

EVALUATION OF DIURETIC ACTIVITY OF SIDDHA HERBAL FORMULATION DHADHU VIRTHI KULIGAI IN WISTAR RATS

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ABSTRACT

The purpose of the present study was to evaluate the diuretic activity of *Dhadhu Virthi Kuligai (DVK)* in experimental animals by following the standard procedure. Randomly selected animals were divided into four groups of six animals each. *DVK* was administered orally at a dose of 100mg/kg b. wt and 200mg/kg b. wt. Parameters like volume of urine, pH of urine and urinary electrolyte concentrations like sodium, potassium and chloride, Lipchitz value and Na^+/K^+ ratio were studied. *DVK* increased the urine output and urinary concentration electrolyte in a dose-dependent manner. There was no significant difference in Lipchitz value and Na^+/K^+ ratio. From the present study, it can be concluded that the root of *Dhadhu Virthi Kuligai* has diuretic property.

KEYWORDS: Dhadhi virthi kuligai, Diuretic, urinary output.

INTRODUCTION

Kidney, the excretory organ of our body serves the important functions of excretion of waste products, regulation of fluid volume and electrolyte content of the extracellular fluid. Diuretic is an agent which increases urine and solute secretion.^[1] Natural diuretics acts by increasing the urine output as well as urinary electrolyte concentration.^[2] *Dhadhu Virthi Kuligai* is a

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herbal formulation which has some potent herbs having diuretic property. A study suggested that the effect of the ethanolic extract of MP aerial parts on the urination of mice was observed for 5 h which revealed that the extract has a marked diuretic effect in the test animals.^[3] Ratnam KV et al study indicates that ethanolic root extract of *Curculigo orchoides* showed dose dependant diuretic activity.^[4] A study on *Hygrophila auriculata* revealed that out of the different fractions and extract, the n-butanol fraction (200mg/kg) significantly and markedly increased the urine output ($p < 0.01$).^[5] A study was carried out with an aqueous extract of the roots of *Asparagus racemosus* utilizing three doses viz 800 mg/kg, 1600mg/kg and 3200mg/kg for its diuretic activity in comparison with standard drug (furosemide) and control (normal saline) rats after doing acute toxicity study. *Asparagus racemosus* showed diuretic activity at a 3200 mg/kg dose without acute toxicity.^[6] Most of the ingredients of the drug DVK has diuretic properties and the combined effect of them is studied in this study.

MATERIALS AND METHODS

Procurement and Authentication of Raw Drugs

The drugs were purchased from authorized country raw drug store in Chennai. All the plant materials, *Mucuna pruriens*, *Curculigo orchoides*, *Hygrophila auriculata*, *Asparagus racemosus* and *Acacia nilotica* were identified and authenticated by Botanist, National Institute of Siddha, Tambaram Sanatorium, Chennai.

Preparation of Dadhu virthi kuligai

All these ingredients were powdered and soaked in lime juice for 24 hours and ground well with the same juice for 3 days and allowed to dry. Again, ground with tender coconut water for 3 days, until it attains a waxy consistency, then it was made into small size pills (5 grains-325mg).^[7]

Experimental animals

Healthy adult Wistar albino male rats of weighing 150-200gm were included in the study. The animals were procured from the animal house of TANUVAS, Madhavaram, Chennai. The animals were maintained in well ventilated room temperature with natural 12 +12 hrs light/dark cycle in the polypropylene cages. The animals were fed with balanced diet that is standard rodent pellet diet Sai meera foods Pvt Ltd, Bangalore and water *ad libitum*. The animals were housed for one week prior to the experiment to acclimatized to the laboratory conditions. Approval for the research work and ethical clearance is obtained from

Institutional Animal Ethical Committee of National Institute of Siddha, Chennai. (Approval No: 1248/AC/09/CPCSEA-9/DEC 2013/12).

Study design

Male albino rats were divided into four groups of six animals each. Group I served as control and administered with vehicle milk. Group II treated as standard received furosamide in the dose of 10mg/ml dissolved in the normal saline. Group III and Group IV were given orally with single dose of 100mg/kg b. wt and 200mg/kg b.wt of *Dhadhu virthi kuligai* suspended in milk. All the doses were administered once a day by oral gavage in the morning.

Procedure

The method of Lipschitz *et al.* was employed for the assessment of diuretic activity. All the animals were hydrated with double distilled water. Food and water were withdrawn 8hrs before the administration of drug. Immediately after dosing, all the animals were placed individually in metabolic cages and urine passed by the animals over a period of 24 hrs was collected in a conical flask. Total urine output, electrolyte and pH were determined.^[8]

Estimation of urine output

Metabolic cage is designed with a stainless-steel circular frame. The upper portion is covered with a lid, provided with a wire mesh bottom and a funnel for collecting the urine. Stainless steel sieves were placed in the funnel to retain the faeces, allowing only urine to flow down for collection and measurement. The whole structure is fixed to a metal frame, which keeps the frame in upright position. Conical flask was kept to collect the urine, at the bottom exit of the funnel for a period of 24hrs. Urine volume is expressed as ml/kg. The room temperature is maintained at 27-29°C.^[9]

Estimation of electrolytes

Electrolyte (Na^+ , K^+ , Cl^-) concentrations were estimated and expressed as (Meq/kg). Analytical estimated was performed according to the procedure provided along with electrolyte estimation-standard-reagents kit(crest Biosystems, India). The Na^+ and K^+ concentration were measured Flame photometer and Cl^- by Spectrophotometer.^[9,10]

PH

Ph was measured with a digital pH meter (MK-VI, Unique instruments & machineries, Calcutta) on fresh urine sample.

For the calculation and presentation of results, urine volume excreted per 100g body weight was worked out. The results are expressed as “Lipschitz value” that is the ratio T/U, where T represents the response of the test compound and U that of urea treatment. The Lipschitz values were calculated for urine excretion. The indices of 1.0 and more indicate a positive diuretic effect of test compounds.

Statistical analysis

For determining the statistical significance, standard deviation, standard error mean and Dunnett's test 1% level significance was employed.

RESULTS AND DISCUSSION

The urinary output and electrolytes were increased in Dhadhu virthi kuligai treated groups. (Table- 1-3). The significant difference were observed in Dhadhu virthi kuligai group IV (200 mg/kg). The lipschitz values of Dhadhu virthi kuligai treated groups were observed, it was near to 1, so the test drug Dhadhu virthi kuligai consider as mild diuretic, According to siddha literature the ingredients of Dhadhu virthi kuligai especially Hygrophilla auriculata (*Neer mulli*) and Asparagus racemosus (*Thannervittan kizhangu*) has diuretic effect, which have been proven by various researched. Hence, this study proved that the test drug Dhadhu virthi kuligai has Diuretic activity.

Table 1: Effect of *Dhadhu virthi kuligai* on urinary output and Ph in rats.

S.no	Drug/dose	Urine Volume(ml/kg/24h)	pH
Group I	Control	14.28±2.03	6.30±0.4
Group II	Standard drug furosemide 10mg/kg	24.32±1.07**	6.20±0.8*
Group III	DVK Dose-I (100mg/kg)	15.31±1.87*	6.10±0.3
Group IV	DVK Dose-II (200mg/kg)	23.07±2.04**	6.20±0.7*

Values are expressed as mean ± SEM, test of significance was done by ANOVA followed by Dunnett test's *P<0.05, **P<0.01 and ***P<0.001.

Table 2: Effect of *Dhadhu virthi kuligai* on urinary electrolyte extraction in rats.

Groups	Na ⁺ extraction (Meq/kg)	Urinary K ⁺ extraction (Meq/kg)	Urinary Cl ⁺ extraction (Meq/kg)
Group-I	83.82±1.72	73.65±2.36	118.06±3.71
Group-II	110.71±6.13**	81.19±1.72**	165.37±2.89**
Group-III	105.32±1.76*	76.36±0.89*	146.78±4.31*
Group-IV	109.72±1.79**	79.96±2.33**	160.37±2.37**

Values are expressed as mean \pm SEM, test of significance was done by ANOVA followed by Dunnett's test * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$.

Table 3: Lipschitz value and Na^+/K^+ ratio.

Groups	Na^+	K^+	Lipschitz value (Na^+) (T/U)	Na^+/K^+ ratio
Group-I	83.82 \pm 1.72	73.65 \pm 2.36	-	1.1380
Group-II	110.71 \pm 6.13	81.19 \pm 1.72	-	1.3635
Group-III	101.32 \pm 1.76	76.36 \pm 0.89	0.915	1.3268
Group-IV	103.72 \pm 1.79	79.96 \pm 2.33	. 0.936	1.2971

Values are expressed as mean \pm SEM, test of significance was done by ANOVA followed by Dunnett's test * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$.

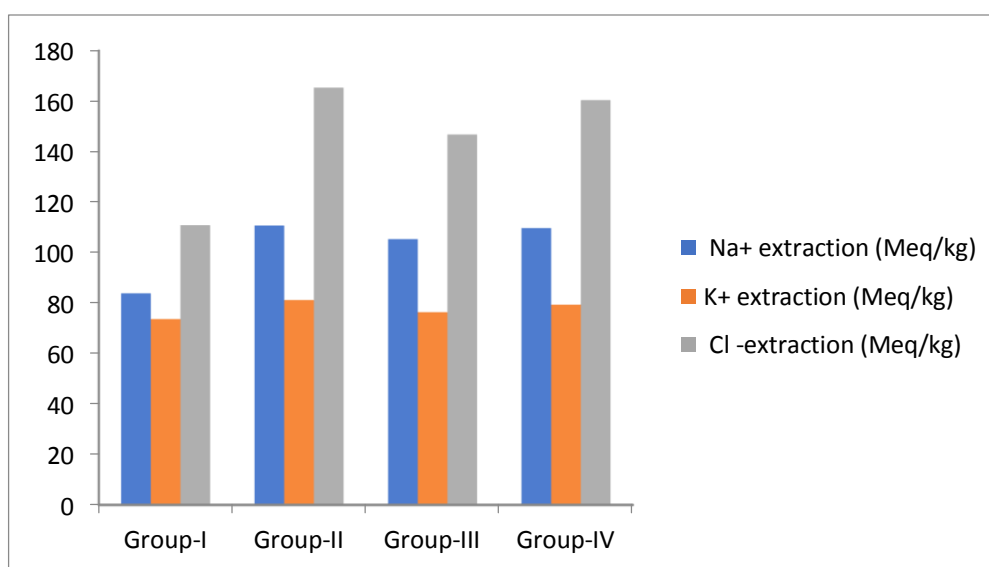


Figure 1: Effect of Dhadhu virthi kuligai on urinary electrolyte extraction in rats.

CONCLUSION

Diuretic property of Dhadhu Virthi kuligai was studied. The results revealed that the drug DVK increased the urinary output, pH and urinary electrolyte concentration which indicates it has significant diuretic activity.

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Conflict of interest

Authors declare that there is no conflict of interest.

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