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Full Length Research Paper

Safety evaluations of the aqueous extract of the leaves of *Moringa oleifera* in rats

A. A. Adedapo[1]*, O. M. Mogbojuri[1] and B. O. Emikpe[2]

[1]Department of Veterinary Physiology, Biochemistry and Pharmacology, University of Ibadan, Nigeria.
[2]Department of Veterinary Pathology, University of Ibadan, Nigeria.

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The aqueous extract from the leaves of *Moringa oleifera* was evaluated for its oral toxicity by the oral route, and for the sub-acute toxicity on haematological, biochemical and histological parameters in rats. In the acute toxicity test, *M. oleifera* extract caused no death in animals even at 2000 mg/kg dose. Oral treatments in rats with this extract at 400, 800 and 1600 mg/kg caused varied significant changes in the total RBC, packed cell volume (PCV), haemoglobin percentage (HB), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), total and differential WBC. The extract did not cause any significant change in the level of platelets. In the biochemical parameters, the extract at different doses also caused varied significant changes in the levels of total proteins, liver enzymes, and bilirubin. Clinico-pathologically, changes were also noted in the body weights, slight dullness at the onset of extract administration and no significant changes were noticed in all the organs examined in the course of this study. The study concluded that the plant is relatively safe both for nutritional and medicinal uses.

Key words: *Moringa oleifera*, haematology, histopathology, serum chemistry, rats, mice.

INTRODUCTION

Moringa oleifera Lam. is the most widely cultivated species of the monogeneric family Moringaceae (order Brassicales), which includes 13 species of trees and shrubs distributed in sub-Himalayan ranges of India, Sri Lanka, North-eastern and South-western Africa, Madagascar and Arabia (Fahey, 2005). *M. oleifera* is one of the most useful tropical trees. The relative ease with which it propagates through both sexual and asexual means and its low demand for soil nutrients and water after being planted makes its production and management easy. Introduction of this plant into a farm which has a biodiverse environment can be beneficial for both the owner of the farm and the surrounding eco-system (Foidl et al., 2001).

The Moringa tree is a multi-function plant. It has been cultivated in tropical regions all over the world for the following characteristics: 1) high protein, vitamins, mineral and carbohydrate content of entire plants; high value of nutrition for both humans and livestock; 2) high oil content (42%) of the seed which is edible, and with medicinal uses; 3) the coagulant of seeds could be used for wastewater treatment (Foidl et al., 2001). This plant has been well documented for its medicinal importance for a long time. The stem bark, root bark, fruit, flowers, leaves, seeds and gum are widely used in Indian folk medicine. The pods and seeds are tastier while they are young and before they turn brown. In Malaysia, the young tender pods are cut into small pieces and added to curries (Abdulkarim et al., 2005).

In terms of phytochemistry, this plant family is rich in compounds containing the simple sugar, rhamnose, and it is rich in a fairly unique group of compounds called glucosinolates and isothiocyanates. For example, components of Moringa preparations that have been reported to have hypotensive, anticancer, and antibacterial activity include 4-(4'-*O*-acetyl-α-L-rhamnopyranosyloxy)benzyl isothiocy-anate**,** 4-(α-L-rhamnopyranosyloxy)benzyl isothiocy-anate**,** niazimicin, pterygospermin**,** benzyl isothiocyanate**,** and 4-(α-L-rhamnopyranosyloxy) benzyl glucosinolate. While these compounds are relatively unique to the Moringa family, it is also rich in a number of vitamins and minerals as well as other more commonly recognized phytochemicals such as the carotenoids,

*Corresponding author. E-mail: adedapo3a@yahoo.co.uk. Tel.: +234 802 3928 512.

as other more commonly recognized phytochemicals such as the carotenoids, including β-carotene or provitamin A (Caceres et al., 1991; Caceres et al., 1992; Akhtar and Ahmad, 1995; Bharah et al., 2003; Fahey, 2005).

The study therefore seeks to assess the leaves of *M. oleifera* for safety or possible toxic effects using haematology, serum chemistry and histopathological changes as indices of toxicosis. Acute toxicity testing in rats will also be employed in this study. It is expected that the findings from this work may add to the overall value of the medicinal and nutritional potential of the plant.

MATERIALS AND METHODS

Plant collection and extract preparation

The pulverized leaves of the plant were obtained from the International Institute of Tropical Africa (IITA), Ibadan, Nigeria. The powder products (IITA/08/894) in sachet are sold commercially to people for medicinal and nutritional purposes. The ground materials (135 g) were dissolved in warm water (2250 ml) for easy dissolution. It was thereafter filtered using a Buckner funnel and Whatman's No. 1 filter paper. It was the filtrate that was administered to the animals in the course of this study.

Animals

The animals used in this study were 54 male Wistar rats (85 - 130 g). Only male rats were used in this study because one was also looking at the possible effects of this extract on the testis. They were maintained at the Experimental Animal House of the Faculty of Veterinary Medicine, University of Ibadan. They were kept in rat cages and fed on commercial rat cubes (Ladokun and Sons Livestock Feeds, Nigeria Ltd.) and allowed free access to clean fresh water in bottles *ad libitum*. At the start of the experiment, all the animals were weighed and subsequently at weekly intervals. All experimental protocols were in compliance with University of Ibadan Ethics Committee on Research in Animals as well as internationally accepted principles for laboratory animal use and care.

Acute toxicity study

The acute toxicity of *M. oleifera* aqueous extract was determined according to the method of Sawadogo et al. (2006). Rats fasted for 16 h were randomly divided into 5 groups of six per group. Graded doses of the extract (400, 800, 1600 and 2000 mg/kg p.o.) corresponding to groups B, C, D and E were separately administered to the rats in each of the 'test' groups by means of bulbed steel needle. The control group (group A) was treated with orally administered distillated water (3 ml/kg p.o.) only. All the animals were then allowed free access to food and water and observed over a period of 48 h for signs of acute toxicity. The number of deaths within this period of time was recorded.

Sub-acute toxicity study

Using a modified method of Cruz et al. (2006), the rats were divided at random into four groups of six rats each per group. While the control group representing group A received distillated water, the experimental groups representing groups B, C, and D received aqueous extract at the doses of 400, 800 and 1600 mg/kg, respectively. The extract was administered orally by means of bulbed steel needle for 21 days. All the animals were weighed on the first day and thereafter weekly till the end of the experiment.

Collection of blood and serum samples

Paired blood samples were collected by cervical decapitation from diethyl ether anaesthetized rats into heparinised bottles for haematological studies; blood samples collected in clean non-heparinised bottles were allowed to clot. The serum was separated from the clot and centrifuged into clean bottles for biochemical analysis.

Determination of haematological and serum biochemical parameters

Packed cell volume (PCV) and haemoglobin concentration were determined by conventional method (Duncan et al., 1994). Erythrocyte count, total leucocytes and leucocytes differential counts were also determined as described by Coles (1986). Erythrocyte indices-mean corpuscular values (MCV), mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular haemoglobin (MCH) were determined from values obtained from RBC count, haemoglobin concentration and PCV values (Duncan et al., 1994).

Total protein was measured using biuret reaction (Lanzarot et al., 2005) while albumin was measured by colorimetric estimation using the sigma Diagnostics albumin reagent (Sigma® Diagnostic, U.K.), which contained bromocresol green (BCG). Globulin was obtained from the difference between total protein and albumin. Aspartate aminotransferase (AST), alkaline phosphatase (ALP) and alanine aminotransferase (ALT) were determined using a photoelectric colorimeter (Gallenkamp® and Sons Ltd.; England) as described by Toro and Ackermann (1975); Duncan et al. (1994); GGT activity was determined using a 747/737 BM/Hitachi autoanalyzer by the method of Bergmeyer et al. (1986), Serum urea and creatinine levels were determined using photoelectric colorimeter (Gallenkamp® and Sons Ltd. England) as described by Toro and Ackermann (1975); Coles (1986).

Histopathology

The liver, kidney, and testes of all the animals were fixed in 10% buffered formalin in labeled bottles. Tissues were processed routinely and embedded in paraffin wax. Sections of 5 µ thickness were cut, stained with haematoxylin and eosin and examined under the light microscope (Figures 1-3).

Statistical analysis

Results were expressed as mean ± standard deviation (S.D.). Where applicable, the data were subjected to one way analysis of variance (ANOVA) and differences between samples were determined by Duncan's Multiple Range test using the Statistical Analysis System (SAS, 1999) program. P values at 5% were regarded as significant.

RESULTS AND DISCUSSION

Acute toxicity studies in rats showed that no mortality was recorded in any of the groups even at 2000 mg/kg dose. The behavioral change noted in these animals following extract administration was slight dullness at the onset of

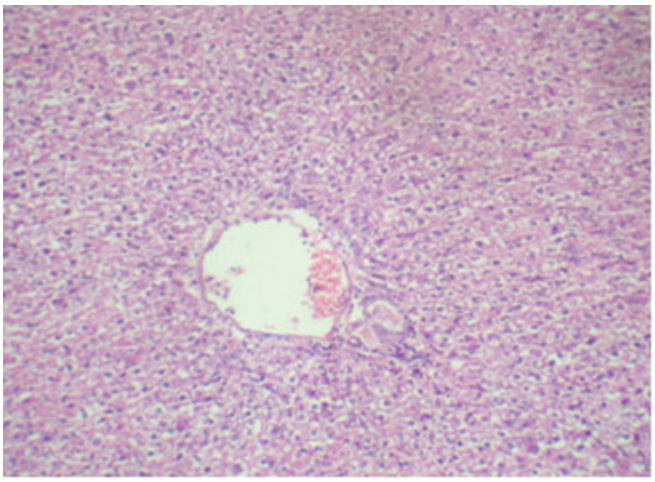

Figure 1. A photomicrograph of the liver of rat showing diffuse hepatic degeneration. Magnification: ×160 H & E.

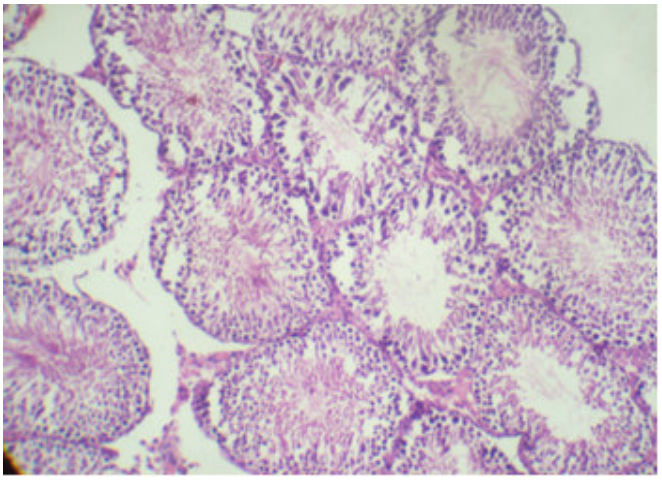

Figure 2. A photomicrograph showing no visible lesion in the testis of rat. Magnification: ×160 H & E.

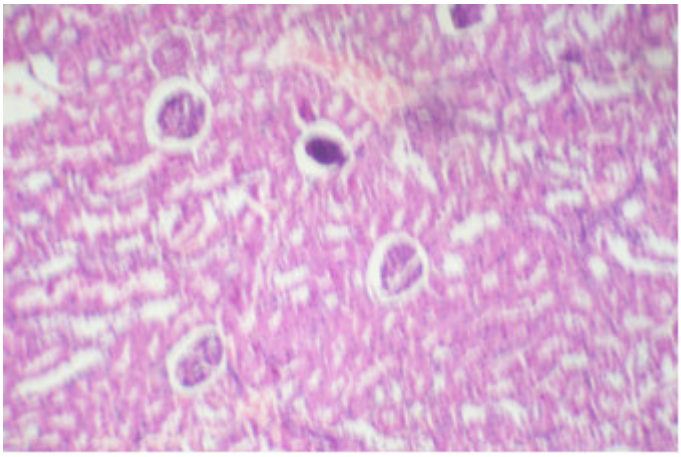

Figure 3. A photomicrograph of the kidney of rat showing no visible lesion. Magnification: ×160 H & E.

extract administration. The animals later become active after some hours of extract administration (Table 1). The acute toxicity study in rats showed that at 2000 mg/kg dose, the plant is safe for consumption and for medicinal uses. At doses above this level however, the animals may exhibit some toxic changes.

In the sub-acute toxicity study, the 400 mg/kg dose of the extract caused significant increase in the level of PCV while the other 2 doses caused significant decrease. The 800 mg/kg dose on the other hand caused significant decrease in the levels of haemoglobin and red blood cell counts while the other 2 doses caused insignificant changes (Table 2). The study therefore showed that the plant could precipitate some level of anaemia if the animals are exposed to this plant for a long period of time. The varied changes of the effects of this plant extract on the haematological parameters may be attributable to the presence of isothiocyanate producing glycosides (Fahey, 2005). Glycosides are ethers that link a sugar to a toxin called aglycone. Either the glycoside or the aglycone alone may be toxic. The glycosides include cyanogenic glycosides. It is generally believed that the toxic properties associated with cyanogenic glycosides, such as linamarin are due to the hydrocyanic acid released from the glycosides by the activity of an enzyme complex. Acute poisoning by hydrocyanic acid (HCN) or prussic acid causes a histotoxic anoxia with a syndrome of dyspnoea, tremor, convulsions and sudden death. Toxicity of hydrogen cyanide (HCN) occurs after ingestion and absorption. Once they are in the bloodstream, there is little difference between toxic and lethal levels of cyanide. HCN has a high affinity for iron and reacts with the trivalent iron of mitochondrial cytochrome oxidase, the terminal respiratory catalyst linking oxygen with metabolic respiration. Cell anoxia is immediate (Cheville, 1988). Treatment of this poisoning is aimed at “fixing” the highly lethal cyanide ion in a harmless form, and then converting it into thiocyanate, which is readily excreted by the kidneys. Sodium nitrite can also be administered intravenously to convert some hemoglobin into methaemoglobin. Cyanide combines readily with methaemoglobin to form the non-toxic cyanomethaemoglobin. Sodium thiosulphate is then administered to act as a sulphur-donor for the conversion of the cyanide moiety of cyanomethaemoglobin to thiocyanate under the action of the enzyme rhodanase (Adedapo, 2002). Since this plant is rich in isothiocyanate, it could therefore play the same role as sodium thiosulphate to act as a sulphur donor for the conversion of cyanide moiety of cyanomethaemoglobin to thiocyanate under the action of the enzyme rhodanase. The plant extract did not produce any significant changes in the platelets.

The study showed that the extract particularly the 400 and 800 mg/kg doses caused significant increase in the level of white blood cell counts and its differentials (Table 2). This observation of increase in the levels of these parameters by this plant extract shows that the principal

Table 1. Acute toxicity study in rats after 48 h of administration of aqueous extract of *M. oleifera*. (n=6).

Group	Dose (mg/kg)	T/D *	Period of signs observation (h)	Signs of toxicity observed
A	3 (distillated water)	6/0	48	-
B	400	6/0	48	No toxic changes observed.
C	800	6/0	48	No toxic changes observed.
D	1600	6/1	48	Slight dullness was observed in the animals in the first 5 h of extract administration, but after this period they became normal.
E	2000	6/2	48	Slight dullness was observed in the animals in the first 5 h of extract administration, but after this period they became normal.

*T/D: number of mice treated/number of deaths.

Table 2. Effects of the graded doses of the aqueous extracts of *M. oleifera* on haematological parameters of rats (n =6).

Parameters	Control (A)	400mg/kg (B)	800mg/kg (C)	1600mg/kg (D)
PCV (%)	41.1 ± 3.2	45.3 ± 1.6[a]	29.5 ± 3.9[a]	35.0 ± 3.7[a]
Hb (g/L)	13.4 ± 1.3	14.0 ± 1.7	9.8 ± 1.3[a]	13.0 ± 0.5
RBC ($X10^{12}$/L)	6.9 ± 0.4	7.4 ± 0.1	4.7 ± 0.4[a]	6.3 ± 0.4
MCV (fl)	60.0 ± 3.0	61.0 ± 1.5	62.7 ± 6.4	55.3 ± 3.8[a]
MCH (pg)	19.5 ± 0.8	18.8 ± 2.2	20.8 ± 2.2	20.6 ± 1.1
MCHC (%)	32.6 ± 2.1	30.8 ± 3.3	33.2 ± 1.8	37.4 ± 3.5[a]
WBC ($X10^9$/L)	9.0 ± 0.3	10.2 ± 1.3[a]	10.1 ± 0.3[a]	9.0 ± 0.1
Lymphocytes($x10^9$/L)	6.3 ± 0.3	6.9 ± 0.7[a]	6.9 ± 0.6[a]	6.2 ± 0.8
Neutrophils ($x10^9$/L)	2.4 ± 0.1	2.9 ± 0.5[a]	2.9 ± 0.5[a]	2.5 ± 0.2[a]
Monocytes ($x10^9$/L)	0.2 ± 0.1	0.3 ± 0.2[a]	0.5 ± 0.2[a]	0.2 ± 0.1
Eosinophils ($x10^9$/L)	0.1 ± 0.01	0.1 ± 0.01	0.1 ± 0.01	0.1 ± 0.01
Platelets (µL)	115875 ± 9623	104574 ± 46219	95708 ± 44397	109000 ± 6066

Superscripted items indicate significant values ($P < 0.05$) from control.
Note: Mean ± S.D

function of phagocytes, which is to defend against invading microorganisms by ingesting and destroying them, thus contributing to cellular inflammatory processes, will be enhanced (Paul, 1993; Swenson and Reece, 1993; Adedapo et al., 2005) which may account for its antibacterial activity. (Caceres, 1991; Fahey, 2005).

The study showed that the 400 and 1600 mg/kg doses of the extract caused significant decrease in the levels of total protein and globulin while the 800 mg/kg dose on the other hand caused a significant increase in the levels of these parameters. It was the 1600 mg/kg that caused a significant decrease in the level of albumin. The other 2 doses caused no significant changes (Table 3). The reduction of serum levels of protein is an indication that toxicants such as isothiocyanate and glycoside cyanides may cause stress-mediated mobilization of protein to cope with the detrimental condition so imposed (Das and Mukherjee, 2000). The protein so mobilized is one of the strategies employed to meet the energy required to sustain increased physical activity, biotransformation and excretion of the toxicants. The significant increase in the level of globulin caused by the 800 mg/kg dose of the extract further support the antimicrobial action of this plant because globulins are principally responsible for both the natural and acquired immunity that an individual has against invading organisms (Lawrence and Amadeo, 1989). The 1600 mg/kg dose of the extract caused significant decrease in the level of albumin. Albumin apart from being a useful indicator of the integrity of glomerular membrane is also important in determining the severity of disease (Adedapo et al., 2005). Decrease albumin may be due primarily to reduction in synthesis by the liver and secondarily to reduced protein intake which further confirms hepatic damage (Luskova et al., 2002; Jyotsna et al., 2003).

The 400 and 1600 mg/kg doses of the plant extract caused significant increase in the levels of ALT and AST but the 800 mg/kg dose actually caused significant decrease in the levels of these liver enzymes. The 1600 mg/kg dose caused significant increase in the level of ALP whereas the other doses caused insignificant changes (Table 3). Aminotransferases (ALT and AST) are produced in the liver and are good markers of damage to liver cells but not necessarily the severity of the damage

Table 3. Effects of the graded doses of *M. oleifera* on the serum biochemical parameters of rats (n = 6).

Parameters	Control (A)	400mg/kg (B)	800mg/kg (C)	1600mg/kg (D)
Total protein (g/dL)	6.6 ± 0.1	5.7 ± 0.2[a]	7.0 ± 0.2 [a]	6.0 ± 0.2 [a]
Albumin (g/dL)	3.2 ± 0.4	3.3 ± 0.4	3.2 ± 0.4	2.9 ± 0.3 [a]
Globulin (g/dL)	3.4 ± 0.3	2.4 ± 0.2 [a]	3.8 ± 0.4 [a]	3.1 ± 0.4 [a]
ALT (U/L)	14.8 ± 3.7	20.7 ± 2.7 [a]	7.1 ± 2.1 [a]	19.4 ± 4.2 [a]
AST (U/L)	13.2 ± 1.9	19.8 ± 2.3 [a]	7.0 ± 1.7 [a]	18.8 ± 1.3 [a]
ALP (U/L)	28.1 ± 14.5	19.0 ± 6.5	42.7 ± 13.9	47.4 ±18.7 [a]
Bilirubin (mmol)	0.1 ± 0	0.1 ± 0	0	0.1 ± 0
Urea (mg/dL)	11.3 ± 1.5	9.5 ± 1.0 [a]	0.1 ± 0 [a]	10.2 ± 1.3 [a]

Superscripted items indicate significant values ($P < 0.05$) from control.
Note: Mean ± S.D

Table 4. Effects of the graded doses of *M. oleifera* on the body weights of rats. X ± S.D. (n = 6)

Parameters	Control	400 mg/kg	800 mg/kg	1600 mg/kg
Weight before extract administration (g)	101.7 ± 4.1	108.3 ± 4.1	121.7 ± 9.8	103.3 ± 5.2
Weight after 21 days	155 ± 13.8	148 ± 7.5	155 ± 13.8	122 ± 19.4
% Difference in weight	52.4	36.7	27.4	18.1

(Rej, 1989). They are normally present at low levels in the blood so if the liver cells are damaged, it would be expected that some of the enzymes leak into the blood and increase in levels. Increase in serum level of AST as observed in this study may reflect damage of liver cells. Serum ALT is known to increase in liver disease and it has been used as a tool for measuring hepatic necrosis (Bush, 1991). Increase in serum ALP as shown by the 1600 mg/kg dose of the extract may be considered as a sensitive indicator of cholestasis in early stages or mild circumstances preceding other indicators such as hyperbilirubinemia (Bush, 1991). The significant decrease in the level of these parameters especially with the 800 mg/kg dose may be an indication that this may be the safest dose to use when administering this extract for medicinal purpose. In this study, the extract did not cause any significant change in the level of bilirubin but caused significant decrease in the level of urea (Table 3). Bilirubin is a breakdown product of the haeme component of the haemoglobin molecule. Total serum bilirubin is elevated in animals with a haemolytic anaemia, and this increase is caused largely by an increase in the indirect-reacting bilirubin. The degree to which bilirubin is elevated in haemolytic anaemia is a function of the rate of red cell destruction and the capacity of the liver to excrete the newly formed bilirubin (Tripathi, 2003). Urea is one of a number of non-protein nitrogenous substances that accumulate in the plasma when renal excretion is reduced. Causes of increased blood urea levels include: high protein diet, intestinal haemorrhage, dehydration, severe haemorrhage, shock, etc. Urea level could be decreased due to the following: liver failure, low protein diet, anabolic steroids, diabetes insipidus, etc (Bush, 1991).

The study showed that all the animals used in the study gained weight (Table 4). It is interesting to note however that the animals in the control group gained more weight compared to the animals in the experimental groups. Weight gained for the experimental animals however decreased with graded doses. This may have implication when it comes to searching for medicinal plants with active compounds that can help reduce weight gain. Consumption of this plant may have tremendous impact on subjects suffering from hypertriglyceridemia. Organ pathology showed that no significant lesions were observed in this study and this may point to the fact that this plant is relatively safe for use nutritionally and medicinally.

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