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Acute toxicity study, phytochemical and elemental analyses of root extract of *Moringa oleifera* Lam (horseradish tree)

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Abstract

Medicinal plants are used for the treatment of many disorders, but a good number of them have been implicated in causing toxicities. This study evaluated the acute toxicity of the root extract of *Moringa oleifera* Lam (horseradish tree) administered via the oral and intra-peritoneal routes, and also conducted phytochemical and elemental analyses of it. Extraction was with methanol, following the Soxhlet extraction method. The acute toxicity and phytochemical and elemental analyses were done following standard procedures. In the first step of the acute toxicity study, albino rats were randomly assigned to 3 groups of three rats for each route and then treated with methanol root extract of *M. oleifera* at doses of 10, 100 and 1000 mg/kg, and the rats were observed for clinical signs of toxicity and mortality for 24 hours. In the second step of the acute toxicity study, the rats received 1600 mg/kg, 2900 mg/kg and 5000 mg/kg and they were also monitored for 24 hours for clinical signs and mortality. The phytochemical analysis was qualitative, while the elemental analysis was done by flame emission spectrometry and atomic absorption spectrometry, as appropriate. The LD_{50} for the oral administration was above 5,000 mg/kg, but the LD_{50} for the intra-peritoneal administration was 1,264 mg/kg. The phytochemical analysis revealed the presence in the extract of carbohydrates, cardiac glycosides, flavonoids, tannins, saponins and terpenoids, while the elemental analysis showed that the root extract contained the following elements at these specified concentrations: Zinc – 1.0184 mg/dl, Magnesium – 0.07227 mg/dl, Iron – 0.05959 mg/dl, Copper – 0.00582 mg/dl; Cobalt – 0.00568 mg/dl, Manganese – 0.00547 mg/dl; Lead – 0.00143 mg/dl; Chromium – 0.00302 mg/dl; and Cadmium – 0.00003 mg/dl. It was concluded that the *M. oleifera* root extract is acutely safe when administered via the oral route, and that it contains some bioactive compounds and essential minerals.

Keywords: Acute toxicity**;** Phytochemical analysis; Elemental analysis; *Moringa oleifera;* Methanolic root extract.

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Introduction

The study of medicinal plants has generated reasonable interest all over the world, hence, the World Health Organization (WHO) encourages and supports countries to identify and provide safe and effective remedies from the use of these plants in the public and private health services (Sofowora, 2008). Medicinal plants are useful for the treatment of many disorders, but a good number of them have been implicated in causing toxicities (Okpako, 2002). Toxicity or poisoning according to Clark and Clark (1979) is the result of adverse effects of chemical substances on living organisms.

Moringa oleifera Lam also known as *Moringa pterygosperma* belongs to the monogeneric family of shrubs and trees (Marcu, 2006), called the Moringaceae – a single genus of 14 known species (Ram, 1994). It is said to have originated from Agra and Oudh in the North West region of India, south of the Himalayan Mountains (Marcu, 2006). It is a common plant in the northeast and middle belt regions of Nigeria and has enormous use traditionally in these areas which earned it the name "Miracle Tree". The *Moringa oleifera* tree has great use medicinally both as preventive and treatment agent. India's ancient tradition stated that the leaves of the Moringa tree can prevent about 300 different diseases. Besides, it has been reported to have various biological activities including blood cholesterol regulation, regulation of thyroid hormone status, blood pressure control, anti-inflammation, and also as therapy for diseases of the liver (Ojo *et al.,* 2012).

The restorative and health benefits of the roots of *Moringa oleifera* were documented by Ayurvedic practitioners in India for centuries, who used it to treat a wide variety of ailments. They are especially useful in controlling disorders of the circulatory system including minor cardiovascular complaints. In small doses, it can be used to stimulate appetite and improve the functions of the digestive system making it useful for individuals with gastric upset and irritable bowel syndrome (Fahey, 2009). Additionally, the roots have been used in controlled doses to treat impotence, sexual dysfunction and female reproductive tract disorders. In poultice, the roots of *Moringa oleifera* are used for cramps and arthritis pains. It has also been shown to have diuretic and antiseptic qualities (Fahey, 2009). Though locally consumed, there is paucity of information in available literature on the root of *Moringa oleifera* with respect to its nutritional and medicinal potentials and toxicity. *Moringa oleifera* has been extensively studied, yet there is a need for more information on the safety/toxicity of the root, and its phytochemical and elemental constituents. This study evaluated the acute toxicity of the methanol root extract of *Moringa oleifera* Lam (horseradish tree) administered via the oral and intra-peritoneal routes, and also conducted its phytochemical and elemental analyses.

Materials and Methods

The roots of *Moringa oleifera* used for the study were collected within the University of Maiduguri Quarters, Borno State, Nigeria. The samples were taken to the Department of Biological Sciences, University of Maiduguri, Nigeria, where they were identified and authenticated by a Taxonomist at Botany Unit and a voucher specimen No. VPP/12/002 was prepared and deposited in the Department of Veterinary Physiology and Biochemistry, University of Maiduguri, Nigeria. The samples were then dried under shade for seven days and then ground into coarse form, and stored at room temperature until extracted.

Extraction of the Plant Materials: The Soxhlet extraction method was adopted in this study. The extraction was carried out using methanol

of analytical grade. One thousand gramme of the ground *M. oleifera* root was extracted using methanol according to the method described by Harwood and Moody (1989), as modified by Usman *et al. (*2007). The extract was then filtered and concentrated at low pressure to obtain a mass called crude methanol extract (CME) and thereafter, kept in a tight container in a refrigerator at 4 °C.

Preliminary Phytochemical Screening: To 4 g of the extract, 50 ml of distilled water was added and boiled on a hot plate for 3 minutes. The mixture was then filtered using Whatman filter paper No. 1 while hot and the resulting filtrates were allowed to cool. The filtrates obtained were used for the following carbohydrate tests:

General test (Molisch's Test): A few drops of Molisch's reagent were added to the extract dissolved in distilled water. This was followed by addition of 1 ml of concentrated sulphuric acid by the side of the test tube so that the acid formed a layer beneath the aqueous layer. The mixture was allowed to stand for two minutes and then diluted with 5 ml distilled water. Formation of a red or dull violet colour at the interphase of the two layers indicated a positive test (Evans, 2009).

Test for Reducing Sugars (Fehling's Test): About 0.2 g of the extract was dissolved in distilled water and filtered. The filtrate was then heated with 5 ml of equal volumes of Fehling's solutions A and B. Formation of a red precipitate of cuprous oxide (CH_20) was an indication of reducing sugars (Evans, 2009).

Test for Combined Reducing Sugars: The extract (0.2g) was hydrolyzed by boiling with 5 ml of dilute hydrochloric acid and the resulting solution neutralized with sodium hydroxide solution. A few drops of Fehling's solution were added to it and then heated on a water bath for 2 minutes. Formation of a brick red precipitate indicated the presence of combined reducing sugars (Evans, 2009).

Test for Tannins: The extract (0.5 g) to be tested was stirred with 10ml of distilled water and then filtered. The filtrate was used for the following tests: to 2 ml of the filtrate, a few drops of 1% ferric chloride solution were added; occurrence of a blue-black, green or blue precipitate indicated the presence of tannins. A mixture of equal volume of 10% lead ethanoate was also added to 2 ml of the filtrate; formation of a white precipitate was an indication of the presence of tannins. The filtrate was boiled with 3 drops of 10% hydrochloric acid and 1 drop of methanol, a red precipitate was taken as evidence of the presence of tannins (Evans, 2009).

Test for Anthraquinones: *Free (Borntragers) test*. The extract (0.5 g) tested was shaken with 10 ml of benzene and then filtered. Five millimeters of 10% ammonia solution was then added to the filtrate. The mixture was shaken and appearance of a pink, red or violet colour in the ammonia (lower) phase was indicative of the presence of free anthraquinones (Evans, 2009).

Test for Combined Anthraquinones (Borntrager's Test): The extract (0.5 g) was shaken with 10 ml of H_2S0_4 and then filtered while hot. The filtrate was shaken with 5 ml of benzene. The benzene layer separated and half its own volume of 10% ammonia solution was then added. The presence of a pink, red or violet coloration in the ammonical (lower) phase was an indication of combined anthraquinones (Evans, 2009).

Test for Cardiac Glycosides: Salkowski's Test - The extract (0.5 g) was dissolved in 2ml of chloroform. Sulphuric acid was carefully added by the side of the test tube to form a lower layer. Appearance of a reddish brown colour at the interphase was an indication of the presence of a steroidal ring (that is aglycone portion of cardiac glycoside) or methylated sterols (Silva *et al.,* 1998).

Test for Cardiac Glycosides: Leiberman-Burchard's Test: To 0.5 g of the extract, 2 ml of acetic anhydride was added. Sulphuric acid was added carefully; colour development from violet to blue or bluish green was an indication of the presence of a steroidal ring i.e. aglycone portion of cardiac glycoside (Silva *et al.,* 1998).

Test for Terpenoids: A little of the extract was dissolved in ethanol. To it 1 ml of acetic anhydride was then added followed by the addition of concentrated H_2S0_4. A colour change from pink to violet showed the presence of terperoids (Silva *et al.,* 1998).

Test for Saponins: One gramme of the extract was added to 5 ml of distilled water, filtered and the filtrate divided into 2 portions. To the first portion, 3 ml of distilled water was added and then shaken for 5 minutes. Frothing which persisted on warming was an evidence for the presence of saponins (Sofowora, 2008). To the second portion, 2.5 ml of a mixture of equal volumes of Fehling's solutions A and B was added; appearance of a brick red precipitate indicated the presence of saponin glycoside (Vishnoi, 1979).

Test for Flavonoids: Shinoda's Test - About 0.5 g of the extract was dissolved, warmed and then filtered. Three pieces of magnesium chips were added to the filtrate followed by a few drops of concentrated hydrochloric acid. A pink, orange or red to purple colouration indicated the presence of flavonoids (Markham, 1982).

Test for Flavonoids: Ferric Chloride Test - Two millilitres (2ml) of the filtrate was prepared and a few drops of 10% ferric chloride solution was added. A green blue or violet colouration indicated the presence of flavonoids (Evans, 2009).

Test for Flavonoids: Lead Acetate Test - To five millilitres (5ml) of the extract, 10% lead acetate solution was added and observed for a coloured precipitate, which indicated the presence of flavonoids (Evans, 2009).

Test for Flavonoids: Sodium Hydroxide Test - Five millilitres (5ml) of $20^{o}/_{o}$ sodium hydroxide was added to 5ml the extract. A yellow colouration indicated the presence of flavonoids (Evans, 2009).

Preliminary Test for Alkaloids: The extract (0.5 g) was mixed with 10 ml of 1% aqueous hydrochloric acid on a water bath and then filtered. 3 ml of the filtrate was taken and divided equally into 3 portions in a test tube. To the first portion, a few drops of Dragendroffs' reagent were added; the occurrence of an orange-red precipitate was taken as positive for alkaloids. To the second portion, 1ml Mayer's reagent was added and appearance of a buff-coloured precipitate was an indication of the presence of alkaloids. To the last 1ml, a few drops of Wagner's reagent was added and a dark brown precipitate indicated the presence of alkaloids (Brain and Turner, 1975).

Experimental Animals: A total of 24 adult Wistar albino rats were used for acute toxicity study for both oral and intra-peritoneal route. They were kept in plastic rat cages at the Animal House of the Department of Veterinary Physiology and Biochemistry, Faculty of Veterinary Medicine, University of Maiduguri. They were allowed to acclimatize to the laboratory environment for one week before the commencement of the experiment. The care and use of the animals for this experiment followed the standard procedure for the care and use of rats for experiments. All the rats were handled according to the International Guiding Principles for Biomedical Research Involving Animals (CIOMS and ICLAS, 2012). Throughout the experimental period, the rats were provided with water *ad libitum and* fed with standard pelletized vital feed, (Grand Cereals Ltd, Jos, Plateau State, Nigeria).

Acute Toxicity Study: The acute toxicity study was done following the two-step procedure as described by Lorke (1983). In this study, the extracts were administered via two different

routes: oral and intra-peritoneal routes. In the first step, nine albino rats were randomly assigned to three groups of three rats, for each route and then treated with methanol root extract of *M. oleifera* at doses of 10, 100 and 1000 mg/kg body weight and observed for 24 hours for mortality and general behavioral changes. Based on the outcome of the first step, three groups containing one rat per group were used in the second step for the same routes of administration. Rats used for the second step were given the extract at a dose rate of 1600 mg/kg, 2900 mg/kg and 5000 mg/kg, respectively for the three groups, and they were also monitored closely for 24 hours for mortality and behavioral alterations. The median lethal dose (LD_{50}) was calculated as the geometric mean of the least dose that killed a rat and the highest dose that did not kill any rat, using the formula: $LD_{50} = \sqrt{a \times b}$, where a = least dose that killed a rat and b = highest dose that did not kill any rat.

Determination of Elemental Contents: Five grams (5) of the dried crude extract was placed in an evaporating dish in an oven at 80 °C and further dried to a constant weight. The sample was then placed in a weighed porcelain crucible and ashed at 500 °C in a hot-spot furnace for three hours. The ashed material was then prepared for determination of the elements (Radojevic and Bashkin, 1999). The cooled, ashed sample (0.5 g) was digested by heating for three hours with a mixture of 10 ml each of concentrated HNO_3, HCl and $HClO_4$ in a 500 ml flask. This was evaporated to 5 ml. This material (aliquot) was mixed with 100 ml of 2 M HNO_3 and 30 ml distilled water in 100 ml volumetric flask (Radojevic and Bashkin, 1999). Blank samples (using the same procedure but omitting the plant material) and standard solutions for the various elements were similarly prepared. All samples were stored in plastic containers in a refrigerator maintained at 4 °C prior to analysis. The levels of the elements Magnesium, Iron, Chromium, Zinc, Lead, Manganese, Cobalt, Cadmium and Copper were determined by atomic absorption spectrophotometry (Radojevic and Bashkin, 1999).

Data Analysis and Presentation: Data obtained was subjected to descriptive statistics and was presented in form of tables

Results

In both steps I and II of the oral acute toxicity test, no death was recorded at the highest dose of 5000 mg/kg body weight (Table 1a). In the step I of intra-peritoneal route of administration of the extract for acute toxicity study, one death was recorded at 1,000 mg/kg. However, in step II of the intra-peritoneal route of administration, one death each was recorded at doses 1,600 mg/kg and 2,900 mg/kg respectively. The LD_{50} for the intra-peritoneal route was calculated to be 1,264 mg/kg body weight (Table 1b).

Table 1a. Mortality pattern in rats given acute oral doses of methanolic root extract of *Moringa oleifera*, following the two-step acute toxicity test.

Steps	No. of Rats	Dose (mg/kg)	Mortality (Number dead / Number in the group)
1	3	10	0/3
1	3	100	0/3
1	3	1000	0/3
2	1	1600	0/1
2	1	2900	0/1
2	1	5000	0/1

Table 1b. Mortality pattern in rats given acute intra-peritoneal doses of methanolic root extract of *Moringa oleifera,* following the two-step acute toxicity test.

Steps	No. of rats	Dose (mg/kg body weight)	Mortality (Number dead/Number in the group)
1.	3	10	0/3
1	3	100	0/3
1	3	1000	1/3
2	1	600	0/1
2	1	1000	0/1
2	1	1600	1/1
2	1	2900	1/1

Table 2: Phytochemical constituents of methanol extract of *Moringa oleifera* root.

Phytochemical Constituents	Type of Test	Results of Tests
Carbohydrates	Molisch's test (General test)	–
	Barfoed's test (monosaccharides)	+
	Fehling's (free reducing sugar)	+
	Standard test for combined reducing sugar	+
	Standard test for ketoses	+
	Standard test for pentoses	–
Tannins	Ferric chloride	–
	Lead acetate	+
Terpenoids	Terpenoids	+
Cardiac Glycosides	Salkowski's (for steroid ring)	+
	Lieberman-Burchard's	+
Flavonoids	Shinoda's	+
	Ferric chloride	–
	Lead acetate	+
	Sodium hydroxide	–
Anthraquinones	Free Anthraquinone (Borntragers)	–
	Combined Anthraquinones	
Saponins	Frothing	+
Phlobatannins	Hydrochloric acid	–
Alkaloids	Dragendoff's	–
	Mayer's	–

Keys: – = Absent; + = Present

Phytochemical analysis of the methanol root extract of *M. oleifera* showed the presence of carbohydrates, cardiac glycosides, flavonoids, tannins, saponins and terpenoids while anthraquinones, phlobatannins and alkaloids were absent (Table 2).

Results of the elemental analysis of *M. oleifera* extract are presented in Table 3. Zinc had the highest concentration (1.0184 mg/dl), followed by Magnesium (0.07227 mg/dl), Iron (0.05959 mg/dl), Copper (0.00582 mg/dl), Cobalt (0.00568 mg/dl) and Manganese (0.00547 mg/dl), while Lead (0.00143 mg/dl), Chromium (0.00302 mg/dl) and Cadmium (0.00003 mg/dl) were detected in trace quantity (Table 3). Apart from Iron, all the other elements were below the WHO standard for elemental concentration (Table 3).

Table 3. Elemental concentration of methanol root extract of *Moringa oleifera.*

Elements	Concentration (mg/dl)	WHO (1996) Standard (mg/dl)
Magnesium (Mg)	0.07227	0.01 – 0.02
Iron (Fe)	0.05959	0.0005 – 0.05
Copper (Cu)	0.00582	0 – 003
Lead (Pb)	0.00143	0.01
Manganese (Mn)	0.00547	0.2
Chromium (Cr)	0.00302	0.0015
Cadmium (Cd)	0.00003	0.01 – 0.035
Cobalt (Co)	0.00568	0.000014 - 000048
Zinc (Zn)	1.0184	0.015 – 004

Guidelines for Elemental Concentration, WHO (1996).

Discussion

The oral LD_{50} above 5000 mg/kg obtained for the *M. oleifera* root extract in this study places the orally administered root extract within the World Health Organization's category of substances "unlikely to present acute hazard in normal use" (WHO, 2001). This implies that when orally administered, the methanol root extract of *M. oleifera* is safe for acute use in the treatment of ailments and diseases for which it is effective (OECD, 2001). The intra-peritoneal LD_{50} of 1264 mg/kg also implies that the root extract is relatively safe/non-toxic via intra-peritoneal injection. This is because dry substances whose intra-peritoneal LD_{50} fall between 50 and 500 mg/kg are regarded as toxic, between 500 mg/kg but less than 1000 mg/kg are moderately toxic and greater than 1000 mg/kg are not toxic (Clark and Clark, 1979; Sodipo *et al*., 2009). Onyeyilli *et al*. (2000) categorized an intra-peritoneal LD_{50} of 1400 mg/kg under low toxicity.

It has been reported that the qualitative presence of the phytochemical constituents such as tannins, terpenoids, cardiac glycosides, flavonoids and saponin were responsible for most physiological and chemotherapeutic effects exhibited by plant extracts both *in vitro* and *in vivo* (Usman, 2012). Some flavonoids possess anti-inflammatory activity while some

possess inhibitory effect against enzymes such as protein tyrosine kinase (Viena *et al.,* 2003). Flavonoids are found in most plant materials. The most important dietary sources are fruits, tea and soya bean (Nita-Bishop, 2003). Flavonoids have antioxidant activity and many health promoting effects (Orlowski *et al*., 2016). Some of the activities attributed to flavonoids include anti-platelet, anti-allergy, anti-cancer, anti-oxidant, anti-inflammatory and anti-viral. These activities are in agreement with the findings of Verma *et al.,* (1976). Also flavonoids have been referred to as nature's biological response modifiers because of their ability to modify the body's reaction to allergies, viruses and carcinogens; they also show anti-microbial activity (Yamamato and Gaynor, 2001). Tannins have astringent properties that hasten wound healing (Orlowski *et al*., 2018). Tannins also decrease bacterial cell proliferation by blocking key enzymes of microbial metabolism (Kaczmarek, 2020). This results of the present study are in agreement with the reports of Ojo *et al*. (2014) even though they reported the presence of alkaloids in *Moringa oleifera* leaf. Cardiac glycosides are known to have cathartic and laxative effects and also used in the treatment of congestive heart failure, constipation, oedema and microbial infections (Frantisek, 1991; Škubník *et al*., 2021). In dogs and cats, cardiac glycosides are indicated for their negative chronotropic effect in supraventricular arrhythmias such as atrial fibrillation. They slow the rate of impulse conduction through the atrioventricular node and allow the ventricular rate to fall below the atrial and so restore more efficient pumping (Aliu and Nwude, 1982). As such the cardiac glycosides found in methanol extract of *Moringa oleifera* root can probably be used in arrhythmia. Saponins are glycosidic in nature with striking physical characteristics of producing foam. They have expectorant action which is very useful in the management of upper respiratory tract infection. They also have characteristic property of haemolysis when injected intravenously even though oral ingestion is comparatively harmless. Most saponins present in plants are cardiotonic in nature (Awe and Sodipo, 2001; Evans, 2009). A high saponin diet can be used in the inhibition of dental caries and platelet aggregation in humans and as an antidote against lead in epidemiological studies (Patrick-Iwuanyanwu and Sodipo, 2007). Carbohydrates in this extract occupy an important position in metabolism, so the method for their detection is useful in phytochemistry. Carbohydrate has no therapeutic actions but they possibly increase the effectiveness of the biologically active principle in the plant, thus most therapeutic principles isolated from plants occur in combination with sugar as glycosides (Morris and Mohiuddin, 2023). Saponins which was identified to be present in the extract, had been described as an active immune booster, and it may be acting in synergy with zinc to promote the immune boosting activity of *Moringa oleifera* root (Gaikwad *et al.*, 2011). The presence in the root extract of *Moringa oleifera* in this study of flavonoids, tannins and saponins may account for the health benefits of *Moringa oleifera*.

Some of the elements have been known to exert beneficial physiological effects. Elements such as Zn, Cu, Mn and Selenium have been postulated to have hypoglycaemic effects (Al-Awadi *et al*., 2004). The presence of essential trace elements is known to influence various body functions due to their direct or indirect action in physiologic and toxic concentrations. Elements such as iron (Fe) and magnesium (Mg) which play an essential role in human health and disease has been highlighted (Moses *et al*., 2002). The presence of iron and copper may be responsible for the plant's renown ability to correct anaemia (Ramachandran *et al*., 1980; Abbaspour *et al*., 2014). Zinc (Zn) is present in every part of the body as a mineral element and it has a wide range of functions, including wound healing

and it is a vital component of many enzymes reactions.

Zinc is important for healthy skin and is essential for a healthy immune systems and resistance to infection. The presence of high concebtration of zinc in *Moringa oleifera* may account for its description as an outstanding immune builder (Ramachandran *et al*., 1980).

The essential elements and minerals analyzed are indispensable for various life processes and are also essential for growth, normal cell function, metabolism, etc. They act as electrolytes, mineral dietary supplements, antidotes, components of several enzymes. These essential elements as components of traditional/orthodox medicines are necessary for strengthening the body's immune system and for therapeutic purposes. The excess or deficiency of these nutritional elements may disturb normal biochemical functions of the body (Olaniyi *et al.,* 1998; Morris and Mohiuddin 2023).

Conclusion: The methanol root extract of *Moringa oleifera* as used in this study, is acutely safe, especially when administered orally, and contained some essential bioactive compounds and elements that may make it useful for treatment against various ailments.

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Conflict of Interest

The authors wish to state that there are no known conflicts of interest associated with this work.

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