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

George Awuku Asare[a,*], Ben Gyan[b], Kwasi Bugyei[c], Samuel Adjei[b], Razak Mahama[a], Phyllis Addo[b], Lydia Otu-Nyarko[a], Edwin Kwame Wiredu[a,d], Alexander Nyarko[b]

[a] University of Ghana School of Allied Health Sciences (SAHS), Korle Bu, Ghana
[b] Noguchi Memorial Institute for Medical Research (NMIMR), Legon, Ghana
[c] Department of Pharmacology, University of Ghana Medical School, Korle-Bu, Accra, Ghana
[d] Department of Pathology, University of Ghana Medical School, Korle-Bu, Ghana

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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 ≤ 1000 mg/kg b.wt.

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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.

* Corresponding author at: Chemical Pathology Unit, Department of Medical Laboratory Sciences, School of Allied Health Sciences, College of Health Sciences, University of Ghana, PO Box KB 143, Korle Bu, Accra, Ghana. Tel.: +233 244 627 456.
E-mail address: gasare@chs.edu.gh (G.A. Asare).

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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

facilitated in these cells because they lack the main nucleus. An increase in the frequency of micronucleated polychromatic erythrocytes in treated animals was an indication of induced chromosome damage.

2.5.1. Plant extract administration/assay procedure (Experiment 2)

Twenty male S-D rats were used for this assay. They were grouped as follows: five for the low dose (LD) group; five for the high dose (HD) group; five for the negative control group and five, the positive control. The LD_{50} of *Moringa oleifera* was previously determined to be ≥3000 mg/kg b.wt (unpublished data). Based on the LD_{50} value, test groups were gavaged with 1000 mg/kg b.wt (LD) and 3000 mg/kg b.wt (HD). The negative and positive control rats received 500 μL saline and *N*-ethyl-*N*-nitrosourea (10 mg/mL) *i.m.*, respectively. Rats were sacrificed after 48 h. Major organs such as the liver, lungs, heart, kidneys and spleen were harvested and immediately immersed in 10% buffered formalin (pH 7.4) for later histopathological examinations. For each rat, the femur bone was removed and the bone marrow gently flushed into a tube containing 0.5 mL of fetal calf serum (FCS).

2.5.2. Preparation of bone marrow slides

The FCS-cell suspension was centrifuged at 1000 rpm for 8 min (HETG, 1998; Zaizuhana et al., 2006) and the sediment resuspended in a drop of the FCS. Thin smears were prepared on slides and air-dried for 24 h.

2.5.3. Fixing and staining

Slides were fixed in absolute methanol for 3 min after which they were stained with a mixture of 12 mL Giemsa, 6 mL Leishman and 2 mL phosphate buffer (pH 5.8) for 5 min. Afterwards, the slides were rinsed with phosphate buffer and air-dried for coded "blind" analyses.

2.5.4. Micronuclei scoring

Each slide was examined for the presence of polychromatic micronucleated erythrocytes (PCEMN) and normochromatic micronucleated erythrocytes (NCEMN) per 1000 cells by two independent microscopists (HETG, 1998; Zaizuhana et al., 2006) and the PCEMN/NCEMN ratio determined.

2.6. Acute toxicity test (Experiment 3)

Five SD rats constituted a group. Three groups comprising a control group (C gp), low dose group (LD gp), and a high dose group (HD gp) were established. A low dose (LD) of 1000 mg/kg b.wt and a high dose (HD) of 3000 mg/kg b.wt extract were reconstituted with distilled water to give a homogenous solution. The route of administration was oral (gavage) in accordance with the main route of intake of *Moringa oleifera* decoction by humans. However, group 1, the control group (C gp) was gavaged with distilled water.

2.7. Clinical observations

The observation period was 14 days post-extract administration. Clinical signs of toxidromes (such as rising fur, draping, tremors, excitability, miosis, twitching, salivation, and morbidity) and mortality were observed. After 48 h, 2 mL of blood was sampled by cardiac puncture for haematological and biochemical parameters. Body weights were measured before dosing on the day of administration and weekly, thereafter.

2.8. Laboratory examinations

2.8.1. Haematological indices

Haematology examinations were done on the 15th day at necropsy. Blood samples were collected into EDTA-2K tubes for immediate analysis using the SYSMEX haematology autoanalyzer (Kobe, Japan). Reagents for the haematology autoanalyzer were obtained from STROMATOLYZER (WH, USA). Leukocyte count (WBC), erythrocyte count (RBC), haemoglobin concentration (HGB), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelet count (PLT) and differential leukocyte counts were determined.

2.8.2. Biochemical analyses

Biochemical examinations were performed using blood collected into plain tubes. Blood samples were centrifuged for 5 min at 3000 rpm. The following biochemical assays were performed using the SELECTRA JUNIOR Version 04 autoanalyzer (Vital Scientific, Spankeren, The Netherlands) for biochemical assays. Total bilirubin, conjugated bilirubin, aspartate amino transferase (AST), alanine amino transferase (ALT), total protein (TP), albumin (ALB), globulin, alkaline phosphatise (ALP), γ-glutamyltranspetidase (γ-GT), urea, creatinine, lactate dehydrogenase (LDH), creatine kinase-R (CK) and creatine kinase-MB (CK-MB) were performed.

2.9. Statistical analysis

The data were analyzed and expressed as mean ± S.E.M. for all groups. For quantitative data the Student's-test was used to test for significant differences between two variables.

Analysis of variance (ANOVA) was used to determine the existence of statistical significance between variables possibly with more than two outcomes and p values ≤ 0.05 were considered significant. The statistical analysis of the data was done using SPSS version 16 (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Clinical observation

Test animals at all dose levels showed no changes in behavior before and after the administration of an oral dose of the aqueous leaf extract of *Moringa*. Toxidromes were therefore recorded as negative.

3.2. Mortality

Mortality is the main criterion in assessing the acute toxicity (LD_{50}) of a drug. There was no mortality recorded even at the highest dose level of 3000 mg/kg b.wt.

3.3. Cytotoxic potential of Moringa oleifera on PBMCs

An increase in *Moringa oleifera* concentration from 5 to 10 mg/mL was capable of inducing the release of LDH by approximately 10 units, but reduced to 8.56 U/mL as the extract doubled to 20 mg/mL. However, further increases above 20 mg/mL also increased the total amount of LDH release proportionally (Fig. 1).

3.4. Genotoxicity assay

The results of the *in vivo* micronucleus assay are presented in Figs. 2 and 3. For the negative control, the amount of Polychromatic erythrocytes micronucleated (PCEMN) and normochromatic erythrocytes micronucleated (NCEMN) cells per 1000 cell count

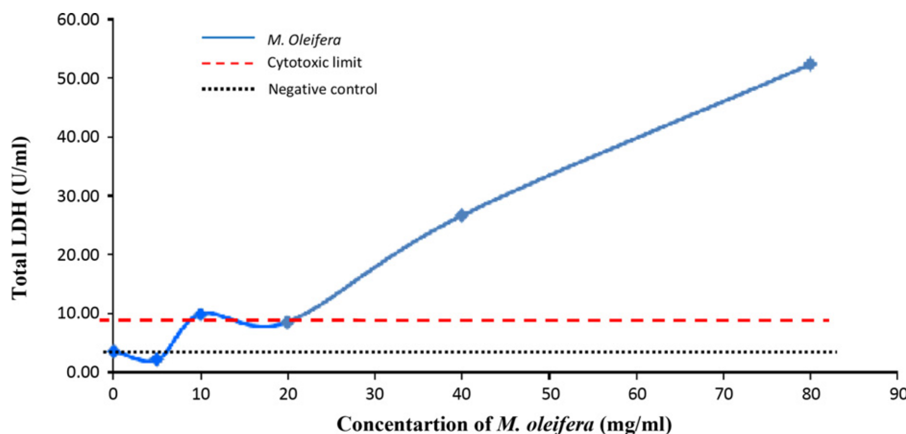

Fig. 1. (Experiment 1) Cytotoxicity of aqueous leaf extracts of *Moringa oleifera* on PBMCs as measured by the LDH cytotoxicity assay at the end of 48 h of exposure.

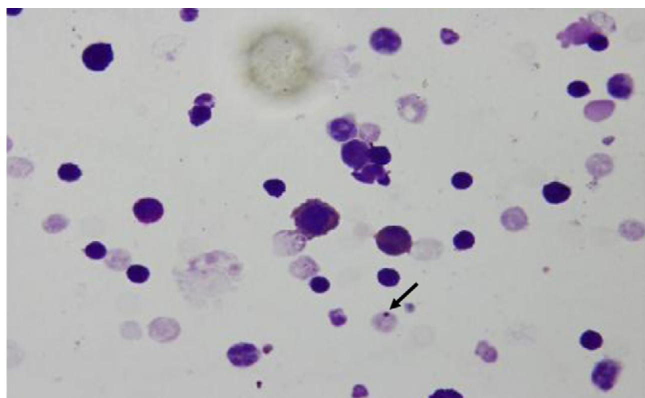

Fig. 2. (Experiment 2) This photomicrograph shows the thin film smear of femur bone marrow aspirate after staining with Giemsa/Leishman stain. The arrow shows a micronucleated erythrocyte (100×).

were 4.8 ± 1.2 and 2.3 ± 0.2 cells, respectively. The LD group PCEMN and NCEMN numbers were 9.8 ± 2.0 and 5.3 ± 1.8 cells, per 1000 cell count, respectively. PCEMN, for positive control and HD groups were 25.4 ± 3.5 and 20.2 ± 4.0 cells per 1000 cell count, respectively. Similarly, NCEMN for HD and positive control

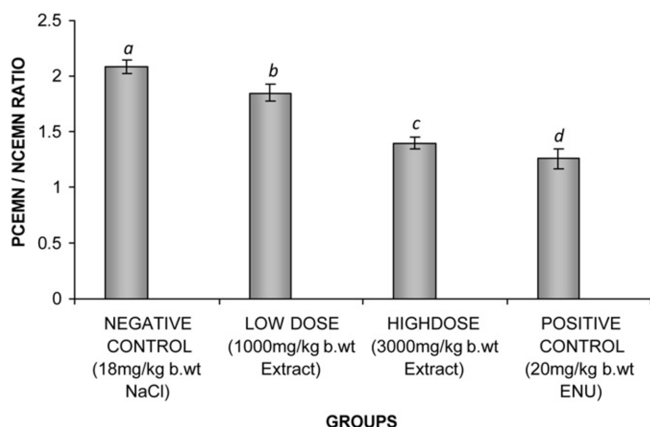

Fig. 3. (Experiment 3) Effect of low and high dose (1000 mg/kg b.wt and 3000 mg/kg b.wt aqueous leaf extract, respectively, *per o.s.*) of *Moringa oleifera* on male S-D rat femur bone marrow (*in vivo* micronucleus test) after 48 h of exposure. ENU and 0.9% normal saline were injected *i.m.* as positive and negative controls, respectively. Columns represent the PCEMN/NCEMN ratio. *a* vs. *b* ($p = 0.134$), *a* vs. *c* ($p = 0.013$), *a* vs. *d* ($p = 0.002$), *b* vs. *c* ($p = 0.020$), *b* vs. *d* ($p = 0.002$). Furthermore, the PCEMN/NCEMN decline from LD to HD is indicative of genotoxicity.

groups were 20.4 ± 0.8 and 14.0 ± 1.3 cells per 1000 cell count, respectively. Statistically, differences between PCEMN negative control and HD were significant ($p = 0.013$). Similarly, LD and HD PCEMN counts were statistically different ($p = 0.013$). Negative control and positive control values were also significant ($p = 0.002$). Furthermore, significant differences were observed in the NCEMN negative control and HD groups ($p = 0.002$), and the LD and HD groups ($p = 0.013$). Positive and negative control values were also significantly different ($p = 0.003$). PCEMN/NCEMN ratios were as follows: negative control = 2.087, LD = 1.849, HD = 1.442, positive control = 1.245. Statistically, LD and HD ratios were significantly different ($p = 0.020$).

3.5. Haematological

After 48 h the total WBC reduced from $7.97 \pm 1.61 \times 10^3$ to $5.14 \pm 0.52 \times 10^3$ for LD and to $6.84 \pm 1.12 \times 10^3$ for HD. These decreases were not significant. However, on day 15, total WBC increased from $7.00 \pm 0.70 \times 10^3$ to $11.14 \pm 1.56 \times 10^3$ (HD). Differences however, were still not significant (Table 2). From Table 1, RBC was slightly lower in the LD group and slightly higher in the HD group compared to the control within 48 h. A similar pattern was observed on day 15 (Table 2). Haemoglobin (HBG) remained virtually unchanged at 48 h when the Control (12.92 ± 0.28 g/dL) was compared to the HD group (12.88 ± 0.44 g/dL) (Table 1). However, HBG for the HD group increased to 13.34 ± 0.37 g/dL by day 15. Differences between the HD and control were however not significant. Slight insignificant changes were observed in HCT at 48 h and on day 15. MCV values were 60.0 ± 0.33 fL (C), 61.26 ± 0.43 fL (LD) and 60.06 ± 1.02 fL (HD) at 48 h (Table 1). On day 15, values remained relatively the same (Table 2). From Table 1, PLT counts dropped from $890.75 \pm 126.34 \times 10^3$ (C) to $703.00 \pm 95.14 \times 10^3$ (HD). A similar reduction was observed on day 15 where PLT dropped from $880.80 \pm 59.47 \times 10^3$ to $601.40 \pm 148.20 \times 10^3$ to $675.60 \pm 131.43 \times 10^3$ (C, LD, and HD, respectively) (Table 2). In both cases, statistical differences at 48 h and day 15 were not significant. Although lymphocyte (LYM) counts did not vary appreciably at 48 h, (Table 1) and on day 15, LYM for C and LD were in the region of $4.90 \pm 0.49 \times 10^3$ compared to $7.70 \pm 1.20 \times 10^3$ for the HD. Differences however, were not significant. SDW values at 48 h and on day 15 remained virtually the same for C, LD and HD groups. Similarly, virtually unchanged results were observed for platelet distribution width (PDW), mean platelet volume (MPV) and platelet large cell ratio (P-LCR) values at 48 h and on day 15. Therefore, all haematological values, both at 48 h (Table 1) and on day 15 (Table 2), were not statistically different.

Table 1
(Experiment 3). Haematology results after 48 h of oral gavage administration of 1000 (low dose) (LD) and 3000 mg/kg b.wt (high dose) (HD) of aqueous leaf extract of *Moringa oleifera* to S-D rats. No statistically significant differences were seen between the control group, the LD group and the HD group [WBC = white blood cells; RBC = red blood cells; HGB = haemoglobin; HCT = haematocrit; MCV = mean corpuscular volume; MCH = mean corpuscular haemoglobin; MCHC = mean corpuscular haemoglobin concentration; PLT = platelet; LYM% = lymphocytes percentage; LYM = lymphocyte count; RDW-SD = standard deviation in red cell distribution width; RDW-CV = coefficient of variation in red cell distribution width; PDW = platelet distribution width; MPV = mean platelet volume; P-LCR = platelet larger cell ratio].

Parameter	Control group	Low dose group (LD)	High dose group (HD)	*F*-value	*p*-Value
WBC × 10^3	7.97 ± 1.61	5.14 ± 0.52	6.84 ± 1.12	1.638	NS
RBC × 10^6	7.08 ± 0.19	7.04 ± 0.18	7.62 ± 0.41	1.291	NS
HBG (g/dL)	12.90 ± 0.21	11.86 ± 0.59	12.88 ± 0.44	1.243	NS
HCT (%)	43.87 ± 1.02	43.14 ± 1.36	45.64 ± 1.89	0.740	NS
MCV (fL)	62.00 ± 0.33	61.26 ± 0.43	60.06 ± 1.02	1.857	NS
MCH (pg)	17.48 ± 0.18	16.82 ± 0.46	17.00 ± 0.47	0.603	NS
MCHC (g/dL)	28.20 ± 0.21	27.46 ± 0.69	28.26 ± 0.34	0.848	NS
PLT × 10^3	890.75 ± 126.34	647.60 ± 156.10	703.00 ± 95.14	0.897	NS
LYM (%)	76.60 ± 2.19	77.70 ± 2.24	73.18 ± 4.44	0.547	NS
LYM × 10^3	6.13 ± 1.24	3.98 ± 0.35	5.08 ± 0.98	1.398	NS
SDW-SD (fL)	31.13 ± 0.39	31.38 ± 0.16	31.04 ± 0.66	0.154	NS
SDW-CV %	12.32 ± 0.35	12.56 ± 0.12	12.74 ± 0.54	0.273	NS
PDW (fL)	5.93 ± 0.17	6.18 ± 0.24	6.12 ± 0.09	0.527	NS
MPV (fL)	5.20 ± 0.07	5.32 ± 0.09	5.32 ± 0.04	0.764	NS
P-LCR (%)	1.30 ± 0.16	1.44 ± 0.19	1.28 ± 0.14	0.280	NS

NS = not significant.

3.6. Biochemical

The biochemical picture however was different. Urea was 11.9 ± 1.0 mmol/L (C) and 9.2 ± 1.5 mmol/L (HD) at 48 h with significant differences ($p = 0.019$) (Table 3). These differences persisted till the 15th day when differences between C (11.1 ± 0.8 mmol/L) and HD (9.7 ± 1.1 mmol/L) were significant ($p = 0.04$) (Table 3). Furthermore, differences between C and LD (12.6 ± 1.2 mmol/L) were significant ($p = 0.05$). Finally, differences between LD and HD were very significant ($p = 0.004$). Albumin concentration decreased from 34.4 ± 1.3 mmol/L (C) to 31.0 ± 1.58 mmol/L (HD) ($p = 0.011$) (Table 3). Similar differences in albumin levels were observed on day 15 where albumin concentration was significantly lower in the HD group compared to C ($p = 0.004$). From Table 4, electrolytes were significantly affected. Sodium was 145.6 ± 2.3 mmol/L (C) and 142.2 ± 1.9 mmol/L (HD) ($p = 0.035$). A reduction in chloride was significant between C and LD; C and HD ($p = 0.13$, $p = 0.005$, respectively). Liver function tests encompassing total bilirubin, conjugated bilirubin and unconjugated bilirubin, did not show statistical differences at 48 h and on day 15. Furthermore, liver enzymes such as AST, ALT, ALP and γ-GT did not show statistical differences throughout. Cardiac enzymes (CK-MB, CK and LDH) measured only on day 15 did not show statistical differences between C, LD and HD (Table 4).

4. Discussion

Medicinal plants have over the years been the lifeline of many indigenes who do have access to, or cannot afford pharmaceuticals. In recent times the shift from synthetic drugs to natural products considered a panacea to wellness is exerting in influence that is cutting across all socio-economic barriers. The inhibition to the widespread use of medicinal plants is gradually being eroded by the inability of orthodox drugs to cure long standing and common ailments such as malaria, diabetes and hypertension. Additionally, anecdotal evidence of efficacy of medicinal plants has boosted confidence in the use of such products. The coupling agent of acceleration to the use of medicinal plants, herbal plants, natural products etc., is the emergence of nutraceutical plants. These are natural plant products that link nutritional plants and medicinal plants. One 21st century plant that has taken center-stage and promises to be the catalyst towards the achievement of the millennium development goals of reducing poverty, disease and malnutrition is *Moringa oleifera*. The present

Table 2
(Experiment 3). Haematology results on day 15 of oral gavage administration of 1000 (low dose) (LD) and 3000 mg/kg b.wt (high dose) (HD) of aqueous leaf extract of *Moringa oleifera* to S-D rats. No statistically significant differences were seen between the control group, the LD group and the HD group [WBC = white blood cells; RBC = red blood cells; HGB = haemoglobin; HCT = haematocrit; MCV = mean corpuscular volume; MCH = mean corpuscular haemoglobin; MCHC = mean corpuscular haemoglobin concentration; PLT = platelet; LYM% = lymphocytes percentage; LYM = lymphocyte count; RDW-SD = standard deviation in red cell distribution width; RDW-CV = coefficient of variation in red cell distribution width; PDW = platelet distribution width; MPV = mean platelet volume; P-LCR = platelet larger cell ratio].

Parameter	Control group	Low dose group (LD)	High dose group (HD)	*F*-value	*p*-Value
WBC × 10^3	7.00 ± 0.77	7.38 ± 2.03	11.14 ± 1.56	2.202	NS
RBC × 10^6	7.61 ± 0.20	7.33 ± 0.39	7.61 ± 0.24	0.312	NS
HBG (g/dL)	12.92 ± 0.28	12.60 ± 0.68	13.34 ± 0.37	0.612	NS
HCT (%)	45.32 ± 0.99	44.56 ± 2.20	46.22 ± 1.35	0.271	NS
MCV (fL)	59.54 ± 0.71	60.86 ± 0.47	60.70 ± 0.56	1.487	NS
MCH (pg)	16.98 ± 0.17	17.18 ± 0.15	17.52 ± 0.16	3.013	NS
MCHC (g/dL)	28.52 ± 0.29	28.24 ± 0.36	28.88 ± 0.15	1.285	NS
PLT × 10^3	880.80 ± 59.47	601.40 ± 148.20	675.60 ± 131.43	1.469	NS
LYM (%)	70.26 ± 3.10	70.24 ± 3.97	68.88 ± 2.07	0.063	NS
LYM × 10^3	4.90 ± 0.49	4.98 ± 1.30	7.70 ± 1.20	2.262	NS
SDW-SD (fL)	28.96 ± 0.11	30.36 ± 0.62	29.94 ± 0.49	2.409	NS
SDW-CV %	11.66 ± 0.20	11.96 ± 0.49	11.70 ± 0.21	0.246	NS
PDW (fL)	6.34 ± 0.11	6.38 ± 0.09	6.40 ± 0.10	0.087	NS
MPV (fL)	5.52 ± 0.08	5.52 ± 0.05	5.52 ± 0.07	0.000	NS
P-LCR (%)	1.74 ± 0.21	2.10 ± 0.06	1.90 ± 0.15	1.344	NS

NS = not significant.

Table 3
(Experiment 3). Biochemical results after 48 h of 1000 (low dose) (LD) and 3000 mg/kg b.wt (high dose) (HD) of aqueous leaf extract of *Moringa oleifera* by oral gavage to S-D rats.

Test	Control groupMean ± SD	Low dose groupMean ± SD	High dose groupMean ± SD	*p*-Value
Urea (mmol/L)	11.9 ± 1.0[b]	13.1 ± 1.2	9.2 ± 1.5[b]	0.019[b]
Creatinine (mmol/L)	65.7 ± 5.2	62.3 ± 3.8	61.5 ± 8.4	NS
Potassium (mmol/L)	7.2 ± 2.5	5.8 ± 0.3	8.3 ± 3.3	NS
Sodium (mmol/L)	141.5 ± 4.4	143.0 ± 3.7	137.8 ± 4.1	NS
Chloride (mmol/L)	97.6 ± 2.6	99.4 ± 1.9	99.0 ± 4.4	NS
Total protein (g/L)	54.4 ± 1.4	53.8 ± 3.2	56.2 ± 8.3	NS
Albumin (g/L)	34.4 ± 1.3[b]	33.1 ± 1.5	31.0 ± 1.58[b]	0.011[b]
Globulin (g/L)	20.0 ± 1.5	20.7 ± 2.4	25.2 ± 8.4	NS
Direct bilirubin (μmol/L)	3.7 ± 3.4	3.6 ± 2.0	8.9 ± 6.1	NS
Indirect bilirubin (μmol/L)	2.5 ± 0.6	3.8 ± 1.3	7.0 ± 3.6	NS
Total bilirubin (μmol/L)	6.1 ± 3.7	7.4 ± 3.2	16.0 ± 9.7	NS
Gamma GT (U/L)	1.08 ± 0.09	1.0 ± 0.06	3.9 ± 3.8	NS
ALT (U/L)	91.2 ± 3.9	82.7 ± 12.1	98.9 ± 45.1	NS
AST (U/L)	135.7 ± 24.2	129.2 ± 25.2	245.1 ± 111.7	NS
ALP (U/L)	556.2 ± 109.5	548.7 ± 120.4	396.4 ± 109.8	NS

b signifies significant statistical differences when the HD group is compared to the control group. NS signifies no statistical differences when the test group is compared to the control group and when the LD group is compared to the HD group.

body of literature focuses largely on the medicinal properties of *Moringa oleifera* such as its antimicrobial, antihypertensive, anti-inflammatory, antidiabetic and anti-cancer activities (Cáceres et al., 1991; Dangi et al., 2002; Nikkon et al., 2003; Chinmoy, 2007; Jabeen et al., 2008). However, most studies done so far do not go beyond *in vitro* and *in vivo* animal studies. The drive to introduce *Moringa oleifera* as a drug is still in abeyance. With literature on clinical trials conspicuously absent, *Moringa oleifera* is largely used as a nutraceutical supplement. Hailed as the "The Miracle Tree" or "The Tree of Life", with an eruptious societal acceptance, one can foresee the over-ambitiousness of supra-supplementation with the notion that "more is better". In this study, supra-supplementation of 3000 mg/kg b.wt aqueous leaf extract did not elicit hepatorenal toxicity after an acute *in vivo* exposure. Similarly, nothing adverse was found haematologically. Phenomenally, *Moringa oleifera* significantly reduced serum chloride levels both at low dose and high dose. Furthermore, sodium was significantly reduced upon the administration of the high dose extract. This reduction seems to suggest that *Moringa oleifera* reduces the effect of aldosterone on sodium reabsorbtion from the renal tubules and thus collaborate evidence of its hypotensive and diuretic properties (Morton, 1991; Caceres et al., 1992; Faizi et al., 1998).

Acute administration at 3000 mg/kg b.wt significantly affected urea levels by reducing serum urea concentration. This reduction was similarly observed by Adedapo et al. (2009) when 1600 mg/kg b.wt was administered orally for 21 days. Conversely, significant urea increases were observed with the 1000 mg/kg b.wt administration. Although renal dysfunction may appear suggestive, serum creatinine level was not affected at 48 h and on day 15, in both LD and HD groups. The increase in urea is therefore not pathological but physiological due to the high level of nitrogenous compounds, especially protein in *Moringa oleifera*. These results are therefore dissimilar to those of Mazumder et al. (1999) who reported that weekly moderate-to-high dose (>46 mg/kg b.wt crude extract) of *Moringa oleifera* methanol root extract adversely affected kidney function in mice.

In this study albumin reduction was observed to be significant at 48 h with the 3000 mg/kg b.wt dose administration ($p < 0.011$). This reduction persisted, proving to be highly significant by day 15 ($p < 0.004$). Similarly, Adedapo et al. (2009) observed significant albumin reduction at a higher dose of 1600 mg/kg b.wt compared to the lower dose of 400 mg/kg b.wt. Reduced albumin caused a reduction in total protein. Furthermore, the reduction of serum albumin levels may be an indication that toxicants such as isothiocyanate and glycoside cyanides that are present in *Moringa oleifera*

Table 4
(Experiment 3). Biochemical results on day 15 of 1000 (low dose) (LD) and 3000 mg/kg b.wt (high dose) (HD) of aqueous leaf extract of *Moringa oleifera* by oral gavage to S-D rats.

Test	Control groupMean ± SD	Low dose groupMean ± SD	High dose groupMean ± SD	*p*-Value
Urea (mmol/L)	11.1 ± 0.8[a,b]	12.6 ± 1.2[a,c]	9.7 ± 1.1[b,c]	0.04[a]/0.05[b]/0.004[c]
Creatinine (mmol/L)	61.0 ± 9.6	75.4 ± 18.2	72.3 ± 9.3	NS
Potassium (mmol/L)	6.1 ± 2.8	5.6 ± 1.5	6.0 ± 2.5	NS
Sodium (mmol/L)	145.6 ± 2.3[b]	143.8 ± 3.7	142.2 ± 1.9[b]	0.035[b]
Chloride (mmol/L)	100.2 ± 1.5[a,b]	94.2 ± 4.0[a]	96.6 ± 1.4[b]	0.013[a]/0.005[b]
Total protein (g/L)	56.2 ± 1.8	60.2 ± 5.8	56.6 ± 8.1	NS
Albumin (g/L)	33.5 ± 1.2[b]	31.5 ± 1.9	30.5 ± 1.2[b]	0.004[b]
Globulin (g/L)	22.7 ± 1.1	28.7 ± 7.5	26.2 ± 7.1	NS
Direct bilirubin (μmol/L)	4.0 ± 3.4	5.7 ± 1.1	4.3 ± 1.7	NS
Indirect bilirubin (μmol/L)	2.5 ± 2.3	2.4 ± 2.1	2.2 ± 0.7	NS
Total bilirubin (μmol/L)	6.5 ± 5.7	8.1 ± 2.4	6.5 ± 2.4	NS
Gamma GT (U/L)	9.0 ± 1.6	9.2 ± 1.4	9.8 ± 0.4	NS
ALT (U/L)	82.8 ± 14.2	88.9 ± 15.3	86.1 ± 24.4	NS
AST (U/L)	162.7 ± 78.1	152.2 ± 68.0	159.5 ± 37.9	NS
ALP (U/L)	367.0 ± 56.7	465.7 ± 88.9	414.3 ± 108.2	NS
CK-MB	690.3 ± 184.5	608.7 ± 60.5	715.2 ± 25.6	NS
CK	749.0 ± 228.4	689.8 ± 121.1	875.6 ± 103.3	NS
LDH	1416.4 ± 89.2	1425.0 ± 97.1	1313.2 ± 174.8	NS

a signifies significant statistical differences between the LD group and the control group. *b* signifies statistical differences between the control group and the HD group. *c* signifies significant differences between the LD group and the HD group. NS signifies no statistical differences when the test group is compared to the control group and when the LD group is compared to the HD group.

may cause stress-mediated mobilization of protein to mitigate the adverse condition elicited (Das and Mukherjee, 2000). This is one of the mechanisms employed to promote biotransformation and excretion of toxicants.

The results of this study showed that levels ≥ 20 mg/mL of *Moringa oleifera* was cytotoxic since the release of total LDH after 20 mg/mL of *Moringa oleifera* increased unabatedly as shown in Fig. 1. These findings agree to some extent with the findings of Monera et al. (2008). In that study, the aqueous leaf extract of *Moringa oleifera* was found to be cytotoxic at a lower concentration (6 mg/mL) using HepG2 human hepatocellular carcinoma cells. Furthermore, even much lower levels of cytotoxicity (0.2 mg/mL) were observed by Pavathy and Umamaheshwari (2007) using *Moringa oleifera* aqueous leaf extract. Differences could result from the extraction and purification processes of the leaf, types of cytotoxicity assays used, types of cells used for culture and even differences in geographical locations of the plant, since soil constituents differ. Mekonnen et al. (2005) investigated cytotoxicity of both ethanol and aqueous extracts of *Moringa stenopetala* leaves at a level of 0.5 mg/mL using Hepa1c1c7 cells. Results obtained in that study indicated that the ethanol leaf extract was cytotoxic at 0.5 mg/mL, while the aqueous leaf extract was not. Care must therefore be given in interpreting cytotoxicity results, since the cells may not only be in a serum-free medium for longer periods, but may also be covered with insoluble particles. These conditions on their own could induce stress and enhance cytotoxicity. Thus, we restricted our results to aqua soluble forms of *Moringa oleifera* leave extracts and an incubation time of 48 h. Teratogenicity of 175 mg/kg dry starting material has been documented in Ayurvedic and Unani drug encyclopedias of Lucknow and Farrukhabad (Uttar Pradesh) dating from March 1987 to July 1987 (Nath et al., 1992).

The most important finding of this study demonstrates that *Moringa oleifera* possess genotoxicity at a high dose (3000 mg/kg b.wt) level by the significant presence of micronucleated polychromatic erythrocytes (Figs. 2 and 3). To the best of our knowledge this is the first of such documentation with regard to *Moringa oleifera*.

Nutraceuticals may therefore be genotoxic depending on the levels consumed. Blackcurrant seeds, for example, are a tocopherol-rich by-product of fruit processing, without any specific commercial value. In a human intervention study, the physiological impact of blackcurrant seed press residue was examined after its incorporation into food and found to demonstrate genotoxicity through an oxidative stress/damage mechanism (Helbig et al., 2009). Similarly, selenium at nutritional levels has been shown to have numerous anti-carcinogenic or preventative effects. Because of the numerous reported advantages, many individuals are supplementing their diets with amounts of selenium that are greater than the recommended daily allowance. Research however, has demonstrated that such supra-supplementation levels are potentially genotoxic (Shamberger, 1985). The need, therefore, for greater understanding between supra-supplementation and toxicity is necessary.

In conclusion, *Moringa oleifera* shows acute toxicity at supra-supplementation levels of ≥3000 mg/kg b.wt. The exact dose of commencement of genotoxicity is yet to be determined.

Conflict of interest

The authors declare that there is no conflict of interest.

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