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# Hematinic Properties and Effects of *MoringaOleifera* Aqueous Leaf Extract on Phenylhydrazine-Induced Bone Marrow Toxicity in Adult Wistar Rats

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## ABSTRACT

This study was aimed at determining the effects of *moringa oleiferia* aqueous leaf on phenylhydrazine-induced anemia and bone marrow toxicity in adult wistar rats. Twenty adult wistar rats weighing between 190 – 230g were used for this study. The animals were obtained and nurtured at the animal house of the Department of Anatomy, Faculty of Basic Medical Sciences, University of Benin. The animals were randomly divided into four groups of five animals each. Group A animals served as the control and were given normal marsh feed. Anemia was induced to animals in groups B, C and D by oral administration of phenylhydrazine for eight (8) days. Animals in group B were left untreated throughout the duration of the experiment while those in groups C and D were treated with 350 mg/kg body weight and 700 mg/kg body weight of the *Moringa oleifera* leaf extract respectively once daily for 28 days. After the completion of the treatment, the animals were anesthetized and sacrificed to collect blood for hematological analysis. Bone marrow aspirates were collected from the pelvis of the animals for histological analyses using hematoxylin and eosin stains. The results showed that phenylhydrazine induced anemia by reducing red blood cell volume, hemoglobin concentration and mean corpuscular volume. It also induced bone marrow toxicity by decreasing the cellularity and myelo-erythroid percentage. Aqueous extract of *Moringa oleifera* leaf reversed these effects in a dose-dependent manner. In concentration. In conclusion, this study showed that aqueous extract of *Moringa oleifera* leaf has dose-dependent reversal capacity against phenylhydrazine bone marrow toxicity.

**Key Words***:* Anemia, Phenylhydrazine, *Moringa Oleifera,* Bone marrow

## INTRODUCTION

Anemia is a blood disorder that is characterized by reduction in red cell mass, reduction in hemoglobin in quantity and quality or both in the blood,[1] thereby reducing the oxygen concentration to the tissues and ultimately affecting cellular metabolism.[2] It can, thus, be classified based on the concentration of hemoglobin, the shape of the red blood cell and size of the red blood cell.[3] It is the commonest nutritional deficiency in the world affecting about a quarter of the population.[4] Anemia can affect our normal day-to-day activities, response to mild and moderate exercises, responses to minor or severe injuries, surgeries and other invasive treatment depending on the onset, degree and duration of the anaemia.[5,6] The etiology of anemia differs and as such it can cut across ethnic group and socio-economic class but those caused by nutritional deficiency is common in the low socio-economic class.[7] Its causes can generally be classified into four main classes which are reduced production of hemoglobin, increased destruction of hemoglobin or blood loss and splenic pooling.[1] Its signs, symptoms and complications depend on the degree of anemia, onset of the anaemia[8] and the age of the individual,[9] which ranges from pallor, dyspnea, tachycardia, cardiomegaly, hepatomegaly, anemic heart failure to renal failure.[10]

Phenylhydrazine (PHZ) is an antipyretic drug which is normally taken for fever.[11] It has been known to induce anemia over decades, with subsequent increase in the numbers of erythrocyte-committed-progenitor-colony-forming units.[12] It may also induce vascular dysfunction and hemodynamic disturbance.[13] PHZ-induced anemia had been used as a model for the evaluation of its influence on some therapeutic effectiveness;[14] as a model of reticulocyte researches or erythrocyte senescence;[15] and as a model for the study of haematinic effects.[16,17] Subchronic intoxication of rats with phenylhydrazine (10 mg/kg body weight/day for 8 days) results in a marked hemolytic anemia characterized by decreased red blood cell, hemoglobin and packed cell volume.[18]

*Moringa oleiferia* is a tree seen in Africa and Asia continent of the world.[19] It is a drought resistant wispy

plant of about 5 meters tall. Its leaves, pods are long slender and triangular giving it the name "drumstick" tree.[20] *Moringa oleiferia* is commonly known as "the tree of life" or "miracle plant" because of its numerous medicinal properties.[21] Its nutritional and chemical composition includes vitamins, fatty acids, polyunsaturated fats, carbohydrates, protein, phenolic acids, isothiocitrate among others.[22] A previous study had shown that *Moringa oleiferia* increases hemoglobin concentration in mice.[23] The plant has also been found to have antibacterial, antidiuretic, antioxidant, anticonvulsant, hypoglycemic, hypolipidemic, hypotensive, mosquito repellant, sedative properties among others.[24]

This study was aimed at determining the effects of *moringa oleiferia* aqueous leaf on phenylhydrazine-induced anemia and bone marrow toxicity in adult wistar rats. A study such as this would be helpful when applied in cases where hematinics cannot be taken due to poverty or in patients who cannot take the conventional hematinics as in cancer patients where the presence of folic acid aggravates the proliferation of cancer cells.[25]

## MATERIALS AND METHODS

**Preparation of leaf extract:** *Moringa oleifera* leaves were harvested from bush farms around Benin City and identified at the hebarium in the Department of Plant Biology and Biotechnology, Faculty of Life Science, University of Benin, Benin City, Edo State, Nigeria. The leaves were dried in shade for seven days and were later taken to an oven to finally dry and totally remove the moisture content. After drying, the leaves were grinded in an electric grinding machine into a powdery form. The powdered leaves were soaked in distilled water for 48 hours at room temperature. The mixture was filtered into conical flask with Watman filter paper. The filtrate was evaporated at a temperature of 30 $^{o}$C for 10 hours to produce a greenish-black semi-solid extract that was stored in the refrigerator at 4 $^{o}$C and used for the experiment. Appropriate concentration of the extract was then subsequently made by dilution with distilled water into 350 mg/kg and 700 mg/kg body weight and administered to the animals. All preparations were performed at the Department of Anatomy, School of Basic Medical Sciences, University of Benin, Benin City, Edo State, Nigeria.

**Animals:** Twenty adult wistar rats weighing between 190 and 230 g were used for this study. The animals were obtained and nurtured at the animal house of the Department of Anatomy, Faculty of Basic Medical Sciences, University of Benin. The rat cages were made of wood and wire gauze. Appropriate ventilation and proper hygiene were ensured. All animals were allowed free access to feed marsh and water throughout the duration of the experiment. The weights of the animals were taken before and after the experiment. The animals were randomly divided into four groups of five animals each. Group A animals served as the control and were given normal marsh feed. Anemia was induced to animals in groups B, C and D by oral administration of phenylhydrazine for eight (8) days. Animals in group B were left untreated throughout the duration of the experiment while those in groups C and D were treated with 350 mg/kg body weight and 700 mg/kg body weight of the *Moringa oleifera* leaf extract respectively once daily for 28 days.

After the completion of the treatment, the animals were anesthetized and sacrificed to collect blood from the aorta after a central median incision into the thoracic cavity and blood samples were subjected to hematological analysis. Bone marrow aspirates were collected from the pelvis of the animals and their smears prepared on glass slides. The slides were allowed to dry and thereafter, stained using the hematoxylin and eosin techniques.

### Statistical Analysis

The data analysis was performed for hematological parameters with statistical package for Social Sciences software version 16 (SPSS, Chicago, Il). Results were presented as mean $\pm$ standard error of mean. The results were compared between groups using One-Way Analysis of Variance (ANOVA) and when statistically significant, Post-Hoc was conducted using Least Square Difference (LSD). Results were considered statistically significant when Probability was less than 5 % ($P<0.05$).

## RESULTS

### Effect on Hematological Profile

The result revealed that red blood cell count was significantly lower ($P<0.05$) in group B when compared with groups A, C and D whereas there was no significant difference in the red blood cell count of group A when compared to groups C and D. More so, hemoglobin concentration was significantly lower ($P<0.05$) in group B when compared with groups A, C and D whereas there was no significant difference in the hemoglobin concentration of group A when compared to groups C and D. There was significant increase in the Mean Corpuscular volume (MCV) in all the experimental groups (group B, C and D) when compared with the control group (group A). There was no statistically significant difference ($P>0.05$) in the Mean Corpuscular Hemoglobin (MCH) between the control group A and the experimental groups (group B, C and D). There was no statistically significant difference ($P>0.05$) in the white blood cell count (WBC) between the control group A and the experimental groups (group B, C and D).

### Histological Findings

Photomicrographs of group A (control group) showed normal cellularity, with a myelo-erythroid percentage of 30% and above and megakaryocytes. Group B (treated with phenylhydrazine only for eight days) showed markedly decreased cellularity and myelo-

erythroid percentage of below 30%. Photomicrographs of group C (treated with phenylhydrazine for eight days, followed by oral administration of 350 mg/kg body of aqueous extract of *Moringa oleifera* for 28 days) showed mildly increased cellularity. Photomicrographs of group D (treated with phenylhydrazine for eight days, followed by oral administration of 350 mg/kg body of aqueous extract of *Moringa oleifera* for 28 days) showed moderately increased cellularity.

**Table 1:** Some Hematological Parameters of the Experimental Animals

| Parameters / Groups | RBC x10/ul | HGB (gd) | MCV (fl) | MCH (pg) | WBC x10/ul |
|---|---|---|---|---|---|
| Group A (n=5) | 7.07±0.43 | 15.60±0.78 | 56.66±9.70 | 21.84±1.05 | 9.73±2.17 |
| Group B (n=5) | 4.83±0.21* | 11.58±0.60* | 96.42±1.77* | 25.20±0.30 | 9.76±3.10 |
| Group C (n=5) | 7.10±0.66 | 16.12±0.18 | 70.04±6.41* | 25.08±1.04 | 10.82±1.72 |
| Group D (n=5) | 6.81±0.33 | 15.74±0.50 | 63.90±4.81* | 22.98±0.76 | 10.64±1.70 |

*=P<0.05

**HISTOLOGICAL FINDINGS**

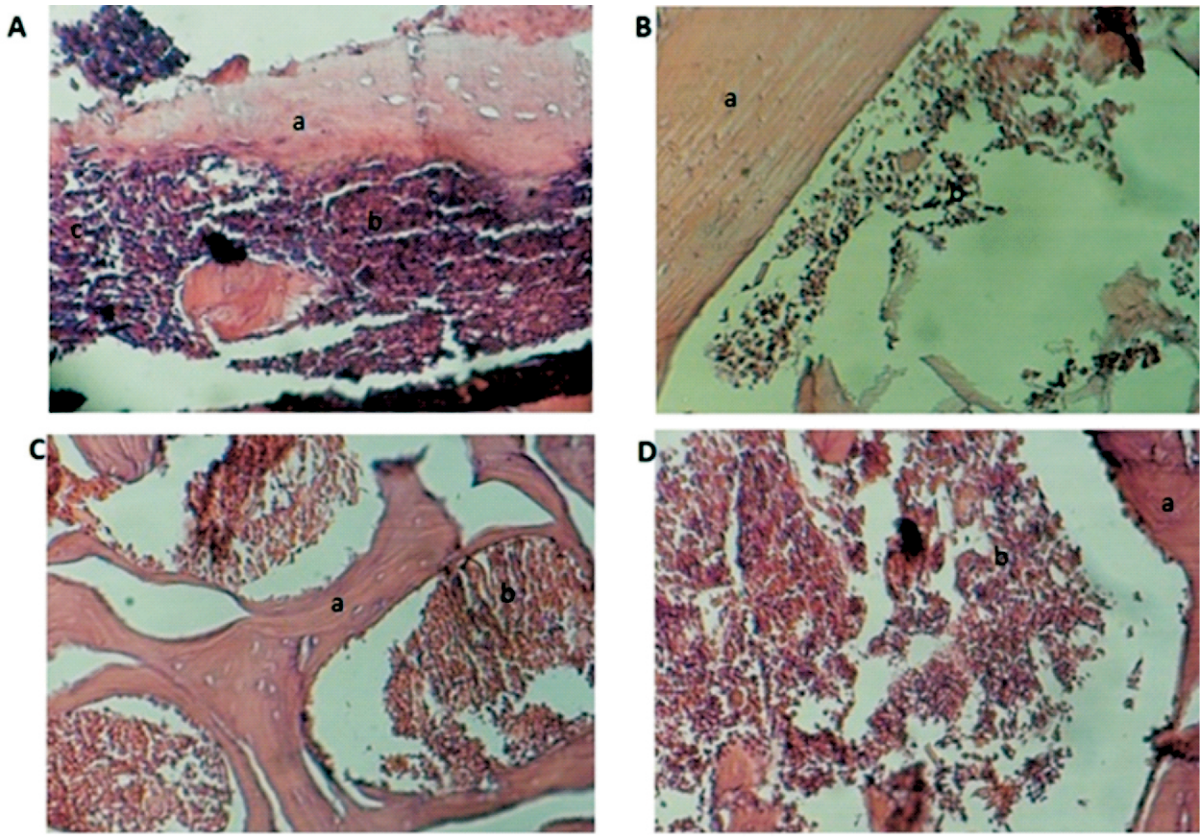


**Figure 1:** Photomicrographs of the experimental animals.

Plate A (Control group) presented normal histology of bone marrow with normal cellularity composed of bone spicules 'a', myelo-erythroid cells 'b' and megakaryocyte 'c'. Plate B (phenylhydrazine-induced anemic group) presented bone marrow with markedly decreased cellularity 'b'. Plate C (anemic rats treated with 350 mg/kg body weight of *Moringa Oleifera*) presented mildly increased cellularity. Plate D (anemic rats treated with 700 mg/kg body weight of *Moringa Oleifera*) showing moderately increased cellularity. (H&E x 100)

## DISCUSSION

Anaemia remains a public health concern in many developing countries. About two billion people around the globe had been estimated to suffer from anemia. In Africa and South-East Asia, report has also shown that it accounts for about seven hundred and fifty thousand deaths a year.[26]

Anemic capacity of phenylhydrazine had been exposed ancient researchers as early as the nineteenth century.[27,28] The present study showed that phenylhydrazine induces anemia by reducing red blood cell volume, hemoglobin concentration and mean corpuscular volume. This is in agreement with previously reported hematotoxic properties of phenylhydrazine.[16,29-32] Studies had pointed at oxidative stress as one of the mechanisms through which phenylhydrazine induces anemia. Other studies indicated that phenylhydrazine induces reticulocytosis, increased osmotic resistance, free plasma hemoglobin, mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and erythropoietin levels, and extramedullar hematopoiesis in the spleen and liver.[33-34]

Furthermore, the present study shows that phenylhydrazine induces bone marrow toxicity by decreasing the cellularity and myelo-erythroid percentage. Säterborg reported marrow abnormality following phenylhydrazine-induced hemolysis in rabbits.[35] This is a confirmation that the bone marrow hematopoiesis is one of the target sites for the action of phenylhydrazine in addition to other extramedullar hematopoietic sites.

Moreau et al. studied bone status and bone marrow erythropoiesis studied in mice with hemolytic anemia induced by phenylhydrazine (PHZ) or Plasmodium infection and in bled mice.[31] Whereas they found no major alterations in cellularity and erythroid cell numbers in the bone marrow of bled mice, reduced bone marrow erythroid precursors and erythroid blast forming units and reduced concentrations of bone remodeling markers were recorded in mice with hemolytic anemia, which also had blunted osteoclastogenesis, in contrast to its enhancement in bled mice.[31]

*Moringa Oleifera* is a plant that has become very popular in modern time due to its vast medicinal properties. Owolabi and Ogunnaike had reported that leaf extract of *Moringa oleifera* has positive effects on some vital organs including the bone marrow.[37] Okwari et al. reported hematologic and hematopoietic effects of *Moringa oleifera* leaf on thermooxized palm oil diet induced toxicity.[38] The present study confirms this by showing a dose-dependent reversal action by aqueous extract of *Moringa oleifera* leaf against phenylhydrazine-induced bone marrow toxicity. It also shows that the extract reversed the reduction in red blood cell and hemoglobin concentration. This is also buttressed by the widely-reported antioxidant properties of *Moringa oleifera*.[39-42]

## CONCLUSION

In conclusion, this study showed that aqueous extract of *Moringa oleifera* leaf has dose-dependent reversal capacity against phenylhydrazine bone marrow toxicity.Further studies are recommended at investigating mechanisms through which *Moringa oleifera* exert this action on the bone marrow.

## REFERENCES


1. Kelly A, Munan L. Hematolytic Profile of Natural Populations: Red Cell Parameters. *British Journal ofHaematology.* 1997;35:153-160.
2. Kasper DL, Braunwald E, Fauci AS, Hauser SL, Longo DL, Jameson JL. *Harrison's Principle of Internal Medicine* (vol. 1) 16th ed. McGraw-Hill Professional; 2004.
3. De Bernoist B, Mclean E, Egli I, Cogswell M. Worldwide Prevalence of Anemia 1993-2005: WHO Global Database on Anaemia. World Health Orgnzation, Geneva, Switzerland; 2008.
4. Bains JW, Crawford DT, Ketcham AS. Effects of Chronic Anemia on Wound Tensile Strength: Correlation with Blood Volume, Total Red Blood Cell Volume and Protein. *Annals of Surgery* 1966; 164(2): 243-246.
5. Kumar V, Abbas AK, Fausto N, Aster JC. *Robbins and Cotran Pathologic Basis of Disease.* 7th ed. Vol. 2., WB Saunders, Philadelphia; 2007.
6. Smith RE Jr. The Clinical and Economic Burden of Anemia. *The America Journal ofManaged Care*. 2010; 16(3): S59-S66.
7. Janz TG, Johnson RL, Rubenstein SD. (2013) Anemia in the Emergency Department: Evaluation and Treatment. *Emergency Medicine Practice*. 2013. www.ebmedicine.net
8. Nabili SN, StöpplerMC. Anemia. *emedicinehealth*. 2014; www.emedicinehealth.com
9. Bhutta ZA, Das JK, Rizvi A, Gaffey MF, Walker N. Evidence-Based Interventions for Improvement of Maternal and Child Nutrition. Lancet. 2013;382(9890):452-77.
10. Silverberg DS, Wexler D, Laina A. The Role of Anemia in the Progression of Congestive Heart Failure. Is There a Place for Erythropoietin and Intravenous Iron? *Journal of Nephrology.* 2004; 17(6):749-61.
11. Dornfest BS, Lapin DM, Adu S, Naughton BA.Dexamethasone Suppresses the Generation of Phenylhydrazine-Induced Anaemia in the Rat. *Proceedings of the Society for Experimental Biology and Medicine. 1992;*199: 491-500.
12. Terszowski G, Waskow C, Conradt P, Lenze D, Koenigsmann J, Carstanjen D et al. Prospective Isolation and Global Gene Expression Analysis of the Erythrocyte Colony-Forming Unit (CFU-E). *Blood*. 2005; 105: 1937-45.
13. Luangaram S, Kukongviriyapan U, Pakdeechote P, Kukongviriyapan V, Pannangpetch P. Protective Effects of Quercetin Against Phenylhydrazine-Induced Vascular Dysfunction and Oxidative Stress in Rats. *Food Chem. Toxicol*. 2007; 45:448-55.
14. Gołab J, Olszewska D, Mróz P, Kozar K, Kamiński R, Jalili A et al. Erythropoietin Restores the Antitumor

Effectiveness of Photodynamic Therapy in Mice with Chemotherapy-Induced Anaemia. *Clinical Cancer Research*. 2002; 8(5): 1265-70.
15. Xie LD, Liu DH, Sun DG, Yao WJ, Gu L, Yan ZY et al. (2002). Study on the Membrane Shear Elastic Modulus and Viscosity of Reticulocytes. *Progress in Biochemistry and Biophysics. 2002*; *29*(5); 776-80.
16. Agbor GA, Oben JE, Ngogang JY. Haematinic activity of *Hibiscus cannabinus*. *Afr J Biotechnol*. 2005; 4:833-7.
17. Biswas S, Bhattacharyya J, Dutta AG. Oxidant Induced Injury of Erythrocyte-Role of Green Tea Leaf and Ascorbic Acid. *Molecular and Cellular Biochemistry. 2005;276*(1-2): 205-10.
18. Unami A, Nishina N, Terai T, Sato S, Tamura T, Noda K et al (1996).Effect of Cisplatin on Erythropoietin Production in Rats. *Journal of Toxicological Sciences.1996;* 21(3):157-8.
19. Maroyi A. Use of Weeds as Traditional Vegetables in Shurugwi District, Zimbabwe. *Journal of Ethnobiology Ethnomedicine* 2013*;* 2(2):130-9.
20. Olson, ME (2010). Flora of North America Editorial Comimitee, ed. Moringaceaceae: Drumstick Family. Flora of North America, North Mexico.7. New York and Oxford.pp.167-169.
21. Abe R, Ohtani K. An ethnobotanical study of medicinal plants and traditional therapies on Batan island, the Philippines. *Journal of Ethnopharmacology.* 2013;142(2): 554-65.
22. Gifoni JM, Oliveira JT, Batista AB, Pereira ML, Gomes AS, Oliveira HP. *et al.* A novel chitin-binding protein from Moringa Oleifera seed with potential for plant disease control. *Biopolymers.* 2012;98(4)*:*406-15.
23. Luqman S, Srivastava S, Kumar R, Maurya AK, Chanda D. Experimental Assessment of Moringa Oleifera Leaf and Fruit for its Anti stress, Antioxidant and Scavenging Potential Using in Vitro and in Vivo Assays. *Evidence- Based Complementary Alternative Medicine.* 2012; 12 pages.
24. Manaheji H, Jafari S, Zaringhalam J, Rezazadeh S, Taghizadfarid R. Analgesic effects of methanolic extracts of the leaf or root of Moringa oleifera on complete Freund's adjuvant-induced arthritis in rats. *Zhong Xi Yi Jie He Xue Bao.* 2011;9(2):216-22.
25. Prabhu K, Murugan K, Nareshkumar A, Ramasubramanian N, Bragadeeswaran S. Larvicidal and repellent potential of Moringa oleifera against malarial vector, Anopheles stephensi Liston (Insecta: Diptera: Culicidae). *Asian Pacific Journal of Tropical Biomedicine.* 2011;1(2):124-9.
26. Osungbade KO, Oladunjoye AO. Anaemia in Developing Countries: Burden and Prospects of Prevention and Control, Anemia. Silverberg D (Ed.), 2012. Available from: http://www.intechopen.com/books/anemia/anaemia-in-developing-countries-burden-andprospects-of-prevention-and-control
27. Heinz R. Morphologische Veranderung der rothen Blutkorperchen durch Gifte. *Virchows Arch. Pathol.* 1890; 122:112-116.
28. Ranvers. Über Pyrodin. Arb. A.d. erst. Med. Klin. Zu Berl. 1981; ii: 471.
29. Berger J. Phenylhydrazine Haematotoxicity. *J Appl Biomed*. 2007; 5:125-30.
30. Akah PA, Okolo CE, Ezike AC. The Haematinic Activity of the Methanol Leaf Extract of *Brillantasia Nitens* Lindau (Acanthaceae) in Rats. *African Journal of Biotechnology*. 2009; 8(10): 2389-93.
31. Moreau R, Tshikudi Malu D, Dumais M, Dalko E, Gaudreault V, Roméro H et al. (2012) Alterations in Bone and Erythropoiesis in Hemolytic Anemia: Comparative Study in Bled, Phenylhydrazine-Treated and Plasmodium-Infected Mice. *PLoS ONE*; 7(9): e46101.
32. Ashour TH. Hematinic and Anti-Anemic Effect of Thymoquinone Against Phenylhydrazine Induced Hemolytic Anemia in Rats. *Research Journal of Medical Science*. 2014; 8(2):67-72
33. Hara H, Ogawa M. Erythropoietic precursors in mice with phenylhydrazine-induced anemia. *Am. J. Hematol.* 1975; 1:453-8.
34. Berger J. Screening of Toxic-Haemolytic Anaemia in Laboratory Rats: A Model of Phenylhydrazine-Induced Haemolysis. *Haematologia* 1985; 18:193-200.
35. Stern A. Drug-Induced Oxidative Denaturation in Red Blood Cells. *Semin. Hematol*. 1989; **26**:301-6.
36. Säterborg NE. Bone Marrow Abnormalities After Phenylhydrazine Induced Hemolysis in Rabbits. *Acta Radiologica: Therapy, Physics, Biology*. 1974; **13(4):**345-56.
37. Owolabi and Ogunnaike. Histological evaluation of the effects of Moringa leaf extract treatment on vital organs of murine models. *Merit Research Journal of Medicine and Medical Sciences*. 2014; 2(10): 245-57.
38. Okwari OO, Emerole CG, Dasofunjo K, Ezugwu HC, Obi J. Haematological Profile of Rats Administered with Aqueous Leaf Extract of *Moringa Oleifera* Following Thermooxidixed Palm Oil Diet Induced Toxicity. *IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS). 2014;9(1): 41-5.*
39. Rao AV, Devi PU, Kamath R. In Vivo Radioprotective effect of *Moringa oleifera* leaves*. Indian Journal of Experimental Biology.* 2001; 39:858-63.
40. Siddhuraju P, Becker K. Antioxidant properties of various solvent extracts of total phenolic constituents from three different agroclimatic origins of drumsticks tree (Moringa oleifera Lam.) leaves. *Journal of Agricultural and Food Chemistry*. 2003; 51(8):44-55.
41. Machado DIS, Cervantes JL, Vázquez NJR. High-performance liquid chromatography method to measure α e γ-tocopherol in leaves, flowers and fresh beans from Moringa oleifera. *Journal of Chromatography A*. 2005;1105(1-2): 111-4.
42. Iqbal S, Bhanger MI. Effect of season and production location on antioxidant activity of Moringa oleifera leaves grown in Pakistan. *Journal of Food Composition and Analysis.* 2006;19(6-7): 544-51.