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Evaluation of diuretic activity of seed and hydro-alcoholic extract of *Blepharis maderaspatensis* ex roth heyne– A folklore plant

Devang Y Joshi[1], Mukesh Kumar B Nariya[1*], Rajesh Barvaliya[2]
[1] Pharmacology Laboratory, Institute of Teaching & Research in Ayurveda (ITRA), Jamnagar, Gujarat, India
[2] Professor, Government Ayurvedic College, Vadodara, Gujarat, India

Abstract
Introduction: *Blepharis maderaspatensis* is used as a diuretic medicine as a folklore plant.
Aim & Objective: The aim of the research study is to evaluate the single dose diuretic activity of seed powder and hydro-alcoholic extract of the *Utigan*.
Materials & Methods: The wistar albino rats were divided into 6 different groups containing 6 animals per group. The normal control group, ref. std. group (10 mg/kg, po), *Utigan* powder at low and high dose (67.5mg/kg and 135mg/kg) and *Utigan* extract at low and high dose (100mg/kg and 200mg/kg po) respectively. 1 hour after the administration of drug animals were kept in metabolic cages and urine of 6hrs and 24 were collected and volume, and electrolytes were evaluated.
Result & Observation: *Utigan* powder at high dose and extract at low dose significantly increase urine volume at 24 hrs. *Utigan* powder at low and high dose significantly increase the excretion of Na^+ and K^+ through urine and have high diuretic index. In the conclusion it is revealed that *Utigan* powder at both doses shows diuretic activity and extract have less diuretic activity when compared with normal control group.
Conclusion: This study shows the diuretic activity of *B. maderaspatensis* possibly mediated through loop diuretic effects, which rationalizes its medicinal use as a diuretic.

Keywords: *B. maderaspatensis*, diuretic, hydro-alcoholic extract, utigan powder

Introduction

Diuretic medicines are useful in many diseases such as thiazides in a heart failure (Musini *et al*., 2009) [7], hypertension patients with magnesium or potassium loss (Mishra, 2016) [6], loop diuretics utilised in the treatment of the salicylate, phenobarbital, and lithium poisoning (Prescott *et al*., 1982) [9], in the syndrome of inappropriate antidiuretic hormone secretion (SIADH) and in restriction of water intake as a second line of therapy (Yasir *et al*., 2021) [8].

Hyponatremia is most uncommon complication occurred during the diuretic therapy (Ayus, 1986) [19]. Administration of thiazide for diuretic effect for prolonged time can lead to the glucose intolerance and occasionally precipitate diabetes mellitus (Furman, 1981) [20]. Administration of thiazide and thiazide like diuretic can decrease libido, erectile dysfunction and difficulty in ejaculation (Chang, 1991) [21]. After considering, the side effects of the drug and/or medicine is necessary to evaluate novel drug for its diuretic effect with minimal or with no side effects. Many herbal plant have been famous for its diuretic activity and with minimal or with side effect apart from its diuretic activity such as *Aerva lanata* Linn. (Goonaratna *et al*., 1993) [22], *Tribulus terrestris* L. (Ali *et al*., 2003) [23] etc.

There is a folklore plant name as *Blepharis maderaspatensis* of Acanthaceae family, which is unexplored plant and known as *Utigan* in local language and used in many diseases such as *Mutrakruccha* (Urolithiasis), *Jwara* (fever), *Prameha* (diabetes), and *Brahma* (hallucination) (Thakar, 1998) [10]. It is known as *Ucchato, Chopani vel* in local language The seed part of the plat has been mainly utilised for treating various ailments from the folk peoples of the Saurashtra region, Gujarat, India. However, till date no pharmacological studies of the plant have been reported to prove its diuretic action as a scientific study. Hence, the present research has been done to evaluate the diuretic activity of its seed powder, and hydro-alcoholic extract of seed powder in animal model.

Materials and Methods

Plant material

The seed of the *Utigan* were collected from Gandhinagar, Gujarat and was authenticated by Pharmacognosy Laboratory, ITRA, Jamnagar, India. A voucher specimen Ph.M. 6298/2020/21 was deposited at department of pharmacognosy, ITRA Jamnagar, India.

Plant extract
The hydro-alcoholic extract of *Utigan* was prepared by taking 5 gm of course seed powder, and was macerated in a closed flask for 18 hrs, shaking frequently during six hours and allowed to stand for 18 hrs. Later, it was filtered and filtrate was evaporated, and obtained extract stored in air tight container under 4 ºC.

Dose
The dose for the experimental study was calculated by extrapolating the prescribed human dose of *Utigan* seed powder two *aani* to *paa pawlabhar* (Rajendar *et al*., 2011) [4] to rat dose based on body surface area ratio (Paget and Barns, 1964) [5]. The suspension of test drugs was prepared in suitable concentration in distilled water and administered orally at constant volume of 5 ml/kg body weight of rats.

Drugs and Chemicals
All the chemicals used during the experimental study were of analytical grade and purchased commercially. And, furosemide was used as reference diuretic drug.

Experimental animals
Wistar albino rats of either sex were procured from the animal house attached to the Pharmacology laboratory, ITRA, Jamnagar. The animals were maintained as per standard husbandry conditions in terms of temperature (22±3ºC), relative humidity (50-60%) and 12-hour light and dark cycle. Animals were fed with VRK brand rat pellet feed supplied by Keval Sales Corporation. The drinking water was given *ad libitum*. The experiments were carried out after approval of Institutional Animal Ethics Committee (IAEC/25/2019/13) in conformity with the guidelines of the CPCSEA, New Delhi.

Diuretic activity in albino rats
Diuretic activity of *Utigan* seed powder and *Utigan* seed extract was evaluated following the procedure described by Al-Shikhan and Ansari, 2016 [1]. The principle of test includes uniformly hydrated rats were subjected to drug administration and kept under metabolic cage. Urine can be collected periodically, measured and collected urine was subjected to estimation of electrolytes, specific gravity, and pH.
Wistar strain of albino rats of either sex weighing between 200±20 g were divided in six groups each consisted of six rats. First (I) group was used as normal control group, received distilled water (5 ml/kg, po). Second group was received furosemide (10 mg/kg, po) as standard drug as diuretic agent (Mazumder *et al*., 2012) [2]. Groups (III) and (IV) were orally treated with *Utigan* seed powder (67.5 and 135 mg/kg) (UPLD and UPHD) respectively. *Utigan* hydro-alcoholic extracts were orally administered at dose of 100 mg/kg and 200 mg/kg body weight of albino rats (UELD and UEHD) to Group (V) and (VI) respectively.
The test drug, reference standard and distilled water were administered to the overnight fasted rats of respective groups All the animals were administered distilled water immediate after drug administration in the dose of 5 ml/100 g body weight to ensure uniform hydration. Immediately after administration, the animals were placed in the metabolic cages separately, specially designed to separate urine and feaces, kept at temperature of 23±0.5ºC throughout the experiment. Urine was collected periodically at the interval of 1, 2, 4 and 6 hours (Maghrani *et al*., 2005). After 6 hours, 10g food pellets were given to the animals to prevent from starvation. Next day after 24 hours of keeping in metabolic cage collected urine was measured.

Collection & analysis of Urine
The urine was collected at 6 h and 24 h intervals. Routine urinalysis such as pH, and specific gravity along with presence of occult blood, bilirubin, urobilinogen, ketone bodies, proteins, nitrite, glucose, and leucocytes in urine was carried out using urocolor test strips (Standard Diagnostics Inc., South Korea) for urine samples of control and extract treated rats. Urine volume (ml/100 g) and Na^+, K^+ and Cl^- concentrations (mg/l/100 g) in the urine were determined (Swain *et al*., 2008) [11] and various indices for diuretic action were calculated (Martin-Herrera *et al*., 2008; Mukherjee, 2008) [12, 13]

Diuretic index = urine volume of test group/urine volume of control group

Saluretic index = urinary excretion of electrolyte of test group/urinary excretion of electrolyte of control group

Natriuretic index = urinary excretion of Na^+/urinary excretion of K^+

CAI Index= urinary excretion of Cl^-/sum of urinary excretion of Na^+ and K^+

Result & Observation
The urine output after acute administration of drug in diuretic study after 6 hrs and 24 hrs are shown in Table 1. *Utigan* seed powder and its extract showed increase in urine volume at both time intervals in comparison with control group. The data revealed that, *Utigan* seed powder at higher dose (UPHD) and *Utigan* seed powder extract at both dose levels (UELD and UEHD) treated groups shows significant increase in urine output when compared with normal control group respectively while, all the other treated groups and standard drug showed

non-significant increase in urine output in ml/100 g body weight. The data of diuretic index shows the least diuretic index in UPLD and UEHD treated groups in diuretic study. However, furosemide treated group showed higher diuretic index followed by UELD and UPHD treated groups (Table 1).

The electrolyte concentrations in urine after acute administration of drug in diuretic study have been shown in Table 2. It revealed that *Utigan* seed powder at lower (UPLD) and higher dose (UPHD) significantly increase sodium and potassium while not affected chloride concentration in urine when compared to normal control group. *Utigan* seed powder extract at both dose levels (UELD and UEHD) treated groups did not show any changes in sodium and potassium concentration while increase the chloride level in urine when compared to normal control group.

The natriuretic index, CAI index, and saluretic index of acute and repeated diuretic study have been shown in table 3. The data showed that UPHD and UPLD treated groups shows dose-dependent natriuretic index when compared with normal control group. While, both the UELD and UEHD extract groups did showed high CAI index when compared with normal control group respectively. All groups show high saluretic index of UPHD, UELD, UEHD followed by UPLD compared to normal control group.

Table 1: Effect of test drugs on urine volume in diuretic study in albino rats

Groups	Urine volume (ml/100g body weight)		
	Acute administration (1 day)		Diuretic Index (24 h)
	After 6 hours	After 24 hours	
NC	3.56±0.26	3.98±0.27	1.00
RS	4.39±0.31	5.03±0.48	1.26
UPLD	3.43±0.55	4.92±0.48	0.57
UPHD	4.14±0.27	6.58±0.61**$^{@@}$	0.77
UELD	4.51±0.30*	7.06±0.55***$^{@@}$	0.82
UEHD	4.39±0.43	5.70±0.48*	0.66

Data: Mean±SEM; *P<0.05, **P<0.01, P<0.001, when compared to control group (Unpaired 't' test); $^{@@}$P<0.01, when compared with control group (Annova followed by Dunnett's multiple 't' test)

Table 2: Effect of test drugs on electrolyte concentrations in urine during diuretic study in rats

Groups	Sodium (mg/L)	Potassium (mg/L)	Chloride (mg/L)
NC	1.463±0.181	5.606±0.452	73.765±2.499
RS	1.306±0.251	5.550±0.950	70.665±3.348
UPLD	6.704±0.696***$^{@@}$	15.620±1.487***$^{@@}$	66.683±3.055
UPHD	6.401±0.680***$^{@@}$	14.142±1.453***$^{@@}$	71.22±8.822
UELD	1.260±0.271	5.207±0.683	81.27±5.138*
UEHD	1.290±0.176	4.693±0.323	76.95±3.761

Data: Mean±SEM; *P<0.05, ***P<0.001, when compared to control group (Unpaired 't' test); $^{@@}$P<0.01, when compared with control group (Annova followed by Dunnett's multiple 't' test)

Table 3: Effect of test drugs on electrolyte concentrations during single dose diuretic study in rats

Groups	Natriuretic index	CAI Index	Saluretic Index
NC	0.25	10.96	1.00
RS	0.24	12.19	1.00
UPLD	0.44	3.14	1.10
UPHD	0.45	3.70	1.13
UELD	0.25	14.37	1.12
UEHD	0.27	13.10	1.03

Discussion

The urine output (ml/100 g body weight), urine electrolytes such as sodium, potassium, and chloride, urine pH measured as a parameter to evaluate the diuretic activity of *Utigan* seed powder and Hydro-alcoholic extract of *Utigan* seed powder in a present study. In the present study the seed powder, and hydro-alcoholic extract of *Utigan* was evaluated for rationalized the medicinal use as diuretic drug which could be used in different types of ailments such as hypertension, cardiopathy activities, *Mutrakruccha* (urolithiasis), stone disease etc.

In the present study, *Utigan* (*B. maderaspatensis*) was evaluated using acute dose administration for diuretic study. The drug sample in a powder and extract of both dose levels increase urine output non-significantly but cannot increase in dose dependent levels. Among all the groups *Utigan* powder at high dose shows highest amount of urine output when compared with all other drug samples.

The extract shows different degrees of urine output, electrolyte in urine, and diuretic index, saluretic index, and CAI index. Na:K ratio is an indirect evaluation of mineralocorticoid activity which is simple and an inexpensive measurement for evaluation of diuretic activity (Milly *et al*., 1961) [14]. Diuretic index is considered to be good if

the values are greater than 1.50, moderate if the values are between 1.00 and 1.50, mild if the values lie between 0.72 and 1.00, and nil if the value is <0.72 (Asif *et al*., 2013) [17]. From the result it is showed that UPLD has lowest diuretic index of 0.57 in diuretic study The UPHD and UELD treated groups have moderate diuretic index.

The result shows that Na:K ratio of all groups is <1 in diuretic study. Which, suggested high urinary potassium loss which can also see in diuretic study (Alexander *et al*., 1971). Natriuretic ratio >2.0 indicates a favourable natriuretic activity (Vogel, 2002) [16]. On the contrary to that the data shows an average natriuretic activity in the present study when compared with normal control group.

The $Cl^{-}/(Na^{+} + K^{+})$ is indicative of carbonic anhydrase inhibitory activity, and substances resulting in ratios between 0.8 and 1.0 can be excluded from this activity (Vogel *et al*., 2008) [18]. All the test group shows more than 1.0 of carbonic anhydrase inhibitory activity in diuretic study which is indicative of diuretic action through carbonic anhydrase inhibition. The increase generation of Na^{+}, K^{+}, and Cl^{-} did the alkalization of urine, showing a strong inhibiting activity of carbonic anhydrase and saluretic.

Utigan seed powder at lower (UPLD) and higher dose (UPHD) treatment excreted high amount of sodium and potassium urine in dose-independent manner. So, it was observed that high amount of Na^{+}, K^{+}, Cl^{-} excretion with alkalinisation of urine with increasing urinary output. These observations suggest that the *Utigan* seed powder acting as a loop diuretic due to non-significant increase of excretion of water, sodium, potassium and chlorine etc. In all the groups the pH, and specific gravity of the urine did not significantly change when compared with normal control group.

Overall, *Utigan* powder at high dose and extract at both dose levels shows diuretic activity on single dose in diuretic study in albino rats. *Utigan* powder shows higher concentration of Na^{+} and K^{+} as well as natriuretic and saluretic index in urine of albino rats. While, CAI index was high in UELD and UEHD treated groups in diuretic study. And, the excretion of Na^{+}, K^{+}, and Cl^{-} is minimum which, suggest that the plant extract have been worked as similar to carbonic anhydrase inhibitor activity. The previous research study of Arul shows the that the plant has significant *in vitro* anti-oxidant activity (Arul-Albert Baskar *et al*., 2012) [26]. Studies have shown that Nitric oxide causes natriuresis, diuresis and inhibits a fluid reabsorption in renal tubules (Garcia *et al*., 1996; Noonan and Banks, 1999) [24, 25]. And, the increase of natriuresis may partly increase the diuresis. The excretion of Na+, K+ causes the decrease in urine osmolarity are of tubular origin for UPLD and UPHD treated groups. So, these findings might explain partially that the powder treated group have diuretic effect in the present study (Tahri *et al*., 2000). The extract of the plant UELD and UEHD have been increase the CAI index and The previous study of Dirar *et al*. shows that the plant may have some secondary metabolites such as glycoside, alkaloids and proteins as of part root is used. Which, increase the GFR rate and increase the urine output and may have work as diuretic as an action. The present study may explain the diuretic activity of the plant in hypertension, and urolithiasis. Nevertheless, further mechanisms may not be ruled out.

Conclusion

In conclusion, our present study is the first scientific study to evaluate the folklore use of *B. maderaspatensis* seed powder, and hydro-alcohlic extract for a diuretic activity. The research study shows that UPLD and UPHD significantly increase 24h urine volume after treatment. And, the diuretic index and urine output *Utigan* powder and extract at high and low dose treated group. There is also significant excretion of sodium and potassium in *Utigan* powder at both doses which suggest use in inflammatory condition associated with renal and cardiac failure.

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Conflicts of Interest

All authors declare that there are no conflicts of interest.

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