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Physico-Chemical Analysis of Shodhit Manashila And To Assess It's Biological Activity on Breast Cancer Cell Lines

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Abstract-

Manashila (realgar/arsenic disulphide) has been used in Ayurvedic medicine since ancient times for the treatment of conditions such as skin diseases, cough, asthma, certain eye disease and psychological disorder etc. Shodhan (purification) is an integral part of Ayurvedic processing especially for poisonous substance before they can be used for therapeutic purpose. In the case of Manashila, which contains the heavy metal arsenic, it is advised that it should be purified optionally by carrying out seven levigations (Bhavana) of Zingiber officinalis Roscoe (Ardraka) juice. A shodhan of Manashila was carried out and both Ashodhita Manashila (AM-unpurified realgar) and Ardrakashodhit Manashila (ASM-realgar purified with ginger juice) were investigated by examination of the relevant physico-chemical parameters, quantitative elemental analysis, including the percentage of arsenic using atomic absorption spectrometry, particle size analysis. A significant change was found in the particle size distribution where 50% of the AM particle were found to be ASM remained as arsenic disulphide.

Key Words : Manashila, arsenic, shodhana, Ayurveda, XRD, laser diffraction.

Introduction :

Manashila is one of the three important arsenical compounds used in Ayurveda as a therapeutic agent (the other two being Haratala-arsenic trisulphide, and gouripashana-arsenic trioxide) in a number of Ayurvedic medicinal formulations including those found in Brihatrayis (Three important ancient classics of Ayurveda viz. Carakasamhita, Sushruta Samhita and Astanga Sangraha/ Astanga Hrudaya). The important therapeutic areas of Manashila use include skin diseases, respiratory diseases, certain ailments of eyes and psychological disorders. It is also used as an ingredients of various processing techniques involved in Rasashastra (a wing of Ayurveda dealing mainly with mercurial, metallic and mineral compounds along with herbal drugs) like shodhana (purification).

Now a day's much number of deaths in women due to breast and cervical cancer. So the present study was undertaken.

Aim And Objectives

Aim :

To prepare and carry out the physico-chemical analysis of Shodhitmanashila and to assess its biological activity on breast cancer cell lines.

Objectives :-

- To study the Literature related to
 - i. ShodhitManashila
 - ii. Breast Cancer Cell lines
- To purify Manashila as per Rasendrachudamani and Rasaratnasamucchya.
- To prepare Shodhit Manashila as per Rasendrachudamani and Rasaratnasamucchya.
- To analyze and provide the physico-chemical standards for ShodhitManashila.
- To study the Biological Activity of Shodhit Manashila on breast cancer cell lines.

VI) Materials And Methods:-**1. Materials:-****A. Review of Literature (Conceptual Study):-**

- Various references regarding ShodhitManashila will be collected from different texts.
- Data regarding cell line experimental study will be thoroughly reviewed.
 1. For the present study references will be taken from:-
 - Hindi Vyakhyakar Acharya Sidhinadanan Mishra :Rasaratnasamucchaya, Sidhiprada Hindiviyakhya Sahit. Chaukhambha Orientalia, Varanasi : 1st Edition 2011 : Page No.77-78.
 - Hindi Vyakhyakar Acharya Sidhinadanan Mishra :Rasendrachudamani, Sidhiprada Hindiviyakhya Sahit. Chaukhambha Orientalia, Varanasi : Reprint Edition 2009 : Page No.181.

● Raw Materials –**● Dravya (Raw Medicinal Ingredients):-****1) Manashila:-**

- Chemical Name: Arsenic Disulphid
- Chemical Formula : AS_2S_2

2) Material for Shodhana (purification):-

- Ardrak Swarasa (Rasendrachudamani)
- Bhrungaraj Swarasa (Rasaratnasamucchaya)
- Cow's Milk (Rasaratnasamucchaya)

B. Yantra's (Instruments) :-

- 1) Khalva
- 2) Thin white Cloth
- 3) Glass container
- 4) DolaYantra

C. Material for Cell Line Study :

- 1) ShodhitManashila
- 2) Breast Cancer Cell Line

2. Methods**I) Pharmaceutical Preparation:-**

- Hindi Vyakhyakar Acharya Sidhinadanan Mishra :Rasendrachudamani, Sidhiprada Hindiviyakhya Sahit. Chaukhambha Orientalia, Varanasi : Reprint Edition 2009 : Page No.181.
- Hindi Vyakhyakar Acharya Sidhinadanan Mishra :Rasaratnasamucchaya, Sidhiprada Hindiviyakhya Sahit. Chaukhambha Orientalia, Varanasi : 1st Edition 2011 : Page No.77-78.

A. Shodhana Procedure:-

- a. Raw material will be identified and examined physico-chemically.
- b. The Shodhana procedure of Manashila will be carried out as- Manashila will be taken in KhalvaYantra and pound it well. Then Bhavana of ArdrakaSwarasa for 7 times will be given to it.

- c. The Shodhana procedure of Manashila will be carried out as- Manashila will be taken in Khalva Yantra and pound it well. Then Bhavana of Bhrungaraj Swarasa for 7 times will be given to it.
- d. Manashila is purified by Swedana with help of Dola Yantra in goat urine up to 1 Prahara.
- e. Shodhana of Ashudha Manashila will be done.
- f. According to other Acharya's I can do Shodhan Procedure with the help of other drugs if required.

II) Method cell lining study :

In vitro testing of compounds on Breast cancer cell lines:-

In vitro testing of compounds would be performed by Sulforhodamine Assay (SRB Assay) as per NCI guidelines. SRB Assay is a rapid, sensitive, and inexpensive method for measuring the cellular protein content of adherent and suspension cultures in 96-well micro-titer plates. Breast Cancer cell line would be maintained in standard culture media containing growth factors. For cytotoxicity assay, breast cancer cells would be dispensed in appropriate plastic plates and allowed to adhere for 24 hours. After 24 hours, compounds would be added to cells at 4 different concentrations (10, 20, 40 and 80 µg/ml). Cells without drug and cells in presence of positive control Adriamycin would serve as negative and positive control, respectively.

After compound addition, plates would be incubated at standard conditions for 48 hours and assay would be terminated by the addition of cold Trichloroacetic acid. Cells fixed are then stained with SRB stain. Cell protein-bound stain is then extracted and read calorimetrically. The absorbance is read on a plate reader at a wavelength of 540 nm with 690 nm reference wavelength.

Percent growth would be calculated for test wells relative to control wells. Data would be represented as Growth Inhibition 50 concentration of the compounds. Compounds exhibiting GI50 of <10 µg/ml would be considered as demonstrating activity against cancer cells in the assay system used.

5. Analytical Methods (Tests) :-

1. Organoleptic Test -

- a. Colour
- b. Odour
- c. Touch
- d. Taste
- e. Appearance

2. Physico-Chemical Analysis -

- a. Moisture Content
- b. Ash Value – i) Acid Insoluble Ash ii) Water Soluble Ash
- c. Solubility Test
- d. P^H test
- e. Particle Size Consistency
- f.

Elemental Analysis by Atomic Absorption Spectrophotometry (AAS).

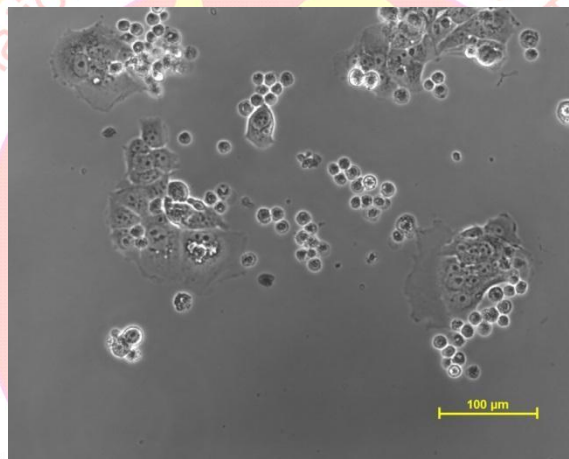
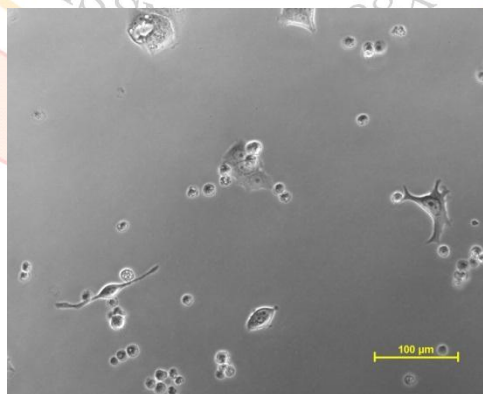
3. Special tests regarding this study will be done accordingly and as needed.

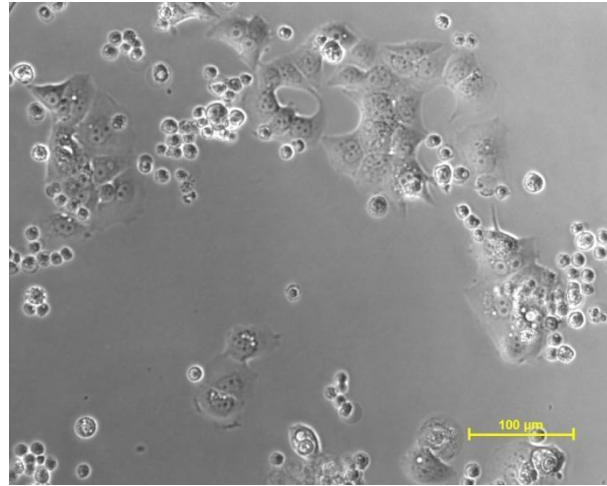
Result :

1. There was no growth change in Breast Cancer cell lines when ShodhitManashila was used.
2. The Sample ShodhitManashila(AS2S2) does not show specific Inhibition on human breast cancer cell.
3. In cell line MCF -7 its action is not prominent and not sustained at the end than with only ADR.
4. These are an in vitro study which shows that ShodhitManashila has no prominent anti -cancer effect.
5. The addition of ShodhitManashila will reduce the dose of anti cancer drug and also the unwanted side effects.
6. The ShodhitManashila in small quantity will make big difference in cost and in adverse effect restriction.

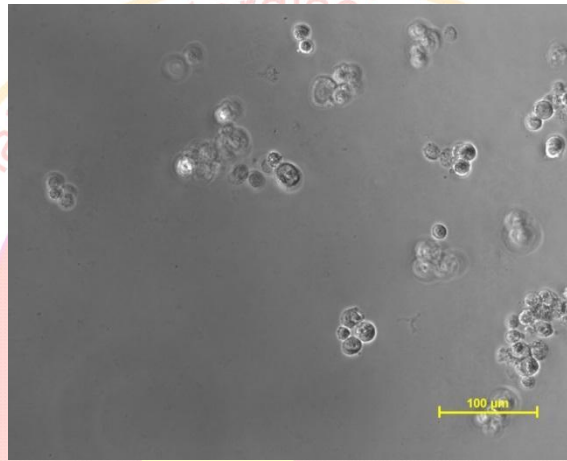
This is a half way result. These results are in vitro, outside our body. The Rasa-kalpa mainly Minerals acts differently in our body. The aim of the shodhan itself is for better absorption, better efficacy of drug in small dosage form -

So the action of ShodhitManashila in our body with Agnisanskar is definitely more precise and highly effective.

**MBCa****MCF-7 Control**



MCF-7 Control-1



MCF-7 Positive Control

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