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Ameliorative Effect of *Moringa oleifera* Leave Extract on Kerosene Induced Hematological, Serum Biochemical and Histological Changes in Wistar Rats

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Abstract: Kerosene is a liquid mixture of chemicals produced from the distillation of crude oil. It is produced on an industrial scale by distilling crude oil in a process similar to that used to produce diesel or petrol. Kerosene is used as a fuel in cooking stoves and heaters, it also has application as a solvent in paints, cleaners, pesticides and some eye medicines. The study is aimed to determine the ameliorative effect of *moringa oleifera* leave extract on kerosene induced hematological, serum biochemistry and histological changes in wistar rats. Several households used kerosene stoves as means of cooking which predisposed them to hydrocarbons. Fumes from petroleum products cause environmental pollution, that have resulted into various health challenges. This study has provided information on how kerosene dynamic change in critical values of hematological and serum biochemical indices for clinical applications. It has also provided updated data on leverage of *Moringa oleifera* in mitigating the damaging effects of kerosene fumes to hematological and serum biochemical indices. An experimental study was conducted and random sampling technique was employed. A total of 20 adult Wister rats were randomly divided into four groups (five each): negative control; exposed to kerosene fume; exposed to kerosene fume later treated with *Moringa oleifera* and treatment with *Moringa* only. The results showed polycythemia, hyponatremia, hyperkalemia and hyperchloremia while the histological findings show no tissue damage. In conclusion, kerosene caused hematological and serum biochemical changes and treatment with *Moringa oleifera* leaf extract showed promising results as observed in this study.

Keywords: Ameliorative Effect, *Moringa oleifera*, Kerosene, Serum Biochemistry, Hematological, Wistar Rats

1. Introduction

Petroleum products are materials derived from crude oil (petroleum). Its by-products contribute to air pollution and global warming [1]. Petroleum products are mixtures of complex compounds, the majority of petroleum is converted to petroleum products, which include several classes of fuels. Such as petrol, diesel, kerosene, heavy gas oil and many others. Kerosene is a liquid mixture of chemicals produced from the distillation of crude oil, it is produced on an industrial scale by distilling crude oil in a process similar to that used to produce diesel or petrol [2]. Kerosene is used as a fuel in cooking stoves and heaters. It has traditionally been the fuel of choice for fire-breathers [2] it's also used as a base for aviation fuel but it also has application as a solvent in paints, cleaners, pesticides and some eye medicines. It was previously a common fuel for stoves, heaters and lamps and is still used today as a fuel for home ('oil') central heating systems [2]. Ingestion of contaminated food and drinking of contaminated water are source of exposure of petroleum hydrocarbons to animals and human [3]. Accidental ingestion of small amounts of kerosene may cause coughing, fever, vomiting, tachypnoea (rapid breathing), constipation, restlessness, drowsiness, abdominal pain, and diarrhea [4]

Ingesting large amounts of kerosene may cause convulsions, coma, or even death [5]. Cooking on gas stoves indoors is associated with an increase in asthma [6] and acute respiratory infections in children [7]. Carbon monoxide (CO) has been shown to increase the formation of blood clots [8] There is also evidence that links CO with an increased risk for a heart attacks, arrhythmias, and an increased risk of readmission to the hospital for heart problems after the first heart attack [9].

Moringa oleifera has high nutritional value because it contained protein, vitamins and various phenolic compounds [10, 11]. Zeatin, quercetin and alkaloids that is present in *Moringa oleifera* have been found to have some ameliorative effects against the petroleum hydrocarbons-linked health hazards [12].

Hematological parameters are considered an important tool in evaluating animal's health. [13]. Blood act as pathological reflection of the status of exposed animal to toxicants and other conditions [13]. Studies has shown that inhalational exposure to kerosene vapours causes decreased packed cell volume (PCV), Hemoglobin (Hb) and red blood cell (RBC) count, as well as increased white blood cell (WBC) count in wistar rats [14]. Red Blood Cells Indices are valuable in the morphological classification of anaemias [15]. Red blood cell indices; Mean Corpuscular Volume (MCV), Mean Corpuscular Heamoglobin (MCH) and Mean Corpuscular Heamoglobin Concentration (MCHC) are calculated from hemoglobin, hematocrit, and red blood cell count.

The electrical activities of the heart are usually as a result of electrolytes distribution in extracellular and intracellular fluid, therefore any change in the concentration of any of the electrolytes will definitely alter the electrical activity of the heart. Calcium plays an essential role in physiology of the cardiovascular system. aberrations from normal serum calcium level are known to be associated with several cardiovascular diseases and it is known to play a crucial role in pathophysiology of many disease [16].

Several households still use kerosene stoves as means of cooking which also predispose them to hydrocarbons. Fumes from petroleum products cause environmental pollution, that have resulted into various health challenges in both animals and humans. The aimed of this study is to determine the ameliorative effect of *moringa oleifera* leave extract on kerosene induced hematological and serum biochemistry changes in wistar albino rats.

This study will provide information on how kerosene dynamic change in critical values of hematological and serum biochemical indices for clinical applications, it will also provide updated data on leverage of *Moringa oleifera* in mitigating the damaging effects of kerosene fumes to hematological and serum biochemical indices.

2. 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. The state is located at the extreme Northwestern Nigeria, it shares common boarders with Niger republic to the north, Kebbi State to the south-west and Zamfara State to the east.

Study Design; An experimental study was conducted and random sampling technique was employed. A total of 20 males and females adult Wister rats of 8–10 weeks, weighing between 130-250g were randomly divided into four groups comprising five rats in each group: negative control (not receiving anything); exposed to kerosene fume only; exposed to kerosene fume later treated with *Moringa oleifera* and treatment with *Moringa* only.

Experimental Animal Acclimatization; A total of 20 males and females adult Wister rats weighing between 130-250g were acquired 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 tap water *ad' libitum*. They were acclimatized for two weeks before the experimental period. Procedures involving animals and their care were performed in accordance with the National Institute of Health (NHI) guideline for the care and use of animals (NRC, 1996). Ethical approval was sought from the faculty Animal Research and Ethics Committee (FAREC) of the Faculty of Veterinary Medicine, Usmanu Danfodiyo University, Sokoto.

Plant Materials/Preparation of Plant Extract; *Moringa oleifera* leaves was obtained from Danchadi village, Bodinga LGA, Sokoto State. The plant was identified at the herbarium unit Department of Biological Sciences, Usmanu Danfodiyo University Sokoto (PCG/UDU/SOR1/0001). 250g of grinded *Moringa oleifera* leaves was soaked in to 80% methanol and 20% distilled water for 3-5 days which was later evaporated. The cold extract was used for this study. 40mg/kg/rat was used as the dosage throughout the study period, this was obtained after toxicity study.

Exposure to Kerosene; A modified human nebulizer nose inhalation exposure method was used as described by [21]. Group A were used asnegative control, group B (Exposed to kerosene fume only), group C (exposed to kerosene fume five minutes daily for 4 weeks later treated with *Moringa oleifera* leaves extract using oral cannula) and group D (treated with *Moringa* only). The exposure dosage was $0.008cm^3$/min/rat.

Evaluations of Hematological and Biochemical Parameters; Blood samples were collected via cardiac puncture into the EDTA bottles for complete hemogram and into the plain sample bottles, centrifuged at 3000 rpm for 5 minutes, serum was obtained within 10 minutes of collection for serum biochemistry analysis.

Statistical Analysis; Data are expressed as means ± standard error of means (SEM); statistical analysis was done using Kruskal's Wallis H Test. $P < 0.05$ was considered significant. All analysis was done using InvivoStat software (Version 4.2.0).

3. Result

The result of the study on the effect of kerosene on hematological parameters of rats is presented on Table 1. kerosene treated group (group B) significantly (P <0.05) increased the packed cell volume (PCV) counts compared to negative control (Group A) and exposed to kerosene fumes later treated with *Moringa oleifera* (Group C), but no difference with *Moringa oleifera* group (Group D). kerosene treated group (group B) significantly (P <0.05) increased the hemoglobin (Hb) counts compared to negative control (Group A) and exposed to kerosene fumes later treated with *Moringa oleifera* (Group C), but no difference with *Moringa oleifera* group (Group D). kerosene treated group (group B) significantly (P <0.05) increased the red blood cells (RBC) counts compared to negative control (Group A) and exposed to kerosene fumes later treated with *Moringa oleifera* (Group C), but no difference with *Moringa oleifera* group (Group D). However, there was no significant (P <0.05) differences in white blood cells, neutrophils, monocytes, eosinophils and basophil in all the groups but eosinophils relatively high in Group C compared to other groups.

Table 1. *Effect of Moringa oleifera leaf extract on hematological parameters in toxicity induced by Kerosene.*

Parameters	A	B	C	D
PCV (%)	38.40 ± 0.87^{a}	42.00 ± 1.14^{b}	35.00 ± 2.32^{a}	42.40 ± 0.93^{cb}
HB (g/dL)	11.84 ± 0.28^{a}	12.94 ± 0.38^{b}	13.19 ± 0.91^{c}	13.09 ± 0.32^{d}
RBC x 10^6/mm^3	4.69 ± 0.41^{a}	6.27 ± 0.25^{b}	4.89 ± 0.32^{a}	5.33 ± 0.06^{a}
MCV (fl)	8.19 ± 0.46	6.70 ± 0.89	7.16 ± 2.00	7.95 ± 0.61
MCH (pg)	2.52 ± 0.13	2.06 ± 0.13	2.70 ± 0.59	2.46 ± 0.26
MCHC (g/dl)	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^{a}	5.32 ± 0.74^{a}	4.70 ± 0.26^{a}	3.90 ± 0.94
N %	16.80 ± 1.24^{a}	17.40 ± 2.68^{a}	16.00 ± 0.71^{a}	18.60 ± 1.96^{a}
L %	81.80 ± 0.92^{a}	80.60 ± 2.66^{a}	83.60 ± 0.75^{b}	79.40 ± 1.63^{a}
M %	1.40 ± 0.60^{a}	1.80 ± 0.37^{a}	0.40 ± 0.24^{a}	1.80 ± 0.37^{a}
E %	0.00 ± 0.00^{a}	0.20 ± 0.20^{a}	0.00 ± 0.00^{a}	0.20 ± 0.20^{a}
B %	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}

KEY: A (Negative Control), B (Exposed to kerosene only), C (exposed to kerosene later treated with *Moringa oleifera* extract) and D (*Moringa* treated 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), N (Neutrophils), L (Leucocytes), M (Monocytes), E (Eosinophils) and B (Basophils). Data is presented as mean ± SEM. Means in a row with a different superscript differ significantly (P < 0.05). Using Kruskal Wallis H test.

The result of the study on effect of kerosene on serum biochemistry parameters of rats is presented on Table 2. There was significant (P <0.05) decrease in Sodium in kerosene treated group (group B) compared to negative control (group A), exposed to kerosene fumes later treated with *Moringa oleifera* extract (Group C) and *Moringa* treated only (group D). There was statistically significant (P <0.05) increased in Potassium in negative control (group A), exposed to kerosene fumes later treated with *Moringa oleifera* (Group C) and Moringa treated only (Group D). There was no statistically significant (P <0.05) difference between chloride in all the groups. There was statistically significant (P <0.05) decrease in Calcium kerosene treated group (Group B) compared to negative control (Group A) and exposed to kerosene fumes later treated with *Moringa* extract (Group C) but no difference with *Moringa* treated only (Group D).

Table 2. *Effect of Moringa oleifera leaf extract on serum biochemistry in toxicity induced by Kerosene.*

Parameters	A	B	C	D
Na^+ (mol/l)	142.80 ± 0.97^{a}	129.80 ± 2.01^{b}	140.40 ± 1.29^{a}	135.60 ± 6.23^{a}
K^+ (mol/l)	4.76 ± 0.17^{a}	23.58 ± 2.72^{b}	5.60 ± 0.44^{a}	15.18 ± 6.36^{a}
Cl^- (mol/l)	106.60 ± 1.33^{a}	104.40 ± 0.68^{a}	103.40 ± 1.57^{a}	106.40 ± 1.29^{a}
Ca2+ (mol/l)	2.54 ± 0.08^{a}	1.99 ± 0.12^{b}	2.23 ± 0.16^{a}	1.93 ± 0.30^{b}

KEY: A (Negative Control), B (Exposed to kerosene only), C (exposed to kerosene later treated with *Moringa oleifera* extract) and D (*Moringa* treated only). Na^+ (Sodium), K^+ (Potassium) and Cl^-(Chloride), Ca^{2+} (Calcium). Data is presented as mean ± SEM. Means in a row with a different superscript differ significantly (P < 0.05). Using Kruskal wali H test.

Histological examination of the longitudinal section of ventricular myocardium of the rats given kerosene appeared normal structure, similar to the structural morphology of myocardium observed in rats from the control group as shown in figure 1.

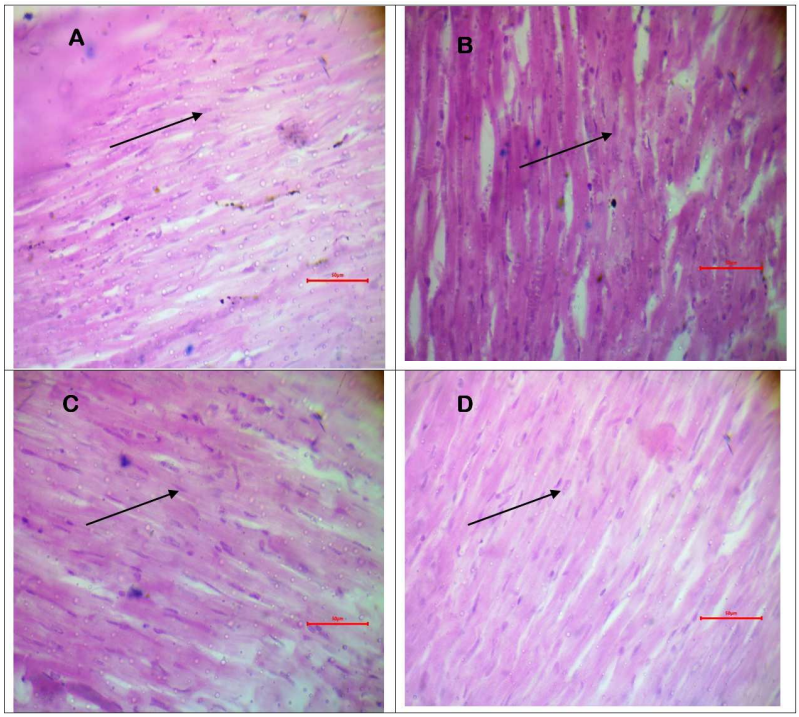

Figure 1. *Photomicrographs of the longitudinal section of ventricular myocardium of the rats at 400 magnification using H & E staining technique, (A) Exposed to kerosene fume only, (B) Given Moringa oleifera later exposed to kerosene fume only and (C) Exposed to kerosene fume and later treated with Moringa oleifera leave extract.*

4. Discussion

Inhalation exposure to kerosene fumes 5 minutes daily for four weeks in rats, causes increased in packed cell volume, hemoglobin and red blood cells count which lead to polycythemia, this is contrary to the findings of [14, 17]. The polycythemia is secondary absolute polycythemia which was caused by a physiologically appropriate release of erythropoietin resulting from chronic hypoxemia as a result of pulmonary and cardiac compromised or anomaly with right to left shunting, or hemoglobinopathy. *Moringa oleifera* seemed to have protected the membrane integrity of the erythrocytes thereby stabilizing the cells and made them osmotically resistant to the redox effect of kerosene as seen in group C (Exposed to kerosene fumes later treated with *Moringa* extract) and is consistent with work of [17].

Decrease in sodium level (hyponatremia) observed in this study, is similar to the finding observed by [18, 19], who reported low level serum sodium in acute myocardial infarction. This could also be due to hydrocarbon component of the petroleum products that interfere with the membrane sodium pump mechanism that maintains low level of sodium ion concentration. Increased of potassium level (hyperkalemia) observed in this study after exposure to kerosene fumes in rats is similar to the findings of [20] who report smoking shows a strong association with serum potassium level increase but is contrary to the findings [18] who reported low level of serum potassium level in acute myocardial infarction. The increase serum potassium level is due to decrease cardiac output/increase in cardiac pressure which caused decreased renal perfusion and subsequently reduced renal filtration and hence hyperkalemia or decreased renal perfusion which activate Renin-Aldosterone-Angiotensin System which in turn increased Angiotensin II and Aldosterone concentration hence hyperkalemia. Similarly, *Moringa oleifera* has able to reverse the effect of kerosene, for both sodium, potassium and calcium (seen in group B, this is similar to the findings of [12, 17, 21]. There is no significant difference between the other calcium groups in this time period.

In this study, the histological findings show no tissue damage. this is contrary to the work of [17] who reported ranging degree of cellular degeneration in rats after exposure to petrol, diesel and kerosene for 10 minutes daily for eight weeks. This is due to the reason that, the rats in this study are exposed to the petroleum products for 5 minutes daily for 4 weeks, probably at this time the tissues damage has not started.

5. Conclusion

Animal and human are exposed to kerosene vapor every day in our environment. increased PCV, Hb and RBC are evidence of polycythemia as seen in this research. Decreased in sodium and increased in potassium and chloride concentration in the blood found in this research is also indicative of acute myocardial injury. Histological findings in this research showed no evidence of architectural damage to the heart tissues. In conclusion, kerosene caused hematological and serum biochemical changes and treatment with *Moringa oleifera* leaf extract showed promising results

as observed in this study. Recommendations; *Moringa oleifera* is locally planted and affordable to get therefore should be considered as alternative remedy in animals and human exposed to kerosene; Further study should carry out to molecularly study the gene that is responsible for the interaction. Further studies should also be carried out to investigate the effect of exposure to kerosene in other organs such as lungs, kidney, liver and brain.

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