

Diuretic effect of chloroform seed extract of *Moringa oleifera* (Linn.) in Wistar rats**Hema Senthil Kumar¹, Lourdu Jafrin A.^{2*}**¹Department of Pharmacology, Government Sivagangai Medical College, Sivagangai, Tamil Nadu, India²Department of Pharmacology, Indira Gandhi Medical College and Research Institute, Pondicherry, India**Received:** 07 September 2016**Revised:** 08 September 2016**Accepted:** 12 October 2016***Correspondence to:**Dr. Lourdu Jafrin A.,
Email: hiwedz@gmail.com**Copyright:** © the author(s), publisher and licensee Medip Academy. This is an open-access article distributed under the terms of the Creative Commons Attribution Non-Commercial License, which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.**ABSTRACT****Background:** Diuretics are widely prescribed group of drugs for mobilizing oedema in a variety of clinical situations, including hypertension, heart failure, renal failure, nephrotic syndrome and cirrhosis. Problems like drug resistance, adverse drug reactions and emerging newer diseases, have necessitated research on the vast untapped potential of herbals as potent drugs. Hence in this study an attempt has been made to evaluate the diuretic property of *Moringa oleifera*.**Methods:** Twenty-four adult wistar rats of either sex were divided into four group of six each and they were fasted for 18 hours. Group I received normal feed and water ad libitum. Group IIa received frusemide 1mg/kg, Group IIb received thiazide 2.5 mg / kg and Group III received chloroform extract of *Moringa oleifera* seed (1000 mg/kg) orally. The diuretic response was assessed by the increase in urine volume and urinary electrolytes.**Results:** The analysis showed an increase in urine volume in the groups IIa, IIb and III.**Conclusions:** The observations of the present study showed, the total volume of urine, natriuretic, kaliuretic effect exerted by the plant extract was similar to that of hydrochlorothiazide (moderate efficacy diuretic).**Keywords:** Diuretics, Chloroform, *Moringa oleifera*, Wistar rats**INTRODUCTION**Diuretics remain one of the most widely prescribed group of drugs for a variety of clinical situations, including hypertension, heart failure, renal failure, nephrotic syndrome and cirrhosis after their introduction about forty years ago.¹ The common basic pathology in all these conditions being the retention of excessive volume of fluid in the interstitial compartment and is invariably associated with renal sodium retention resulting in oedema.Three fundamental strategies exist for mobilizing oedema:²

1. Correction of underlying disease
2. Restriction of Na⁺ intake
3. Administration of diuretics

Diuretics remain the cornerstone for the treatment of oedema by increasing the rate of urine flow and sodium excretion and are used to adjust the volume and composition of body fluids.³Many diuretic agents like loop diuretics, thiazides, amiloride and triamterene, exert their effects on specific membrane transport protein in the luminal surface of renal tubular epithelial cells. Other diuretics exert osmotic effects by preventing water reabsorption (mannitol), inhibiting carbonic anhydrase enzymes (acetazolamide), acting on hormonal receptors in renal epithelial cells (spironolactone). Most diuretics act up on a single anatomic segment of the nephron. The action of each diuretic agent can be best understood in relation to its site of action in the nephron and the normal physiology of the segment, because the segments have distinctive transport functions.⁴

Problems like drug resistance, adverse effects with available drugs and emerging newer diseases where no medicines are available, have stimulated, a striking increase in the use of herbals in both developing and developed countries due to their natural origin and minimal (or) no side effects. The world over, the pharmaceutical companies and research organization are focusing on the vast untapped potential of herbals as potent drugs.⁵

Plants are a rich source of a variety of chemicals with nutritive and therapeutic properties. All plants produce chemical compounds as a part of their normal metabolic activities. There are primary metabolites and secondary metabolites. Secondary metabolites have therapeutic actions in humans, which can be refined to produce drugs.

Many herbal remedies are being used for oedematous conditions. Among them *Moringa oleifera* was found to have diuretic property along with antispasmodic, anti-ulcer, anti-inflammatory, hypoglycemic effects, antidiabetic, antioxidant, antihypertensive, immunomodulatory, chemo protective, radio protective, antipyretic, antiepileptic, antitumor, antibacterial, and antifungal activities which have been reported in literature.^{6,7} Hence, in this present study an attempt has been made to evaluate the diuretic property of *Moringa oleifera*.

METHODS

This study was carried out in the central animal house, institute of pharmacology, Madurai Medical College (MMC), Madurai. It was an experimental study conducted for a period of six months from October to March 2007. Ethical clearance was obtained from the institutional ethical committee, MMC, Madurai.

Table 1: Various groups and its diuretic effects.

Group	Category	Diuretic effect (drugs administered)
I (n=6)	Control	Normal Feed and water
II a (n=6)	Standard I	Furosemide 1 mg/kg normal feed and water
II b (n=6)	Standard II	HCT 2.5 mg/kg normal feed and water
III (n=6)	Test	Chloroform extract (seed) 1000mg/kg normal feed and water

Experimental animals

Albino rats⁸

Healthy adult wistar rats, 6-8 months old, weighing around 200 to 250 gm of either sex were used. The

animals were inbred colony maintained in the central animal house, MMC. They were fed with commercially available standard pellet diet obtained from Amrut feeds, Pranav agro industries limited and water ad libitum.

Drugs and chemicals

Preparation of Extract of *Moringa oleifera*⁹

The seeds of *Moringa oleifera* were collected and dried in shade for 10 days. It was then coarsely powdered. 200 gm of the powdered seed was soaked in sufficient quantity of chloroform overnight. The contents were transferred to a soxhelt apparatus and extracted for about two hours using hot water bath. The process was repeated several times with fresh seed powder to get sufficient quantities of an extract. The semisolid extract obtained was weighed accurately and utilized for experimental studies. The extract was dissolved in distilled water to yield the required concentration. The dose of the extract was 1000 mg/ kg of body weight.

Hydrochlorothiazide^{10,11}

Tablet hydrochlorothiazide 25 mg was powdered and mixed with 10ml of distilled water and the suspension prepared to provide 2.5 mg/ml and it was administered orally at the dose of 2.5 mg/kg of body weight.

Diuretic effect

24 albino rats weighing 200 – 250 grams of either sex were divided into four groups of 6 each and they were fasted for 18 hours. Group I (Control) received normal feed and water. Group II a (Standard 1) received Furosemide 1 mg/kg Group IIb (Standard 2) received Thiazide 2.5 mg/kg. Group III (Test) received a chloroform extract of *Moringa oleifera* seed (1000 mg/kg). All drugs were administered orally. After 24 hours, urine output and electrolytes were assessed for diuretic efficacy. Metabolic cages were used in the analysis of diuretic effect.

RESULTS

Table 2: Analysis of urinary volume.

Group	Volume of urine (ml/24 hours)
I Control	4.98 ± 0.26
IIa Standard I	9.4 ± 0.39
IIb Standard II	8.07 ± 0.27
III Test	7.72 ± 0.25

Group III vs Group I, IIa and IIb - p <0.001, <0.001 and <0.05 respectively.

24 adult albino rats weighing 200-250 gms of either sex were divided into four groups of six each. Every day the animals were assessed after the administration of drugs. On close follow up of the animals which received

hydrochloro thiazide, Frusemide and chloroform extract of *Moringa oleifera*, did not show any behavioural abnormalities (or) weight loss. These indicated that the extract did not have any systemic toxicity.

Table 3: Analysis of urinary pH.

Group	Volume of urine (ml/24 hours)
I Control	7.1 ± 0.06
IIa Standard I	7.6 ± 0.06
IIb Standard II	8.17 ± 0.05
III Test	9 ± 0.06

Group III vs Group I, IIa and IIb - p <0.001, <0.001 and <0.01 respectively.

Table 4: Analysis of urinary specific gravity and albumin.

Group	Specific gravity	Albumin mg/dl
I Control	1.011 ± 0.0009	20 ± 0.63
IIa Standard I	1.011 ± 0.0006	20 ± 0.53
IIb Standard II	1.011 ± 0.0003	20 ± 0.63
III Test	1.011 ± 0.0008	20 ± 0.43

Group III vs Group I, IIa and IIb; p >0.05.

Diuretic effect

The diuretic effect of control, standard I and II and the extract were estimated using metabolic cage and the results were statistically analysed by using a student's unpaired 't' test.

Table 5: Analysis of urinary electrolytes.

Group	Urinary electrolytes		
	Sodium meq/l	Potassium meq/l	Chloride meq/l
I Control	70.62 ± 0.29	57.12 ± 0.23	90.92 ± 0.56
IIa Standard I	118.25 ± 0.105	81.15 ± 0.27	120.08 ± 0.68
IIb Standard II	106.95 ± 0.27	76.15 ± 0.21	108.22 ± 0.50
III Test	105.13 ± 0.29	74.2 ± 0.35	102.43 ± 0.56

Sodium: Group III vs Group I, IIa and IIb; p <0.001, <0.001, >0.05 respectively; Potassium: Group III vs Group I, IIa and IIb; p <0.001, <0.001, >0.05 respectively; Chloride: Group III vs Group I, IIa and IIb; p <0.001.

Urine volume

The mean 24 hour urinary volume in Group I (control), Group IIa (Standard I), Group IIb (Standard II) and Group III (Extract) were 4.98±0.26, 9.4±0.39, 8.07±0.27 and 7.72±0.25 ml respectively. There was a highly significant increase in urinary volume in groups which

received standard drugs and the extract when compared to control (p <0.001).

When the urinary volume of the extract was compared with the standard I (Frusemide) it was highly significant (p <0.001) and with standard II (hydrochloro thiazide) it was significant (p <0.05) (Table 1).

PH

The mean pH in Group I, Group IIa, IIb and Group III after 24 hours was 7.1±0.06, 7.6±0.06, 8.17±0.05 and 9±0.06 respectively. There was a highly significant increase in urinary pH in groups which received standard II (hydrochloro thiazide) and the extract when compared to control (p <0.001).

When the urinary pH of the extract was compared with the standard I (frusemide) it was highly significant (p <0.001) and with standard II (hydrochloro thiazide) it was moderately significant (p <0.01) (Table 2).

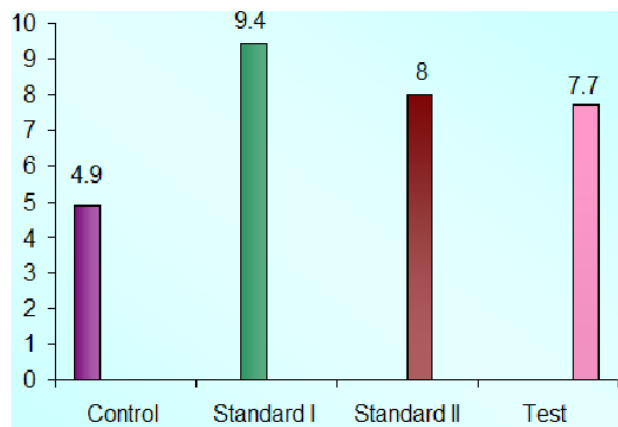


Figure 1: Urine output.

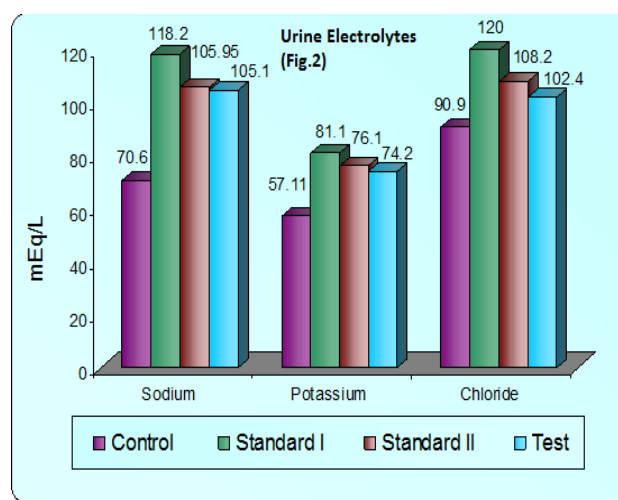


Figure 2: Urine electrolytes.

Specific gravity

No significant change in urinary specific gravity and albumin were noted in Group IIa, Group IIb and Group III after 24 hours ($p > 0.05$) (Table 3).

Analysis of urinary electrolytes

a. Sodium

The mean urinary sodium level in Group I, Group II a, IIb and Group III after 24 hours were 70.62 ± 0.29 , 118.25 ± 0.105 , 106.95 ± 0.27 and 105.13 ± 0.29 meq/l respectively. There was a highly significant increase in urinary sodium in groups which received standard drugs and the extract when compared to control ($p < 0.001$).

When the urinary sodium of the extract was compared with the standard I (frusemide), it was highly significant ($p < 0.001$) and with standard II (hydrochloro thiazide) it was not significant ($p > 0.05$) (Table 4).

b. Potassium

The mean urinary potassium levels in Group I, Group IIa, IIb and Group III after 24 hours were 57.12 ± 0.23 , 81.15 ± 0.27 , 76.15 ± 0.21 and 74.2 ± 0.35 meq/l respectively. There was a highly significant increase in urinary potassium in groups which received standard drugs and the extract when compared to control ($p < 0.001$).

When the urinary potassium of the extract was compared with the standard I (frusemide), it was highly significant ($p < 0.001$) and with standard II (hydrochloro thiazide) it was not significant ($p > 0.05$) (Table 4).

c. Chloride

The mean urinary chloride levels in Group I, Group IIa, IIb and Group III after 24 hours were 90.92 ± 0.56 , 120.08 ± 0.68 , 108.22 ± 0.50 and 102.43 ± 0.56 meq/l respectively. There was a highly significant increase in urinary chloride in groups which received standard drugs and the extract when compared to control ($p < 0.001$).

When the urinary chloride of the extract was compared with the standard I (frusemide) and standard II (hydrochloro thiazide) it was highly significant ($p < 0.001$) (Table 4). Thus the extract of *Moringa oleifera* (seed) showed significant diuretic, natriuretic and kaliuretic effects.

DISCUSSION

Diuretics are considered as the ideal pharmacotherapeutic agents for the correction of excess sodium and water load. *Moringa oleifera* (Drumstick) commonly used and widely cultivated perennial tree has both diuretic effect in addition to other properties¹².

In this present study, diuretic property of *Moringa oleifera* was compared with standard drugs. The observations emanated in the present study indicated that the total volume of urine and urinary excretion of sodium, potassium, and chloride were significantly increased in groups which received standard drugs and the extract (Group IIa + IIb and Group III). The possible explanation for significant sodium, potassium, chloride excretions are

- Sodium excretion is always associated with water excretion.¹³
- The urinary chloride excretion could be attributable to sodium and potassium excretion.

Interestingly, the urinary pH in groups which received hydrochloro thiazide and the extract, showed significant alteration in pH (alkaline pH).

There was no alteration in the urinary specific gravity and the albumin content in Group IIa, Group IIb and Group III. Physiologically, albuminuria indicates glomerular pathology or injury. Urine specific gravity depends upon the tubular system of the nephron during physiological situations. As albuminuria and alteration in specific gravity were not noticed in animals which received standard drugs and the extract (Group IIa + IIb and Group III), it is likely that the plant extract is safe. From the above data the effect of the extract was similar to that of hydrochloro thiazide (moderate efficacy diuretic). However, further evaluation is required to understand the molecular mechanism, and site of action of *Moringa oleifera*. The results were statistically analysed and proved to be significant. Hence *Moringa oleifera* possess diuretic (moderate efficacy) effect.

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Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Ethics Committee

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