Hepatoprotective and Anti-anemic Effects of Aqueous Leaf Extract of *Moringa oleifera* in Wistar Rats

**ABSTRACT**

**Introduction** *Moringa oleifera* and related species are commonly used in folk medicine for various human diseases. The aim of this study is to evaluate the hepatoprotective and anti-anemic effects of aqueous leaf extract of *M. oleifera* in Wistar rats.

**Methods** Fresh leaves of *M. oleifera* were dried and extracted with distilled water. Thirty male albino Wistar rats, weighing between 150 and 250 g, which were kept under uniform laboratory conditions, and were randomly divided into five groups (A–E), were used for this study. The control group (group A) was given 0.5 ml of distilled water orally while the treatment groups (groups B–E) were given 250, 450, 650, and 850 mg/kg body weight, respectively, of the extract orally, once a day, for 56 days. Blood sample was collected from each rat by cardiac puncture at the end of extract administration for laboratory analysis of red blood cell indices and the animals' livers harvested for histological examination.

**Results** Phytochemical analysis revealed that tannins, saponins, salkowski, free anthraquinone and flavonoids were moderately present, while steroids, phenobtannins, combined anthraquinone, Lieberman's and Keller kiliani were slightly present. The quantitative analysis revealed the presence of polyphenols, flavonoids, tannins, alkaloids and glycosides in the following proportions: 2.70, 4.10, 8.00, 15.00, and 2.50%, respectively. Significant (*P* < 0.05) increase in red blood cells was noted for high dose of extract. Hemoglobin concentration increased significantly (*P* < 0.05) for the dose of 650 mg/kg administered; while a non-significant change was seen in the level of packed cell volume and red cell indices, and non-significant dose-dependent reduction was observed in the level of immature reticulocyte fraction (IRF) and reticulocyte when compared to the control. The histological architecture and features of the animals' liver were normal following extract administration when compared to the control.

**Discussion** It can be concluded that the aqueous leaf extract of *M. oleifera* may possess a possible anti-anemic and hepatoprotective potential, owing to the presence of phytochemicals indicated in the study. Reticulocyte count with IRF can be routinely and widely used in the laboratory to evaluate erythropoietic activity.

**KEYWORDS** *Moringa oleifera*, hepatoprotective anti-anemic, Wistar rats, erythropoietin

**INTRODUCTION**

Natural products from plants provide an important source of new drugs and potential pharmaceutical compounds. Natural products include about 28% of all new chemical entities. Furthermore, many blood boosting agents in current clinical use are of natural origin.

Leaves, roots, and flowers of *Moringa oleifera* have been used in the treatment of rheumatism, cardiac and circulatory disorders in folk medicine and also possess antitumor, anti-inflammatory, anti-hypertensive, anti-diabetic, hepatoprotective, cholesterol lowering, antioxidant, antibacterial and antifungal properties, as well as liver tonic activity. Seeds of this plant are reported to have immunosuppressant activity. Important chemical constituents identified in *M. oleifera* are alkaloids, moringine, vitamin C, flavonoids like quercetin and kaempferol, tocopherols, benzyl isothiocyanate derivatives and glycosides.

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**Author’s contribution:** Author Ojeka Sunday Ogbu conceived the study, designed the protocol and coordinated the experiment. The animal feeding, laboratory procedures and manuscript writing were performed by author Zabbey Victor Zigabelbari while author Njoku Bestman performed statistical analysis, data interpretation, contributed in the manuscript writing. All authors read through and approved the final manuscript.

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Moringa has been used in traditional medicine for centuries in many cultures around the world, for skin infections, anemia, anxiety, asthma, blackheads, blood impurities, bronchitis, cystitis, chest congestion, cholera, conjunctivitis, cough, diarrhea, eye and ear infections, fever, glandular swelling, abnormal blood pressure, hysteria, pain in joints, pimples, psoriasis, respiratory disorders, scurvy, semen deficiency, sore throat, sprain, tuberculosis, for intestinal worms, lactation, diabetes and pregnancy. Moringa oil has tremendous cosmetic value and is used in body and hair care as a moisturizer and skin conditioner. The oil has also been used in skin preparations and ointments since Egyptian times.

Blood parameters namely: Packed cell volume (PCV), hemoglobin (Hb), white blood cell count and differentials and platelets were also found to be positively affected by using this plant.

A reticulocyte is defined as a RBC containing at least two granules of reticulum. Reticulocytes are immature red blood cells (RBCs). They are released into the peripheral blood after a period of maturation in the bone marrow and undergo further differentiation into mature RBCs. Immature reticulocyte fraction (IRF) is defined as the ratio of immature reticulocytes to the total number of reticulocytes. Immature reticulocytes are larger, having the greatest light scatter properties due to their very high concentration of ribonucleic acid. Immature reticulocytes normally constitute less than 5% of the total number of reticulocytes. They are released into the peripheral blood during periods of intense erythropoietic stimulation. An increase in the proportion of reticulocytes greater than 1% is an indicator of erythroid regeneration.

This study attempts to evaluate the hepatoprotective and anti-anemic effects of the aqueous leaf extract of M. oleifera in Wistar rats.

MATERIALS AND METHODS

Thirty male albino Wistar rats weighing between 120 and 300 g were used for the experimental work. The animals were obtained from the animal care facility of the University of Port Harcourt and were housed in wooden cages and were allowed to acclimatize for a period of 14 days, to observe for any signs of illness before the experiment started. They were kept under standard laboratory conditions in well ventilated and clean wooden rat cages, with proper bedding (saw dust). The animals were given standard rat feed that contained groundnut, wheat bran, maize grains, palm kernel and fish meal, bought from the animal feed store in Choba, Rivers State, Nigeria, and tap water. The feeding and water troughs were thoroughly cleaned and the beddings changed regularly to ensure proper hygiene and healthy living conditions.

The rats were randomly divided into five groups (groups A–E), comprising six rats in each group. A calculated amount of the aqueous extract of M. oleifera was constituted in 20 ml of distilled water to give doses of 250–850 mg/kg body weight. Administration of the aqueous leaf extract of M. oleifera was performed orally once daily, between 7.30 and 9.30 am, using a 2-ml syringe. The various groups were administered with the aqueous extract as follows:

(A) Group A served as the control, with no extract administered; instead, 2 ml of distilled water was given.

(B) Groups B–E received 2 ml of the Moringa extract, in doses of 250, 450, 650, and 850 mg/kg, respectively, using a syringe.

These administrations were performed daily, for 56 days after which the animals were sacrificed and the blood samples collected in an EDTA bottle. The blood samples were collected by cardiac puncture, after each rat had been anaesthetized in a desiccator, using diethyl ether. The liver of each rat was also harvested for histological analysis.

Chemical tests were performed on the aqueous extract and on the powdered specimens using standard procedures, to identify the constituents.

STATISTICAL ANALYSIS

The results were subjected to statistical analysis using statistical package for social sciences (SPSS) version 20.0. Data are presented as mean ± SEM. Difference of means were considered significant at P value less than 0.05.

RESULTS

The results of the qualitative phytochemical analysis indicate that alkaloids were most abundantly present while tannins, saponins, Salkowski, free anthraquinone and flavonoids were moderately present. Steroids, phlobatannins, combined anthraquinone, Lieberman’s and Keller kiliani were slightly present. Cyanogenetic glycosides were observed to be absent (Table 1).

The results of the quantitative phytochemical analysis indicate the presence of polyphenols, flavonoids, tannins, alkaloids and glycosides in the following percentages: 2.70, 4.10, 8.00, 15.00, and 2.50%, respectively (Table 2).

The result of M. oleifera extract on erythrocyte indices shows a significant (P < 0.05) increase in the level of RBC
for the high dose administered when compared with the control. A non-significant reduction in the level of RBC was seen for the 250 and 450 mg/kg doses administered when compared. However, a dose-dependent non-significant increase in the level of RBC was observed for the doses administered. There was a non-significant change in the percentage level of reticulocyte when compared to the control (Figs. 2–4).

A significant \((P < 0.05)\) reduction was observed in the level of mean corpuscular hemoglobin (MCH) for the 450 mg/kg administered dose of the extract when compared with the control. However, a non-significant reduction was recorded in the level of MCH for the doses administered. There was a non-significant reduction in the levels of percentage reticulocyte and IRF following administration of leaf extract of \(M. \text{oleifera}\), especially at high doses when compared to the control (Figs. 6 and 7).

Table 1 Qualitative phytochemical analysis of aqueous leaf extract of \(M. \text{oleifera}\) in Wistar rats.

<table>
<thead>
<tr>
<th>Phytochemical</th>
<th>Observation</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>+++</td>
<td>Heavily present</td>
</tr>
<tr>
<td>Tannins</td>
<td>++</td>
<td>Moderately present</td>
</tr>
<tr>
<td>Saponins</td>
<td>++</td>
<td>Moderately present</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>++</td>
<td>Moderately present</td>
</tr>
<tr>
<td>Steroids</td>
<td>+</td>
<td>Slightly present</td>
</tr>
<tr>
<td>Phlobatanins</td>
<td>+</td>
<td>Slightly present</td>
</tr>
<tr>
<td>Combined anthraquinone</td>
<td>+</td>
<td>Slightly present</td>
</tr>
<tr>
<td>Free anthraquinone</td>
<td>++</td>
<td>Moderately present</td>
</tr>
<tr>
<td>Cyanogenetic glycosides</td>
<td>−ve</td>
<td>Absent</td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td>++</td>
<td>Moderately present</td>
</tr>
</tbody>
</table>

+, slightly present; ++, moderately present; ++++, heavily present; −ve, absent; *, Significant at \(P < 0.05\) when compared to control.

Table 2 Quantitative phytochemical analysis of \(M. \text{oleifera}\) leaf extract.

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>Percentage abundance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyphenols</td>
<td>2.70</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>4.10</td>
</tr>
<tr>
<td>Tannins</td>
<td>8.00</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>15.00</td>
</tr>
<tr>
<td>Glycosides</td>
<td>2.50</td>
</tr>
</tbody>
</table>

*, Significant at \(P < 0.05\) when compared to control.

Fig. 1 Effect of \(M. \text{oleifera}\) leaf extract on red blood cell count. *Significant at \(P < 0.05\) when compared to control.

Fig. 2 Effect of \(M. \text{oleifera}\) leaf extract on hemoglobin concentration. *Significant at \(P < 0.05\) when compared to control.

Fig. 3 Effect of \(M. \text{oleifera}\) leaf extract on packed cell volume (%).

Fig. 4 Effect of \(M. \text{oleifera}\) leaf extract on mean corpuscular hemoglobin.
Hepatoprotective and anti-anemic effects of aqueous leaf extract

Normal liver architectures were observed in both the control and the test groups (plates 1–5) in Fig. 8.

DISCUSSION

The hepatoprotective and anti-anemic effects of the aqueous leaf extract of *M. oleifera* was investigated in Wistar rats.

The findings of the preliminary phytochemical investigations, anti-anemic and hepatoprotective effects of the extract were depicted in the respective Tables 1 and 2 shown above. The preliminary phytochemical tests performed were of qualitative and quantitative types. From the qualitative analysis it was observed that alkaloid was heavily present, tannins, saponins, Salkowski, free anthraquinone and flavonoids were moderately present, while steroids, phlobatannins, combined anthraquinone, Liebermanns and Keller kiliani were slightly present. Cyanogenetic glycosides was observed to be absent. This review is consistent with report of Edoga et al.\(^{12}\)

The results obtained for the quantitative investigation of leaf extract of *M. oleifera* depicts the presence of polyphenols, flavonoids, tannins, alkaloids and glycosides in the following percentages 2.70, 4.10, 8.00, 15.00, and 2.50% respectively. These review shows the possible multifaceted potency of the extract and further established the heavy presence of alkaloid as shown in both the qualitative and quantitative screenings of the aqueous leaf extract of *M. oleifera*.

The results suggest that the administration of aqueous extract of *M. oleifera* leaves from our studies on the blood components of the studied rats is attributed to its effect on the hemopoeitic system. This argument is supported by the increased red blood, hemoglobin and PCV of rats treated with aqueous extract of *M. oleifera* leaves. This may implicate hematopoietic pathways\(^{13}\). Therefore, this study provides evidence that aqueous extract of *M. oleifera* may have potential for influencing anemic conditions in rats.

The values of MCH and MCH concentration does not appropriately contribute to the clinical evaluation of anemia\(^{14,15}\).

Fig. 5 Effect of *Moringa oleifera* leaf extract on MCH concentration (MCHC).

Fig. 6 Effect of *Moringa oleifera* leaf extract on percentage reticulocyte.

Fig. 7 Effect of *Moringa oleifera* leaf extract on immature reticulocyte fraction.

Fig. 8 Photomicrographs of histological architecture of rats’ liver following extract administration (400×). Plate 1: Control group – normal histological features. Plates 2–5; 250, 450, 650, and 850 mg/kg of extract – normal liver histological architecture when compared to the control group.
Immature reticulocyte fraction is a new routine parameter in the hematology analyzer that can give the idea of the earliest morphologic change of bone marrow recovery before other test become positive after chemotherapy. The non-significant increase in IRF count observed for the low doses of the extract administered, may be a useful predictor of early hematologic boost, owing to the stimulatory effect of high IRF concentration on erythropoietin production, which may have reduced the concentration as the doses of the extract is increase as observed in this study. This conforms to the report of Linda et al.3 This suggests that the aqueous extract of the leaf of *M. oleifera* may increase the rate of erythropoiesis and could probably be used as a therapeutic agent in the treatment of anemia.

The observed effect of the aqueous leaf extract of *M. oleifera* from this study, reticulocyte count and IRF correlates to the elevated level of erythropoietin parameters recorded. This further exposes the likely anti-anemic activity of the extract, which may implicate one or more of the phytochemical constituents reviewed in this study.13,15,16

The effect observed in the hematological parameters where stronger at high doses of the extract administered, suggesting possible better potentiation as the doses increases.

The liver is an organ involved in many metabolic functions and is prone to xenobiotics injury because of its central role in xenobiotics metabolism.16,17

The result from this study further reveals normal histological architecture of the liver of the studied rats following administration of aqueous leaf extract of *M. oleifera* relative to the control. This observation depicts a possible hepatoprotective effect of the extract that might have resulted due in part to the presence of some chemical constituents in the extract, especially tannins and flavonoids as reviewed in the qualitative and quantitative investigations from this study.

This result corroborates with the work of others which establish that the leaf of *M. oleifera* has a hepatoprotective action.17,19

**CONCLUSION**

From the result of this study, it can be concluded that the aqueous leaf extract of *M. oleifera* may possess a possible anti-anemic and hepatoprotective potential, owing to the presence of phytochemicals indicated in the study.

This study concluded that reticulocyte count with IRF can be routinely and widely used in the laboratory to evaluate erythropoietic activity.

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