International Journal of Medicinal Plant Research ISSN: 2169-303X Vol. 1 (6), pp. 075-081, November, 2012. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Sub-acute toxicity evaluation of *Moringa oleifera* leaves aqueous and ethanol extracts in Swiss Albino rats

Josephine. N. Kasolo[1]*, Gabriel. S. Bimenya[1], Lonzi Ojok[2] and Jasper W. Ogwal-Okeng[1]

[1]School of Biomedical Sciences, Makererere University College of Health Sciences. Kampala, Uganda.
[2]School of Veterinary Medicine, Makerere University, Kampala, Uganda.

Received 13 August, 2012; Accepted 09 November, 2012

This study is set out to establish the sub-acute toxicity profile of the aqueous and ethanol extracts of *Moringa oleifera* leaves in Swiss Albino rats. *M. oleifera* leaves contain important phytochemicals, such as gallic tannins, catechol tannins, steroids and triterponoids, flavonoids, saponins, anthraquinones, alkaloids and reducing sugars. It also contains proteins, vitamins, beta-carotene and amino acids. When they are taken in large quantities can cause adverse effects in rats. Many families worldwide consume the leaves over varying periods of time, without knowing the possibility of causing organ toxicity. This laboratory based study had *M. oleifera* leaves harvested during dry season, air dried and pulverized. Serial extraction was done using ether, ethanol and water as solvents. The control group received no extract, but 16.1, 8.05, 4.02, 2.01 g/kg of aqueous extract and 39.8, 19.9, 9.95, 4.97 g/kg of ethanol extracts; were given in a single daily oral dose to groups of 5 male and 5 female Swiss Albino rats for 30 days. On the last day, blood was taken for hematological and biochemical analysis. The liver, kidney, and heart tissues had histopathology examination done under light microscope. All aqueous extract groups had a significant increase ($p<0.002$) in the mean of total white blood cells, Cl^-, K^+ and Ca^{++}, alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and serum bilirubin while histopathology analysis showed mild features of hepatitis, glumerulonephritis and myocarditis. The ethanol extract values were close to the control group. In conclusion, *Moringa oleifera* leaves aqueous extract given to rats orally in a single lethal dose of full LD_{50} for 30 days is associated with mild organ toxicity. However the ethanol extract showed no features of organ toxicity.

Key words: *Moringa oleifera*, sub-acute toxicity, medicinal plant extracts, plant toxicity, Swiss Albino rats.

INTRODUCTION

Earlier studies have shown that M. *oleifera* leaves ether, ethanol and aqueous extracts contain a profile of important phytochemicals, (Kasolo et al., 2010, Kasolo et al., 2011), which are potential toxins to animals and insects depending on the amount ingested. *M. oleifera* leaves also contain a variety of micronutrients (Fuglie, 1999; Nambiear and Seshadri, 2001) and macronutrients (Seshadri, 2003), trace elements (Barminas et al., 1998, Lockelt et al., 2000). The ingestion of above nutrients and phytochemicals in large amounts and over a period of time may cause animal organ toxicity. It is also documented that the acute toxicity (LD_{50}) of the aqueous extract of 16.1 g/kg and ethanol extract 39.6 g/kg are within the safe range (Kasolo et.al., 2012).

In Senegal and Benin *M. oleifera* leaf powder is dispensed at health facilities to treat moderate malnutrition in children with great success (Fuglie, 1999) and the leaf powder is now recommended in Africa and Asia as a nutritional supplement for lactating mothers and

*Corresponding author. E-mail: josephinekasolo@yahoo.com. Tel: 256-772-553088.

moderately malnourished children. In the Department of Food Science Nutrition and Biotechnology, Makerere University, Uganda, *M. oleifera* leaf powder is being used as nutritional additive.

M. oleifera leaf preparations have been cited in the scientific literature as having medicinal values which communities take advantage of (Fahey, 2005). The anti-oxidant properties of *M. oleifera* leaves have been evaluated and recommended for use (Sharma, 2009; Siddhuraju and Becker, 2003; Atawodi et al., 2010), including beta-carotin, (Nambiear and Seshadri, 2001; Subadra et al., 1997).

While the plant is consumed for long periods of time by many families worldwide due to its attributes, its sub-acute toxicity profile is not well documented. This study therefore set out to determine sub-acute toxicity profile of aqueous and ethanol extracts of *M. oleifera* leaves in Swiss Albino rats.

Objectives

This study was undertaken to determine hematological parameters, serum electrolytes, serum enzymes and end products which indicate liver, kidney and heart toxicity; and identify the histo-pathological changes of the liver, kidney and heart of the rats that received different doses of *M. oleifera* aqueous and ethanol extracts in a single oral daily dose for 30 days.

METHODS

This was an experimental study that used *M. oleifera* leaf aqueous and ethanol extracts given orally in single daily dose to Swiss Albino rats for 30 days.

Botanical identification of the plant

The plant was identified by Ms. Olive Wanyanna the botanist working in the department of Botany, School of Biological Sciences, Makerere University, who confirmed the kingdom, family and species of the *M. oleifera.* Plant parts were stored in the Makerere University, Botany Department Herbarium, voucher numbers 41302 and 41303. The plants were about two years old, grown on the hillside loam soil, had their dark green mature leaves and mature root bark harvested during the wet season between 9.00 and 11.00 a.m. The plants were grown in the district of Wakiso located in Uganda's central region.

Extraction process

The extraction was done following the already established extraction procedure of plant material (Cowan, 1999; Ciulei, 1964). Serial extraction methods in which the powder was first soaked in ether; then followed by ethanol and lastly distilled water as solvents were used. Two hundred grams of plant powder was soaked soaked in 500 ml diethyl ether (98%) in Ehlnmeyer flask (Cowan, 1999; Ciulei, 1964). The mixture was shaken at two hourly intervals during day-time for 3 days. The mixture was decanted and filtered using Whatman number 1 filter paper in Buchner funnel using a suction pump. The residue was air dried at room temperature for 3 days.

The same procedure was repeated on the residue using ethanol (95% V/V) for 3 days. Rotary evaporator (BUCHI Rotavapor R-205) was used to recover the ether and ethanol. The dried residue was soaked in 700 ml distilled water at 96°C to prevent fungal attack and cooled at room temperature. The mixture was shaken hourly to ease extraction for 12 h. The filtrate was freeze dried at pressure 32 Pa, original temperature was set at -47°C and then maintained at 0°C for 36 h to dry the extract. The yield from aqueous extract was 2.5% while that from the ethanol extract was 3.7%.

The dry ethanol and aqueous extracts were weighed, and volume of distilled water added to make a stoke solution from which the daily doses were calculated according to the rats' body weight and the allocated daily dose.

Sub-acute toxicity determination

The study used 8 to 10 weeks old Swiss Albino rats, reared in Physiology Department, which received 12 h of light and 12 h of darkness. They were fed on commercial rat pellets at leisure with tap water available all the time. Five rats of the same sex were put in one cage until a total of 9 cages for males and 9 cages for females were obtained. One cage of males was paired with another of females and labeled groups: I, II, III, IV, V, VI, VII, VIII, IX consecutively. Group I received distilled water and acted as the control group. Group II, III, IV, and V received aqueous extract, 16.1, 8.05, 4.02, 2.01 g/kg consecutively. Groups IV-IX received doses; 39.8, 19.9, 9.95, 4.97 g/kg of ethanol extracts consecutively. The doses given corresponded to full LD_{50}, ½ $LD_{50,\ 1/4}$ LD_{50} and 1/8th LD_{50} consecutively. The extracts were given in a single daily dose for 30 days at 9.00 am. having been starved overnight. On day 0, the rats were weighed and repeated on a weekly basis. On day 31, the animals were sacrificed, blood withdrawn from the heart ventricles, half of it put in a heparinised vacutainer glass tubes and the other in a general vacutainer glass tubes. Heamatological parameters were measured using Nihon Kohden Celltac F Coulter counter and serum was anlysed using COBAS INTEGRA 400 Plus clinical chemistry analyzer for electrolytes, enzymes, and end products. The electrolytes included: calcium, chloride, sodium and phosphate, while the enzymes included: alanine aminotransferase, alkaline phosphotase, aspartate aminotransferase, creatin phosphokinase, gamma-glutamly transpeptidase and lactate dehydrogenase. The end metabolites included: blood urea, bilirubin and creatinine. Kidney

Table 1. Mean weight gain by the rats that received different doses of the extracts in 30 days.

Extract	Control	Full LD_{50}	½ LD_{50}	¼ LD_{50}	1/8 LD_{50}
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Leaves aqueous	30.3 ± 10.78	12.7 ± 7.90*	10.8 ± 5.67*	13.4 ± 6.72*	17.3 ± 4.83
Leaves ethanol	30.3 ± 10.78	27.3 ± 22.56	27.3 ± 22.56	37.2 ± 23.03	42.7 ± 16.70

* Statistically significant values with p<0.002.

liver and heart tissues were immersed in 10% formalin, slides were made and tissue stained with haematoxylin and Eosin and examined viewed under light microscope of magnification ×10.

Data management

Data from blood chemistry and heamatology measurements were entered into Microsoft Excel 2007 and exported to STATA version 10.0 for analysis. The variables were summarized using means and standard deviations and experimental groups were compared against controls. Bivariate Analysis (Pearson Correlation) and the One-way Anova of STATA 10 was done and the level of significance was fixed at P=<0.002. Photographs of the informative slides were taken.

RESULTS AND DISCUSSION

All the rats in this study gained an average weight of 17.3 g to 30 g in the 30 days. However, the control group gained more weight than the intervention groups (Table 1).

The rats that received aqueous extract gained less weight than those that received the ethanol extract.

This may suggest possible reduction in food intake or less body fat deposition in the intervention group. *M. oelifera* leaves have demonstrated the ability to cause hypocholestronemia *in vitro*, (Broadhurst et al., 2000). This low weight gain while the rats were taking the aqueous extract may be useful for the development of herbal remedies for weight loss.

Hematological values

There was a significant increase in the white blood cells (WBC) in the animals that ingested various doses of *M. oleifera* leaves aqueous extract (Table 2).

The changes in the hematological indices for the rats in both extract groups were not significant, (Tables 2 and 3). This may be due to an immunological response to tissue inflammation or due to the presence of steroids in the leaves extracts. Such effect of steroids on WBC production was reported in patients with asthma on steroid treatment (Chirag M. Pandya, 2007). The increase in WBC could also have been caused by the reducing sugars in *Moringa oleifera* leaves (Antai AB, 2009, Kasolo J.N et al., 2010). There were no significant change in WBC values in the rats that received ethanol extract, Table 3.

Serum electrolytes

There was a statistically significant increase in the mean concentration of chloride, potassium and calcium ions (Cl^-, K^+ and Ca^{++}) in all the doses of aqueous extract when compared with the control group, Table 2.

The increased chloride ion concentration attracts hydrogen ions into plasma making the blood acidic. The lower pH does not favor majority of metabolic processes that take place in the body and therefore contributes to the toxicity profile. Metabolic acidosis is associated with increased morbidity and mortality because of its depressive effects on cardiovascular function, facilitation of cardiac arrhythmias, stimulation of inflammation, and suppression of the immune response,(Kraut JA, 2012). The increase in Ca^{++} and K^+ may be a result of the observation that 1gm of *Moringa oleifera* leaves contain 17 times the calcium in 1gm milk and 15 times the potassium found in bananas (Fuglie, 1999). These electrolytes in the extracts may be bioavailable and are responsible for their rise. However metabolic acidosis reduces calcium excretion and may have contributed to serum C^{++} rise, (Testerman SK et al., 1995).

Maintenance of potassium ions (K^+) balance depends primarily on excretion by the kidneys. Increase in serum K^+ in the intervention groups may be caused by reduced renal tubule function.

However, metabolic acidosis has been associated with increased extracellular potassium concentration, mainly in relation to diabetic keto-acidosis, (Adrogué HJ et al., 1986).

Serum enzymes

There was a statistically significant increase in the mean of alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransaminase (AST) in all the doses of of the animals that received leaves aqueous extractt, when compared to the control group (Table 2).

A rise in alkaline phosphatase, alanine aminotransferase and aspartate aminotransaminase indicate damage to the liver including the biliary system.

Table 1. Hematological and serum chemical values of rats that received aqueous extract for 30 days.

Parameter	Control (0.0 g/kg) Mean ± SD	Full LD_{50} (16.1 g/kg) Mean ± SD	½ LD_{50} (8.05 g/kg) Mean ± SD	¼ LD_{50} (4.02 g/kg) Mean ± SD	1/8 LD_{50} (2.01 g/kg) Mean ± SD
Hematological values					
WBC(x1000/µL)	5.14 ± 1.96	12.56 ± 3.62*	13.74 ± 2.29*	18.11 ± 4.670*	9.96 ± 4.48*
RBC(x1000/µL	8.60 ± 2.12	7.24 ± 0.95	7.500 ± 0.72	8.12 ± .290	7.41 ± 0.63
HGB (g/dl)	15.39 ± 3.76	14.29 ± 0.9	14.41 ± 0.76	17.96 ± 7.95	14.58 ± 1.84
HCT (%)	41.26 ± 9.67	37.95 ± 2.17	40.28 ± 3.61	23.64 ± 11.56	39.56 ± 4.49
MCV(fl)	48.08 ± 2.11	51.84 ± 2.67	52.55 ± 3.47	50.96 ± 0.63	52.68 ± 1.82
MCH (pg)	17.96 ± 0.791	19.23 ± 1.31	18.20 ± 2.70	18.51 ± 0.440	19.42 ± 0.96
MCHC (g/dl)	37.25 ± 0.646	36.75 ± 0.43	33.18 ± 1.33	34.13 ± 7.59	29.66 ± 9.28
PLT (x1000/µL)	52.49 ± 24.2	43.07 ± 10.75	41.13 ± 6.407	45.15 ± 16.56	37.70 ± 14.76
Electrolytes					
Cl^- (mmol/dil	93.34 ± 9.1	124.54 ± 7.7*	114.38 ± 6.0*	102.67 ± 7.6*	122.71 ± 11.3*
K^+ (mmol/dl)	23.84 ± 7.5	5.11 ± 0.5*	3.74 ± 1.1*	6.30 ± 1.3*	5.29 ± 0.3*
Na^+ (mmol/dl)	137.25 ± 8.4	161.69 ± 2.8*	156.33 ± 11.0	141.07 ± 6.0	148.9 ± 4.3
Ca^{++} (mmol/dl)	0.21 ± 0.62	3.14 ± 0.70*	2.82 ± 0.65*	2.63 ± 0.15*	2.69 ± 0.219*
$P0_4^{3-}$ (mmol/dl)	2.84 ± 1.79	3.83 ± 1.67	3.09 ± 1.35	2.28 ± 0.23	2.89 ± 0.30
Enzymes					
CPK(U/L)	501.82 ± 1.79	5389.8 ± 3894.5*	4336.0 ± 3330.4*	4190.6 ± 1836.*	6564.4 ± 1573*
LDH (U/L)	508.90 ± 1.79	1791.2 ± 1838.2*	8003.0 ± 1513.9*	4394.4 ± 1498*	3306.3 ± 606.9*
ALP (U/L)	7.37 ± 1.79	110.40 ± 18.19*	101.64 ± 17.12*	91.62 ± 41.37*	146.75 ± 37.14*
ALT (U/L)	93.58 ± 1.79	174.95 ± 30.29	180.78 ± 54.02	106.44 ± 10.54	170.21 ± 22.95
AST (U/L)	241.24 ± 1.79	349.81 ± 176.30	342.60 ± 176.78	291.77 ± 77.01	321.07 ± 69.51
GGT (U/L)	2.77 ± 1.791	8.370 ± 10.03	3.86 ± 2.49	3.83 ± 1.25	6.21 ± 3.85
End product					
Creatinine (mg/dl)	39.15 ± 22.1	32.42 ± 10.4	30.42 ± 7.6	22.73 ± 4.2	27.7 ± 1.98
Total Bilirubin (mg/dl)	0.55 ± 1.79	4.31 ± 1.07*	3.29 ± 1.37*	3.12 ± 0.58*	3.35 ± 0.39*
Urea (mg/dl)	5.89 ± 1.79	4.79 ± 2.85	3.42 ± 1.95	7.76 ± 0.71	7.19 ± 1.53

*Statistically significant values with $p<0.002$; aspartate aminotransaminase (AST); alanine aminotransferase (ALT); creatine phosphokinase (CPK): alkaline phosphatases (ALP); lactate dehydrogenase (LDH); gamma-glutamyl transpeptidase (GGT).

Adedapo et.al (2009), reported a significant increase in alkaline phosphate having given *M. oleifera* leaves aqueous extract to rats in quite a low dose of 1.6 g/kg. Increase in serum alkaline phosphate may be considered as a sensitive indicator of cholestasis which is also supported by significant increase in total bilirubin in rats that received aqueous extract (Table 2). Serum ALT is known to increase in liver disease and it has been used as a tool for measuring hepatic necrosis (Bush BM, 1991).

Serum end products

There was a statistically significant increase in the mean of total bilirubin in all the groups that received different doses of leaves aqueous extract when compared with the control group (Table 2). The rats showed no visible jaundice indicating that most of the bilirubin was un-conjugated. However the difference in mean total bilirubin was not significant in the rats that received leaves ethanol extract compared to the control group (Table 3). In all the experimental groups none of them had a significant difference in serum urea and creatinine (Tables 2 and 3).

Histo-pathology of liver, kidney and heart

This study examined the slides of H and E stained tissues of the liver; kidney and heart of all the study animals that received a single daily dose of *M. oleifera* leaves aqueous and ethanol extracts. The major histopathological changes occurred in organs of animals that received the LD_{50} doses. There were minimal histopathological changes in the organ tissues of the rats that received ethanol extracts including those that received doses below LD_{50} of the aqueous extract. Having some animals that received the LD_{50} dose also showing minimal changes, indicated that high doses of

Table 3. Hematological and serum chemical parameters of rats that received ethanol extract for 30 days.

Parameter	Control (0.0g/kg) Mean ± SD	Full LD_{50} (39.8 g/kg) Mean ± SD	½ LD_{50} (19.9 g/kg) Mean ± SD	¼ LD_{50} (9.95 g/kg) Mean ± SD	1/8 LD_{50} (4.97 g/kg) Mean ± SD
WBC(x1000/µL)	5.14 ± 1.96	3.14 ± 1.29	2.94 ± 0.89	2.65 ± 0.64	2.71 ± 0.613
RBC(x1000/µL	8.60 ± 2.12	7.52 ± 0.63	6.94 ± 0.6.60	6.06 ± 1.38	5.20 ± 1.83
HGB (g/dl)	15.39 ± 3.76	13.59 ± 1.10	12.78 ± 1.41	11.26 ± 1.37	11.33 ± 0.91
HCT (%)	41.26 ± 9.67	35.95 ± 1.77	33.92 ± 3.18	41.56 ± 5.00	41.91 ± 4.34
MCT(fl)	48.08 ± 2.11	47.99±1.80	49.24±1.51	46.42±6.52	46.62±5.80
MCH (pg)	17.96 ± 0.79	17.82 ± 0.76	17.71 ± 1.31	16.41 ± 2.53	16.57 ± 2.64
MCHC (g/dl)	37.25 ± 0.646	37.00 ± 0.98	33.24 ± 1.00	35.00 ± 0.84	35.40 ± 1.70
PLT(x1000/µL)	52.49 ± 26.7	51.8 ± 25.9	58.62 ± 22.2	64.34 ± 25.3	35.37 ± 27.6
Electrolytes					
Cl^- (mmol/dl	93.34 ± 9.1	87.2100 ± 7.5	80.34 ± 7.2	91.47 ± 6.5	89.13 ± 6.6
K^+ (mmol/dl)	23.84 ± 7.5	37.11 ± 7.6	27.65 ± 5.3	21.70 ± 6.6	35.10 ± 0.3
Na^+ (mmol/dl)	137.25 ± 8.4	137.45 ± 8.4	138.51 ± 11.4	134.6 ± 10.1	134.01 ± 8.3
Ca^{++} (mmol/dl)	0.21 ± 0.6	0.010 ± 0.03	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
$P0_4^{3-}$ (mmol/dl)	2.84 ± 1.8	9.42 ± 4.7	4.84 ± 1.9	4.84 ± 1.9	10.24 ± 7.6
Enzymes					
CPK (U/L)	501.82 ± 18.	573.20 ± 22.0	460.78 ± 12.0	596.0 ± 82.6	498.3 ± 16.1
LDH (U/L)	508.90 ± 18.	527.00 ± 26.6	546.00 ± 28.1	856.5 ± 77.2	522.10 ± 23
ALT (U/L)	93.58 ± 1.8	175.35 ± 81.3	162.48 ± 26.2	98.53 ± 8.6	105.49 ± 16.4
AST (U/L)	241.24 ± 1.8	401.33 ± 16.4	311.07 ± 14.4	167.49 ± 16.4	272.71 ± 25.8
GGT (U/L)	2.77 ± 1.791	4.05 ± 4.104	1.49 ± 4.92	0.73 ± 4.92	1.05 ± 0.69
End products					
Cretinine (mg/dl)	39.15 ± 22.0	41.2500 ± 8.0	40.55 ± 13.2	39.68 ± 6.5	43.35 ± 14.3
Total Bil (mg/dl)	0.55 ± 1.79	0.56 ± 0.14.9	0.47 ± 0.26	0.52 ± 0.26	0.38 ± 0.64
Urea (mg/dl)	5.89 ± 1.79	6.19 ± 1.23	5.26 ± 2.57	4.35 ± 0.5.10	4.91 ± 2.32

*Statistically significant values with $p<0.002$

M. oleifera leaves aqueous extract are needed to cause significant histo-pathology changes in the liver, kidney and heart of rats that receive a single oral daily LD_{50} dose for 30 days.

The liver tissue of aqueous extract showed congestion with scattered focal necrosis, peri-vascular lymphocytosis, and scattered mononuclear cell infiltration, with normal hepatocytes (Figure 1). These are features of mild chronic tissue inflammation. However, (Adedapo et al., 2009), observed no abnormal features in the histopatholoy examination of the liver tissue. This could have been caused by the low doses used in their study, whereby the highest dose was 2 g/kg, yet our study's highest dose was 16.1 g/kg.

The kidneys tissue showed expanded and congested glomeruli, mononuclear cellular infiltration; with fibrosis around mildly atrophied tubules; there was also a hemorrhagic interstitium with lymphocytocis (Figure 2). These are features of mild nephritis caused kidney tissue damage. This suggests that high doses of *M. oleifera* leaf extract can cause mild kidney tissue damage when given to rats for 30 days.

There was mild damage to the heart that was shown by patchy myocardial cell degeneration (apoptosis) with mild lymphocytosis (Figure 3). This finding suggests that *M. oleifera* leaves aqueous extract may be useful in conditions where there is heart muscle hypertrophy.

Conclusion

M. oleifera leaves aqueous extract given to rats orally in a single LD_{50} for 30 days is associated with moderate liver necrosis, glomerular nephritis and interstitial nephritis, chronic myocarditis, which are features of mild organ toxicity. The ethanol extract indicates safer outcomes. Therefore an oral daily LD_{50} dose of *M. oleifera* leaves aqueous extract shows features of mild sub-acute toxicity

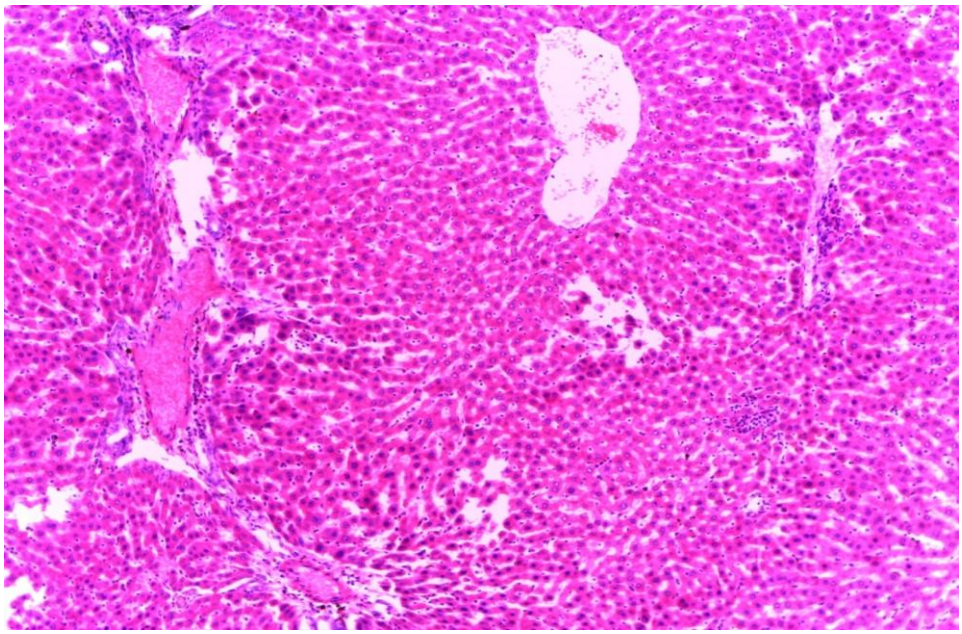

Figure 1. Photomicrograph showing histopathology of the liver of a rat after taking full lethal dose of *Moringa oleifera* leaf aqueous extract for 30 days (haematoxylin and Eosin staining, magnification x 10). There is vascular congestion with scattered focal necrosis, peri-vascular lymphocytosis, and scattered mononuclear cell infiltration.

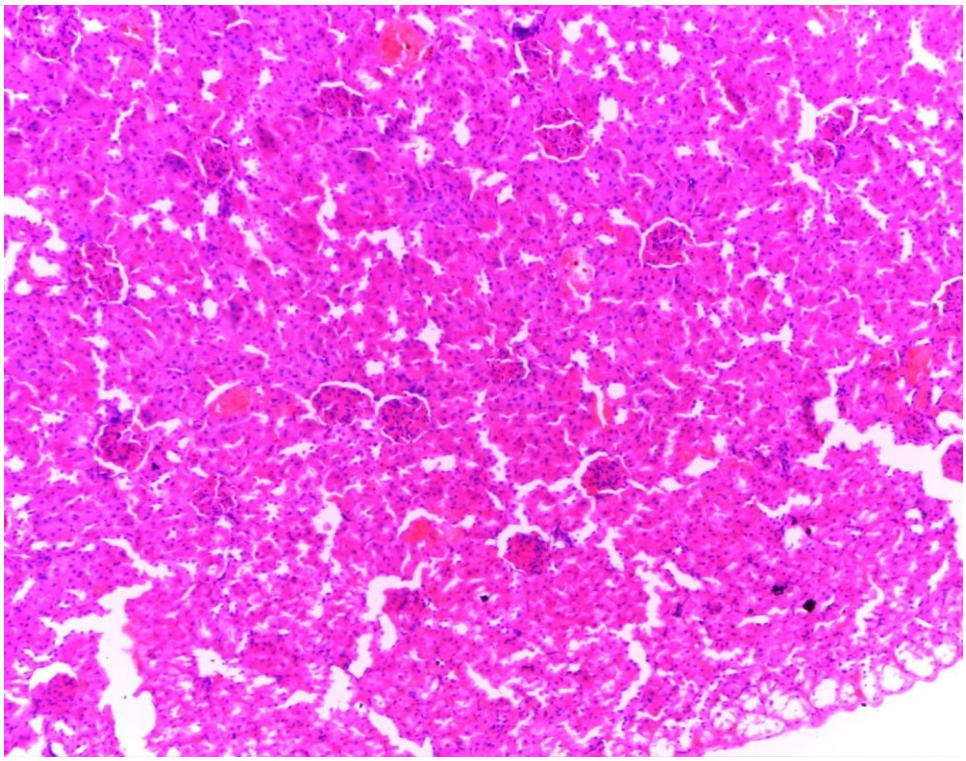

Figure 2. Photomicrograph showing histopathology of the kidney of a rat after taking after taking full lethal dose of *M.oleifera* leaf aqueous extract for 30 days (haematoxylin and Eosin staining, magnification ×10). There are features of glomerular congestion and expansion, plus mononuclear cellular infiltration; fibrosis around mildly atrophied tubules; hemorrhagic interstitium with lymphocytocis and mild fat degeneration.

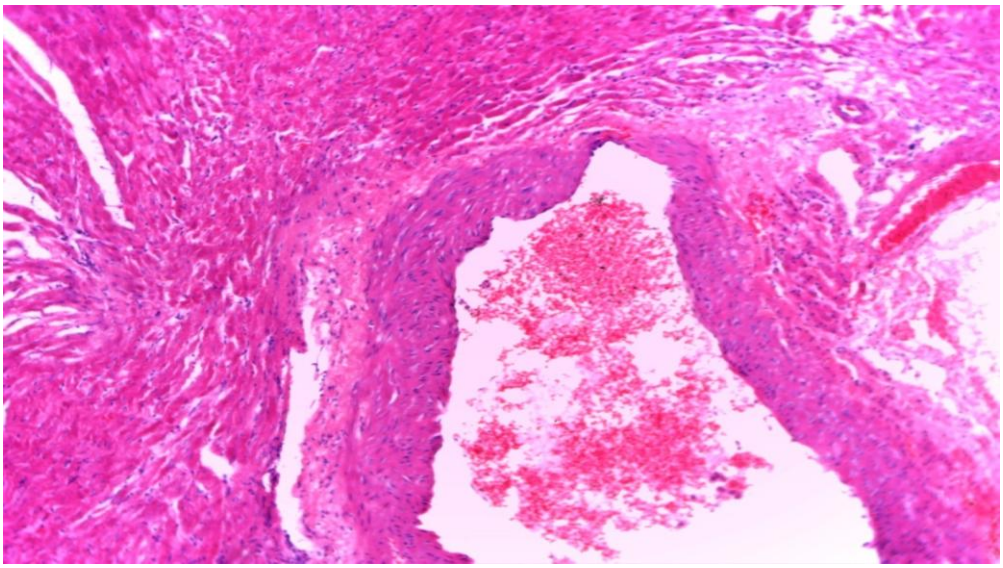

Figure 3. Photomicrograph showing histopathology of the heart after taking full lethal dose of *M. oleifera* leaf aqueous extract for 30 days (haematoxylin and Eosin staining, magnification ×10). There was myocardial cell degeneration (apoptosis) in patches with mild lymphocytosis.

in rats.

ACKNOWLEDGMENTS

Many thanks go to the members of Physiology Department Makerere University College of Health Sciences for the technical support and Carnegie Cooperation of New York for funding the study.

REFERENCES

Adedap AA, Mogbijuri OM, Emikpe BO (2009). Safety evaluations of the Aqueous extract of the Leaves of Moringa Oleifera In Rats. J. Med. Plants Res., 3: 586-591.

Adrogué HJ, Lederer Ed, Suki WE (1986). Determinants of Plasma Potassium Levels in Diabetic Ketoacidosis. Medicine, 65: 163-172.

Antai AB, Ikpi DE, Ukafia S, Agiang EA (2009). Phytochemistry and Some Haematological Changes Following Oral Administration Of Ethanolic Root Extract Of Gongronema Latifolium In Rats. Niger. J. Physiol. Sci., 24: 79-83.

Atawodi Se, Atawodi Jc, Idakwo Ga, Pfundstein B, Haubner R, Wurtele G, Bartsch H, Owen Rw (2010). Evaluation Of The Polyphenol Content And Antioxidant Properties of Methanol extracts of the leaves, Stem, and root barks of Moringa Oleifera Lam. *J Med Food.,* 13**,** 710-6.

Barminas Jt, Charles M & Emmanuel D (1998). Mineral Composition of Non-Conventional Leafy Vegetables. Plant Foods Human Nutr., 53: 29-36.

Broadhurst C, Leigh PMM Anderson R (2000). Insulin-Like Biological Activity of Culinary and Medicinal Plant Aqueous Extracts *in vitro*. J. Agric. Food Chem., p. 48.

Bush BM (1991). Interpretation of Laboratory results for Small Animal Clinicians. Oxford, Blackwell Scientific Publications.

Chirag M, Pandya M, Andrei BMD (2007). White blood cell dynamics in copd and asthma patients treated with corticosteroids. CHEST, 132: 505c-506.

Ciulei (1964). Practical Manuals on the Industrial Utilization of Medicinal And Aromatic Plants, Romania, University of Bucharest.

Cowan MM (1999). Plant products as Antimicrobial Agents. Clin. Microbiol. Rev., 12: 564-582.

Fahey J (2005). A Review Of The Medical Evidence For Its Nutritional, Therapeutic And Prophylactic Properties. Trees Life J., 1: 5.

Fuglie L (1999). The Miracle Tree: The Multiple Attributes of *Moringa*., Dakar.

Fuglie LJ (1999). The Miracle Tree: Moringa Oleifera: Natural Nutrition For The Tropics. Church World Service, Dakar. 68 Pp; Revised In 2001 And Published As: The Miracle Tree: The Mult Atributes of Moringa, p. 172.

Kasolo JN, Bimenya GS, Ojok L, Ochieng J, Ogwa-Okeng JW (2010). Phytochemicals And Uses Of Moringa Oleifera Leaves In Ugandan Rural Communities. . *J. Med. Plants Res.,* 4: 753-757.

Kasolo JN, Bimenya GS, Ojok L, Ogwal- Okeng JW (2011). Phytochemicals and Acute Toxicity of *Moringa Oleifera* Roots in Mice. J. Pharm. Phytother., 3: 38-42.

Kasolo JN, Bimenya GS, Okwi Al, Othieno EM, Ogwal-

Okeng JW (2012). Acute Toxicity Evaluation of *Moringa*

Kasolo JN, Bimenya GS, Okwi AL, Othieno EM, Ogwal-Okeng JW (2012) Oleifera Leaves Extracts of Ethanol and Water in Mice. Afr. J. Anim. Biomed. Sci., 7(1): 40-44.

Kraut JA, Madias NE (2012). Treatment of Acute Metabolic Acidosis: A Pathophysiol. Approach Nat Rev. Nephrol., 2012. 8(10): 589-601

Lockelt CT Calvert CC, Grivetti L (2000). Energy And Micronutrient Composition Of Dietary And Medicinal Wild Plants Consumed During Drought. Study of Rural Fulani, Northeastern Nigeria. Int. J. Food Sci. Nutr., 51: 195-208.

Nambiear VS, Seshadri S (2001). Bioavailability Trials of Beta-Carotine from fresh and Dehydrated Drumstick Leaves (Moringa Oleifera) In a Rat Model. Plant Foods Hum. Nutr., 56: 83-95.

Seshadri SNVS (2003). Kanjero (Digera Arvensis) And Drumstick Leaves (*Moringa Oleifera*): Nutrient Profile And Potential For Human Consumption. World Rev. Nutr. Diet., 91**,** 41-59.

Sharma RK, Rai DKCS, Mehta S, Rai PK, Singh RK, Watal G, Sharma B (2009). Antioxidant Activities And Phenolic Contents Of The Aqueous Extracts Of Some Indian Medicinal Plants. J. Med. Plants Res., 3: 944-948.

Siddhuraju PK Becker (2003). Antioxidant Properties Of Various Solvent Extracts of Total Phenolic Constituents From Three Different Agroclimatic Organs of Drugstick Tree (*Moringa Oleifera* Lam) Leaves. J. Agric. Food Chem., 51: 2144-2155.

Subadra S, Monicaj ET (1997). Comparison of Antmacrobial Activity of Beta-Carotine In Dehydrated Drumstic Leaves (*Moringa Oleifera*). Int. J. Food Sci Nutri., 48: 373-379.

Testerman SK, Mitchell CO, Smith MA (1995). The Effect of Correction of Metabolic Acidosis on Calcium and Protein Metabolism In Patients on Hemodialysis. J. Am. Diet. Assoc., 95: A20.