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Original Research Article

Evaluation of Toxicity Effect of Graded Doses of *Moringa oleifera* Leaf Extract on Blood Indices Using 20 Adult Wistar Rats

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Abstract

Epidemiological studies have indicated that *Moringa oleifera* leaves are a good source of nutrition and exhibit antioxidant activities. *Moringa oleifera* is being used for many medicinal and nutritional purposes, since it is widely consumed by all for nutritional and varying medicinal purposes; however there is limited scientific data available regarding excessive consumption of this plant. This study is carried out to evaluate the effect of *Moringa oleifera* leaf extract on some blood indices. 20 adult wistar rats were used for the study. They were divided into five groups (A, B, C, D & E) of four animals each. Group A served as the control and was orally administered distilled water; the experimental groups B, C, D, & E were orally administered different graded doses of *Moringa oleifera* leaf extract as follows 2000mg/kg, 3000mg/kg, 5000mg/kg and 7000mg/kg respectively for twenty one days. Twenty four hours after the last administration, blood samples for haematological analysis were collected through cardiac puncture into sterilized glass tubes containing ethylene diamine tetra acetic acid (EDTA) as anticoagulant. Packed cell volume estimation was done using microhaematocrit method. Sample dilution was done using Turk's fluid and cell count using improved neubauer counting chamber. Platelet count was estimated with ammonium oxalate and cell count using counting chamber. The result of this study suggests that excessive consumption of aqueous *Moringa oleifera* leaf extract may have little effect on blood parameters with slight changes in white blood cells.

Keywords: *Moringa oleifera*, Blood indices, Platelet count, Packed cell, Wistar rats.

1.Introduction

Medicinal plants have been identified and used throughout human history. Plants have the ability to synthesize a wide variety of chemical compound that are used to perform important biological functions and to defend against attack from predators such as fungi, insect and herbivorous mammals. Chemical compound in plants mediate their effects on the human body through processes identical to those already well understood for the chemical compound in conventional drugs; thus herbal medicines do not differ greatly from conventional drugs in terms of how they work. This enables herbal medicines to be as effective as conventional medicines, but also gives them the same potential to cause harmful side effects[1][2].

Generally, the chemical substances differ from plant to plant, thus the plant kingdom provides a large store of various chemical substances with potential therapeutic properties which has been utilized in the treatment and cure of human and other animal diseases including urinary infection, relieving of pains, convulsion, and cardiovascular diseases[3].

Among these medicinal plants is *Moringa oleifera*. The World Health Organization (WHO) has been studying the use of *Moringa oleifera* for many decades as a low cost supplement enhancer in the poorest countries around the world. This organization has been promoting the use of this plant to help those countries suffering from malnutrition, which is one of the major causes of death worldwide.

United Nations Food and Agriculture reported that one in twelve people worldwide is malnourished, including 160 million children under the age of five[4].

The medicinal effect of *Moringa oleifera* has been attributed to its possession of anti-oxidant which is known to have suppressive effects on formation of reactive oxygen species (ROS) and free radicals[5][6].

It has been reported by Bureau of plant industry that *Moringa oleifera* is an outstanding source of nutritional components. Besides, *Moringa oleifera* is also suggested as a viable supplement of dietary minerals. The pods and leaves of *Moringa oleifera* contain high amounts of calcium, magnesium, potassium, phosphorus, zinc, sodium, iron etc. The levels of these phytochemicals (bioactive compounds) reveals that the leaves sample showed higher levels of these bioactive compounds than the seeds sample. Although, mineral content of *Moringa oleifera* shows variation in composition with changes in location[7][8].

Haematology has been defined as the study of blood and an important part of clinical pathology as well as diagnostic process. It includes not only the examination of the cellular and fluid portions of blood, but also includes a study of the tissues that form, store and circulate blood cells. The result of haematology analysis is usually used to assess the health status of an animal. Haematological parameters have been observed as good indicators of the physiological status of animal and its changes are important in assessing the response of animal to various physiological situations[9].

Therefore, with the essential properties discovered from *Moringa oleifera* leaf extract, there is need to evaluate its effects on the blood indices in adult male wistar rats.

2. Materials and Method

2.1 Materials for the Study

The materials used included; Distilled water, *Moringa oleifera* leaf powder, Standard cages, Rats, Electronic weighing balance, Syringes, Normal growers mesh, Sieve, Light microscope, slides, Bouin's fluid, 10% formalin.

2.2 Drug Extraction

Fresh *Moringa oleifera* leaves were plucked from Okofia in Nnewi, Anambra State. It was identified at herbarium unit, Botany Department, Nnamdi Azikiwe University, Anambra State. They were dried in an oven and grinded using laboratory blender. Ground sample of about 10g was extracted with 100ml of deionised water by boiling. The boiled mixture was shaken vigorously for 10-15 seconds and allowed to stand for about 30 minutes and then filtered through a 150 aperture sieve to obtain the aqueous extract. The aqueous extract was thereafter freeze-dried and then lyophilized to give a 10.9%. The sample was then placed in air-tight containers and refrigerated. 2000mg/kg, 3000mg/kg, 5000mg/kg and 7000mg/kg was dissolved in 10ml of distilled water and administered to the animals.

2.3 Experimental Animals and Design

The research was done with 20 adult male wistar rats. The rats were bred at a local farm at Nnewi. They were transferred to the Animal House of the Department of Anatomy, College of Health Sciences, Nnamdi Azikiwe University, Nnewi. They were housed in five standard cages and were divided into 5 groups (A to E) according to their weights. The animals were provided with food and water *ad-libitum*. Prior to the commencement of the experiment, the animals were pre-conditioned for two weeks. The animal care and handling was conducted in compliance with the National Regulations for Animal Research. University Ethical committee reviewed the protocols, which were consistent with International Animal Welfare Guidelines. The animals were divided into five groups of four animals each. Group A served as the control and were administered only distilled water while groups B, C, D and E served as the test groups and were administered 2000mg/kg, 3000mg/kg, 5000mg/kg and 7000mg/kg of Moringa oleifera leaf extract respectively for twenty one days subcutaneously using syringes. Twenty four hours after the last administration, blood samples for haematological analysis were collected through cardiac puncture into sterilized glass tubes containing ethylene diamine tetra acetic acid (EDTA) as anticoagulant. Packed cell volume estimation was done using microhaematocrit method. Sample dilution was done using Turk's fluid and cell count using improved Neubauer counting chamber. Platelet count was estimated with ammonium oxalate and cell count using counting chamber.

2.4 Data Analysis

Data were analysed using ANOVA and student's t-test of SPSS version 16 software package and $P<0.05$ was considered as the level of significance.

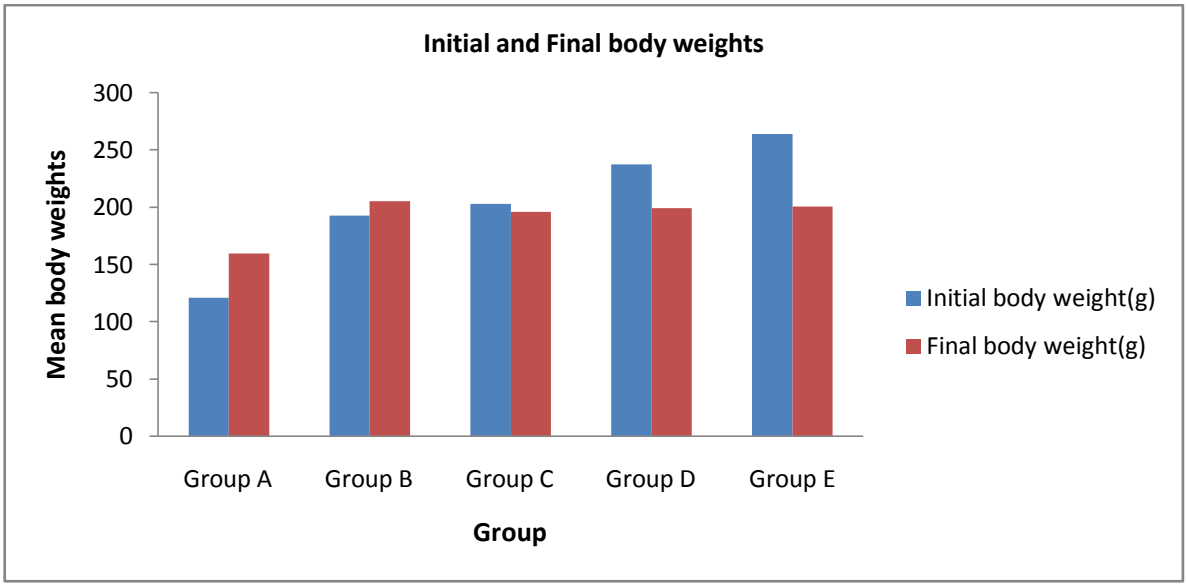
3. Result

3.1 Morphometric Analysis of Body Weight

Table 1: Comparison of mean initial and final body weight in all the groups (A, B, C, D & E) (Mean ± SEM given for each measurement)

Group	Group A	Group B	Group C	Group D	Group E
Initial body weight(g)	120.78±7.73	192.85±6.78	202.90±32.40	237.68±9.66	263.85±25.55
Final body weight(g)	159.78±5.07	205.26±17.32	195.92±7.01	199.10±0.58	200.65±0.39

Figure 1: Bar Chart showing the initial and final mean body weights of the five groups.



3.2 Morphometric Analysis of Haematological Parameters

Table 2: Morphometric Analysis of Haematological Parameters

Haematological parameters	(A)Control	B(2000mg)	C(3000mg)	D(5000mg)	E(7000mg)
PCV	0.23±0.01	0.30±0.00*	0.26±0.02≠	0.25±0.00≠	0.24±0.00≠
PLTC x 10^9/L	224.25±32.10	158.75±2.21*	279.75±28.26≠	200.75±0.75≠	192.34±0.96≠
WBCT /mm^3	4137.50±23.94	6149.75±742.32≠	7599.75±1414.24*	8412.50±119.68*	9554.00±21.51*
Neutrophil %	50.50±0.96	48.75±0.75≠	51.25±3.07≠	46.75±0.75≠	45.75±1.31≠
Lymphocyte %	48.00±0.71	48.25±0.48≠	47.75±2.78≠	49.25±0.48≠	55.25±0.48*

*Significant; ≠ Not significant; Values are mentioned in MEAN±SEM

3.3 Haematological Result

Pack Cell Volume (PCV) of the group B increased significantly (P>0.05) when compared with the control while there is slight increase in PCV of other groups (C, D& E) but not statistically significant. There is also increase in total white blood cell count (WBCT) in all the test groups when compared with the control group. This increase is statistically significant in all the test groups except the group B. Other haematological parameters showed slight changes but are not significant.

4. Discussion

Currently, medicinal herbs as a whole were reported to be used against a wide range of health problems such as cough, cold, stomach, cataract, constipation and many other ailments[10].

Herbs are generally recognized as safe when used in therapeutic doses, but researches have shown that herbal extracts have the potential to produce adverse effects, especially when used in toxic forms[11]. There may be some effects when consumed in the small levels that typify culinary "spicing", and some herbs are toxic in larger quantities. For instance, some types of herbal extract, such as the extract of St. John's-wort **(***hyperium perforatum*) or of kava (*piper methysticum*) can be used for medical purposes to relieve depression and stress. However, large amounts of these herbs may lead to toxic overload that may involve

complications, some of a serious nature, and should be used with caution[11].

The belief in the efficacy of *Moringa oleifera* to heal and cure many diseases both scientifically proven and otherwise has given it the name "miracle tree" or a "wonder tree". This has made so many people to consume it at different doses ranging from moderate dosages to excessively high dosages[12].

In this study, 20 adult male albino rats were used as working models to test the potential toxicity effect of high doses of aqueous extract of *M. oleifera* on the blood indices.

The level of increase in the blood parameters could have been caused by the presence of some harmful elements present in the leaves such as those elements reported by Aminu *et al*[13]. Such elements include Sr, Rb, and Zr, although they are within the least detectable limits. These authors reported also that *Moringa* leaves contained among others the following elements in parts per million: Calcium (1.29 x104 x 104 ±500); potassium (7.2 x 103± 600); Sodium (3.8 x 104 ± 500); Iron (4.53 x 102 ± 21); and Chlorine (1.44 x 102 ± 15) and that the presence of some of these constituents could be responsible for the observable microscopical lesions.

The observed increase in white blood cell of this study could have being caused by necrosis observed in the histology of the organs. This increase is in line with the study done by Adedapo *et al*[14], who reported that the extract particularly the 400 and 800 mg/kg doses caused significant increase in the level of white blood cell counts and its differentials. This observation of increase in the levels of these parameters by this plant extract shows that the principal function of phagocytes, which is to defend against invading micro organisms by ingesting and destroying them, thus contributing to cellular inflammatory processes, will be enhanced which may account for its antibacterial activity[15][16][17].

Moreover, a significant increase in pack cell volume (PCV) by 400mg/kg in addition to a significant decrease in the level of haemoglobin and red blood cell (RBC) were reported by Adedapo *et al*[14].

More so, the observed decrease in body weight of the animals used in this study is not in accordance with the study done by Ghebreselassie D *et al*[18], who reported that Mice treated with 900 mg/kg of the extract per kg of body weight showed a significant increase in body weight compared to the controls (P=0.014). However, the decrease in body weight as observed in this study may be as a result of high dose of *Moringa oleifera* leaf extracts.

5. Conclusion

The result of this present study suggests that excessive consumption of aqueous *Moringa oleifera* leaf extract may have little effect on blood parameters with slight changes in white blood cells.

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