WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 9, Issue 12, 772-783. Research Article ISSN 2277– 7105

EVALUATE THE STABILITY OF *MEDHYA CHURNA* (AN AYURVEDIC POLY HERBAL DRUG) BY MICROBIOLOGICAL MEASURES IN THE MANAGEMENT OF ATTENTION - DEFICIT / HYPERACTIVITY DISORDER (ADHD) IN CHILDREN

Kingsila Manohari[1]*, Dr. Cholera M. S.[2], Prof. K. S. Patel[3] and Dr. V. K. Kori[4]

[1]MD final Year Scholar, Dept. of Kaumarbhritya, I.P.G.T. & R. A., G.A.U., Jamnagar, Gujarat. India.

[2]Head, Microbiology Laboratory, Dept. of Microbiology, I.P.G.T.& R. A., G.A.U., Jamnagar, India.

[3]Professor & Head of Dept. of Kaumarbhritya, I.P.G.T.& R. A., G.A.U., Jamnagar, Gujarat. India.

[4]Assistant Professor, Dept. of Kaumarbhritya I.P.G.T. & R. A., G.A.U., Jamnagar, Gujarat. India.

Article Received on
10 August 2020,

Revised on 31 August 2020,
Accepted on 20 Sept. 2020,

DOI: 10.20959/wjpr202012-18601

***Corresponding Author**
Kingsila Manohari
MD final Year Scholar,
Dept. of Kaumarbhritya,
I.P.G.T. & R. A., G.A.U.,
Jamnagar, Gujarat. India.

ABSTRACT

Background: ADHD is the most common neurodevelopmental, behavioral disorder in childhood characterized by persistent pattern of inattention and /or hyperactivity and impulsivity, it affects 5% of Children all over the world. This disorder is mainly managed by multimodal approach with psychostimulant medicines in modern medical system. There is no exact correlation in Ayurvedic classics to ADHD, but it's etio-pathogenesis can correlate with *Manasa Roga* in Ayurveda. **Aim:** To study the stability of *Medhya Churna* in different time interval, different climatic conditions, different temperature and different humidity set ups. **Materials and Methods**: Sample of *Medhya Churna* was prepared and check the microbial and fungal contamination at different climatic conditions, temperature and humidity from the date of preparation to date of last microbiological study. **Results:** No any microbial and fungal contaminations was found in microbiological study of *Medhya Churna*. **Discussion:** The present study was carried out to observe the stability study of *Medhya Churna* with respect to Microbial Contamination of sample prepared, stored in different climatic conditions and

temperature. Thus a baseline Microbial profile was studied at different interval for total 11 months of period. (i.e. time for consumption of prepared drug). At the end of study, the sample was not showed presence of any Microbes. **Conclusion**: In microbiological study of the *Medhya Churna* it was showed that there were not found any growth of bacterial or fungal microorganisms till 20th Feb 2020 i.e. 11 months from the date of preparation, its shows stability and good shelf life of the drug. Hence in the present study the stability test of *Medhya Churna* with respect to microbiological findings was negative at room temperature, warm and cold, dry and humid conditions.

KEYWORDS: Attention –Deficit /Hyperactivity Disorder(ADHD), *Medhya Churna*, Microbial contamination.

INTRODUCTION

ADHD is the most common neurobehavioral disorder in childhood [1]which affects 5%[2] of children in all over the world and 11.32% primary school children in India.[3] The medicine which was used to treat in the clinical study was *Medhya churna* is Ayurvedic poly herbal drug which is *Anubhuuti* Ayurvedic formulation commonly used to treatment of various Neurological and Psychological illnesses like CP, Developmental Delay, ADHD and Autism etc. in Department of Kaumarbhritya I.P.G.T.& R.A. hospital Gujarat Ayurveda University, Jamnagar in India. This formulation contains *Shankhpushpi*, *Brahmi*, *Guduchi*, *Yashtimadhu Vacha* and *Pippali* which are responsible for *Medhya*, *Smritiprada*, Nootropic, Antioxidant and Neuroprotective actions on brain and mind. Those properties included *Medhya Churna* has proved its effectiveness and safeness in the management of Attention- Deficit /Hyperactivity Disorder (ADHD) in children. As well as there is a need to assess the stability of the medicine for prove the its good quality to proper effectiveness and safeness.

The drug was prepared at Department of R.S.B.K., I.P.G.T.& R.A., Gujarat Ayurved University, Jamnagar, India. No any preservative was added to the test drug and preparation of drug was finished on 18th March 2019. Finished product was stored in airtight plastic containers at room temperature. Thus in the present study on attempt was taken to assess the stability of *Medhya Churna* with respect to its Microbial profile at different climatic conditions and temperature setups at different time interval for a period of 11 months.

AIM

To study the stability of *Medhya Churna* in different time interval, different climatic conditions, different temperature and different humidity set ups.

MATERIAL AND METHODS

The prepared *Medhya Churna* was stored at room temperature and finished product was studied to check microbial contamination at different intervals for a period of 11 months up to drug used. Microbiological study has been carried out at Microbiology Laboratory, I. P. G. T. & R. A., Jamnagar, India. Mainly two 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 21st day of preparation, before giving drug to the patients. Then sample from all the bags was subjected to the microbiological study regularly with random intervals during different seasons.

Materials of drug

Majority ingredients was obtained from Pharmacy of Gujarat Ayurved University, Jamnagar, India and only *Shankahpushpi* was found from local market at Jamnagar.

Date of Drug Preparation

18th March 2019

Storage

Finished product of *Medhya Churna* was stored in air-tight 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 which are 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.

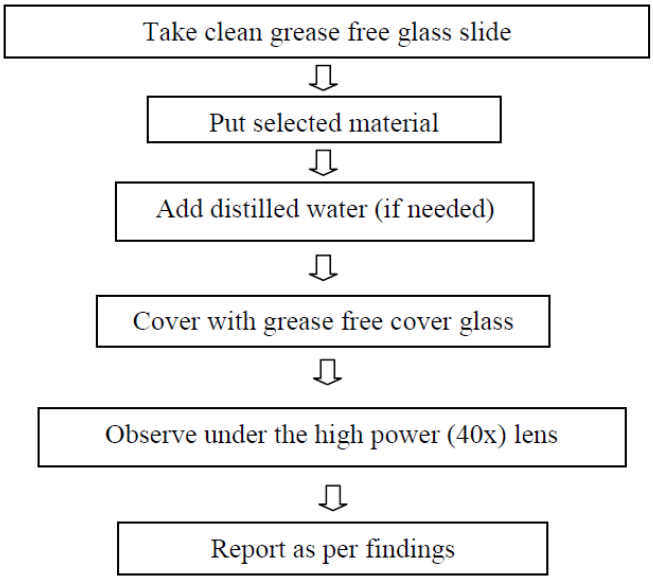
1. Smear Examination

A. Wet mount /10% K.O.H. Preparation

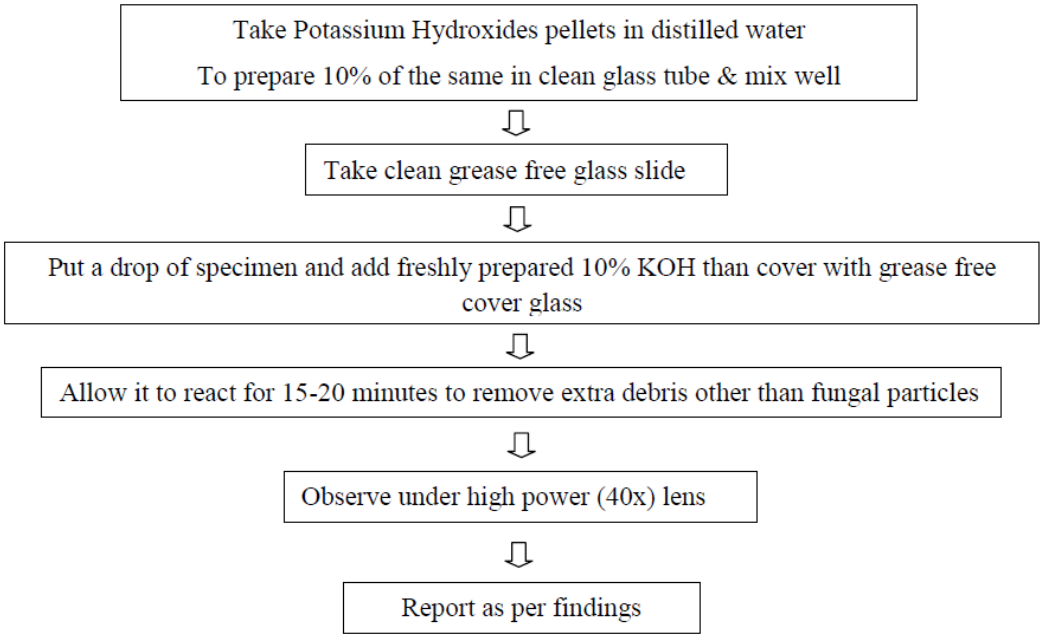
Aim: To rule out any mycological findings.

Specimen: *Medhya Churna*

Procedure for Wet Preparation



Procedure for 10% KOH Preparation



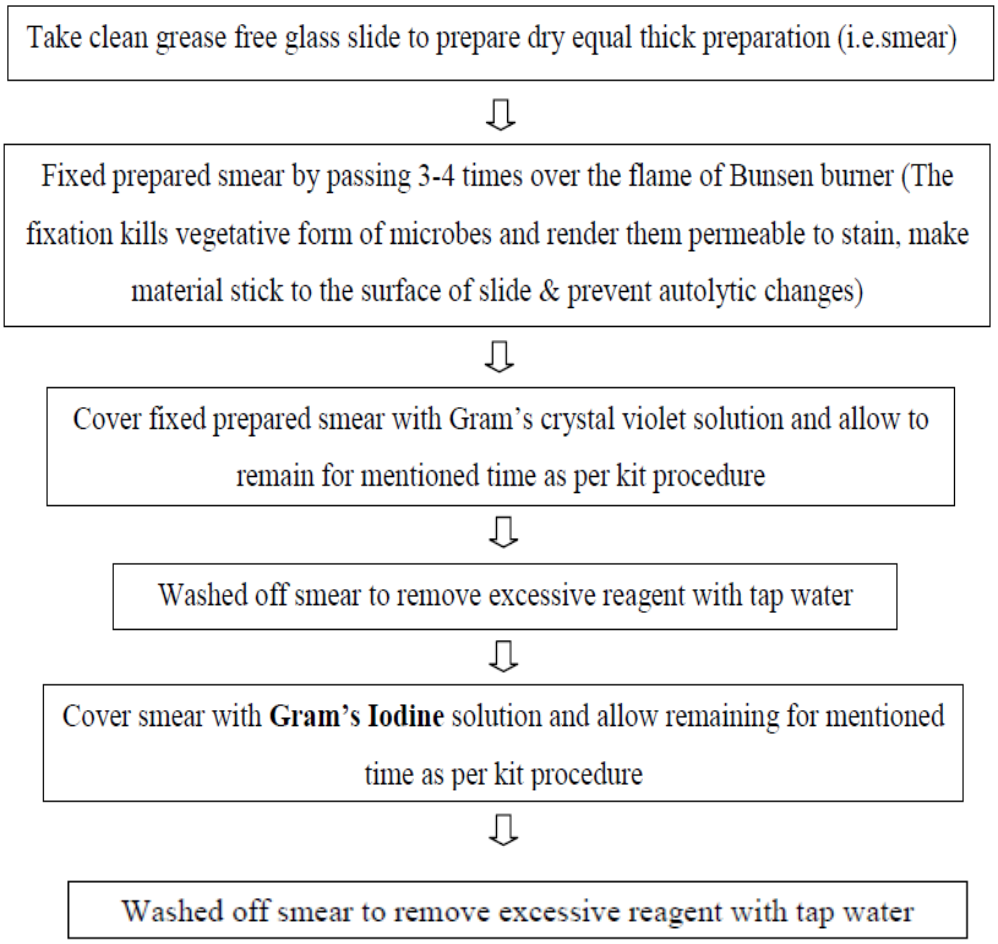
B. Gram's stain test

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: *Medhya Churna*

Procedure for Gram's Stain



⇩

Decolourize 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 colour of primary dye (i.e. Gram's Crystal Violet) or as per kit procedure

⇩

Washed off smear to remove excess acetone with tap water

⇩

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

⇩

Blot and allow to dry smear

⇩

Examine under oil immersion lens and report as per findings

Figure 1 & 2 Smear staining Procedure.

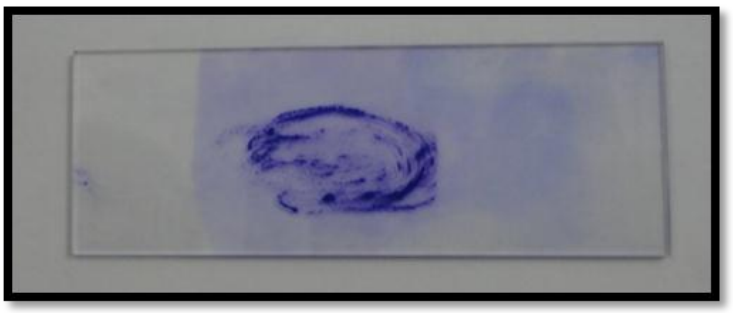


Figure 3: Stained smear ready for examination.

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.

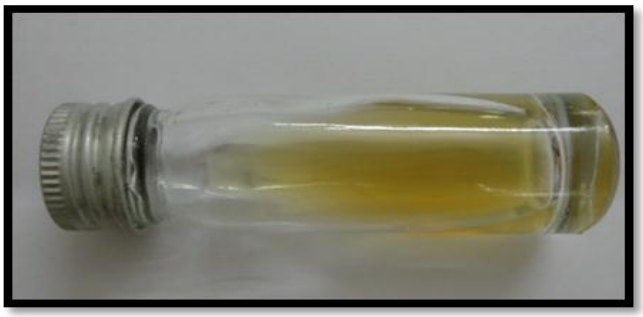


Figure 4: **Sabouraud Dextrose Agar Base (SDA) bottle.**

Procedure For Fungal CultureAerobic culture method

In the clinical microbiology laboratory culture method are employed for isolation of organisms (The lawn / 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 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 aliow it cool than loop is charged with selected specimen to be cultured. One loopful 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 arial growth and confirm growth by performing different related biochemical reactions and different related staining procedures. After that report isolates.

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 : 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.

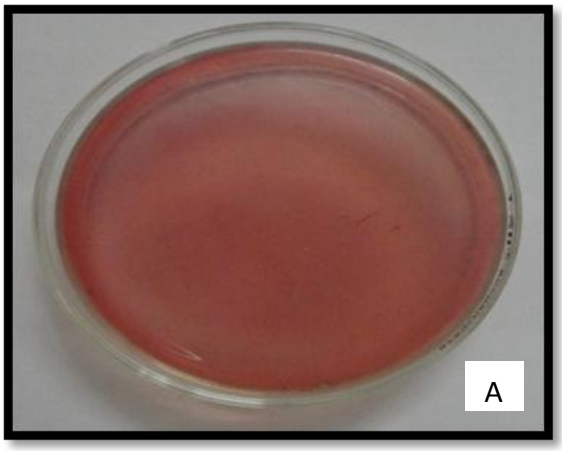


Figure 5A: Aerobic culture media (MA)

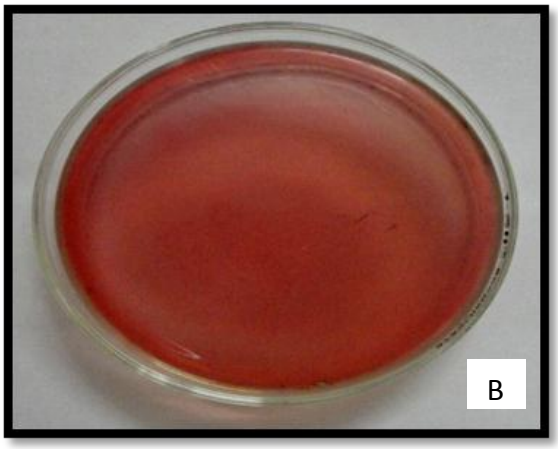
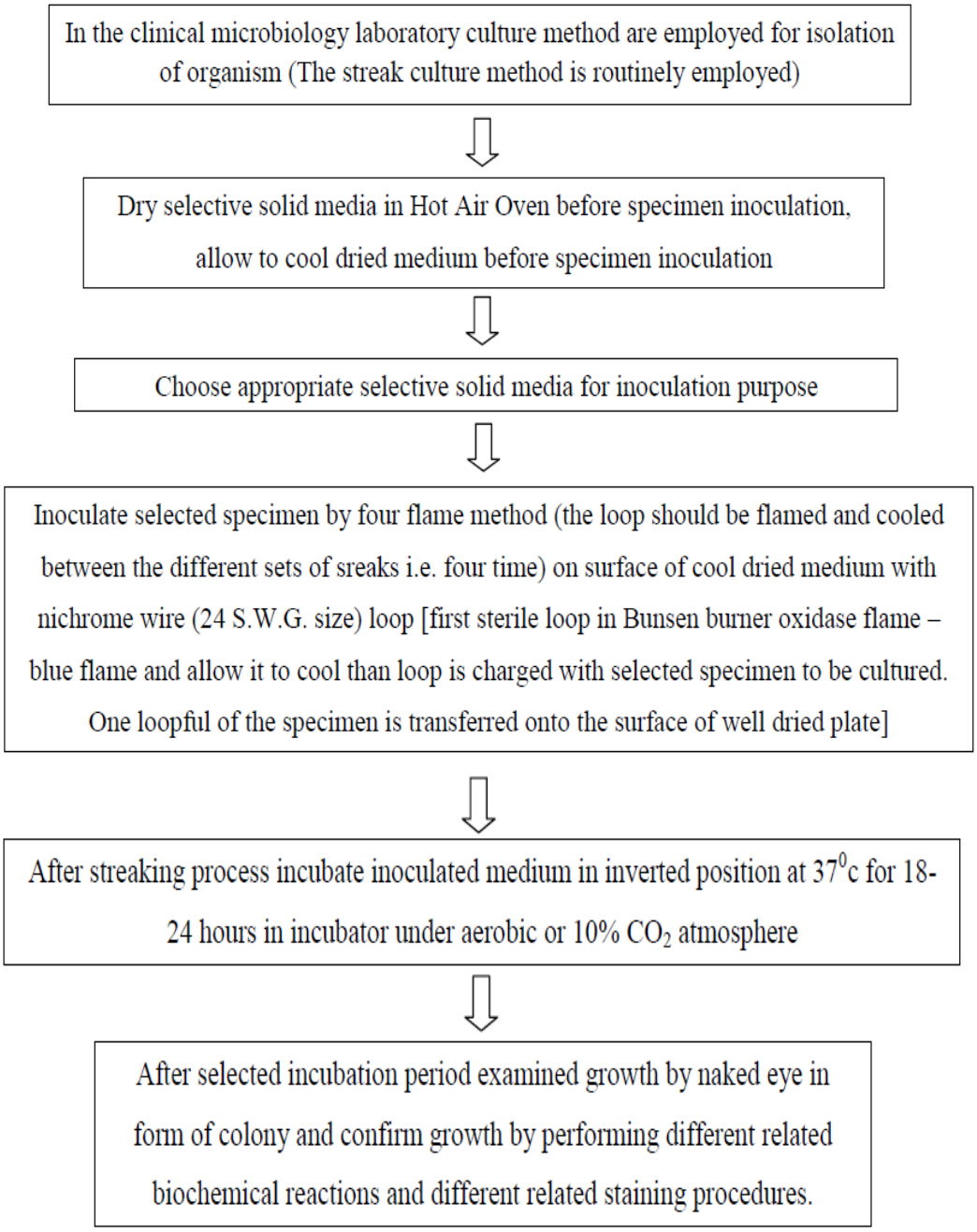


Figure 5 B: Aerobic culture media (BA)

Procedure for Aerobic Culture



OBSERVATIONS AND RESULTS

Every time samples were subjected to the microbiological study to rule out stability of prepared drug up to consumption of the same. Results are shown in Table no:1.

Table No.1: Observations of *Medhya Churna.*

Preserved at room temperature - Drug preparation date - 18/03/2019

Sr. No.	Microbiological tested date	Days of investigations After preparation of the sample	Temp. & Humidity	Observations of sample			
				Gram's Stain	Aerobic culture	Wet mount/ 10% KOH Preparation	Fungal culture
1.	25/03/2019	21 Days	26˚ C 56%	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen	No Fungal Pathogen Isolated
2.	09/05/2019	51 Days	27˚ C 74%	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen	No Fungal Pathogen Isolated
3.	10/06/2019	82 Days	37˚ C 54%	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen	No Fungal Pathogen Isolated
4.	10/07/2019	121 Days	28˚ C 82%	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen	No Fungal Pathogen Isolated
5.	07/08/2019	139 Days	27˚ C 90%	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
6.	09/09/2019	171 Days	26˚ C 96%	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
7.	11/10/2019	203 Days	27˚ C 81%	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
8.	19/11/2019	271 Days	21˚ C 75%	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
9.	17/12/2019	269 Days	29˚ C 47%	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
10.	09/01/2020	291 Days	13˚ C 72%	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
11.	12/02/2020	324 Days	28˚ C 55%	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated

DISCUSSION

For better safety and efficacy, drug should be free from any type of microbial contamination. Stability of drug is expressed in term of its Shelf life. The factors affecting stability of prepared drug are categorized under intrinsic and extrinsic factor. 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. Microbial contamination should have avoided to increase drug stability and storage time.[5]

Medhya Churna was prepared and stored at room temperature. Sample was selected randomly for study of microbiological contamination. Changes in temperature and humidity of environment was observed during the study period.

During this study minimum room temperature was reported 13^0C in month of January and the maximum room temperature was reported 37^0C in month of March (Table No **:**1). Optimum temperature for microbial growth and multiplication is variable even though in this study of *Medhya Churna* there was not found any microbiological growth between 13^0C - 37^0C temperature range.

In this study highest Humidity was observed 96% in month of September while lowest humidity was observed 47% in month of December (Table No**:** 1). High Humidity may allow the growth of microbes.[6] The Humidity percentage was variable during the whole study period. Wet mount, fungal culture, gram stain and aerobic culture tests were used to study fungal and bacterial contamination in the sample of different intervals from 25th March 2019 to 12th Feb 2020. During this study period of *Medhya Churna* humidity percentage were changes between 47% - 96% but within this range of humidity there were not isolated any microbial growth and fungal growth (Table No**:** 1).

CONCLUSION

The period between production time to consume time of the drug is called as Shelf- life. It ranges from organoleptic qualities to microbiological safety. During this 11 months of shelf life period temperature between 13^0C -37^0C range and Humidity between 47% -96% range there were not found growth of any kind of bacterial or fungal microorganisms in Microbiological study of the *Medhya Churna*. It proved that the quality of the *Medhya Churna* was in the standard condition to better efficacy and safety of the medicine.

REFERENCES

1. https://www.webmd.com/add-adhd/childhood-adhd/qa/what-are-the-two-most-common-neurodevelopmental-disorders-in-kids -Retrieved on dated- 15/06/2020
2. APA, Diagnostic and Statistical Manual of Mental Disorders, 5th Ed., Washington, DC, 2013; 61.

3. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3890923/- Retrieved on 15/06/2020
4. Alfred E Brown, Benson: Microbiological Application, 8th Edition, the McGraw – Hill Companies, 2001; 64.
5. https://www.who.int/traditional-complementary-integrative-medicine/publications/ Retrieved on 15/06/2020
6. Bruce J., Drysdale E.M. Trans shell transmission. Microbiology of Avian egg. Chaman and Hall, London, 1994; 63-91.