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Experimental evaluation of diuretic effect of *Usheera Moola - Vetiveria Zizanioides* (Linn.) Nash

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ABSTRACT

By literary survey *Usheera* is mentioned from *Vedakala*. Some *Nighantus* and some Ayurvedic modern books mention that, it has *Mootravirechaniya* action used in *Mootrakricchra*. This drug is easily available and grows all over India. Hence roots of *Vetiveria zizanioides* (Linn) Nash i.e. *Usheera* was selected for this study. Most of Ayurvedic drugs or formulations are known for their safety and efficacy. Hence literary research is done to specify a medicine that can act as a diuretic. Lots of drugs are mentioned as *Mootravirechaniya* (diuretic) in Ayurvedic classics. Here *Mootravirechaniya* is also called as *Bastishodhana* and *Mootrala*. They are having *Sheeta Veerya* or *Ushna Veerya*, *Madhura*, *Amla*, *Lavana Rasas*, *Drava* and *Upakledi* properties. The selected drug *Usheera* is having *Sheeta* and *Madhura* properties. Diuretics are the drugs which cause a net loss of sodium and water in urine. These are widely prescribed drugs. They are used whenever there is need to eliminate excess of water and ion accumulated in tissues.

Key words: *Usheera, Vetiveria zizanioides, Mootravirechaniya, Diuretics.*

INTRODUCTION

Ayurveda, the knowledge of life science bestowed health and longevity in the form of preventive and curative measures. The curative aspects are mainly covered by *Dravya Chikitsa* (Treatment using drugs). As diseases are born with human there is always a search for safest and curative drugs.

In the present era, the attraction towards Ayurveda is increasing day by day due to less unwanted side effects. On account of increasing urbanization, increasing demand of medicine for population, shortage of authentic material and also tendency of profiteering, there is a need for statutory control and development of pharmacopoeal standards.

According to Charaka qualities of drug should be free available, effectiveness, capability of being subjected to various forms of pharmaceutical processing and it should be in excellent condition i.e. without insect etc and which required *Rasapanchakas* etc. The term *Sampat* is very important to make many formulations of many drugs or single drug. It should go through the standardization parameters along with its authentic sources.

The roots of *Vetiveria zizanioides* (Linn) Nash. were taken for present study. Analysis of sample was done to evolve suitable parameters for checking the quality. The main objective of present work is to evaluate the diuretic effect of *Usheera* in healthy albino rats.

OBJECTIVES OF THE STUDY

To evaluate the effect of extract of roots *Vetiveria zizanioides* (Linn) Nash for diuretic activity on the selected animals (Albino rats).

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DRUG REVIEW

Sanskrit Name

Usheera - The fragrant root of the plant.[1]

Botanical Name[2]

Vetiveria zizanioides (Linn) Nash. - *Vetiveria* = the root i.e. dug up and *zizanioids* = zizania like.

The generic name *Vetiveria* comes from Tamil word *Vetiver* meaning "root that dug up". The specific name *zizanioides* was first given by the Swedish taxonomist Carolus Linnaeus in 1771, meaning "by the river side". Or *Andropogon muricatus* Retz - *Ander* = man, Pogon = bear, *Murcatus* = covered with sharp short points or small spikes.

Morphology [3]

A densely tufted perennial grass, root stock, branching with spongy aromatic roots, culms stout, upto over 1.8 cm high, usually sheathed all along, leaf sheaths compressed, especially the lower which are sharply keeled and fan like, imbricate, very smooth, firm, ligules reduced to a scarious rim: blades narrowly linear, acute, 30-90 cm long 4.2 -10.6 mm wide erect, rigid, firm or some what spongy, usually glabrous, rarely more or less hairy downwards on the face, pale green, midrib slender, lateral nerves close, 6 or more on each side, rather stout slightly prominent, margin spinously rough, pamcle oblong, upto over 30 cm, long, usually contracted rhachis stout, smooth; whorls 6-10 with upto 20 rays branches oblique to suberect, naked for upto 5 cm filiform slightly rough, racemes upto 5 (rarely 7.5) cm long, very slender, joints about a slong as the sessile spikelets or sometimes distinctly exceeding them, smooth or more or les rough, minutely and unequally ciliolate at the slightly oblique tips, pedicels similar but shorter, sessile spikelet linear – lanceolate to almost linear acute or subacute 4.2-4.8 mm long; Yellowish, olive or violet – brown or purplish to almost black; callus obtuse, under 1mm long glabrous, involueral glumes, acute, coriaceous, lower muriculate, all over the black, 5 nerved, lateral nerves close, very fine, upper spinulously muricate on the keel; lower floral glume as long as the involucral glums, acute, reversedly ciliolate upper upto 3.3mm long, narrow ablong – lanceolate mucronulate; ciliolate, lodicules 2, quadrate and conspicuous, though, small, styles and stigmas short stigmas purple, anthers 2.33 mm long pedicelled spikelet sparingly aculeolate or almost smooth, upper floral glume entire acute.

Table 1: Properties od drug *Usheera*.

Book Name	*Rasa*	*Guna*	*Veerya*	*Vipaka*	*Doshaghnata*
Raj-Nighantu[4]	*Tikta*	*Sheeta*	*Sheeta*	-	*Pittashamaka,*
Dhanvantari Nighantu[5]	*Tikta*	*Snigdha*	*Sheeta*	-	*Vataghna* *Pittaghna*
Kaiyadeva Nighantu[6]	*Madura Tikta*	*Laghu Ruksha*	*Sheeta*	-	*Vatadi, Doshaghna*
Priya Nighantu[7]	*Tikta*		*Sheeta*	-	
Bhavaprakasha Nighantu [8]	*Tikta Madhura*	*Laghu*	*Sheeta*	-	*Kapha Pitta Shamaka*
Dravyaguna Vijnana[9]	*Tikta Madhura*	*Ruksha Laghu*	*Sheeta*	*Katu*	*Kapha Pitta Shamaka*
Ayurvedic Pharmacology and Therapeutic [10]	*Tikta Madhura*	*Ruksha Laghu*	*Sheeta*	*Katu*	*Kapha Shamaka, Pitta Shamaka*
Indian Materia Medica [11]	*Tikta Madhura*	*Laghu*	*Sheeta*	-	*Kapha, Pitta Hara*

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Table 2: Useful parts of *Usheera*.

Name of the text	Useful parts
Raj-Nighantu	Root
Priya-Nighantu	Root
Bhavaprakash-Nighantu	Root
Dravyaguna Vijnana	Root
Indian Materia Medica	Fibrous wiry roots from therhizomes

MATERIALS AND METHODS

Materials

Animals: Female albino rats of body weight 160gm to 190gm and 3-4 months of age were used for testing.

Chemical Record: Frusemide, Normal Saline, root extract of *Vetiveria zizanioides* (Linn) Nash.

Equipments: Special cages.

Collection of Rats: Animals were obtained from the BLDE Medical College Animal House, Bijapur.

Selection of animals

Female albino rats of body weight 160gm to 190gm and 3-4 months of age were kept in standard cages. They were exposed to natural day and night cycles with ideal laboratory conditions in terms of suitable temperature and humidity, and fed laboratory diet i.e. balanced rat pallet food and free excess to drinking water.

Sample size

To study diuretic activity, eighteen female albino rats were taken for study. They were distributed in three groups containing six each.

Preparation of Animals

24 hours before testing, the animals were transferred to metabolic cages then only water was made accessible without food.

Extract Used: *Vetiveria zizanioides* (Linn) Nash extract.

Dose selection: Drug was given as, dose per kg body weight calculated by using the formula,

Animal dose = Human dose X 0.018

For trial drug, animal dose = 3gms X 0.018 = 0.054gms or 54 mg. = 54 X 5 = 270 mg/kg bd wt.

For standard drug, Animal dose = 20mg X 0.018 = 0.36 mg = 0.36X5 = 1.80 mg/kg bd wt.

For control group, Normal saline = 25ml/kg bd wt.

Route of drug administration

To the trial, standard and control groups, drugs were administered by oral route with the help of metallic syringe.

Preparation of drug

The drugs were prepared for different groups with normal saline.

Group - I: Extract was weighed and dissolved in normal saline 25ml/kg bd wt. Given orally in respective doses.

Group - II: Frusemide was weighed and dissolved in normal saline 25ml/kg bd wt. Given orally in respective doses.

Group - III: Measured normal saline according to body weight i.e. 25 ml/kg bd wt. and given orally.

Method: Lipschitz et.al. method[12]

Immediately after administration of drugs, rats were placed in metabolic cages and kept at room temperature for 5 hours, during this period no food or water were made available to them. At the end of 1st, 2nd, 3rd hours urine was collected and measured, at the end of 5th hour urine expelled from the body by pulling the base of the tail. Then animals were taken out of the cages and the total volume of urine extracted by each group was noted.

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Laboratory investigations

Urine samples were sent to laboratory to analyze sodium, potassium and chlorine concentration.

Statistical analysis

The data collected were statistically compared by using Histogram to see difference in urine volume and sodium, potassium and chlorine concentration. The data analyzed by using unpaired students 't' test with the consultation of Biostatistician.

OBSERVATIONS

Observation on selection of animals

- The healthy and nutritional status of the experimental albino rats were observed and selected.
- Maintained experimental animals, to keep them in physical comfort and good health and permitted them to grow and behave normally.
- Weighed all albino rats with identification marking and recorded.

Sample size

As per the bifurcation of each albino rats in their groups, they were observed and recorded.

Dose

- The dose was divided corresponding to the weight of the albino rats.
- The dose was kept in different sides and keenly observed up to its internal administration.
- The related divided dose was given to the different groups and their changes were observed.
- During selection of drug dosage all the parameters were taken into consideration and their harmful effects were also observed.

Route of drug administration

- During oral administration of drug in all groups precautionary measures were taken and the exact dose initiation through the oral route was observed.
- After administration of drug oral cavity was observed keenly.

Preparation of drug

- **Group I -** It was observed that the required dose of the roots extract of *Vetiveria zizanioides* (Linn) Nash was diluted with Normal saline at the dose of 25 ml / Kg body wt was given orally.
- **Group II -** The required dose of fine powder of standard drug Frusemide was added with normal saline (25 ml / Kg body wt) and given orally.
- **Group III -** The same amount of Normal saline (25 ml / Kg body wt) was given orally to this group. The respective changes were compared with other groups and considered as vehicle.

Observation during treatment

- All the healthy albino rats were kept starving up to 24 hrs.
- After completion of 24 hrs, the extract of *Vetiveria zizanioides* (Linn) Nash (test drug) was administered to group I. Their change in urine out put was observed, collected and recorded hourly.
- In the same way the standard drug was administered to group II their changes in urine out put was observed, collected and recorded hourly.
- The Normal saline was administered to group III and their changes in urine out put was observed, collected and recorded hourly.
- At the end of the 5th hour, total volume of the urine extracted by each group was observed.

RESULTS

Urine output of 3 Groups hourly in ml.

Table 3: In 1st hour

No of Albino rats	Gp-I	Gp-II	Gp-III
1	0.4	0.6	0.3
2	0.3	0.5	0.4

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3	0.3	0.6	0.0
4	0.2	0.7	0.2
5	0.4	0.6	0.1
6	0.3	0.5	0.3

Table 4: In 2nd hour

No of Albino rats	Gp-I	Gp-II	Gp-III
1	0.7	1.0	0.5
2	0.7	0.9	0.5
3	0.6	0.9	0.6
4	0.6	0.8	0.5
5	0.7	0.9	0.6
6	0.5	1.0	0.6

Table 5: In 3rd hour

No of Albino rats	Gp-I	Gp-II	Gp-III
1	0.8	1.3	0.7
2	0.9	1.4	0.6
3	0.9	1.3	0.7
4	0.8	1.2	0.7
5	0.9	1.4	0.6
6	0.9	1.4	0.6

Table 6: In 5th hour

No of Albino rats	Gp-I	Gp-II	Gp-III
1	1.3	1.1	0.8
2	1.2	1.3	0.8
3	1.4	1.1	1.0
4	1.4	1.2	1.1
5	1.3	1.2	0.8
6	1.4	1.0	1.0

Laboratory Reports

Urine Sodium, Potassium and Chloride concentration measured in mEq/L

Table 7: Urine sodium

No of Albino rats	Gp-I	Gp-II	Gp-III
1	52	180	34
2	66	178	35
3	63	170	36
4	58	169	34
5	59	179	33
6	67	181	39

Table 8: Urine potassium

No of Albino rats	Gp-I	GpII	Gp-III
1	56	85	15
2	48	88	12
3	47	81	14
4	51	90	17
5	52	82	16
6	54	87	14

Table 9: Urine chloride

No of Albino rats	Gp-I	Gp-II	Gp-III

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1	118	124	115
2	108	125	113
3	116	134	118
4	111	128	120
5	109	121	109
6	110	133	114

Table 10: Mean Urine Output

Time	Group I	Group II	Group III
1st hour	0.3166	0.5833	0.2166
2nd hour	0.6333	0.9166	0.55
3rd hour	0.8666	1.3333	0.65
5th hour	1.3333	1.15	0.9166

Table 11: Mean Urine Sodium

Rats	Group I	Group II	Group III
Rat 1	52	180	34
Rat 2	66	178	35
Rat 3	63	170	36
Rat 4	58	169	34
Rat 5	59	179	33
Rat 6	67	181	39

Table 12: Mean Urine Potassium

Rats	Group I	Group II	Group III
Rat 1	56	85	15
Rat 2	48	88	12
Rat 3	47	81	14
Rat 4	51	90	17
Rat 5	52	82	16
Rat 6	54	87	14

Table 13: Mean Urine Chloride

Rats	Group I	Group II	Group III
Rat 1	118	124	115
Rat 2	108	125	113
Rat 3	116	134	118
Rat 4	111	128	120
Rat 5	109	121	109
Rat 6	110	133	114

DISCUSSION

Diuretic effect on albino rats was carried out in 3 groups of 18 Female albino rats 6 in each group. Here we used female albino rats because they give more uniform responses. According to Lipschitz et. al. method, For Group I - Trial drug was administered with Normal saline, for Group II - Standard drug was administered with Normal saline and for Group III - only Normal saline was administered.

On the basis of body weight after drug administration rats were kept in special cages. These cages were specially made for evaluating diuretic activity. They separate the feces from urine because fecal contamination of urine would distort the apparent electrolyte excretion. For the same reason the animals were denied access to food overnight before the test.

The Urine was measured 1st, 2nd, 3rd and 5th hourly then sodium, potassium and chloride concentration was analyzed in laboratory. After this urine output and ion concentrations were statistically compared using histogram and significance was seen in unpaired student 't' test.

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The mean urine output in 1st hour was in Group I 0.3166ml, in Group II 0.5833ml and in Group III 0.2166ml. The results when compared with Trail group I and control group III 't' value was 1.4816 and p value was 0.1693 showed statistically non significant and compared with standard group II and control group III 't' value was 5.4325 and p value was 0.0003 showed statistically highly significant.

The mean urine output in 2nd hour was in Group I 0.6333 ml, in Group II 0.9166ml and in Group III 0.55ml. The results when compared with Trail group I and control group III t value was 2.0761 and p value is 0.0646 showed statistically significant and compared with standard group II and control group III t value was 9.6476 and p value was 0.000002 showed statistically highly significant.

The mean urine output in 3rd hour was in Group I, 0.8660ml, in Group II, 1.3333ml and in Group III, 0.65ml. The results when compared with Trail group I and control group III t value was 7.0502 and p value was 0.00003 showed statistically highly significant and compared with standard group II and control group III t value was 17.0243 and p value was 0.0000 showed statistically highly significant.

The mean urine output in 5th hour was in Group I 0.3333 ml, in Group II 1.15ml and in Group III 0.9166ml. The results when compared with Trail group I and control group III t value was 6.5428 and p value was 0.00007 showed statistically highly significant and compared with standard group II and control group III t value was 3.3757 and p value was 0.0071 showed statistically highly significant.

The mean urine Sodium concentration in group I was 60.8333, in group II 176.1667 and in group III 35.1667. The results when compared with trial group I and control group III t value was 10.4302 and p value was 0.0000 showed statistically highly significant and compared with standard group II control group III t value was 6.70393 and p value was 0.0000 showed highly significant.

The mean urine potassium concentration in group I was 51.3333, in group II 85.5000 and in group III 14.6667. The results when compared with trial group I and control group III t value was 23.2417 and p value was 0.0000 showed statistically highly significant and compared with standard group II control group III t value was 44.2612 and p value was 0.0000 showed highly significant.

The mean urine chloride concentration in group I was 11.0000, in group II 127.5000 and in group III 114.8333. The results when compared with trial group I and control group III t value was 1.2397 and p value was 0.2434 showed statistically non significant and compared with standard group II control group III t value was 4.8067 and p value was 0.0007 showed highly significant.

Probable mode of drug action

The *Dravyas* perform their actions in body by virtue of their qualities and configuration of *Mahabhoota*. But correlating the modern concept with Ayurvedic drug action is very difficult. Hence the drug action may be assessed on the basis of effects by their *Rasapanchakas* on body constituents on the basis of *Samanya Vishesha Siddhanta*.

Among so many drug actions diuretic is one of the action which increases rate of urine volume and used for reduction of excess fluid and sodium and diuretics are very necessary in acute and chronic conditions.

By literary survey *Usheera* has diuretic action (*Mootravirechaniya* action) and *Rasapanchakas* are *Ruksha Laghu Gunas*, *Tikta*, *Madhura Rasas*, *Sheeta Veerya* and *Katu Vipaka*.

According to, *"Kinchid rasen kurute karma veeryena chaaparam, Dravyam gunen pakena prabhavena cha kinchan."* Some drugs act due to *Rasa*, some due to *Vipaka*, some due to *Guna*, others due to *Veerya* and still other due to *Prabhava*. Whenever there is association of dissimilar qualities, the more potent quality will supersede the quality of lower potency and exhibit its own effects or qualities. Like this *Madhura Rasa* and *Sheeta Veerya* may act as potent and may supersede the *Katu Vipaka*.

Veerya is pharmacologically active ingredient of the drug or it is efficacy of drug. According to *"Veeryam tu kriyate yena ya kriya"*, *Sheeta Veerya* having *Saumya*

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Guna or it is *Jalamahabhoota* predominated one. On the bases of *Samanya Siddhanta* and by its *Santarpaka* action, it increases the quantity of *Jala* in *Mootra*.

Due to *Shoshana Guna* of *Ruksha Guna*, it may help in absorption of fluid from the extra cellular compartments to intracellular compartments. This leads to *Jaleeymsa Vriddhi* in *Mootra*.

The modern concept reveals the trial drug *Vetiveria zizanioides* (Linn) Nash has active constituents which possess pharmacological action. They are sterols, saponins, flavonoids and volatile oil which are having diuretic action. These may help in diuretic action of *Vetiveria zizanioides* (Linn) Nash.

CONCLUSION

Vetiveria zizanioides (Linn) Nash shows a significant diuretic effect in experimental study.

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