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Assessing toxicity potential of dried *Moringa oleifera* leaves

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ABSTRACT

Moringa oleifera is a highly valued plant, distributed in many countries of the tropics and subtropics. It has an impressive range of medicinal uses with high nutritional value. Different parts of this plant contain a profile of important minerals. Results of the study revealed the presence of elements such as sodium (Na), magnesium (Mg), phosphorus (P), potassium (K), calcium (Ca), manganese (Mn), chromium (Cr), iron (Fe), copper (Cu) and zinc (Zn). Hazard quotients (HQ) and Hazard Index (HI) were calculated for samples from Otiakrom and Techiman. The HI values for both study areas were less than unity (HI<1) meaning no chronic risks are likely to occur when three tablespoonfuls of dried Moringa powder is consumed daily. It is recommended that intake of the powder should be based on advice from a nutritionist to avoid the risk of toxicity increasing in vulnerable groups including, children, pregnant women and the aged.

Introduction

Minerals are necessary for health and as such are part of all aspects of cellular function (Shenkin, 2004). They are involved in structural components. Example calcium and phosphorous are structural components in bone and acts as cofactors for enzyme activities. Minerals also form an integral part of enzyme or protein structure (metalloproteins). Minerals are essential for growth, development and maintenance of tissues and are also linked to the expression of genetic information, the effectiveness of immune system, the prevention of cell damage. In general, minerals increase resistance to many chronic and some infectious diseases. Minerals are nutritionally important components in food. They could be classified as essential or non essential elements. Essential elements are not synthesized internally and must be consumed from its environment. These include the major elements and minor elements. Major elements are those elements that are required in large quantities. The body requires more than hundred (100mg/day) of each major elements (Mader, 2006). The major elements are components of cells and body fluids and are structural components of cells (Mader, 2006). Examples of major elements include calcium, chloride, magnesium, phosphorous (present as phosphates), potassium, sodium.

Trace elements essential for life generally occur in the body in micrograms per gram of tissue and are usually required by humans in milligrams per day. Thus the body requires less than twenty (20mg/day) of each trace elements (Mader, 2006). These trace elements include copper, iron, manganese, zinc, selenium and iodine. The newer trace elements are ones that are possibly essential. Boron, chromium, manganese, nickel, tin, vanadium, molybdenum, arsenic, lithium, aluminium, strontium, cesium and silicon are regarded as new trace elements in the sense that they have only recently been considered essential in human diets. Non-essential components of foods can still have significant impact on health and can either be beneficial or toxic. These non essential elements are however frequently consumed and accumulated in living organisms, though are not required

Among the roles played by essential elements are growth and production of bones, teeth, hair, blood, nerves, skin, vitamins, enzymes and hormones. Essential elements also play a major role in nerve transmission, blood circulation, cellular integrity, energy production and muscle contraction. It is now well recognized that several trace elements are essential constituents of enzymes and play vital role in human metabolism. All the nutrient elements are primarily supplied through diet. The amount needed depends on age, sex, health status, geographical and climatic conditions (O_Dell&Sunde, 1997).

There exists a range of intake over which the supply of essential elements is adequate for the body. However, above and below this range, toxic and deficiency effects are observed respectively (Merian, 1991). As a result of this, it is essential to determine elemental contents of food items and to estimate their daily dietary intake. Essential elements can be systemic toxins with specific neurotoxic, nephrotoxic, fetotoxic and teratogenic effects. Essential elements can influence behavior by impairing immune, mental and neurological function, influencing neurotransmitter production and utilization and altering other metabolic processes in the body (Allesio, 1992). Uptake of elements from both the atmosphere, leaf surfaces and from soil through roots may account for the elevated levels of elements in plants.

A major source of human exposure to trace elements (as well as heavy metals) from the environment is from food

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(Goyer, 1991). Elements can also find their way into humans by direct absorption via air or drinking water. Other elements find their way into food either naturally or through anthropogenic activities such as agricultural practices, industrial emission and exhaust fumes. *Moringa* like most plants is a cheap source of essential trace metals. However, little attention has been given to their exact concentrations present in different preparations of the plants. Studies have shown that high intake of elements can lead to metal poisoning whereas low intake levels can lead to deficiency effects. This study was therefore conducted to investigate the toxicity potential in leaf powder of *Moringa oleifera*

Materials and methods

Sample collection and preparation

Leaves of *Moringa oleifera* were collected from ten different plants into clean polythene containers and transported to the laboratory. Samples were then washed with de-ionized water and divided into three portions for drying.

Description of drying methods used

The samples were divided into three portions and the drying methods; freeze drying, air drying and oven drying were employed to dry the various portions. A portion of the samples were air dried at (room temperature ie.25^0C±4^0C) for seven (7) days. A portion of the samples were freeze dried for 24 hrs using CHRist LMC-1 manufactured in Germany. The last portion of samples were Oven dried at (45^0C) for sixteen hours. Dried samples were ground to fine powder using an electronic laboratory blender. The pulverised samples were secured in zip-loc bags, well labelled and stored at 4ºC for analysis.

Results and Discussions

Mineral concentrations were determined by acid digestion of the dried and pulverized samples of moringa leaves using Milestone laboratory protocol (MLS, 1996-200). 6 mL of HNO_3 as well as 1mL of H_2O_2 were added to 0.50 g moringa sample, mixture was kept in a programmed microwave oven to achieve the desired digestion, digestate was allowed to cool followed by transfer into a 15 mL test tube where digested sample was made to the 10 mL mark with distilled water. The mineral concentrations were then measured using the fast sequential Atomic Absorption Spectrometer (AAS) technique (Varian AA240FS). Determination of the concentrations of minerals such as Na, Mg, P, K and Ca involved wet preparation of 5.0 g of moringa sample in dilutions using appropriate solvents, followed by leaching, filtration and centrifugation processes. Atomic absorption spectrometry analysis of Ca^{2+}, Mg^{2+}, Na^+ and K^+ were carried out using the flame Sherwood aspirator at 440 nm while the vis-uv spectrophotometer (Shimadzu Corp., Tokyo, Japan) was employed for the determination of PO_4^{3-} at 780 nm. The final concentrations of all analysed minerals were calculated using the following formula:

$$Concentration = \frac{(AAS\ reading \times Nominal\ volume)}{Initial\ sample\ weight}$$

The final phosphorus composition however was estimated from a standard calibration curve followed by the multiplication of the phosphate concentration by a common factor. Thus, P = $[PO_4^{3-}] \times 0.3261$.

Calculating hazard quotient and hazard index

Exposure assessment involves quantifying the estimated intake of contaminants by humans for each exposure pathway identified. The following assumptions were made for Moringa daily intake (MDI) of leaf powder: (i) ingestion is the pathway for Moringa intake by consumers (ii) a tablespoonful of Moringa leaf powder weighs 0.004 kg, thus a three tablespoonful daily dosage weighs 0.012 kg. Hazard quotients (HQ) and Hazard Index (HI) were calculated for the minerals whose concentrations were determined using the formula:

That is, $Harzard\ Quotient\ (HQ) = \frac{MDI}{RDI}$

But, $MDI = \frac{C \times CR \times EF \times ED \times 10^{-3}}{BW \times AT}$

Where, MDI = moringa leaf powder daily intake (0.012 kg by mineral content, mg/kg/day);
RDA= recommended daily allowance (mg/kg/day);
C = concentration of individual mineral in 3tablespoonful, 0.012 kg, of moringa leaf (mg/kg),
CR = contact rate adopted from spinach exposure (4.068 mg/day);
EF = exposure frequency (365 days/year);
ED = exposure duration (1year);
BW = body weight (70 kg) and;
AT = averaging time (70 years) and
10^{-3} = unit conversion factor.

The hazard quotient (HQ) assumes that there is a level of exposure (i.e., RfD) below which it is unlikely to cause adverse health effects, even for sensitive populations. If the HQ exceeds unity (a value of 1), there may be a concern for potential noncarcinogenic effects. To assess the overall potential for non-carcinogenic health effects posed by more than one chemical, the HQs calculated for each chemical are summed (assuming additivity of effects), and expressed as a Hazard Index (HI) (US EPA ,1989).

$$HI = \sum_{i=1}^{n} HQi$$

The Hazard Index (HI) = $HQ_{Ca} + HQ_{Mg} + HQ_P + HQ_K + HQ_{Mn} + HQ_{Na} + HQ_{Cu} + HQ_{Cr} + HQ_{Fe} + HQ_{Mn} + HQ_{A\ l} + HQ_{Cl} + HQ_V + HQ_{Zn}$
Where, HQ=DI/RDA

$$HI = \sum_{i=1}^{n} HQi$$

HI= 0.255
Where, HQ=DI/RDA

$$HI = \sum_{i=1}^{n} HQi$$

HI=0.214

Conclusion

In this work non-cancerous effect was estimated for adults using ingestion exposure. The additive effects of the mineral ions which were evaluated as hazard index (HI) from Hazard quotient (HQ) values revealed 0.214 and 0.255 for Otiakrom and Techiman respectively.

Based on this quantity the Hazard Index (HI) for both regions does not exceed unity.ie. (HI <1), hence it is assumed that no chronic risks are likely to occur when three tablespoonful of *Moringa* powder is consumed daily. The major elements (Ca, Mg, P, K, Na) contributed over 90% to the HI for both study areas. The most vulnerable groups in risk assessment studies are children, the aged and pregnant women. It is also likely that, the potential risk may worsen for vulnerable group. Sadly, the RDA for such categories is not readily available for conclusive evaluation. Also, Ghana lacks a comprehensive data on reports

which would serve as the biological indicators for characterizing the risk from food products consumed in the country.

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Table 1 Amounts of elements in moringa consumed by participants and hazard quotient for techiman

Element	C(mg/kg)	I(mg/kg/day)	RDA	HQ
Ca	141.5600	42.8949	1000	0.0429
Mg	243.9600	73.9259	400	0.1848
P	0.1427	0.0043	700	0.0009
K	100.8000	30.5449	4700	0.0065
Na	0.1337	0.0405	1500	0.0002
Cu	0.1334	0.0040	900	0.0009
Cr	0.0161	0.0049	35	0.0001
Fe	0.2951	0.0894	8	0.0112
Mn	0.0005	0.0001	23	0.0006
Al	0.0215	0.0065	6	0.0007
Br	0.1383	0.0419	5	0.0052
Cl	0.0107	0.0033	2300	0.0001
V	0.0148	0.0045	10	0.0025
Zn	0.0155	0.0043	11	0.0004

Table 2 Amounts of elements in moringa consumed by participants and hazard quotient for otiakrom

Element	C(mg/kg)	I(mg/kg/day)	RDA	HQ
Ca	83.800	25.394	1000	0.0254
Mg	232.440	70.435	400	0.1761
P	0.121	0.037	700	0.0001
K	6.780	2.055	4700	0.0004
Na	0.111	0.034	1500	0.00002
Cu	0.016	0.047	900	0.0001
Cr	0.021	0.006	35	0.0002
Fe	0.149	0.045	8	0.0057
Mn	0.001	0.004	23	0.0002
Al	0.012	0.004	6	0.0004
Br	0.116	0.035	5	0.0044
Cl	0.023	0.006	2300	<0.0001
V	0.014	0.001	10	0.0001
Zn	0.014	0.004	11	0.0004