05
A vs C	-4.800	4.879*	P<0.05
E vs C	-0.8000	0.8131ns	P>0.05

Key: A: Negative Control; B: Exposed to diesel fume at 0.008 cm^3/min/rat only; C: Treated with 40 mg/kg *Moringa oleifera* leaf extract then Exposed to diesel fume at 0.008 cm^3/min/rat; D: Exposed to diesel 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. While ns: not significant and *: Significant

Table 3: Effect of diesel fumes on haemoglobin Level in Wistar rat and its Mitigation by Moringa oleifera Leaf Extract. (N = 25)

Comparison	Mean Difference	Q	P value
B vs D	-1.810	4.522 *	P<0.05
B vs A	0.4920	1.229 ns	P>0.05
B vs E	-0.7660	1.914 ns	P>0.05
B vs C	-1.018	2.543 ns	P>0.05
D vs A	2.302	5.751 **	P<0.05
D vs E	1.044	2.608 ns	P>0.05
D vs C	0.7920	1.978 ns	P>0.05
A vs E	-1.258	3.143 ns	P>0.05
A vs C	-1.510	3.772 ns	P>0.05
E vs C	-0.2520	0.6295 ns	P>0.05

Key: A: Negative Control; B: Exposed to diesel fume at 0.008 cm^3/min/rat only; C: Treated with 40 mg/kg *Moringa oleifera* leaf extract then Exposed to diesel fume at 0.008 cm^3/min/rat; D: Exposed to diesel 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. While ns: not significant and *: significant

Table 4: Means ± standard deviation of Na^+, K^+, Cl^-, $Ca^{2+}$and cTn-I of rats exposed to diesel and administered *Moringa oleifera* leaf extract. (N = 25)

Parameters	A	B	C	D	E
Na^+ (mmol/l)	142.80 ± 0.97^a	129.20 ± 1.36^b	132.20 ± 2.01^{cb}	144.60 ± 1.12^a	135.60 ± 6.23^{abc}
K^+ (mmol/l)	4.76 ± 0.17^b	24.54 ± 1.14^a	22.32 ± 1.93^a	5.60 ± 0.62^b	15.18 ± 6.36^{ab}
Cl^- (mmol/l)	106.60 ± 1.33^b	107.00 ± 0.84^b	111.40 ± 1.21^a	102.80 ± 1.24^b	106.40 ± 1.29^b
Ca^{2+} (mmol/l)	2.54 ± 0.08	2.11 ± 0.14	1.93 ± 0.20	2.72 ± 0.25	1.93 ± 0.30
cTn-I (Hg/l)	0.67 ± 0.20^b	0.99 ± 0.09^a	0.36 ± 0.11^b	0.94 ± 0.14^a	0.60 ± 0.03^b

Key: A: Negative Control; B: Exposed to diesel fume at 0.008 cm^3/min/rat only; C: Treated with 40 mg/kg *Moringa oleifera* leaf extract then Exposed to diesel fume at 0.008 cm^3/min/rat; D: Exposed to diesel 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; Ca^{2+}: Calcium and cTn-I: Cardiac troponin I. ^{abc}Means in a row with a different superscript differ significantly (P < 0.05). statistical analysis was done using a one-way ANOVA (Tukey-Kramer Multiple Comparisons Test)

greater than expected by chance. Tukey-Kramer Multiple Comparisons Test. If the value of q is greater than 4.232, then the P value is less than 0.05. The P values of 0.2684 (RBC), 0.6761 (WBC), 0.6406 (Lymphocytes), 0.7971 (Neutrophils), 0.5910 (Monocytes), 0.1875 (MCV), and 0.7753 (MCH) are considered not statistically significant. The variation among column means is not significantly greater than expected by chance. Post-tests were not calculated because the P value was greater than 0.05.

Table 4 shows the results of the effects of diesel on some serum biochemical parameters in rats. There was a significant (P < 0.05) decrease in Sodium in groups B, C, and E compared to groups A and D. There was a statistically significant (P < 0.05) increase in Potassium in groups B and C compared to groups A, D, and E. There was also a statistically significant (P <0.05) increase in Chloride in group C compared to groups A, B, D, and E. There was a slight statistically significant (P > 0.05) decrease in Calcium in all the groups. There was a statistically significant (P < 0.05) increase in cTn in groups B and D compared to groups A, C, and E.

Plate II showing the longitudinal section of ventricular myocardium of rats in group A (Negative control). Histological examination of the longitudinal section of ventricular myocardium of the rats exposed to diesel fumes showed normal structure.

Plate III showing the longitudinal section of ventricular myocardium of rats in group B (Exposed to diesel fume only). Histological examination of the longitudinal section of ventricular myocardium of the rats given diesel showed normal structure, similar to the structural morphology of myocardium observed in rats from the control group.

Plate IV showing the longitudinal section of ventricular myocardium of rats in group C (Given *Moringa* oleifera and later exposed to diesel fume).

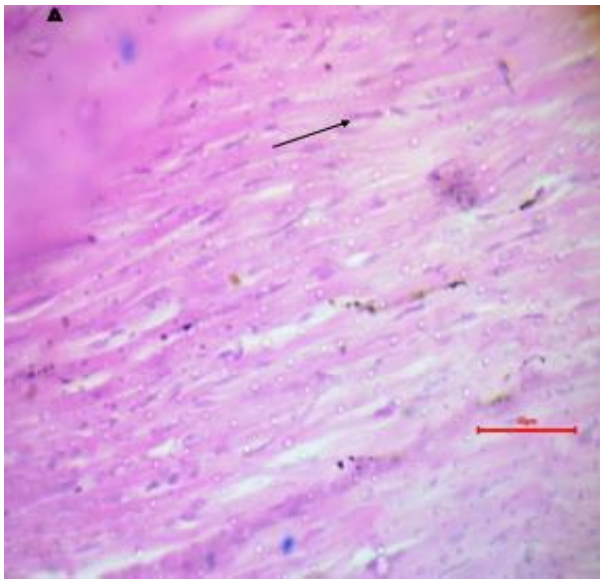

Plate II: Longitudinal section of the ventricular myocardium of the rats showing normal (H & E). X400

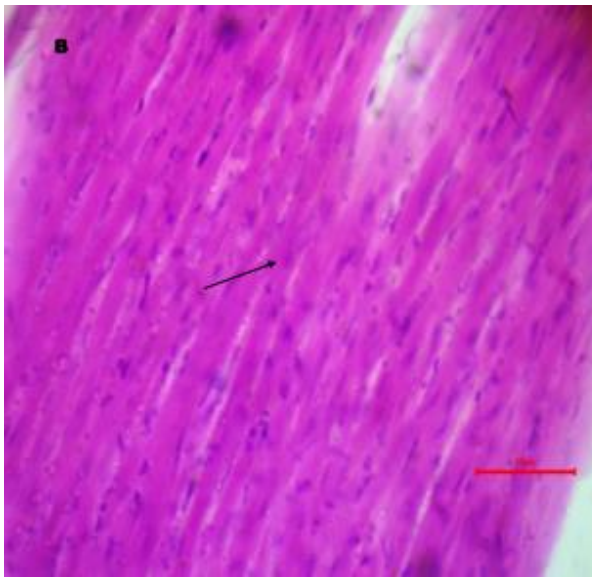

Plate III: B: Longitudinal section of the ventricular myocardium of the rats exposed to diesel fume only showing normal tissue structure (Black arrow) (H & E). X400

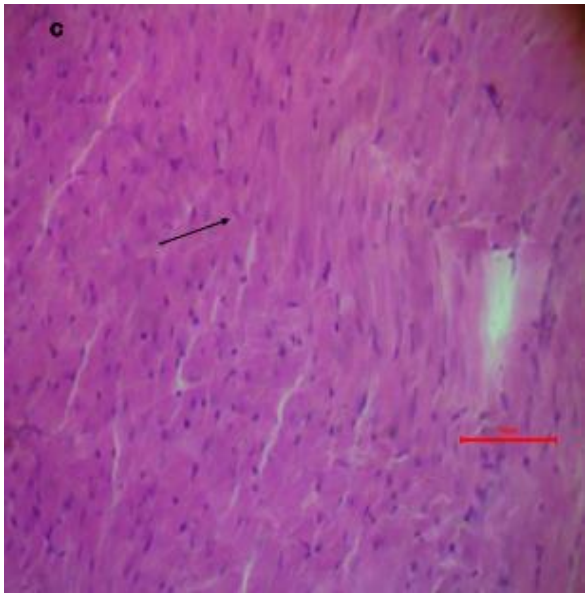

Plate IV: C: Longitudinal section of ventricular myocardium of the rats given *Moringa oleifera* and later exposed to diesel fume showing normal tissue structure (Black arrow). (H & E). X400

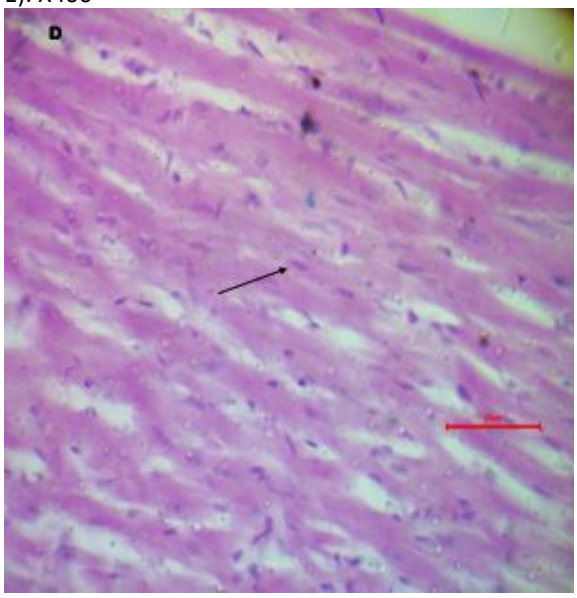

Plate V: D: longitudinal section of ventricular myocardium of the rats exposed to diesel fume and later treated with *Moringa oleifera* leave extract showing normal tissue structure (Black arrow) (H & E). X400

Histological examination of the longitudinal section of the ventricular myocardium of the rats given diesel showed normal structure, similar to the structural morphology of myocardium observed in rats from the control group.

Plate V: Showing the longitudinal section of the ventricular myocardium of rats in group D (Exposed to diesel fume and later treated with *Moringa oleifera* leave extract). Histological examination of the longitudinal section of the ventricular myocardium of the rats given diesel showed normal structure, similar to the structural morphology of myocardium observed in rats from the control group.

Plate VI shows the longitudinal section of ventricular

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

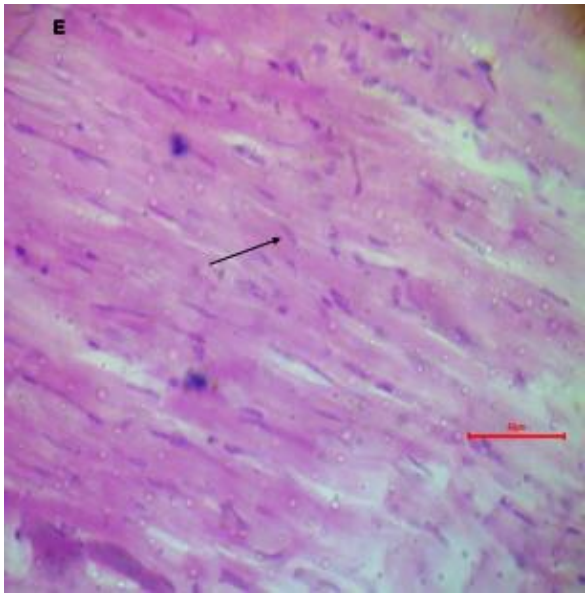

Plate VI: E: Longitudinal section of the ventricular myocardium of the rats given moringa only showing normal tissue structure (Black arrow) (H & E). X400

Discussion

Diesel products have become an essential constituent of human life due to their industrial and domestic values. However, clinical and experimental studies on exposure to diesel hydrocarbons have shown that they are among the risk factors of cardiovascular disease (Azeez *et al*., 2012a). Thus, this study investigated the effect of exposure to diesel on cardiovascular parameters and possible preventive and therapeutic effect by *Moringa oleifera* 80% methanolic leaf extract. The finding of this study have shown that exposure to diesel fumes causes changes in cardiovascular parameters.

The polycythemia seen in this study might be secondary absolute polycythemia, which was brought about by a physiologic release of erythropoietin due to chronic hypoxia, that might be induced due to a cardiac condition, pulmonary disease, haemoglobinopathy, or an anomaly with right to left shunting due to diesel fumes, which is consistent with the work of Ubah *et al.* (2010). From the study, 40 mg/kg *Moringa oleifera* leaf extract seemed to have protected the membrane integrity of the erythrocytes thereby stabilizing the cells and made them osmotically resistant to the redox effect of diesel. This is similar to the findings of Azeez *et al*. (2015) who reported that *Moringa oleifera* is rich in various phytochemical compounds such as Zeatin, quercetin and alkaloids which have some ameliorative effects against the petroleum hydrocarbons-linked health hazards.

The study's findings on hyponatremia following diesel exposure are comparable to those of Wali & Yatiraj (2014), who found low levels of serum sodium in acute myocardial infarction. This drop in sodium levels could be brought on by hypoxia and ischemia, which makes the sarcolemma more permeable to sodium. The hyponatremia might also be caused by the hydrocarbon components of petroleum products, which alters the membrane sodium pump function and keep the concentration of sodium ions low. Hyperkalemia observed in this study after exposure to diesel in rats is similar to the findings of Wali & Yatiraj (2014) and Wannamethee *et al.* (1997) who reported that smoking showed a strong association with increase in serum potassium level. They also reported an increase in mortality with increasing serum potassium levels in cardiovascular disease. The results were however contrary to the findings of Wali and Yatiraj (2014) who reported low levels of serum potassium in acute myocardial infarction. The increase in serum potassium level in this study might be due to decrease cardiac output or increase in cardiac pressure which resulted in decreased renal perfusion and subsequently reduced renal filtration and hence hyperkalemia or decreased renal perfusion which activate Renin-Aldosterone-Angiotensin System which in turn increases Angiotensin II and Aldosterone concentrations, hence hyperkalemia. In cases of severe hyperkalemia, it might be due to calcium retained through cardiac muscle damage to stabilize the heart cell membrane against depolarization.

The renin-angiotensin-aldosterone system is directly linked to kidney failure, which may be the cause of the hypochloremia seen in this study. This research supports the findings of Hajime (2021), who found that chloride is a known critical electrolyte for tubule-glomerular feedback in the kidney and a potential regulatory electrolyte for body fluid distribution in the pathophysiology of heart failure. Hypercalcemia might be due to cardiac muscle damage, that leads to the release of intracellular contents into the bloodstream, including calcium or due to the disruption of calcium regulation when the heart muscle is damaged, this regulation may be disrupted,

leading to abnormal calcium levels in the bloodstream. Likewise, 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 hypercalcemia. This finding is similar to the work of Basale *et al.* (2012) who reported that the primary causes of acute kidney injury include ischemia, hypoxia, or nephrotoxicity. Action potentials and extracellular calcium influx, both control the contraction and relaxation of cardiac muscle. The release of extra calcium from the sarcoplasmic reticulum is potentiated by the action potential, which causes an inward influx of calcium. Intercalated discs transmit the contraction of one heart muscle cell to the neighboring cells, enabling the synchronized contraction of cardiac muscle. Interestingly, *Moringa oleifera* was able to reverse the effect of diesel in the levels of sodium, potassium, chloride, and calcium, this is similar to the findings of (Azeez *et al*., 2012b; Azeez *et al*., 2015; Azeez *et al*., 2017).

This study revealed an increase in cardiac troponin I level after exposure to diesel which might be due to the damage of cardiac muscle as shown in other parameters. Originally the rationale behind the cTn-I assay was relatively due to myocardial hypoxia, ischemia or necrosis which leads to membrane disruption causing troponin release which is detected in serum. Troponins have been used to diagnose acute myocardial injury and such use has become engrained in the universal definition of acute myocardial infarction (Kyung *et al*., 2017). However, from this study, *Moringa oleifera* has no effect on the cTn-I level.

The histology results of this study revealed no evidence of tissue injury. This runs counter to research by Azeez *et al.* (2015), who found that rats exposed to diesel for 10 minutes a day for eight weeks experienced varying degrees of cellular damage. This may be because the rats in this study were exposed to petroleum products for five minutes every day for eight weeks, during which the tissue damage was most likely not yet present.

In conclusion, exposure to diesel fumes causes changes in haematological parameters, serum biochemical parameters and the cardiovascular system. The rise in cardiac troponin concentration seen in this research is evidence of acute myocardial injury. Prophylactic and therapeutic administration of *Moringa oleifera* leaf extract showed a promising result.

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Conflict of Interest

The authors declare that there is no conflict of interest.

References

Abalaka ME, Olonitola OS & Onaolapo JA (2009) *Momordica charantia* using Wistar rats to determine safety level and usefulness of the plant ethnochemotheraphy. *International Journal of Pure and Applied Sciences,* **3**(4): 1-6.

Abdulazeez N, Yusuf HI, Shekwaye AA, Bello MB, Abubakar AA & Baraya YS (2020). Changes in haematological parameters following toxicity study with 80% methanol extract of *Moringa oleifera* in Wistar rats. *American Journal of Applied Chemistry*, **8**(6): 143-151.

Agewall S, Giannitsis E, Jernberg T & Katus H (2011). Troponin elevation in coronary vs. non-coronary disease. *European Heart Journal,* **32**(4):404–411.

Anwar F, Latif S, Ashrat M & Gilam AH (2007). *Moringa oleifera*: a food plant with multiple medicinal uses. *Phytotherapy Research,* **21**(1): 17-25.

Arti Verma & Chandana VR (2009). Invitro and Invivo antioxidant properties of different fraction of *Moringa oleifera* leaves. *Food and Chemical Toxicology,* **47**(9): 2196-2201.

Azeez OM, Adah SA, Adekunle AY, Ameen SA & Biobaku KT (2015). Modulatory Effects of Ascorbic acid (Vitamin C) and *Moringa oleifera* on Erythrocyte Osmotic Rigidity Following Acute Exposure to Premium Motor Spirit (petrol fume) in Wistar rats; Organization of Women in Science in the Developing world (OWSD) Conference Proceeding Federal University of Technology Abeokuta. Abstract number 31.

Azeez OM, Adah SA, Olaifa FH, Basiru A & Abdulbaki R (2017). The ameliorative effects of *Moringa oleifera* leaf extract on cardiovascular functions and osmotic fragility of Wistar rats exposed to petrol

vapor. *Sokoto Journal of Veterinary Sciences*, **15**(2): 36-42.

Azeez OM, Akhigbe RE & Anigbogu CN (2012a). Exposure to petroleum hydrocarbon: Implication on lung lipid peroxidation and antioxidant defense system in rat. *Toxicology International,* **19**(3): 306-309.

Azeez OM, Akhigbe RE, Anigbogu CN, Ige SF & Saka WA (2012b). Variability in cardiovascular functions and baroreflex sensitivity following inhalation of petroleum hydrocarbons. *Journal of Cardiovascular Disease Research,* **3**(2): 99-103.

Bai N, Kido T, Suzuki H, Yang G, Kavanagh TJ, Kaufman JD Rosenfeld ME, Van Breemen C, van & Eeden SF (2011). Changes in atherosclerotic plaques induced by inhalation of diesel exhaust. *Atherosclerosis*, **216**(2): 23-35.

Basale DP, Anderson MD & Sutton TA (2012). Pathophysiology of acute kidney injury. *Comparative Physiology,* **2**(2):1303-1353.

Bertrand ME, Simoons ML & Fox KA (2000). Management of acute coronary syndromes: acute coronary syndromes without persistent ST segment elevation. Recommendations of the Task Force of the European Society of Cardiology. *European Heart Journal,* **21**(17): 1406–1432.

Bradley JM, Cryar KA, El Hajj MC, El Hajj EC & Gardner JD (2013). Exposure to diesel exhaust particulates induces cardiac dysfunction and remodelling. *Journal of Applied Physiology*, **115**(7): 343-363.

Chilcott RP (2006). Chemical Hazards and Poisons Division (HQ). *Health Protection Agency Compendium of Chemical Hazards*. Chilton, Didcot, Oxfordshire, OX11 0RQ, United Kingdom. Prepared by R P Chilcott CHAPD HQ, HPA Version 1. Pp 1-31.

Chumark P, Khunawat P, Sanvarinda Y, Phornchiraslip S & Morales NP (2008). The *in vivo* and *ex vivo* antioxidant properties, hypolipidaemic and atherosclerotic activities of water extracts of *Moringa oleifera* leaves. *Journal of Ethnopharmacology,* **116**(3): 439-446.

Hajime K (2021). Chloride in heart failure: its pathophysiologic role and therapeutic implication. *Cardiology and Therapy,* **10**(2): 407-428.

ICT Directorate Sokoto State Government (2023). https://sokotostate.gov.ng/history-of-sokoto/the-land/, retrieved 15-07-2023.

Kafoury RM & Madden MC (2005). Diesel exhaust particles induce the over-expression of tumor necrosis factor alpha (TNF-alpha) gene in alveolar macrophages and failed to induce apoptosis through activation of nuclear factor kappa B (NF-kappa B). *International Journal of Environmental Respiratory Public Health,* **2**(1): 107-113.

Klein BG (2020). Cunningham Textbook of Veterinary Medicine. Sixth edition, Elsevier, United States. Pp 172-176.

Koike E, Hirano S, Furuyama A & Kobayashi T (2004). cDNA microarray analysis of rat alveolar epithelial cells following exposure to organic extract of diesel exhaust particles. *Toxicology and Applied Pharmacology.* **201**(2): 178-185.

Kyung CP, David C, Gaze, Paul O, Collinson & Michael SM (2017). Cardiac troponins: from myocardial infarction to chronic disease. *European Society of Cardiology*. **113**(14): 1708-1718.

Melanson SE, Tanasijevic MJ & Jarolim P (2007). Cardiac troponin assays: a view from the clinical chemistry laboratory. *Circulation,* **116**(18): e501–e504.

Munguro LO & Lemma P (2007). Phenolics of *Moringa oleifera* leaves. *Natural Product Research,* **21**(1): 56-68.

NRC (National Research Council) (2011). *Guide for the Care and Use of Laboratory Animals*. Committee for the Update of the Guide for the Care and Use of Laboratory Animals, Institute for Laboratory Animal Research, Division on Earth and Life Studies. The National Academies Press. doi.10.17226/12910.

Oliveira JTA, Silveira SB, Vasconcelos IM, Cavada BS & Moreira RA (1999). Compositional and nutritional attributes of seeds from the multipurpose tree *Moringa oleifera* Lamarck*. Journal of the Science of Food and Agriculture.* **79**(6): 815-820.

Rankin GD, Kabéle M, Brown R, Macefield VG, Sandström T & Bosson JA (2021). Acute exposure to diesel exhaust increases muscle sympathetic nerve activity in humans. *Journal of the American Heart Association*, **10**(10): 243-274.

Schwarze PE, Totlandsdal AI, Låg M, Refsnes M, Holme JA & Øvrevik J (2013). Inflammation-related effects of diesel engine exhaust particles: Studies on lung cells *in vitro*.

BioMed Research International, doi. 10.1155/2013/685142.

Sreelatha S & Padma PR (2009). Antioxidant activity of total phenolic content of *Moringa oleifera* leaves in two stages of maturity. *Plant Foods Human Nutrition,* **64**(4): 303-311.

Stuart JA, Murray KM, Ursano RJ & Wright KM (2002). The Department of Defense's Persian Gulf War registry year 2000: An examination of veterans' health status. *Military Medicine.* **167**(2): 121-128.

Sun HJ, Wu ZY, Nie XW & Bian JS (2020). Role of endothelial dysfunction in cardiovascular diseases: The link between inflammation and hydrogen sulfide. *Frontiers in Pharmacology*, doi.10.3389/fphar.2019.01568.

Thygesen K, Alpert JS, Jaffe AS, Simoons ML, Chaitman BR & White HD (2012). Third universal definition of myocardial infarction. *European Heart Journal*, **33**(20): 2551–2567.

Thygesen K, Mair J, Katus H, Plebani M, Venge P, Collinson P, Lindahl B, Giannitsis E, Hasin Y, Galvani M & Tubaro M (2010). Recommendations for the use of cardiac troponin measurement in acute cardiac care. *European Heart Journal*, **31**(18): 2197–2204.

Ubah FE, Akpanabitu MI, Ndem JI, Alozie Y & Ebong PE (2010) Comparative nephrotoxic effect associated with exposure to diesel and gasoline vapours in rats. *Journal of Toxicology and Environmental Health Sciences,* **1**(4): 68-74.

Wali V & Yatiraj S (2014). Study of serum sodium and potassium in acute myocardial infarction. *Journal of Clinical and Diagnostic Research*, **8**(11): CC07-CC09.

Wannamethee SG, Lever AF, Shaper AG & Whincup PH (1997). Serum potassium, cigarette smoking and mortality in middle aged men. *American Journal of Epidemiology,* **145**(7): 598-601.