Changes in Haematological Parameters Following Toxicity Study with 80% Methanol Extract of *Moringa oleifera* in Wistar Rats

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Abstract: To determine the acute toxicity effects of *Moringa oleifera* leaf extract on haematological parameters, twenty albino Wistar rats weighing between 108-254 g were split into four groups of fives rat each. Different doses of the extract were administered to the 3 groups and one group is used as control. *Moringa oleifera* 80% methanol leaf extract was administered at 2000 mg/kg to group, 1000 mg/kg to group, and 500 mg/kg while one group was used as control. The result recorded 4 deaths (60% mortality) in groups that received 2000 mg/kg and 1 death (20% mortality) in groups that received 1000 mg/kg. Blood samples were collected from the survived rats for hematological analysis. The result showed variation in Park cell volume (PCV), hemoglobin concentration (Hb), Red blood cells (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC). There are significant differences at p > 0.05, p > 0.01 and p > 0.001 between groups that exposed to different doses *Moringa oleifera* extract and the control group. The result also shows slight variation in total and differential leucocyte count, but all the values obtained from both treated and control groups are within the normal range. There are no recorded values of eosinophil, basophil and band cells in both the control and the experimental groups. From this result, it can be concluded that high dose of this extract caused alteration in normal red cells indices with significant differences, but have mild or no effect on total and differential leucocyte count. Hence, low toxicity of this plant made it safe and good medicinal agent and or supplement. Histopatological screening as well as phytochemical study to identify the compounds that affect the blood chemistry is highly recommended.

Keywords: Hemoglobin Concentration, Leucocyte, Mean Corpuscular Hemoglobin, Mean Corpuscular Hemoglobin Concentration, Mean Corpuscular Volume, Park Cell Volume, Red Blood Cells

1. Introduction

*Moringa oleifera* is a tropical Indian tree that has been cultivated and used in diverse regions of the world. It is referred to as “drum stick tree” or the “horse riding tree.” Its consumption both in the raw and as processed preparations has increased a great deal, hence making the plant a highly valued and cultivated one in the tropics and sub-tropics like Africa, Malaysia, Mexico, tropical America, Sri Lanka and the Phillipine Islands [1]. The plant is a slender, fast-
2. Materials and Methods

2.1. Materials Used

*Moringa oleifera* leaf, white plastic bucket, cotton silk, measuring cylinder, beaker, weighing balance, needle and cannula, syringe, EDTA bottle, centrifuge machine, spectrophotometer, glass slide, pasteur pipette, micropipette, micropipette tip, gloves, capillary tube, microscope, hemocrit reader, plastacine, neubauer chamber, cotton wool, turk’s solution, methanol, DRABKINS solution, diluting, pipette, hayem’s solution, cover slip, leishmann stain and distilled water.

2.2. Plant Collection and Identification

Fresh leaf of *Moringa oleifera* found around Sokoto State Central Market was collected. The plant’s leaf was identified and authenticated at the botany unit of Department of Biological Sciences in Usmanu Danfodiyo University, Sokoto, Nigeria.

*Plant dose Preparation*

The leaf was prepared according to the procedure described by [22]. Briefly, the procedure involved drying the leaf in the laboratory to constant weight at room temperature. The leaf was then washed and grounded to semi powdered form using an electric blender. 5 g of the extract was mixed with 50 mL distilled water. The prepared solution was left to stand for two days and then filtered using muslin cloth.

2.3. Animal

Twenty four (24) Wistar rats weighing between (120-300g) of both sexes were used. They were obtained from the Animal House of the Department of Pharmacology and Clinical Pharmacy, Usmanu Danfodiyo University Teaching Hospital. They were kept in plastic cages under laboratory condition (25-27°C) placed on standard feed and allowed free access to water.

2.3.1. Animal Grouping

Twenty four (24) Wistar rats were divided randomly into four groups (I-V) of six animals in each group (n=6).

- **Group I:** Control group (Normoglycemic animals)
- **Group II:** Untreated group
- **Group III:** Group that received *Moringa leaves* extract at

2.3.2. Blood Sample Collection and Preparation

A 5 ml syringe and 21 gauge needle was used to collect 3 mL of blood directly from the heart following proper restrain. The needle was then capped and disengaged from the syringe and transferred the blood into a sample bottle containing Ethylene diamine tetraacetic (EDTA) and rocked gently. The sample bottles were immediately placed in a cooler packed with ice and the transported to laboratory for analysis. Packed Cell Volume (PCV), Red Blood Cell (RBC), Hemoglobin counts, Leucocyte (WBC), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), Mean Corpuscular Volume (MCV), and Red Cell Distribution Width (RDW) were determined.
2.3.3. Determination of Haematological Parameters

(i) Determination of Packed Cell Volume (PCV)

The PCV was estimated by micro-hematocrit method [23]. The blood was drawn into capillary tubes of length 7.5 cm and 0.1 mm diameter, with capillary action to 2/3 of the capillary tube was filled with blood sample. The outside of the capillary tubes were wiped with cotton wool to free it from blood. Tubes were then sealed at one end using plasticine to prevent the flow of blood and to provide sufficient space from the opposite ends. The capillary tubes were placed in the centrifuge with the sealed ends pointing out ward and centrifuged at 10,000 revolutions per minute (rpm) for 5 minutes. PCV was determined by rolling the capillary tubes in a PCV reader until the top of the plasma column was aligned with 100% line and the bottom of the packed erythrocytes was on the zero line. The line that crossed the top of the packed erythrocyte column represented the PVC in percent.

(ii) Determination of Erythrocyte Count (RBC)

Erythrocytes count was determined according to the method used by [24]. Blood sample was sucked through the diluting pipette to 0.5 ml mark held at horizontal level. The diluting fluid (Hayem’s Solution) and was sucked from the watch glass until the mixture reached the 101 mark. The mixture was then tilted up and down to allow for proper mixing which was facilitated by the presence of red beads in the bulb. A cover slip was placed on a cleaned moistened shoulder of a counting chamber; this makes a volume of 0.1 mm between the cover slip and counting chamber. The diluted blood was then introduced into the 0.1 mm space of counting chamber and allowed to settle for a minute. This was then mounted on the microscope under low magnification and the central squares of the 9 squares were focused. Finally, the counting was done under a high magnification and the central squares of the 9 squares were counted, i.e. 1 square from each corner and the central square of the counting chamber. The sums of the cells in the 9 squares (small squares) X 10,000 = Total erythrocyte in µL.

(iii) Determination of Hemoglobin (Hb) Concentration

Determination of hemoglobin concentration carried out according to the method described by [25]. To 0.02 ml of blood, 5 ml of reagent (Drapkin’s solution) was added, mixed well and allowed to stand for 10 min. spectrophotometer was used to the samples at 540 nm together with the standard solution cyanmethemoglobin against a blank containing 5.0 ml of the reagent. The hemoglobin content was expressed as g/dl of blood.

(iv) Determination of Mean Corpuscular Volume (MCV)

Determination of MCV was carried out according to the method described by [26]. The mean corpuscular volume was calculated using the formula below

\[ MCV = \frac{PCV(\%)}{RBC (x 10^{12}/L)} \times 10 \]

(v) Determination of Mean Corpuscular Haemoglobin (MCH)

Determination of mean corpuscular hemoglobin (MCH) was carried out according to the method described by [27]. The mean corpuscular haemoglobin was calculated using the formula below

\[ MCH = \frac{HB (g/L)}{RBC (x 10^{12}/L)} \times 10 \]

(vi) Determination of Mean Corpuscular Haemoglobin Concentration (MCHC)

Determination of mean corpuscular hemoglobin concentration (MCHC) was carried out according to the method used by [28]. The mean corpuscular haemoglobin concentration was calculated using the formula below

\[ MCHC = \frac{HB (g/L)}{PCV (\%)} \times 100 \]

(vii) Determination of Red Cells Distribution Width

Determination of red cell distribution width was carried out according to the method used by [29]. The red cells distribution width was calculated using the formula below

\[ RDW = \frac{Standard \ deviation \ of \ MCV \ of \ group \times 100}{MCV} \]

(viii) Determination of Total Leucocyte (WBC) Count

Determination of leucocyte count was carried out as described by [30]. Blood was drawn up to 0.5 marks on the stem of the diluting pipette and held horizontally; the external surface of tip was wiped out clearly using a clean cotton wool. WBC diluting fluid (Turk’s solution) was drawn by the same pipette till the 101 mark above the bulb. The content of the pipette was mixed well by rolling the pipette in between the palms. The neubauer chamber was covered by a thick cover slip leaving a 0.1 mm space. After 5 minutes, the content of the WBC diluting pipette was mixed well again and few drops of the content were expelled out. The blood mixture was then tilted up and down to allow for proper mixing which was facilitated by the presence of red beads in the bulb. The mixture was then diluted by the drop by drop addition of buffered distilled water and allowed for a period of 7-10 minutes. The stain was then mounted on the microscope under low magnification. The white blood cells were counted, i.e. 1 square from each corner and the central square of the counting chamber. The sum of the cells counted in the 4 diagonal squares X 100 = Total leucocyte mm³.

(ix) Determination of Differential Leukocytes (WBC) Count

A drop of blood was placed on a clean grease-free glass slide about 1-2 cm from one end with the help of a pinpoint dropper. Another clean grease-free spreading slide was placed at an angle of 45° approximately. The drop of blood was spread out quickly along the line of control of the spreader with the slide. The slide was placed flat on two glass rods over a sink and allowed to dry. The glass slide was then covered with Leishman stain for about 2 minutes. The stain was then diluted by the drop by drop addition of buffered distilled water and allowed for a period of 7-10 minutes. The stain was then mounted on the microscope under low magnification. The white blood cells were counted, i.e. 1 square from each corner and the central square of the counting chamber. The sum of the cells counted in the 4 diagonal squares X 100 = Total leucocyte mm³.
observed under microscope. Counting was started under high power oil immersion objective from the edge of the smear moving the smear towards center. Leucocytes were identified and the movement was repeated till a total 100 cells were counted. The values of different morphological types were expressed as the percentage.

Statistical Analysis

The data obtained were analyzed using Microsoft office Excel 2007 and Statistical Package used was Graph prism version 5.0. Results were expressed as mean ± SD and presented. The result of hematological changes from the experimental groups were compared with that of control groups using paired two-tailed student’s t-test for matched samples and analysis of variance (ANOVA) was used for comparisons of three (3) or more values of the parameters in the various groups.

3. Results

3.1. Result of Percentage Park Cell Volume (PCV%)

Effect of Moringa oleifera leaf extract on PCV of Wistar rats 24 hours post treatment. The result shows variation between the control groups and the groups that were exposed to different concentration of the extract. There is significance difference at p> 0.05 between the control groups (CTR) and the groups that received Moringa oleifera leaf extract (MLE) 1000 mg/kg. Significance difference at p> 0.001 was also observed between the control groups (CTR) and the groups that were exposed to Moringa oleifera leaf extract (MLE) 2000 mg/kg. The result also recorded significant difference at p> 0.01 between the groups that were treated with 500 mg/kg and the groups that received 2000 mg/kg of Moringa oleifera leaf extract (MLE) figure 1.

Figure 1. Effect of Moringa oleifera (leaf) extract at 500–2000 mg/mL on PCV in experimentally treated rats model after 24 hours of treatment. There is variation in PCV between the control group and the experimentally exposed groups (500–2000 mg/mL). Result is shown as concentration of PCV versus treatment and control. The values represent mean ± SD from two independent experiments with (n=6).

3.2. Result of Total Red Blood Cell Counts (RBC (×10⁶ cells/mm³))

Effect of Moringa oleifera leaf extract on RBC of Wistar rats 24 hours post treatment. The result shows variation between the control groups and the groups that were exposed to different concentration of the extract. There is significance difference at p> 0.001 between the control groups (CTR) and the groups that received Moringa oleifera leaf extract (MLE) 2000 mg/kg. Significance difference at p> 0.01 was also observed between the groups that received 500 mg/kg and the groups that were exposed to 2000 mg/kg of Moringa oleifera leaf extract (MLE) figure 2.

Figure 2. Effect of Moringa oleifera (leaf) extract at 500–2000 mg/mL on Rbc in experimentally treated rats model after 24 hours of treatment. There is variation in Rbc between the control group and the experimentally exposed groups (500–2000 mg/mL). Result is shown as concentration of Rbc versus treatment and control. The values represent mean ± SD from two independent experiments with (n=6).

3.3. Result of Total Haemoglobin Concentration (Hb (g/dl))

Effect of Moringa oleifera leaf extract on Hb of Wistar rats 24 hours post treatment. The result shows variation between the control groups and the groups that were exposed to different concentration of the extract. There is significance difference at p> 0.01 between the control groups (CTR) and the groups that received Moringa oleifera leaf extract (MLE) 2000 mg/kg. Significance difference at p> 0.01 was also observed between the groups that received 500 mg/kg and the groups that were exposed to 2000 mg/kg of Moringa oleifera leaf extract (MLE) figure 3.

Figure 3. Effect of Moringa oleifera (leaf) extract at 500–2000 mg/mL on Hb in experimentally treated rats model after 24 hours of treatment. There is variation in Hb between the control group and the experimentally exposed groups (500–2000 mg/mL). Result is shown as concentration of Hb versus treatment and control. The values represent mean ± SD from two independent experiments with (n=6).

3.4. Result of Mean Corpuscular Volume (MCV (×10⁹fl))

Effect of Moringa oleifera leaf extract on MCV of Wistar rats 24 hours post treatment. The result shows variation
between the control groups and the groups that were exposed to different concentration of the extract. There is significance difference at $p>0.05$ between the groups that are exposed to 500 mg/kg and the groups that received *Moringa oleifera* leaf extract (MLE) 2000 mg/kg. Significance difference at $p>0.01$ was also observed between the control (CTR) groups were exposed to 2000 mg/kg *Moringa oleifera* leaf extract (MLE) figure 4.

**3.6. Result of Mean Corpuscular Haemoglobin Concentration (MCHC (g/dl))**

Effect of *Moringa oleifera* leaf extract on MCHC of Wistar rats 24 hours post treatment. The result shows variation between the control groups and the groups that were exposed to different concentration of the extract. There is significance difference at $p>0.05$ between the control groups (CTR) and the groups that received *Moringa oleifera* leaf extract (MLE) 500 mg/kg. Significance difference at $p>0.05$ was also observed between the groups that received *Moringa oleifera* leaf extract (MLE) 500 mg/kg and the groups that are exposed to 1000 and 2000 mg/kg of *Moringa oleifera* leaf extract (MLE) figure 6.

**3.7. Result of Red Cells Distribution Width (RDW)**

Effect of *Moringa oleifera* leaf extract on RDW of Wistar rats 24 hours post treatment. The result shows variation between the control groups and the groups that were exposed to different concentration of the extract. There is significance difference at $p>0.001$ between the control groups (CTR) and the groups that received *Moringa oleifera* leaf extract (MLE) 500, 1000 mg/kg. Significance difference at $p>0.001$ was also observed between the control groups (CTR) and the groups that received 2000 mg/kg of *Moringa oleifera* leaf extract (MLE) figure 7.
1000 mg/kg. Significance difference at p > 0.001 was also observed between the groups that received *Moringa oleifera* leaf extract (MLE) 1000 mg/kg and the groups that are exposed to 500 and 2000 mg/kg of *Moringa oleifera* leaf extract (MLE). Changes was also recorded with significant difference at p > 0.01 between the control groups and the groups that received 2000 mg/kg of *Moringa oleifera* leaf extract (MLE) and also between the groups that were treated with 500 mg/kg of of *Moringa oleifera* leaf extract (MLE) and the groups that are exposed to 2000 mg/kg of of *Moringa oleifera* leaf extract (MLE) figure 7.

3.8. Result of Total White Blood Cells Counts (TWBC (×10^3 cells/mm^3))

Effect of *Moringa oleifera* leaf extract on TWBC of Wistar rats 24 hours post treatment. The result shows slight variation between the control groups and the groups that were exposed to different concentration of the extract. There is no significance difference between the control groups (CTR) and the groups that received different doses of *Moringa oleifera* leaf extract (MLE) figure 8.

![Figure 8. Effect of Moringa oleifera (leaf) extract at 500–2000 mg/mL on TWBC in experimentally treated rat’s model after 24 hours of treatment. There is no variation in TWBC between the control group and the experimentally exposed groups (500–2000 mg/mL). Result is shown as concentration of TWBC versus treatment and control. The values represent mean ± SD from two independent experiments with (n=6).](image)

3.9. Result of Percentage Neutrophil Count (%)

Effect of *Moringa oleifera* leaf extract on neutrophil of Wistar rats 24 hours post treatment. The result shows slight variation between the control groups and the groups that were exposed to different concentration of the extract. There is significance difference at p > 0.001 between the control groups (CTR) and the groups that received different doses of *Moringa oleifera* leaf extract (MLE) figure 9.

![Figure 9. Effect of Moringa oleifera (leaf) extract at 500–2000 mg/mL on neutrophil in experimentally treated rat’s model after 24 hours of treatment. There is variation in neutrophil between the control group and the experimentally exposed groups (500–2000 mg/mL). Result is shown as concentration of neutrophil versus treatment and control. The values represent mean ± SD from two independent experiments with (n=6).](image)

3.10. Result of Percentage Lymphocyte Count (%)

Effect of *Moringa oleifera* leaf extract on lymphocyte of Wistar rats 24 hours post treatment. The result shows slight variation between the control groups and the groups that were exposed to different concentration of the extract. There is no significance difference between the control groups (CTR) and the groups that received different doses of *Moringa oleifera* leaf extract (MLE) figure 10.

![Figure 10. Effect of Moringa oleifera (leaf) extract at 500–2000 mg/mL on lymphocyte in experimentally treated rat’s model after 24 hours of treatment. There is variation in lymphocyte between the control group and the experimentally exposed groups (500–2000 mg/mL). Result is shown as concentration of lymphocyte versus treatment and control. The values represent mean ± SD from two independent experiments with (n=6).](image)

3.11. Result of Percentage Monocyte Count (%)

Effect of *Moringa oleifera* leaf extract on monocyte of Wistar rats 24 hours post treatment. The result shows variation between the control groups and the groups that were exposed to different concentration of the extract. There is significance difference at p > 0.001 between the control groups (CTR) and the groups that received different doses of *Moringa oleifera* leaf extract (MLE) figure 11.

![Figure 11. Effect of Moringa oleifera (leaf) extract at 500–2000 mg/mL on monocyte in experimentally treated rat’s model after 24 hours of treatment. There is variation in monocyte between the control group and the experimentally exposed groups (500–2000 mg/mL). Result is shown as concentration of monocyte versus treatment and control. The values represent mean ± SD from two independent experiments with (n=6).](image)
groups (CTR) and the groups that received *Moringa oleifera* leaf extract (MLE) 500, 1000 and 2000 mg/kg. Significance difference at p > 0.001 was also observed between the groups that received *Moringa oleifera* leaf extract (MLE) 1000 mg/kg and the groups that are exposed to 2000 mg/kg of *Moringa oleifera* leaf extract (MLE). A change was also recorded with significant difference at p > 0.01 between the groups that are exposed to 500 mg/kg and the groups that received 1000 mg/kg of *Moringa oleifera* leaf extract (MLE) figure 11.

### 4. Discussion

An acute toxicity study was carried out in Wistar rats to observe it concentration effects in blood parameters. At the begging 60% mortality was recorded in the group that was exposed to 2000 mg/kg of *Moringa oleifera* leaf extract after 24 hours. Mortality of up to 20% was also observed in group that received 1000 mg/kg while no mortality was recorded in group that was treated with 500 mg/kg of *Moringa oleifera* leaf extract after 24 hours. After the complete analysis of blood samples obtained from the survived rats from each groups following doses administration, hematological parameters such as PCV, Hb, RBC, MCV, MCH, MCHC, RDW, WBC and DLC were analyzed.

Present study shows slight decrease in PCV% between control groups and the group that are exposed to different concentration of the crude extract. Control groups shows a normal PCV 39.6 to 52.5 as established by [31], there is severe decrease in PCV in groups that received 1000 and 200 mg/kg than the groups that are exposed to 500 mg/kg crude extract. Although, variation in values was observed between the different groups in this experiment, but all values are within the normal range [31]. Most of literature so far, reported increased in PCV in Wister rat following exposure to different concentration of *Moringa oleifera* extract (figure 1). Hence, this result disagree with the finding reported by some scientist which shows increase in PCV of Wister rat following treatment with different concentration of this extract [32, 33]. Result of total Rbc count revealed variation between the control groups and the groups that were exposed to different concentration of the extract (figure 2), but the values are still within the normal range as established by [31]. This finding did not similar with the one reported by [32, 33] who shows increase Rbc value following Wister rat exposure to different concentration of *Moringa oleifera*, but agree with the finding documented by [34] whose reported decreased MCV, MCH, MCHC and RDW count after exposing rat to different concentration of the extract. *Moringa oleifera* leaf extract shows no changes in both the relative and absolute value of total as well as differential leucocyte count between the control groups and the groups that were exposed to different concentration of the extract. There is no significance difference between the control groups (CTR) and the groups that received different doses of *Moringa oleifera* leaf extract (MLE) figures 8-10. Variations in blood parameters may be associated with bioactive compounds present in the extract. Although most bioactive compound are of medicinal important, but high concentration of the compounds may be detrimental to cells and tissues [35, 36]. Several studies reported the benefit of bioactive compound present in plant and food. Some of it advantages include antioxidant and anti-inflammatory potentials, others may have bioactive compound with unknown mechanism of action but yield positive result when use as medicinal agent [37, 38]. Several valuable reviews of the ethnobotanical uses of *M. oleifera* reported it as a good source of polyphenols and antioxidants [39, 40]. There are no observed changes in both total and differential leucocyte changes. The result shows no recorded value of eosinophil, basophil and band cells.

Phytochemicals such as vanillin, omega fatty acids, carotenoids, ascorbates, tocopherols, beta-sitosterol, moringine, kaempferol, and quercetin have been reported in its flowers, roots, fruits, and seeds. The leaf, in particular, have been found to contain phenolics and flavonoids [41, 42]; these compounds have various biological activities, including antioxidant, anticarcinogenic, immunomodulatory, anti-diabetic, antiatherogenic, and hepato protective functions and the regulation of thyroid status [43, 44]. Moreover, leaf contains trace elements that are essential to human health. For instance, magnesium, iron, selenium, and zinc play an important role in metabolism, and interest in these elements is increasing together with reports relating trace element status and oxidative diseases [45, 46]. Despite all the benefit of these biochemical constituents, it also caused allowed of side effect especially in blood tissues. Reports showed that some extracts contains an agent with profound effects on blood parameter. Several studies reported the potentials of Moringa extracts to elicits increase in blood parameters especially Rbc and PCV [47]. Effect of Gonglonema latifolium root extract contains polyphenols in abundance with moderate amounts of alkaloids, glycosides and reducing sugars while saponins, tannins, flavonoids, phlobatanins, anthraquinones and hydroxymethyl anthraquinones. The finding also reported severe decrease in blood parameters such MCV, MCH, MCHC and RDW after administration of this extract [48].

### 5. Conclusion and Recommendations

In conclusion, this study showed variation in haematological parameters especially red cell indices following oral administration of *Moringa oleifera* 80% leaf
extract at dose 500, 1000 and 2000 mg/kg. Phytochemical studies to reaffirm it effect in several body tissues and organs as well as identifying the bioactive lead compounds are highly recommended.

References


