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Protective Effect of Ethanolic Leaf Extract of *Moringa oleifera* on Haematological Indices of Rats Fed with Crude Oil-Treated Diet

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ABSTRACT

Oil pollution in the Niger Delta area of Nigeria has poised a major health challenge to inhabitants of the area. The government had paid little or no attention to the plight of these people as regards to their health. This study sought to assess the protective effect of ethanolic leaf extract of *Moringa oleifera* on haematological indices of rats fed with crude oil-treated diet. Twenty adult Wistar rats were used for this study. They were divided into four groups and fed accordingly: animals in group A was feed with 100 g of standard feed only (Control group), Group B with 100 g of standard feed +10 g of extract of *M. oleifera*, group C with 100 g of standard feed + 4 ml of crude oil and group D with 100 g of standard feed + 10 g of 4 ml of crude oil + extract of *M. oleifera* leaves. At the end of thirty days treatment, animals were fasted overnight and anaesthetized using diethyl ether. Blood samples were collected by cardiac puncture. Haematological parameters were determined using standard methods. Crude oil significantly reduced the haematological indices of animals used in this experiment when compared with the control group at $p<0.05$ but this effect was alleviated by *M. oleifera* leaf extract. This indicates that crude oil treat-diet down-regulated the haematological parameters of animals after thirty days treatment. This might be what happens in humans and animals living in the Niger Delta area of Nigeria who are continuously exposed to crude oil. However, this negative effect was annulled by *M. oleifera* leaves.

Keywords: *Moringa oleifera*, haematological parameters, crude oil treat-diet, ethanolic leaf extract, anaemia

1. INTRODUCTION

In the Niger Delta area of Nigeria some rural dwellers are exposed to crude oil because they use the chemical in various forms to treat a variety of ailments. They do this either by ingesting the crude oil or taken in combination with other substances [1]. In addition, humans get exposed to crude oil by consuming contaminated food, either directly or through the food chain [2]. Crude oil is injurious to animal's health [3, 4], which can be acute or chronic. Acute exposure of animals to crude oil usually result in eye irritation, nausea, vomiting, diarrhea and confusion, while chronic effects of petroleum hydrocarbon include decreased immune function, organ damage, biochemical and physiological abnormalities [5, 6, 7]. In fact, petroleum hydrocarbon causes metabolic imbalances in experimental animals [7]. In addition, crude oil had been implicated in the alteration of haematological parameters in animal models [8, 9]. Moreover, researchers have shown that antioxidants such as vitamins [10], palm oil [11], and honey [4, 12]. can be used to attenuate petroleum hydrocarbon toxicity in animals.

Moringa oleifera Lam. is the most widely cultivated species of the mono-generic family Moringaceae, which includes 13 species of trees and shrubs distributed in sub Himalayan ranges of India, Sri Lanka, North-eastern and South-western Africa, Madagascar and Arabia. Moringa is also native to parts of West Africa particularly Nigeria [13]. The whole *Moringa oleifera* plant is used in the treatment of psychosis, eye diseases, fever and as an aphrodisiac, the aqueous extracts of roots and barks were found to be effective in preventing implantation [14]. The Moringa tree is a multifunction plant. It has been cultivated in tropical regions all over the world for the following characteristics: high protein, vitamins, mineral and carbohydrate content of entire plants; high value of nutrition for both humans and livestock; high oil content (42%) of the seed which is edible, and with medicinal uses; the coagulant of seeds could be used for wastewater treatment [13].

Different parts of the *Moringa oleifera* (Mo) tree have been established as being good sources of unique glucosinolates, flavonoids and phenolic acids [15, 16], carotenoids [17], tocopherols [18], polyunsaturated fatty acids (PUFAs) [19], highly bioavailable minerals [20], and folate [21]. Among glucosinolates, 4-O-(a-L-rhamnopyranosyloxy)-benzylglucosinolate (glucomoringin) is the most predominant in the stem, leaves, flowers, pods and seeds of *M. oleifera* [15]. Although in the roots, benzyl glucosinolate (glucotropaeolin) is the most prominent. The highest content of glucosinolate is found in the leaves and seeds. The enzymatic catabolism of glucosinolates by the endogenous plant enzyme myrosinase produces isothiocyanates, nitriles, and thiocarbamates that are known for strong hypotensive (blood pressure lowering) and spasmolytic (muscle relaxant) effects [22]. In the leaves, the amount of quercetin and kaempferol was found to be in the range of 0.07–1.26 and 0.05–0.67 %, respectively. The potent antioxidant activity of Moringa is attributed to the high concentration of these polyphenols. Medicinally, the antioxidant, wound healing, hypotensive, and diuretic effects of this plant have been reported [23, 24].

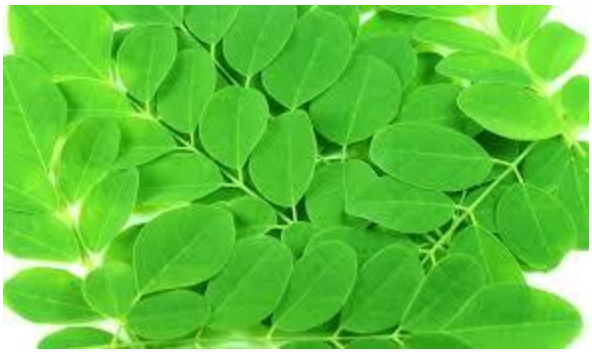

Figure 1: *Moringa oleifera* Leaves [13]

Previous studies have reported the antioxidant [25], anti-inflammatory [26] and pharmacological [15] properties of *M. oleifera*. Furthermore, Awodele *et al*., [27] worked on the toxicological evaluation of the aqueous extract of *Moringa oleifera* Lam (Moringaceae). Oyedepo *et al*., [28] evaluated the anti-hyperlipidemic effect of aqueous leaves extract of *Moringa oleifera*, while Choudhary *et al*., [29] assessed the antiulcer potential of *Moringa oleifera* root bark extract in rats. *Moringa oleifera* leaf has been reported to be potent in the prevention of peptic ulcer [30]. However, Airaodion et al. [31] reported that the combination of *M. oleifera* leaves and turmeric root is more potent in the prevention of peptic ulcer.

2. MATERIALS AND METHODS

2.1 Collection and Extraction of Plant Materials

Fresh plants of *M. oleifera* were from Institute of Agricultural Research and Training, Moor Plantation, Ibadan, Nigeria and were identified by a botanist. The leaves were carefully removed from the stem and washed in running water to remove contaminants. They were air dried at room temperature in an open laboratory space for ten days and milled into powder using an electronic blender (Moulinex). The extraction was done using soxhlet apparatus and ethanol as the solvent according to the methods described by Airaodion et al [32]. About 25 g of the powder was packed into the thimble of the soxhlet extractor and 250 mL of ethanol was added to a round bottom flask, which was attached to the soxhlet

extractor and condenser on a heating mantle. The solvent was heated using the heating mantle and began to evaporate moving through the apparatus to the condenser. The condensate dripped into the reservoir housing the thimble containing the sample. Once the level of the solvent reached the siphon, it poured back into the round bottom flask and the cycle began again. The process was allowed to run for a total of 18 hours. Once the process was completed, the ethanol was evaporated in a rotary evaporator at 35 ^{o}C with a yield of 2.55 g which represents a percentage yield of 10.20%. The extract was preserved in the refrigerator until when needed.

2.2. Experimental Design

Twenty adult male Wistar rats with body weight between 200 and 220 g were purchased from a private animal house in Osogbo, Nigeria. They were acclimatized for seven (7) days during which they were fed *ad libitum* with standard feed and drinking water and were housed in clean cages placed in well-ventilated housing conditions (under humid tropical conditions) throughout the experiment. All the animals received humane care according to the criteria outlined in the 'Guide for the Care and Use of Laboratory Animals' prepared by the National Academy of Science and published by the National Institute of Health. They were randomly divided into four groups of five rats each and treated as follows:

Group A: 100 g of standard feed only (Control group)

Group B: 100 g of standard feed +10 g of extract of *M. oleifera*

Group C: 100 g of standard feed + 4 ml of crude oil.

Group D: 100 g of standard feed + 10 g of 4 ml of crude oil + extract of *M. oleifera*

At the end of thirty days treatment, animals were fasted overnight and anaesthetized using diethyl ether. Blood samples were collected by cardiac puncture into heparinized bottles. The blood samples were centrifuge for 10 minutes using a bench-top centrifuge (Centromix) and the supernatant plasma was then used for the determinations of the haematological parameters.

2.3. Determination of Haematological Parameters

The red blood cells (RBC) and white blood cells (WBC) counts were determined by the improved Neubauer haemocytometer method. The haemoglobin (Hb) concentration was determined according to Jain [33], using the cyanomethaemoglobin method. The packed cell volume (PCV) was determined by the microhaematocrit method according to Dacie and Lewis [34]. Schilling method of differential lecukocyte count was used to determine the distribution of the various white blood cells [35]. Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were computed according to Jain [33].

2.4 Statistical Analysis

Data were subjected to analysis of variance using Graph Pad Prism. Results were presented as Mean ± standard deviation. One way analysis of variance (ANOVA) was used for comparison of the means followed by Tukey's (HSD) multiple comparison test. Differences between means were considered to be significant at $p<0.05$.

2. RESULTS

Results of this study are presented in tables 1 and 2.

Table 1: Effect of Ethanolic Leaf Extract of *M. oleifera* on Erythrocyte Parameters in Crude Oil Treated-Wistar Rats after 30 days of Treatment

Parameters	Control	*M. oleifera* only	Crude Oil only	Crude Oil + *M. oleifera*
PCV (%)	38.24±1.20^{a}	45.98±4.33^{b}	29.19±2.98^{c}	36.94±3.83^{a}
Hb (g/dL)	10.22±2.39^{a}	17.23±2.45^{b}	6.34±1.78^{c}	11.63±1.05^{a}

RBC ($X10^{12}$/L)	7.88±1.35[a]	12.34±1.46[b]	5.04±1.02[c]	7.03±1.83[a]
MCV (FL)	50.03±4.24[a]	51.34±6.48[a]	42.82±5.89[c]	48.99±6.03[a]
MCH (pg)	16.00±2.37[a]	16.79±1.32[a]	12.93±1.73[c]	13.06±2.62[c]
MCHC (g/dL)	24.02±1.44[a]	30.87±2.43[b]	19.67±2.63[c]	25.82±3.72[a]

Values are presented as Mean±standard deviation, where n = 5. Values with different superscript along the same row are significantly different at $p<0.05$.

LEGEND: PCV = Packed Cell Volume; Hb = Haemoglobin; RBC = Red Blood Cell; MCV = Mean Corpuscular Volume; MCH = Mean Corpuscular Haemoglobin; MCHC = Mean Corpuscular Haemoglobin Concentration

Table 2: Effects of Ethanolic Leaf Extract of *M. oleifera* on White Blood Cells Parameters and Platelets in Crude Oil Treated-Wistar Rats after 30 days Treatment

Parameters	Control	*M. oleifera* only	Crude Oil only	Crude Oil + *M. oleifera*
WBC ($X10^9$/L)	14.18±1.67[a]	21.00±2.91[b]	8.12±1.78[c]	15.22±1.33[a]
Lymphocyte (%)	40.52±4.82[a]	52.45±6.29[b]	29.92±4.82[c]	38.82±4.03[a]
Neutrophil (%)	59.21±6.79[a]	68.78±4.93[b]	48.48±3.48[c]	60.38±8.23[a]
Platelet ($X10^9$/L)	422.62±22.03[a]	436.92±19.37[b]	399.03±23.82[c]	419.19±16.63[a]

Values are presented as Mean±standard deviation, where n = 5. Values with different superscript along the same row are significantly different at $p<0.05$. WBC = White Blood Cell

4. DISCUSSION

Anaemia increases in prevalence and severity as renal function decreases, it becomes much more common at reduced glomerular filtration rate. Depending on the severity, some of the symptoms of anaemia may include: pale skin, fatigue, weakness, loss of appetite, low haematocrit and hemoglobin in a RBC etc. Factors likely to contribute to anaemia in chronic kidney diseases include blood loss, shortened red cell life span, vitamin deficiencies, the "uremic milieu," erythropoietin (EPO) deficiency, iron deficiency and inflammation [36]. However, the typical "anaemia of chronic renal insufficiency" is a result of a decreased production of red blood cells by the bone marrow. This defect in red blood cell production is largely explained by the inability of the failing kidneys to secrete hormone erythropoietin. This hormone is a necessary stimulus for normal bone marrow to produce red blood cells. Several researchers have reported the beneficial effect of *M. oleifera* leaves. Crude oil is a potential industrial, domestic and environmental toxicant [37, 38]. This study is therefore aimed at assessing the protective effect of ethanolic leaf extract of *M. oleifera* on haematological indices of Wistar rats fed with crude oil treated diet.

In this study, a significant increase was observed when the blood levels of erythrocyte parameters of control animals were compared with animals treated with leaf extract of *M. oleifera* only at $p<0.05$ as presented in table 1. This is an indication that there may be increased production of red blood cells therefore, suggesting the non-toxic nature of the plant to red blood cells at this period of administration. This is in agreement with the reports of Airaodion et al. [39, 40] who treated animals with *T. triangulare* and *T. occidentalis* respectively for twenty-eight days. However, when animals fed with hydrocarbon treated-diet were compared with the control animals at $p<0.05$, the erythrocyte parameters were observed to have significantly reduced. Previous studies indicated that certain organic and inorganic substances can mitigate the toxic effect of petroleum hydrocarbon [7, 12]. This is consistent

with the result of the present study. The addition of *M. oleifera* to diets pretreated with crude oil reduced the deleterious effect of hydrocarbon on the exposed animals. This is not suprising as *M. oleifera*, has been reported to treat Alzheimer's disease that was caused by Al accumulation [41]. The leaves of this plant which contain vitamins and Fe in significant amount were mentioned to improve iron and blood status of rats [42]. Furthermore, an earlier study by Airaodion et al. [30] revealed that *M. oleifera* leaves were able to prevent peptic ulcer due to its phytochemical content and antioxidant potentials. Its protective effect on erythrocyte parameters against hydrocarbon treated-diet might also be due to these phytochemicals and antioxidants.

The decrease in the blood levels of erythrocyte parameters observed in animals treated crude oil treated-diet in this study might be suggestive that crude inhibits erythropoietin release from the kidneys, which is the humoral regulator of RBC production and also affect the oxygen-carrying capacity of the blood and the amount of oxygen delivered to the tissues since red blood cells and haemoglobin (Hb) are very important in transferring respiratory gases [43, 44]. Thus, continuous exposure to crude oil may lead to anaemia.

The increase in the blood levels of erythrocyte parameters observed animals pretreated with *M. oleifera* leaf extract only when compared with the control at $p<0.05$ in this study might be suggestive that *M. oleifera* leaves have possible potentials to enhance erythropoietin release from the kidneys and also affect the oxygen-carrying capacity of the blood and the amount of oxygen delivered to the tissues since red blood cells and haemoglobin (Hb) are very important in transferring respiratory gases [40]. This may be due to the high content of iron and proteins in the plant. It is therefore possible that consumption of *M. oleifera* by humans can help prevent anaemia especially in menstruating and pregnant women. It has also been reported that values of RBC and associated parameters lower than normal ranges are indicative of anemic conditions while higher values are suggestive of polycythemia [45], thus, the 30-day treatment with *M. oleifera* leaves extract may not have the potential to induce anaemia nor polycythemia but was able to protect the animals against the effect of crude oil-induced anaemia.

The results of this study revealed a significant difference in the white blood cells parameters and platelet of control animals when compared with those treated with leaf extract of *M. oleifera* at $p<0.05$ as presented in table 2. White blood cells, platelet, neutrophil, and lymphocytes are used to provide useful information for diagnosis in routine clinical evaluation of the state of health of a patient. Changes in the haematological system have a higher predicative value for human toxicity [46]. The increase in WBC parameters and platelet counts may be due to the presence of anti-nutritional compounds such as saponins, flavonoids and steroid glucosides in *M. oleifera* leaves. It has been emphasized that the high percentage of WBC especially lymphocytes are associated with the ability of the animals to perform well under very stressful conditions [47]. This increase in the WBC and percentage lymphocyte counts suggests that the phytochemical compounds present in the extracts elicited stress responses. The effect of this plant on the total WBC count could be due to the presence of glycosides. This compound has an anti-inflammatory property and so has vital effect on inflammatory processes of some pathological states such as bacterial infection, malaria and liver diseases [48]. This might also imply that *M. oleifera* leaves may strengthen the immune system through many cytokines regulation.

The reduction in WBC parameters caused by crude oil treated-diet might be an indication that exposure to crude oil may reduce the ability of the animals to perform well under very stressful conditions as well as compromised the body's ability to attack and destroy invading bacteria, viruses and other injurious agents (Phagocytosis) [40]. However, this effect was remedied by *M. oleifera* leaf extract.

The significant decrease in platelet count at $p<0.05$ observed in animals treated with crude oil-diet only in this study may be an indication that hydrocarbon has inhibits the actions of platelet activating factor (PAF) and thus the blood clotting. It could also be an indication that it has the potential to inhibit

thrombopoietin production [49]. However, this effect was again alleviated by *M. oleifera* leaf extract.

5. CONCLUSION

The result of this study indicates that crude oil treat-diet down-regulated the haematological parameters of animals after thirty days treatment. This might be what happens in humans and animals living in the Niger Delta area of Nigeria who are continuously exposed to crude oil. However, this negative effect was annulled by *M. oleifera* leaves.

6. ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

7. COMPETING INTERESTS

Authors have declared that no competing interests exist.

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