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SHORT COMMUNICATION

Evaluation of Diuretic Activity of *Siddha* Poly Herbal Formulation *Vithu Vagai Chooranam* in Rodents

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Abstract

The current pharmacological investigation was performed to study the diuretic activity of *Siddha* polyherbal formulation *Vagai Chooranam* (VVC) in Wistar albino rats using the Lipschitz method. The animals were divided into four Groups (I, II, III, and IV) of six rats (n = 6) each. Group I served as control treated with milk (1ml/kg p.o), and Group II received Standard furosemide (10 mg/kg, p.o). Groups III and Group IV were treated with test drug at a dose of 70mg/kg and 90mg/kg, p.o, respectively. The urine volume, pH, and electrolyte concentration (Na^+, K^+ and Cl^-) were estimated at the end of 24 hours. Diuretic index, Lipschitz values, and natriuretic index were also calculated from the results to make a comparison with control and furosemide-treated groups. Statistical analysis was carried out using a one-way analysis of variance, followed by Dunnett's '*t*' test. Oral administration of VVC at both doses significantly increased the urine output and concentration of urinary electrolytes ($p < 0.01$). The VVC-induced diuresis pattern was almost like that of frusemide. The findings revealed that the *Vithu vagai chooranam* possessed significant diuretic activity in experimental rats.

Keywords: Diuretic, Frusemide, Urinary Electrolytes, *Vithu Vagai Chooranam*, Wistar Albino Rats

1. Introduction

A diuretic is a substance that enhances the excretion of urine and solutes[1]. Diuretic compounds are used to treat a variety of conditions, including Chronic congestive cardiac failure, cardiac or renal edema, nephritis, Renal stones, and hypertension. Diuretics are classified into different types and work by increasing water excretion through different mechanisms. Several diuretics like furosemide, ethacrynic acid, chlorothiazide, clopamide, and Mannitol are used in current practice. Diuretics such loop diuretics, thiazides, and potassium-sparing diuretics now on the market have side effects like metabolic alkalosis, electrolyte imbalance, weakness, fatigue, and hyperglycemia[2,3]. However, there is a need for safe, orally active, inexpensive, and less toxic diuretics, preferably from herbal sources. Many unidentified chemical compounds in medicinal plants may have therapeutic properties[4]. The *Siddha* system of medicine, with its vast heritage, is strengthened with many formulations that enhance urine output and are useful in the treatment of renal disorders, hypertension, ascites, and jaundice. Many indigenous drugs in the *Siddha* system were suggested to have diuretic properties, although they were not scientifically validated. Therefore, an attempt was undertaken to investigate the diuretic effect of Polyherbal *Siddha* formulation *Vithu Vagai Chooranam* (VVC) in the animal model. All medicinal preparations are divided into 64 categories according to the *Siddha* system. Thirty-two types of dosage forms are mentioned as internal medicines and thirty-two are laid down for external therapies, that are provided based on the form of medicine, method of preparation, or application. *Chooranam* is one among the 32 types of internal

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medicines, the drugs should be separately treated, powdered, sieved, and homogeneously mixed[5]. As per *Siddha* literature, VVC is a polyherbal formulation consisting of ten herbs that are mentioned in *Noigalukku Siddha Parigaram* – Part 1 by Dr. M. Shanmugavelu. This formulation is indicated for dropsy, ascites, retention of urine, and venereal diseases like gonorrhea and acts as an aphrodisiac[6]. Most of the ingredients of *Vithu vagai chooranam* have already been proven the diuretic potential in various scientific studies. However, the diuretic potential of VVC has not been scientifically validated or refuted. Hence, the study was designed to evaluate the diuretic potential of VVC in Wistar albino rats using the Lipschitz test.

2. Materials and Methods

The ingredients of VVC are shown in Table 1.

2.1 Identification and Authentication of the Drug

The collected raw materials were identified and authenticated by the Assistant Professor, Department of Medicinal Botany, National Institute of *Siddha*, Tambaram sanatorium. (Certificate No: NISMB4812021).

2.2 Method of Preparation of Drug: *Vithu Vagai Chooranam*

The ingredients, after purification, were ground separately to powder. The powder was sieved through a white cloth. All these powdered ingredients were mixed thoroughly in a stone mortar. The prepared test drug was stored in a clean, air-tight glass container.

Drug dosage: *Mooviral alavu* (800-1000 mg), twice a day. **Adjuvant:** Milk

2.3 Evaluation of Diuretic Activity by Lipschitz Model in Male Wistar Rats[7-9]

2.3.1 Procurement and Rearing of Experimental Animal

The inbred animals weighing 160-200 gm were procured from the animal house of TANUVAS, Madhavaram, Chennai. The experiment was carried out at the National Institute of *Siddha*, Chennai. The animals were housed in six per cage in polypropylene cage at ambient temperature (22 ± 2 °C), relative humidity (55 ± 5 %), and 12 hrs/12 hrs light-dark cycles. The animals were fed with standard rodent pellets and water ad libitum. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) of the National Institute of *Siddha* – project proposal no. NIS/IAEC-22/R02/E6.

2.3.2 Animal Grouping and Interventions

A total of 24 male Wistar albino rats were used for this experiment. The animals were divided into four groups of six rats (n = 6) each. Group I served as normal control (Milk-1 ml). Group II was treated with the standard drug Furosemide (10 mg/kg, p.o). Group III and Group IV received the test drug at doses of 70 mg/kg and 90 mg/kg, respectively. After 30 min of test drug administration, All the rats were fastened overnight and were given distilled water (2 ml/100 g). The animals were then individually placed in metabolic cages with netted floors, and urine was collected in conical flasks placed beneath

Table 1. Ingredients of *vithu vagai chooranam*

S. No.	Name of the drug	Scientific name	Common name	Quantity
1	*Ulunthu Maavu* (Fabaceae)	*Vigna mungo* L.	Black gram	*1 palam* (35g)
2	*Ellu* (Pedaliaceae)	*Sesamum indicum* L.	Sesame	*1 palam* (35g)
3	*Poonaikkalivithu* (Fabaceae)	*Mucuna pruriens* L.	Velvet bean	*1 palam* (35g)
4	*Neermullivithu* (Acanthaceae)	*Hygrophila auriculata* (Schumach.) *Heine*	Marsh Barbel	*1 palam* (35g)
5	*Nilappanai Kizhangu* (Amaryllidaceae)	*Curculigo orchioides* Gaertn.	*Golden Eye Grass*	*1 palam* (35g)
6	*Thanneervittan Kizhangu* (Lilliaceae)	*Asparagus racemosus* Willd.	Wild asparagus	*1 palam* (35g)
7	*Chukku* (Zingiberaceae)	*Zingiber* officinale Rosc.	Ginger	*½ palam* (17.5g)
8	*Milagu* (Piperaceae)	*Piper nigrum* L.	Black Pepper	*½ palam* (17.5g)
9	*Thippili* (Piperaceae)	*Piper longum* L.	Long pepper	*½ palam* (17.5g)
10	*Vellai sarkkarai* (Poaceae)	*Saccharum officinarum* L.	White Sugar	*½ palam* (17.5g)

the polyethylene funnel of the metabolic cages. Precautions were made to prevent the contamination of urine with faecal matter. Urine was collected in a measuring cylinder up to 24 hrs after dosing for all control and drug-treated groups. Room temperature was maintained up to 25 ± 0.5 °C. No food or water was provided to the animals during this time. Total urine volume and urine electrolyte concentrations were measured for 24 hrs to determine the diuretic activity and it was compared and tabulated for all the groups. The urine volume is expressed in mL/kg/24 h and the concentration of electrolytes in urine is expressed in terms of mEq/L. The samples were filtered to remove debris and shedding before estimating the electrolyte levels. Urinary electrolytes concentration (Na^+ and K^+) were measured by flame photometer from the freshly obtained urine and Cl^- concentration was estimated by titration with silver nitrate solution (N/50) using three drops of 5% potassium chromate as an indicator. pH of the fresh urine samples was estimated by a calibrated pH meter. Diuretic index, Lipschitz values, and natriuretic (Na^+/K^+ ratio) were calculated from the results. Different urine parameters were calculated using the following formulas:

Formula 1:

$$\text{Diuretic index} = \frac{\text{Urine volume of the test group}}{\text{Urine volume of the control group}}$$

Formula 2:

$$\text{Lipschitz value} = \frac{\text{Urine output in test animal}}{\text{Urine output in Standard drug-treated animal}}$$

Formula 3:

$$\text{Natriuretic index} = \frac{\text{Urinary excretion of } Na^+}{\text{Urinary excretion of } K^+}$$

2.4 Statistical Analysis

The results were expressed as mean ± SEM. The difference between the groups was statistically analyzed using One-way Analysis of Variance (ANOVA), followed by Dunnett's '*t*' test. $P < 0.05$ was considered statistically significant.

3. Results and Discussion

3.1 Effect on Urine Volume

The diuretic effect of VVC was found to be dose-dependent, i.e., among the two doses studied, dose level at 90mg/kg produced a significant (**P < 0.01) increase in urinary volume and urine p^H in the treated animals when compared with control. As indicated in Table 2, Furosemide, a well-known diuretic drug, significantly increased (**P<0.01) the urine output (Urine volume of 23.99 mL/kg body weight). Significant diuretic activity was observed with VVC at the dose level 90mg/kg (urine volume of 24.11 mL/kg body weight) than the VVC at the dose level 70mg/kg (urine volume of 15.87 mL/kg body weight) (Tables 2 and 3).

3.2 Effect on Urinary Electrolyte Excretion

The trial drug VVC at 90mg/kg showed an increase in electrolyte excretion (Na^+ - 108.91 ± 1.93, K^+ - 80.11 ± 2.67, Cl^- - 161.23 ± 2.69) compared to 70mg/kg (Na^+ - 106.71 ± 1.58, K^+ - 77.25 ± 0.65, Cl^- - 149.22 ± 3.87). This was found to be relatively more when compared to the standard drug (Na^+ 109.93 ± 5.98, K^+ - 80.89 ± 1.63, Cl^- - 159.89 ± 2.65). The increased sodium excretion in experimental animals after VVC administration indicated that this formulation could be employed as an antihypertensive agent. However, it was noted that potassium excretion in the interventional group was relatively less when compared to standard which may

Table 2. Effect of VVC on urinary output, pH, and Diuretic index in rats

Groups	Treatment	Urine volume (ml/kg/24h)	pH	Diuretic index
Group I	Control (1 ml/kg p.o)	14.34 ± 2.13	6.30 ± 0.3	-
Group II	Standard (furosemide 10 mg/Kg)	23.99 ± 1.73**	6.20 ± 0.5*	1.67
Group III	VVC Dose-I (70 mg/kg)	15.87 ± 1.32*	6.10 ± 0.4	1.10
Group IV	VVC Dose-II (90 mg/kg)	24.11 ± 1.80**	6.20 ± 0.6*	1.68

Values are expressed as mean ± SEM, the test of significance was done by ANOVA followed by Dunnett's '*t*' test
*P <0.05, **P<0.01, ***P<0.001.

Table 3. Effect of VVC on urinary electrolyte excretion in rats

Groups	Treatment	Na^+ excretion (mEq/L)	Urinary K^+ excretion (mEq/L)	Urinary Cl^- excretion (mEq/L)
Group I	Control (1 ml/kg p.o)	82.74 ± 2.04	74.55 ± 2.57	117.68 ± 3.71
Group II	Standard (furosemide 10 mg/Kg)	109.93 ± 5.98**	80.89 ± 1.63**	159.89 ± 2.65**
Group III	VVC Dose-I(70 mg/kg)	106.71 ± 1.58*	77.25 ± 0.65*	149.22 ± 3.87*
Group IV	VVC Dose-II (90 mg/kg)	108.91 ± 1.93**	80.11 ± 2.67**	161.23 ± 2.69**

Values are expressed as mean ± SEM, the test of significance was done by ANOVA followed by Dunnett's '*t*' test *P <0.05, **P<0.01, ***P<0.001

Table 4. Effect of VVC on Lipschitz value and Natriuretic index

Groups	Treatment	Urinary electrolyte concentration (mEq/24h)		Lipschitz value [Na^+] [T/U]	Natriuretic index (Na^+/K^+ ratio)
		Na^+	K^+		
Group I	Control (1 ml/kg p.o)	82.83 ± 1.83	75.68 ± 2.87	.....	1.0944
Group II	Standard (furosemide 10 mg/Kg)	109.63 ± 5.89	80.86 ± 1.89	.....	1.3558
Group III	VVC Dose-I(70 mg/kg)	103.56 ± 1.54	77.11 ± 0.78	0.922	1.3430
Group IV	VVC Dose-II (90 mg/kg)	105.32 ± 1.88	78.85 ± 2.55	0.940	1.3357

decrease the risk of hypokalaemia. So, it may suggest that the VVC has potassium-sparing properties[10,11]. Fractional excretion of chloride (FE_{CL}) can be used as an indirect marker of effective circulatory volume. An elevated level of urine chloride damages the kidney, causing salt loss (salt-losing nephropathy) and potassium loss from the blood or body[12]. However, VVC at the dose level of 90 mg/kg excreted chloride level (Cl^- - 161.23 ± 2.69) when compared to the standard drug (Cl^- 159.89 ± 2.65). The 'diuretic index', which was used as a measure of the degree of diuresis, was defined as the ratio of urine volume of the test group and the control group. The diuretic index indicates the diuretic potential of any substance, is good if the values are greater than 1.50. If the values are between 1.00 and 1.50 is considered moderate. mild if the values lie between 0.72 and 1.00, and nil if the value is <0.72[13]. The drug VVC has good diuretic activity at 90mg/kg, as revealed by the high diuretic index value of 1.68 (Table 2). The Lipschitz values and natriuretic activity values (ratio of Na^+/K^+) are shown in Table 4.

Diuretics are commonly used to treat clinical conditions such as nephrotic syndrome, liver cirrhosis, and high blood pressure. Uncontrolled hypertension causes the advancement of kidney disorders, and blood pressure regulation is an effective technique for preventing this progression. Furosemide, as a standard drug, is a sulfonamide derivative that belongs to high-ceiling or loop diuretics. It significantly enhances Na^+ and Cl^- excretion of urine. Loop diuretics are quite effective because a large amount of NaCl- is absorbed in this segment[1]. Our findings demonstrated that the trial drug VVC significantly increased urine volume while also excreting a substantial quantity of Na^+, K^+ and Cl^- load. Most of the ingredients in this formulation have been proven to have diuretic potential in various in-vitro and in-vivo studies.

Mucuna pruriens L. seeds were traditionally used as diuretic and hypotensive. At doses of 200 and 400 mg kg^{-1}, the electrolyte loss ratio (Na^+/K^+ excretion ratio) of these seeds is 1.48 and 1.45, respectively, as compared to the standard drug furosemide (1.47)[14]. *Hygrophila auriculata* is an indigenous medicine that is commonly used to treat urinary infections, gout, hepatic damage obstruction, and as a diuretic[15]. In a study, it is speculated that sesamin and its active metabolites can induce antihypertensive effects in experimental rats[16]. *Curculigo orchioides* Gaertn. are used as demulcent, *diuretic*, and restorative[17].Previous studies by Kumar *et al.,* reported that at a dose of 3200 mg/kg, *Asparagus racemosus* Willd. exhibited diuretic activity without acute toxicity[18]. Ethanolic extract of *Zingiber officinale* exhibited a promising diuretic effect when compared to standard diuretic furosemide[19]. Taking all

factors into account, our study has generated a scientific background for the use of *vithu vagai chooranam* as a diuretic. However, more research studies on the active phytoconstituents responsible for diuretic activity are required.

4. Conclusion

In the current investigation, VVC exhibited promising diuretic activity in a dose-dependent manner in Wistar rats. It could be used to avoid the adverse effects of diuretics that are available on the market. The data gleaned from the study confirms and justifies the rationale for the trial drug's traditional use as a diuretic. So, further studies have to be carried out to identify the active principle and explore the exact mechanism of action behind diuresis. However, extensive clinical studies must be conducted to validate the therapeutic value of this formulation in humans.

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6. References

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