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Metal constituents and effect of *Moringa oleifera* leaf extract on some haematological parameters in rats

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Abstract

The air–dried powdered leaf of *Moringa oleifera* was analysed using energy dispersive X-ray fluorescence (EDXRF) transmission emission technique. It was found to contain Ca ($1.29X\ 10^4$±500ppm), K ($7.2X10^3$±600ppm), S ($3.8X101^4$±500ppm), Fe ($4.53\ X\ 10^2$±21ppm) and Cl ($1.44X10^2$±15 ppm). A total of 24 albino rats (Wistar strain) *Rattus norvegecus,* were grouped into four A, B, C, D and fed orally with modified diet containing 25% w/ w, 50% w/w and 75% w/w powdered leaves of *Moringa oleifera,* mixed with standard livestock feed (Feed Master® grower mash) for 93 days. The control group D, was fed with standard diet alone. At the end of the experiment, it was observed that the mean parked cell volume (%) were 54.2 and 47.6 for 50%and 75% group respectively and the control was 51.5; the mean Haemoglobin (gm/ dl) were 17.9 and 15.8 for 50% and 75% groups respectively, the control group was 17.1; the mean total protein (mg/ 100ml) were 8.2 and 7.3 for the 50% and 75% groups respectively with 9.0 for the control group; the mean neutrophils (%) were 19.8 and 24.6 for 50% and 75% respectively and the control was 16.7. Even though these results revealed no significant difference between the treated and the controlled group animals (P<0. 05), it is still not enough justification to conclude that the plant is safe for long term consumption both as food and as medicine.

Keywords: *Moringa oleifera,* Albino rats, energy dispersive X-ray fluorescence (EDXRF) transmission emission

Introduction

Moringa oleifera Lam. (Syn. *M. pterygosperma* Gaetn.) is of the family Moringaceae. It is a small tree with sparse foliage, often planted in compounds or used as fence in northern Nigeria. It is a deciduous plant and could grow up to 8m high (Keay, 1989). The plant is commonly called horse-radish tree and is locally known as '*Zogalegandi*' in Hausa, '*Eweigbale*' in Yoruba and '*Okweoyibo*' in Igbo (Dalzell, 1956). *M. oleifera* is well known for its nutritional as well as medicinal values by many communities in Northern Nigeria. The leaf is used as vegetable for soup preparation and mixed with milled groundnut cake and other spices and then eaten as food. The plant has been reported to exhibit anti-inflammatory, anti-hypertensive and anti-ulcer activities (Ezeamuzie *et al*; 1996; Pal *et*

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al; 1995). It is known to possess anti-bacterial activity against *Bacillus subtilis, Mycobacterium phlei, Staphylococcus aureus, Salmonella* and *Shigella* species (Caceres *et al;* 1991, Sofowora, 1993). The plant is known in traditional therapies as abortifacient and fertility control (Oommachan and Khan, 1981; Watt and Breyer-Brandwijk, 1962). Phytochemical investigation on the plant revealed the presence of moringine and moringinine alkaloids in the root, pterygospermine alkaloid in the flower, fatty acids and fixed oils in the seed. Enzymes and bassorin substances have been found to be present in the exudates of this plant. In view of the common use of the plant as food and as medicine, the present study investigates the metal constituents of the leaf of the plant and their effects on some hematological parameters in order to recommend the continued usage or otherwise of the plant.

Experimental

Plant material. The plant material was collected from around Dambo village of Sabon Gari Local Government area, Kaduna State, Nigeria in the month of June, 2002. The herbarium keepers at the Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria identified it as *Moringa oleifera* via taxonomic means, where a voucher specimen (No.3317) was deposited. The leaves of the plant were handpicked, dried under shade and ground to powder form using pestle and mortar. It was then kept in plastic container under a dry condition for further use.

Elemental analysis. 0.061 g/cm^2 of the powered leaf sample of *Moringa oleifera* was used for the elemental analysis by energy dispersing X-ray fluorescence (EDXRT) transmission emission technique, at the Centre for Energy Research and Training, Ahmadu Bello University, Zaria, Nigeria using the method outlined by· Igile *et al.* (1994).

Experimental animals. Albino rats (Wistar strain) were housed in a cleaned environment at the animal facility, Department of Pharmacology and Clinical Pharmacy Ahmadu Bello University, Zaria. The animals were provided with food and water *ad libitum*. Prior to the commencement of the experiment the animals were acclimatized for two weeks and screened for diseases. 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.

Treatment of animals. Twenty-four Albino rats of both sexes weighing between 80 and 120 g were divided into four groups of six animals each and housed in separate cages. The animals were allowed free access to drinking water and standard livestock feed (Feed Master®, Grower Mash). The rats were kept under room temperature with day and night cycles. Group A rats were fed with 25% (w/w) modified diet, group B rats were fed with 50% (w/w) modified diet, group C rats were fed with 75% (w/w) modified diet, while group D rats served as the control and was fed with the standard diet only.

Formulation of animal diet. Three different concentrations of the diet were prepared using Feed Master® Grower Mash livestock feed as the standard feed. This was carried out by mixing 25% (w/w), 50% (w/w) and 75% (w/w) powder plant material with the standard diet.

Collection and processing of blood. The blood samples were obtained by nipping the tip of the tail and then squeezing to collect about 2-3 drops of the blood from each animal. The tail was first disinfected with 75% methanol and the tip cut with a sterilized sharp pair of scissors. Packed cell volume was estimated following collection of blood into heparinised capillary tube. Haemoglobin was

determined after diluting blood with Drabskin fluid leading to the conversion of haemoglobin to cyanomethemoglobin (Coles, 1974). Total protein was estimated by the Biuret method (Kaplan *et al.*, 1988). Red blood cell, white blood cell, neutrophil and lymphocyte counts were carried out as outlined by Barbara and Brown (1980).

Statistical analysis. Tests of significance difference between the means were carried out using analysis of variance (ANOVA) single factor.

Results

The result of the elemental analysis of the powdered leaf of *M. oleifera* revealed the presence of thirteen different elements with varied concentration, (table 1). The full blood count/differential was conducted on both the treated and the control animals as shown in table 2. Table 3 showed blood glucose concentration of the albino rats fed with different concentrations of modified diet that contained *Moringa oleifera* leaf.

Table 1: Result of the elemental analysis of powdered leaf of *M. oleifera* (in ppm)

Elements	Concentrations
S	3800 ± 300
K	7200 ± 600
Ca	12,900± 500
Cl	144 ± 15
Cr	126 ± 33
Mn	83 ± 22
Fe	453 ± 21
Br	6 ± 2
Rb	12 ±2
Sr	69 ± 3
Zr	11 ± 2
Mo	5 ± 1
Ar	10.9 ± LDL

LDL = Least detectable limit

Table 2: Full blood count/differential of the albino rats fed with the modified diet containing *M. oleifera* leaf

Amended diet	Animal Group	Mean values of PCV (%)	Mean value of Hb (gm/dl)	Mean value of TP mg/100ml	Mean value of RBC	Mean value of WBC ($x10^9$/L)	Mean value of Neut. (%)	Mean value of Lym. (%)
25%	Group A	52	17.2	8.2	7.0	12.9	21.8	77.4
50%	Group B	54.2	17.9	8.2	7.2	10.7	19.8	78.8
75%	Group C	47.6	15.8	7.3	7.1	10.9	24.6	74.2
Control	Group D	51.5	17.1	9.0	7.3	10.8	16.7	76.7

Table 3: Blood glucose concentration of the albino rats fed with the modified diet containing *M. oleifera* leaf

Animals	Amended diet (%)	Mean value (meg/dl)
Group A	25%	77.0
Group B	50%	76.7
Group C	75%	61.8 n
Group D	Control	74.0

Discussion

Poisoning with metal is one of the oldest forms of toxicities known to man. However, it is only recently that the mechanisms of toxicity have become known. The symptoms of poisoning are related to the amount of the metals ingested or absorbed and to the duration of exposure (Allan *et al,* 1995). The leaf of *M. oleifera* is consumed in large quantities because of its medicinal and nutritional values in northern Nigeria. This study described the evaluation of safety of the plant in relation to the elements present in the leaf.

Elemental analysis is normally carried out to determine the nature and types of elements in a powdered sample of crude drug or plant. Thirteen elements were detected in the powdered sample of the plant ranging from trace/essential element to heavy metals. The plant is rich in calcium ($1.29 \times 10^4 \pm$ 500ppm), potassium ($7.2 \times 10^3 \pm$ 600ppm), sulphur ($3.8 \times 10^{14} \pm$ 500ppm), iron ($4.53 \times 10^2 \pm$ 21ppm) and chlorine ($1.44 \times 10^2 \pm$ 15ppm). Theses are essential elements and are needed in minute quantities in the body for normal physiological functions. Arsenic (10.9 ± LDL), is a heavy metal, though it exists in its least detectable limit. Heavy metals are very toxic to the living tissues because they can chronically accumulate in various tissues and organs when ingested.

Albino rats (Wistar strains) fed with modified diets containing powdered leaves at concentration of 25% (w/w), 50% (w/w) and 75% (w/w) in the feed showed that, the mean blood glucose concentrations (mg/dl) were 76.7 and 61.8 for 50% and 75% groups respectively, while the control group was 74 The mean packed cell volume (%) were 54.2 and 47.6 for 50% and 70% groups respectively and the control was 51.5; the mean Haemoglobin (gm/dl) were 17.9 and 15.8 for 50% and 75% respectively, the control group was 17.1. The mean total protein (mg/100ml) were 8.2 (at 25% & 50%) and 7.3 (75%) while control was 9.0. The mean neutrophils (%) were 19.8 and 24.6 for 50% and 75% respectively and the control was 16.7, These results revealed that there was no significant difference between the treated and the control animals ($P<0.05$). The animals fed with 25% w/w modified diet containing powdered leaf of *Moringa oleifera* appeared healthier than all the three groups including the control. This observation lead us to suggest that, up to 25% w/w of *Moringa oleifera* leaf could be successfully utilized as substitute in the feed formulation for the rearing of albino rats to reduce costs.

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