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# Effects of *Moringa oleifera* Leaves Extract on Haematological Parameters of Phenylhydrazine Anaemia Induced Wistar Rats

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## Abstract

*Moringa oleifera* is known for its therapeutic effects as it has been long used in treatment of many diseases. The haematinic effect of this plant was investigated in this work. The work was carried in Madonna University Elele Campus Rivers State. A total of 15 rats were procured and grouped into three groups and allowed to acclimatize for one week. Phenyl hydrazine was used to induce the anaemia in all the groups; the extract was prepared and administered orally using oral gavage. The rats were grouped into three groups. Group A served as control while groups B and C served as test group and were administered with 200 mg per body weight of *Moringa oleifera* leaf extract and 300 mg per body weight of same extract respectively. Blood samples were collected through ocular puncture after 28 days and analysed for haematological parameters using standard manual methods. Results showed that there was significant ($P<0.05$) increase in red blood cell count, hemoglobin count, packed cell volume and white blood cell count. Oral administration of *Moringa oleifera* leaf extract irrespective of the dose has the tendency to increase blood parameters such as WBC, RBC, Hb and PCV in anaemic rats.

**Keywords:** *Moringa oleifera;* Anaemia; Traditional medicine

## Introduction

The practice of traditional medicine is as old as the origin of man [1]. The use of plants in traditional medicine referred to as herbalism or botanical medicine [2] falls outside the mainstream of the Western or Orthodox medicine. It has been estimated that about two third of the world's population (mainly in the developing countries) rely on traditional medicine as their primary form of health care. The use of traditional medicine in the treatment and management of diseases in the African continent cannot fade away and this could be attributed to the socio-cultural, socio-economic, lack of basic health care and qualified personnel [3]. Plants contain active components such as anthraquinones, flavonoids, glycosides, saponins, tannins, etc., which possess medicinal properties that are harnessed for the treatment of different diseases. The active ingredients for a vast number of pharmaceutically derived medications contain components originating from phytochemicals. These active substances that contain the healing property are known as the active principles and are found to differ from plant to plant. Among these plants are the vegetables whose part(s) are eaten as supporting food or main dishes and which could be aromatic, bitter or tasteless [4]. Vegetables vary considerably in their nutrient contents and are good sources of vitamins, essential amino acids, proteins, as well as minerals and antioxidants [5]. They are included in meals mainly for their nutritional value although some are reserved for the sick due to their medicinal properties. Generally, the active principles found in vegetables can be extracted and used in different forms which include infusions, syrups, concoctions, decoctions, infusion oils, essential oils, ointments and creams in the treatment/management and prevention of some diseases.

The human body is known to produce billions of new red blood cells, and other blood components which replace blood cells that are lost due to normal cell turnover processes, illness or trauma [6]. All the mature blood cells in the body are generated from a relatively small number of haematopoietic stem cells (HSCs) and progenitors. Each blood cell, red blood cells, white blood cells, and platelets play important roles in the body's normal physiological functions. However, certain diseases and conditions such as malaria, malnutrition, protozoan infections and pregnancy are among various conditions that could disrupt normal haematopoiesis thus predisposing one to anaemia.

Anaemia results in the decrease of the oxygen-carrying capacity of the blood due to reduction in circulating haemoglobin. The normal quantity of haemoglobin in humans is greater than 13 g/dl for males and 12 g/dl for females [7]. World Health Organisation (WHO) estimated in the year 2004 that about two billion people, representing 30% of the world's population were anaemic [8]. Also over 50% of pregnant women and over 40% of infants worldwide are anaemic with a prevailing significant morbidity and mortality particularly in the developing world. Iron deficiency is the most common cause of nutritional anaemia which affects over 600 million people throughout the world, particularly in developing countries. The vulnerable groups are infants, young children and women of child-bearing age. Hence anaemia is one of the leading health disorders posing great threat to global healthcare. Medicinal plants are currently being used in various parts of the world especially in the tropics for the treatment of various forms of anaemia. Most vegetables and plants have been found to contain haematinic agents such as folic acid, vitamin B 6, iron which could stimulate the erythropoietic pathway [9]. Pumpkin leaves have been shown to possess haematinic effect [10].

*Moringa oleifera* leaves, used as vegetables in various countries of the world have been shown to have positive effects on some

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haematological parameters [9]. However, due to their ability to increase blood parameters, their polyherbal formulations are been used in several localities among housewives without any scientific investigation of their effect on haematological indices. It is therefore expedient to compare the blood boosting capacities of the individual extracts with their polyherbal formulation. Against this background, we examined the effects of *Moringa oleifera* leaf extract on some haematological parameters using experimental rats.

## Materials and Methods

### Preparation of *Moringa oleifera* leaves extract

*Moringa oleifera* leaves were obtained from a farm in Rivers State, Nigeria. The leaves were cleaned and made free from sand and other impurities. The fresh leaves were air-dried and powdered in an electric kitchen blender. About 200 g of the powdered *M. oleifera* leaf was soaked in 1000 ml (1 litre) of distilled water for about 24 hours. The mixture was then filtered with a white cotton (satin) material, followed with filter paper (Whatman, 24 cm) into beakers and placed in an oven. The filtrate was evaporated to dryness using a rotary evaporator with temperature set at 50°C. The extract was then collected into a sample bottle and preserved in a refrigerator.

### Animal model

A total of 15 Wister strain albino rats with weight range of (100.5-202.6 g) obtained from the animal house, Madonna University Elele, Nigeria were used for the study. The rats were housed in wire meshed cage under standard conditions (temperature 25-29°C, 12 hours light and 12 hours darkness cycles) and fed with standard rat pelleted diet and water. The animals were given a period of one week for acclimatization. They were nursed under control of environmental conditions in accordance with international standard.

### Placement/Inducement

After one week, the rats were weighed and randomly separated into three groups (n=5 in each group). Anaemia was induced in all groups by intraperitoneal injection of phenylhydrazine at 40 mg/kg bodyweight for 2 days and 2 groups (experimental groups; B and C) were treated with *Moringa oleifera* leaves extract. This study lasted for 4 weeks.

Group A (Control); no *Moringa oleifera* leaf extract was administered and rats were fed with only Growers mash and water for 28 days.

Group B (Experimental 1) rats were administered 200 mg/body weight of *Moringa oleifera* leaf extract and fed with Growers mash and water for 28 days.

Group C (Experimental 2) rats were administered 300 mg/body weight of *Moringa oleifera* leaf extract and fed with Growers mash and water for 28 days.

### Ethical approval

The research was approved by the ethical committee of the institution. The standard, rules and regulations of use of animal for research purposes was strictly adhered to as approved by the committee.

### Sample collection/Analysis

All experimental animals were anaesthetized using chloroform fumes 24 hours after the last administration of the extract. 4 ml of blood sample was collected by ocular puncture from each of the animal model using capillary tube and was dispensed into commercially prepared concentrations of ethylene diamine tetra acetic acid containers. Blood samples collected were analyzed within six hours of collection using standard manual methods.

## Result

From the result of the research work there was a significant ($p<0.05$) difference in packed cell volume, haemoglobin concentration, total white cell count and platelet count when the test groups was compared with control group (Table 1).

## Discussion

This study was designed to investigate the effect of extract of *Moringa oleifera* leaves on haematological parameters in Phenylhydrazine induced-anemia in Wistar rats. *Moringa oleifera* leaves have been reported to have antitumor and anticancer activity and increases blood cell production, but its action on blood disorder has not been reported and there is paucity of information on its effects on anaemia. Since this poses a serious health risk to humans especially in the tropical region of Nigeria, it becomes expedient to evaluate the effect of this extract on anaemia using some hematological parameters (WBC, RBC, Hb, PCV, platelets and differential count) (Chinwe and Isitua, 2010). The result showed that there was a significant increase ($p<0.05$) in mean values of packed cell volume, when group B (31.2 ± 5.93%) and C (38.8 ± 3.96%) were compared with the control group A (28.2 ± 3.27%). No significant ($p>0.05$) increase was seen when group A (28.2 ± 3.27%) was compared with B (31.2 ± 5.93%) but significant ($p<0.05$) increase was seen when group A (28.2 ± 3.27%) was compared with group C (38.8 ± 3.96%).

There was also significant increase ($p<0.05$) in mean values of haemoglobin concentration, when group B (9.8 ± 1.26 g/dl) and C (13.8 ± 1.12 g/dl) were compared with the control group A (9.8 ± 1.26 g/dl). No significant increase ($p>0.05$) was seen when group A (9.8 ± 1.26 g/dl) was compared with B (9.8 ± 1.26 g/dl) but significant increase ($p<0.05$) was seen when group A (9.8 ± 1.26 g/dl) was compared with group C (13.8 ± 1.12 g/dl). There was a significant increase ($p<0.05$) in mean values of red blood cell count when group B (4.9 ± 1.05 × $10^{12}$/L)

| Parameters | Control (A) | Low dose (B) | High dose (C) | P-value |
|---|---|---|---|---|
| PCV (%) | 28.2 ± 3.27 | 31.2 ± 5.93 | 38.8 ± 3.96 | P<0.05 |
| HB (g/dl) | 9.8 ± 1.26 | 9.8 ± 1.26 | 13.8 ± 1.12 | P<0.05 |
| RBC (× $10^{12}$/L) | 4.4 ± 0.95 | 4.9 ± 1.05 | 6.1 ± 0.66 | P<0.05 |
| TWBC (× $10^{9}$/L) | 9.4 ± 1.4 | 6.3 ± 0.82 | 5.9 ± 1.08 | P<0.05 |
| Platelet (× $10^{9}$/l) | 227.6 ± 27.36 | 254.8 ± 22.39 | 269.2 ± 27.28 | P>0.05 |
| Neutrophil (%) | 52.4 ± 8.47 | 52.4 ± 6.39 | 43.8 ± 6.61 | P>0.05 |
| Lymphocytes (%) | 41.8 ± 6.91 | 41.6 ± 6.46 | 50.0 ± 5.15 | P>0.05 |
| Monocytes (%) | 4.2 ± 1.64 | 4.4 ± 1.34 | 4.4 ± 1.82 | P>0.05 |
| Eosinophil (%) | 1.6 ± 0.54 | 1.6 ± 0.89 | 1.8 ± 0.84 | P>0.05 |

**Table 1:** Mean ± S.D of haematological parameters of the control group (A) and the test groups (B and C).

and C ($6.1 \pm 0.66 \times 10^{12}$/L) were compared with the control group A 6.1 ± 0.66. No significant increase ($p>0.05$) was seen when group A ($4.4 \pm 0.95 \times 10^{12}$/L) was compared with B ($4.9 \pm 1.05 \times 10^{12}$/L) but significant increase ($p>0.05$) was seen when group A ($4.4 \pm 0.95 \times 10^{12}$/L) was compared with group C ($6.1 \pm 0.66 \times 10^{12}$/L).

RBC, haemoglobin concentration and PCV in anaemic group showed a significant decrease when compared with extract-fed groups [11]. This could be due to toxicity caused by Phenylhydrazine by the involvement of aryl and hydroxyl radicals it generates. It could also be due to poor affinity of oxygen to haemoglobin molecules since the tendency of haemoglobin to bind to oxygen enhances blood flow to the tissues [12]. In extract-treated groups, there was also a significant increase in these parameters when compared with the anaemic groups. This could be due to the phytochemical constituents in the extract and also presence of minerals and vitamins. These constituents are well known haemopoietic factors that have direct influence on the production of blood in the bone marrow [13]. The findings of this study seem to support this claim and restoration of these functions could entail the presence of *M. oleifera* extract. The principal role of *M. oleifera* extract seems to facilitate iron absorption, as adequate amount of this element is necessary for Haemoglobin synthesis and for the animal tissues such as the kidneys and bones to take part in manufacture of RBCs. The normal levels of WBC, Platelets and lymphocytes could further confirm this claim as their production does not necessitate iron absorption.

There was a significant decrease ($p<0.05$) in mean values of total white blood cell count, when group B ($6.3 \pm 0.82 \times 10^{9}$/L) and C ($5.9 \pm 1.08 \times 10^{9}$/L) were compared with the control group A ($9.4 \pm 1.4 \times 10^{9}$/L). No significant ($p>0.05$) increase was seen when group A ($9.4 \pm 1.4 \times 10^{9}$/L) was compared with B ($6.3 \pm 0.82 \times 10^{9}$/L) but significant increase ($p<0.05$) was seen when group A ($9.4 \pm 1.4 \times 10^{9}$/L) was compared with group C ($5.9 \pm 1.08 \times 10^{9}$/L). However, rats fed with moringa extract showed increase in total white blood cells as supported by the work. This can be attributed to the anti-bacterial properties of the extract although the mechanism of this action is yet unknown.

## Conclusion

The effect of oral administration of *Moringa oleifera* leaf extract irrespective of the dose has the tendency to increase blood parameters such as WBC, RBC, Hb and PCV in anemic rats. This possibly could the case in humans, hence justifying its use in traditional medicine practice.

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