Stability study of Vijaysaradi ghanvati used in the management of type 2 diabetes (Madhumeha) - with respect to baseline microbial diagnostic modalities

Sachin Kumar Sharma, Alankruta R Dave, MS Cholera and Vasundhara Sharma

Abstracts

Background: Diabetes Mellitus is one of the most serious health problems in the 21st century. In Ayurveda disease Diabetes Mellitus can be correlated with Madhumeha. Madhumeha (Type 2 Diabetes) is included in the Ashtamahagadhas caused by the involvement of all three Doshas and ten Dushyas. Lots of single and compound drugs have been described in Ayurveda for the management of Madhumeha (Type 2 Diabetes). From such mentioned medicines a compound formulation has been made named Vijaysaradi Ghanvati which is found to be very effective in the management of Madhumeha (Type 2 Diabetes). In this formulation Heart wood of Vijaysar (Pterocarpus marsupium), Fruit of Harihaki (Terminalia chebula), Fruit of Amukti (Emblica officinalis), Fruit of Vibihiaki (Terminalia belerica), Panchang of Kiratitika (Swertia chirata), Leaves of Patolpatra (Trichosanthes dioica), Rhizome of Katuki (Picrorhiza kurroa), Fruit of Gokshur (Tribulus terrestris), Root of Musta (Cyperus rotundus), Heart wood of Swetcandan (Santalum album), Stem and Root of Durbaridra (Berberis aristata), Root of Usira (Vetiveria zizanoides) were taken.

Aim: In present study, stability with respect to its microbial profile of Vijaysaradi Ghanvati was carried out.

Materials and Methods: Sample of Vijaysaradi Ghanvati was prepared and studied to check microbial contamination at regular time intervals. Vati was stored in plastic container during different climatic conditions were studied at regular intervals of 1 month for a period of 11 months to analysis Mycological findings and presence of bacteriological findings by Wet mount preparation and Gram stain test respectively.

Results: Sample was subjected to the microbiological study from the date of the preparation to the date of last microbiological study. No any contaminations were found in microbiological study.

Discussion: Hence the present Study was carried out to observe the stability study of Vijaysaradi Ghanvati with respect to Microbial Contamination of sample prepared and store in different climatic conditions and temperature. Thus a baseline Microbial profile was studied at regular interval of 1 month for total 1 year & 2 month (i.e. time for consumption of prepared drug). At the end of study it was found that sample was not showed presence of any Microbes.

Conclusion: In microbiological study, Vati container has not present of microbes after 14 months of preparation sample, even in different climate and temperature, shows its stability and good shelf life. Hence in present study the stability test of Vijaysaradi Ghanvati with respect to microbiological findings was negative at room temperature, warm and cold, dry and humid conditions.

Keywords: Climate conditions, microbial profile, stability, type 2 diabetes (Madhumeha)

Introduction

Diabetes mellitus is a group of metabolic disorder in which a person has a high blood sugar, because body does not produce sufficient amount of insulin or lack of conversion of glucose into glycogen in the cells and tissues. It has been estimated that the number of diabetes sufferers in the world will double from the current value of about 190 million to 325 million during the next 25 years. In Ayurveda disease type 2 diabetes can be correlated with Madhumeha. According to Ayurveda excess Asyasukham (Sedentary life style), Swapasukham (excess sleeping), Dadhi (Excessive consumption of Curds and its preparations), Gramyoudaka-anupamamsa ( Flesh or meat soup of animals living in water and marshy regions), Payamsi (Excessive consumption of milk, its derivatives and preparations), Navaannapanam (Food, drinks and dishes prepared from new grains etc.), Guddavaikruti (Jaggery, its derivatives and dishes made out of it) life style activities which increase Kapha, use of Guru (heavy to
The Pharma Innovation Journal

digest). Snigdha (unctuous), Atinidra (excess sleep), Ayuyama (lack of exercise), Achinta (lack of mental exercise) are the causes of Madhumeha. Ayurvedic management of Madhumeha (Type 2 Diabetes) aims not only to achieve a good glycaemic control but also to treat the root cause of disease and its prevention. For the first time the research work carried out for its authentication and microbial profile. The drug was prepared in pharmacy of Gujarat Ayurved University, Jamnagar by adopting standard operative procedure for Ghanvati formation. No any preservative was added to the test drug. Drug preparation was finished on 12/01/2017. Finished product was stored in airtight plastic containers at room temperature. It was necessary to prepare the formulation in a better form which is also free from microbial contamination, stability of a pharmaceutical product is the capability of a particular formulation in a specific container or closure system, to remain within its physical, chemical, microbiological therapeutic specifications. Thus in the present study on attempt was taken to check stability of Vati with respect to its Microbial profile at different climatic conditions and temperature setups at regular interval for a period of 11 months.

**Aim:** To study the stability of finished product and to check microbial contamination in the finished product at different time interval- at different climatic conditions, temperature and humidity set ups.

**Materials and Methods**
Sample of Vijaysaradi Ghanvati was prepared (stored at room temperature) and finished product studied to check microbial contamination at regular intervals of 1 month for a period of 11 months (upto drug used). Microbiological study has been carried out in Microbiology Laboratory, IPGT & RA, Jamnagar. Mainly 02 studies have been carried out to rule out that presence of any bacteria or fungi in the prepared drug as a final finished product. The initial microbiological study was done on 90th day of preparation. Then samples from same container were subjected to the microbiological study regularly with intervals of 1 months during different seasons.

**Drug material**
All the raw drugs were obtained from Pharmacy of Gujarat Ayurved University, Jamnagar. The ingredients and the part used are given in table 1.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Botanical Name</th>
<th>Parts Used</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vijaysar</td>
<td>Pterocarpus marsupium Roxb.</td>
<td>Heart wood (dry)</td>
<td>11 part</td>
</tr>
<tr>
<td>Haritaki</td>
<td>Terminalia chebula Retz.</td>
<td>Fruit (dry)</td>
<td>1 part</td>
</tr>
<tr>
<td>Amlaki</td>
<td>Emblica Officinalis Gaertn.</td>
<td>Fruit (dry)</td>
<td>1 part</td>
</tr>
<tr>
<td>Vibhutaki</td>
<td>Terminalia belerica Roxb.</td>
<td>Fruit (dry)</td>
<td>1 part</td>
</tr>
<tr>
<td>Kiratikutki</td>
<td>Swertia chirata (Wall) C.B.Cl</td>
<td>Panchang (dry)</td>
<td>1 part</td>
</tr>
<tr>
<td>Patolpatra</td>
<td>Trichosanthes dioica Roxb.</td>
<td>Leaves (dry)</td>
<td>1 part</td>
</tr>
<tr>
<td>Katuki</td>
<td>Picrorhiza Kurroo ex Benth.</td>
<td>Rhizome (dry)</td>
<td>1 part</td>
</tr>
<tr>
<td>Gokshur</td>
<td>Tribulus terrestris Linn.</td>
<td>Fruit (dry)</td>
<td>1 part</td>
</tr>
<tr>
<td>Musta</td>
<td>Cyperus Rotundus Linn.</td>
<td>Root (dry)</td>
<td>1 part</td>
</tr>
<tr>
<td>Swetcandan</td>
<td>Santalum album Linn.</td>
<td>Heart wood(dry)</td>
<td>1 part</td>
</tr>
<tr>
<td>Daruharidra</td>
<td>Berberis aristata DC</td>
<td>Stem &amp; Root(dry)</td>
<td>1 part</td>
</tr>
<tr>
<td>Usira</td>
<td>Vetiveria zizanoides (Linn.) Nash</td>
<td>Root(dry)</td>
<td>1 part</td>
</tr>
</tbody>
</table>

Date of drug preparation: 12 January 2017

Storage:
Finished product of Vijaysaradi Ghanvati was stored in airtight food grade, plastic containers, stored in the open light area in the department at room temperature. Clean and dry stainless steel spoon was used to take medicine.

**Microbial profile**
Microbial contamination was assessed by two methods to check any mycological findings and bacteriological findings.

1. **Smear examination**
   A) Wet mount/10% K.O.H. Preparation
   B) Gram’s stain

2. **Culture Study**
   A) Fungal culture
   B) Aerobic culture

The details of the procedures followed are given below:
1. **Smear Examination:**
   A. Wet mount /10% K.O.H. Preparation
   **Aim:** To rule out any mycological findings.
   **Specimen:** Vijaysaradi Ghanvati
   **Procedure for Wet Preparation:**
   - Take clean grease free glass slide
   - Put selected material
   - Add distilled water (if needed)
   - Cover with grease free cover glass
   - Observe under the high power (40x)
   - Report as per findings

2. **Culture Study:**
   A) Fungal culture
   B) Aerobic culture

...
Procedure For 10% KOH Preparation

Take Potassium Hydroxides pellets in distilled water
To prepare 10% of the same in clean glass tube & mix well

Take clean grease free glass slide

Put a drop of specimen and add freshly prepared 10% KOH,
then cover with grease free cover glass

Allow it to react for 15-20 minutes to remove extra debris other than fungal

Observe under high power (40x) lens

Report as per findings

B. Gram’s stain test
Gram staining is a differential staining technique that differentiates bacteria into two groups: gram positive and gram negative. The procedure is based on the ability of microorganisms to retain color of the stains used during the gram stain procedure. Gram negative bacteria are decolorized by any organic solvent (acetone or Gram’s decolorizer) while Gram positive bacteria are not decolorized as primary dye retained by the cell and bacteria will remain as purple. After decolorization step, a counter stain effect found on Gram negative bacteria and bacteria will remain pink. The Gram stain procedure enables bacteria to retain color of the stains, based on the differences in the chemical and physical properties of the cell wall (Alfred E Brown, 2001) [4].

Aim: To rule out any bacteriological findings.

Specimen: Vijaysaradi Ghanvati

Procedure for Gram’s Stain

Take clean grease free glass slide to prepare dry equal thick preparation (i.e. smear)

Fixed prepared smear by passing 3-4 times over the flame of Bunsen burner (The fixation kills vegetative form of microbes and render them permeable to stain, make material stick to the surface of slide & prevent autolytic changes)

Cover fixed prepared smear with Gram’s crystal violet solution and allow to remain for mentioned time as per kit procedure

Washed off smear to remove excessive reagent with tap water

Cover smear with Gram’s Iodine solution and allow remaining for mentioned time as per kit procedure
2. Culture study

A. Fungal culture method
Respected materials collected with sterile cotton swab for inoculation purpose on selected fungal culture media (i.e. an artificial preparation).

Name of media : Sabouraud Dextrose Agar Base (SDA), Modified (Dextrose Agar Base, Emmons)  
Company : HIMEDIA Laboratories Pvt. Ltd.  
Required time duration : 05 to 07 days  
Required temperature : 37 ºC  
Use of media : For selective cultivation of pathogenic fungi.
Procedure for fungal culture
B. Aerobic culture method
Respected materials collected with sterile cotton swab for inoculation purpose on selected aerobic culture media (i.e. an artificial preparation).
Name of media: MacConkey agar (MA) and Columbia blood agar (BA)

Company: HIMEDIA Laboratories Pvt. Ltd.
Required time duration: 24 to 48 hours
Required temperature: 37 °C
Use of media: for selective cultivation of pathogenic bacteria.
Procedure for aerobic culture

In the clinical microbiology laboratory culture method are employed for isolation of organism (The streak culture method is routinely employed)

Choose appropriate selective solid media for inoculation purpose

Dry selective solid media in Hot Air Oven before specimen inoculation, allow to cool dried medium before specimen inoculation

Inoculate selected specimen by four flame method (the loop should be flamed and cooled between the different sets of streaks i.e. four time) on surface of cool dried medium with nichrome wire (24 S.W.G. size) loop (first sterile loop in Bunsen burner oxidase flame - blue flame and allow it to cool than loop is charged with selected specimen to be cultured. One loopful of the specimen is transferred onto the surface of well dried plate)

After streaking process incubate inoculated medium in inverted position at 37°c for 18-24 hours in incubator under aerobic or 10% CO2 atmosphere

After selected incubation period examined growth by naked eye in form of colony and confirm growth by performing different related biochemical reactions and different related staining procedures. After that report isolates

Observations and Results
Every time sample (In which drug preserved) were subjected to the microbiological study from the date of the preparation to the date of last microbiological study. Results are shown in table 2.

Table 2: Showing observations of sample preserved at room temperature

<table>
<thead>
<tr>
<th>S.N.</th>
<th>Months of study</th>
<th>Temperature</th>
<th>Humidity</th>
<th>Gram’s Stain</th>
<th>Aerobic culture</th>
<th>Wet mount/ 10% KOH Preparation</th>
<th>Fungal culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>3rd month (13/04/2017)</td>
<td>41 °C</td>
<td>30%</td>
<td>Microorganisms not seen</td>
<td>No organisms isolated</td>
<td>Fungal filaments not seen.</td>
<td>No fungal pathogen isolated</td>
</tr>
<tr>
<td>2.</td>
<td>4th month (17/05/2017)</td>
<td>43 °C</td>
<td>35%</td>
<td>Microorganisms not seen</td>
<td>No organisms isolated</td>
<td>Fungal filaments not seen.</td>
<td>No fungal pathogen isolated</td>
</tr>
<tr>
<td>3.</td>
<td>5th month (13/06/2017)</td>
<td>41 °C</td>
<td>38%</td>
<td>Microorganisms not seen</td>
<td>No organisms isolated</td>
<td>Fungal filaments not seen.</td>
<td>No fungal pathogen isolated</td>
</tr>
<tr>
<td>4.</td>
<td>6th month (18/07/2017)</td>
<td>32 °C</td>
<td>74%</td>
<td>Microorganisms not seen</td>
<td>No organisms isolated</td>
<td>Fungal filaments not seen.</td>
<td>No fungal pathogen isolated</td>
</tr>
<tr>
<td>5.</td>
<td>7th month (23/08/2017)</td>
<td>30 °C</td>
<td>80%</td>
<td>Microorganisms not seen</td>
<td>No organisms isolated</td>
<td>Fungal filaments not seen.</td>
<td>No fungal pathogen isolated</td>
</tr>
<tr>
<td>6.</td>
<td>8th month (21/09/2017)</td>
<td>33 °C</td>
<td>69%</td>
<td>Microorganisms not seen</td>
<td>No organisms isolated</td>
<td>Fungal filaments not seen.</td>
<td>No fungal pathogen isolated</td>
</tr>
<tr>
<td>7.</td>
<td>9th month (12/10/2017)</td>
<td>33 °C</td>
<td>61%</td>
<td>Microorganisms not seen</td>
<td>No organisms isolated</td>
<td>Fungal filaments not seen.</td>
<td>No fungal pathogen isolated</td>
</tr>
<tr>
<td>8.</td>
<td>10th month (16/11/2017)</td>
<td>34 °C</td>
<td>30%</td>
<td>Microorganisms not seen</td>
<td>No organisms isolated</td>
<td>Fungal filaments not seen.</td>
<td>No fungal pathogen isolated</td>
</tr>
<tr>
<td>9.</td>
<td>12th month (17/01/2018)</td>
<td>29 °C</td>
<td>35</td>
<td>Microorganisms not seen</td>
<td>No organisms isolated</td>
<td>Fungal filaments not seen.</td>
<td>No fungal pathogen isolated</td>
</tr>
<tr>
<td>10.</td>
<td>13th month (15/02/2018)</td>
<td>32 °C</td>
<td>24%</td>
<td>Microorganisms not seen</td>
<td>No organisms isolated</td>
<td>Fungal filaments not seen.</td>
<td>No fungal pathogen isolated</td>
</tr>
<tr>
<td>11.</td>
<td>14th month (26/03/2018)</td>
<td>42°C</td>
<td>28%</td>
<td>Microorganisms not seen</td>
<td>No organisms isolated</td>
<td>Fungal filaments not seen.</td>
<td>No fungal pathogen isolated</td>
</tr>
</tbody>
</table>
Discussion
Ayurveda as an adjuvant therapy is widely used in management of Madhumeha (Type 2 Diabetes). Vijaysaradi Ghanvati is never used before for the research work at IPGT & RA and elsewhere in India. In present study, it has shown a very good and promising result in management of Madhumeha (Type 2 Diabetes). Hence the present Study was carried out to observe the stability study of Vijaysaradi Ghanvati with respect to Microbial Contamination of sample prepared and preserved in different climatic and temperature conditions. Thus a baseline Microbial profile was studied at regular interval of 1 month for total 11 months. At the end of study it was found that sample has not shown presence of any Microbes.

Stability is usually expressed in term of shelf-life, which is the time period from when the product is produced until the time it is intended to be consumed or used. The factors affecting stability of prepared drug are categorized under intrinsic and extrinsic factor (FDA report 2001). Intrinsic factors include moisture content, acidity, nutrient content, biological structure, redox potential, naturally occurring and added antimicrobials. Extrinsic factors include types of packaging, effect of time/temperature on microbial growth, storage/holding conditions and processing steps (FDA report 2001). Microbial contamination should avoid increasing drug stability and storage time.

Vijaysaradi ghanvati was prepared and stored at room temperature. Sample was selected randomly for study of microbiological contamination. Changes in temperature and humidity of environment were observed during study period. The region where the drug was prepared and sample was stored was very proximal to sea coast, this area has longest sea shore and maximum number of sea ports, so relative humidity (RH) remains high in all the seasons of the year. Highest RH observed was 80% in month of August while lowest relative humidity was 24% observed in month of February (As shown in Table 2). High RH may allow the growth of microbes [5], RH remain variable during whole study period. Wet mount, fungal culture, gram stain and aerobic culture tests were used to rule out any fungal and bacterial contamination in the sample of monthly interval from 13th April 2017 to 26th March 2018. During this study period no any microbes were isolated as a result of aerobic culture and no any fungal pathogen were isolated as a result of fungal culture (As shown in Table 2). Moisture content of drug play important role in its long term storage. Moisture contents main causative factor in drug deterioration, it also act as an enzymatic activator which slowly decompose the drug resulting in its degradation [6].

Conclusion
Shelf-life is the time period from when the product is produced until the time it is planned to be consumed or used. Several factors are used to determine a product’s shelf-life, ranging from organoleptic qualities to microbiological safety. Hence Microbiological study of the Vijaysaradi Ghanvati showed that the quality of vati in standard condition. There were no growth of microorganisms (bacterial or fungal) found, till 26th March 2018 i.e. 14th month from the date of preparation of Vijaysaradi Ghanvati, which shows their good shelf life.

References