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ACUTE AND SUBACUTE TOXICITIES OF DEFATTED ETHANOLIC EXTRACT OF MORINGA OLEIFERA SEED IN ALBINO RATS

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SUMMARY

Moringa oleifera seeds are widely accepted as a nutritional supplement. The seeds are consumed and are sold on the shelf of nature, herbal shops, pharmacy and supermarkets. They are consumed as herbal remedy for various diseases. This study was designed to evaluate the acute and sub-acute toxicity of defatted ethanolic extract of *Moringa oleifera* seed (DESMOL) in albino rats using standard methods. The extract at 400, 800, 1600mg/kg caused no significant changes ($P>0.05$) in haematological parameters such as PCV, RBC, Hb, MCV, MCH, WBC, lymphocyte and platelet counts. In contrast, the extract, at 800 and 1600mg/kg caused significant changes in the levels of total protein ($P<0.05$), total and conjugated bilirubin, ALT, AST, ALP and GGT ($P<0.05$). The results of the histopathology showed that the extract at doses above 400mg/kg caused kidney damage by inducing widespread necrosis of tubular epithelial cells. Findings from this study revealed that grave consequences attend the chronic consumption of *Moringa* seeds as this could lead to kidney damage.

Keywords: *Moringa oleifera*, Sub-chronic toxicity, Safety limits

Introduction

Moringa oleifera Lam (syn. *M. pterygosperma* Gaertn.) is a widely distributed and naturalized species of the monogeneric family *Moringaceae*. *Moringa oleifera*, native to the western and sub-Himalayan tracts, India, Pakistan, is now widely distributed in Africa and Arabia, Central America, North and South America and the Caribbean Islands (Qaiser, 1973). In some parts of the world *Moringa oleifera* is referred to as the 'drumstick tree' or the 'horse radish tree', whereas in others it is known locally by other names such as the kelor tree, shigru, Igi Iye, igbale, zogalle and by several other names in the different communities of the world where the tree is found (Dhar and Gupta, 1982; Kadashi, 2008). This multipurpose tree has an impressive profile of high nutritional value, medicinal uses, and compelling water purifying attributes (Anwar *et al*, 2007, Suarez *et al*, 2003). Various parts of the plant such as the flower, seed, immature pod, stem bark, leaf and root have been reported to possess anti-oxidant, anticancer, antimicrobial, anti-ulcer, anti-hepatotoxic, antihypertensive, anti-hyperlipidaemic, antidiabetic, antispasmodic, antiepileptic, antipyretic, anti-inflammatory and analgesic properties (Songpol *et al*., 2012, Anwar and Rashid 2007; Fahey, 2005; Goyal *et al*., 2007)

Moringa oleifera is an important food commodity which has had enormous attention as the 'natural nutrition of the tropics', the leaves, fruit, flowers and immature pods of this tree are used as highly nutritive vegetable in many countries. Various parts of the plant have been reported to be rich sources of natural antioxidants, â-carotene, vitamin C, tocopherol, and to also contain proteins (Atawodi *et al*., 2010). High levels of iron, calcium, phosphorus, potassium, copper, riboflavin, folic acid, pyridoxine and essential amino acids such as methionine, cysteine, tryptophan ,lysine from the seed of this plant make it an ideal dietary supplement (Asiedu-Gyekye *et al* ., 2014; Anwar et al, 2007).

Increase in the consumption of herbal remedies worldwide has been stimulated by several factors, including the notion that all herbal products are safe and effective. Concerns over long-term toxicity in man are exacerbated by the practice of self-medication and uncontrolled *Moringa oleifera* use. Recent undocumented cases of hypoglycaemia, fainting spells and other toxicities have generated research interest on the safety and toxicity of *Moringa oleifera* seeds (Asiedu-Gyekye *et al*., 2014, Ojo *et al*., 2013, Songpol *et al*., 2012). The use of herbal

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remedies from leaves, seeds, flowers and roots of plants or extracts require precautions to ensure that these are not only efficacious but safe. Therefore the main objective of this study was to evaluate the toxicity and safety of *Moringa oleifera* seed extract using white albino rat model.

Materials and Methods

Plant Materials

Moringa oleifera seed was bought from Avuco farms company Ltd. Kaduna, Kaduna State. The plant materials was identified and authenticated by Professor F.A Oladele of the Plant Biology Department, University of Ilorin. The plant was then deposited at University of Ilorin herbarium where a voucher specimen UIH011/1011 was assigned. The seed shell was removed and the seed was crushed using clean non-ferrous crushing material. The crushed seed was quartered to obtain representative samples of 400g used for the safety evaluation study.

Seed Extraction

Moringa oleifera seed was extracted by the methods described by Pavial *et al.*, (1982) and Harbone (1998). One hundred grams (100g) of the crushed seeds of *Moringa oleifera* was defatted by macerating with 1L of Petroleum ether (BDH) at room temperature and extracted for 72h with intermittent shaking. The solution was filtered and the defatted seed was then re-extracted with absolute ethanol (BDH), filtered, concentrated using a rotary evaporator and freeze-dried. Different concentrations of the seed extract (400, 800, 1600 and 2000mg/ml) were prepared and used for the toxicity assay.

Animals

Twenty five albino rats weighing between 190 and 260g were obtained from the Animal Facility Center, of the Obafemi Awolowo University, Ile-Ife, Nigeria. The animals were acclimated to housing conditions for at least one week prior to commencement of the experiment. Animals were housed singly at an ambient temperature of 25°C, and 12 hours light, 12h dark cycle. Water and food were provided *ad libitum* to the animals.

Acute Toxicity Test

Acute toxicity study of ethanolic extract of *Moringa oleifera* seed (DESMOL) for safety evaluation was carried out by standard methods (Lorke ,1983 and Adedapo *et al.*, 2009). Twenty-Five albino rats were fasted for 16 hours , and randomly assigned into 5 groups of five rats each (n=5). Graded doses of the seed extract (400, 800, 1600 and 2000 mg/kg) were orally administered to the rats in each of the test groups while the control group received no extract but was treated with orally administered distilled water (3ml/kg p.o). All the animals were allowed free access to food and water. They were initially observed for 24h for any mortality and subsequently for 14 days for signs of acute toxicity.

Sub-acute Toxicology Study

Toxicity study of *Moringa oleifera* seed extract was carried out using a modified method of Cruz *et al.*, (2006). Albino rats were randomly divided into four groups of five rats each and given oral administration of 400, 800 and 1600 mg/kg/day of DESMOL respectively for 21 days. Groups B, C and D were orally administered with 400, 800 and 1600mg/kg of DESMOL respectively. Group A (the control group) received no extract, only 3ml/kg of distilled water was administered orally. All the animals were allowed free access to food and water and at the end of the 21 days exposure period; the animals were sacrificed under diethyl ether anesthesia.

Blood samples were collected by cardiac puncture from the rats and dispensed into sample bottles containing Ethylene Diamine Tetra Acetic Acid (EDTA) for haematological assay or Lithium heparin for clinical chemistry assay. Haematologic parameters were assessed using the Sysmex Automated Hematology Analyzer (Sysmex Corp. Tokyo, Japan) using the method described by Dacie and Lewis (2006).

Analysis of the serum for aspartate transaminase (AST), plasma alanine transferase (ALT), alkaline Phosphatase (ALP) and gamma glutamyl transferase (GGT) was done using spectrophotometric method and the absorbance was read at 405nm. The serum was also assayed for total protein, albumin and bilirubin by colorimetric method, using kits from Agappe Diagnostics Limited, Kerala, India.

For histopathology examination, samples of the liver and kidney of all the animals were excised, weighed, and fixed in 10% formalin for at least 48hours and embedded in paraffin wax. 5mm thick sections were cut on a rotary microtome and stained with haematoxylin and eosin. Stained slides were examined by light microscopy, and photographed.

Statistical analysis

Statistical analyses of values were presented as mean ±SEM. Haematological and biochemical parameters were analyzed with the SPSS statistical software Version 20 (Product of Microsoft). ANOVA test was used for comparison of differences between individual treatment groups, and P value d" 0.05 was considered statistically significant.

RESULTS

Acute Toxicity

At 2000mg/kg mild toxicity was observed, including raised hair coat, dimmed eyes and dullness. No mortality was observed at 48 hours and 14 days post administration of the seed extract.

Sub-Acute Toxicity Study

Haematological parameters

The mean values and standard deviation of the various haematological parameters (PCV, HB, RBC, MCV, MCH, WBC, Lymphocyte and Platelet Counts) in the control and the treated groups are shown in Table I. No significance difference was observed in red blood cell (RBC) count, haemoglobin (Hb) concentration, pack cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), white blood cell count(WBC), lymphocyte and platelet counts in the treated rats that received varied concentrations of DESMOL when compare to the control group($p>0.05$)

Table I: Effect of graded doses of Ethanolic extract of *Moringa oleifera* seeds on Haematological Parameters

Parameters	PCV (%)	RBC (µ/L)	Hb (g/dL)	MCV fL	MCH (g/dL)	PLT (µL)	WBC (i/L)	LYMPH (%)
Group 1 Control	42.3±2.9	7.2 ±0.8	13.2±0.9	62.8 ±5.6	29.2 ±0.4	873.6 ± 158.8	13.2 ±5.3	74.8 ±7.2
Group 2 (400mg/kg)	43.0 ±7.2	8.5 ± 1.4	14.9 ± 1.2	58.3 ±1.0	30.4 ± 3.6	795.2 ± 240.7	7.9± 2.6	72.0±6.0
Group 3 (800mg/kg)	45.9 ±8.4	7.7 ± 1.5	13.2± 1.8	57.9± 1.4	30.1 ± 1.5	824.8 ±703.6	10.2 ±4.8	70.8± 3.0
Group 4 (1600mg/kg)	46.7 ±2.5	7.9 ± 0.8	13.4± 0.5	58.2 ±0.7	29.2 ± 1.4	617.4 ±347.3	7.9 ±1.4	73.9± 0.6
P-value	0.082	0.380	0.086	0.055	0.713	0.785	0.121	0.587

Biochemical parameters

The effects of graded doses of the seed extract on biochemical parameters are depicted in tables II and III. Table II shows the effect of graded doses of ethanolic extract of *Moringa oleifera* seed on liver enzymes. There was a significant decrease ($P<0.05$) in the serum levels of the hepatic enzymes (ALT, AST, ALP and GGT) at the various doses of the extract administered. The effects of graded doses of ethanolic extract of *Moringa oleifera* seed on serum total protein, albumin and bilirubin are depicted in Table III. There was a significant decrease ($p<0.05$) in the levels of total bilirubin and conjugated bilirubin essentially in all the groups as the doses of the extract increased. However, there were no significant difference ($p<0.05$) in the values total protein and albumin in the test group when compared with the controls.

Table II: Effect of graded doses of Ethanolic extract of *Moringa oleifera* seed on Serum Hepatic enzymes

Parameters	ALT(u/L)	AST(u/L)	ALP(u/L)	GGT(u/L)
Group 1 Control	134.2±77.1	235.8±48.6	386 ± 193.6	317 ± 160.6
Group 2 (400mg/kg)	131.0 ± 76.8	85.1 ± 67.9	434.3 ± 414.4	475.3 ± 160.6
Group 3 (800mg/kg)	91.7 ± 67.1	65.5 ± 32.8	361.9± 181.0	183.6 ± 69.8
Group 4 (1600mg/kg)	85.1 ± 17.9	45.8 ± 18.0	301.5 ± 165.2	217 ± 45.7
P-value	0.011	0.000	0.000	0.000

Discussion

The results of acute toxicity study of defatted ethanolic extract of *Moringa oleifera* seed (DESMOL) on albino rats showed no mortality at a dose range of 400 to 2000mg/kg DESMOL. However there was some evidence of acute toxicity such as starry hair coat, dimmed eyes and dullness at a dose the highest dose of 2000 mg/kg. This observation supports previous reported studies of Songpol *et al.*, (2012) and Adedapo *et al.*, (2009).

Histopathological examination of the livers and kidneys of rats subjected to sub-acute administration of *Moringa* seeds demonstrated deleterious adverse effects only on the kidneys. The histopathological examination of the organs showed widespread tubular necrosis of the kidney cells in the test animals. This observation is consistent with the reported work of Ojo *et al.*, (2013). Songpol *et al.*, (2012) observed that administration of 5.1-10 g/kg DESMOL caused lethality and mortality in mice, but did not cause significant histopathological lesions in the organs. Haematological findings in this study showed that the *Moringa oleifera* seed extract (DESMOL) did not produce significant effect on blood indices in the treated groups. The observed absence of any significant changes in these blood parameters could possibly indicate that the extract is relatively safe in the rats with no toxic effects on the haematological indices. This may also be an indication that the extract did not exert deleterious effects on the erythroid, myeloid and megakaryocytic progenitor cells in the bone marrow. Although some studies have reported either an increase or decrease in some or all of the blood indices (Osman *et al.*, 2012; Auwal *et al.*, 2013), our observation of little or no significant change in blood parameters in this study is in agreement with the findings of Ajibade *et al.*, (2012) where *Moringa oleifera* seed extract used at high doses of 400, 800 and 1600mg/kg produced no resultant negative effect on the haematological indices of the female Wistar rats. The study of Jahn (1988) also reported that the aqueous extract of the seed of *Moringa oleifera* did not induce any toxic effect in Wistar rats. At the three different doses of DESMOL used in this study, there was no significant difference ($P>0.05$) in the platelet counts of treated groups when compared to the control. This may also suggest that the blood coagulation factors were not significantly impaired at the doses used. However, Ajibade *et al.* (2012) recorded a reduction in platelet counts with increasing doses of seed extract of *Moringa Oleifera* with no visible bleeding in the animals. Further evaluation of the extract may therefore be necessary in future studies to ascertain the safety margin on haematologic parameters following prolonged administration.

Effects of the extract on biochemical parameters showed that the activities of the hepatic enzymes (ALT, AST, ALP, GGT and Bilirubin) reduced with increasing doses of the *Moringa* seed extract. The levels of liver enzymes serve as an instrument normally used to assess the integrity of the liver. The inhibitory effect of the seed extract on both the total and conjugated bilirubin levels may suggest an inhibition of the excretory function of the liver. The histological sections of the liver however, showed no deleterious morphological effect of the extracts on the liver.

Findings from this study do not support chronic, indiscriminate use of *Moringa oleifera* seeds in humans. Our results have revealed that grave consequences may attend the chronic consumption/use of *Moringa* seeds, as this could lead to damage to the kidneys.

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