Journal of Medicinal Plants Studies
www.PlantsJournal.com

ISSN (E): 2320-3862
ISSN (P): 2394-0530
NAAS Rating 2017: 3.53
JMPS 2017; 5(5): 180-185

Received: 23-07-2017
Accepted: 24-08-2017

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Acute toxicity of *Moringa oleifera* leaf powder in rats

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Abstract
Moringa oleifera is widely used as a source of food and in traditional medicine for the treatment of a variety of diseases. Since the dried powder is consumed by people almost daily it is important to evaluate the toxicity and safety in recognized animal models used for allopathic medicines. Sprague Dawley rats were given up to 2000mg/kg of the dried powder according to the acute Toxic class method described in the OECD Guideline 423. Animals were observed every hour for the first four hours and thereafter daily for 14 days for the animals in step1. The observation period for the step 2 animals were 7 days because no adverse reaction were seen in step 1 animals. This study indicated that oral administration of *Moringa oleifera* dried leaf powder up to 2000mg/kg showed no changes in clinical signs or gross pathology and that the LD_{50} was greater than 2000mg/kg.

Keywords: *Moringa oleifera*, acute toxicity, pathology, clinical signs, rats

Introduction
Moringa oleifera is widely used as a source of food and has been used for centuries in traditional medicine for the treatment of skin diseases, respiratory illnesses, ear and dental infections, hypertension, diabetes, cancer treatment, water purification and as nutrient supplementation (Anwar *et al*., 2007; Fahey, 2005) [2]. Some studies showing the potential therapeutic benefits in humans are summarized below.
Tété–Bénissan *et al*. 2013) [16], evaluated the mineral composition of "Togolese ecotype" of *Moringa oleifera* leaves and its effect on anthropometric parameters, atherogenic lipids and glycaemia during nutritional recovery in HIV negative and HIV positive malnourished patients in Togo after daily use of the leaves powder. The study reported significant decrease of serum levels of total cholesterol, triglycerides, LDL-C correlated with significant increase in HDL-C. These results suggest that *Moringa oleifera* leaves has potential hypolipidemic, hypocholesterolemic properties which induced a decrease of atherogenic lipids. A reduction of the glycaemia values among HIV positive and HIV negative patients was also reported
Similarly, (Rajanandh *et al*., 2012) evaluated the leaves of *Moringa oleifera* for its hypolipidemic. antioxidant, anticoagulant, platelet antiaggregatory and anti-inflammatory activity in experimental animals. The results reported the therapeutic potential of the hydroalcoholic extract of *Moringa oleifera* against vascular damage and atherogenesis that leads to various types of cardiovascular complications. Furthermore, the study suggests that *Moringa oleifera* can be used by patients with coronary artery disease along with their regular medicine.
In six type 2 diabetic subjects, 50 g of a *M. oleifera* leaf powder was include in a standard meal and it was found that on a one-time basis it decreased blood glucose levels by 21% (William *et al*., 1993) [17].
Kumari (2010) [8] administered to 46 type 2 diabetic subjects with 8 g of powdered *M. oleifera* leaf in a tablet form. At the end of the study, in the treated subjects, fasting blood and postprandial blood glucose levels were 28% and 26% lower, respectively. Additionally, total cholesterol, triglycerides, Low density lipoprotein and very low density lipoprotein were 14%, 14%, 29%, and 15% lower relative to the control group. Nambiar *et al*. (2010) [11] administered a lower amount of 4.6 g of a leaf powder in a tablet to 35 type 2 diabetic persons daily for 50 days... Relative to the control group, the diabetic subjects showed a 1.6% decrease in total plasma cholesterol and a 6.3% increase in HDL.
Ghiridhari *et al*. (2011) [7] administered two *M. oleifera* leaf powder tablets (doses not specified) to 60

Type 2 diabetic subjects per day or placebo for up to 3 months... At the end of 3 month period, postprandial blood glucose had decreased by 29% relative to the control group, while hemoglobin A1C, decreased by 0.4%.

Monera and Maponga, (2012) [10] conducted a cross-sectional survey to determine the prevalence and patterns of *Moringa oleifera* use by HIV positive people in Harare, Zimbabwe. The study established evidence of its use as a nutritional supplementation among HIV positive patients as a large percentage of the study participants that have commenced antiretroviral therapy consume *Moringa oleifera*. The study also reported that friends or relatives were the most common source of a recommendation for use of *Moringa oleifera* to the HIV patients. Also in Oyo State in Nigeria (Osewa *et al*., 2013) reported a study to determine the perception of rural dwellers on the nutritional and medicinal values of *Moringa oleifera*. The study reported that the health benefit of Moringa is significant. Majority of the respondents are aware that Moringa leaves has highly protein content and rich in vitamins and minerals. The respondents had access to information through friends or relatives on a regular basis on the utilization of Moringa. The leaves are either taken fresh, dried and cooked. The study also reported that Moringa does not have any religious taboos while its acceptability cuts across both religious and cultural beliefs.

In a study of 30 postmenopausal women, Kushwaha *et al*. (2012) [9] reported that those who were given 7 g of *M. oleifera* leaf powder daily with for a period of 3 months showed significant increases in serum glutathione peroxidase (18.0%), superoxide dismutase (10.4%), and ascorbic acid (44.4%), with decreases in malondialdehyde (16.3%; lipid peroxidation), as well as a significant decrease in fasting blood glucose levels (13.5%) and w an increase in hemoglobin (17.5%).

These studies suggest that whole leaf powders of *M. oleifera* given orally shows marked anti-hyperglycemic, anti-dyslipidemic, and antioxidant effects in human subjects and is also consumed by people living with HIV and AIDS without any observable adverse effects.

If *Moringa oleifera* is consumed by people daily on a long term basis it is important to evaluate the toxicity in and safety in recognized animal models use for allopathic medicines.

A number of studies have examined the safety of an aqueous or ethanolic leaf extracts given orally to rats or mice. Apart from the use of extracts, these studies do not give a clear indication of how administered doses we calculated, making it difficult to interpret the results with unambiguity. None of these studies actually studied the administration of moringa leaf powder, the form which it is actually consumed. However, it is suggested that these when the doses if they could be estimated they would be far in excess of the actual amounts of leaf powder consumed by humans (Stokes and Hartman, 2015). In the only reported study found, the actual dose moringa leaf powder, the actual dose administered was not reported (Ambi *et al*, 2011) [1].

We therefore set out to evaluate in the first instance the acute toxicity of Moring oleifera powder in accordance with the Organisation of Economic Co-operation and Development Guidelines (OECD Guideline 423) for animal toxicity studies for medicines.

Materials and Methods

Plant Material

Dried leaf powder of *Moringa oleifera* were obtained from Grenera Nutrients Pvt Ltd. 37-B, Pudupalayam, Avalpoondurai, Tamilnadu, India The dried leaf powder was analysed for amino acid, mineral (including heavy metals and vitamin content and for microbial load at the Food Science laboratory of the Council for Scientific and Industrial Research in Durban. The leaf powder conformed in all respects to reported values for *Moringa oleifera*.

Animals

Sprague Dawley nulliparous and non-pregnant Rats (6 Females and 6 males) were obtained from South African Vaccine Producers PO Box 28999, Sandringham, and Johannesburg

The animals were acclimatized in the animal unit of La–Bio Research for a period of 5 days before the start of the study at a room temperature of between 19-23°C and a humidity of 40 -75 %.

A 12-hour day/night light cycle is a constant in the animal unit. The light intensity was kept between 70-100 Lux. The animals were housed in cages in accordance with European standards.

Water, food and bedding were sterilized before use in the cages and fed with pellets procured from Epol®. Each animal will be assigned a unique identification number.

Experimental design

The acute Toxic class method described in the OECD Guideline 423 was used for this study.

The method enabled a judgement with respect to classifying the test substance to one of a series of toxicity classes defined by fixed LD_{50} cut-off values.

Animals were observed daily for the duration of the study.

Description of the method

Test groups

The acute toxic class method is a stepwise procedure with the use of 3 animals per step. A starting dose level of 2000 mg/kg was used because of the indication of low toxicity of the test substance. A higher oral dosage of 5000 mg/kg was practically not possible because of the physical property of the test substance that would require a too high dosage volume. The flow charts from *Annex2D* of the OECD guidelines Figure 1 describes the procedure followed by starting with a dose of 2000 mg/kg. Three female rats were used in step 1 and three males in step 2.

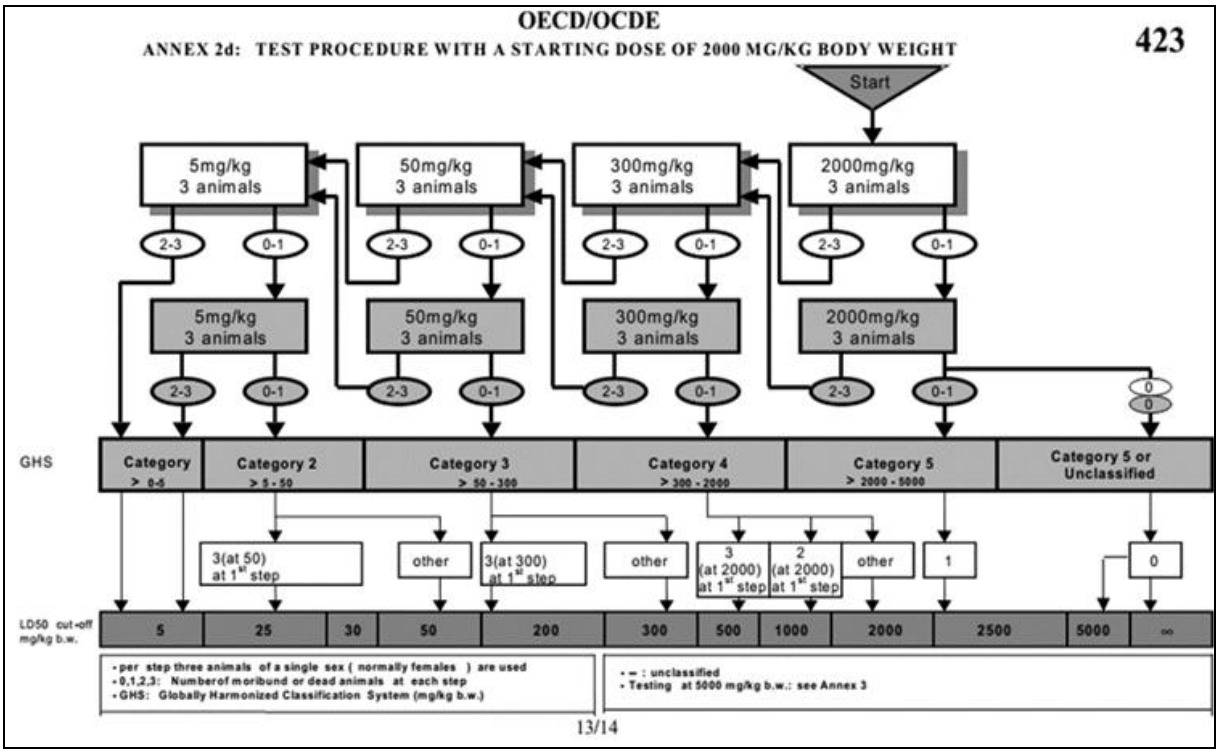

Fig 1: OECD Guidelines Procedure

The experimental procedure

Dosage

Food and water were withheld 12 hours prior to dosing and for a further 3-4 hours thereafter. Animals were weighed before dosing. The required volume of test substance was administered orally as a single dose with a plastic 15 gage oral gavage cannula (SOP: LBR-A-052). Dosages were calculated for males and females for 2000 mg per kg for each group according to the average body weight of each sex and group.

Test Group 2000 mg/kg:

Step 1: Females:

- Rat no1uc (240g) – 480 mg in 2.4 ml Distilled water
- Rat no 2cl (232g) – 464 mg in 2.3 ml Distilled water
- Rat no 1cr (228g) – 460 mg in 2.3 ml Distilled water

Step 1: Males:

- Rat no1uc (316g) – 632 mg in 3.2 ml Distilled water
- Rat no 2cl (335g) – 670 mg in 3.4 ml Distilled water
- Rat no 1cr (295g) – 600 mg in 3.0 ml Distilled water

The test substance was diluted to the total dosage volume using SABAX distilled water,

Observation Period

Animals were observed every hour for the first four hours and thereafter daily for 14 days for the animals in step1. The observation period for the step 2 animals were 7 days because no adverse reaction were seen in step 1 animals.

Observation of Animals

Clinical Examination

Observations were recorded in detail, using explicitly defined scales according to LBR standard operating procedure (SOP: LBR-A-0011). LBR observation sheets LBR-A-011-F01 were used. Observations included the following evaluation criteria:

- the skin and fur
- the eyes
- mucus membranes
- respiratory and circular effects and
- autonomic effects.

Animal Weights

Individual weights of the animals were determined shortly before the test substance was administrated, weekly thereafter and at termination of the study.

Pathology

At the end of the study, surviving animals were weighed and euthanized with CO_2 (SOP: LBR-A-007). A gross necropsy was performed on all animals in the study (SOP: LBR A-009). Necropsies were preformed immediately after euthanasia.

Histopathology

No samples were taken because no gross pathology were present

ETHICS.

The ethics committee of the Tshwane University of Technology verified that the animal facility operated within the standards and rules of the National Laboratory Animal Ethical Code of Conduct and that the animals was kept according to recognized international standards in animal husbandry practice.

The protocol was submitted to the Animal Ethics Committee of Tshwane University of Technology for notification.

Results

Clinical Observation.

Below is a summary of the clinical symptoms observed?

Group 2 000 mg/kg:

Step 1 Female Rats.

No changes in clinical signs were observed at any stage during the 14 day observation period.

Step 2 Male Rats.

No changes in clinical signs were observed at any stage during the 7 day observation period (Figure 2)

Fig 2: Observation of rats in their cages

Animal Weights
Animal weights in grams.
Group 2 000 mg/kg:

Step 1 Female Rats
All three animal showed normal weight gain

Step 2 Male Rats
All three animal showed normal weight gain

Post-mortem
Post-mortem findings (Figure 3)
Group 2 000 mg/kg:

Step 1 Female Rats
All three animals showed no signs of gross pathology

Step 2 Male Rats
All three animals showed no signs of gross pathology.

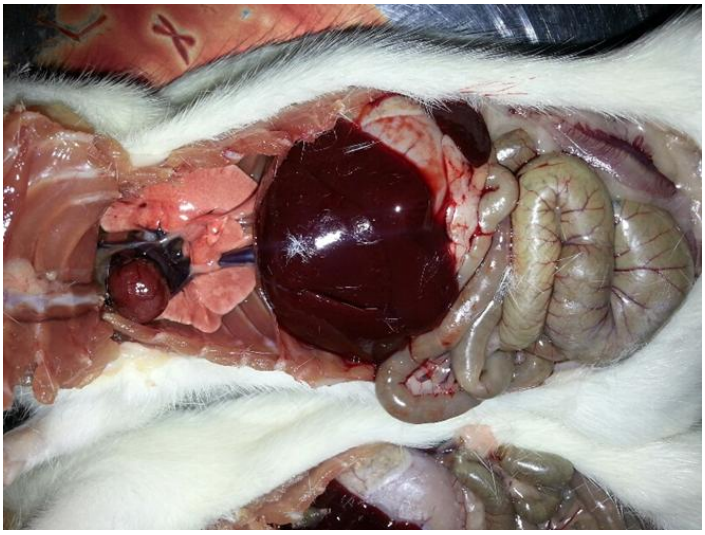

Fig 3: Post mortem observations

Conclusion
This study indicated that oral administration of *Moringa oleifera* dried leaf powder up to 2000mg/kg showed no changes in clinical signs or gross pathology and that the oral toxicity (LD_{50}) of the *Moringa oleifera* Dried Leaf Powder (Batch number: 0001/2014) is higher than 2 000 mg/kg

Discussion
This study undertook to evaluate the acute toxicity of moringa leaf powder which is reported to be the form in which it extensively consumed. The only other study evaluating the effects of moringa leaf powder was in rats (Ambi *et al*. (2011) [1] that were fed with varying amounts of powdered leaves mixed with standard livestock feed (25%, 50%, 75%, and control) for 93 days.. However, the results which showed some microscopic lesions in organs of some rats that ingested the mixture could not be unambiguously interpreted as the total amount of leaves consumed could not be quantified. If the amount was estimated at 15–20 g of leaves per kilogram for an adult rat, this would equate to an very high consumption of 195–260 g for an 80-kg human (Stohs and Hartmann, 2015).
However, a number of other studies have evaluated the safety of an aqueous or ethanolic leaf extract given orally to rats or mice. Although this is not the manner in which *Moringa oleifera* is consumed, these concentrated extracts may contain a different or higher concentrations of potentially toxic compounds and is therefore of interest. Regrettably, the authors of these studies do not show how they calculated the doses which also seems to be extraordinarily high given that these are liquid extracts
In an acute study, mice were administered an aqueous leaf

extract up to 6400 mg/kg orally and 1500 mg/kg intraperitoneally. In the sub chronic study, mice received 250, 500, and 1500 mg/kg orally for 60 days (Awodele *et al*., 2012) [5] the lethal dose of 50 % (LD_{50}) was estimated to be 1585 mg/kg. No significant effects were observed with respect to hematological or biochemical parameters or sperm quality. The authors conclude that the aqueous extract was safe to use.

The safety of an aqueous leaf extract given orally to rats at doses of ranging from 400, to 2000 mg/kg body weight was evaluated (Adedapo *et al*., 2009). The doses were administered either as an acute single or daily dose for 21 days except for the highest dose. Having assessed various parameters including blood cell counts and serum enzyme levels, the authors concluded that consumption of leaf extract at doses of up to 2000 mg/kg was safe. However, it was noted that a dose-dependent decrease in body weights of the rats occurred over the 21 days of the study.

Rats that were given 1000 and 3000 mg/kg of an aqueous extract, were assessed for up to 14 days (Asare *et al*. 2012) [4]. At 3000 mg/kg the leaf extract was shown to be genotoxic based on blood cell analysis dose, A dose of 1000 mg/kg which is also a dose in excess of commonly used doses did not produce genotoxicity and was deemed safe,

The potential toxicological effects of 50, 100, 200, or 400mg/kg of a methanol extract of Moringa. oleifera for 8 weeks was evaluated in 30 rats (Oyagbemi *et al*., 2013). Animals that received Moringa. oleifera had a significant dose-dependent increase in body weight contrary to the findings of (Adebayo *et al*., 2009 who studied an aqueous extract). At the higher doses (200 and 400 mg/kg), rats showed a significant increase in serum alanine aminotransferase, aspartate aminotransferase, blood urea nitrogen, and creatinine.

Bakre *et al*. (2013) determined that the lethal dose of 50% of an orally administered ethanol extract of *M. oleifera* leaves in mice was greater than 6.4 g/kg *M. oleifera*.

The safety of the of the aqueous and ethanolic extracts of the seeds and roots of *Moringa oleifera* were also evaluated in a few studies in rats and mice.

The genotoxicity of an aqueous *M. oleifera* seed extract was assessed using three separate assay systems including the Ames assay (Rolim *et al*., 2011) [12]. The authors concluded that seed extract was not genotoxic, and did not appear to pose a risk to human health.

The effect of a hexane extract of *M. oleifera* leaves on reproductive organs of male rats was examined (Cajuday and Pocsidio, 2010) [6]. The extract was given orally at doses of 17, 170, and 1700 mg/kg body weight for 21 days. A dose-dependent increase in testis and epididymis weights, in seminiferous tubule diameter, and epididymal epithelium thickness without change in plasma gonadotropin levels was observed. The authors concluded that the changes were associated with an increase in spermatogenesis.

Cytotoxicity of an aqueous extract of the seeds was evaluated by Araújo *et al*. (2013) [3]. Following 14 days of the extract administration (500 and 2000 mg/kg) in mice, no signs of systemic toxicity were observed, and all the animals survived. There were no changes in organ indices between treatment and control groups. Small but insignificant changes were observed in erythrocytes, platelets, hemoglobin,

And hematocrit.

The consumption of mooring leaf powder seems to be relatively safe as shown by the acute toxicity study in rats. This conclusion appears to be supported by other studies as indicated above. A sub chronic study of the administration of moringa leaf powder needs to be undertaken.

Acknowledgements

This study was carried out under the supervision of Dr. Daan Goosen at Lab Bio Research based at Tshwane University of Technology campus175 Nelson Mandela drive, Pretoria

This study was funded by the Department of Science & Technology, Pretoria, South Africa

Declaration of Interest

The author has no conflict of interest

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