EVALUATION OF DIURETIC POTENTIAL OF DRAKSHARISHTA 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 potential of Draksharishta-T and Draksharishta-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 Draksharishta-T, Draksharishta-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. Both types of Draksharishta as Draksharishta-T and Draksharishta-M prepared by traditional and modern methods respectively and its marketed formulation showed significant increase 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 Draksharishta as Draksharishta-T and Draksharishta-M and its marketed formulation showed significant diuretic, natriuretic and kaliuretic effects.

Key words: Diuretic potential, furosemide, Draksharishta, natriuretic effect, kaliuretic effect.
Introduction

Draksharishta is a polyherbal hydroalcoholic ayurvedic preparation and is advised as blood purifier, in the treatment of anaemia and advised as a choice of remedy in respiratory problems. The chief ingredient of Draksharishta is draksha, fruits of *Vitis vinifera*\(^1\). The composition and properties of fruits of *Vitis vinifera*, have been extensively investigated and it was reported that they contain large amount of phenolic compounds as catechins, epicatechin, quercetin, gallic acid, dimeric, trimeric and tetrameric procyanidins\(^2\)\(^-\)\(^3\). These compounds have many favourable effects on human health such as lowering of human low density lipoproteins, reduction of heart diseases as myocardial infarction, hypertension etc. because of having their antioxidant property\(^4\)\(^-\)\(^11\).

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

Material and Methods

Preparation of Draksharishta-T

This was prepared by the method as given in The Ayurvedic Formulary of India, Part-I\(^1\). All the ingredients of Draksharishta were procured from local market, Jamnagar while jaggery was procured from local market, Mehsana. Authentification of all the ingredients of Draksharishta was done by Dr. G. D. Bagchi, Scientist, Department of Taxonomy and Pharmacognosy, Central Institute of Medicinal and Aromatic Plants, Lucknow. Prepared herbarium has been deposited in the Central Institute of Medicinal and Aromatic Plants, Lucknow for future reference. Identification of all the individual plant material was done as per The Ayurvedic Pharmacopoeia of India. Quantity of ingredients taken for the preparation of batch size 3.25 l of Draksharishta has been calculated according to the formula as given in The Ayurvedic Formulary of India, Part-I, 2000. The formula used for the preparation of batch size 3.25 l of Draksharishta has been shown in Table 1.

According to this method, dried fruits of *Vitis vinifera* after proper crushing were placed in polished vessel of brass along with prescribed quantity of water (13 l), and allowed to steep overnight. After overnight steeping, this material was warmed at medium flame until the water for decoction reduced to one fourth of the prescribed quantity (3.25 l), then the heating was stopped and it was filtered through unstarched muslin cloth in cleaned and fumigated vessel and after that jaggery was added and mixed properly. Then dhataki flowers (*Woodfordia floribunda*) and prescribed quantity of coarsely powdered prakshepa dravyas as mentioned in the formula given in Table 1 were added and this sweet filtered fluid was placed for fermentation in incubator for fifteen days at 33\(^\circ\)C±1\(^\circ\)C. After fifteen days completion of fermentation was confirmed by standard tests\(^12\). The fermented preparation was filtered with unstarched muslin cloth and kept in cleaned covered vessel for further next seven days. Then, it was poured in clean amber coloured glass bottles previously rinsed with ethyl alcohol, packed and labelled properly.
Table 1: Formula used for batch size 3.25 l of Draksharishta

<table>
<thead>
<tr>
<th>S no.</th>
<th>Name</th>
<th>Botanical name</th>
<th>Part (AFI)</th>
<th>Weight (AFI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Draksha</td>
<td><em>Vitis vinifera</em></td>
<td>Dr. Fr.</td>
<td>635 g</td>
</tr>
<tr>
<td>2</td>
<td>Water for decoction</td>
<td>-----</td>
<td>----</td>
<td>13 l</td>
</tr>
<tr>
<td>3</td>
<td>Water reduced to</td>
<td>-----</td>
<td>----</td>
<td>3.25 l</td>
</tr>
<tr>
<td>4</td>
<td>Jaggery</td>
<td>-----</td>
<td>----</td>
<td>2.54 Kg</td>
</tr>
</tbody>
</table>

Prakshepa dravyas

<table>
<thead>
<tr>
<th>S no.</th>
<th>Name</th>
<th>Botanical name</th>
<th>Part (AFI)</th>
<th>Weight (AFI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>Tvak</td>
<td><em>Cinnamomum zeylanicum</em></td>
<td>St. Bk.</td>
<td>12.7 g</td>
</tr>
<tr>
<td>6</td>
<td>Ela (Sukshamaila)</td>
<td><em>Eletteria cardamomum</em></td>
<td>Sd.</td>
<td>12.7 g</td>
</tr>
<tr>
<td>7</td>
<td>Patra (tejpatra)</td>
<td><em>Cinnamomum tamala</em></td>
<td>Lf.</td>
<td>12.7 g</td>
</tr>
<tr>
<td>8</td>
<td>Kesara (nagakesara)</td>
<td><em>Mesua ferrea</em></td>
<td>Stmn.</td>
<td>12.7 g</td>
</tr>
<tr>
<td>9</td>
<td>Priyangu</td>
<td><em>Callicarpa macrophylla</em></td>
<td>Fl.</td>
<td>12.7 g</td>
</tr>
<tr>
<td>10</td>
<td>Marich</td>
<td><em>Piper nigrum</em></td>
<td>Fr.</td>
<td>12.7 g</td>
</tr>
<tr>
<td>11</td>
<td>Krishna (pippali)</td>
<td><em>Piper longum</em></td>
<td>Fr.</td>
<td>12.7 g</td>
</tr>
<tr>
<td>12</td>
<td>Vidanga</td>
<td><em>Embelia ribes</em></td>
<td>Fr.</td>
<td>12.7 g</td>
</tr>
<tr>
<td>13</td>
<td>Dhataki</td>
<td><em>Woodfordia floribunda</em></td>
<td>Fl.</td>
<td>12.7 g</td>
</tr>
</tbody>
</table>

Preparation of Draksharishta-M

Method for the preparation of Draksharishta-M was same as followed for Draksharishta-T only dhataki flowers were replaced by yeast for inducing fermentation.

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°C±2°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 Experimentals on Animals (CPCSEA) of the Government of India were followed and prior permission was granted from the Institutional Animals 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. 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 Draksharishta-T (2 ml/kg, p.o.)
Group IV: Animals received Draksharishta-M (2 ml/kg, p.o.)
Group V: Animals received marketed Draksharishta (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 Draksharishta-T, Draksharishta-M and marketed Draksharishta 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 25 ºC±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 have been 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 Draksharishta as Draksharishta-T and Draksharishta-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 produced by furosemide (Fig.1).

**Urinary electrolyte concentration**

**Urinary sodium:** All the test formulations of Draksharishta as Draksharishta-T, Draksharishta-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 types of Draksharishta as Draksharishta-T and Draksharishta-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 Draksharishta showed significant kaliuretic effect (Fig.2).

**Urinary chloride:**
All the test formulations of Draksharishta as Draksharishta-T, Draksharishta-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 Draksharishta-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 Draksharishta-T, Draksharishta-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 Draksharishta as Draksharishta-T and Draksharishta-M prepared by traditional and modern methods respectively and its marketed formulation produced striking increase in total urine output over a period of 5 h. All these test formulations of Draksharishta 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 Draksharishta as Draksharishta-T and Draksharishta-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 in all the test formulations of Draksharishta as Draksharishta-T, Draksharishta-M and its marketed formulation, promoting the hypothesis that these type of polar compounds may also be responsible for the diuretic effects. It is known that this type of compounds increase renal circulation, and thus the rate of glomerular filtration which promotes increased urine formation$^{15-17}$. Thus, presence of self generated alcohol helps in the faster absorption of biologically active compounds as tannins, flavonoids and steroidal saponins which by their chemical nature are antioxidants, might contribute to the prevention of cardiac diseases as hypertension by acting as diuretics$^{18}$.

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References