

EVALUATION OF DIURETIC ACTIVITY OF *MORINGA OLEIFERA* (*Lam.*) POD EXTRACT IN RATS

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Summary

Moringa oleifera is used in various clinical conditions in Ayurveda system of medicine and also in other alternative medicines. In the present study the aqueous extract of *Moringa oleifera* pod was tested in two oral doses (400mg/kg and 800mg/kg) for its diuretic activity in albino rats and its diuretic activity was compared with that of a standard drug hydrochlorothiazide. In our study aqueous extract of *Moringa oleifera* showed a significant increase in urine volume and it was comparable to that of hydrochlorothiazide. It also increased the excretion of sodium, potassium, chloride and phosphate but not that of calcium and uric acid. Thus the authors conclude that *Moringa oleifera* has diuretic action.

Key words: *Moringa oleifera*; diuretic action.

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Introduction

Moringa oleifera is an important medicinal herb, belonging to the family Moringaceae. It is considered as **miracle tree** as all the parts of the plant are useful for human health. It is also known as drum stick tree, horseradish tree, clarifier tree and *mother's best friend* in different parts of the world, based on their appearance and unique uses. It is mostly cultivated all over the plains of India, in the old world tropics and it is used medicinally in Guinea, Madagascar, Guiana and Burma.^{1,2,3}

The various parts of the plant used include flower, pod, leaves, bark and roots. The *flowers*, which must be cooked, are eaten either mixed with other foods or fried in butter and have been shown to be rich in potassium and calcium⁴. The *leaves* are eaten as greens, in salads, in vegetable curries, as pickles and for seasoning. The *Bark* is regarded as an antiscorbic, and it exudes a reddish gum sometimes used for the treatment of diarrhoea. The *roots* are bitter, act as a tonic to the body and lungs, and have an expectorant activity. They are a mildly diuretic and are used as a stimulant in paralytic afflictions, epilepsy and hysteria. The pods are the most valued and widely used of all the tree parts. The pods are extremely nutritious, containing all the essential amino acids along with many vitamins and other nutrients. The immature pod can be eaten raw or prepared like green peas or green beans, while the mature pods are usually fried and possess a peanut-like flavour. The pods also yield 38 - 40% of non-drying, edible oil known as Ben Oil which is clear, sweet and odourless, and never becomes rancid. Overall, its nutritional value most closely resembles olive oil⁴.

In recent decades, many scientific studies using the extracts of leaves, pods and roots of *Moringa oleifera* are being carried out to confirm many potential uses including wound healing^{5,6} anti-tumour⁷, anti-hepatotoxic⁸, anti-fertility⁹, hypotensive¹⁰, antiurolithiatic¹¹ acute anti-inflammatory^{6,12,13}, and analgesic activity¹⁴.

Even though the root extracts of *Moringa oleifera* have been studied for diuretic activity¹¹, there is no scientific study showing diuretic activity of *Moringa oleifera* pod extract. Therefore this study was conducted in order to evaluate its diuretic activity in rats.

Materials and Methods

Approval for the project: Approval for the experiment was obtained from the Institutional animal ethics committee (IAEC), Kasturba Medical College, Manipal, vide letter No. IAEC/KMC 07/2008-2009.

Plant material: The pods of *Moringa oleifera* Lam. Available locally were collected between February and May in and around Udupi. The botanical identity has been confirmed by department of botany, Sri Poornapagna College, Udupi. The voucher specimen is preserved in our laboratory for future reference.

Method of extraction: The fresh pods were cut into small pieces, partially crushed and soaked in water overnight which was later subjected to boiling for 6 hours. The resultant extract was then drained and concentrated in a water bath to get the concentrated extract. The yield of the extract was 8.5%. The extract was stored in desiccators and used for further experiments after dissolving it in distilled water.

Animals: Inbred albino rats of Wistar strain, of either sex, aged around 2 to 3 months and weighing 150 – 200g were used. They were housed in standard conditions of temperature (25±2°C), relative humidity of 45-55%, and maintained on 12-hour light: 12-hour dark cycle in animal house of Kasturba Medical College, Manipal. They were fed standard pellet diet (Hindustan Lever rat pellets) and water *ad libitum*.

Acute toxicity study: The aqueous extract of *Moringa oleifera* pod was administered orally in the escalating dosages, up to 8 g to different groups of rats (n=6, in each). The animals were observed for behavioural and physiological variations initially continuously for 4 hours, followed by 4th hourly for 12 hours and there after once daily for 14 days.

Diuretic study: Albino rats of either sex, were fasted overnight before initiating the study. They were divided into 4 groups, with each group containing 6 rats. Group I contains control animals to which 5ml of normal saline was administered. Group II animals were administered standard drug (hydrochlorothiazide – 25mg/kg) and Group III and IV were administered with the test extract at the dosage of 400 and 800mg/kg respectively. They were placed individually in a metabolic cage and urine was collected in tubes containing 2 drops of liquid paraffin (to minimize the evaporation). The urine collected over a period of 24 hours was measured and concentration of electrolytes, sodium, potassium, chloride, phosphate, calcium and uric acid were determined using a suitable biochemical analysis. All the results were later tabulated and analysed for statistical significance.

Statistical analysis: The data obtained from the study was statistically evaluated using a parametric test ANOVA (Analysis of Variance) and Turkey as post hoc test. This was done with SPSS (Statistical package for social science) software.

Results

The results of various parameters in the study are shown in table 1 and table 2. The aqueous extract of *Moringa oleifera* pod when given orally to rats, revealed significant increase (p<0.05) in urine output compared to control at both doses 400mg/kg and 800mg/kg orally (table 1).

Table 1: Effect of aqueous extract of *Moringa oleifera* pod on 24 hour urine output in rats.

Group	Treatment	Urine output (in ml) (Mean ± SEM)
I	Control (Normal Saline – 5ml-oral)	12.33 ± 1.20
II	Hydrochlorothiazide (25mg/kg-oral)	18.6 ± 0.66*
III	Moringa (400mg/kg -oral)	16.83 ± 1.01*
IV	Moringa (800mg/kg-oral)	17.33 ± 0.71*

SEM – Standard Error of Mean, n = 6, * p ≤ 0.05 vs control

The excretion of sodium, potassium, phosphate and chloride was significantly ($p < 0.05$) increased in the treated groups which were administered with 400mg/kg or 800mg/kg of *Moringa oleifera* extract in comparison to control group (table 2). The excretion of other electrolytes like calcium and uric acid were increased in rats receiving 800mg/kg but the excretion was not significant in comparison to control.

Table 2: Effect of aqueous extract of *Moringa oleifera* pod on urinary electrolyte concentration.

(Results expressed in Mean±SEM)

Drug dose (mg/kg)	Sodium (mmol/L)	Potassium(m mol/L)	Chloride (mmol/L)	Phosphate (mmol/L)	Calcium (mg/dl)	Uric acid (mg/dl)
Control (Normal saline)	30.8±1.51	40.5±2.89	87.66±1.05	39.5±1.67	0.53±0.40	0.23±0.95
Hydrochlorothiazide (25mg/kg)	47.8±1.16*	62.17±1.01*	121± 1.50*	59.83±3.62*	0.63±0.40	0.40±0.43
M.oleifera (400mg/kg)	45.16±1.8 *	58±2.17*	115.83±3.01*	44.83±2.13	0.42±0.14	0.18±0.11
M.oleifera (800mg/kg)	47.33±1.8*	58.33±1.6*	119±3.58*	54.67±2.61*	0.56±0.48	0.31±0.39

SEM – Standard Error of Mean, n = 6, *P < 0.05 vs control

Discussion

In our study, the effects of the vehicle (normal saline), aqueous suspension of the test drug at two different doses or a standard drug were given orally, on urine volume and electrolyte content of the urine collected for 24 hrs were measured. The test drug showed a significant increase in urine volume at both 400mg and 800mg/kg over controls. A significant increase in urinary electrolyte concentration was also observed. In particular sodium, potassium and chloride were increased with 400 and 800mg/kg dose when compared to control levels. However the extract did not show significant increase in uric acid and calcium excretion over controls. The maximum increase in urine volume (17.33 ml) and electrolyte concentration was seen at 800mg/kg of extract. This increase is comparable to that of the effect of standard drug like hydrochlorothiazide. Diuretics are used to increase urine volume and to increase net output of electrolytes. In this study our data, indicate that *Moringa oleifera* pod extract has a significant diuretic and natriuretic effect in our rat model. These findings from our study support the use of extract of *Moringa oleifera* pods as diuretic agent in traditional medicine. Identifying the nature of the active compound in the extract is yet to be performed.

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