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APPLICATION OF DIFFERENT DIAGNOSTIC MODALITIES TO RULE OUT ANY MICROBIAL CONTAMINATION IN *PANCHENDRIYAVIVARDHANA TAILA*- A PREPARED AYURVEDIC FORMULATION FOR CEREBRAL PALSY

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ABSTARCT

Background: Cerebral Palsy includes a group of conditions that are characterized by chronic disorders of movement or posture. In present study *Panchendriyavivardhana Taila* described in *Shatkapladhaya* in Kashyapa Samhita was used for *Nasya*. **Aim:** To carry out stability study with respect to its Microbial profile of *Panchendriyavivardhana Taila*. To prove the concept of shelf life as given in Sharangdhara Samhita for *Taila* i.e. 16 Months. **Material and Method:** Sample of *Panchendriyavivardhana Taila* was prepared and studied to rule out any microbial contamination at regular and random time intervals. **Result:** At the end of study *Taila* has no presence of microbes after 15 months of preparation of sample, even in different climate conditions. **Discussion:** The present study was carried out to observe the stability study of *Panchendriyavivardhana Taila* with respect to microbial contamination of sample was prepared. *Panchendriyavivardhana Taila* was stored in plastic container during different climacteric conditions for a period of 15 months (452 days). Sample was studied at regular and random intervals to analysis mycological findings and presence of bacteriological findings by two methods i.e. smear examination and culture study. **Conclusion:** In the microbiological study of *Panchendriyavivardhana Taila*, there was no any growth found of microorganisms for a period of 15 months (452 days) and it proves the shelf life of *Taila* as

per given in Samhita i.e. 16 months. Hence the stability test of *Panchendriyavivardhna Taila* with respect to microbiological findings was negative at room temperature, warm and cold, dry and humid conditions.

KEYWORDS: Climate conditions, Microbial profile, *Panchendriyavivardhana Taila*, Shelf-life and Stability.

INTRODUCTION

Cerebral palsy (C.P.) is characterized by the inability to normally control motor functions, and it has the potential to have an effect on the overall development of a child by affecting the child's ability to explore, speak, learn, and become independent.[1] Population based studies from around the world report prevalence estimates of CP ranging from 1.5 to 4 per 1000 live births.[2] In Ayurveda classics there is no exact description of the disease entity which exactly matches the feature of CP. Few conditions and diseases that have some similarity in etiopathogenesis and clinical presentation. These include *Vyadhijafakka,*[3] *Vata Vyadhi,*[4] and *Nanatmaja Vata Vikara*. In this context Aacharya Kashyapa described *Panchendriyavivardhana Taila* in *Shatkapladhaya* for *Nasya* (Drug given through nasal route). It is also known as *Panchbhoutika Taila.*[6] For the first time the research work carried out for its authentication in form of stability of prepared drug (i.e. shelf life)

Concept of Shelf- Life

Taila (Oil) and *Ghrita* (Ghee) preparations were used for different Panchkarma procedure and they have different shelf-life. In Ayurvedic literatures, '*Saviryata Avadhi'* term is mentioned in context of the time period during which the *Virya* (potency) of any drug remains unaffected due to environmental/microbial deterioration; whereas in the contemporary system, the term 'Shelf- life' is used to indicate the time period during which an API (Active Pharmaceutical Ingredient) or FPP (Finished Pharmaceutical Product) is expected to remain within the approved stability specification, provided that it is stored under the conditions defined on the container label.[7] The shelf-life of *Taila* and *Ghrita* formulations were different according to different Acharya. According to Sharangdhara, Yogaratnakara and Vanga Sen the shelf-life of Oil & Fat based preparation are 16 months, 12 months and 06 months respectively.[8]

Preparation of drug

The drug was prepared in pharmacy of Gujarat Ayurved University, Jamnagar by adopting procedure given in Kashyapa Samhita for *Taila* preparation.

No any preservative was added to the test drug. Drug preparation was finished on 06/01/2017. Final product was stored in airtight plastic containers at room temprature.

It was necessary to prepare the *Panchendriyavivardhana Taila* as per SOP mentioned for *Taila* preparation in Samhita which is also free from microbial contamination. Stability of a pharmaceutical product is the capability of a perticular formulation in a specific container or closure system, to remain within its physical ,chemical, microbiological therapeutic specifications. Thus in the present study 14 attempts was made to study the stability of *Taila* with respect to its microbial profile at different climatic conditions and temperature setups at regular interval for a period of 15 months.

Aim

- To study the stability of *Panchendriyavivardhana Taila* at different climatic conditions (temperature and humidity set ups) to rule out any microbial contamination.
- To prove the concept of shelf life as given in Sharangdhara Samhita for *Taila* i.e. 16 months

MATERIALS AND METHODS

Sample of *Panchendriyavivardhana Taila* was prepared by following guidelines giving in Kashyapa Samhita i.e. all ingredients were taken in equal quantity and mixed with four times of milk. For *Taila* preparation *Taila Paka* has done till it gets *Sneha Siddhi Lakshana*. After preparation *Taila* was filtered and stored at room temperature and final product studied to rule out any microbial contamination at regular and random intervals for a period of 1 year 3 months (upto drug used for nasya in last patient). Microbiological study has been carried out in Microbiology Laboratory, of Institute. Sample was studied at regular and random intervals to analysis mycological findings and presence of bacteriological findings by two methods i.e. smear examination and culture study to rule out that presence or absence of any bacteria or fungi in the prepared drug.

Before enrolling the first patient in Nasya group the initial microbiological study was done on 39th day of preperation, Before giving *Nasya* to the patients. Then samples from same container were subjected to the microbilogical study regularly with random intervals during different seasons, before giving to the patients.

Drug material

All the raw drugs were obtained from Pharmacy of Gujarat Ayurved University and identified for its authentication at Pharmacognosy lab, IPGT & RA, Jamnagar.[9] The ingredients and the part used are given in table no. 1.

Date of drug preparation: 06thJanuary 2017.

Storage

Final product of *Panchendriyavivardhana Taila* was stored in air-tight food grade, plastic containers, stored in the open light area in the Kaumarbhritya department at room temperature. Clean and dry stainless steel spoon was used to take medicine.

Method

Microbial profile

Microbial contamination was assessed by two methods (Smear examination and Culture study) 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

Objective: To rule out any mycological findings

Specimen: ***PanchendriyavivardhanaTaila***

Procedure for wet preparation

Take a clean grease free glass slide then put selected material and add distilled water (if needed). Cover with grease free cover glass, than Observe under the high power (40x) lens. Report as per findings (if found).

Procedure for 10% KOH preparation

Take Potassium Hydroxides pelletsin distilled water to prepare 10% of the same in clean glass tube & mix well. Take a clean grease free glass slide and put a drop of specimen and add freshly prepared 10% KOH then cover with grease free cover glass. Allow it to rest for 15-20 minutes to remove extra debris other than fungal particles. Observe under high power (40x) lens and report as per findings (if found).

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).[10]

Aim: To rule out any bacteriological findings in the specimen.

Specimen: ***Panchendriyavivardhana Taila***

Procedure for gram' stain

Take a 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 the material stick to the surface of slide & prevent autolytic changes). Then 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 than cover smear with Gram's Iodine solution and allow remaining for mentioned time as per kit procedure Washed off smear to remove excessive reagent with tap water. Decolorize smear with Gram's decolourizer by holding the slide at slope position and pour gram's decolourizer – acetone from its upper end up to removal of color of primary dye (i.e. Gram's Crystal Violet) or as per kit procedure. Washed off smear to remove excess acetone with tap water than cover smear with Safranin solution and allow remaining for mentioned time as per kit procedure.

Washed off smear to remove excessive reagent with tap water than blot and allow to dry smear. Blot and allow to dry smear Examine under oil immersion lens and report as per findings (if found).


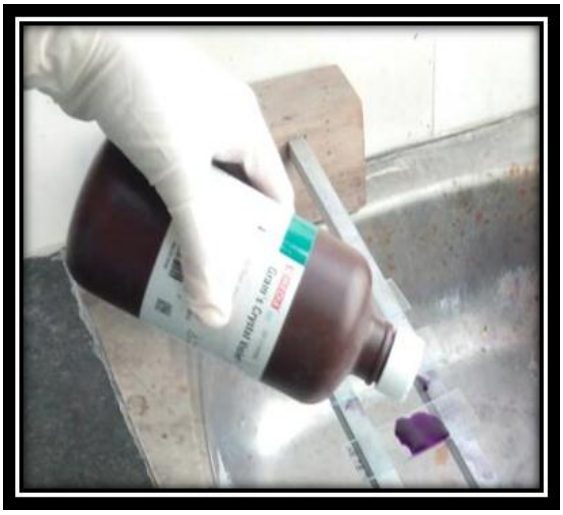

Figure 1 & 2: Smear staining procedure.

2. Culture Study

A. Fungal culture

Sample 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.

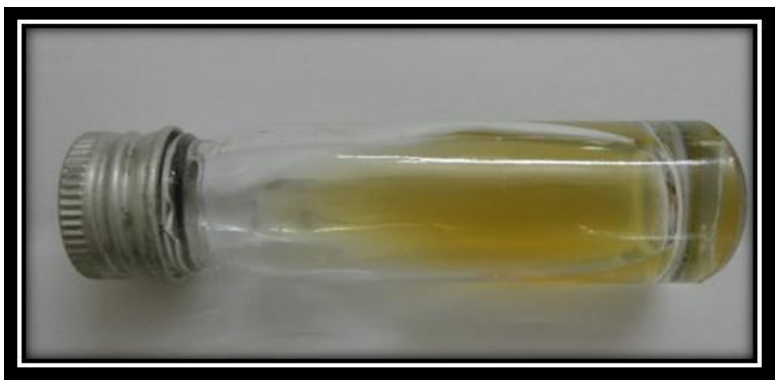

Figure 3: Sabouraud dextrose agar base media used for cultivation of any fungal contamination (SDA) bottle.

Procedure for fungal culture

In the clinical microbiology laboratory culture method are employed for isolation of organisms (The lawn / streak culture method is routinely employed) by choosing appropriate selective solid media for inoculation purpose. Dry selective solid media in Hot Air Oven before specimen inoculation and allow it to cool dried medium before Specimen inoculation Inoculate selective specimen by Sterile cotton swab or by Nichrome wire (24 S.W.G. size) loop [First sterile loop in Bunsen burner oxidase flame-blue flame and allow it cool than loop is charged with selected specimen to be cultured. One loop full of the specimen is transferred onto the onto the surface of well dried culture media] After inoculation / streaking process incubate inoculated medium in inverted position at 37^0 c for 05 to 07 to 21 days in incubator (incubation days are as per growth requirement) under aerobic atmosphere. After selected incubation period examined growth by naked eye in form of colony or aerial growth and confirm growth by performing different related biochemical reactions and different related staining procedures. After that report isolates (if found).

B. Aerobic culture method

Sample of *Panchendriyavivardhana Taila* collected with sterile cotton swab for inoculation purpose on selected aerobic culture media (i.e. an artificial preparation)

Name of media: Mac Conkey Agar (MA) and Columbia Blood agar (BA)

Company: HIMEDIA Laboratories Pvt. Ltd.

Required time duration: 24 to 48 hours

Required temperature: 37 ºC

Use of media: for selective cultivation of pathogenic bacteria.

Figure 4: Mac conkey agar (MA).

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, then dry selective solid media in Hot Air Oven before specimen inoculation and allow it to cool dried 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 loop full of the specimen is transferred onto the surface of well dried plate]. After streaking process incubate inoculated medium in inverted position at 37^0c for 18-24 hours in incubator under aerobic or 10% CO_2 atmosphere. After that incubation period is examined the growth in form of colony and confirm growth by performing different related biochemical reactions and different related staining procedures. After that reports the isolates (if found).

OBSERVATIONS AND RESULTS

Each and every time before giving *Nasya* to the patients sample of *Panchendriyavivardhana Taila* were subjected to the microbiological study from the date of the preperation to the date of last consumption of *Panchendriyavivardhana Taila*. Observation and results are shown in table no. 2.

Table no. 1: Ingredients of *Panchendriyavivardhana Taila.*[5]

Content	Botanical name	Part used	Ratio
Jiwaka	*Leptidinia reticulata* W&R	Root	1 part
Rishbhaka	*Melaxis mucifera*	Root	1 part
Draksha	*Vitis vinifera* Linn.	Fruit	1 part
Madhuka	*Glycrrhiza glabra* Linn.	Root	1 part
Pippali	*Piper longum* Linn.	Fruit	1 part
Bala	*Sida cordifolia* Linn.	Root	1 part
Prapaundarika	*Nelumbo nucifera* Gaeris.	Whole part	1 part
Brihati	*Solanum indicum* Linn.	Root	1 part
Manjishtha	*Rubia cordifolia* Linn.	Root	1 part
Tvaka	*Cinnamomum zeylanicum* Breyn.	Bark	1 part
Punarnava	*Boerhavia diffusa* Linn.	Whole part	1 part
Anshumati	*Desmodium gengeticum*DC	Whole part	1 part
Meda	*Poligonatum multiflorus*		
Vidanga	*Embelicaribes* Burm.f.	Fruit	1 part
Saindhava	*Rock salt*		1 part
Neela Kamala	*Nymphaea nouchali*	Whole part	1 part
Swadanshatra	*Tribulusterrastris* Linn.	Fruit	1 part

Rasna	*Pluchea lanceolata* C. B. clarke	Bark	1 part
Nidigdhika	*Solanum surratensa* Burm. f.	Whole part	1 part
Tila Taila	*Sesamum indicum*Linn.		4 part
Godugdha			16 part
Sharkara	Sugar		1 part
Substitute have taken of below drugs			
Main Drug	**Substitute**	**Botanical Name**	**Part Used**	1 part
Jivaka	*Vidarikanda*	*Puararia tuberosa* DC	*Kanda*	1 part
Rishabhaka	*Vidarikanda*	*Puararia tuberosa* DC	*Kanda*	1 part
Meda	*Shatavari*	*Asparagus recemosus* Willd	Root	1 part

Table No. 2: Observations of sample preserved at room temperature.

Sr. No.	Days of study at	Date of investigations After preparation of the sample	Temp. of Environment	Humidity of Environment	Observations of sample			
					Gram's Stain	Aerobic culture	Wet mount/ 10% KOH Preparation	Fungal culture
1.	39 Days	16/02/2017	26˚ C	17%	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
2.	68 Days	17/03/2017	28˚ C	20%	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
3.	103 Days	21/04/2017	35˚ C	73%	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
4.	135 Days	24/05/2017	38˚ C	34%	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
5.	165 Days	23/06/2017	32˚ C	75%	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
6.	196 Days	24/07/2017	27˚ C	89%	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
7.	233 Days	30/08/2017	29˚ C	90%	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
8.	255 Days	21/09/2017	30˚ C	59%	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
9.	280 Days	16/10/2017	28˚ C	26%	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
10.	322 Days	27/11/2017	24˚ C	28%	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
11.	353 Days	28/12/2017	28˚ C	17%	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
12.	395 Days	08/02/2018	25˚ C	20%	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
13.	421 Days	06/03/2018	33˚ C	16%	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
14.	452 Days	05/04/2018	28˚ C	70%	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated

DISCUSSION

As an adjuvant therapy, Ayurveda is comprehensively used in neurological disorders like Cerebral Palsy. Hence, the present Study was carried out to observe the stability study of *Panchendriyavivardhana Taila* with respect to Microbial Contamination of sample in different climatic conditions (temperature and humidity set ups). Thus a baseline Microbial

profile was studied at regularly in random intervals for total of 15 months (from 16/02/2017 to 05/04/2018).

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. Microorganisms needs suitable environmental conditions like water, humidity and temperature to grow and develop in any media, surface or article. According to Acharya Sharangdhara, *Saviryata Avadhi* of *Taila* is 16 months.[02] The oleaginous preparations were mentioned comparatively good shelf-life rather than other preparations like *Kwatha* (Decoction), *Churna* (Powder), *Vati* (Tablet) and *Avaleha* (Medicated semisolid preparation), it may be because of the relatively low environmental oxidation and rancidity rate. These are also remaining unaffected by microbial infestation until it develops some moisture which can facilitate the microbial growth. In present study, as mentioned in observation table no. 2; at different climatic conditions i.e. humidity and temperature stability study of prepared drug was checked out and found no microbial contamination as a result up to 15 month of consumption (i.e. total duration of completion of drug; usage for enrolled patients). So Stability of *Panchendriyavivardhana Taila* was found at the range of temperature of environment was 24˚C-38˚C and the humidity of environment was 16%-90% during different seasons throughout the year.

Hence, Standard mentioned in Samhita (as mentioned in Aim); Stability study data of present work supported the same.

CONCLUSION

Shelf-life of drugs depends on several factors; ranging from organoleptic qualities to microbiological safety. Hence Microbiological study of the *Panchendriyavivardhana Taila* showed that the quality of *Taila* is in a standard condition. There were no any growth of bacterial and fungal microorganisms found, from the date of preparation i.e. 06thJanuary 2017 till last consumption i.e. 06th April 2018 for total of 15 months, shows its good shelf life.

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