African Journal of Laboratory Haematology and Transfusion Science

Vol 3, Issue 2, page 107 - 113| June 2024 | www.ajlhtsonline.org
ARTICLE DOI: doi.org/10.59708/ajlhts.v3i2.2408
DOI URL: 10.59708/ajlhts.v3i2.2408

ORIGINAL ARTICLE

Effect of aqueous leaf extract of Moringa oleifera on some complete blood count and some oxidative stress markers of stress-induced Albino Wistar rats.

Odozi, E. B. and Ayeni, E. E.

Department of Medical Laboratory Science, School of Basic Medical Sciences, University of Benin, Benin City, Edo State, Nigeria.

Corresponding Author:
Bright Efeota Odozi
efeota.odozi@uniben.edu,
efeotaodozi@gmail.com

Received 29-02-2024
Accepted 30-04-2024
Published 30-06-2024

Abstract

Introduction: *Moringa oleifera* is a nutritive plant that is used to treat malnutrition in underdeveloped nations due to its nutritional benefits. It contributes significantly to humans' diet and is a good source of protein, carbohydrates, vitamins, and minerals. The leaves of *Moringa oleifera* contain phytochemicals that may impact one's health. This study aimed to determine the effect of administering feed formulated from Moringa oleifera leaves on some hematological Parameters and oxidative stress markers of Gasoline vapour stress-induced *Albino Wistar* rats.

Materials and methods: Fifty-eight (58) age-matched, apparently healthy male *Albino Wistar* rats were obtained from the animal holding unit of the Anatomy Department, University of Benin, Benin City, Nigeria. The rats were housed in stainless steel cages measuring 4 ft x 2 ft x 2 ft, partitioned into four compartments at the same facility. Automated ERMA haematology autoanalyzer PCE-210 was used to analyze the haematological parameters while Enzyme- linked immunosorbent assay (ELISA) was used to analyze Malondialdehyde (MDA), Catalase (CAT) and Superoxide dismutase (SOD) for oxidative stress.

Results: Findings from the study revealed that exposure of animals to Gasoline vapour alone significantly ($p< 0.05$) increased malonaldehyde (MDA) levels and decreased superoxide dismutase (SOD) level and catalase (CAT) levels. There was a significant decrease in the haemoglobin and haematocrit values ($p= 0.027$ and 0.008 respectively) in the stressed groups when compared to the corresponding values in the control group. Monocyte concentration also increased in the group stressed and administered *Moringa oleifera* when compared to the group that was stressed only.

Conclusion: This study's results show that an extract from the leaf of Moringa oleifera fed to Albino Wistar rats might affect some haematological parameters, such as monocyte, haemoglobin,

haematocrit, and platelet count. The gasoline vapour might be connected with the decrease observed in some groups.

Keywords: *Moringa Oleifera*, haematological parameters, oxidative stress markers, *Albino Wistar rats.*

Introduction

Moringa oleifera leaf is a nutritive plant that is ideal for treating malnutrition in underdeveloped nations (1). Its rich amino acid and flavone content provide nutritional benefits that are useful in food supplements and certain cosmetics. Due to its rich amino acid and flavone content, it provides nutritional benefits that are useful in food supplements and in certain cosmetics. M. *oleifera* earned the moniker "Miracle Tree" and commercial attention (2).

Oxidative stress is caused by an imbalance between the system that scavenges free radicals and the system that produces them. Free radical-mediated damage leads to various processes, including chemical injury, ischemia-reperfusion injury, mitochondrial dysfunction, membrane phospholipid loss, and cytoskeleton anomalies (3).

It has been claimed that medicinal herbs are used to treat different illnesses, including coughs, colds, stomach aches, cataracts, constipation, and many other conditions (4). One of these herbs, M. *oleifera*, has been shown to effectively prevent morphological alterations and oxidative damage in rats' lenses by increasing the activities of antioxidant enzymes, lowering the severity of lipid peroxidation, and preventing the production of free radicals (5). A relatively small amount of haematopoietic stem cells (HSCs) and progenitors give rise to all of the adult blood cells in the body. The different blood cells all have significant functions in the body's typical physiological processes. However, several illnesses and situations, including malaria, starvation, protozoan infections, and pregnancy, can interfere with normal hematopoiesis, predisposing a person to anemia (5). Most plants have been shown to contain substances known as haematinic agents, including folic acid, vitamin B6, iron, and other substances that could encourage the development of erythropoietic cells and other blood components (6).

Study Population/Area

Fifty-eight (58) age-matched, apparently healthy male *Albino Wistar* rats were obtained from the animal holding unit of the Anatomy Department, University of Benin, Benin City, Nigeria. The rats were housed in stainless steel cages measuring 4 ft x 2 ft x 2 ft, partitioned into four compartments at the same facility. Testing was conducted at the medical laboratory services unit of the University of Benin Teaching Hospital and the Department of Biochemistry. The rats were divided into four groups (n = 10) for the study. They were provided with a basal diet of grower's mash (Bendel Feeds and Flour Mill, Ewu Edo State) and had access to tap water provided in open bowls at regular intervals.

Collection of plant materials

Fresh *Moringa oleifera* leaves were collected in Benin and identified by a plant taxonomist in the Department of Plant Biology and Biotechnology as *Moringa oleifera* and assigned a voucher number: UIH-10847.

Preparation of *Moringa oleifera* plant extract

With the aid of a blender, 3.65kg of leaves were pulverized (after drying), and 665g of powder was obtained. 1g of the powder was dissolved in 4 ml of distilled water and allowed to stand for 24 hours at room temperature (25OC).

The extract was filtered using Whatman's (Nitrocellulose 45; 0.45µm pore size) filter paper; the filtrates were concentrated to dryness at 100°C in a water bath, and a stock solution was made. Thereafter, it was put in an airtight container and refrigerated until use.

2.4. Inclusion criteria

Apparently, healthy adult male Wistar rats weighing 150-200 g were employed in the study.

2.5. Exclusion criteria

Some rats were excluded due to excessive breathing, reduced appetite, immobility and low weight (<150g).

Experimental Design

Fifty-eight (58) *Albino Wistar* rats weighing 150-200g were used for this study. After seven days of acclimatization, the acute toxicity and baseline hematological parameters were determined before the extracts were administered.

Acute toxicity: Eighteen rats were randomly distributed into six groups of three (3) Wistar rats each and administered 10, 100, 1000, 1600, 2900, and 5000mg/kg of the aqueous extract, respectively, for 7 days. The test involved two phases. In phase one, the animals were grouped into three (3) groups of three rats each and were given 10, 100, and 1000mg/kg of the extracts daily for 7 days. In the second phase, the animals were grouped into three (3) rats each and were given 1600, 2900, and 5000mg/kg of the extract for 7 days.

Treatment with *Moringa oleifera*: Forty (40) Mature Wistar rats were randomly selected and divided into four groups (n = 10 per group). Animals in Group A served as unexposed controls, while animals in Group B were exposed to gasoline vapor (GV) alone for 28 days. Animals in group C were exposed to GV but were co-administered 250mg of aqueous *M. oleifera* leaf extract (low dose), and animals in group D were exposed to GV and co-administered 1000mg of aqueous *M. oleifera* leaf extract (high dose) for the same time period.

Exposure to gasoline vapour

A calibrated 100 mL beaker measured 50 mL of petrol, which was used to soak cotton wool. The gasoline-soaked cotton wool was placed in the rat cages of the test groups for one hour each day for twenty-eight (28) consecutive days.

Sample collection

The rats were sacrificed by anesthetizing with chloroform. Blood was collected by cardiac puncture into a Tripotassium ethylene diamine tetraacetic acid (K_3EDTA) anticoagulant bottle for haematological analysis (Full blood count) and a Lithium Heparin anticoagulant bottle for oxidative stress analysis (MDA).

Oxidative stress tests

Oxidative stress tests for catalase, malondialdehyde, and superoxide dismutase were carried out using commercially prepared enzyme-linked immunosorbent assay (ELISA) techniques (Thermo Fisher Scientific Inc, Waltham, Massachusetts, United States).

Complete blood count

Complete blood counts were done on the Mythic 18 fully automated haematology bench-top analyzer using impedance technology for a complete blood count (CBC).

Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS), version 20.0. The data obtained was analyzed using descriptive statistics and reported as the mean standard deviation (SD). Independent t-tests were carried out on both the test and

control groups. Analysis of variance (ANOVA) was also used. Differences with p-values less than 0.05 were considered statistically significant.

Ethical Approval

Approval for this research was sought and obtained from the Edo State Research Ethics Committee, Ministry of Health (Ref. Number: HM.1208/146).

Result

Table 1 shows the acute toxicity phases of the aqueous leaf extract of *Moringa oleifera*. The result revealed no death in both phases (1 and 2), as seen in the mortality outlook. An increase in weight was recorded in the rats when compared to the starting weight in different groups except for phase 2 and group 3, which neither had a weight increase or decrease. Table 2 shows that the values of controls in catalase, malondialdehyde, and superoxide dismutase were significantly lower than the test groups. Table 3 shows that monocyte concentration of the stressed group and administered low dose of *Moringa oleifera* was significantly ($p< 0.05$) higher (1.06 ± 0.17) when compared to the stressed group (0.52 ± 0.41). Table 4 shows that haemoglobin concentration was significantly higher ($p < 0.02$) in the stressed group (13.56 ± 1.73) and the group stressed and administered low dose of *Moringa oleifera* (12.84 ± 1.44) when compared to the control group (15.78 ± 0.62). Haematocrit was significantly higher ($p< 0.005$) in the control group (43.06 ± 1.78) when compared to the group that was stressed (35.68 ± 3.78), and also the group stressed and administered a low dose of *Moringa oleifera* (36.60 ± 2.22).

Table 1: The acute toxicity phases of aqueous leaf extract of *Moringa oleifera.*

	Group	Dosage (mg/ kg.bw)	Weight initial (g)	Weight After (g)	Mortality
Phase 1	1	10	160	170	0/3
	2	100	160	170	0/3
	3	1000	150	155	0/3
Phase 2	1	1600	150	155	0/3
	2	2900	150	155	0/3
	3	5000	150	150	0/3

Table 2. The oxidative stress markers of control and test groups

Groups	Parameters		
	MDA (μ/ml)	CAT (μ/g)	SOD (μ/ml)
Controls	7.5 ± 1.27	8.64 ± 0.58	223.24 ± 4.26
Stressed	33.9 ±4.26a	1.16 ± 0.15a	102.94 ± 3.12a
Stressed + Low dose	17.4 ± 1.85ab	3.64 ± 0.32ab	126.78 ± 4.95ab
Stressed + High dose	7.42 ± 0.46bc	7.57 ± 0.43bc	187.76 ± 7.29abc
p-value	<0.0001	<0.0001	<0.0001

Values are shown in Mean ± SD, a represents Significance with control, b represents Significances with stressed group, c represents Significances with stressed + low dose, d represent Significances with stressed + high dose

Table 3. White blood cells parameters of test and control groups

Groups	Parameters			
	WBC ($10^3\mu l$)	**LYM ($10^3\mu l$)**	**MON ($10^3\mu l$)**	**GRA ($10^3\mu l$)**
Controls	9.44 ± 2.26	8.60 ± 1.97	0.60 ± 0.23	0.28 ± 0.11
Stressed	9.44 ± 6.30	9.20 ± 6.36	0.52 ± 0.41	0.34 ± 0.36
Stressed + Low dose	8.42 ± 0.59	7.08 ± 0.50	0.80 ± 0.27	0.34 ± 0.11
Stressed + High dose	12.16 ± 1.98	10.42 ± 2.10	1.06 ± 0.17b	0.42 ± 0.11
p-value	0.399	0.599	0.029	0.761

Values are shown in Mean ± SD, a represents significance with control, b represents Significances with stressed group, c represents Significances with stressed + low dose, d represent significances with stressed + high dose

Table 4. Red blood cells, red cell indices and platelets of test and control groups

Groups	Parameter						
	RBC ($10^6\mu l$)	**HGB (g/dl)**	**HCT (%)**	**MCV (μm^3)**	**MCH (pg)**	**MCHC (g/dl)**	**PLT ($10^3/\mu l$)**
Controls	7.07 ± 0.15	15.78 ± 0.62	43.06 ± 1.78	61.00 ± 2.06	24.70 ± 6.00	36.36 ± 0.72	675.20 ± 234.49
Stressed	7.61 ± 3.30	13.56 ± 1.73a	35.68 ± 3.78a	61.60 ± 6.15	23.76 ± 5.37	36.44 ± 1.76	793.20 ± 376.37
Stressed + Low dose	6.83 ± 0.82	12.84 ± 1.44a	36.60 ± 2.22a	58.30 ± 2.32	22.28 ± 1.60	33.34 ± 1.23	1107.60 ± 138.32
Stressed + High dose	6.97 ± 0.85	14.68 ± 1.72	38.54 ± 3.54	59.62 ± 3.16	22.24 ± 0.93	37.44 ± 1.59	666.80 ± 247.20
p-value	0.901	0.027	0.005	0.534	0.740	0.545	0.058

Values are shown in Mean ± SD, a represents significance with control, b represents significances with stressed group, c represents significances with stressed + low dose, d represent significances with stressed + high dose

Discussion

The acute toxicity of the aqueous leaf extract of *Moringa oleifera* was estimated in two (2) phases on eighteen (18) *Albino Wistar* rats, three (3) per concentration 7 using the standard method of Lorke. There was neither sign of toxicity nor mortality. As such, the median lethal dose of the aqueous extract of *Moringa oleifera* was estimated to be over 5000mg/kg. In agreement with the findings of (7, 8, 9), results from this study showed that exposure to gasoline vapour caused significant increase in Malondialdehyde (MDA) levels and also caused a significant decrease in Superoxide Dismutase (SOD) and Catalase (CAT) level. Exposure to vapours of gasoline could result in increased Malondialdehyde (MDA) concentration as a result the presence of reactive oxygen species (ROS) in vapors, which might promote lipid peroxidation, resulting in the generation of MDA. In addition, vapors from gasoline exposure could reduce the amount of Superoxide Dismutase (SOD) and Catalase (CAT), which are the antioxidant enzymes linked with neutralizing ROS. This reduction suggests that exposure to gasoline

vapor impairs the body's antioxidant defense system, leading to oxidative stress and damage to cellular makeup.

Apart from haemoglobin concentration (g/dl), Haematocrit (%) and Monocyte that had lower values after exposure to gasoline vapour, there was no significant change in other haematological parameters measured. This means exposure to gasoline vapour did not cause severe haematoxocity in the rats. Perhaps, this might be due to the time of exposure of the gasoline vapour. Accordingly, changes in haematological parameters in *Albino Wistar* rats were higher in test groups that were exposed for a longer period. In this study, the rats were exposed to gasoline vapour for 1 hour daily. This present study provides additional evidence to support previous epidemiological and clinical studies that demonstrated a close association of exposure to gasoline compounds with haematotoxicity (10). It was noted that after the administration of Moringa oleifera leaf extracts, the gasoline vapor-induced changes in oxidative stress markers were reversed. This indicated that *Moringa oleifera* leaf extracts ameliorated the effect of gasoline vapour-induced stress in the rats. The aforementioned changes in Oxidative stress parameters (specifically significant increases in MDA, and decrease in SOD and CAT) of albino Wistar rats exposed to GV alone indicate GV-induced lipid peroxidation leading to an increased MDA level. It also indicated SOD and CAT reacting with the reactive oxygen species (ROS) from the inhalation of gasoline vapor. All these are evidence of oxidative stress caused by the inhalation of gasoline vapor. Oxidized lipids produce MDA as a decomposition product. Hence the increase in MDA levels, decrease in SOD, and Catalase was a result SOD dismutating O2- into H2O2 to avoid accumulation to toxic levels. Catalase, which is one of the most abundant peroxisomal proteins in mammalian cells, converts H_2O_2 into H_2O and O_2 (11). Decrease in haemoglobin content could be attributed to shortened life span of RBC and impairment of heme synthesis by the metabolic end product of free radicals of benzene and other aliphatic hydrocarbon constituents of gasoline vapour. These free radicals can alter bone marrow's erythrocyte membrane and heme protein synthesis (12). A Decrease in haematocrit (%) is associated with decrease in red blood cell concentration, however, it is unclear why contrasting result was observed in this study. The ameliorative effect of *Moringa oleifera* leaves extract on gasoline vapour-induced oxidative stress observed in this study may be due to its antioxidant activity. Evaluation of the phytochemical and nutritional constituents of fresh *Moringa oleifera* leaf extract in a previous study showed that several *Moringa oleifera* constituents, including phytochemicals (saponins, tannins, phenolic acids, flavonoids, and carotenoids); vitamins (vitamins A, C, E, folate, thiamine, niacin, pyridoxine, and riboflavin); minerals and trace elements (potassium, calcium, magnesium and Iron), electrolytes, show antioxidative effects in human and animal cells (13). Other important nutrients identified in *Moringa oleifera* extract include carbohydrates, protein, and fat. These antioxidative constituents can alleviate GV-induced oxidative stress and suppress other pathophysiological processes.

Conclusion

This study demonstrated that aqueous leaf extract of *Moringa oleifera* might have ameliorative effects on stress-induced oxidative stress in *Albino Wistar* rats owing to its anti-oxidative activities. It was observed that oxidative stress had a significant effect on haemoglobin and haematocrit values which were subsequently ameliorated after administration of *Moringa oleifera* leaves. Further studies are required to identify the active ingredient(s) responsible for the antioxidant activities of *Moringa oleifera* and the resultant impact of gasoline vapour is needed.

References

1. Popoola JO, Obembe OO. Local knowledge, use pattern and geographical distribution of *Moringa oleifera* Lam. (Moringaceae) in Nigeria. *Journal of Ethnopharmacology* 2013; 150 (2): 682-691.
2. Zongo U, Zoungrana SL, Savadogo A., Traoré AS. Nutritional and clinical rehabilitation of severely malnourished children with *Moringa oleifera* Lam. leaf powder in Ouagadougou, Burkina Faso 2013.
3. Scheibmeir HD, Christensen K, Whitaker S. H., Jegaethesan, J, Clancy R, Pierce JD. A review of free radicals and antioxidants for critical care nurses. *Intensive and Critical Care Nursing* 2005; 21 (1): 24-28.
4. Jimenez-Arellanes A, Meckes M, Ramirez R, Torres J. and Luna-Herrera J. Activity against multidrug-resistant Mycobacterium tuberculosis in Mexican plants that treat respiratory diseases. *Phytotherapy Research* 2003; 17(8): 903-908.
5. Sreelatha S, Padma PR. Antioxidant activity and total phenolic content of *Moringa oleifera* leaves in two stages of maturity. *Plant Foods for Human Nutrition* 2009; 64 (1): 303-311.
6. Adedapo AA, Mogbojuri OM, Emikpe BO. Safety evaluations of the aqueous extract of the leaves of *Moringa oleifera* in rats. *Journal of Medicinal Plants Research* 2009; 3(8): 586-591.
7. Freiberger CE, Vanderjagt DJ, Pastuszyn A, Glew RS, Mounkaila G, Millson M, Glew RH. Nutrient content of the edible leaves of seven wild plants from Niger. *Plant foods for Human nutrition* 1998; 53 (1): 57-69.
8. Anwar F, Ashraf M, Bhanger MI. Interprovenance variation in the composition of *Moringa oleifera* oilseeds from Pakistan. *Journal of the American Oil Chemists' Society* 2005; 82 (1): 45-51.
9. Ekpenyong CE, Akpan EE. Use of Cymbopogon citratus essential oil in food preservation: Recent advances and future perspectives. *Critical reviews in food science and nutrition* 2017; 57 (12):2541-2559.
10. Yoon BI, Hirabayashi Y, Kawasaki Y, Kodama Y, Kaneko T, Kim DY, Inoue T. Mechanism of action of benzene toxicity: cell cycle suppression in hemopoietic progenitor cells (CFU-GM). *Experimental Haematology* 2017; 29 (3): 278-285.
11. Sedky A, Elsawy H. Protective Effect of Vitamins C and E against Gasoline Vapors Induced Haematological and Biochemical Changes in Male Rats. *Journal of Scientific Research* 2015; 7(3): 139-149.
12. Ross D. The role of metabolism and specific metabolites in benzene-induced toxicity: evidence and issues. *Journal of toxicology and environmental health* 2000; 61 (5): 357-372.
13. Amaglo NK, Bennett RN, Curto RBL, Rosa EA, Turco VL, Giuffrida A, Timpo GM. Profiling selected phytochemicals and nutrients in different tissues of the multipurpose tree *Moringa oleifera* L. grown in Ghana. *Food Chemistry* 2010; 122(4): 1047-1054.

How to cite this article

Odozi EB, Ayeni EE. Effect of aqueous leaf extract of Moringa oleifera on some complete blood count and some oxidative stress markers of stress-induced Albino Wistar rats. *Afr J Lab Haem Transf Sci* 2024;3(2):
DOI: https://doi.org/10.59708/ajlhts.v3i2.2408