



Proximate analysis and hematological evaluation of ethanol leaf extract of *Moringa oleifera* (Lam)

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ABSTRACT

Increasing interest in medicinal plants has increased scientific scrutiny of their therapeutic potentials and safety. This work evaluated the proximate analysis and toxicological effects and its consequences on hematological parameters of *Moringa oleifera* leaf. Proximate analysis showed that the leaf contains carbohydrate, fats, protein and fibre. It also shows the presence of nitrogen, calcium, magnesium, phosphorus, potassium, zinc, iron, copper, and sulphur. Subacute toxicity was done on Wistar albino rats for 28 days. There were no changes in body and organ weight except a statistical decrease in the spleen weight. Hematological investigation revealed an increase in mean corpuscular volume, granulocytes, and monocytes. There was a decrease in white blood cells and lymphocytes. The work shows that the leaf of the plant is nutritious and it is relatively safe at low dose. However, further evaluation of its long term effects may be necessary.

KEYWORDS: Toxicity, Analysis, *Moringa oleifera*, Herbal, Haematology

INTRODUCTION

Medical plants have been used for centuries as remedies for human disease because they contain components of therapeutic value [1]. Increasing interest in medicinal plants has increased scientific scrutiny of their therapeutic potentials and safety thereby providing physicians and patients with data to help them make wise decision before using them [2]. Although plant based natural medicines are popularly acclaimed to be safe, scientists advocate for proper toxicological studies in order to ensure safety in the use of natural medicine [3].

Moringaceae is also known as the horseradish tree family with single genus *Moringa* [4] including 13 species of dicotyledonous tropical and sub-tropical flowering trees with gummy bark and gum canals in bark and pith. The alternate leaves are pinnate.

They may produce an unpleasant odour when crushed. The irregular flowers are grouped in panicles and have 5 sepals and 5 petals often reflexed. Fruits are angular dehiscent capsules containing 3 winged or wingless seeds [5].

Moringaceae are widely distributed. *Moringa oleifera* from India is the most widely grown, for its nutritious pods, edible leaves and flowers. The mature seeds can be roasted or used to prepare oil. The fleshy roots are grated and used as a seasoning [6].

The leaves are rich in protein, vitamin A, vitamin B, vitamin C and minerals. A 100 g portion of fresh *Moringa* leaves has 9.3 g protein, 434 mg calcium, 404 mg potassium, 738 mg vitamin A, and 164 mg vitamin C [7]. Feeding the high protein leaves to cattle has been shown to increase weight gain by up to 32% and milk production by 43 to 65% [8]. The seeds contain 30 – 40% oil that is high in oleic

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acid, while degreased meal is 61% protein [9]. The defatted meal is a flocculant and can be used in water purification [10].

Moringa cultivation is on the increase in Honduras and across South America. Since 2012 support for *Moringa* is being offered to farmers by the Honduras Federal Government through the secretary of Agriculture and by private foreign investment firms [11]. The plants market potential is widespread given its easy growth and high nutrient content.

Several compounds of pharmaceutical importance have been isolated from *Moringa oleifera* leaf. These include 4-hydroxycinnamic acid, 3, 4-dihydroxy cinnamic acid, quercetin, isoquercetin, kaempferol, niazirin, gallic acid, ellagic acid and tocopherol [12]. Although moringa leaves have been extensively studied, there is a need to determine the composition of the plant from different localities and their effects. This work focused on the proximate analysis of the leaf and its hematological effects when administered to wistar albino rats.

MATERIALS AND METHODS

Materials

Wistar Albino rats, ethanol, 0.9% normal saline, chloroform, formalin, heparin, hydrochloric acid, mixed selenium tablet catalyst, Erichrome black T.

Plant collection and authentication

The leaves of *Moringa oleifera* were collected from home garden in University of Benin, Benin City, Edo State, Nigeria on the 8th of August, 2014. The fresh leaves were identified by the plant curator in the Department of Pharmacognosy Herbarium, University of Benin, Benin City, Edo State, Nigeria. The leaves were air-dried at room temperature for four days, then further dried in an oven at 40 °C for 6 h. The crispy leaves were ground into powder and filtered using a sieve aperture of 1.0 mm. The fine powder was preserved in moisture free, airtight container and used for proximate analysis and toxicological evaluation.

Proximate analysis

Moisture content determination

The sample (1 g) was dried in an oven until constant weight was obtained.

Moisture percentage was calculated thus:

$$\text{Moisture percentage} = \frac{\text{loss of weight} \times 100}{\text{weight of sample used}}$$

Protein determination

To 1 g of crude in clean desiccation flask was added 2 ml of concentrated sulphuric acid and selenium tablet. This was properly mixed before placing in the auto digester, first at low temperature and later the temperature was increased when frosting had ceased. Digestion continued until the sample became clear to milk colour. 10 ml liquor of the sample was pipetted into the distillate, with dilute HCl (0.01 N) until the milk color disappeared. A blank determination was carried out and the crude protein values were determined using the formula.

% N of sample

$$= \frac{1R \times SR \times FV \times CD \times 100 \text{ loss of weight} \times 100}{\text{Aliquot} \times WT \times 1000,000}$$

IR = Instrument reading, SR = Slope reciprocal from the standard reading, FV = Final volume, CD = Colour developed, WT = Weight of sample used.

Crude protein = % N of sample x 6.25

Fat Determination

The sample (1 g) was extracted with n-hexane using a soxhlet extractor for 8 hours and the solvent was removed.

The lipid content was then calculated:

$$\frac{\text{Initial weight} - \text{final weight} \times 100\%}{\text{Weight of sample}}$$

Ash content determination

One (1) g of sample and bagasse were placed in an already weighed crucible in a muffle furnace to ash at a temperature of 500 °C for about 3 h. The ash obtained was then weighed.

Crude Fibre Determination

The sample (2 g) and 100 ml of freshly boiled crude fibre reagent were placed on the crude fibre apparatus and boiled. The content was filtered under suction after refluxing for 1 hour. The residual was rinsed with boiling water until they were free of acid. Sodium hydroxide (NaOH) was added and the solution was filtered while hot. The residue was cooled and weighed; the loss in weight was calculated as the crude fiber content.

Crude fibre was calculated thus:

$$\% \text{ of crude fiber} = \frac{y-z \times 100}{x}$$

Where y = Weight of sample before washing and
 z = Weight of sample after washing
 x = weight of Sample

Determination of Carbohydrate Content

Basically, the percentage moisture, ash, fat, protein and crude fibre were added and subtracted from 100. The difference was taken as the carbohydrate content.

Toxicological evaluation

Preparation of extract

The powdered leaf sample (950 g) was macerated in 6 litres of ethanol for 72 hours. The suspension was filtered and the resulting filtrate was evaporated over a water bath. The extract was made into different concentrations.

Subacute toxicity study

Thirty healthy Wistar albino rats of either sex weighting between 183 and 235 g were purchased from animal house, University of Ibadan and acclimatized in the animal house of the University of Benin, for 3 weeks. The rats were divided into 5 groups of 6 animals per group and were fed with standard rat pelleted diet (Ewu feed®) and had free access to water ad libitum. Coloured marker pen was used to distinctly label each animal for easy identification. *Moringa oleifera* extract was administered for 28 days at doses of 100, 250, 500 and 1000 mg/kg body weight and control group (group 1) received 10 ml/kg of the vehicle alone (30% ethanol in 0.9% normal saline). Toxic manifestation and mortality were monitored daily and body weight changes were recorded every 7 days till the end of the study.

Clinical test parameters

At the 28th day, animals were fasted for 12 hours, weighed, anaesthetized with chloroform anesthesia, and sacrificed. Blood sample (5 ml) was collected from the abdominal aorta or by cardiac puncture, and kept in EDTA (ethylenediaminetetraacetic acid) tube for immediate analysis of hematological parameters.

Parameters investigated

The parameters investigated in the study were: hemoglobin (HB), red blood cells (RBC), white blood cells (WBC), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH), platelet (PLT), lymphocyte (LYMP), mixed (MXD) and neutrophils (NEU) were performed using an

automatic multichannel blood counter (sysmex Kx 21 Hematology Analyzer).

Statistical analysis

Graph pad instant version 2.05 software (UK) was used. The values were expressed as mean \pm standard deviation. Statistical analysis was performed by one way variance followed by Turkey Kramer multiple comparison tests. *P*-values <0.005 were considered as significant.

RESULT

Proximate analysis

The result of the proximate analysis is shown in Table 1. The leaf is mainly made up of carbohydrate. Table 2 shows the percentage of dietary minerals in the leaf.

Nitrogen and Calcium are the most abundant elements present. Table 3 shows the effect of ethanol extract of *M. oleifera* on body and organ weight changes in control and treated rats while Table 4 shows the effect of *Moringa oleifera* on haematological parameters.

TABLE 1: Proximate analysis of *Moringa oleifera* leaf

NUTRIENT	COMPOSITION (g/100g)
Carbohydrates	57.01 \pm 0.01
Fats	2.74 \pm 0.03
Protein	18.92 \pm 0.02
Fiber	9.31 \pm 0.02
Moisture	9.83 \pm 0.27
Ash	17.50 \pm 2.32
Acid insoluble ash	9.1 \pm 0.01
Water soluble ash	2.5 \pm 0.03

TABLE 2: Percentage content of some dietary mineral in leaf of *Moringa oleifera*

MINERALS	Percentage content (%)
Nitrogen	3.03 \pm 0.02
Calcium	2.09 \pm 0.02
Magnesium	0.48 \pm 0.00
Potassium	1.62 \pm 0.02
Phosphorus	0.44 \pm 0.01
Zinc	0.05 \pm 0.01
Iron	0.03 \pm 0.00
Copper	0.01 \pm 0.00
Sulphur	0.85 \pm 0.01

Values are expressed as mean \pm SEM for three replicates

TABLE 4: The effect of *Moringa oleifera* on haematological parameters

Parameters	Group1 (control)	Group 2 (100 mg/kg)	Group 3 (250 mg/kg)	Group 4 (500mg/kg)	Group 5 1000 (mg/kg)
WBC (10 ³ cell/mm ³)	8.15±0.64	3.25±1.06	3.00±0.99	4.90±0.28	4.90±0.14*
RBC (10 ⁶ cell/mm ³)	8.67±0.77	7.44±0.34	7.70±0.70	8.61±0.15	8.40±0.14
Hb (g/dl)	17.11±0.13	15.26±0.22	15.45±1.06	18.10±0.28	17.31±0.13
PCV (%)	43.65±1.91	37.95±1.06	38.5±1.41	45.75±2.90	44.10±2.26
MCV(FL)	47.85±1.34	50.25±0.07	52.25±0.35	54.08±088	53.36±0.48*
MCH(Pg)	18.15±0.64	18.75±1.20	20.05±0.50	20.40±0.71	20.05±0.50
MCHC (g/dl)	37.55±0.92	37.85±1.63	38.40±1.13	37.85±0.50	37.75±0.35
PLT (10 ³ cells/mm ³)	427±22.63	356±100.4	487.5±38.89	463±56.57	417±9.90
LYMP (%)	51.45±5.45	40.60±2.69	42.45±14.78	32.85±7.43	31.25±5.73*
MO (%)	14.7±3.54	18.05±2.33	20.35±10.25	21.10±5.51	17.80±3.25*
GR (%)	28.85±1.91	39.55±2.90	37.20±4.53	43.05±6.15	41.10±4.95*

Values are expressed as mean ± SD for ten albino Wistar rats.

Comparisons were made between groups. $P < 0.05$.

WBC = White blood cells, RBC = Red blood cells, Hb = Hemoglobin, PCV = Packed cell volume, MCV = Mean corpuscular volume, MCH = Mean corpuscular haemoglobin, MCHC = Mean corpuscular haemoglobin concentration, PLT = Platelet, LYMP = lymphocyte, MO = Monocytes, GR = Granulocyte

DISCUSSION

The powdered leaf of *Moringa oleifera* is rich in carbohydrate (57.01%). This might be the reason why the plant is cultivated as a major source of food ingredients in many developing countries [13]. The moisture content of the *Moringa oleifera* leaf was found to be 9.83%. This low moisture content would hinder the growth of microorganisms and also the storage life will be increased.

The ash content of the leaf was found to be 17.50% while the protein content was found to be 18.92% which are both relatively moderate. However the fat content was found to be low 2.74% which indicates that the vegetable is not a source of lipid accumulation, which can cause arteriosclerosis [14].

Also the fibre content was found to be 9.31% which might be responsible for the easy digestible nature of the leaf [15]. *Moringa oleifera* was also found to

contain various dietary mineral such as nitrogen, potassium, phosphorus, zinc, iron, copper and sulphur in varying percentages. Herbal medicines have received greater attention as an alternative to clinical therapy and the demand of these remedies has currently increased [16].

Experimental screening method is important to ascertain the safety and efficacy of traditional and herbal products and also to establish the active component of the herbal products [17]. Observation of behavioral and hematological parameters has been employed in toxicological studies [18]. In the subacute toxicity study, changes in body weight have been used as an indicator of adverse effect of *Moringa oleifera* and chemicals. Since there are no changes in the average body weight at all doses ($P > 0.05$) of the treated rats when compared with the control group and between treatment groups. It suggests that at the oral doses administered, *Moringa oleifera* may be considered safe [19-21].

There were no significant changes in the average weight of the organs at doses administered to the treated rats when compared with the control group and between treatment groups ($P > 0.05$) with the exception of a slight decrease in the weight of the spleen. This may suggest that at the oral doses administered, *Moringa oleifera* may be safe [22]. The significant decrease ($P < 0.05$) in the spleen weight is from 0.76 ± 0.06 to 0.44 ± 0.11 in Group 3 and Group 5 respectively. It has been established that the enlargement of spleen could occur as a result of destruction of red blood cells and platelet as well. Higher concentration of *Moringa oleifera* significantly reduces spleen weight which is an indication that the plant may be cytoprotective [23-24].

There was marked significant differences ($P < 0.05$) in White blood cell (WBC) and Mean corpuscular volume (MCV) in Group 2, 3, 4, and 5. There was also significant differences ($P < 0.05$) in lymphocytes, monocytes, and granulocytes in Group 3, 4, and 5. While there was no significant differences ($P > 0.05$) in red blood cell (RBC), haemoglobin (Hb), packed cell volume (PCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), and platelet (PLT). Increase in the value of mean corpuscular volume as observed in different groups may be an indication of increase in the average red blood size [25] while the differences not observed in RBC, Hb, PCV, MCH, MCHC, and platelet may indicate that *Moringa oleifera* may not have a direct effect on the haemoglobin concentration of RBC and therefore may not be useful in the management of various form of anaemia [26-28]. Also a decrease in WBC and lymphocytes value may be an indication of immunosuppression [29] which may involve an inhibition of the activation of the body immune system. Increase in monocytes and granulocytes value may be an indication of inflammatory response [30-31].

CONCLUSION

Moringa oleifera leaf may be considered relatively safe at low oral doses of administration. However at higher doses there were observable detrimental effects caused by its administration in subacute toxicity study in rat model. Therefore care should be taken with its use at higher doses over a long period of time as it causes damages to the body tissues. Proximate analysis established that the plant contains carbohydrate, fats, protein, moisture, fibre and ash. It also contains minerals such as nitrogen, calcium,

magnesium, phosphorus, potassium, zinc, iron, copper and sulphur.

CONFLICT OF INTEREST

The authors declare no conflict of interest. The authors alone are responsible for the content and writing of this article.

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