



DIURETIC AND LAXATIVE ACTIVITIES OF *MORINGA OLEIFERA* SEEDS AND PODS IN MICE

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Abstract: *Moringa oleifera* (Family- Moringaceae) is a well-known vegetable for its health benefits for thousands of years having diverse nutritional bioactive constituents. Phytochemical analysis of ethanolic extract of this plant indicated the presence of carbohydrate, reducing sugar, combined reducing sugar, glycosides, tannins (absent in seeds), alkaloids, flavonoids, saponins and steroids. In diuretic activity test, the extract showed a significant ($p < 0.001$) effect at the dose of 200 & 400 mg/kg body weight in production of total urine volume in comparison with standard drug furosemide (5 mg/kg). Upon electrolyte analysis of excreted urine, the extract was found to increase Na^+ , K^+ , Cl^- excretion and may act as loop diuretics. The pods and seeds extract showed significant ($p < 0.01$) laxative effects in mice in terms of stool production with soft consistency at the dose of 200 & 400 mg/kg in comparison with standard drug bisacodyl (10 mg/kg). The most edible part of this plant is pods which showed better diuretic and laxative effects than seeds. So, the pods may be a good source of bioactive constituents which may be used as parent compound(s) for discovering better diuretic or laxative drugs.

Keywords: *Moringa oleifera*, Pods, Seeds, Laxative, Diuretic, Electrolytes, Acute Toxicity.

Introduction

Diuretics are drugs that promote the output of urine. The primary action of most diuretics is the direct inhibition of Na^+ transport at one or more of the four major anatomical sites along the nephron, where Na^+ reabsorption takes place (Wile 2012). The increased excretion of water and electrolytes by the kidneys is dependent on three different processes viz., glomerular filtration, tubular reabsorption (active and passive) and tubular secretion (Friis 1983). Diuretic compounds stimulate the excretion of water that is potentially useful in many disorders including congestive heart diseases, nephritis, toxemia of pregnancy, premenstrual tension, hypertension (Sayana *et al.* 2014) and also play an important role in patients with pulmonary congestion. Diuretics like mannitol, thiazides, furosemide, and ethacrinic acid are choice of drugs at present to manage the above pathological conditions. Conventional diuretics have some limitations such as fluctuated potassium level in the blood (for potassium-sparing diuretics), low sodium levels, headache, dizziness, thirst, elevated blood sugar and muscle cramps etc. (Das *et al.* 2005). Plants may serve as the alternative natural sources for the development of new diuretic agents which may overcome such limitations.

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Laxatives are medicines that promote bowel movements and relieve constipation. The causes of constipation are two types: obstructed defecation and colonic slow transit (or hypomotility). Obstructed defecation has mechanical and functional causes and colonic slow transit constipation occurs due to diet, hormones, side effects of medications, and heavy metal toxicity (Golla *et al.* 2014). The consumption of conventional laxative drugs (bisacodyl, lactulose, mannitol etc.) is sometimes limited due to side effects such as bloating, cramping, diarrhea and metabolic disturbances include hypercalcemia, hyperphosphatemia, hyponatremia and hypokalemia (Harris 2006). Cardiotoxic and arrhythmogenic effects have been reported with magnesium purgatives and cisapride (Arezoomandan *et al.* 2011). The use of stimulant laxatives such as Senna (Glycoside containing plant) compounds and bisacodyl may be associated with colonic neoplasia or skin blistering (Vilanova-Sanchez *et al.* 2018). Therefore, the search for novel safe laxative drugs is important.

Moringa oleifera belonging to the family Moringaceae is native to tropical and subtropical regions of South Asia. Regionally it is known as moringa, drumstick tree, horseradish tree, and ben oil tree or benzolive tree. It is a fast-growing, deciduous tree that can reach a height of 10–12 m (32–40 feet) and trunk diameter of 45 cm (1.5 feet) (Parrotta 1993). The bark has a whitish-grey color and is surrounded by thick cork. Young shoots have purplish or greenish-white, hairy bark. The tree has an open crown of drooping, fragile branches and the leaves build up feathery foliage of tripinnate. The seeds have three whitish papery wings and are dispersed by wind and water. In cultivation, it is often cut back annually to 1–2 m (3–6 feet) and allowed to re-grow so the pods and leaves remain within arm's reach (Zhigila 2014). Moringa roots, leaves, flowers, gum and seeds have been found to possess diuretic activity and such diuretic components are likely to play a complementary role in the overall blood pressure lowering effect of this plant (Morton 1991; Caceres *et al.* 1992; Kumar *et al.* 2016). From literature review it has been found that different parts of this plant have many medicinal importance and some parts such as seeds and leaves or barks have already been investigated by some earlier researchers except pods (the most edible part of its fruit). So, here in this study, we have attempted to investigate the relative efficacy of pods and seeds as diuretic and laxative.

Materials and methods

Plant collection: The pods and seeds of *M. oleifera* were collected from Batiaghata, Khulna, Bangladesh during March, 2018 and was identified by experts at Bangladesh National Herbarium, Mirpur, Dhaka (DACB Accession number: 46497).

Extract preparation: The collected parts of *M. oleifera* (pods and seeds) were then dried under shade and grounded in powder using a blender. The powder of pods (750 gm) and seeds (270 gm) was taken in two different clean, flat-bottomed glass containers and soaked in 1800 mL and 1000mL of 96% ethanol, respectively. The containers with its contents were sealed and kept for a period of 14 days accompanying occasional shaking and stirring. The whole mixtures were then underwent a coarse filtration by a piece of clean cloth & cotton. Then those two parts were filtered through Whatman filter paper and the filtrate were concentrated using a rotary evaporator and was marked as crude ethanolic extract. It rendered concentrate of greenish black (pods-1.07% yield) and yellowish black (seeds-1.85% yield) colored gum.

Chemicals and reagents: As standard, furosemide and bisacodyl were collected from Beximco Pharmaceuticals Ltd. Sodium chloride, potassium chloride, silver nitrate, sodium bicarbonate and potassium chromate reagents were purchased from Merck, Germany.

Experimental animal: For animal study, young Swiss-albino mice aged 4-5 weeks; average weight 21-25 gm was purchased from Jahangirnagar University, Bangladesh. They were then kept under standard environmental condition for one week in the animal house of Pharmacy Discipline, Khulna University, Khulna-9208, for adaptation and provided with standard laboratory food and tap water.

All animal experiments were carried out following animal ethics guidelines (Ethical approval number: KU/PHARM/AEC/15/006/027), set up by Pharmacy Discipline, Khulna University, Bangladesh.

Evaluation of Diuretic activity: Diuretic test was carried out following an established protocol adopted by Golla and coworkers with slight modification (Golla *et al.* 2014). Twenty-four mice of both sexes (21-25 g) were randomly divided into four groups of six each. Each group was fasted and deprived of water for 18 h prior to the experiment. The first group was provided with normal saline (12 mL) as control. The second group received standard drug furosemide (5 mg/kbw) in normal saline as positive control. The third and fourth group was provided with pods or seeds extract of *M. oleifera* at a dose of 200 mg/kbw and 400 mg/kbw in normal saline, respectively. Immediately after dosing, the animals were placed in metabolic cages. Food and water were withheld, and the cages were maintained at (25.0 ± 0.5) °C throughout the experiment (6 hours). The urinary output (V_o) was collected every hour and the urine was then stored in freeze ($0-4$ °C) for further electrolyte analysis. The urinary excretion was calculated as ratio of total urinary output by total liquid administered (V_i). The diuretic action was calculated as the ratio of urinary excretion in test group (U_{ET}) and that of control group (U_{EC}). Diuretic activity was calculated as the ratio of diuretic action in test group (D_{AT}) and that of standard group (A_{AF}). The electrolyte content (Na^+ , K^+) of collected urine sample was measured using a flame photometer and Cl^- was measured titrimetrically. pH, conductivity and density were also determined using appropriate methods.

Evaluation of laxative activity: Laxative test was carried out according to earlier methods (Golla *et al.* 2014) with slight modification. Before starting the experiment, 24 mice of 22 to 24 gm weight were taken and fasted for 12 hours period. The mice were divided in four groups containing 6 in each. The first group was administered with normal saline (2 ml) as control. The second group was administered with bisacodyl in saline (10 mg/kg) as positive control; the third and fourth groups received ethanolic extract of *M. oleifera* pods or seeds at a dose of 200 mg/kg and 400 mg/kg body weight in saline, respectively. Then they were housed in a cage lined with clean and white filter paper and observed for 16 hours. No food and water were given at that time. After 16 hours, the faeces were collected and quantified. The faeces consistency was also felt. The experiments were duplicated every time. All experimental data have been analyzed using Microsoft excel 2010 and the GraphPad Prism software.

Results

M. oleifera pods exhibited significant diuretic activity on experimental mice as compared with the control group. The cumulative volume of urine was found to be more (4.89 ml and 5.94 ml for dose 200 mg/kbw and 400 mg/kbw, respectively) for pods extract treated mice than seeds extract treated mice (3.85 ml and 4.35 ml, respectively, at same doses after 6th hour) whereas for control group mice urine volume was found to be 3.25 ml for pods and 3.45 ml for seeds (Table 1).

Table 1. Comparison of urinary output between ethanolic extract of *M. oleifera* pods and seeds.

Group	Cumulative Volume of urine(V ₀): mL/6hr		Urinary excretion (V ₀ /V _i) × 100		Diuretic action (U _{ET} /U _{EC})		Diuretic activity (D _{AT} /D _{AF})	
	pods	seeds	pods	seeds	pods	seeds	pods	seeds
Control (Normal saline)	3.25	3.45	27.08 ±0.59	28.75 ±0.42	-	-	-	-
Furosemide (5 mg/kbw)	7.96	8.25	66.33 ±0.59***	68.75 ±0.42***	2.45	2.39	-	-
Test extract (200 mg/kbw)	4.89	3.85	40.71 ±0.77**	32.08 ±0.41*	1.5	1.11	0.61	0.47
Test extract (400 mg/kbw)	5.94	4.35	49.5 ±0.82**	36.25 ±0.42**	1.83	1.26	0.75	0.53

Values are expressed as Mean ± S.E.M (n = 2); * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared with control group.

The concentration of Na⁺(82.85-167.53 mEq/L), K⁺(29.33-93.78 mEq/L) and Cl⁻(380.00-406.67 mEq/L) ions in collected urine samples for extract treated mice was found to be elevated significantly ($p < 0.05$) as compared to control [89.36 mEq/L (Na⁺), 11.10 mEq/L (K⁺), 106.67 mEq/L (Cl⁻)] (Table 2).

Table 2. Comparison between determination of concentration of Na⁺, K⁺, Cl⁻ in test samples of *M. oleifera* pods and seeds.

Group Ion Conc.	Control	Positive Control (Furosemide 5 mg/kbw)	Pods (200 mg/kbw)	Seeds (200 mg/kbw)	Pods (400 mg/kbw)	Seeds (400 mg/kbw)
	Na ⁺ (mEq/L)	89.36 ±6.52	161.02±6.51**	154.5±6.51**	161.02±11.28**	82.85 ±0.00*
K ⁺ (mEq/L)	11.1 ±0.00	27.39±1.94**	29.33±0.00**	75.18±2.06***	37.97 ±2.07*	93.78±2.07***
Cl ⁻ (mEq/L)	106.67±6.67	193.33±6.67**	380±11.55***	396.67±3.33***	400±0.00***	406.67±6.67***

Values are expressed as Mean ± S.E.M (n = 2); * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared with control group.

For predicting the mechanism of diuretic action, different saluretic indices i.e., natriuretic index, kaluretic index and carbonic anhydrase inhibition index) were calculated using appropriate formula (Table 3) where the natriuretic values were found from 1.78 to 5.27; kaluretic index from 0.19 to 0.56 and CAI index from 1.56 to 3.31 for the tested groups but for control group it was found to be 8.05, 0.12 and 1.06, respectively.

Table 3. Effect of oral administration of *Moringa oleifera* pods ethanol extract and furosemide on urinary electrolytes excretion in mice

Group	Cumulative Concentrations of ions (mEq/L/6h)			Saluretic Index			Na ⁺ /K ⁺	K ⁺ /Na ⁺	CAI [Cl ⁻ / (Na ⁺ +K ⁺)]
	Na ⁺	K ⁺	Cl ⁻	Na ⁺	K ⁺	Cl ⁻			
Control (Normal saline)	89.36 ±6.52	11.1 ±0.00	106.67 ±6.67	-	-	-	8.05	0.12	1.06
Furosemide (5 mg/kg)	161.02 ±6.51**	27.39 ±1.94**	193.33 ±6.67**	1.8	2.47	1.81	0.83	0.01	1.03
Pods (200 mg/kg)	154.5 ±6.51**	29.33 ±0.00***	380 ±11.55***	1.73	2.64	3.56	5.27	0.19	2.14
Pods (400 mg/kg)	82.85 ±0.00*	37.97 ±2.07**	400 ±0.00***	0.93	3.42	3.75	2.18	0.46	3.31
Seeds (200 mg/kg)	161.02 ±11.28**	75.18 ±2.06***	396.67 ±3.33***	1.8	6.78	3.72	2.14	0.47	1.68
Seeds (400 mg/kg)	167.53 ±6.51**	93.78 ±2.07***	406.67 ±6.67***	1.87	8.45	3.81	1.78	0.56	1.56

Values are expressed as Mean ± S.E.M, (n = 2); * p < 0.05, ** p < 0.001, *** p < 0.0001 compared with the control group (Student's unpaired t-test).

Measured pH for the urine of extract treated mice was found to be alkaline pH (7.53-8.03) in nature and conductivity was also found to be elevated (17.89 mS/cm -22.65 mS/cm) in test sample urine (Table 4) which indicated the presence of more electrolytes but for control group pH was 7.11 and conductivity was 13.5 mS/cm. A significant (p < 0.01) laxative activity was observed both for the pods and seeds extracts tested group as compared with the control group where pods showed better laxative activity than seeds.

Table 4. Effects of oral administration of *Moringa oleifera* pods and seeds ethanol extract, normal saline and furosemide on urinary volume, diuretic index, conductivity, pH and density in mice.

Group	Urine Volume (mL/6h)	Diuretic Index	pH	Conductivity (mS/cm)	Density (g/mL)
Control (Normal saline)	3.25±0.05	1	7.11±0.01	13.5±0.05	0.95±0.01
Furosemide (5 mg/kg)	7.95±0.07***	2.44	7.47±0.03*	12.21±0.09**	0.97±0.01
Pods (200 mg/kg)	4.88±0.16**	1.5	8.03±0.01***	22.65±0.35**	0.81±0.1
Pods (400 mg/kg)	5.94±0.07**	1.83	7.66±0.04*	18.60±0.40**	0.95±0.01
Seeds (200 mg/kg)	3.85±0.05**	1.12	7.66±0.04**	25.9±0.4**	0.81±0.1
Seeds (400 mg/kg)	4.35±0.05**	1.26	7.53±0.03**	17.89±0.12**	0.95±0.01

Values are expressed as Mean ± S.E.M, (n = 2); Diuretic index = Urine volume of test group/ Urine volume of control group; * p < 0.05. ** p < 0.001, *** p < 0.0001, compared with the control group (Student's unpaired t-test).

The highest dose (500 mg/kbw) of pods and seeds could produce 56.08% and 37.83% more faeces production, respectively, compared with control (Table 5).

Table 5. Comparison between laxative effect of crude extract *M. oleifera* pods and seeds in mice.

Group	Average weight of faeces (gm) \pm S.E.M		% of increment \pm S.E.M	
	<i>M. oleifera</i> pods	<i>M. oleifera</i> seeds	<i>M. oleifera</i> pods	<i>M. oleifera</i> seeds
Control	0.63 \pm 0.02	0.66 \pm 0.01	-	-
Bisacodyl (5 mg/kbw)	1.12 \pm 0.02	1.2 \pm 0.05**	80.12 \pm 3.2	81.74 \pm 4.82
Test extract (200 mg/kbw)	0.91 \pm 0.06	0.85 \pm 0.04*	46.21 \pm 4.5	31.53 \pm 6.92
Test extract (400 mg/kbw)	0.97 \pm 0.02	0.91 \pm 0.03**	56.08 \pm 2.24	37.83 \pm 2.45

Value expressed in Mean \pm SEM, ** $p < 0.001$, * $p < 0.01$ compared with control group.

Discussions

The pods extract exhibited more diuretic activity compared to seed extract. The magnitude of Na⁺, K⁺ and Cl⁻ was determined as a parameter for saluretic activity. The ratio of Na⁺/K⁺ was calculated as an indicator for natriuretic activity. Natriuretic values greater than 2.0 indicate a favorable natriuretic effect, while values greater than 10.0 indicate potassium sparing effect (Kane *et al.* 2009). From the result it is found that pods at the dose of 200 mg/kg had a greater natriuretic index than seeds. With regard to K⁺ excretion almost all doses of the extracts showed increase in K⁺ excretion which indicates that the ethanolic extracts are not acting as a potassium sparing agents. Observing the experimental result, it can be explained that the extracts of *Moringa oleifera* exerts its diuretic effect by inhibiting loop of Henle's tubular reabsorption of water and enhanced excretion of electrolytes. So, one of the mechanisms may be assumed as loop diuretic. Again, the [Cl⁻]/ [Na⁺+K⁺] ratio i.e. carbonic anhydrase (CA) index was calculated. CA index is an indicator of carbonic anhydrase enzyme inhibition (Amuthan *et al.* 2012). The lower CA index indicates higher carbonic anhydrase inhibition (Tirumalasetty *et al.* 2015). The CA index for experimental extracts (seeds) is comparable to that of standard drug furosemide (Table 3) which indicates that another possible mechanism of diuretic activity may be carbonic anhydrase enzyme inhibition. Diuresis occurs mainly by two pathways including net increase in urine volume and elevated the excretion of electrolytes in the urine (Bhavin *et al.* 2011). There are two factors on which urine volume depends; rate of glomerular filtration and the degree of tubular reabsorption. The observed effect may be attributed to the mechanism like increasing the renal blood flow and the attendant increase in glomerular filtration rate. Another possible mechanism involved may be stimulation of release of endogenous natriuretic peptides, which promotes sodium and water secretion (Shulman *et al.* 1989). Two main types of diuretics are thiazide diuretics and loop diuretics. Thiazide diuretics inhibit Na⁺/Cl⁻ symporters in the distal convoluted tubule by competing for the Cl⁻ binding site, thereby increasing the excretion of Na⁺ and Cl⁻ ions. Loop diuretics (Furosemide) that was used in our experiment as standard increases urinary excretion of Na⁺ by inhibiting Na⁺/K⁺/Cl⁻ symporters in the thick ascending limb of loop of Henley (Gadge *et al.* 2011).

Previous studies have shown that there are several compounds which could be responsible for diuretic effects of different plant extracts. These include flavonoids (Jouad *et al.* 2001), tannins (Patel *et al.* 2011) and steroids (KP *et al.* 2009). These constituents might cause diuretic effect by stimulating regional blood flow or initial vasodilation by producing inhibition of tubular reabsorption of water and electrolytes, or by increasing renal circulation (Martin-Herrera *et al.* 2008). The preliminary phytochemical analysis of *M. oleifera* crude extract revealed the presence of alkaloids, carbohydrates,

proteins, tannins, phenolic compounds, flavonoids, terpenoids and glycosides in pods and seeds extract of *M. oleifera*. And these may be the probable reasons for diuretic effects of *Moringa oleifera* plant extracts.

During laxative test, both pods and seeds extract of *M. oleifera* demonstrated significant laxative activity but pods were found to be more active as laxative. Moreover, the consistency of the faeces was soft more as the dose increased. Fecal consistency is correlated to the ratio of the water holding capacity of the insoluble solids, such as those that derived from dietary fiber, and to the total water in lumen (Saito *et al.* 2002). Many conventional laxatives, especially the stimulant and saline laxatives affect water absorption and/or secretion in the gut. The intestinal transit is controlled by both neural and myogenic mechanisms (Huizinga *et al.* 1998). In general, an increase in the contractile activity of the smooth layers is responsible for acceleration of intestinal propulsion. Several mediators and neurotransmitters govern these motor patterns. Acetylcholine is the main excitatory neurotransmitter in the enteric nervous system (Waterman *et al.* 1994). Thus, the possible presence of cholinomimetic constituents in the plant extract could explain the potent laxative activity of the crude extracts. Moreover, the presence of phyto-constituents such as flavonol, phenolics and alkaloids are also reported to be responsible for laxative activities (Yang *et al.* 2008; Otshudi *et al.* 2000).

Previously, the *Moringa oleifera* plant's roots, leaves, flowers and seeds have been reported in literature as diuretic and antihypertensive by some scientists (Gadge 2006; Fernandes *et al.* 2016; Kumar *et al.* 2016). But no report could be retrieved about the causative constituents responsible for such activities. Here, we did the phytochemical tests following the appropriate literally available methods which indicated the presence of alkaloids, steroids, flavonoids and phenolic compounds. It is hypothesized that those compounds present in this plant are working as the chemical protagonist to work as diuretic or laxative agent.

In summary, it can be suggested that pods of *M. oleifera* possess promising diuretic activity in experimental mice. The calculated saluretic index demonstrates the sample to act as loop diuretic or carbonic anhydrase enzyme inhibitory. Further exploration may be helpful in isolating and identifying pure compound as potent diuretic or laxative drug lead(s).

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