Pharmaceutical study of Lohasava With Special Reference to Its Iron Content

Vijay Gupta, K.R.C.Reddy

Pharmacopoeia Commission for Indian Medicine and Homeopathy, Ghaziabad, ^1^Department of Rasa Shastra, Faculty of Ayurveda, IMS, BHU, Varanasi, Uttar Pradesh, India

ABSTRACT

Ayurveda has a veritable abundance of useful drugs belonging to vegetal, animal and mineral origin, which are used as a single drug or in compound formulations. The treatises of Ayurveda mention a number of formulations useful for promotion and preservation of health in the form of Rasayana (rejuvenation), and also for curative purposes in a wide range of clinical conditions. Asava and Arishta (self-generated alcoholic medicinal preparations) are two such potent ancient therapeutic products described under “Sandhana Kalpana” (self-fermented dosage forms). Because of the presence of self-generated alcohol content, these preparations occupy a unique place amongst all other Ayurvedic and modern medicines. These are traditionally prepared by natural fermentation process, but since Vedic period to till date, many developments have been made in the field of fermented preparations, which has opened newer dimensions in the standardization of these products. One such important Asava is Lohasava, which is primarily indicated in anemia. A pharmaceutical study was conducted to analyze it on the different parameters and also to assess the content of Loha (Iron) in Lohasava in the form of Shodhít Loha Churna (purified iron in powder form) [as per classical Ayurvedic texts] or Loha Bhasma (Incinerated Iron).

Key words: Arishta, Asava, Lohasava

INTRODUCTION

Asava-Arishta preparations (self-generated alcoholic medicinal preparations) have occupied unique place, amongst all the Madhya Kalpanas (alcoholic preparations) and all other Kalpana (dosage forms) mentioned in Ayurveda. These medicinal preparations are Sandhana Kalpana (self-fermented dosage forms),[1] and are more popular and appreciated because of their quick action and high preserving quality. Usually herbal medicine lose their potency after some time, hence ancient Ayurvedic scholars evolved this preparation, by which the active principle of the medicinal drugs could be preserved for prolonged periods in alcoholic media.[2] In addition to longer shelf life, these dosage forms also possess palatable taste, quick action and can be easily administered to the patients. Asava and Arishta are the medicinal preparation made by admixture of drug either in Churna (powder) or in Kashaya (decoction) form, with the sugar or jaggery for the specified period of time during which it undergoes a process of self-fermentation. Some amount of the sugar which is present in the sweetening agent will be reduced and converted into alcoholic media. Generated alcohol thus facilitates the dissolution of active principle of drugs into liquid media. The alcohol so generated also serves as a preservative for the formulae.[3] Moreover, while describing the shelf life of various dosage forms, Sharangadhara explained that Asava and Arishta preparations become more effective as they get older,[4] which can be linked to the alcoholic content of these preparations.

A large population of world is suffering from Pandu Roga (anemia) because of multitude of factors like poverty, malnutrition, pregnancy, infections etc. So there is need of safe and effective Ayurvedic preparations which also have quicker action, longer shelf life, better palatability and cost effectiveness too. Lohasava is one such preparation which is being used since long by Ayurvedic physicians in management of Pandu Roga.[5] But for promoting its judicious use in anemia in this scientific era, standardization of the Lohasava with respect to its iron content is of paramount importance. Taking all above facts into consideration, an attempt has been made for pharmaceutical study of Lohasava and to assess its iron content by incorporating Loha Churna vs Loha Bhasma in the different samples of Lohasava.

Review of Lohasava

The first reference of Lohasava found in the Gada Nighraha[6] Later on, Sharangadhara Samhita described the Lohasava,[7] which was followed by Bhaishajya Ratnavali,[7] and now the same has been included in Ayurvedic Formulary of India, Part – I,[8] as the standard reference. For the present study, Lohasava has been made as per the Sharangadhara Samhita.

Address for correspondence: Dr.Vijay Gupta,
Email: gupta.drvijay@gmail.com
MATERIALS AND METHODS

Aims and objectives

1. Preparation of four different samples of Lohasava, adopting different pharmaceutical procedures.
2. To standardize each and every step of preparation of Lohasava.
3. To study the effect of different containers on the fermentation.
4. To find out the solubility of Loha, is better in the form of Bhasma or in the form of Churna.

Preparation of Lohasava

Pharmaceutical preparation of Lohasava was made as per the aforesaid reference in following steps:

Collection of raw drugs

The herbal drugs and jaggery are obtained from Ayurvedic Pharmacy, Institute of Medical Science, Banaras Hindu University, Varanasi; the Shodhita Loha Churna & Loha Bhasma was made in the Department of Rasa Shastra, IMS, BHU, Varanasi; and the honey was purchased from Khuadi Gramoudyog, Varanasi.

Preparation of the coarse powder of drugs

First of all, herbal drugs [except Dhataki Pushpa (flowers of Woodfordia fruticosa (L.) Kurz.)] were taken individually in prescribed quantity. They were then cleaned to remove visible physical impurities, washed and then dried. After that, they were separately subjected to coarse grinding.

Preparation of the containers

For the preparation of four different samples of drug, different containers were taken for each sample; viz. one stainless steel, one of china clay and two plastic containers were taken.

Before use, all these containers were properly washed with detergent and hot water, and sun dried. After that, the inner surface of containers was smeared with Ghee and then all of them were fumigated with Guggulu. [9]

Fermentation

The specific amount of powdered jaggery was added in the prescribed quantity of water in each container and it was made dissolve by proper stirring. The solution was then filtered through a muslin cloth to remove the physical impurities. It was then mixed with the prescribed quantity of the powdered drugs taken individually in each container [Table 1]. Lastly, a definite quantity of Dhataki Pushpa was added into the containers and mixed thoroughly. The containers were then tightly closed with cloth ribbon smeared with clay, and then kept in a room of the aforesaid pharmacy, where the temperature was maintained all throughout the fermentation process.

The mouth of the containers were opened from time to time to affirm the onset of fermentation; and after few days from the onset, the completion of the fermentation was ascertained by flame (burning candle test) and lime water test. [10]

Filtration and packaging of the samples

After completion of fermentation, the liquid was filtered through a muslin cloth. The filtered liquid was further allowed to settle for two days, after which the supernatant liquid was collected and filled in amber colored glass bottles and sealed. It was these packed samples that were further subjected to analytical and other targeted studies.

Table 1: Ingredients and their quantity used in the preparation of different samples of Lohasava.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Ingredients</th>
<th>Part used</th>
<th>Quantity (gm)</th>
<th>Lohasava</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>I</td>
<td>II</td>
</tr>
<tr>
<td>1.</td>
<td>Amalaki (Emblica officinalis Gaertn.)</td>
<td>Pencarp</td>
<td>96</td>
<td>+</td>
</tr>
<tr>
<td>2.</td>
<td>Haritaki (Terminalia chebula Retz.)</td>
<td>Pencarp</td>
<td>96</td>
<td>+</td>
</tr>
<tr>
<td>3.</td>
<td>Vibhuti (Terminalia bellerica Roxb.)</td>
<td>Pencarp</td>
<td>96</td>
<td>+</td>
</tr>
<tr>
<td>4.</td>
<td>Chitraka (Plumbago zeylanica Linn)</td>
<td>Root</td>
<td>96</td>
<td>+</td>
</tr>
<tr>
<td>5.</td>
<td>Sonthi (Zingiber officinale Rosb.)</td>
<td>Rhizome</td>
<td>96</td>
<td>+</td>
</tr>
<tr>
<td>6.</td>
<td>Pippali (Piper longum Linn.)</td>
<td>Fruit</td>
<td>96</td>
<td>+</td>
</tr>
<tr>
<td>7.</td>
<td>March (Piper nigrum Linn.)</td>
<td>Fruit</td>
<td>96</td>
<td>+</td>
</tr>
<tr>
<td>8.</td>
<td>Favari [Trachyspermum ammi (Linn.) Sprague ex Turril.]</td>
<td>Fruit</td>
<td>96</td>
<td>+</td>
</tr>
<tr>
<td>9.</td>
<td>Mustaka (Cyperus rotundus Linn.)</td>
<td>Rhizome</td>
<td>96</td>
<td>+</td>
</tr>
<tr>
<td>10.</td>
<td>Vudanga (Emblica ribes Burn.f.)</td>
<td>Fruit</td>
<td>96</td>
<td>+</td>
</tr>
<tr>
<td>11.</td>
<td>Dhataki [Woodfordia fruticosa (L.) Kurz.]</td>
<td>Flower</td>
<td>480</td>
<td>+</td>
</tr>
<tr>
<td>12.</td>
<td>Shodhita Loha Churna</td>
<td></td>
<td>96</td>
<td>-</td>
</tr>
<tr>
<td>13.</td>
<td>Loha Bhasma (20 putas)</td>
<td></td>
<td>96</td>
<td>+</td>
</tr>
<tr>
<td>14.</td>
<td>Honey</td>
<td></td>
<td>1.53</td>
<td>+</td>
</tr>
<tr>
<td>15.</td>
<td>Jaggery</td>
<td></td>
<td>2.4</td>
<td>+</td>
</tr>
<tr>
<td>16.</td>
<td>Water</td>
<td></td>
<td>12.50</td>
<td>L</td>
</tr>
</tbody>
</table>

Note: ‘+’ and ‘-’ respectively stand for incorporation or absence in the samples of Lohasava

OBSERVATIONS AND RESULTS

During preparation of Lohasava, a number of observations were made [Table 2 & 3]. After completion of fermentation & filtration, the samples were analyzed on different parameters prescribed by Central Council for Research in Ayurveda & Siddha (CCRAS) for standardization of this oral dosage form. [11] Apart from these, several other tests (e.g., Atomic Absorption Spectrophotometric Analysis, Thin Layer Chromatography etc.) relevant to quality control of the products were also performed to set the standards of the prepared drugs. The results of various pharmaceutical studies are as follows:
Phyto-Chemical Analysis

The phyto-chemical tests were done to ascertain the presence of different chemical constituents present in various samples of Lohasava (I-IV) [Table 6].

Table 6: Presence or absence of various chemical constituents in different samples of Lohasava

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Name of different chemical constituents</th>
<th>Lohasava I</th>
<th>Lohasava II</th>
<th>Lohasava III</th>
<th>Lohasava IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Alkaloids</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
</tr>
<tr>
<td>2.</td>
<td>Amino acids</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>3.</td>
<td>Glycosides</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>4.</td>
<td>Saponins</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>5.</td>
<td>Sterols</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>6.</td>
<td>Terpenoids</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>7.</td>
<td>Tannins</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
</tr>
</tbody>
</table>

Thin Layer Chromatographic Analysis (TLC)

Procedure: The different samples of prepared drug were spotted over TLC plate (20x8cm). The plate was marked by a line at the other end, about 15cms above the spot and run with CHCl₃: MeOH (1:1) solvent in chromatography chamber till the solvent reached the marked line. The plate was then removed, air dried and sprayed with LB reagent to visualize the spots. Subsequent heating in oven at 100°C for 15 minutes showed spots [Table 7].

Table 7: Rₜ value of different samples of Lohasava

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Samples</th>
<th>Distance travelled by solute(A) in (cm)</th>
<th>Distance travelled by solvent (B) in (cm)</th>
<th>Rₜ (A/B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Lohasava I</td>
<td>8</td>
<td>11.7</td>
<td>0.68</td>
</tr>
<tr>
<td>2.</td>
<td>Lohasava II</td>
<td>8.3</td>
<td>11.7</td>
<td>0.71</td>
</tr>
<tr>
<td>3.</td>
<td>Lohasava III</td>
<td>8.4</td>
<td>11.7</td>
<td>0.72</td>
</tr>
<tr>
<td>4.</td>
<td>Lohasava IV</td>
<td>8.1</td>
<td>11.7</td>
<td>0.69</td>
</tr>
</tbody>
</table>

Atomic Absorption Spectrophotometric Analysis

It is generally used for measuring the relative concentrations of metallic and semi metallic elements in solution samples.

Procedure: 0.5 ml of each sample was taken in a beaker and diluted to 100 ml by adding distilled water little by little. This solution was used for the determination of iron in different concentrations by Atomic Absorption Spectrophotometric Analysis. All the samples prepared were analyzed by this technique and results are as depicted in following table [Table 8].

Table 8: Iron content in different samples of Lohasava

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Name of the sample</th>
<th>Fe (in mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Lohasava I</td>
<td>364.60</td>
</tr>
<tr>
<td>2.</td>
<td>Lohasava II</td>
<td>341.70</td>
</tr>
<tr>
<td>3.</td>
<td>Lohasava III</td>
<td>315.00</td>
</tr>
<tr>
<td>4.</td>
<td>Lohasava IV</td>
<td>225.00</td>
</tr>
</tbody>
</table>
DISCUSSION

The Lohasava preparation in different containers showing that slower onset and completion of the fermentation process occurred in the sample prepared using china clay container, may be attributed to the least effect of outer climatic variation of temperature in this container. It was also observed that during the fermentation process, the temperature of the samples raised from initial point, which is because of the anaerobic fermentation process during which the glucose/fructose of the media breaks down, get converted into alcohol, along with generation of energy and evolution of carbon dioxide gas (CO₂) by the yeast. The odor of the samples also changed from aromatic to alcoholic, and the pH values also decrease due to generation of alcohol during fermentation. The solubility of the Loha Bhasma is found to be maximum in the sample I (in stainless steel container), followed by sample II (prepared and plastic container). Analytical parameters done on the samples of prepared drug are found to be within the same range except that lowest iron content was seen in the sample IV which was prepared incorporating the Shodhit Loha Churna; this may be due to fineness and better solubility of Loha bhasma in comparison to Shodhit Loha Churna.

CONCLUSIONS

The procedure adopted in the present study, namely adding Loha Bhasma in the samples of Lohasava (I-III) in place of Shodhit Loha churna (Lohasava IV) as suggested in Sharangadhara Samhita, has proved to be more effective in having more iron content in the Lohasava. It is also observed that a slower onset of fermentation was observed in the Lohasava III prepared in China Clay container, as compared to other samples. The pharmaceutical studies show that the all the samples are nearly showing same analytical findings, except the iron content which was least in the Lohasava IV containing Shodhit Loha Churna.

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How to cite this article: Gupta Vijay, Reddy KRC. Pharmaceutical Study of Lohasava wsr to Its Iron Content. Int J Ayurveda & Med Sc 2016; 1(4): 83-86.

Source of Support: Nil  Conflict of Interest: None