MPRI-JMBP | INTERNATIONAL JOURNAL OF MEDICAL, BIOLOGICAL AND PHAR. SCI.
VOL. 11 NO.3 JUN-2020 ISSN: 1999-5650

# EFFECTS OF MORINGA OLEIFERA LEAVES EXTRACT ON SOME HAEMATOLOGICAL PARAMETERS IN PHENYLHYDRAZINE INDUCED ANAEMIC ALBINO RATS

*ZAINAB HASSAN BELLO[1], SHAMSUDEEN MUHAMMAD[1], SALE AMINU[1], SUMAYYA AHMED AYUBA[1], FARIDA BASHAR[2] AND ZULKALLAINI SHEHU[1]

[1]Department of Biochemistry, Sokoto State University, Sokoto, Nigeria [2]Department of Biochemistry, Usmanu Danfodiyo University, Sokoto, Nigeria

**Abstract**

This research was conducted to investigate the effect of ethanol leaf extract of Moringa oleifera in phenylhydrazine-induced anaemic albino rats on some blood parameters (Packed Cell Volume (PCV), Red Blood Cell Count (RBC), Haemoglobin (Hb) Concentration, Mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC) White Blood Cell Count (WBC) and Differential Leucocyte Count (DLC)). Twenty (20) rats of both sexes were randomly divided into 5 groups after acclimatization for three weeks. Group 1 (normal control), Group 2 (negative control) was administered with Phenylhydrazine (40 mg/kg, interpretoneally) without treatment. 0.6ml of Moringa oleifera extract was given to Group 3. Phenylhydrazine (40 mg/kg) were administered to Groups 4 and 5 and were treated with 300 and 600 mg/kg of Moringa oleifera respectively. All treatments with the extract were given orally for 14 days. Anaemia was comfirmed after 24 hours through PCV and Hb estimation. After treatment period, blood samples were collected from the rats via their heart and was analysed.

**KEYWORDS:**
Moringa olifera, Phenylhydrazine, Heamatological parameters, Anemic, Albino rats.

*Results showed that, there was significant (P<0.05) increase in some blood parameters (PCV, Hb, RBC count). Therefore, oral administration of ethanolic extract of Moringa oleifera may increase some blood parameters and may be essential in the treatment and management of anaemia.*

## INTRODUCTION

*Moringa oliefera commonly referred to simply as "Moringa" belongs to the kingdom Plantae, Order Brassicales, Family Moringacacae, Genus Moringa and Species Moringa oliefera. Moringa oliefera is native to the Indian subcontinent and has become naturalized in the tropical and subtropical areas of the world. It is the most widely cultivated species of the Genus Moringa, which is the only genus in the Family Moringacacae. It is an exceptionally nutritious vegetable tree with a variety of potential uses. Moringa oliefera trees are well naturalized in the northern parts of Nigeria where the leaves are popularly known as 'zogala' and widely consumed by populace (Pallavi and Dipika, 2010).*

*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. 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 (Adedapo et al., 2009). 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 (Atawodi et al., 2010).*

*The rationale behind this research is in-view of nutritional importance of Moringa oleifera as well as its therapeutic values in having antihypertensive effect, anticancer, analgesic, antiinflammatory, antidiabetic, antispasmodic, antiulcer and antibacterial activity and hepatoprotectivity. Until now, to the best of my knowledge there is no research that used phenylhydrazine in inducing anaemia, moringa extract as the anti-anaemia agent and monitored haematological parameters such as RBC, PCV, Hb WBC, and Differential leucocyte count in this catchment area (sokoto).*

## *MATERIALS AND METHODS*

*All chemicals and reagents used for the study were of analytical grade. The list of materials and reagents used include: Potassium Cyanide 500mg, Potassium 200mg, Potassium dihydrogen phosphate 140mg, Distilled water 1 Liter, pH 7.4. Stored in a dark bottle (for Hb estimation), Sodium citrate 3.0 g, Formaldehyde 1.0 ml Distilled water 99.0 ml (for RBC count), leishman's stain, methanol, ethanol, 2% glacial acetic acid (for WBC). Weighing balance, beakers, test-tubes, colorimeter, microscope, glass slides, cover-slips, hemocytomete, cuvette, mortar and pestle, spatula, Microhematocrit centrifuge, Microhematocrit capillary tube, Microhematocrit reader, plasticine, cotton wool, blood sample.*

### *Source/Preparation of Moringa oleifera Leaves Extract*

*Moringa oleifera leaves were obtained from a farm garden in Sokoto state, Nigeria, it was identified at Pharmacognocy and Ethno Pharmacy, Usmanu Danfodiyo University Sokoto and batch number (PCG/UDUS/MORI/0001) was issued. The leaves were cleaned with water and freed from sand and other impurities. The fresh leaves were shade dried and then powdered using pestle and mortal. Powdered Moringa oleifera leaf (200 g) was soaked in 1000 ml (1 litre) of ethyl alcohol for about 24 hours. The mixture was then filtered using filter paper (Whatman 24 No 1) in 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 in a sample bottle and preserved in a refrigerator.*

### *Animal Model*

*A total of 25 strains of albino rats weighing about 200 g were obtained from the animal house, Ahmadu Bello University Zaria, Nigeria. The rats were housed in plastic rubber cage in Pharmacology and Toxicology Animal House, Usmanu Danfodiyo University Sokoto under standard conditions (temperature 25-29°C, 12hours light and 12hours darkness cycles) and fed with standard rat pelleted diet and water. The animals were given a period of three*

*weeks for acclimatization. They were nursed under control of environmental conditions in accordance with international standard.*

***Experimental Design***

***Placement/ Inducement***

*After three weeks of acclimatization, the rats were weighed and randomly separated into five groups (n=5 in each group). Anaemia was induced in group 2 (negative control), group 4 and group 5 by intraperitoneal injection of phenylhydrazine at 40 mg/kg bodyweight for 24 hours. Anaemia was comfirmed by collection of blood sample from the heart of one animal in each group after 24 hours by PCV and Haemoglobin determination.*

***Treatment Groups***

*Group 1: Normal control (distilled water), Group 2: Negative control (PHZ without treatment), Group 3: MOE (0.6 ml/200 g/day), Group 4: Anemic + MOE (300 mg/kg daily), Group 5: anemic + MOE (600 mg/kg daily).*
*MOE = Moringa oleifera leaves extract. The extracts were dissolved in distilled water and treatment was given orally which lasted for 14 days*

***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 through the heart of each animal into EDTA bottles. Blood samples were analyzed manually. Parameters determined were Hb count, RBC count, PCV, MCV, MCH, MCHC. Blood samples collected were analyzed within six hours of collection using standard manual methods.*

***RESULTS***

*The results indicated that Packed cell volume, Haemoglobin concentration and Red blood cell count of Moringa oleifera treated groups significantly increased ($P<0.05$) when compared to the positive control group and significant reduction ($P<0.05$) was observed in negative control group.*

***Table 1:*** *Effect of Moringa oleifera on some Haematological Parameters*

| GROUPS (n = 4) | CTRL | NTAC | MO | AM (300mg/kg) | AM (600mg/kg) |
|---|---|---|---|---|---|
| PCV (%) | 33.00±0.91 | 19.75±0.48*** | 39.00±0.82*** | 37.25±0.63** | 34.75±0.25 |
| Hb (g/dl) | 10.83±0.35 | 7.40±0.24*** | 12.95±0.25*** | 12.40±0.21** | 11.60±0.10 |
| RBC (/mm³) | 5.81±0.20 | 2.69±0.12*** | 7.20±0.12*** | 6.79±0.14** | 6.27±0.06 |

***Values are expressed as Mean ± SEM***

****Represents highly significant difference (P<0.05) compared to the control***

*****Represents extremely significant difference (P< 0.01) compared to the control***

***CTRL= cntrol, NTAC= non- treated anaemic control, MO = moringa only, AM = anaemia + Moringa.***

***The effect of Moringa oleifera on erythrocytic indices was assessed. The results indicated that there is no significant difference in group 3, 4, and 5 (P>0.05) when compared with normal control but there was significant difference in group 2 (P<0.05) when compared with normal control.***

***Table 2 :*** *Effect of Moringa oleifera on Erythrocytic Indices*

INTERNATIONAL JOURNAL – MBP
VOL. 11 NO.3 JUN-2020 ISSN: 1999-5650

| ***GROUPS (n = 4)*** | *CTRL.* | *NTAC* | *MO* | *AM (300mg/kg)* | *AM(600mg/kg)* |
|---|---|---|---|---|---|
| ***MCV (fl)*** | *56.83±0.51* | *73.68±1.45*** | *54.18±0.23* | *54.88±0.32* | *55.45±0.13* |
| ***MCH (pg)*** | *18.63±0.11* | *27.50±0.31*** | *17.98±0.09* | *18.28±0.11* | *18.50±0.04* |
| ***MCHC (g/dl)*** | *33.03±0.19* | *37.30±0.31*** | *33.18±0.09* | *33.25±0.03* | *33.35±0.05* |

***Values are express in Mean ± SEM***

****Represents extremely significant difference (P<0.001) compared to the control***

***CTRL= cntrol, NTAC= non treated anaemic control, MO = moringa only, AM = anaemia and Moringa, MCV = means cell volume, MCH = means cell haemoglobin and MCHC = mean cell haemoglobin concentration***

*Table 3**: The effect of Moringa oleifera on some white blood cells parameters was assessed. The results indicated that Moringa oleifera significantly increased (P<0.05) white blood cell count in anaemia non treated and anaemia treated with Moringa at 600mg/kg groups when***

***compared to the positive control and no significant difference when Moringa only and anaemia treated with Moringa at 300mg/kg was compared with control group(P<0.05) in negative control.***

| *GROUPS (n=4)* | *CTRL* | *NTAC* | *MO* | *AM(300mg/kg)* | *AM(600mg/kg)* |
|---|---|---|---|---|---|
| *WBC (/mm³)* | *8.67±0.427* | *13.47±0.60*** | *8.85±0.613* | *7.89±0.91* | *10.37±0.71** |
| *Neutrophils (%)* | *22.25 ±0.469* | *18.75 ±3.065** | *25.5 ±0.848** | *23.0 ± 1.080* | *21.75 ± 0.479* |
| *Lymphocytes (%)* | *74.25 ±0.853* | *74.25± 0.854* | *70.25±0.562** | *73.25 ± 1.797* | *74.25 ± 1.031* |
| *Monocytes (%)* | *3.5 ± 0.289* | *3.5 ± 0.866* | *4.75 ± 0.854* | *2.75 ± 0.854* | *2.5 ± 0.646* |

*Values are expressed as Mean ± SEM*

**Represents highly significant difference (P< 0.05) compared to the control*

***Represent extremely significant difference (P< 0.001) compared to the control*

*CTRL= control, NTAC= anaemia non- treated, MO = moringa only, AM = anaemia + moringa.*

## DISCUSSION

This research investigated the effect of ethanolic extract of Moringa oleifera leaves on haematology in phenylhydrazine-induced anaemic rats. Moringa oleifera leaves has been reported to have antitumor and anticancer activity and increases blood cell production. Since this constitute a serious health risk to humans especially in Nigeria, it becomes vital to evaluate the effect of this extract on anaemia using some haematological parameters (RBC, Hb, PCV, MCV, MCH, MCHC WBC and DLC). The result showed Red blood cell count (RBC), haemoglobin concentration (Hb) and packed cell volume (PCV) in anaemic group significanty (P < 0.05) decreased when compared with normal control group and this support the work done by Nku-Epang et al. (2015) and Anslem et al. (2017). This could be due to toxicity

caused by phenylhydrazine induced. It could also be due to poor affinity of oxygen to haemoglobin molecules since the ability of haemoglobin to bind to oxygen enhances blood flow to the tissues. In extract-treated groups, there was also a significant increase ($P< 0.05$) in these parameters when compared with the control and 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 hemopoesis in the bone marrow.

MCV, and MCH increased significantly ($P<0.05$) in the anaemic group when compared with the normal control and extract-treated groups. This supports the earlier works of Nku-Epang et al. (2015) and Hisham (2012) in which phenylhydrazine decreased Hb, RBC and PCV levels but increased MCV, MCH, MCHC and extramedular hematopoiesis in the spleen and liver. Sembulingam, Murakami et al showed that MCV, MCH and MCHC increases in pathological conditions like liver cirrhosis and haemolytic anaemia. The increase observed in the white cells count (WBC, neutrophils and lymphocytes) may be as a result of the toxicity caused by phenylhydrazine which lead to the immune cells response.

## CONCLUSION

The effect of oral administration of Moringa oleifera leaf extract irrespective of the dose has the tendency to increase blood parameters such as RBC, Hb and PCV in anemic rats. It will be useful in the treatment of anaemia since traditional medicine has become highly integrated in the world of medicine today. However, caution should be taken when administering the extract as it could lead to increase above normal (polycythemia) or abnormal high blood cell production when taken in high doses.

## REFERENCES

Adedapo, A.A., Mogbojuri, O.M. and Emikpe, B.O. 2009. Safety evaluations of the aqueous extract of the leaves of Moringa oleifera in rats. Journal of Medicine Plants Research. 3:586-591.

Adias, T.C., Ajugwo, A.O., Erhabor, T. and Nyenke, C.U. 2013. Effect of pumpkin extract (Telfairia occidentalis) on routine haematological parameters in acetone-induced oxidative stress albino rats. American Journal of Food Science and Technology.1:67-69.

Ali, A., Yusof, Y.A., Chin, N.L., Ibrahim, M.N. 2017 "Processing of Moringa leaves as natural source of nutrients by optimization of drying and grinding mechanism". Journal of Food Process Engineering. 40(e12583).

Atawodi, S.E., Atawodi, J.C., Idakwo, G.A., Pfundstein, B., Haubner, R., Wurtele, G., Bartsch, H. and Owen, R.W. 2010 "Evaluation of the polyphenol content and antioxidant properties of methanol extracts of the leaves, stem, and root barks of Moringa oleifera Lam". Journal of Medicinal Food.13(3):710-6.

Catassi, C., Bai, J.C., Bonaz, B., Bouma, G., Calabrò, A., Carroccio, A., Castillejo, G., Ciacci, C., Cristofori, F., Dolinsek, J., Francavilla, R., Elli, L., Green, P., Holtmeier, W., Koehler, P., Koletzko, S., Meinhold, C., Sanders, D., Schumann, M., Schuppan,. D, Ullrich, R., Vécsei, A., Volta, U., Zevallos, V., Sapone, A., Fasano, A. 2013. "Non-Celiac Gluten sensitivity: the new frontier of gluten related disorders". Nutrients (Review).5(10):3839-53.

Chinwe, C., and Isitua, N. (2010). Studies on the haematological impact of Moringa oleifera in rabbits. A poster presented at 2nd Internationals Conference on Applied Biotechnology, October 25-27, Khartoum, Sudan.

Divi, S.M., Bellamkonda, R., Dasireddy, S.K. 2012. "Evaluation of antidiabetic and antihyperlipedemic potential of aqueous extract of Moringa oleifera in fructose fed insulin resistant and STZ induced diabetic wistar rats" a comparative study. Asian Journal of Pharmacology and Clinical Research. Pp. 67-7.

Hara, H., and Ogawa, M. 1975. Erythropoietic precursors in mice with Phenylhydrazine-induced anemia. American Journal of Hematology. 1:453-458.

Iqbal, S, Bhanger, M.I. 2006. "Effect of season and production location on antioxidant activity of Moringa oleifera leaves grown in Pakistan". Journal of Food Composition and Analysis.19(6–7):544-551.

Kumar, H.D. 2004. "Management of Nutritional and Health Needs of Malnourished and Vegetarian People in India". Complementary and Alternative Approaches to Biomedicine. Advances in Experimental Medicine and Biology. 546. Springer US. Pp:311-321.

Mahajan, S.G, Mali, R.G, Mehta, A.A. 2007. "Protective effect of ethanolic extract of seeds of Moringa oleifera Lam. against inflammation associated with development of arthritis in rats". Journal of Immunotoxicology. 4(1):39-47.