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Evaluation of Sub Chronic Toxicity of *Moringa Oleifera* Leaf Powder in Mice

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Abstract

Background: 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. Recently moringa leaf powder is reported to be widely used by HIV and AIDS patients inZimbabe nad Nigeria and there is evidence of its use as a nutritional supplementation among HIV positive patients who have commenced antiretroviral therapy.

Objective: To evaluate the sub chronic toxicity of Moring oleifera in mice.

Materials & Methods: Balb c mice were given 1000 mg/kg per day in their drinking water for 90 days in this repeated dose, oral toxicity study. During the 90-day period the animals were observed daily and once weekly thereafter, detailed clinical observations were made in all animals for signs of any adverse effects

Results: All animals, in both the test and control groups showed a normal weight gain profile over the 90 day period. No macro-pathology was observed in any of the major organs of any of the test or control animals. There was no significant difference between the clinical biochemistry profile of the test group and control group for either male or female animals. Although the average blood glucose levels of the test group vs. the control group is lower, there is no statistical significant

Conclusion: This study indicated that oral administration of Moringa oleifera dried leaf powder at a 1000mg/kg daily showed no changes in clinical signs or gross pathology over a prolonged chronic exposure period of 90 days.

Keywords: Moringa oleifera; Sub acute toxicity; Pathology; Clinical signs; Mice

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 [1,2]

Recently two studies reported that moringa leaves powder or moringa leaves are used by HIV and AIDS patients [3]. Monera and Maponga, [3] 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 a large percentage HIV positive patients participants that have commenced antiretroviral therapy. 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. [4] reported a study that conducted to 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 oleifera was percievd to be significant. Majority of the respondents are aware that Moringa leaves have high protein content and is 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 oleifera does not have any religious taboos while its acceptability cuts across both religious and cultural beliefs.

While the actual benefits of Moringa oleifera in HIV patents have not been studied it is reported that lipodystrophy is a frequent and disfiguring adverse effect of antiretroviral therapy (ART) in patients with HIV. It affects the quality of life of the patient and adherence to treatment [5]. Moringa oleifrea has been shown to lower total cholesterols and triglycerides.

Tété–Bénissan et al. [6], evaluated the mineral composition of "Togolese ecotype" of Moringa oleifera leaves as well as 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.

Similar studies in experimental animal models seem to support the notion that Moringa oleifera can have similar effects. Rajanandh et al., [7] 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.

Some other studies showing the potential therapeutic benefits in humans are summarized below.

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% [8]). Kumari [9] administered 8 g of M. oleifera leaf powdered in a tablet form to 46 type 2 diabetic subjects. 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. [10] 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. [11] 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%.

In a study of 30 postmenopausal women Kushwaha et al. [12] 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

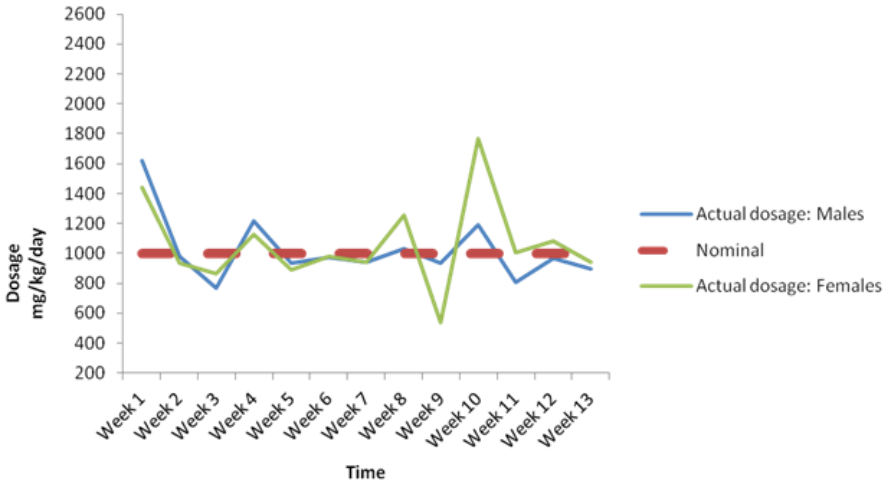

Figure 1: Illustration of the actual vs. nominal dosage for the duration of the study. Both males and females actual dosage is illustrated with the nominal dosage (1000 mg/kg/day being set as a reference).

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 an increase in hemoglobin (17.5%).

These studies suggest that whole leaf powders of Moringa 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 were 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 if estimated the doses of theses extracts, would by far, exceed the actual amounts of leaf powder consumed by humans [13]. In the only reported study found, on moringa leaf powder, the actual dose administered was not reported [14].

In the first instance the acute toxicity of Moringa oleifera powder in rats [15] was evaluated and showed no evidence of any adverse effects. We therefore set out to investigate if there was any observable adverse effects when the dose was given by repeated administration. and report on the safety and toxicity of Moringa oleifera in a 90 day repeat dose study in mice.

Materials and Methods

Plant material

Dried leaf powder of *Moringa oleifera* were obtained from Genera Nutrients (Grenera Nutrients Pvt Ltd. 37-B, Puthupalayam, Valpoondurai, Tamilnadu, India) The dried leaf powder was analysed for amino acid, mineral (including heavy metals and vitamin content and for microbial load at the CSIR (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

Nulliparous and non-pregnant Balbc mice (15 Females and 15 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 where the study was conducted 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% with a constant 12-hour day/night light cycle is 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 the mice were fed with pellets procured from Epol®. Each animal will be assigned a unique identification number.

Experimental design

The Repeated Dose 90-day Oral Toxicity method described in the OECD Guidelines [www.oecd.org/test/monos/htm] was used in this study. Results from the acute oral toxicity test [14] indicated that the "limit test" at a dose of 1000 mg/kg per day may be used for the 90-day study. During the 90-day period the animals were observed for signs of adverse effects,

Dosage

Only one dosage level was used for the test group (10 male and 10 female mice). Dosage calculations were performed to assure animals consumed the correct amount of test substance. The control group (5 males and 5 females) was administered with the vehicle (water) only. The actual vs. nominal dosage is illustrated in Figure 1 and Figure 2. The average dosage is shown in Table 1.

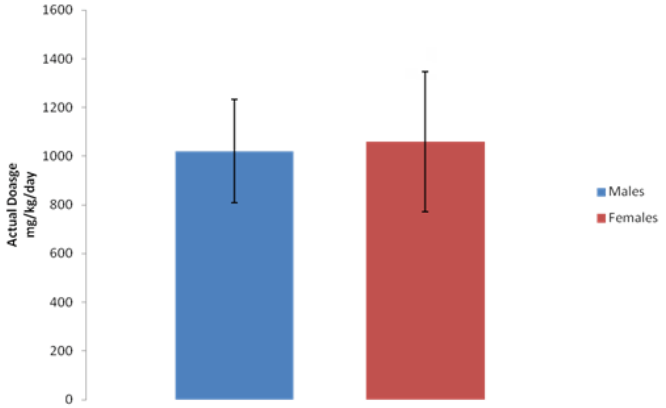

Figure 2: Illustration of the average and variance of the actual dosage for the duration of the study for both the male and female animals. The error bars indicate the standard deviation. Both males and females actual dosage is illustrated with the nominal dosage (1000 mg/kg/day being set as a reference).

Table 1: The average actual dosage that both male and female animals received over the 90 day period.

	Males	Females
Average Dosage mg/kg/day	1020	1059
Standard deviation	212	288

Administration of doses

The test substance was administered for 90 days via the drinking water. Other than treatment with the test substance the control group was treated identically to the test group.

Observation of animals

Observations were made daily and weekly. Daily observations were made to detect any general adverse effects. Weekly observations were more detailed and were aimed to detect a wide range of symptoms associated with toxicity.

Daily observation

General clinical observations were made once a day. At least twice daily animals were inspected for mortality and morbidity. Data from the daily observations was recorded on the at Lab Bio Research (LBR) animal laboratory lab book.

Weekly observations

Animals were weighed at weekly intervals. Water intake was also measured on weekly bases and recorded on an animal weight and dosage chart. Once prior to the first exposure, and once weekly thereafter, detailed clinical observations were made in all animals. These observations were made outside the cage on a standard operation table. Attempts were made to perform these observations on the same time of day as the previous occasion. The observations were recorded on a clinical observation chart using a scoring system which is defined on the scoring chart. Signs noted on the chart included but weren't limited to: respiratory, motor activity, convulsion, ocular symptoms, salivation, piloerection, oedema, erythema, righting reflex and startle reflex.

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 since this was a continuation study following on from the acute toxicity study. .

Results

Actual dosage vs nominal dosage

The route of administration of the test substance (via drinking water) makes it difficult to control the dosage to exact levels. The variables such as the variance in the water consumption rate from day to day and the changing of weight of the animals results in an actual dosage that will differ from the calculated dosage. The dosages were recalculated weekly with updated animal water consumption rates and updated animal weights. The actual dosage, on average, was slightly above the 1000 mg/kg/day level as is shown in Table 1. Figure 1 illustrates the variation from week to week. Overall the dosage was suitable to be considered as an intake level that approximates to 1000 mg/kg/day (Figure 2).

Animal weights

All animals, in both the test and control groups showed a normal weight gain profile over the 90 day period. There is no significant difference ($p>0.05$) between the profile of the test group and control as shown in Figure 3.

Clinical biochemistry

Heamatology and clinical biochemistry: Blood samples were taken via cardiac puncture. Animals was fasted overnight prior to euthanasia. The following haematological examinations were made at the end of the test period: blood glucose, haematocrit, haemoglobin concentrations, erythrocyte count, reticulocytes, total and differential leucocyte count, platelet count and a measure of blood clotting time/ potential. All blood from individual test groups were pooled together and all blood from individual control groups were pooled together. The test group and control group blood samples were analysed separately for comparison. Blood were collected in heparin tubes. Blood tubes were stored at 4°C until it was sent for analysis within 3 hours. A blood clotting profile test was performed with capillary tubes. Clinical Biochemistry determinations to investigate major toxic effects in tissues were performed on blood samples obtained from all animals. Investigations on plasma or serum includes: sodium, potassium, glucose, total cholesterol, urea, creatinine, total protein and albumin at least two enzymes indicative of hepatocellular effects and bile acids were measured.

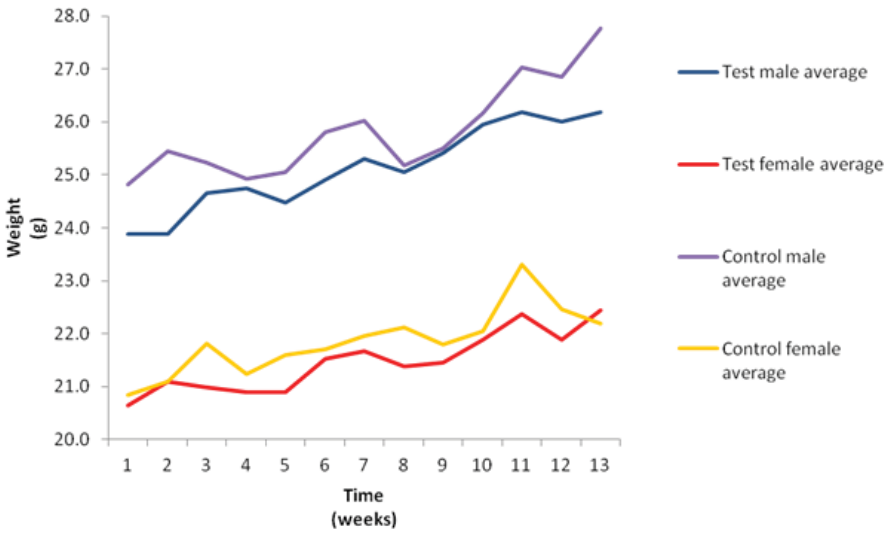

Figure 3: Illustration of the animal weight gain/loss profile, by group, over the 90 day dosage period. Shown is the weight gain/loss profile of the male test groups, the female test groups, the male control group and the female control group.

Table 2 and Table 3 show the clinical biochemistry comparison between the 2 male test groups (TMGr1 & TMGr2) and the 2 female groups (TFGr1 & TFGr2) respectively. There was no significant difference between the clinical biochemistry profile of the test group and control groups for either male or female animal respectively (Figures 4 and 5).

The average blood glucose levels of the test group vs. the control group is lower for both male and female animals (Table 4). However, this difference was not statistical significant (Figures 6 and 7)).

There was no statistical significant difference between the haematological profile of the test group and control group for either male or female animals (Tables 5 and 6 and Figures 8 and 9). Although the average blood clotting time of the test group vs. the control group was lower (Table 7), there was no statistical significant difference (Figure 10 and 11).

At the end of the study, all mice were placed under sedation with diethyl ether and killed by terminal blood collection via cardiac puncture. A gross necropsy was performed on all animals in the study

Table 2: Clinical biochemistry comparison between 2 male test groups (TMGr1 & TMGr2) and the male control group.

Test	TMGr1	TMGr2	Test Mean	Control	Units
Albumin	34.6	35.5	35.1	32.7	g/L
Globulin	20.6	26	23.3	28.3	g/L
Total serum protein	55.2	61.5	58.4	61,0	g/L
Alanine aminotransferase	38	41	39.5	46	U/L
Alkaline phosphatase	110	94	102.0	61	U/L
Aspartate aminotransferase	110	195	152.5	151	U/L
Urea nitrogen	8.5	7.5	8.0	9.8	mmol/L
Creatinine	24	27	25.5	22	umol/L
Cholesterol	3.3	3.3	3.3	3.9	mmol/L
Bile acid single/pre sample	2	27	14.5	3	umol/L
Sodium	155	155	155.0	153	mmol/L
Potassium	7.64	8.7	8.2	7.69	mmol/L

Table 3: Clinical biochemistry comparison between 2 female test groups (TFGr1 & TFGr2) and the female control group.

Test	TFGr1	TFGr2	Test mean	Control	Units
Albumin	40.0	36.8	38.4	40.0	g/L
Globulin	20.0	19.2	19.6	13.7	g/L
Total serum protein	60.0	56.0	58.0	53.7	g/L
Alanine aminotransferase	39	39	39.0	23	U/L
Alkaline phosphatase	129	122	125.5	150	U/L
Aspartate aminotransferase	140	167	153.5	104	U/L
Urea nitrogen	8.9	9.1	9.0	9.3	mmol/L
Creatinine	21	20	20.5	20	umol/L
Cholesterol	2.8	2.6	2.7	2.6	mmol/L
Bile acid single/pre sample	16	20	18.0	>350	umol/L
Sodium	153	150	151.5	153	mmol/L
Potassium	7.87	8.26	8.1	5.82	mmol/L

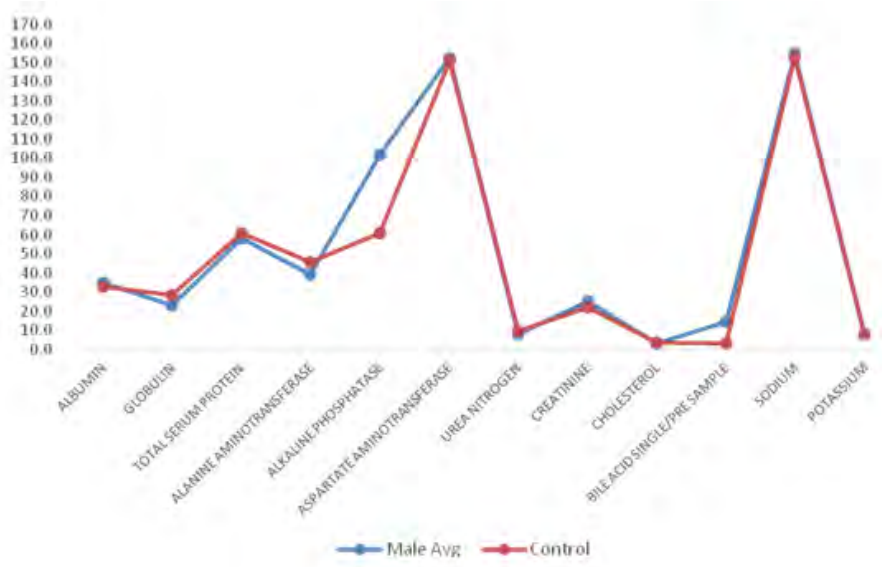

Figure 4: Illustration of the clinical biochemistry profile comparison of all male animals. Shown is the comparison between the test and control male groups for a range of clinical biochemistry markers.

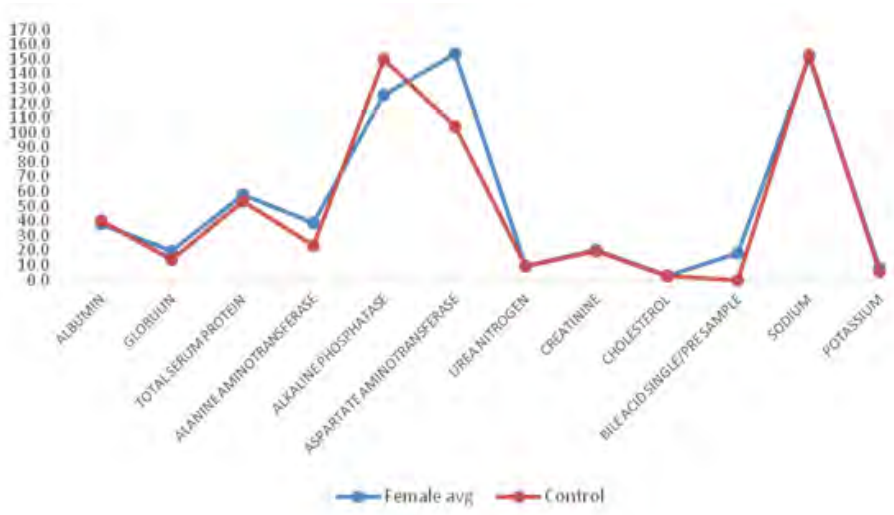

Figure 5: Illustration of the clinical biochemistry profile comparison of all female animals. Shown is the comparison between the test and control female groups for a range of clinical biochemistry markers.

Table 4: Blood glucose comparison between the test and control groups is shown in mmol/L.

Males			Females		
Animal no	**Test**	**Control**	**Animal no**	**Test**	**Control**
1	5.4	8.3	1	6.3	5.8
2	5.8	6.3	2	5.6	8
3	-	8.4	3	7.2	6.9
4	4.8	7	4	7.2	6.2
5	5.7	6.4	5	7.1	5.7
6	5.9		6	6	
7	6.2		7	5.6	
8	8.1		8	7	
9	8.8		9	4.7	
10	7.2		10	6.8	
Mean	6.4	7.3	**Mean**	6.4	6.5
Max	8.8	8.4	**Max**	7.2	8
Min	4.8	6.3	**Min**	4.7	5.7
Range	4	2.1	**Range**	2.5	2.3
Std dev	1.25	0.91	**Std dev**	0.81	0.85

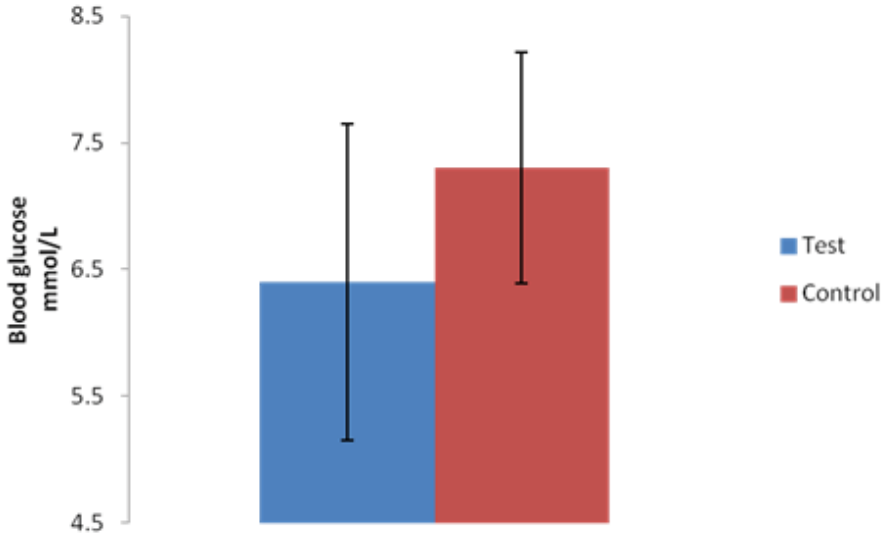

Figure 6: Comparison of the average blood glucose levels between the male test group and the male control group. The error bars indicate the standard deviations within each group.

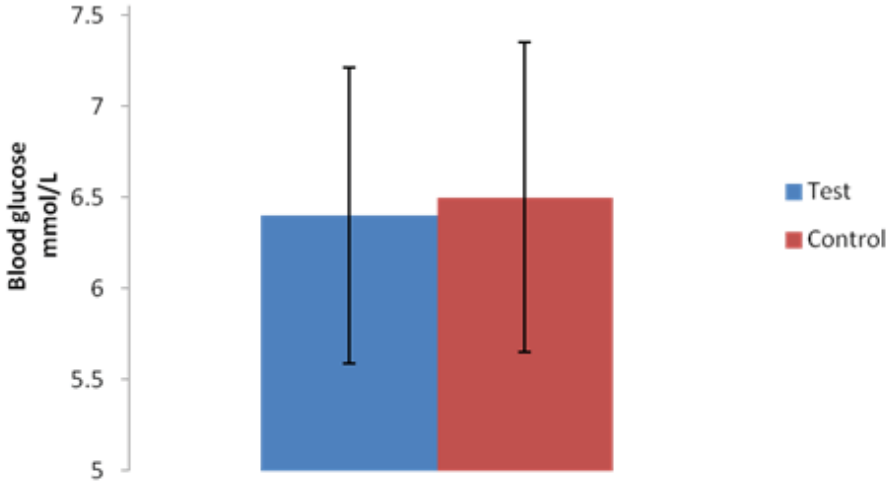

Figure 7: Comparison of the average blood glucose levels between the female test group and the female control group. The error bars indicate the standard deviations within each group.

Table 5: Haematology comparison between the male test group average and the male control group average.

Test	Males	Control	Unit
Haemoglobin	174	172	g/L
Red cell count	11.22	11.07	$x10^{12}$/L
Haematocrit	0.535	0.56	L/L
Mean corpuscular volume	47.7	50.6	fL
Mean corpuscular haemoglobin	15.45	15.5	Pg
Mean corpuscular haemoglobin conc.	32.5	30.7	g/dL
Red cell distribution width	13.95	13.9	%
White cell count	4.175	2.43	$x10^{12}$/L
Segmented neutrophil	1.425	1.09	$x10^{12}$/L
Band neutrophil	0	0	$x10^{12}$/L
Lymphocyte	2.57	1.22	$x10^{12}$/L
Monocyte	0.115	0.05	$x10^{12}$/L
Eosinophil	0.07	0.07	$x10^{12}$/L
Basophil	0	0	$x10^{12}$/L
Platelet count	358	346	$x10^{12}$/L

Table 6: Haematology results of all female animals. The table shows the Haematology comparison between the female test group average and the female control group average.

Test	Females	Control	Unit
Haemoglobin	169	168	G/l
Red cell count	10.93	11	$x10^{12}$/L
Haematocrit	0.51	0.54	L/l
Mean corpuscular volume	46.9	48.8	Fl
Mean corpuscular haemoglobin	15.45	15.3	Pg
Mean corpuscular haemoglobin conc.	33.05	31.4	G/dl
Red cell distribution width	12.6	13	%
White cell count	4.18	4.88	$X10^{9}$/l
Segmented neutrophil	1.03	1.22	$X10^{9}$/l
Band neutrophil	0	0	$X10^{9}$/l
Lymphocyte	2.99	3.42	$X10^{9}$/l
Monocyte	0.125	0.15	$X10^{9}$/l
Eosinophil	0.085	0.1	$X10^{9}$/l
Basophil	0	0	$X10^{9}$/l
Platelet count	79	608	$X10^{9}$/l

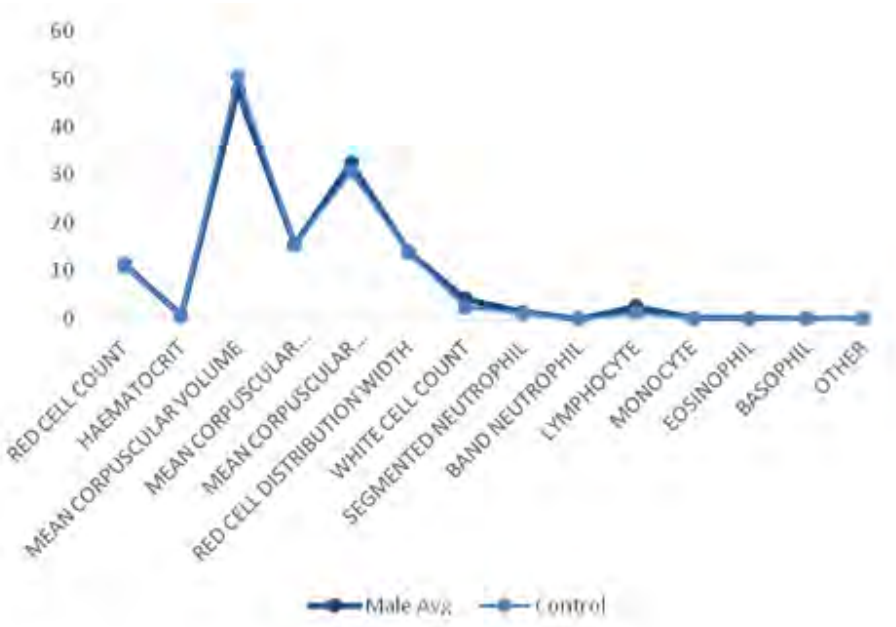

Figure 8: Illustration of the haematology profile comparison of all male animals. Shown is the comparison between the test and control male groups for a range of haematological markers.

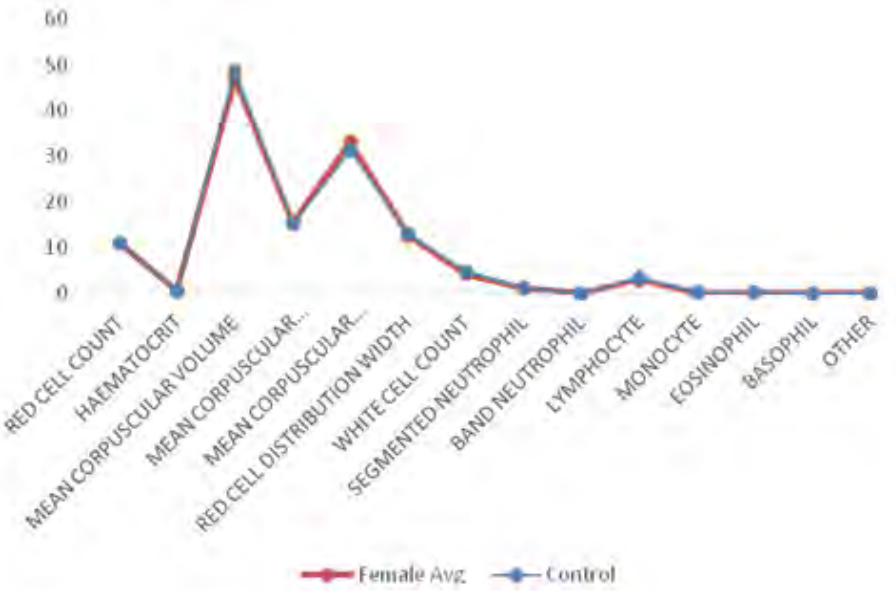

Figure 9: Illustration of the haematology profile comparison of all female animals. Shown is the comparison between the test and control female groups for a range of haematological markers.

Following a detailed necropsy, the different organs listed below were examined, fixated in 10% formalin and stored:

The organs include, all gross lesions, brain, spinal cord, pituitary, thyroid, parathyroid, thymus, oesophagus, kidneys, adrenals, spleen, heart, trachea, lungs, aorta, gonads, uterus, accessory sex organs, female mammary gland, prostate, urinary bladder and lymph nodes, small intestines, large intestines, stomach and liver. All samples taken were stored for histopathology if required

Gross necropsy

No macro-pathology was observed in any of the major organs of any of the test or control animals.

Conclusion

The data obtained and analysed in this repeated dose 90-day oral toxicity study showed that there were no adverse effects at a level of at least 1000 mg/kg/day of Moringa oleifera Dried Leaf Powder over a prolonged chronic exposure period of 90 days.

Discussion

An earlier study showed that acute oral administration of *Moringa oleifera* dried leaf powder in rats up to 2000mg/kg showed no changes in clinical signs or gross pathology and that the LD50 was greater than 2000mg/kg [14]. This study undertook to evaluate the sub chronic 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 [15] 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, but no other signs of toxicity. The total amount of leaves consumed 6ccording to an analysis Stohs and Hartman [13] t was estimated at 15–20 g of

Table 7: Blood clotting time comparison between the test and control groups is shown in min.

Males			Females		
Animal no	Test	**Control**	**Animal no**	**Test**	**Control**
1	1.0	1.5	1	0.75	2.0
2	5.5	1.5	2	0.75	2.0
3	1.0	3.5	3	2.0	2.5
4	1.5	3.0	4	0.33	2
5	1.0	2.0	5	0.75	2
6	1.0		6	0.5	
7	1.5		7	2.75	
8	1.0		8	5.0	
9	0.5		9	2.0	
10	1.0		10	0.5	
Mean	1.5	2.3	Mean	1.5	2.1
Max	5.5	3.5	Max	5.0	2.5
Min	0.5	1.5	Min	0.33	2.0
Range	5	2	Range	4.7	0.5
Std dev	1.4	0.81	Std dev	1.4	0.2

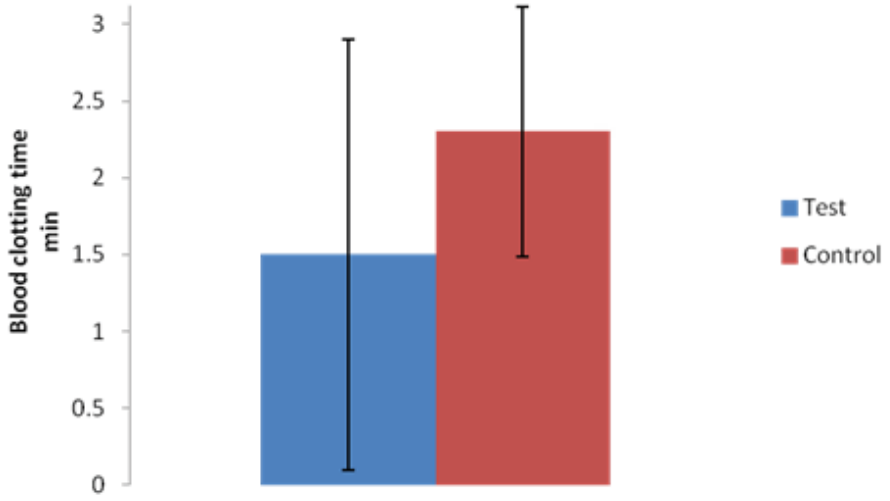

Figure 10: Illustration of the blood clotting profile comparison of all male animals. Shown is the comparison between the test and control male groups for their blood clotting profiles.

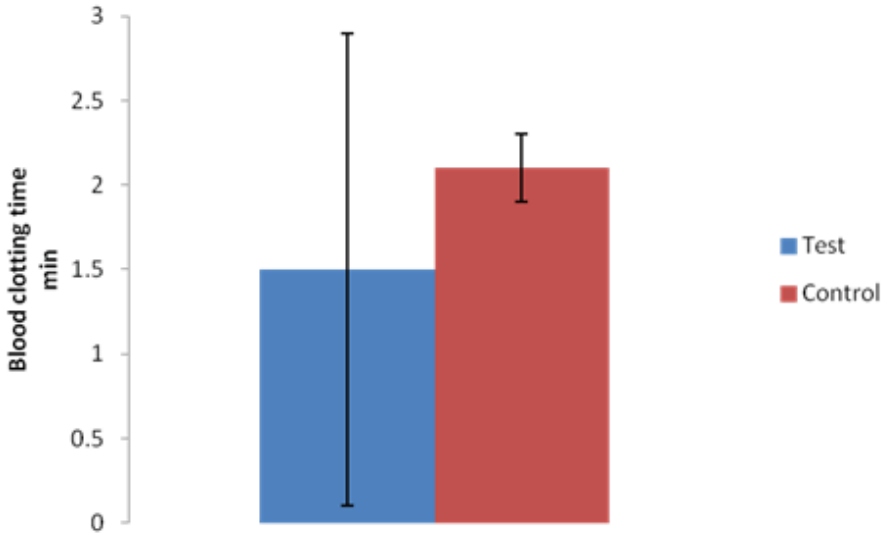

Figure 11: Illustration of the blood clotting profile comparison of all female animals. Shown is the comparison between the test and control female groups for their blood clotting profiles.

leaves per kilogram for an adult rat, which equate to a very high consumption of 195–260 g for an 80-kg human.

However, a number of other studies have evaluated the safety of an aqueous or ethanolic leaf extract given orally to rats or mice. While this is not the usual manner in which Moringa oleifera is consumed, these concentrated extracts may contain a different or higher concentrations of potentially toxic compounds and therefore may be of interest. Even though the doses which seem to be extraordinarily high given that these are liquid extracts, very few studies showed any overt toxicity.

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 [16]. 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 [17] .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 [18] .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 [19] Animals that received Moringa .oleifera had a significant dose-dependent increase in body weight contrary to the findings of (Adebayo et al.,[20] 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. [21] 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 [22]. 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 [23]. 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. [24]. 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 subchronic consumption of moringa leaf powder in rodents seems to be relatively safe. Further, aqueous or ethanolic extracts even at high doses seems to be relatively safe as shown by either acute or sub chronic toxicity study in rodents. This conclusion appears to be supported by studies as indicated above.

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