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Research Article

DIURESIS: EXPERIMENTAL EVIDENCE OF POLYHERBALS IN ALBINO RATS

Kumawat Mukesh K*, Kamble Mahesh K, Gumate Dipak S, Naikwade Nilofar S, Mali Prabha R
Appasaheb Birnale College of Pharmacy, Sangli, Maharashtra, India

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*Mukesh K Kumawat, Appasaheb Birnale College of Pharmacy, Sangli, Maharashtra, India, 416416.
Email: mukesh.kums@gmail.com

ABSTRACT
Numerous medicinal plants and their formulations are used for various disorders in ethno medical practices as well in the traditional system of medicine in India. The Vrukkadoshantake vati (VV) and Nephrovin (NEP) are ayurvedic polyherbal formulations and are used in alternative system of medicine for treatment of urinary disorder. No data is available for its efficacious use in urinary disorders. The Lipschits method was used for collection of supportive data for diuretic action of the Vrukkadoshantake vati and Nephrovin. Wistar albino rats were fasted for 18 h prior to experiment and aqueous suspensions of the formulations were administered at the graded doses of 200, & 400mg/kg body weight. After the treatment, urine was collected for 24h and diuretic activity was assessed by evaluation of the total volume of urine, Na^+, K^+, Cl^- concentration and also the Diuretic index, Nariuretic effect, & saluretic effect were calculated. The total urine volume of the rats treated with the aqueous suspensions of the formulations (400 mg/kg) were found to be nearly two fold ($p<0.05$) when compared with the control (saline treated) group. Excretion of cations (sodium and potassium ions) and anions (chloride ions) was also found to be increased significantly ($p<0.01$) with respect to the control group. The diuretic effect was comparable with that of the standard drug Furosemide.

Keywords: Vrukkadoshantake vati, Nephrovin, Diuretic activity.

INTRODUCTION

Diuretics are drugs able to increase urine volume and used to treat the congestive heart failure, hypertension, acute oedema of the lung, nephritic oedema syndrome and diseases related with the retention of fluids.[1,2] Medicinal plants may have important chemical substances with potential therapeutic effects. Numerous medicinal plants and their formulations are used for various disorders in ethno medical practices as well in the traditional system of medicine in India.[3,4] According to an estimate, 80% of the world's population relied upon plants for their medication.[5] The use of the medicinal plants is increasing in many countries where 35% of drugs contain natural products.[6]

Although most of the modern medicines are synthetic compounds, but in many cases modern drugs have originated from the nature, more especially from the plant, animal and mineral sources. Plants are considered as natural chemical factory[7], because presence of diversified chemical compounds as steroids, terpenoids, flavonoids, chalcones, alkaloids and glycosides in the plants has already been reported.[8]

The VV and NEP are ayurvedic polyherbal formulations and used in alternative system of medicine for treatment of dysuria, hesitancy, renalcolic, urolithiasis, albuminuria, UTIs, and many other renal disorders. These formulations may have diversified chemical compound as flavonoids, glycosides, alkaloids, terpenoids and may evoke diuresis. Out of the constituents of ployherbal formulations some are medicine and reported as diuretic, antibacterial and anti-inflammatory activity (Gokharu),[9,10] diuretic, anti-inflammatory activity (Punarnava),[10,11] anti-inflammatory activity (Guggul)[12] as active constituents and all in combination manifest significant results for diuretic, anti bacterial and anti-inflammatory activity and make purposeful in the treatment of urinary disorders. Hence, in the present study the ployherbal formulations Vrukkadoshantake vati and Nephrovin were comparatively evaluated for their diuretic effect.

MATERIALS AND METHODS

Drug Samples

Vrukkadoshantake vati tablets (Vyas pharmaceuticals) and Nephrovin tablets were (Sharangdhar Pharmaceuticals Pvt. Ltd.) purchased from local market in Sangli, Maharashtra and Furosemide (Aventis, Mumbai) was obtained as gift ample.

Experimental Animals

Wistar strain albino rats having a weight range of 160-200 g were used for the study. The animals were well housed in polypropylene cages under hygienic conditions and kept in 12 hr light dark cycle and maintained at 23±2

ºC temperature. The animals were allowed to have food and water ad libitum. All the animals were acclimatized to laboratory condition for a week before commencement of experiment. The Institutional Animal Ethics Committee approved all the experiment protocols (843/ac/2004). The required number of animals (wistar albino rats) for the study was obtained from research laboratory animal house Appasaheb Birnal College of Pharmacy, Sangli and animal study was carried out in their Pharmacology research laboratory.

Method

The method described by Lipschits et al. (1943)[13], Mukherjee et al. (1996)[14] and Murugesan et al. (2000)[15] was employed for the assessment of diuretic activity. Healthy albino rats of either sex were divided into six groups of six animals each. They were fasted 18 hours prior to the test, with free access to water. On the day of the experiment, animals were given 25ml/kg of body wt. normal saline orally. Group I received vehicle and served as control group. Test groups formulations received in a dose equivalent to 200 mg/kg and 400 mg/kg body weight to respectively groups[16]. Group II as standard treated orally with Furosemide 20 mg/kg body weight. Groups III & IV received normal saline containing VV at doses of 200 & 400 mg/kg body wt. respectively. Groups V & VI were received normal saline containing NEP at doses of 200 & 400 mg/kg body wt. respectively. Immediately after dosing, the rats were placed in the metabolic cages with special provision to collect faeces and urine. Animals were kept at room temperature of 25±5 °C throughout the experiment. The urine was collected in measuring cylinders up to a period of 24 hours. During this period no food or water was made available to the animals. The parameters taken to study were total urine volume, Na^+, K^+ (cation) and Cl^- (anion) excreted in urine.

The concentration of Na^+ and K^+ were analyzed by flame photometer[17] and the concentration of Cl^- was determined titrimetrically by silver nitrate solution (2.906 g/l, dissolved in double distilled water), using two drop of 5% potassium chromate solution as an indicator.[18] The sum of Na^+ and Cl^- excretion as a measure of salurertic effect, ratio of the concentration of Na^+/K^+ calculated for natriuretic activity and diuretic index at the end of 24h were calculated to assess the diuretic potential of the formulations.[19]

Diuretic index = Urine volume in test group/ Urine volume in control group.

Statistical Analysis

Results are expressed as the mean values ± SEM (standard error of mean). The statistical evaluation was carried out by one way analysis of variance (ANOVA) followed by Dunnett's test for multiple comparisons, using Graphpad Instat 3.0 trial version. When comparing with control group, values of $P < 0.05$ were considered significant

RESULTS

The results of diuretic study indicate that marketed polyherbal formulations VV and NEP show the significant effect on the urine excretion volume, and electrolyte excretion in comparison with the control group in the tested dose levels of 200 and 400 mg/kg body weight orally. Results of diuretic study shows that the urine volume collected for 24 hours for vehicle and formulations treated groups and Furosemide treated group were found to be 7.33 ± 0.81(VV 200 mg/kg), 12.33 ± 0.97(VV 400 mg/kg), 15.17 ± 1.47 (NEP 200 mg/kg), 12.18 ± 0.82(NEP 400 mg/kg), 13.4 ± 0.93 and 22.25 ± 1.82 ml (20 mg/kg furosemide) respectively [Figure. 1]. These formulations VV and NEP at a dose of 400 mg/kg at 24 h have diuretic index 66.00% and 60.40% respectively, in the percentage of furosemide treated group. After the treatment with formulations VV and NEP at different dose levels and standard drug excretion of urinary cations (Na^+ & K^+) and anion (Cl^-) content were increased in respectively groups [Table.1]. The calculated data of all groups for natriuretic and saluretic effect were shown in figure.1 and figure.2 respectively. All the results were comparable with those for vehicle treated (control) and observed significant diuretic activity.

DISCUSSION

The experimental results of the study demonstrated that VV and NEP act as a diuretic in rats, with increased excretion of total volume of urine as well as of cations and anions. The aqueous suspensions of the VV and NEP have shown significant ($p<0.05$) increase in the urine volume at 200 and 400 mg/kg dose levels as compared to control and standard drug treated groups under the same condition (Figure1). Diuretic study results also shows significant and increasing diuretic activity with increased two step dose levels.

After 24h, total urine volume output of the formulations (400 mg/kg) treated rats were evaluated nearly two fold ($p<0.01$) when compared with the control (saline treated) group. The diuretic effect was quite comparable with that of the standard drug furosemide and VV has quite potent diuretic effect when compared with NEP at similar dose level.

The most clinically useful diuretics are directed towards reducing extracellular fluid volume by decreasing total sodium chloride (NaCl) content. Sodium chloride (NaCl) is the major determinant of the extracellular fluid volume in the body. As like many other herbal diuretics, VV and

NEP also exert their action by directly affecting electrolyte balance of minerals. Natriuresis and chlorouresis are significantly ($p<0.01$) contributing in the fluid metabolism [Table.1]. These polyherbal formulations at dose of 400mg/kg reveal significant ($p<0.01$) natriuretic effect, which is comparable with the efficacious natriuretic effect of furosemide. Furosemide act as loop diuretic and inhibit the sodium absorption in the ascending limb of loop of henle. However, it produces significant hypokalemia and metabolic acidosis. Contrast of results, polyherbal formulations displayed increase the natriuretic and saluretic activity with increasing dose but kaliuresis was not increased as like natriuresis [Table.1 and figure 2 & 3].

The present study justifies the traditional basis use of these ayurvedic formulations as diuretic and as urinary antiseptic with experimental data. Further study required on these formulations for determination of active chemical constituents which is evokes the therapeutic use in urinary disorders.

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Table. 1: Effect of VV & NEP on excretory parameters.

Groups (n=6)	Urine volume in ml (mean±SEM)	Measured parameters of experimental groups (mean ±SEM)			
		Total sodium (µmoles/kg body wt.)	Total potassium (µmoles/kg body wt.)	Total chloride (µmoles/kg body wt.)	Diuretic Index (T/C)
Control (normal saline)	7.33 ± 0.81	247.91 ± 9.54	208.15 ± 13.43	1533.33 ±17.97	---
Standard (Furosemise 20 mg/kg)	22.25 ± 1.82**	1843 ± 24.92**	811.72±11.26**	5448 ± 20.66**	3.035
VV 200 mg/kg	12.33 ± 0.97*	833.97 ± 23.04**	440.09 ± 17.48**	2314.33 ± 16.28**	1.68
VV 400 mg/kg	15.17 ± 1.47**	1075.9 ± 29.06**	537.36± 14.99**	4198.66 ± 6.93**	2.07
NEP 200 mg/kg	12.18 ± 0.82*	785.10 ± 14.94**	429.24± 12.70**	2080 ± 32.45**	1.66
NEP 400 mg/kg	13.4 ± 0.93**	963.26 ± 15.96**	511.71 ± 8.99**	4016.33 ± 16.18**	1.83

(n=6), results expressed as mean ±SEM. *p<0.01 *p<0.05 statistically significant when compared with control by ANOVA followed by Dunnett test

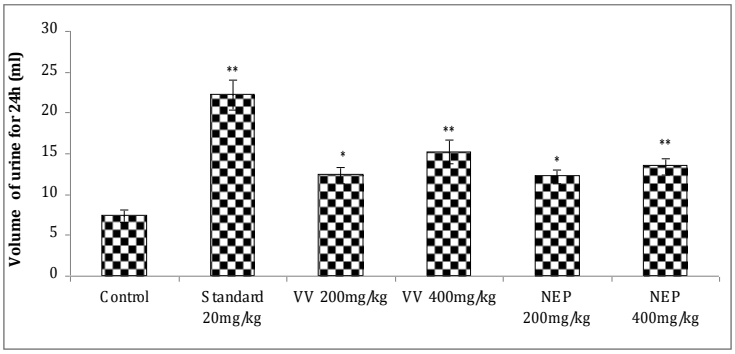

Figure 1: The effect of VV and NEP of 200 and 400 mg/kg orally on urine output for 24 h (ml) results expressed as Mean ± SEM, (n=6). **p<0.01 and *p<0.05 statistically significant when compared with control by ANOVA followed by Dunnett test

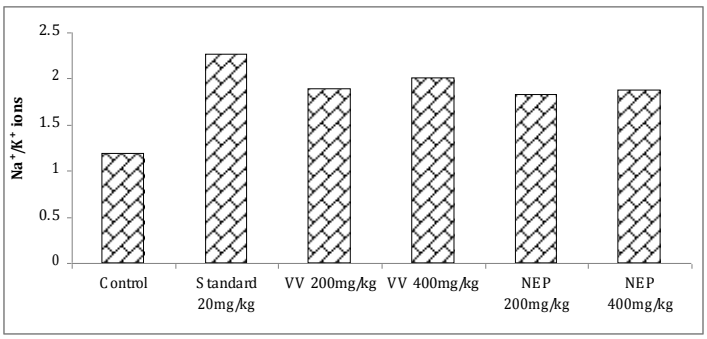

Figure 2: Natriuretic effect of VV and NEP at 200 and 400 mg/kg orally dose levels. (n=6)

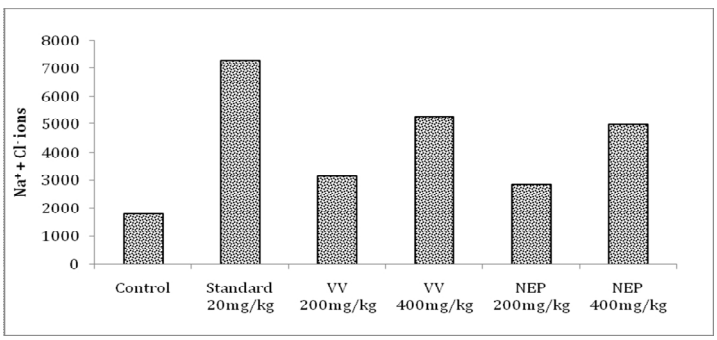

Figure 3: Saluretic effect of VV and NEP at 200 and 400 mg/kg orally dose levels. (n=6)

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