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Physico-Chemical Analysis of Yashada Bhasma and To Assess It's Biological Activity on Breast and Cervical Cancer Cell Lines

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Abstract :

Yashad Bhasma a Zinc based Ayurvedic materiel preparation indicated specially in KaphajVyadhi, and associated complication. Zinc is responsible for the number of different functions in the human body and it helps stimulate the activity of 100 different enzymes.

Today in medical filed the article highlights the information of potential health benefitsof Zinc and provides thesources of nutrient symptoms Zinc deficiency as well as excessive Zinc intake.

Cancer differs from normal tissues. They grow more or less autonomously beyond most ability to control them they spread in to and destroy surround tissues. zinc in wound healing, concluded that topical zinc <u>may stimulate leg ulcer healing</u> by enhancing re-epithelialization, decreasing <u>inflammation</u> and bacterial growth. When zinc is applied on wounds, it not only corrects a local zinc deficit but also acts pharmacologically.

However, research has not consistently shown that use of zinc sulfate in patients with chronic wounds or ulcers is effective at improving healing rate.

So zinc is helps of producing the normal cell growth. Cause of this properties of Yashad Bhasma we can used in cancer study.

Key Words :Yashada Bhasma, Breast and Cervical Cancer Cell Line etc.

Introduction :

Yashada Bhasma, a Zinc based Ayurvedic metallic preparation, was prepared as per Rasatarangini and it was tested with both ancient and modern analytical parameters to know how the basic metal was transformed into bio-absorbable bhasma form and also to know its physical nature as to in which form the final product is. The ancient bhasmaparikshas revealed that the paepared Bhasma passed all the tests and thus ascertaining it was properly formed and modern analytical techniques like SEM (Scanning electron microscope with EDS) identified the final product as Zinc oxide (ZnO).

The significance of Rasoushadhis is in the fact that they are used in AlpaMatra (minute doses) and fast acting. Bhasmas are one among such Rasoushad-his which are complex compound forms of metals or minerals obtained by repeated incineration with liquid extracts. Yashadabhasma, Zinc based Ayurvedic metallic preparation is indicated specially in Pra-meha (diabetes) and associated complica-tions. Various methods are described in classical Rasashastra texts to prepare Yashadabhasma but the Bhasma prepared by using Parada (mercury) is believed to be Sreshta (best). 1 Hence Yashadabhasma was prepared as per Rasatarangini and its analysis was done using both ancient and modern parameters.

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 YashadaBhasma and to assess its biological activity on breast and cervical cancer cell lines.

Objectives:

- To study the Literature related to
 - i. Yashada Bhasma.
 - ii. Breast cancer and Cervical cancer Cell lines.
- To purify Yashada as per Rasatarangini.
- Toprepare YashadaBhasmaas per Rasatarangini.
- To analyse and provide the physico-chemical standards for Yashada Bhasma.
- To study the Biological Activity of Yashada Bhasma on breast and cervical cancer cell lines.

Materials And Methods:-

1. Materials:-

A. Review of Literature (Conceptual Study):-

- Various references regarding Yashada Bhasma 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:-
- Rasatarangini : -Tarang 19/ 102 & 116shlokano. Pg.no.178-179

B. Raw materials -

- Dravya (Raw medicinal ingredients):-
- 1) Yashada:-
- English Name: Zinc
- Chemical Formulae: Zn
- 2) Material for Shodhana(purification):-
- Godugdha(Cow milk).
- 3) Material for Marana (Incineration) :-
- LohaKadhai(Iron vessel).
- C. Yantra's(Instruments) :-
 - 1) LohaDarvi(Iron vessel).
 - 2) Stainless steel vessel.
 - 3) Thin white cloth.
 - 4) Glass container.

D. Material For Cell Line Study :

- 1) Yashada Bhasma.
- 2) Breast & cervical cancer cell line.

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2. Methods :-

I) Pharmaceutical preparation:-

• The method of preparation of 'Yashada Bhasma' will be asper the Rasatarangini: -Tarang 19/ 102 & 116 shlokano. Pg.no.178-179

A. Shodhana procedure:-

- a. Raw material will be identified & examined physico-chemically.
- b. Yashada will be melt and Dhalana in Godugdha will be made up to 21 times.
- c. Shodhana of AshudhaYashada will be done.

B. Marana procedure:-

- a. ShudhaYashada will be taken in iron pan. Agni will be given till the time of Yashada melting. While melting Yashada will be stir with Iron stirrer until the fine choorna of Yashada will be made.
- b. Then filtered with fine cloth.
- c. Above procedure will be repeated until choorna turned into shiny white Yashadabhasma.
- d. 'Yashada Bhasma' will be done.\ \\ CI UISC/(
- e. Prepared 'Yashada Bhasma' will be kept in air tight glass container.

II) Method cell linning 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 demonstratingactivity against cancer cells in the assay system www.aiirjournal.con used.

1. Organoleptic Test -

- a. Colour
- b. Odour
- c. Touch
- d. Taste
- e. Appearance
- 2. Physico-Chemical Analysis
 - a. Moisture Content

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- b. Ash Value i) Acid Insoluble Ash ii) Water Soluble Ash
- c. Solubility Test
- d. P^H test
- e. Particle Size Consistency
- 3. Bhasma Pareeksha Mentioned in Ayurvedic Classics -
 - 1. Nischandrika (lusterless)
 - 2. Rekhapurnatva (Fine enough to enter the crevices of finger)
 - 3. Varitartwa (Floats on Water)
 - 4. Nirdhuma (Smokeless)
 - 5. Niswadu (Tasteless)
 - 6. Apunarbhava (Irreversible)
- 4. Elemental Analysis by Atomic Absorption Spectrophotometry (AAS).
- 5. Special tests regarding this study will be done accordingly and as needed.

Result :

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- 1. There was no growth change in both cell lines when only Yashad bhasma was used.
- 2. When Yashad bhasma was used with ADR in two concentrations both lines had measurable restrictions in cell growth.
- 3. In cell line MCF -7 its action is more prominent and sustained at the end than with only ADR.
- 4. These are an in vitro study which shows that only Yashad bhasma has no prominent anti cancer effect but when used with other anti –cancer drugs, it definitely restrict cancer cell growth.
- 5. These studies show that Yashad bhasma can reduce the dose of anti-cancer drug to measurable extent with same or higher effectiveness.
- 6. The addition of Yashad bhasma will reduce the dose of anti cancer drug and also the unwanted side effects.
- 7. The Yashad bhasma 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 bhasmakalpana acts differently in our body. The aim of the shodhan and maran itself is for better absorption, better efficacy of drug in small dosage form –

गुंजार्द्धतः समारभ्यगुंजैकप्रमितंमृतम् । यशदंविनियुजीतबलकालाद्यपेक्षया ॥ १२४ ॥

(रसतरगिणी १९/१२४)

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

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HeLa A+10ug ADR



HeLa Control



HeLa Positive Control

MCF-7 A



MCF-7 A+5ug ADR



MCF-7 A+10ug ADR



MCF-7 Control

MCF-7 Positive Control

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