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Full Length Research Paper

Phytochemicals and acute toxicity of *Moringa oleifera* roots in mice

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The phytochemicals used by plants to protect themselves against predators in *Moringa oleifera* roots were qualitatively identified in the aqueous and ethanol extracts. Its acute toxicity in 24 h was evaluated in Swiss albino mice. *M. oleifera, a* native plant of the sub-Himalayan tracts of India, Pakistan, Bangladesh and Afghanistan is used in folk medicine. It is claimed to have nutritional, medicinal, socio-economic and industrial values. Many individuals and families consume the roots for their medicinal properties. Despite wide use the roots, the phytochemicals and toxicity profile are not well documented. This study set out to determine the phytochemicals and acute toxicity of *M. oleifera* roots in mice. The roots were harvested during dry season and air dried. Serial extractions using ether, ethanol and water were done. The harvested phytochemicals were qualitatively identified using standard chemicals procedures. The phytochemicals identified were: gallic tannins, catechol tennins, steroids and triterponoids, saponins, anthraquinones, alkaloids, and reducing sugars. Acute toxicity was determined by giving a single oral dose to Swiss albino mice and observed for 24 h. The LD_{50} was calculated using the probit tables. The LD_{50} of ethanol extract was 17.8 g/kg and that of aqueous extract was 15.9 g/kg. In conclusion, *M. oleifera* roots contain protective phytochemicals and are relatively non-toxic when given in a single dose.

Key words: *Moringa oleifera* roots, phytochemicals, medicinal plant extracts, herbal medicine, acute toxicity, mice.

INTRODUCTION

The scientific classification of *Moringa oleifera* shows that it comes from Kingdom: Plantae, Division: Magnoliphyta, Class: Magnoliopsida, Order: Brassicales, Family: Moringaceae, Genus: Moringa, Species: *M. oleifera*. It is a fast growing tree, native to the sub-Himalayan tracts of India, Pakistan, Bangladesh and Afghanistan where it is used in folk medicine (Fahey, 2005), which is at the moment distributed all over the world (Lockelt et al., 2000). The roots of this plant are used in folk medicine to treat a number of ailments (Fahey, 2005).

A number of phytochemicals exist to protect plants from predators such as insects and animals. These phytochemicals are secondary plant metabolites which act on animals cells and tissues to inhibit membrane bound enzymes, affects DNA formation, destroy cell membranes (Cowan, 1999). Many of them are strong antioxidants, effective antimicrobials, possess substantial anti-carcinogenic and anti-mutagenic properties (Li-Weber, 2009; Nandakumar et al., 2008; Hausteen, 2005). They are also active in reducing high blood pressure (Ayinde et al., 2007; Dhawan and Jain, 2005). They can cause local tumors (Kapadia et al., 1978), inactivate and kill

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microorganisms (Cowan, 1999; Taylor et al. 1996; Hausteen, 2005).

Although some phytochemicals cause cancers after prolonged use, some are capable of preventing cancer (Raju et al., 2004), while others have anti-carcinogenic effects (Yun, 1996) and anti-oxidants (Rausch et al., 2006). Moringa family is rich in compounds containing the simple sugar, rhamnose, and rich in glucosinolates and isothiocyanites.

Because of its several nutritional, pharmacological (Caceres et al., 1991; 1992; Fuglie, 2001) and industrial applications (Makkar and Becker, 1997; Foidl, 2001) plus lowering blood pressure and cholesterol (Kelble, 2005; Broadhurst et al., 2000), *M. oleifera* has gained popularity in many communities. This study therefore set out to determine the protective phytochemicals constituents and establish the acute toxicity levels by determining LD_{50} of *M. oleifera* root peel.

MATERIALS AND METHODS

Plant collection and extracts preparation

M. oleifera roots were harvested during the dry season from trees grown on loam soil. The family and species of *M. oleifera* were confirmed by a Makerere University botanist and leaves were kept in the University Herbarium. *M. oleifera* roots peel were air-dried at room temperature in the Department of Physiology until constant weight was attained. They were kept away from direct sun light to avoid destroying active compounds. They were then pounded to powder using metallic motor and pestle to ease the extraction of active compounds.

Extraction process

The already established extraction procedure on the plant materials was used (Harborne, 1984). *M. oleifera* root peel powder was soaked in ether for 72 h, filtered using a Whatman No. 1 filter paper in Buchner funnel and solvent removed by evaporation. The residue was left to dry at room temperature, away from direct light for 48 h. It was soaked in ethanol (96% V/V) for 72 h, a suction pump was used to dry the extract. The residue was left to air dry at room temperature for 72 h. The dry residue was put in hot water (96°C) to prevent being attacked by fungi and allowed to cool while being shaken at intervals for 6 h. The filtrate was freeze dried.

Qualitative tests on the ether, ethanol and water root extracts

The qualitative methods already established to test for classes of compounds in plant extracts by Ciulei (1964) and Chitravadivu et al. (2009) were used. The substances that were tested for included: alkaloids, steroids and triterpenoids, tannins, anthracenosides, reducing sugars, flavones, saponins and coumarins which are reported to protect plants against predators. The dry *M. oleifera* root peel, ether, ethanol and water extracts were used to determine the compounds.

Hydrochloric acid, Dragendoff's reagent and Meyer's reagent were used, presence of yellowish precipitate indicated the presence of alkaloids. To detect the presence of steroids, triterpenoids, acetic anhydride, Chloroform and concentrated sulphuric acid were used, a brown-red ring at the interface between the two liquids and a green supernatant indicated their presence.

Methanol and ferric chloride were used to indicate the presence of catechol and gallic tannins. A blackish blue color indicated the presence of gallic tannins while green blackish color, indicated the presence of catechol tannins. Adding the dry extract to 25% ammonia solution a cherish-red solution indicated the presence of emodols (aglycones of anthracenosides in oxidized form).

Foam formation appearing after shaking for 15 min a test tube containing 1 mg Dimethylsulfoxide, ethanol and distilled water indicated presence of saponins. Ammonia solution added to 1 mg dry extract and observed under ultra violet light indicated the presence of coumarins and its derivatives. One milligram of dry extract was dissolved in 1 ml of methanol at 50°C, metallic magnesium and 4 to 5 drops of concentrated hydrochloric acid added. A red or orange color indicates the presence of flavones aglycones.

One milligram of the extract was dissolved in 2 ml of water. 1 ml of Fehling's solution
(I and II), added and the mixture heated. A brick red precipitate denoted the presence of reducing sugars.

Chemicals used in this study were purchased from: Tomas Baker (Chemicals) Ltd, 4/86 Bharat, Mahal, Marine Drive, Mumbai- 400 002, India; *ALPHA*Chemicals, 18 Inman Road, Crorner, NSW 20999 Australia; LOBA CHEMIE PVT. LTD. Jehangir Villa, 107, Wode House Road, Colaba, Mumai, 400 005. India, and BDH Laboratories VWR International Ltd, 14 Media Village, Liscombe Park, Soulbury, Leighton Buzzard, LU7 0JL, UK.

Establishing the LD_{50}

Swiss albino mice, aged 6 to 8 weeks, weighing 15 to 20 g, were used to determine the percentage death of animals 24 h after an oral dose. Ethanol and water extracts were orally (through a gastric tube) given in single doses to 5 male and 5 female mice "test groups", having been starved for 12 h. The test doses indicated that for aqueous extract, the test animals received; 10, 15, 20, 25 g, and 35 g/kg body weight per a group respectively, while that of ethanol extract received; 10, 15, 20, 25 g and 30 g/kg body weight. The control group of 5 males and 5 females received 1 ml distilled water. The animals behaviors were observed for 12 h and the number of animals that died in 24 h were recorded.

Ethical clearance

In relation to the use of laboratory animals, the protocol used in this study was approved by the Makerere University Faculty of Medicine, Research and Ethics committee. The mice were kept in mice cages and fed on commercial rat pellets and allowed to freely access tap water in bottles at reassure. They enjoyed 12 h of light and same hours of darkness.

Data analysis

Graphs of probit against log dose of the ethanol and aqueous extracts were drawn. The log dose that responded to probit 5 (50% deaths) was calculated from the graph equation and its ant-log gave the LD_{50}.

RESULTS

The phytochemical screening of aqueous and ethanol root peel extracts of *M. oleifera* reviled that water

Table 1. Phytochemicals present in *Moringa oleifera* roots.

Phytochemical	Ether extract	Ethanol extract	Aqueous extract
Gallic Tannins	+	_	_
Catechol Tennins	_	+	_
Coumarins	_	_	_
Steroids and Triterponoids	++	+++	_
Saponins	_	+++	+
Anthraquinones	+	+	+++
Alkaloids	_	+++	++
Reducing sugars	_	+	+

-: not detected; +: present in low concentration; ++: present in moderate concentration; +++ present in high concentrations.

Table 2. Determination of LD_{50} values by Miller and Tainter method of the ethanol and aqueous extracts of *Moringa oleifera* root in mice.

Oral dose (g/kg)	Log dose	Dead/total	% Death	Probit
Aqueous extract				
10	1.0	1/10	10	4.48
15	1.18	2/10	20	4.75
20	1.30	3/10	30	5.25
25	1.40	6/10	60	5.52
35	1.54	8/10	80	5.84
LD_{50} Value from the group = 15.9 g/kg				
Ethanol extract				
10	1.0	2/10	20	4.16
15	1.18	3/10	30	4.48
20	1.30	6/10	60	5.25
25	1.40	7/10	70	5.52
30	1.54	8/10	80	5.84
LD_{50} Value from the group = 17.8 g/kg				

The aqueous extract of *M. oleifera* roots had a lower LD_{50} than the ethanol extract.

extracted more phytochemicals than ether. None of the solvents extracted coumarins from the root powder (Table 1). The table also shows that all the three solvents were able to extract Anthraquinones with water giving the strongest response.

Ether extract

Ether was able to extract gallic tannins, steroids and triterponoids, anthraquinones.

Ethanol extract

Ethanol was able to extract catechol tannins, steroids and triterponoids, saponins, anthraquinones, alkaloids and reducing sugars. The greatest response for ethanol was observed in steroids and triterponoids, plus saponins.

Water extract

Water was able to extract saponins, anthraquinones, alkaloids and reducing sugars. The greatest response for that of water was in anthraquinones and alkaloids.

Lethal dose (LD_{50})

The animals having received aqueous or ethanol extract did not exhibit marked behavior change. The ones that died just become weak and less active followed by gradual death.

The LD_{50} of *M oleifera* roots peel administered intra-gastric in a single dose was higher in ethanol extract than aqueous extract (Table 2). The probit-log dose of the extracts and the equations used in calculating the LD_{50} are shown in Figures 1 and 2.

The LD_{50} of *M. oleifera* roots peel aqueous extract was

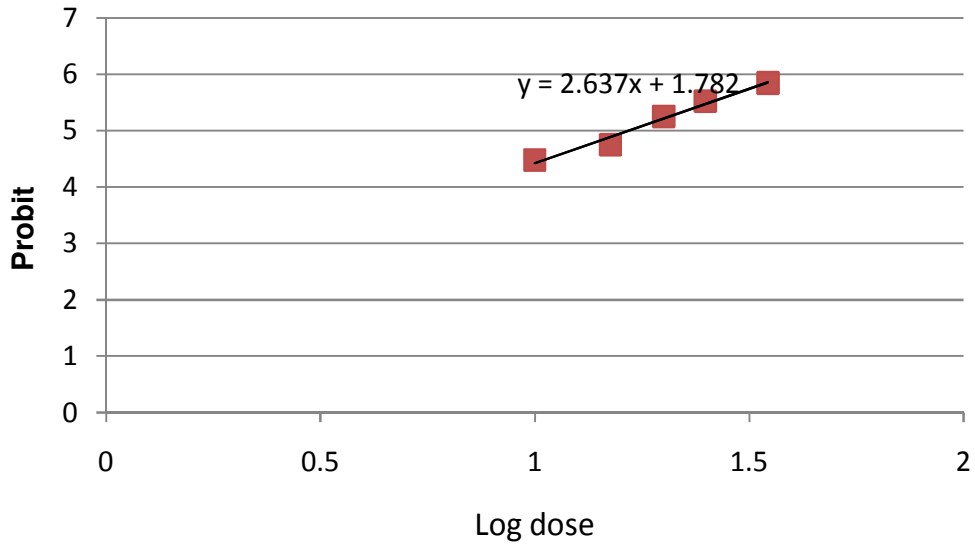

Figure 1. Graph of probit Vs log dose *Moringa oleifera* roots aqueous extract. The equation of this graph Was used to calculate the log dose for probit 5 (50%) animal death whose anti log gave the LD_{50} for the *M. oleifera* roots aqueous extract.

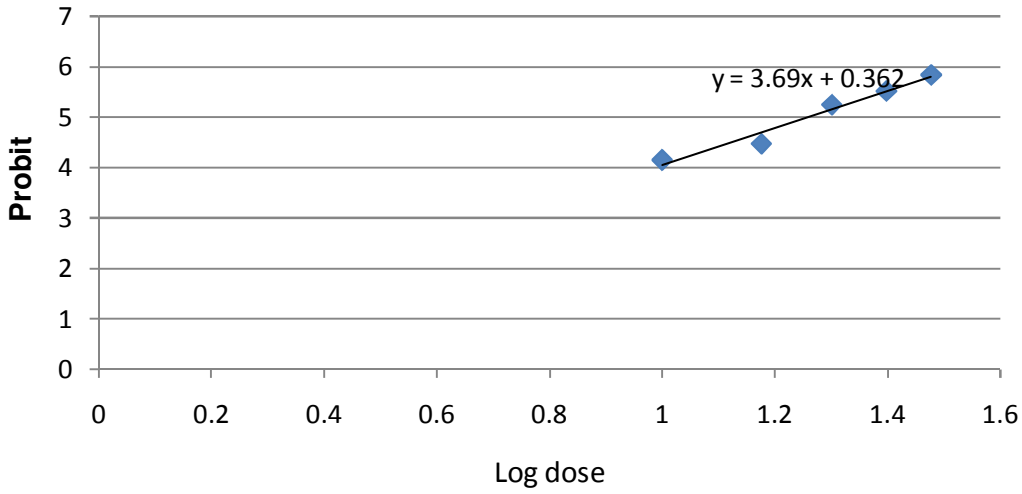

Figure 2. Graph of probit Vs log dose of *Moringa oleifera* roots ethanol extract. The equation of this graph was used to calculate the log dose for Probit 5 (50%) animal death whose anti log gave the LD_{50} for the *M. oleifera* roots ethanol extract.

15.9 g/kg while that of ethanol extract was 17.8 g/kg.

DISCUSSION

This study indentified the protective phytochemicals in *M. oleifera* roots, ether, ethanol and aqueous extracts which included: gallic tannins, catechol tennins, steroids and triterponoids, flavonoids, saponins, anthraquinones, alkaloids and reducing sugars. These phytochemicals partialy explains some actions of *M. oleifera* roots as herbal medicine. Componds like niazini A, niazinin B, niziamicin, niazinin A+B and niazimin A+B (4+5) were isolated from *M. oleifera* leaves, (Anwar et al., 1993), and some alkaloids have been found in *M. oleifera* roots, (Mazumder et al., 1999). The toxicity effect of some vegetables has been attributed to their phytochemicals' composition rather than the variations in the test animals (Orech et al., (2005). The fact that water and ethanol exhibited stronger reactions thus being able to extract more phytochemicals means that there are more non-polar phytochemicals in the roots.

Results of acute toxicity study tests with aqueous and ethanol extracts of *M. oleifera* roots showed a safe range. The LD_{50} for the aqueous extract was 15.9 g/kg body weight while that of ethanol extract was 17.8 g/kg. The results are in agreement with those of Adedapo et al. (2009), which reported the plant leaves being relatively safe for both nutritional and medicine uses. However this study identified the lethal dose that can assist those who wish to standardize the *M. oleifera* root peel as herbal medicine. Using commonly used terms for toxicities along the dose equivalent for rats/mice, *M. oleifera* root peels are relatively harmless = > 1 kg for probable lethal single dose for man (Gosh, 1984).

Conclusion

M. oleifera roots, ether, ethanol and aqueous extracts contain gallic tannins, steroids and triterponoids, anthrax-quinones, catechol tennins, saponins, alkaloids and reducing sugars.

The LD_{50} *M. oleifera* root peel aqueous extract is 15.9 g/kg while that of ethanol extract is 17.8 g/kg. *M. oleifera* root peel is relatively non-toxic when given as a single dose.

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