EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 3
Issue: 6
505-510
Year: 2016

STABILITY STUDY OF *MADHU-GHRITA* AND *SWARNA PRASHANA YOGA* WITH RESPECT TO BASELINE MICROBIAL PROFILE

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Article Received on 17/04/2016 **Article Revised on 07/05/2016** **Article Accepted on 27/05/2016**

ABSTRACT
Swarna Prashana (Administration of Gold) is a unique and one of the best example of nano-medicine applied for preventive health care in Ayurveda. *Swarna Prashana* described in *Kashyap Samhita* is intended to boost memory, growth and immunity in children. In the present study, stability with respect to the microbial profiles of *Madhu-Ghrita* (honey-ghee), *Swarnaprashana Yoga* prepared with *Swarna Bhasma*, *Madhu* and *Ghrita* in a specific proportion were carried out. Two groups named group B (*Madhu-Ghrita*) and group C (*Swarnaprashana Yoga*) prepared in 2 batches in the month of March 2015 and September 2015, stored under different climatic and temperature conditions were studied at regular intervals in different climatic seasons for a period of 11 months and 5 months respectively to analyze mycological findings and presence of microorganisms by Wet mount test and Gram stain test respectively. Sample B_1, B_3 and Sample C_1, C_3 were stored at room temperature while sample B_2, B_4 and sample C_2, C_4 stored in refrigerator. At the end of the study, all sample of group B and group C did not reveal presence of microbes after 11 months (1^{st} batch) and 5 months (2^{nd} batch) of preparation of the drug. The findings of the present study indicates that warm, dry and less humid climatic conditions does not favour any microbial contamination, it reveal that the stability of *Swarnaprashana Yoga* and mixture of *Madhu-Ghrita* with respect to microbiological findings are nearly 1 years when handled hygienically.

KEYWORDS: Microbial Profile, *Swarnaprashana Yoga*, *Madhu-Ghrita*, Stability, Climatic conditions.

INTRODUCTION
Swarna Prashana has been traditionally practiced as a recipe for child growth and enhancement of the immunity. *Swarna* (Gold) is the tonic for the nervous system and increases intellect, memory and tones up tissue. It merely indicates the metal Gold which is considered to be one among the *Shuddhaloha*[1] (pure metal). The word *Prashana* means to eat, consume or lick.[2] Thus, in short, the word *Swarna Prashana* denotes the consumption of gold by means of licking. The description of *Swarnaprashana* in detail has been given in *Kashyap Samhita*[3] one of the ancient book of *Kaumarbhritya* (one of the eight branches of Ayurveda dealing with mother and child health care). *Acharya* Kashyapa described that consuming of gold increases intellect, digestive and metabolic power, physical strength given long life; is auspicious, increase complexion and immunity in infants. *Acharya* Charaka has mentioned only mixture of *Ghrita* and *Madhu*.[4]

The efficacy and safety of *Swarnaprashana* therapy entirely depends upon the quality of *Swarna bhasma*, ghee and honey. Hence standardized *Swarna bhasma* along with superior quality of ghee and honey should be used for the preparation of *Swarnaprashana yoga*. Standardization of *Swarnaprashana* preparation is needed to prevent toxicity and malpractice. The administration of this *Yoga* has been told for children with specific indications and contraindications (Hemaraja Sharma).[5] Many other texts of Ayurveda have also explained internal administration of gold (Harisastri Paradakara, 2002 & Shivaprasad Sharma, 2006).[6,7] From the above cited references it can be understood that this specific formulation has to be freshly prepared and administered.

As *Swarnaprashana* is administered in children even from new born period, it was necessary to prepare the formulation in a better dosage form which is also free from any microbial and fungal contamination. Thus the

present study was designed to study the stability of *Swarna Prashana Yoga* with respect to microbial contamination in the four selected samples of each groups prepared and stored in two different climatic conditions and temperature.

MATERIALS AND METHODS

Samples of *Madhu-Ghrita* labeled as B_1, B_3 (stored at room temperature) and sample B_2, B_4 (stored at refrigerator). Sample of *Swarna Prashana* labeled as C_1, C_3 (stored at room temperature) C_2, C_4 (stored at refrigerator) were prepared and studied to check microbial contamination at different climatic conditions. The study was conducted at Microbiology laboratory, Institute for Post Graduate Teaching and Research in Ayurveda, Jamnagar, Gujarat, India.

Contents of samples

Group B contained unequal quantities of 27g of ghee and 110 g of honey and group C contained 315mg of *Swarna Bhasma* (Ash of gold) in addition to Group B contents, triturated well for about 8 hours in *Akika Khalvayantra* (Mortar and pestle made of semi- precious stone, *Akika*). This specific proportion was followed to fix the dosage form as drops which will be easier in administration in children. *Swarna Bhasma* was procured from the Department of *Rasashastra* and *Bhaishajya kalpana*, Institute for Post Graduate Teaching and Research in Ayurveda, Gujarat Ayurved University, Jamnagar, Gujarat, India. Honey (Agmark 'A' Grade) was procured from IPD of Kaumarbhritya, IPGTRA Jamnagar, and Ghee (Agmark Cow ghee) from standard local market which were also evaluated for microbial contamination before the preparation of *Madhu-Ghrita* and *Swarna yukta Madhu-Ghrita (Swarnaprashana Yoga)*.

Figure 1: *Swarnaprashana Yoga*

Figure 2: *Madhu-Ghrita*

Preparation time

Group B and group C were prepared in two different batches. 1st batch (group B and group C) was prepared and preserved during the months of March 2015 to Jan 2016 and 2nd batch (group B and group C) were prepared and preserved during the months of September 2015 to January, 2016. The preparation was done with utmost care to avoid any sort of contamination by using sterile vessels and gloved hands. Cap and mask were worn during the entire preparation period. To ensure further safety, glass bottles for storage were sterilized by rinsing with 10% sodium hypochlorite (chemical sterilization method) and then kept in hot air oven (physical sterilization method).

Storage

The 1st batch sample was stored in two parts; one part at room temperature (sample B_1 and sample C_1) in a dry and dark place to avoid exposure to direct sunlight and wind and the other in refrigerator (sample B_2 and C_2).

The 2nd batch sample was also stored in two parts; one part at room temperature (sample B_3 and sample C_3) and the other in refrigerator (sample B_4 and sample C_4). No preservatives were added to the samples for storage. All the eight samples were subjected to stability study with respect to microbial and fungal contamination at regular intervals in different season for a period of 11 months (1st batch) and 5 months (2nd batch) the details of which are cited below.

Microbial profile

Microbial contamination was assessed by two methods to check any mycological findings and bacteriological findings. The details of the procedures followed are given below.

1. Wet mount test and Fungal culture

Aim: To rule out any mycological findings.
Specimen: Group B (Sample B_1, B_2, B_3, B_4) and Group C (sample C_1,C_2,C_3,C_4)

Procedure

Smear examination: A drop of selected samples were taken on grease free glass slides + 10% KOH and covered with clean cover slips for microscopic examination.

Fungal culture

Respected material 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.

Figure 3: Picture shows Fungal culture media

2. Gram 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 colour of the stains used during the gram stain procedure. Gram-negative bacteria are decolourized by any organic solvent (acetone or Gram's decolourizer) while Gram-positive bacteria are not decolourized as primary dye retained by the cell and bacteria will remain as purple. After decolourization step, a counter stain effect found on Gram negative bacteria and bacteria will remain pink. The Gram stain procedure enables bacteria to retain colour of the stains, based on the differences in the chemical and physical properties of the cell wall (Alfred E Brown, 2001).[8]

Aim: To rule out any bacteriological findings.

Specimen: Group B (Sample B_1,B_2,B_3,B_4) and Group C (sample C_1,C_2,C_3,C_4)

Procedure

The smear was covered with crystal violet and allowed to remain for mentioned time as per kit procedure. Then the stain was washed off, using a wash bottle of distilled water/tap water. Excess water was drained off.

In second step the smear was covered with Gram's iodine solution and allowed to remain for mentioned time as per kit procedure. Gram's iodine was later poured off and the smear was washed off, using a wash bottle of distilled water/tap water.

In third step the smear was flooded with Gram's decolourizer i.e. acetone for mentioned time as per kit procedure. The excess acetone was removed by rinsing the slide with distilled water/tap water.

In fourth step the smear was covered with saffranin for mentioned time as per kit procedure followed by distilled water/tap water wash and allowed to air dry. The slide was examined under oil immersion.

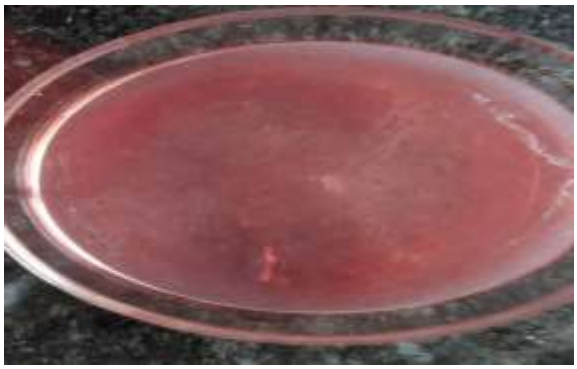

Figure 4: Picture showing Aerobic culture media

Figure 5: Picture showing Smear Examination

OBSERVATIONS

Following findings were observed at the end of the study. The tables 1, 2, show the observations of 1st batch samples i.e. B_1, C_1 and B_2, C_2 respectively while tables 3, 4, show the observations of 2nd batch samples i.e. B_3, C_3 and B_4, C_4 respectively during the tests at regular intervals in different climatic seasons for a period of 11 months (1st batch) and 5 months (2nd batch).

i) Observation of 1st batch samples

Sample B_1, C_1 (Table 1) and sample B_2, C_2 (Table 2) preserved at room temperature and refrigerator respectively during the month of March 2015 to Jan 2016, did not show presence of any mycological or bacteriological findings at the end of 11 months after preparation of the samples.

ii) Observation of 2nd batch samples

Sample B_3, C_3 (Table 3) and sample B_4, C_4 (Table 4) preserved at room temperature and refrigerator respectively during the month of September 2015 to Jan 2016, did not show presence of any mycological or bacteriological findings at the end of 5 months after preparation of the samples.

All the samples of 1st and 2nd batch show negative finding of bacterial as well as mycological contamination.

Table 1: Observation of Sample B_1 and C_1 preserved in room temperature.

SI No.	Months of investigations After preparation of the sample	Observation of sample B_1 and sample C_1			
		Gram's Stain	Aerobic culture	Wet mount/ 10% KOH Preparation	Fungal culture
1.	May (2015)	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
2.	Aug (2015)	Microorganisms Not Seen	No organisms Isolated	Fungal filaments not seen	No Fungal Pathogen Isolated
3.	Nov (2015)	Microorganisms Not Seen	No organisms Isolated	Fungal filaments not seen	No Fungal Pathogen Isolated
4.	Jan (2016)	Microorganisms Not Seen	No organisms Isolated	Fungal filaments not seen	No Fungal Pathogen Isolated

Table 2: Observation of Sample B_2 and C_2 preserved in refrigerator-

SI No.	Months of investigations After preparation of the sample	Observation of sample B_2 and sample C_2			
		Gram's Stain	Aerobic culture	Wet mount/ 10% KOH Preparation	Fungal culture
1.	May (2015)	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
2.	Aug (2015)	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen	No Fungal Pathogen Isolated
3.	Nov (2015)	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen	No Fungal Pathogen Isolated
4.	Jan (2016)	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen	No Fungal Pathogen Isolated

2nd Batch-

Table 3: Observation of sample B_3 and C_3 preserved in room temperature.

SI No.	Months of investigations After preparation of the sample	Observation of sample B_3 and sample C_3			
		Gram Stain	Aerobic culture	Wet mount/ 10% KOH Preparation	Fungal culture
1.	Oct (2015)	Microorganisms Not seen	No organisms isolated	Fungal filament not seen	No Fungal pathogen isolated
2.	Dec (2015)	Microorganisms Not seen	No organisms isolated	Fungal filament not seen	No Fungal Pathogen isolated
3.	Jan (2016)	Microorganisms Not seen	No organisms isolated	Fungal filament not seen	No Fungal Pathogen isolated

Table 4: Observation of sample B_4 and C_4 preserved in refrigerator.

SI No.	Months of investigations After preparation of the sample	Observation of sample B_4 and sample C_4			
		Gram Stain	Aerobic culture	Wet mount/ 10% KOH Preparation	Fungal culture
1.	Oct (2015)	Microorganisms Not seen	No organisms isolated	Fungal filament not seen	No Fungal pathogen isolated
2.	Dec (2015)	Microorganisms Not seen	No organisms isolated	Fungal filament not seen	No Fungal Pathogen isolated
3.	Jan (2016)	Microorganisms Not seen	No organisms isolated	Fungal filament not seen	No Fungal Pathogen isolated

DISCUSSION

Traditionally *Shuddha Swarna* has been advised to be rubbed on water washed stone and then emulsified with *Madhu* and *Ghrita* to achieve a fine colloidal suspension. This process of administration for certain period of time to achieved health and immunity in infants and children. The appearance, smell and most importantly the taste of the drug play a major role in acceptance of a drug by the children. The method of preparation, potency of ingredients and the dosage form also play a major role. It is clear from the explanation of *Swarna Prashana* that the Acharya was very specific about the method of

administration of gold wherein all the necessary parameters of drug administration in children, specifically in neonates are taken care of such as hygiene, palatability and bioavailability.

It is need of the modern era that this unique formulation should be prepare and store in specific environment for a reasonable time period which will be helpful for the safe usage in infants and children. *Swarnaprashana Yoga* prepared in the present study contained *Swarna Bhasma*, ghee and honey in a specific proportion which was selected for a better dosage form in children.

The present study was carried out to observe the stability of *Madhu-Ghrita* and *Swarna prashana Yoga* with respect to microbial contamination of the samples prepared and preserved in two different climatic and temperature conditions. Thus a baseline microbial profile was studied at regular intervals in different climatic seasons for a period of 11 months (1st Batch) and 5 months (2nd Batch). At the end of the study it was observed that 1st Batch and 2nd Batch containing both groups i.e. group B(*Madhu-Ghrita*) and group C (*Swarna -Madhu-Ghrita*) preserved at room temperature as well as in refrigerator during the months of March 2015 to Jan 2016 and September 2015 to Jan 2016 did not show presence of any microbes or fungal filaments at the end of 11 months and 5 month after preparation of drug.

The stability parameters of a drug dosage form can be influenced by environmental conditions of storage (temperature, light, air and humidity), as well as the package components.[9] In a previous research work (Bhaskaran Jyothy K. et all)[10] *Swarna Prashana Yoga* (*Swarna-Madhu-Ghrita)* preserved in room temperature during the months of September to November 2013 showed presence of many gram negative pleomorphic rods on 37th day of the Gram stain test. But no mycological finding was observed in it. While the formulation preserved in refrigerator showed negative results in wet mount test at the end of 3 months. But in gram stain test it showed presence of many gram negative rods arranged singly on 86th day of preparation. The month of September in 2013 received heavy rain fall in Jamnagar district and during October to December 2013 received average 27% higher rainfall in Jamnagar district during which, low temperature, high humidity and moisture content of the atmosphere might have also influenced the positive microbial contamination findings in the samples during those period.[10]

The prepared *Swarnaprashana Yoga* in this present study contained *Swarna Bhasma*, ghee and honey. No preservatives were added to the formulation. The factors which might have influenced the findings of the tests in the samples are discussed here in detail.

Microorganisms need water to grow and will die without water sources. Moist areas are particularly prone to bacterial growth. Water content in any medicinal product also provides an excellent environment for microorganisms to grow.[11]

Microorganisms generally have optimum and minimum levels of a_W for growth. Gram negative bacteria are generally more sensitive to low a_W than Gram positive bacteria (FDA report, 2001).[12] Water activity is defined as the ratio of water vapor pressure of the prepared product to the vapor pressure of pure water at the same temperature (James M. Jay, 2000).[13] Thus the a_W describes the degree to which water is bound in the product, its availability to participate in chemical or biochemical reactions and its availability to facilitate growth of microorganisms.

In the present study of *Swarna Prashana Yoga* and mixture of *Madhu-Ghrita, Madhu* (Honey) an ingredient, *was* of comparatively higher quantity than ghee. Approximate a_W value of honey is < 0.60 (Bibek Ray, 2004).[14] Honey is hygroscopic in nature. The amount of water absorb in honey is dependent upon the relative humidity of the air. Although the water activity value of honey is high, a change in that value might have taken place in the samples due to change in climatic conditions and temperature. Negative findings of any microbial and fungus formation in all samples of both batches at room temperature as well as at refrigerator, during the month March 2015 to Jan 2016 and September 2015 to Jan 2016 which may be due to low degree of humidity which depends on natural rainfalls and the flow of moist wind and also depends upon proper hygienic conditions during preparation and storage. In 2015 there is low rain fall in Jamnagar Gujarat city and average minimum and maximum temperature was noticed 21.7ºC and 38.9 ºC respectively.[15] The level of microorganisms in the air is controlled by degree of humidity, size and level of dust particles, temperature and air velocity and resistance of microorganisms to drying. Generally dry air with low dust content and higher temperature has low microbial level.

It is scientific truth that the decomposition or formation of fungi (fungus) is directly proportional to degree of humidity present in the atmosphere. The degree of humidity depends on natural rainfalls and the flow of moist winds. During the year of 2015, Jamnagar was one of among the drought area. In said environment the test carried out on the samples of both batches, the observation obtained are shown in the above mention table 1,2,3 and 4.The results indicate that the prepared drugs samples are free from any microbial and fungus formation (fungi).

CONCLUSION

Hence it is being proved that if *Madhu-Ghrita* and *Swarna Prashana yoga* are kept in ideal packages, after making appropriate specified materials and then further it

is preserved under normal temperature, pressure, light and dry (damp-less) air (below normal degree of humidity), the shelf life of these formulations are nearly one year.

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