

Evaluation of diuretic activity of ethanolic and petroleum ether extracts of *Nardostachys jatamansi* DC roots in rats

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ABSTRACT

Nardostachys jatamansi DC is important plant of the family Valerianaceae. *Nardostachys jatamansi* DC is classified as hypno-sedative in Ayurveda. It is used in treatment of insomnia, hysteria and depressive illness. The plant abounds in sesquiterpenes predominantly; jatamansone and nardostachone. The plant has demonstrated several pharmacological activities including hepatoprotective, cardio protective and hypolipidemic and antifungal. The aim of present study was to investigate the diuretic activity of ethanolic and petroleum ether extracts of *Nardostachys jatamansi* DC roots in rats. Ethanolic and petroleum ether extracts were administered orally at a dose of 200mg/kg and 500mg/kg. The results were analysed by One Way Analysis Of Variance (ANOVA). The results showed that both the extracts of roots showed significant diuresis relatively ethanolic extract has shown more activity than petroleum ether extracts. The findings concluded that *Nardostachys jatamansi* DC roots exhibit diuretic activity and further studies are suggested to isolate the active principles responsible for the activity.

Keywords: *Nardostachys jatamansi* DC, Ethanolic extract, petroleum ether extracts, Diuretic, Urine volume.

INTRODUCTION

Since the time immemorial our traditional system of medicine and folklore claiming that medicinal plants as a whole or their parts are being used in all types of diseases successfully [1]. About 65% of world populations have access to local medicinal plant knowledge system [2]. Traditional systems of medicine are popular in developing countries and upto 80% of population relies on traditional medicines or folk remedies for their primary health care needs [3]. India has about 45000 plant species and among them, several thousands have been claimed to possess medicinal properties [4] [5].

Diuretics are drugs that increase the rate of urine flow, sodium excretion and are used to adjust the volume and composition of body fluids in a variety of clinical situations. Drug-induced diuresis is beneficial in many life threatening disease conditions such as congestive heart failure, nephritic syndrome, cirrhosis, renal failure, hypertension, and pregnancy toxemia. Most diuretic drugs have the adverse effect on quality of life including impotence, fatigue, and weakness [6]. Two widely used diuretics, thiazides and frusemide, the high-ceiling loop diuretic, have been associated with numerous adverse effects, such as electrolyte imbalance, metabolic alterations, development of new-onset diabetes, activation of the renin-angiotensin and neuroendocrine systems, and impairment of sexual function [7]. Hence, there is a requirement for novel diuretics such as plant-based substances, which are considered to be relatively safe, possessing lower potential for adverse effects.

Nardostachys jatamansi DC is important plant of the family Valerianaceae. It is commonly known as Indian spikenard and found in Himalayas. In Ayurveda, *Nardostachys jatamansi* is used for nervous headache, excitement, menopausal symptoms, flatulence, epilepsy and intestinal colic. Externally, the oil is added to a steaming bath to

treat inflammation of the uterus. It is used in treatment of insomnia, hysteria and depressive illness. The plant abounds in sesquiterpenes predominantly; jatamansone and nardostachone. The plant has demonstrated several pharmacological activities including hepatoprotective, cardio protective and hypolipidemic and antifungal. The significant effect is on the central nervous system, as diverse pharmacological actions, ranging from sedative to nootropic have been reported. Animal and clinical research with jatamansone, the active principle of the plant, has justified hypno-sedative claim of Ayurveda [8].

Nardostachys jatamansi DC is claimed for its diuretic activity but the systematic work was not carried out on this. So, this is to confirm the potential use of *Nardostachys jatamansi* DC roots extracts have diuretic properties in vivo. This study will be a prospective study for the development of new diuretic agents.

MATERIALS AND METHODS

Plant material

The roots of *Nardostachys jatamansi* DC is collected from local sources and with the help of expert taxonomist and it was identified by studying its various morphological and microscopic characters and the plant was authenticated by Dr. B. D. Gachande (Associate Professor) of P.G. Department of Botany, Science College, Nanded.

Preparation of the Extracts

Preparation of ethanolic and petroleum ether extracts of *Nardostachys jatamansi* DC roots

The dried powder material was successfully extracted with ethanol and petroleum ether by hot continuous percolation method in Soxhlet apparatus till the solution become colourless. The residue obtained was then utilized for pharmacological screening. Approximately 300g of powdered drug material was extracted using ethanol and petroleum ether in the ratio of 1:3 (w/v) in a Soxhlet apparatus. The extracts obtained and the dried mass was weighed and recorded. The percentage of yield was calculated [9].

$$(\%) \text{ yield} = \frac{\text{Wt. of extract}}{\text{Wt. of powdered drug}} \times 100$$

Phytochemical analysis

The ethanolic and petroleum ether extracts of *Nardostachys jatamansi* DC roots were subjected to preliminary phytochemical screening.

Drugs and chemicals

Ethanol and petroleum ether (Fine Chem Industries, Mumbai), furosemide (Aventis pharma Ltd).

Experimental animals

Male albino Wistar rats weighing 150-200 g were used in the present study. The experimental animals were maintained under standard laboratory conditions in an animal house of Nanded Pharmacy College approved by the committee for the purpose of control and supervision on experiments on animals (CPCSEA) (Reg. No. 1613/PO/a/12/CPCSEA) under 12 h light/dark cycle and controlled temperature ($24 \pm 2^\circ\text{C}$) and fed with commercial pellet diet and water *ad libitum*. All animals were acclimatized to the laboratory environment for at least one week before the commencement of experiment. The experimental protocol for the study was followed according to the norms of Institutional Animal Ethics Committee.

Acute toxicity studies

Acute toxicity study was carried out on ethanolic and petroleum ether extract of *Nardostachys jatamansi* DC roots on male Swiss albino mice. The mice were fasted overnight and the weight of each mouse was recorded just before use. Animals were divided randomly in to ten treatment groups; each group consisting of three mice; each treatment group received orally the ethanolic and petroleum ether extract of *Nardostachys jatamansi* DC roots in a dose of 5, 50, 300, 2000 and 5000 mg/kg. For each dose two groups of animals were used. Animals were kept under close observation for 4 hours after administering the extract, and then they were observed daily for three days for any change in general behaviour and/or other physical activities. Acute toxicity study was done as per OECD, 2006 Guidelines. Hence we selected 200 mg/kg and 500 mg/kg as low and high doses.

Diuretic activity in rats (Lipschitz test)

The method of Lipschitz et al [10] was employed for the assessment of diuretic activity. According to this method, the animal should be deprived of food and water for 18 hours prior to the experiment, and were randomly divided into six groups of six animals each as follows:

Group 1(Control) - received saline 25ml/kg p.o.
 Group 2(Standard) - received furosemide 20mg/kg p.o.
 Group 3(Test)- received ethanolic extract of *Nardostachys jatamansi* DC 200mg/kg p.o.
 Group 4(Test)- received ethanolic extract of *Nardostachys jatamansi* DC 500mg/kg p.o.
 Group 5(Test)- received petroleum ether extract of *Nardostachys jatamansi* DC 200 mg/kg p.o.
 Group 6 (Test)- received petroleum ether extract of *Nardostachys jatamansi* DC 500mg/kg p.o.

Immediately after administration, animals were placed in metabolic cages specially designed to separate urine faecal matter. During the period of study no food, water was made available to the animals. The total volume of urine was collected and measured from control, standard and extract treated groups up to 5hours of administration. The parameters monitored for the each individual rat were total urine volume and urine concentration of Na⁺, K⁺ and Cl⁻. Concentration of Na⁺ and K⁺ were determined using flame photometer while Cl⁻ concentration was estimated titrimetrically using 0.02N AgNO₃ with 5% potassium chromate as an indicator. Appearance of brick red precipitate was taken as the end point.

Statistical Analysis

The results were expressed as a mean \pm S.E.M. The differences were compared using One Way Analysis of Variance (ANOVA) and subsequently followed by Bonferroni's test.

RESULTS

Table 1: %Yield, texture and colour of ethanolic and petroleum ether extracts of *Nardostachys jatamansi* DC roots

Plant part	Type of extract	%yield	texture	Color of extract
<i>Nardostachys jatamansi</i> DC roots	Ethanolic extract	6.03	Sticky	Dark brown
	Petroleum ether extract	1.6	Non sticky	Greenish

Ethanolic extract gave more yield (6.03%) than petroleum ether extract while petroleum ether extract gave least yield (1.6%).

Preliminary Phytochemical Analysis

Table No. 2 Phytochemical analysis of Ethanolic and Petroleum Ether extracts of *Nardostachys jatamansi* DC roots

Chemical constituents	Ethanolic Extract	Petroleum Ether extracts
Flavonoids	—	—
Coumarins	+	+
Proteins and amino acids	+	+
Starch	—	+
Tannins	+	+
Resins	+	—

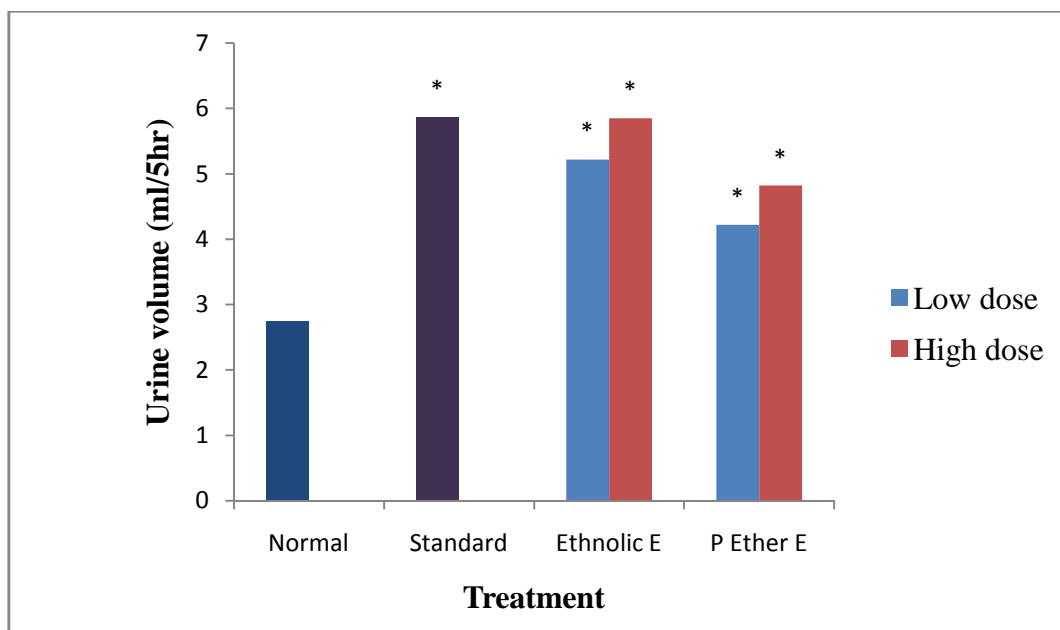
(+) present, (-) absent

Diuretic activity in rats (Lipschitz test)

Table No. 3. Effect of Ethanolic and Petroleum Ether extracts of *Nardostachys jatamansi* DC roots on urine volume in treated rats

Treatment	Urine volume (ml/5hr)	Diuretic index
Normal control	2.75 \pm 0.05	1.00
Standard Frusemide (20 mg/kg p o)	5.87 \pm 0.025	2.13
Ethanolic extract (200 mg/kg p o)	5.22 \pm 0.025**	1.89
Ethanolic extract (500 mg/kg p o)	5.85 \pm 0.05**#	2.13
Petroleum ether extract (200 mg/kg p o)	4.22 \pm 0.025**	1.53
Petroleum ether extract (500 mg/kg p o)	4.82 \pm 0.025**	1.75

Each value represents the mean \pm S.E.M. (n=6), *P<0.05, ** P<0.001 compared with control and #p>0.05 compared with standard.

Figure No. 1. Effect of Ethanolic and Petroleum Ether extracts of *Nardostachys jatamansi* DC roots on urine volume in treated rats

Each value represents the mean \pm S.E.M. (n=6), * $P < 0.05$, ** $P < 0.001$ compared with control and # $p > 0.05$ compared with standard.

Table No. 4. Effect of ethanolic and petroleum ether extracts of *Nardostachys jatamansi* DC roots on urine volume and electrolyte excretion in rats

Treatment	Urine volume (ml/5hr)	Na ⁺ (mEq/L)	K ⁺ (mEq/L)	Cl ⁻ (mEq/L)
Normal control	5.05 \pm 0.05	90.59 \pm 0.16	28.52 \pm 0.32	146 \pm 1
Frusemide(20mg/kg po)	9.1 \pm 0.1	199.98 \pm 1.75	65.17 \pm 0.2	221.5 \pm 1.5
Ethanolic extract (200 mg/kg p o)	8.65 \pm 0.05**	104.83 \pm 0.49**	52.35 \pm 0.21**	231.9 \pm 1.3**
Ethanolic extract (500 mg/kg p o)	9 \pm 0.1**#	139.58 \pm 0.45**	58.31 \pm 0.62**	290.37 \pm 3.37**
Pet. ether extract (200 mg/kg p o)	5.85 \pm 0.05*	94.05 \pm 0.14*	32.85 \pm 0.8*	159 \pm 1*
Pet. ether extract (500 mg/kg p o)	6.95 \pm 0.05**	100.93 \pm 0.07**	36.31 \pm 0.43*	224.4 \pm 0.6**#

Each value represents the mean \pm S.E.M. (n=6), * $P < 0.05$, ** $P < 0.001$ compared with control and # $p > 0.05$ compared with standard.

DISCUSSION

In traditional medicine system, many plants and herbs are claimed to have diuretic and antioxidant efficacy without any significant scientific study. *Nardostachys jatamansi* DC is used traditionally as folk medicine to treat a number of illnesses including some disorders where its diuretic potential is claimed to be useful. However, there is no scientific evidence in diuretic activity of Roots of *Nardostachys jatamansi* DC. In the present study so it was selected for evaluation of its diuretic activity and its effect on electrolytes level. Two extracts were prepared with ethanol and petroleum ether (J B Harbone, 1998). Preliminary phytochemical evaluation of two extracts was carried out for the determination of presence of phytoconstituents [11]. Ethanolic extract showed presence of tannins, resin, coumarins, protein, amino acids. Petroleum ether extract showed presence of starch, tannins, coumarins, protein and amino acid. The presence of sesquiterpenes in ethanolic extracts was further confirmed by Thin Layer Chromatography. Acute oral toxicity study was carried out for extracts of *Nardostachys jatamansi* DC roots as per OECD guideline No. 423 (Acute Oral Toxicity-Class method) which reveals LD₅₀ is greater than 5000mg/kg. Such study is useful for interpreting safety of test product and to calculate safe dose for experimental purpose. Accordingly 1/10 of LD₅₀; that is \leq 500mg/kg dose of extracts was considered for pharmacological screening as maximum dose.

This study investigated the diuretic potential of extracts of *Nardostachys jatamansi* DC roots. The results showed that the highest dose (500mg/kg) of both extracts of roots possesses strong diuretic activity when given orally in a single dose. The findings suggest effect of two different doses of both extracts (200 mg/kg, and 500 mg/kg) of *Nardostachys jatamansi* DC roots is probably mediated through its ability to cause a significant increase in urine volume, sodium and potassium excretion, without interfering with other parameters related to renal functions. Both the extracts have induced strong diuresis and were not accompanied with a reduction in urinary K⁺ levels. Further, there was no alkalization of urine. Collectively, these observations suggest that the extracts are not acting as potassium-sparing diuretics. In this study, both urinary Na⁺ and K⁺ levels were increased without any significant alteration in the Na⁺/K⁺ ratio [12].

On the other hand, the extracts of *Nardostachys jatamansi* DC roots showed a notable dose dependent increase in the urinary volume and urinary electrolyte (Na^+ and K^+) ions excretion. The diuresis induced by the extracts of *Nardostachys jatamansi* DC roots was strong and the intensity was similar to that of frusemide and accompanied by marked increases in both urinary Na^+ and K^+ levels at higher doses. These features strongly suggest that the extracts may act as a loop diuretic [13].

Ethanollic extracts showed more diuretic activity than petroleum ether extract. Ethanollic extracts showed presence of sesquiterpenes and they may be responsible for strong diuretic activity. Loop diuretics are clinically used in patients with salt and water overload due to conditions such as pulmonary oedema, heart failure ascites and hypertension. Loop diuretic type of mode of action of the different extracts of the *Nardostachys jatamansi* DC roots indicate that it may be useful as a non toxic natural therapeutic agent in the treatment of such conditions by traditional practitioners; although further mechanism based investigation is needed to confirm exact mechanism.

CONCLUSION

Ethanollic and petroleum ether extracts of *Nardostachys jatamansi* DC roots have showed dose dependent increase in urine and electrolyte excretion. Relatively ethanollic extract showed more activity when compared to petroleum ether extract.

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