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Effect of *Moringa oleifera* Lam. Ethanol Leaf Extract on Hematology in Phenylhydrazine-induced Anemic Albino Wistar Rats

Nku-Ekpang Okot-Asi T.[1], Nwaehujor Chinaka O.[2,3,*], Ofem Ofem E.[1], Ezekiel Josiah I.[1]

[1]Department of Physiology, Faculty of Basic Medical Sciences, University of Calabar, Calabar, Cross River State, Nigeria
[2]Department of Biochemistry, Faculty of Basic Medical Sciences, University of Calabar, Calabar, Cross River State, Nigeria
[3]Department of Biochemistry, Faculty of Biological Sciences, University of Nigeria, Nsukka, Enugu State, Nigeria
*Corresponding author: chinaka_n@yahoo.com

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Abstract The study was designed to investigate the effect of ethanol leaf extract of *Moringa oleifera* Lam. in phenylhydrazine-induced anemic albino Wistar rats. Twenty five (25) rats of both sexes were randomly assigned to 5 groups. Group 1 (normal control), Group 2 (negative control) was challenged with Phenylhydrazine (40 mg/kg, i.p.) without treatment. Group 3 received *M. oleifera* extract at 300 mg/kg. Groups 4 and 5 were challenged with phenylhydrazine (40 mg/kg) and treated with 300 and 600 mg/kg of *M. oleifera* respectively. All treatments with the extract were *per os*. All animals were allowed free access to food and water pre and post treatment for 21 days. At the end of the treatment period, blood samples were collected from the rats via the retro-orbital plexus of the eye. The hematological parameters assayed for were red blood cell count, hemoglobin count, white blood cell count, packed cell volume, platelet count, mean cell volume, mean cell hemoglobin, mean cell hemoglobin concentration and lymphocytes count. Changes in body weight of the rats were also determined. Results showed that there was significant ($P<0.05$) increase in some blood parameters (red blood cell count, hemoglobin count, white blood cell count). Body weights were also found to increase with increasing doses of the extract in the PHZ-challenged animals. In conclusion, the oral administration of ethanol extract of *M. oleifera* has the tendency to increase some blood parameters and may be important in the treatment and management of anemia especially hemolytic and hemorrhagic anemia.

Keywords: *Moringa oleifera, anemia, phenylhydrazine (PHZ), ethanol extract, hematology, albino Wistar rats*

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1. Introduction

In the last few decades, there have been an exponential growth in the field of herbal medicine [1]. It is getting popularized in developing countries and developed countries owing to its natural origin and lesser side effects [1]. Herbal drugs constitute a major share of all the officially recognized systems of health in India, including Ayurveda, yoga, Unani, Siddha homeopathy and naturopathy, excepting allopathy ([1]. More than 70 % of India's 1.3 billion population still use these non-allopathic system of medicine and herbal drugs are integral to the Indian and African system of medicine (Ayurveda) which is ancient and mainstream [2]

Moringa oleifera Lam. belongs to the family, Moringaceae, commonly known as "Sahajan" in Hindi, "Horseradish tree", "drumstick tree", "ben tree". "mother's best friend in English", "Zogeli", "Zogalagandi" in Hausa, "Ewê Ilê", "Ewê Igbale", "idagbo mononye" (the tree that grows crazily) in Yoruba, "odudu oyibo", "okwe oyibo", "okwe olu", "uhe", "oku-ghara-ite", "okochi egbu" (cannot be killed by dry season) in Igbo and Efik call it "Mbubik ikong" [3]. *Moringa oleifera* is a small fast growing, deciduous tree that usually grows up to 10 to 12m high [4]. It is distributed among sub-Himalayan tract, Assam, Bengal and Penisular India [4]. Various properties are attributed to it like antispasmodic, diuretic, expectorant and abortifacient [5]. Many pure compounds have been isolated from the leaves of *Moringa oleifera* including niazirin, niazirinin and three mustard oil glycosides 4-(4-O-acetyl-alpha-L- rahamnosyloxy-benzyl] isothiocyanate, niaziminin A and niaziminin B [6]. The leaves also contain aspartic acid, glutamic acid, glycine, threonine, alanine, valine, leucine, isoleucine, histidine etc. [7]. *Moringa oleifera* contain a number of flavonoids, saponins, triterpenes, steroids, alkaloids and many other chemical constituents [8]. The flavonoid, quercetin present in the leaves is a well-known anti-ulcer agent [9]. *Moringa oleifera* has numerous medicinal uses which have long been recognized in the Ayurvedic and Unani systems of

medicine [3]. The medicinal attributes and pharmacological activities ascribed to the various parts include – anti cancer and anti-tumor, anti-hypertensive, cholesterol-lowering [10], anti-bacterial and anti-fungal [11], hepatoprotective and water-purifying/coagulant activities [12].

The aim of this study is to investigate the effect of ethanol crude extract of *Moringa oleifera* leaves on hematological parameters in phenylhydrazine-induced anemic albino Wistar rats since it is used in Nigerian ethnopharmacology to treat anemia and blood-loss-related diseases.

2. Materials and Methods

2.1. Drugs and Chemicals

Dimethyl sulfoxide (DMSO) (Sigma-Aldrich, USA), phenylhydrazine chloride (Sigma, Germany), EDTA, chloroform, ethanol were from Sigma-Aldrich, Germany. All drugs and reagents were of analytical grade

2.2. Collection and Extraction of Plant Material

Fresh leaves of *Moringa oleifera* were obtained from the University of Calabar staff quarters in March, 2014 and were identified by Mr. Ekpo of Botany Department, University of Calabar, Nigeria. They were washed under running tap water and allowed to dry under air and at room temperature. The dried leaves were pulverized using an electric mill. 670 g of the pulverized leaves were macerated in ethanol for 48 h. This was filtered with Whatman No. 1 filter paper and dried using a rotary evaporator at 40 °C. % yield was calculated.

2.3. Animals

Albino Wistar rats of both sexes (120-150 g) were purchased from the Laboratory Animal Facility of the Department of Physiology, University of Calabar and used for the experiments. They were kept in clean cages, maintained at normal room temperature and natural daylight/night conditions and were allowed free access to standard commercial pelleted feed and clean drinking water.

2.4. Experimental

All experiments with animals were approved by the Ethical Committee of the University of Calabar, Nigeria prior to the commencement of the various tests.

2.4.1. Experimental Design

2.4.1.1. Induction of anemia and determination of hemoglobin (Hb) concentration

Before anemia was induced, Hb concentration was determined. This was done by blood collection from the tail vein of each animal. Blood sample was added into a graduated tube containing 5 drops of 0.1N HCl ad was placed in a color comparator and allowed to stand for 5 min.

After determination of Hb concentration, anemia was induced in rats by intraperitoneal administration of phenylhydrazine (PHZ) at 40 mg/kg and 48 h intervals. The animals were allowed 2 days to recover [19].

2.4.1.2. Treatment Groups

A total of 25 Wistar rats of both sexes (140 – 280 g) were used for the study. They were randomly divided into 5 groups as follows:

Group 1: Normal control (distilled water)
Group 2: Negative control (PHZ without treatment)
Group 3: MOE (0.3 ml/100 g/day)
Group 4: Anemic + MOE (300 mg/kg daily, low dose (LD))
Group 5: anemic + MOE (600 mg/kg daily, high dose (HD))

The extracts were dissolved in distilled water and treatment was *per os* which lasted for 21 days. At the end of the treatment period, animals were anesthetized and blood was collected from each animal through the retrobulbar plexus of the media canthus after an overnight fast into EDTA bottles. Blood samples were analyzed using automated hematology cell counter (Coulter electronic, Lutonbed Fordshire, UK). Parameters determined were Hb count, WBC count, RBC count, PCV, platelet count, MCV, MCH, MCHC and lymphocytes count.

2.4.2. Statistical Analysis

Data were analyzed using a one way ANOVA followed with a post hoc test (least square division test) using the SPSS 18 version. Results were presented as mean±SEM and p value of less than 0.05 was accepted as statistically significant.

3. Results

3.1. Description of the Extract

The ethanol extract of MOE was green in color and with a peculiar fragrance. The extraction process gave a yield of 32.76 % w/w.

3.2. Acute TOXICITY TEST

Acute toxicity studies revealed no extract-induced mortality or overt serious clinical manifestation even at the highest test dose of 2000 mg/kg. However, transient restlessness was observed in animals treated with the extract doses above 1500 mg/kg.

3.3. Effect of Ethanol Extract of MOE on Body Weight

3.3.1. Body Weights and Feed Consumption - Comparison of Weekly Body Weights of the Different Experimental Groups

There was a significant ($p<0.05$) and time dependent increase in the mean body weight of all rats that received the extract (300 and 600 mg/kg) relative to negative control. The 12.5% lose in weight of the experimental rats on day 21 was most remarkable compared to 5.4 and 6.4% weight loses on days 7 and 14 respectively (Figure 1). The mean daily feed consumption of the test rats was however,

comparable with that of the control value within the overall duration of study (21 days).

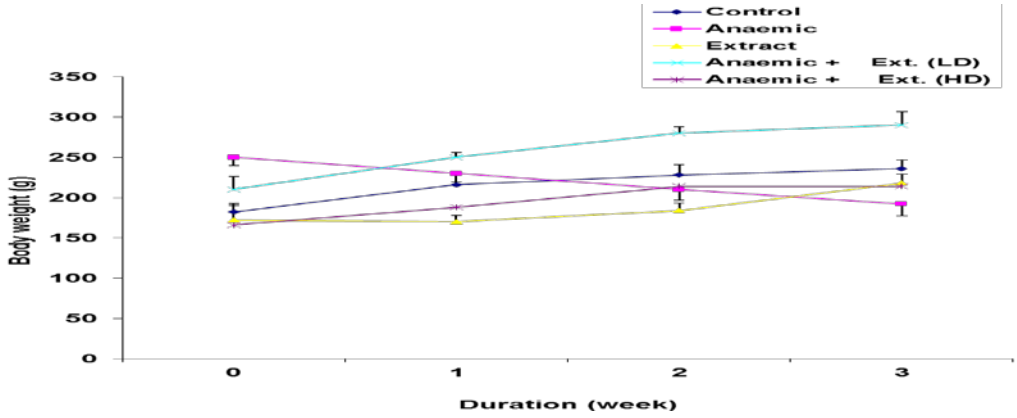

Figure 1. Comparison of weekly body weights of the different experimental groups. Values are mean ± SEM, n = 5

3.3.2. Comparison of Mean Total Body Weights of the Different Experimental Groups

The result showed that there was a significant difference ($p<0.05$) across the groups. The mean total body weight change decreased significantly in the negative control (anemic group) compared to the control group while there was a significant increase in group 3, 4 and 5 when compared to the control and negative control groups (Figure 2).

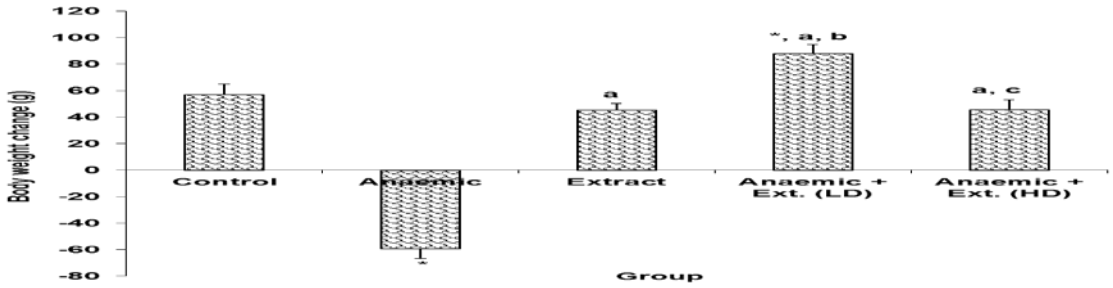

Figure 2. Comparison of mean total body weights of the different experimental groups. Values are mean ± SEM, n = 5. *significantly different from control at $p<0.05$; a = $p<0.05$ vs anemia; b = $p<0.05$ vs extract; c = $p<0.05$ vs anemia + extract (LD)

3.4. Effect of Ethanol Extract of MOE on Blood Hematology

3.4.1. Comparison of Total White Blood Cell Count

A significant ($p<0.05$) change was observed in the total white blood cell count in the extract-treated groups when compared with the normal control and the anemic groups. This is most obvious with Group 5 (anemic + high dose of extract) (Figure 3)

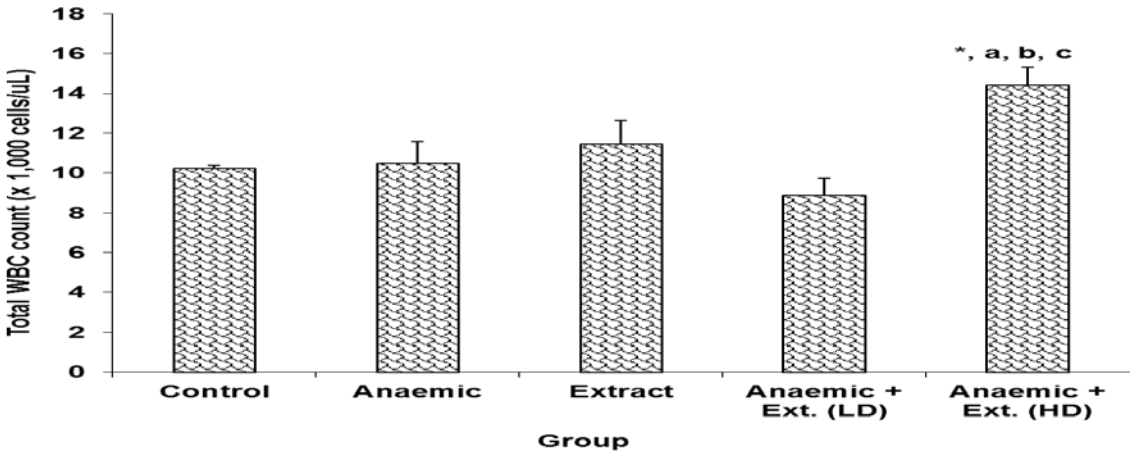

Figure 3. Comparison of total white blood cell count in the different experimental groups. Values are mean ± SEM, n = 5. *significantly different from control at $p<0.05$; a = $p<0.05$ vs anemia; b = $p<0.05$ vs extract; c = $p<0.05$ vs anemia + extract (LD)

3.4.2. Comparison of Red Blood Cell Count

Result showed that there was a significant ($p<0.05$) decrease in RBC count in the negative control group when compared to the control. Group 3 showed a significant increase in RBC count when compared to the negative and normal control groups. Group 4 showed a significant increase when compared to the negative control while

Group 5 showed a significant increase when compared to the negative control but showed a significant decrease when compared to Group 3 (Figure 4)

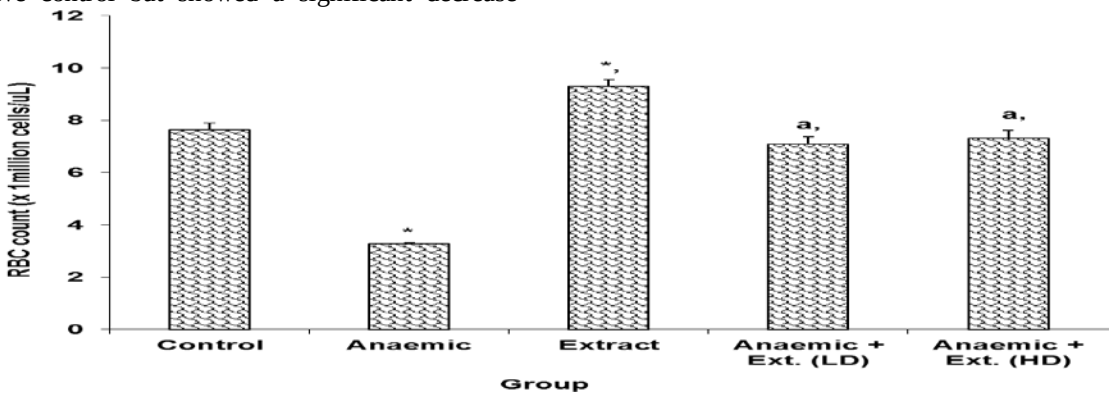

Figure 4. Comparison of red blood cell count in the different experimental groups. Values are mean ± SEM, n = 5. *significantly different from control at p<0.05; a = p<0.05 vs anaemia; b = p<0.05 vs extract

3.4.3. Comparison of Hemoglobin Concentration

The negative control group showed a significant (p<0.05) reduction in Hb concentration when compared to the normal control. Significant increase (p<0.05) in hemoglobin concentration was observed in Groups 3, 4 and 5 when compared to the negative control. However, Hb concentration in Group 4 significantly reduced when compared to Group 3 (Figure 5).

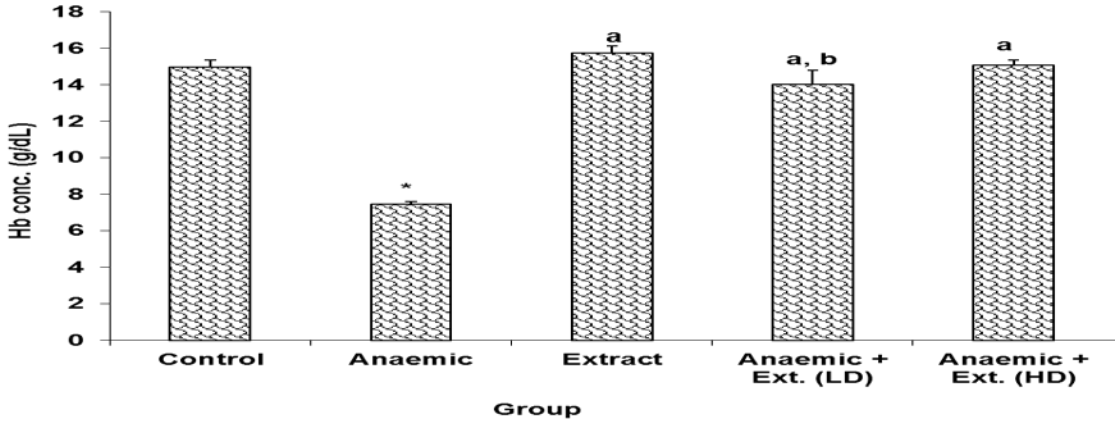

Figure 5. Comparison of hemoglobin concentration in the different experimental groups. Values are mean ± SEM, n = 5. *significantly different from control at p<0.05; a = p<0.05 vs anemia; b = p<0.05 vs extract

3.4.4. Comparison of Packed Cell Volume

Result showed that there was a significant (p<0.05) reduction in % PCV in the negative control when compared to the normal control. While Groups 3, 4, 5 showed significant increase in % PCV when compared to the normal and negative groups (Figure 6).

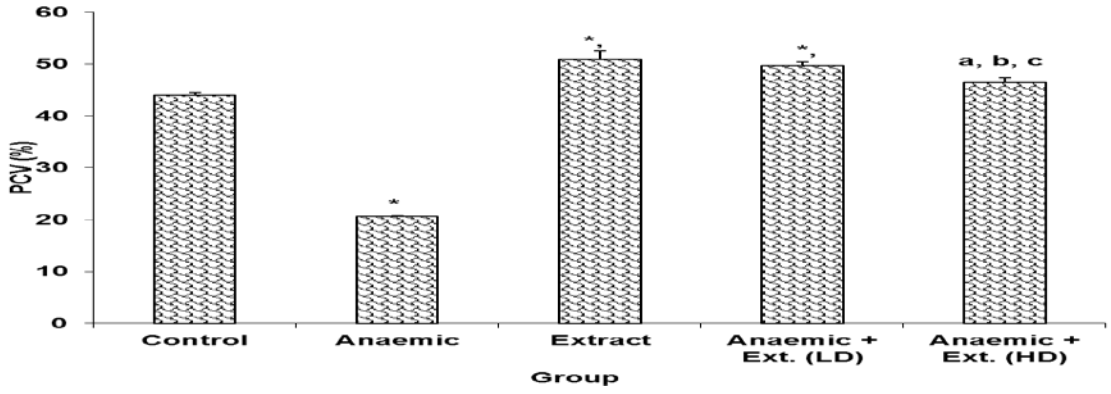

Figure 6. Comparison of packed cell volume in the different experimental groups. Values are mean ± SEM, n = 5. *significantly different from control at p<0.05; a = p<0.05 vs anemia; b = p<0.05 vs extract; c = p<0.05 vs anemia + extract (LD)

3.4.5. Comparison of Mean Corpuscular Volume

Result showed a significant (p<0.05) increase in MCV in the negative control compared to the normal control while there was significant (p<0.05) reduction in Group 3 when compared to Group 2. However, there was significant increase in MCV in Groups 4 and 5 when compared to group3 and significantly reduced when compared to Group 2 (Figure 7).

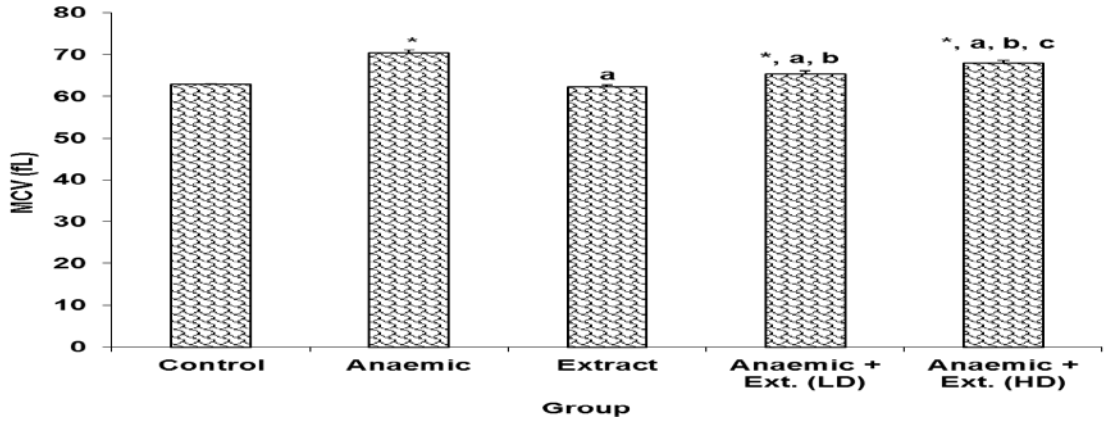

Figure 7. Comparison of mean corpuscular volume in the different experimental groups. Values are mean ± SEM, n = 5. *significantly different from control at p<0.05; a = p<0.05 vs anaemia; b = p<0.05 vs extract; c = p<0.05 vs anaemia + extract (LD)

3.4.6. Comparison of Mean Corpuscular Hemoglobin (MCH)

MCH significantly (p<0.05) increased in the negative control compared to the normal control. Group3 showed a significant reduction when compared to the negative control while Groups 4 and 5 showed significant reduction when compared to the negative control and significant increase when compared to the normal control and Group 3 (Figure 8).

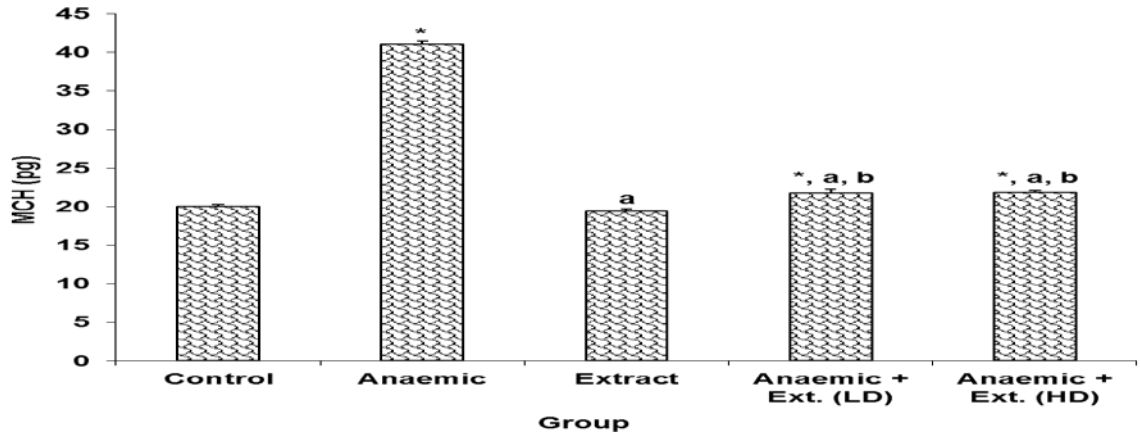

Figure 8. Comparison of mean corpuscular hemoglobin in the different experimental groups. Values are mean ± SEM, n = 5. *significantly different from control at p<0.05; a = p<0.05 vs anaemia; b = p<0.05 vs extract

3.4.7. Comparison of Mean Corpuscular Hemoglobin Concentration (MCHC)

Result showed that there was significant increase in MCHC in the negative control compared to the normal control. Group 3 showed significant reduction when compared to the normal and negative control groups while Groups 4 and 5 showed significant increase when compared Group 3 and significant reduction when compared to the negative control (Group 2) (Figure 9).

Results of platelet count and lymphocyte count showed no significant difference amongst the test groups when compared to the control groups (Figure 10 and Figure 11).

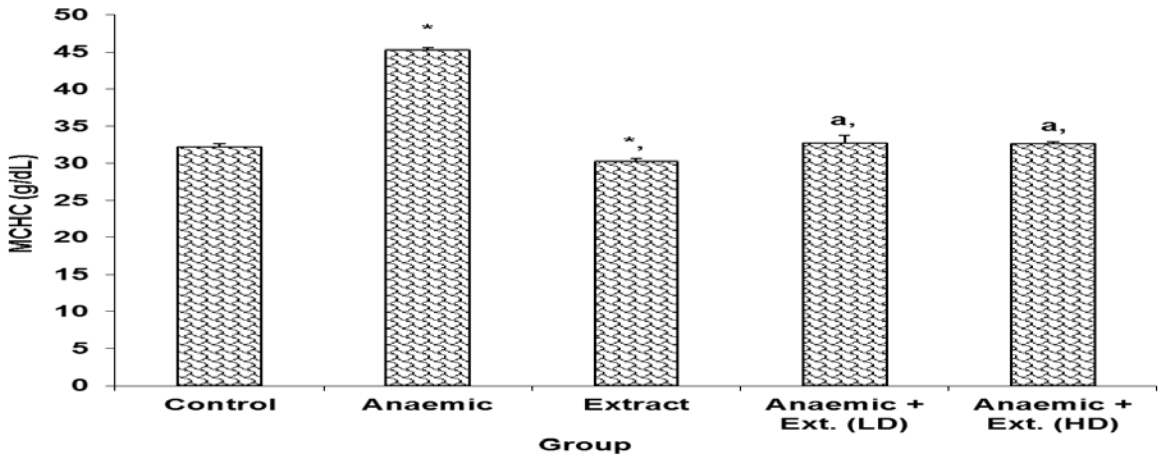

Figure 9. Comparison of mean corpuscular hemoglobin concentration in the different experimental groups. Values are mean ± SEM, n = 5. *significantly different from control at p<0.05; a = p<0.05 vs anemia; b = p<0.05 vs extract

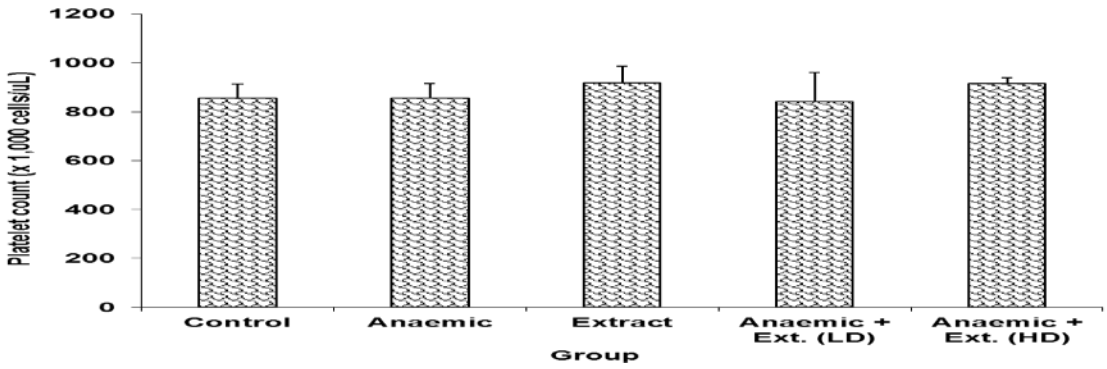

Figure 10. Comparison of platelet count in the different experimental groups. Values are mean ± SEM, n = 5. No significant differences among groups

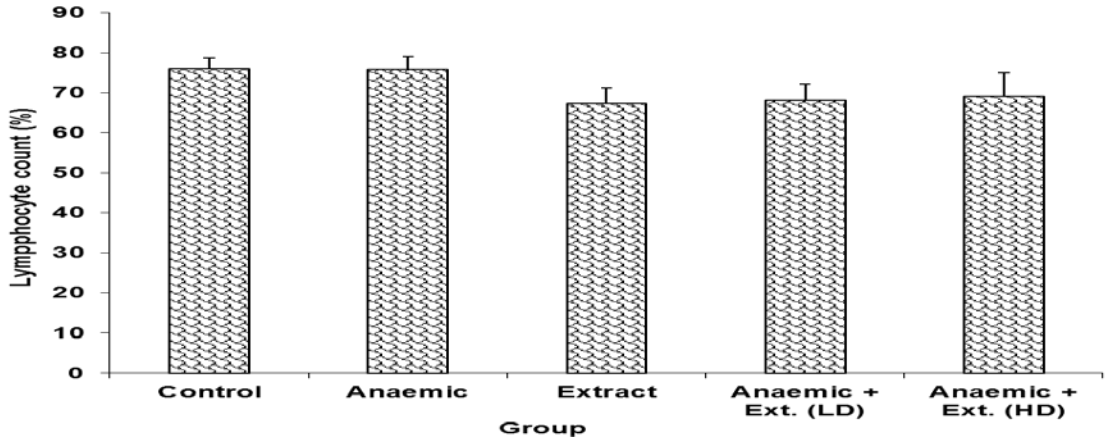

Figure 11. Comparison of lymphocyte count in the different experimental groups. Values are mean ± SEM, n = 5. No significant differences among groups

4. Discussion

This study was designed to investigate the effect of ethanol extract of *Moringa oleifera* leaves on hematology and serum lipid profile in PHZ-induced anemia in Wistar rats. *Moringa oleifera* leaves has been reported to have antitumor and anticancer activity [13] and increases blood cell production [14], but its action on blood disorder has not been reported and there is paucity of information on its effects on anemia. 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 anemia using body weight and some hematological parameters (WBC, RBC, Hb, PCV, MCV, MCH, MCHC, platelets and lymphocytes).

The result showed that there was a significant ($P<0.05$) increase in mean body weight of the Wistar rats that were treated with ethanol leaf extract of *Moringa oleifera* (300 and 600 mg/kg) when compared with the negative control group.

In the body weight change, rats treated with ethanol leaf extract of Moringa oleifera also showed a significant increase ($P<0.05$) in body weight when compared with normal control and anemic (Group 2). While the anemic group showed a significant reduction ($P<0.05$) in body weight when compared with control and treated groups. These increases observed in the treated groups could be due to the presence of amino acids, vitamins and minerals particularly iron found in the leaf extract [15]. On the other hand, the reduction in mean body weight and body weight change in the anemic group could be due to impaired erythrocytes deformities and oxidative damage from free radicals caused by PHZ [16,17].

RBC, hemoglobin concentration and PCV in anemic group showed a significant decrease when compared with normal control group. This could be due to toxicity caused by PHZ by the involvement of aryl and hydroxyl radicals it generates. It could also be due to poor affinity of oxygen to hemoglobin molecules since the tendency of hemoglobin to bind to oxygen enhances blood flow to the tissues [18]. In extract-treated groups, there was also a significant increase in these parameters when compared with the control and anemic groups. This could be due to the phytochemical constituents in the extract and also presence of minerals and vitamins. These constituents are well known hemopoietic factors that have direct influence on the production of blood in the bone marrow.

MCV, MCH and MCHC increased significantly ($P<0.05$) in the anemic group when compared with the normal control and extract-treated groups. This supports the earlier works of Flanagan and Lessler [19] in which PHZ 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 hemolytic anemia [20,21]. The results showed that there was no significant difference in platelet and lymphocyte counts amongst the groups when compared with the control.

The effect of oral administration of *Moringa oleifera* ethanol leaf extract irrespective of the dose has the tendency to increase blood parameters such as WBC, RBC, Hb, PCV etc. as well as alleviate blood disorders. It will be useful in the treatment of anemia 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 polycythemia or abnormal high blood cell production when taken in high doses.

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