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PHARMACEUTICO-ANALYTICAL STUDY OF RASAKARPURA W.R.T. RASA TARANGINI AND ITS IN VITRO CYTOTOXIC ACTIVITY ON A431 SKIN CANCER CELL LINE

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ABSTRACT

Skin cancer, particularly squamous cell carcinoma (SCC), remains a significant health concern worldwide. Despite advances in surgical and pharmacological interventions, recurrence and treatment-related complications continue to challenge clinicians. Ayurveda describes various skin disorders under the broad category of Kushtha Roga and recommends several herbo-mineral formulations for their management. Rasakarpura, a classical Kupipakwa Rasayana formulation described in Rasa Tarangini, has traditionally been used for skin diseases, wounds, and blood disorders. The present study aimed to prepare Rasakarpura according to the classical method, evaluate its physicochemical properties, and investigate its in vitro cytotoxic activity against the A431 human squamous cell carcinoma cell line. The formulation was prepared using purified mercury, sulphuric acid, and rock salt through the Kupipakwa process. Analytical evaluation was conducted to assess quality and composition, followed by cytotoxicity assessment using the A431 cell line. The study provides preliminary scientific evidence regarding the pharmaceutical standardization and potential anticancer activity of Rasakarpura, highlighting the need for further preclinical and clinical investigations.

KEYWORDS: Rasakarpura, Rasa Tarangini, Kupipakwa Rasayana, Ayurveda, Skin Cancer, Squamous Cell Carcinoma, A431 Cell Line, Cytotoxicity.

INTRODUCTION

Ayurveda, the traditional system of Indian medicine, emphasizes maintaining equilibrium among the three doshas—Vata, Pitta, and Kapha—to preserve health and prevent disease. Rasashastra, an important branch of Ayurveda, deals with the therapeutic application of metals, minerals, and herbo-mineral formulations. Among its numerous preparations, Kupipakwa Rasayanas are regarded as highly potent due to their enhanced bioavailability, low dosage requirements, long shelf life, and rapid therapeutic action.

Rasakarpura is a classical mercurial formulation categorized under Nirgandha Sa-Agni Kupipakwa Rasayana. It is described in Rasa Tarangini and other Ayurvedic texts for the treatment of skin disorders (Twakroga), chronic wounds, syphilis, diarrhea, blood disorders, and related conditions. Traditionally, it is prepared through controlled heating of purified mercury in the presence of sulphuric acid and rock salt within a specially designed glass apparatus.

Skin cancer is one of the most common malignancies worldwide. Squamous cell carcinoma (SCC), a major form of non-melanoma skin cancer, is particularly significant because of its invasive nature and potential for metastasis. Although surgical excision, radiotherapy, chemotherapy, and immunotherapy remain the standard treatment modalities, recurrence and adverse effects continue to necessitate the search for alternative therapeutic approaches.

The A431 human epidermoid carcinoma cell line is widely employed in oncology research due to its overexpression of epidermal growth factor receptors (EGFR), making it an ideal in vitro model for evaluating anticancer potential. The present study was undertaken to explore the pharmaceutical standardization and cytotoxic activity of Rasakarpura against A431 skin cancer cells.

AIMS AND OBJECTIVES

Aim

To prepare and analyze *Rasakarpura* and to study its cytotoxic activity on A431 skin cancer cell line (in-vitro).

Objectives

1. To prepare *Rasakarpura* as mentioned in *Rasatarangini*.

Secondary Objectives

To identify and authenticate raw materials.

To analyze *Rasakarpura* physico-chemically.

To evaluate the cytotoxic action of *Rasakarpura* on A431 skin cancer cell line.

MATERIAL AND METHODS

The entire study is divided into three stages

- Pharmaceutical work i.e. Preparation of *Rasakarpura*
- Analytical study
- Experimental study i.e. In vitro cell line study

1. Pharmaceutical Preparation of Rasakarpura

(a) Collection and Authentication of Raw Materials

The raw materials used in the study, namely Parada (Mercury), Gandhakamla (concentrated H_2SO_4), Saindhava Lavana (Rock Salt), and Rasona (Garlic), were procured from authenticated Ayurvedic raw drug suppliers. All ingredients were examined for quality and authenticated in a recognized analytical laboratory before their use.

(b) Shodhana of Parada (Purification of Mercury)

Purification of mercury was carried out according to the classical reference of *P. Samhita (30/85)*. Five hundred grams of crude Parada was triturated with fresh garlic paste in a *Khalva Yantra* continuously for seven days. During the process, the mercury gradually lost its globular nature and blended uniformly with the garlic paste, changing in colour from light grey to dark grey and finally black. After completion, the mixture was washed thoroughly with lukewarm water to recover purified mercury, which appeared bright and lustrous. A final yield of 470 g of purified mercury was obtained. Continuous trituration, careful washing, and the use of protective equipment were maintained throughout the procedure.

(c) Preparation of Parada Churna (Mercuric Sulphate)

Parada Churna was prepared following the method described in *Rasa Tarangini (6/65–67)*. Purified mercury and concentrated sulphuric acid were mixed in the proportion of 1:1.5 and heated gradually in a glass vessel placed in a *Valuka Yantra*. Initial heating produced brown coloration, white fumes, and foaming, followed by evaporation of the acid. Continued heating resulted in the formation of a stable white mercuric sulphate powder. The procedure was

carried out in a well-ventilated area using only glass apparatus and appropriate safety measures.

(d) Mixing with Saindhava Lavana

The prepared mercuric sulphate powder was triturated with an equal quantity of finely powdered Saindhava Lavana for 30 minutes, as described in *Rasa Tarangini (6/68)*. The process yielded a homogeneous white powder with a characteristic pungent odour. Proper care was taken to prevent material loss and avoid inhalation of dust.

(e) Preparation of Rasakarpura (Kupipakwa Process)

Rasakarpura was prepared according to the *Kupipakwa Rasayana* method described in *Rasa Tarangini (6/68–71)*. The mixture of Parada Churna and Saindhava Lavana was filled into a glass Kupi coated with seven layers of Multani mitti-smeared cloth and placed in a *Valuka Yantra*. Controlled heating was applied in stages, beginning with *Mandagni* followed by *Madhyamagni*. White crystalline deposits started appearing in the neck of the bottle at around 260°C, and complete neck blockage occurred by the sixth hour. Heating was continued until completion, after which the apparatus was allowed to cool naturally. On the following day, the bottle was carefully broken, and Rasakarpura crystals were collected from the neck and body of the Kupi. Throughout the process, standard precautions were followed to ensure safety and prevent contamination.

2. Analytical Study

The prepared Rasakarpura was evaluated for its organoleptic characteristics, including appearance, colour, odour, taste, and texture. Physicochemical analysis was performed to determine parameters such as moisture content, total ash, acid-insoluble ash, water-soluble extractive, alcohol-soluble extractive, pH, specific gravity, and volatile matter using standard analytical methods. In addition, advanced analytical studies were carried out using X-ray fluorescence (XRF) for elemental composition, X-ray diffraction (XRD) for crystalline phase identification, and particle size analysis to assess particle size distribution and polydispersity index.

3. Experimental Study i.e. In vitro cell line study

The in vitro cytotoxic activity of Rasakarpura was assessed against the A431 skin cancer cell line using the MTT assay. Cells were exposed to varying concentrations of Rasakarpura (50, 25, 12.5, 6.25, and 3.12 µg/mL) and incubated for 48 hours at 37°C in a humidified atmosphere containing 5% CO_2 . Following incubation, MTT reagent was added, and the resulting formazan crystals were dissolved in DMSO. The absorbance was measured using a microplate reader, and the percentage of cell viability and IC_{50} value were calculated. All experiments were performed in triplicate to ensure reproducibility.

RESULTS

The observations and results obtained from the present study, which include the pharmaceutical study of Rasakarpura, the Analytical study, and the Experimental (in vitro) study of the cytotoxic activity of Rasakarpura on A431 skin cancer cell line of Rasakarpura, are presented below under the following headings:

- Pharmaceutical study result
- Analytical study result
- Experimental study result

1. Pharmaceutical study result

- The pharmaceutical preparation of Rasakarpura was carried out successfully through sequential stages, and the observations confirmed the proper progression of each process.
- During Parada Shodhana, mercury gradually lost its globular nature, mixed uniformly with garlic paste, and after purification appeared bright and lustrous.
- In the preparation of Parada Churna, gradual heating of mercury with concentrated sulphuric acid produced transient brown coloration, white fumes, foaming, and finally a stable white powder, indicating complete conversion.
- The Matkapad (Kacha Kupi covering) was prepared uniformly by applying and drying successive layers of Multani mitti-coated cloth, resulting in a crack-free and stable coating suitable for Kupipakwa processing.
- During the mixing of Parada Churna and Saindhava Lavana, a pungent odour and fumes were observed, while the pinkish rock salt gradually transformed into a homogeneous fine white powder.
- In the Kupipakwa process using Valuka Yantra, the appearance of white fumes, crystalline deposits in the Kupi neck, bluish-white sublimation, and positive Siddhi Lakshana confirmed successful pharmaceutical transformation.

- After self-cooling and careful collection, the final product was obtained as white, needle-shaped crystals with a smooth texture and satisfactory yield (35%), demonstrating proper sublimation and successful preparation of Rasakarpura.

Table: Temperature profile recorded during the preparation of Rasakarpura in *Valuka Yantra*.

2. Analytical Study Result

- The prepared Rasakarpura was subjected to organoleptic, physicochemical, and advanced analytical evaluation at an accredited laboratory.
- Organoleptic examination revealed that the formulation was a white, crystalline compound with a characteristic odour and a slightly bitter but pleasant taste.
- Physicochemical analysis showed a pH of 7.1, total ash of 29.04%, acid-insoluble ash of 16.23%, loss on drying of 0.19%, specific gravity of 0.991 g/ml, and mercury content of 71.04%, indicating good quality and stability.
- Particle size analysis demonstrated a relatively uniform crystalline structure with an average particle size of 1197.9 nm (≈1.2 µm) and a polydispersity index of 0.830, suggesting effective pharmaceutical processing and favourable bioavailability.
- XRF analysis confirmed the presence of Mercury (78.9%) and Chlorine (21.0%), identifying the formulation as Mercury Chloride ($HgCl_2$) with only a negligible trace of arsenic (0.0558%). Furthermore,
- XRD analysis established 100% phase purity of $HgCl_2$, confirming the crystalline nature and successful pharmaceutical transformation of the raw materials into Rasakarpura.

3. Experimental Study Result

Cytotoxic Activity of Rasakarpura on A431 Cell Line.

The test formulation was evaluated for cytotoxicity against A431 human epidermoid carcinoma cell line.

- Different concentrations (50, 25, 12.5, 6.25, and 3.12 µg/mL) were tested.
- Cell viability decreased with increasing concentration, indicating a dose dependent cytotoxic effect.
- At the highest concentrations (50 µg/mL and 25 µg/mL), cell viability was reduced to ~2–10%.
- At lower concentrations (6.25 µg/mL and 3.12 µg/mL), cell viability remained relatively higher (~54–90%).

- The calculated IC_{50} value was 7.809 µg/mL, confirming significant cytotoxic potential of the test formulation.

DISCUSSION

The successful preparation of Rasakarpura according to the method described in *Rasa Tarangini* highlights the significance of classical Ayurvedic pharmaceutical techniques in producing a standardized formulation. The sequential *Shodhana* of Parada with Rasona facilitated purification and detoxification, while the *Kupipakwa* process in *Valuka Yantra* enabled proper sublimation and crystallization. Organoleptic evaluation showed that the final product possessed the classical characteristics of a white, crystalline compound with a characteristic odour.

Physicochemical analysis further confirmed the quality and stability of the formulation, with a near-neutral pH (7.1), total ash of 29.04%, acid-insoluble ash of 16.23%, and low moisture content (0.19%). Particle size analysis demonstrated a relatively uniform crystalline structure with an average particle size of approximately 1.2 µm, which may enhance bioavailability. XRF analysis identified the formulation as Mercury Chloride ($HgCl_2$), containing 78.9% mercury and 21% chlorine with only trace amounts of arsenic (0.0558%), while XRD confirmed 100% phase purity of $HgCl_2$. These findings provide modern scientific validation for the classical preparation of Rasakarpura and support its reproducibility, quality, and pharmaceutical standardization.

The cytotoxicity evaluation of Rasakarpura against the A431 human epidermoid carcinoma cell line demonstrated significant anticancer potential. The MTT assay showed a clear dose-dependent reduction in cell viability, with higher concentrations producing greater cytotoxic effects. At concentrations of 50 µg/mL and 25 µg/mL, almost complete inhibition of cancer cell growth was observed, while moderate activity was noted at 12.5 µg/mL. Lower concentrations (6.25 and 3.12 µg/mL) showed partial cell survival, indicating that a threshold concentration is required for marked therapeutic activity. Microscopic examination revealed characteristic features of cytotoxicity, including cell shrinkage, detachment, and reduced cell density. The IC_{50} value of 7.809 µg/mL further confirmed the potency of the formulation, suggesting effective anticancer activity at relatively low concentrations. Overall, the findings indicate that Rasakarpura possesses strong cytotoxic and anti-proliferative properties against A431 skin cancer cells and support its potential as a candidate for further in vivo and mechanistic studies.

CONCLUSION

The present study successfully prepared Rasakarpura according to the classical method described in *Rasa Tarangini*. The raw materials were properly identified and authenticated, and *Shodhana* of Parada was carried out as per the guidelines of *Parada Samhita*. The pharmaceutical process, involving the conversion of mercury to mercuric sulphate followed by its reaction with Saindhava Lavana and subsequent *Kupipakwa* processing in *Valuka Yantra*, facilitated the formation of Mercuric Chloride ($HgCl_2$) as white needle-like crystals in the neck of the Kupi. Strict safety measures, including the use of glassware and protective equipment, were maintained throughout the procedure. Analytical studies, including XRF and XRD, confirmed the formation of pure $HgCl_2$ with excellent pharmaceutical standardization. The in vitro cytotoxicity study demonstrated that Rasakarpura exhibited strong anticancer activity against the A431 skin cancer cell line, showing marked cytotoxicity at 50 µg/mL and 25 µg/mL, with partial cell survival at lower concentrations. Furthermore, the low IC_{50} value of 7.809 µg/mL indicates significant pharmacological potency, suggesting that Rasakarpura may have promising therapeutic potential and merits further in vivo and mechanistic investigations.

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