DIURETIC ACTIVITY OF ASHWAGANDHARISHTA PREPARED BY TRADITIONAL AND MODERN METHODS IN EXPERIMENTAL RATS

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Summary

The objective of the present study was to evaluate the diuretic effect of Ashwagandharishta-T and Ashwagandharishta-M prepared by traditional and modern methods respectively and its marketed formulation in experimental rats using Furosemide (10 mg/kg p.o) as a standard diuretic drug. Oral administration of Ashwagandharishta-T, Ashwagandharishta-M and its marketed formulation at the dose of 2.0 ml/kg over a period of 5 h showed a significant increase in urine volume as compared to control group. All the test formulations of Ashwagandharishta as Ashwagandharishta-T, Ashwagandharishta-M and its marketed formulation showed significant rise in sodium, potassium and chloride level in urine sample as compared to control group. The maximum diuretic effect was produced by Furosemide. Thus, both types of Ashwagandharishta as Ashwagandharishta-T and Ashwagandharishta-M showed significant diuretic, natriuretic and kaliuretic effects.

Key words: Diuretic activity, Furosemide, Ashwagandharishta, natriuretic effect, kaliuretic effect

Introduction

Ashwagandharishta is an Ayurvedic polyherbal hydro-alcoholic preparation and is used as rasayana[1]. Rasayanas are used to promote health and longevity by increasing defence against disease, arresting the ageing process and revitalizing the body in debilitated conditions[2]. The chief ingredient of Ashwagandharishta is roots of Ashwagandha, *Withania somnifera*, is known for its varied therapeutic uses in Ayurvedic and Unani practices in India[3,4]. Roots of *Withania somnifera*, commonly known for its usefulness in the treatment of hypercholesterolemia, arthritis in combination with other drugs, is also credited to be hypoglycaemic and diuretic[5]. The pharmacological effect of the roots of *Withania somnifera* is attributed to withanolides, a group of steroidal lactones[6]. Earlier studies have reported the absence of any side effects of *Withania somnifera* in animals[7,8].

Besides roots of *Withania somnifera*, the other ingredients of Ashwagandharishta as Arjuna (Bark of *Terminalia arjuna*), Liquorice (roots of *Glycyrrhiza glabra*), Majith (roots of *Rubia cordifolia*), Rasna (roots of *Alpinia chinensis*), Taj (inner bark of *Cinnamomum zeyleynicum*), Nagarmotha (rhizomes of *Cyperus rotundus*), haritaki (fruits of *Terminalia chebula*), turmeric (rhizomes of *Curcuma longa*), Nagakesara (stamens of *Messua ferrea*) etc. contain a rich quantity of phenolic compounds and flavonoids and possess significant antioxidant activity [9-13]. The presence of polar compounds as flavonoids and steroidal saponins may be responsible for the diuretic property of both types of Ashwagandharishta as Ashwagandharishta-T and Ashwagandharishta-M prepared by traditional and modern methods respectively and its marketed formulation.

However no study has been carried out for the diuretic activity of Ashwagandharishta, in order to confirm its assumed beneficial property. Therefore, the present study was undertaken to verify the efficacy of all the test formulations of Ashwagandharishta as Ashwagandharishta-T and Ashwagandharishta-M prepared by traditional and modern methods respectively and its marketed formulation as diuretic agent in experimental albino rats.

Material and Methods

Preparation of Ashwagandharishta-T

This was prepared by method as given in Ayurvedic Formulary of India [1]. Formula of Ashwagandharishta has been given in the **Table-1**.The ingredients of Ashwagandharishta were procured from Local market, Jamnagar. Identification of all the individual plant material was done as per Ayurvedic Pharmacopoeia of India. Authentification of all these ingredients was done in the Botany Department of Central Institute of Medicinal and Aromatic plants (CIMAP), Lucknow.

Prepared herbarium has been deposited in the CIMAP for future reference. According to this method, coarsely powdered Ashwagandha roots (*Withania somnifera*) with prescribed ingredients were placed in polished vessel of brass along with prescribed quantity of water (24.576L), and allowed to steep. After 12 h of steeping, this material was warmed at medium flame until the water for decoction reduced to one eighths of the prescribed quantity (3.072 L), then the heating was stopped and it was filtered in cleaned vessel and after that honey was added. Then, Dhataki flowers (*Woodfordia floribunda)*, and Prakshepa dravyas as Sonth, marich, pippali, tvak, Tejpatra, priyangu and nagakesara were added and this sweet filtered material was placed for fermentation in incubator for fifteen days at $33^{o}C \pm 1^{o}C$. After 15 days, completion of fermentation was confirmed by standard tests[14].The fermented preparation was filtered with cotton cloth and kept in cleaned covered vessel for further next seven days. Then, the liquid was poured in amber colored glass bottles, packed and properly labelled.

Table1: Prescribed Formula of Ashwagandharishta as per Ayurvedic Formulary of India for Batch Size 12.288L and used for Batch size 3.072 L

S. No.	Vernacular name of Drugs	Botanical name	Plant part	Prescribed Quantity for Batch size 12.288 L	Quantity Taken for Batch size 3.072 L
1.	Ashwagandha	*Withania somnifera*	Rt.	2.4 Kg	600 g
2.	Kali musali	*Curculigo orchioides*	Rt.	960 g	240 g
3.	Manjistha	*Rubia cordifolia*	Rt.	480 g	120 g
4.	Haritaki	*Terminalia chebula*	Fr.	480 g	120 g
5.	Haridra	*Curcuma longa*	Rz.	480 g	120 g
6.	Daruharidra	*Berberis aristata*	St.	480 g	120 g
7.	Yasti	*Glycyrrhiza glabra*	Rt.	480 g	120 g
8.	Rasna	*Alpinia chinensis*	Rt.	480 g	120 g
9.	Vidari	*Pueraria tuberosa*	Rt.	480 g	120 g
10	Arjuna	*Terminalia arjuna*	StBk	480 g	120 g
11	Mustaka	*Cyperus rotundus*	Rz.	480 g	120 g
12	Trivrt	*Ipomoea turpethum*	Rt.	480 g	120 g
13.	Svet Sariva	*Hemidesmus indicus*	Rt.	384 g	96 g
14.	Krisna sariva	*Ichnocarpus frutescens*	Rt.	384 g	96 g
15.	Svet Chandan	*Santalum album*	Htwd	384 g	96 g
16.	Rakta Chandan	*Pterocarpus santalinus*	Htwd	384 g	96 g
17.	Vacha	*Acorus calamus*	Rz.	384 g	96 g
18.	Chitraka	*Plumbago zeyleynica*	Rt.	384 g	96 g
19.	Water for decoction Water reduced to	-	-	98.304 L 12.288 L	24.576 L 3.072 L
Prakshepa Dravyas					
20.	Madhu (Honey)	-	-	14.4 Kg	3.6 Kg
21.	Dhataki	*Woodfordia floribunda*	Fl.	768 g	192 g
22.	Sonth	*Zingiber officinalis*	Rz.	96 g	24 g
23.	Marica	*Piper nigrum*	Fr.	96 g	24 g
24.	Pippali	*Piper longum*	Fr.	96 g	24 g
25.	Tvak	*Cinnamomum zeyleynicum*	StBk	192 g	48 g
26.	Elaichi (Chhoti)	*Eletteria cardamomum*	Sd.	192 g	48 g
27.	Tejpatra	*Cinnamomum tamala*	Lf.	192 g	48 g
28.	Priyangu	*Callicarpa microphylla*	Fl.	192 g	48 g
29.	Nagakesara	*Mesua ferrea*	Stmn	96 g	124 g

Preparation of Ashwagandharishta-M

Method of preparation was same as followed with Ashwagandharishta-T, only Dhataki flowers were replaced with Yeast for inducing fermentation [15].

Animals

Adult Wistar albino rats, weighing between 200-220g of either sex were acclimatized to normal environmental conditions in the animal house for one week. The animals

were housed in standard polypropylene cages and maintained under controlled room temperature (22 ^{o}C±2^{o}C) and humidity (55±5%) with 12:12 hour light and dark cycle. All the animals were given a standard chow diet (Hindustan Lever Limited), and water *ad libitum*. The guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) of the Government of India were followed and prior permission was granted from the Institutional Animal Ethics Committee (CPCSEA No. 07/09).

Experimental Procedure

The method of Lipschitz *et al.*, (1943) was employed for the assessment of diuretic activity. Twenty four hours before testing the animals were transferred to metabolic cages[16]. Then only water was made accessible *ad libitum* without food.

All the animals were randomly divided into the five groups with six animals in each group as follows:

Group I : Control group received normal saline as vehicle (25 ml/kg, p.o.)
Group II : animals received Furosemide (10 mg/kg, p.o.)
Group III : animals received Ashwagandharishta-T (2 ml/kg, p.o.)
Group IV : animals received Ashwagandharishta-M (2 ml/kg, p.o.)
Group V : animals received marketed Ashwagandharishta (2 ml/kg, p.o.)

The second group received same volume of normal saline (25 ml) in which Furosemide (10 mg/kg bw) was dissolved. The animals of Group III, IV and V received Ashwagandharishta-T, Ashwagandharishta-M and marketed Ashwagandharishta at the dose of 2 ml/kg bw orally, after diluting to all of them up to 25 ml with normal saline to maintain the fluid intake same in all the cases. Immediately after dosing the rats were placed in metabolic cages and kept at room temperature of 25ºC±0.5 ºC for 5 h. During this period, no food and water was made available to them. At the end of 5 h the animals were taken out of the cages and the total volume of urine excreted by each group was noted. Urine samples were analysed thereafter for Na^{+} and K^{+} concentration by flame photometer while Chloride (Cl^{-}) was determined by using standard kit containing chloride reagent from Span Diagnostics, Surat, India.

Statistical analysis

The results are expressed as mean ± SEM. Statistical analysis of data among the various groups was performed by using one way analysis of variance (ANOVA) followed by the Tukey's test using Graph Pad Prism software of Statistics. Significance value (P<0.05) was considered statistically significant.

Results

Diuretic effect

Total urine output

Both types of Ashwagandharishta as Ashwagandharishta-T and Ashwagandharishta-M were prepared by traditional and modern methods respectively showed significant (P<0.001) increase in urine volume, as compared to control group. The diuresis was almost equal to that induced by Furosemide **(Fig.1)**.

Urinary electrolyte concentration

Urinary sodium: All the test formulations of Ashwagandharishta as Ashwagandharishta-T, Ashwagandharishta-M and its marketed formulation were found to produce significant (P<0.001) increase in natriuresis but the maximum natriuresis was produced by Furosemide **(Fig.2)**.

Urinary potassium: Both Ashwagandharishta-T and Ashwagandharishta-M have been shown to cause significant (P<0.001) increase in the excretion of potassium in urine as compared to the control group. Furosemide also significantly increased the excretion of potassium. Thus, all the test formulations of Ashwagandharishta showed significant kaliuretic effect **(Fig.2)**.

Urinary chloride: All the test formulations of Ashwagandharishta as Ashwagandharishta-T, Ashwagandharishta-M and its marketed formulation showed significant (P<0.001) increase in the excretion of chloride in urine as compared to control. Furosemide also showed significant increase in the excretion of chloride in urine **(Fig.2)**.

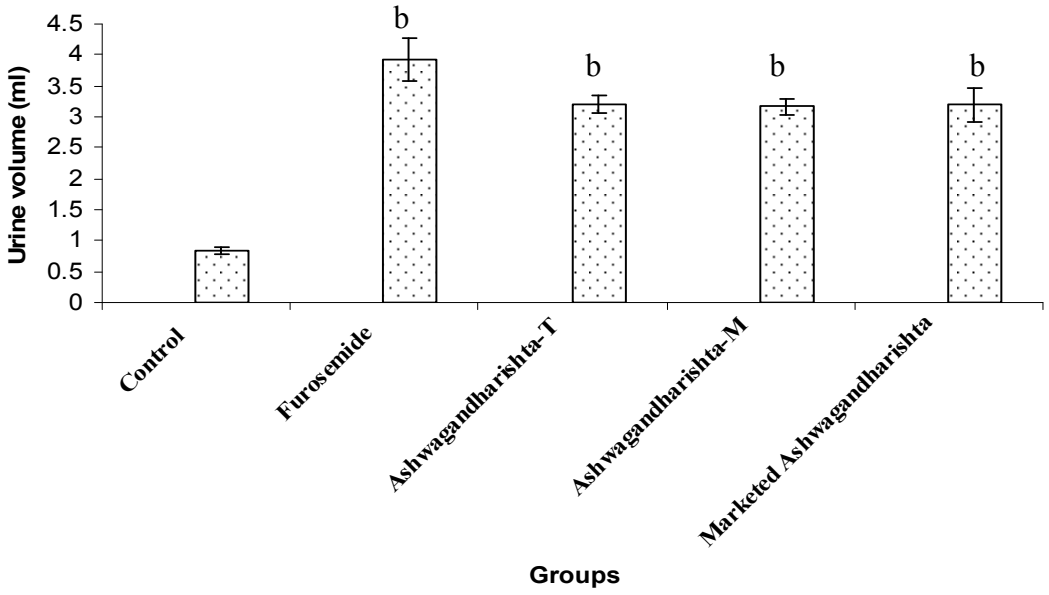

Fig.1 Effect of Ashwagandharishta-T, M and its marketed formulation on urinary volume
All values are expressed as mean±SEM; b- P<0.001 as compared to control

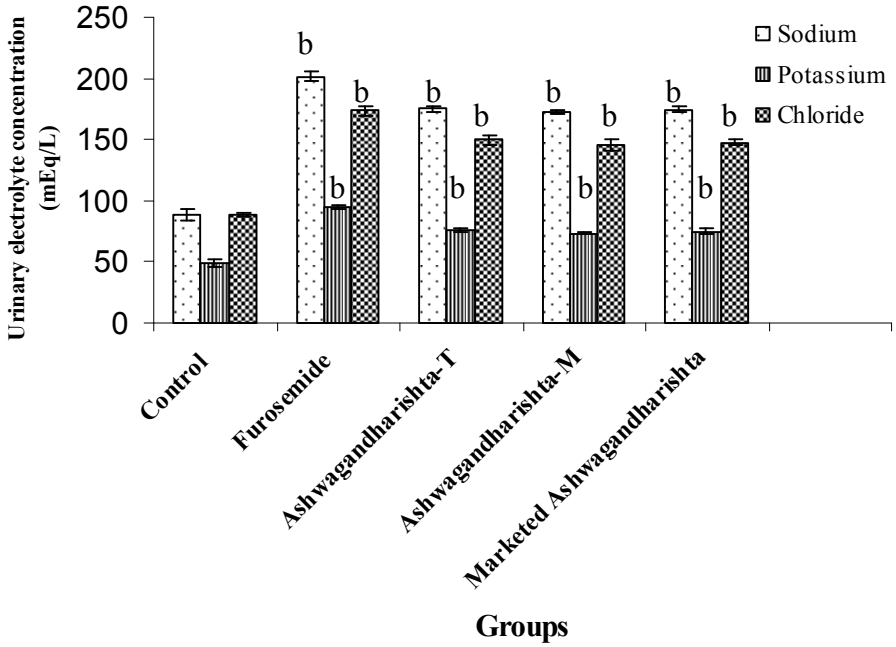

Fig.2 Effect of Ashwagandharishta-T, M and its marketed formulation on urinary electrolyte concentration
All values are expressed as mean±SEM; b- $P<0.001$ as compared to control

Discussion

This study shows that both types of Ashwagandharishta as Ashwagandharishta-T and Ashwagandharishta-M prepared by traditional and modern methods by carrying slight modifications in the traditional method respectively and its marketed formulation produced striking increase in total urine output over a period of 5 h. All these test formulations of Ashwagandharishta also showed significant ($P<0.001$) increase in the excretion of sodium, potassium and chloride in urine as compared to control group. Therefore, both types of Ashwagandharishta as Ashwagandharishta-T and Ashwagandharishta-M have been shown to possess significant diuretic, natriuretic and kaliuretic effects which may be one of the basis of their therapeutic application in various ailments, such as nephritis, burning micturation etc. and different oedematous diseases. Their diuretic effects have been shown to be more or less equal to that produced by Furosemide.

Preliminary phytochemical studies have confirmed the presence of phenolics, particularly hydrolysable tannins and flavonoids and other nonphenolic constituents as steroidal saponins and steroidal lactones as withanolides in all the test formulations of Ashwagandharishta as Ashwagandharishta-T, Ashwagandharishta-M and its marketed formulation, promoting the hypothesis that these types of polar compounds may also be responsible for the diuretic effects. It is known that these types of compounds increase renal circulation, and thus the rate of glomerular filtration which promotes increased urine formation[17-19]. Thus, presence of self generated alcohol helps in the faster absorption of biologically active compounds as tannins, flavonoids and steroidal saponins, glycowithanolides which by their chemical nature are antioxidants, might contribute to the prevention of cardiac diseases as hypertension by acting as diuretics[20].

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