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Toxicity potentials of the nutraceutical *Moringa oleifera* at supra-supplementation levels

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ABSTRACT

Moringa oleifera Lam. (order – *Moringales*, family – *Moringaceae* and genus – *Moringa*) is a well known nutraceutical used in the treatment of hypercholesterolemia and hyperglycemia, and also, as a nutritional supplementation. Its popularity use raises the question of possible toxicity at supra-supplementation levels. The objective of the study was to ascertain possible acute toxicity with supra-supplementation using Sprague-Dawley (S-D) rats. In experiment 1, human peripheral blood mononuclear cells were given graded doses of *Moringa oleifera* aqueous leaf extract to induce cytotoxicity. In experiment 2, two groups of rats received low and high dose (LD and HD, respectively) levels (1000 and 3000 mg/kg b.wt, respectively) *per o.s.* alongside negative and positive control rats (0.9% saline and 10 mg/mL *N*-ethyl-*N*-nitrosourea – administered *i.m.*, respectively). Each group consisted of five rats. Rats were killed after 48 h and the femur bone marrow aspirate examined for polychromatic micronucleated erythrocytes (PCEMN)/normochromatic micronucleated erythrocytes (NCEMN) ratios after Giemsa/Leishman staining. In experiment 3, control, LD and HD groups were established. The LD and HD extracts were administered *per o.s.* to the respective groups and observed for 14 days. Each group consisted of five rats. Blood was sampled after 48 h and 14 days and examined biochemically and haematologically for acute toxicity. Experiment 1 showed that *Moringa oleifera* was cytotoxic at 20 mg/mL. In experiment 2, PCEMN/NCEMN ratios were: negative control = 2.087; LD = 1.849; HD = 1.397; positive control = 1.257. Statistically, LD and HD ratios were significant ($p = 0.020$). Experiment 3 showed that hepatonephro-toxicity was nil with no abnormal haematology results. Genotoxicity results have hitherto not been shown. *Moringa oleifera* is genotoxic at supra-supplementation levels of 3000 mg/kg b.wt. However, intake is safe at levels $\leq$ 1000 mg/kg b.wt.

1. Introduction

Nutraceuticals have been used for health purposes for many years. The WHO estimates that about 5.6 billion people, representing 80% of the world's population, depend on medicinal plants as part of the repertoire of their primary health care needs (Gias, 1998). Vast knowledge of the use of these medicinal plants for various ailments is still prevalent in areas where the plants are of great importance.

Medicinal plants are used for a wide range of acute and chronic conditions (Diallo et al., 1999). In some cases a clear-cut line cannot be defined between an herb that is medicinal and one which is a dietary supplement. The medicinal and/or nutritional value, in most cases, lie in several chemicals within the plant of which the substance that has a direct action may not yet be fully elucidated. Furthermore, the synergy of the various compounds is arguably the

Abbreviations: ALT, alanine amino transferase; AST, aspartate amino transferase; AIN-93G, American Institute of Nutrition; ALB, albumin; ALP, alkaline phosphatise; ANOVA, analysis of variance; C, control; EDTA-2K, ethylenediamine-*N*,*N*,*N*′,*N*′-tetraacetic acid dipotassium; GAFCO, Ghana Agriculture Food Company; γ-GT, γ-glutamyltranspetidase; HCT, haematocrit; HD, high dose; HGB, haemoglobin; LD, low dose; LD_{50}, lethal dose; LYM %, lymphocytes percentage; LYM, lymphocyte count; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; MCV, mean corpuscular volume; MPV, mean platelet volume; NCEMN, normochromatic erythrocytes micro nucleated; PCEMN, polychromatic erythrocytes micro nucleated; PDW, platelet distribution width; P-LCR, platelet larger cell ratio; PLT, platelet; RBC, red blood cells; RDW-CV, coefficient of variation in red cell distribution width; RDW-SD, standard deviation in red cell distribution width; S-D, Sprague-Dawley; TP, total protein; WBC, white blood cells.

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mechanism for efficacy. Alkaloids, flavonoids, tannins and phenolic compounds are the most commonly isolated and extensively studied bioactive compounds (Edeoga et al., 2005). Plant-based food supplements are beginning to emerge and are labeled as nutraceutical products. A wide variety of these exist in tropical Africa and other parts of the world. One medicinal plant which has gained widespread popularity in recent times is *Moringa oleifera* Lam. (order – *Moringales*, family – *Moringaceae* and genus – *Moringa*).

Moringa oleifera is one of the most useful tropical trees with a variety of uses (Jahn, 1986; Adedapo et al., 2009). Different parts are used in the indigenous systems of human medicine for the treatment of a variety of ailments. The ethanol leaf extract of *Moringa oleifera* is used for hypertension (Siddiqui and Khan, 1968; Kirtikar and Basu, 1984; Nikkon et al., 2003). The leaves are used as hypocholesterolemic and hypoglycemic agents (Siddiqui and Khan, 1968; Ghasi et al., 2000; Dangi et al., 2002). Additionally, the leaves have been reported for its antitumour (Aruna and Sivaramakrishnan, 1990), antioxidant (Diallo et al., 2001; Sreelatha and Padma, 2009, 2010; Atawodi et al., 2010), radio-protective (Rao et al., 2001; Arora et al., 2005), anti-inflammatory/diuretic properties (Cáceres et al., 1991), antihepatotoxic (Ruckmani et al., 1998), antifertility (Prakash, 1988), antiurolithiatic (Karadi et al., 2006) and analgesic activities (Rao et al., 2003).

Moringa oleifera leaf powder is said to give a child the following recommended daily allowances: protein 42%, calcium 125%, magnesium 61%, potassium 41%, iron 71%, vitamin A 272%, and vitamin C 22%. Gram for gram, *Moringa oleifera* leaves contain seven times the vitamin C in oranges, four times the calcium in milk, four times the β-carotene in carrots, twice the protein in milk and three times the potassium in bananas (Ramachandran et al., 1980; Fuglie, 1999a,b). In Ghana and other parts of the world *Moringa oleifera* was used as a nutritional supplement and remained popular among the lower socio-economic class for more than twenty years. However, it is beginning to gain popularity in the entire society irrespective of one's socio-economic background and health status.

Despite the aforementioned nutraceutically beneficial properties, different parts of the plant have different pharmacological actions and toxicity profiles, which have not yet been completely elucidated (Chinmoy, 2007). Furthermore, international regulations relating to human health require that all new pharmaceutical and nutraceutical products are tested for their safety, and key to ensuring this is to conduct toxicity tests in appropriate *in vitro* and *in vivo* models (Robinson et al., 2008).

Toxicity studies on *Moringa oleifera* are scarce. The question therefore arises, are the high levels of vitamins and trace elements in *Moringa oleifera*, and the non-existence of standard doses necessarily safe for users who, without giving thought to the precarious consequences of "over-supplementation", consider it a nutraceutical. The aim of this study therefore was to determine whether supra-supplementation of *Moringa oleifera* poses any health risk.

2. Materials and methods

2.1. Plant material

Moringa oleifera leaves were collected from Accra in the Greater Accra region of Ghana in June, 2010. The plant was identified in its vernacular names by farmers in the locality and confirmed by the herbarium at the University of Ghana Botany Department to be the same as those previously authenticated. A sample was deposited and the voucher specimen number Voc. No. GC1010 documented.

2.2. Method of extraction

The leaves were air dried to attain a constant weight at room temperature and ground into powder. The powder (250 g) was boiled in 4.5 L of water under atmospheric pressure for 15 min. The solution was subsequently filtered. The filtrate was thereafter lyophilized using a freeze drying system, and a yield of 60.6 g freeze-dried sample recorded. The freeze-dried sample was stored in a cool dry place for the various experiments.

2.3. LDH cytotoxicity assay (Experiment 1)

2.3.1. Culture preparation

Lactate dehydrogenase (LDH) is a soluble enzyme located in the cytosol. The enzyme is released into the surrounding culture medium upon cell damage or lysis. These are processes that occur during apoptosis and necrosis. LDH activity in the culture medium can therefore be used as an indicator of cell membrane integrity, and thus a measure of cytotoxicity. Since the activity of intracellular LDH corresponds to the number of cells in the culture, quantification of LDH in cell lysates can be used as a measure of cell growth (Haslam et al., 2000; Wolterbeek and van der Meer, 2005).

Human peripheral blood mononuclear cells (PBMC) were seeded at a density of 10^5 cells/well in 120 μL of culture medium into the 96-well culture plate. Descending concentrations (80.0, 40.0, 20.0, 10.0, 5.0 mg/mL) of *Moringa oleifera* solution (80 μL each) were prepared under sterile conditions and added in duplicates to wells. Cells were then incubated for 48 h under the following conditions: 37 °C, 5% CO_2 and 90% humidity. After centrifugation, the supernatant was analyzed for the level of cytotoxicity using a Cayman (MI, USA) cytotoxicity test kit, according to the manufacturer's instructions.

2.4. Experimental animals

The protocol was reviewed and approved by the Institutional Animal Care and Use Committee of the Noguchi Memorial Institute for Medical Research (NMIMR) according to the Guidelines for Animal Experimentation. Ethical clearance number STC 1 (3)/2008-9 was subsequently issued.

Thirty-five male Sprague-Dawley (S-D) rats (weighing 150–200 g) were obtained from NMIMR and housed at the University of Ghana Medical School Animal Experimentation and Care Unit and treated humanely. During the acclimatization period clinical observations on the animals were conducted as well as body weight measurements, and the rats were found healthy. According to their body weights, rats were assigned into groups, including a control group, by the stratified random method. S-D rats were housed in metal cages with stainless steel tops in the animal care facility, where room temperature, humidity and ventilation were controlled. S-D rats were fed *ad libitum* a standard chow diet (AIN-93G formulation, obtained from GAFCO – Ghana). Rats were maintained at a 12-h light-cycle and prepared for various experiments. The rats were anesthetized and later euthanized. All visible organs and tissues were macroscopically examined and harvested after blood sampling by cardiac puncture.

2.5. Genotoxicity assays (micronucleus assay)

The mammalian *in vivo* micronucleus test was used for the detection of damage induced by the test substance to the chromosomes or the mitotic apparatus of erythroblasts by analysis of erythrocytes sampled in the bone marrow using rodents. Furthermore, the test was used to ascertain whether *Moringa oleifera* could cause cytogenetic damage resulting in the formation of micronuclei containing lagging chromosome fragments or whole chromosomes. When bone marrow erythroblasts develop into a polychromatic erythrocyte, the main nucleus extrudes and any micronucleus that have been formed may remain behind in the otherwise anucleated cytoplasm. Visualization of micronuclei is