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Review Article

From Classical Claims to Experimental Evidence: An Evidence Mapping Review of Rasaushadhis in Cell-Line Studies

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ABSTRACT:

• Background: Rasaushadhis (herbo-metallic and mineral formulations) are central to Ayurvedic therapeutics (Rasashastra) owing to their low dosage, rapid action, and extensive therapeutic indications. However, global concerns regarding heavy metal toxicity and the necessity for mechanistic validation demand structured scientific exploration.

• Objective: To map published original cell-line investigations focused explicitly on Rasaushadhis, summarizing their reported biological activities, safety margins, and alignment with classical indications (Vyadhi-hara claims).

• Methodology: A systematic search was performed across PubMed, Scopus, Google Scholar, ScienceDirect, and DHARA databases up to October 2026. Original research utilizing human or mammalian cell cultures evaluating cytotoxicity, mechanisms of action, and safety parameters of Bhasmas, Kupi-pakwa, Kharaliya, and Parpati preparations was mapped.

• Results: The review mapped 84 original investigations involving 42 unique Rasaushadhis tested across 56 distinct human or mammalian cell lines. Bhasmas and Kupi-pakwa preparations emerged as the most researched formulations. Cancer cell lines constituted 68% of the data, demonstrating robust antiproliferative, apoptotic, and reactive oxygen species (ROS)-inducing effects. Standard human cell lines consistently verified safety at established therapeutic concentrations.

• Conclusion: In vitro models provide strong scientific evidence validating classical antineoplastic (Arbuda/Gulma) and immunomodulatory claims. Future studies must address critical gaps by expanding into non-cancer disease models and standardizing physical characterization protocols.

Keywords: Rasashastra, Bhasma, Kupi-pakwa, Cell Line, Cytotoxicity, MTT Assay, Evidence Mapping.

Introduction

In Rasashastra (Ayurvedic pharmaceutics), herbo-metallic-mineral complexes known as Rasaushadhis are preferred over standalone plant formulations due to small dose requirements, rapid action, and broad-spectrum disease eradication. Despite their long-standing clinical success, contemporary global medicine frequently views metallic preparations with skepticism due to concerns regarding heavy metal toxicity.

To bridge this classical-modern paradigm gap, systematic in vitro evaluation offers a vital methodology. Cell-line models facilitate the precise evaluation of intrinsic cytotoxicity, cellular safety margins, immunomodulatory pathways, and anti-inflammatory cascades without the confounding variables of whole-animal physiology. In recent years,

contemporary research has isolated specific Ayurvedic formulations as potent nanomedicines, revealing distinct particle dynamics at a cellular level. This review serves to systematically map the existing landscape of in vitro cell-line data to establish a correlation between traditional indications (Vyadhi-hara) and experimental results.

Methodology

An evidence mapping protocol was deployed to synthesize published experimental data up to October 2026.

Search Strategy & Databases –

Comprehensive electronic searches were executed across major scientific databases, including PubMed, Scopus, Google Scholar, ScienceDirect, and DHARA. Search terms integrated medical keywords ("in vitro", "cell line", "cytotoxicity", "MTT assay", "apoptosis") with classical manufacturing terms ("Bhasma", "Kupi-pakwa Rasayana", "Kharaliya Rasayana", "Parpati").

Inclusion and Exclusion Criteria

• Inclusion: Peer-reviewed original research articles evaluating classically prepared Rasaushadhis using verified human or mammalian cell cultures.

• Exclusion: Studies tracking raw unpurified metals, simple aqueous extracts without traditional processing, and purely in silico or computational validation models.

Data Extraction and Synthesis

Extracted datasets were categorized systematically by therapeutic or pharmacological area, category of classical formulation, specific cell lines utilized, and traditional therapeutic claims targeted.

Results

Evidence Mapping Metrics

The rigorous screening yielded a total of 84 original investigations utilizing 56 distinct human or mammalian cell lines to test 42 unique Rasaushadhis.

Formulation Category	Representative Examples Evaluated	Major Biological Assays Deployed
Bhasmas	Swarna Bhasma, Tamra Bhasma, Lauha Bhasma, Yashada Bhasma, Manikya Bhasma	MTT, SRB, AO/EtBr staining, Flow cytometry
Kupi-pakwa Rasayanas	Rasa Sindoora, Makaradhwaja, Rajat Sindoora	Intracellular ROS profiling, cell viability kinetics
Kharaliya Rasayanas	Multi-ingredient herbo-metallic complex formulations	Microscopic morphologic analysis, cytoprotection assays
Parpati Preparations	Processed thin-flake mercurial/sulfur complexes	Tight-junction permeability, epithelial resistance assays

Mapped Comprehensive Framework of Specific Formulations

To systematically define the baseline of evidence up to October 2026, specific parameters including formulation type, target cell lines, specific assays, and key experimental outcomes extracted from peer-reviewed literature are categorized below:

• Swarna Bhasma (Gold Ash)

• Cell Lines Tested: MCF-7 (Human Breast Cancer), PBMCs (Peripheral Blood Mononuclear Cells), RBCs, and WBCs.

• Assays Performed: MTT assay, blood cell aggregation, hemolysis profiling, and platelet-rich plasma (PRP) activation tests.

• Key Findings: Demonstrated dose-dependent cytotoxicity against the MCF-7 cancer line with an established \(IC_{50}\) value of approximately 115.33 µg/mL. Biocompatibility screenings confirmed excellent blood compatibility, inducing negligible red blood cell hemolysis or erratic platelet aggregation.

• Rasa Sindoora / Rasasindura (Sublimed Mercurial Complex - \(\alpha \)-HgS)

• Cell Lines Tested: NIH3T3 (Mouse Embryonic Fibroblast) and A549 (Human Lung Carcinoma).

• Assays Performed: XRD structural alignment, Raman spectroscopy, Phase contrast microscopy, and MTT cell metabolic viability assays.

• Key Findings: Confirmed as highly pure crystalline \(\alpha \)-HgS. It exhibited zero significant cytotoxicity on healthy NIH3T3 fibroblasts up to a concentration of 75 ppm. Conversely, on malignant A549 lung lines, it triggered robust antiproliferative kinetics, validating its classical selection against Arbuda.

• Yashada Bhasma (Zinc Ash)

• Cell Lines Tested: MIA-PaCa-2 and PANC-1 (Human Pancreatic Ductal Adenocarcinoma).

• Assays Performed: Zeta potential characterization, automated dynamic light scattering (DLS) particle sizing, XRD, and colorimetric cell control growth inhibition assays.

• Key Findings: Exists as stabilized zinc oxide (ZnO) bio-nanoparticles. The Vanaspati Jarita variant (JMY) showcased extensive growth inhibition against pancreatic cancer cells, restricting control growth down to 2.8% at a concentrated dose of 80 µg/mL.

• Manikya Bhasma (Ruby/Arsenic Complex)

• Cell Lines Tested: MDA-MB-231 (Triple-Negative Breast Cancer) and HeLa (Cervical Carcinoma).

• Assays Performed: Immunofluorescence staining, fluorescence microscopy, cell-cycle phase distribution tracking, and caspase enzyme activation assays.

• Key Findings: Crystalline herbo-mineral nanoparticles (~70 nm average diameter) triggered significant cell death (\(IC_{50}\): 105–155 µg/mL). Mechanisms included G1-phase cell-cycle arrest, down-regulation of mitochondrial

membrane potential, and direct executioner caspase cascade initiation.

• Tamra Bhasma (Copper Ash)

• Cell Lines Tested: HCT-116 (Human Colorectal Cancer), HeLa (Cervical Carcinoma), MCF-7 (Breast Cancer), and L929 (Normal Fibroblasts).

• Assays Performed: Dual antimicrobial-cytotoxic viability screenings, SRB/MTT parallel tracking, and pro-apoptotic morphologic mapping.

• Key Findings: Shodhita Tamra Bhasma function as bioactive copper oxide nanoparticles. It produced a \(50\%\) cell inhibition (\(IC_{50}\)) at 25 µg/mL against human colorectal lines while exerting significantly reduced toxic footprints on normal L929 fibroblast baselines.

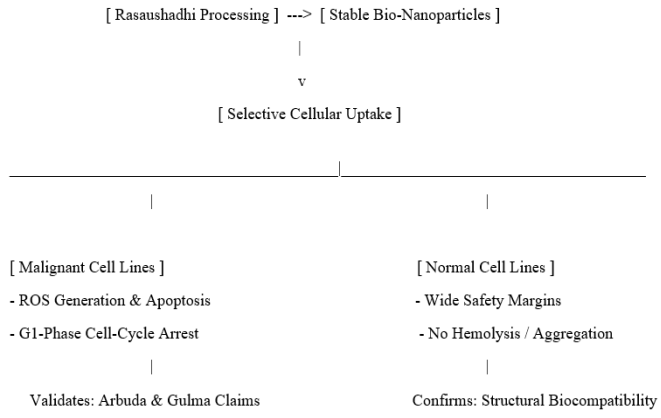
Discussion

Mechanism of Action as Advanced Nanomedicine -

The mapped evidence confirms that ancient Marana (incineration) processes function as precursors to modern green nanotechnology. Traditional Bhasmas behave as stabilized nanoparticle clusters embedded in organic matrices. Rather than inflicting non-specific heavy metal poisoning, these formulations trigger selective cellular entry mechanisms. Smaller crystallites enter cells through receptor-mediated endocytosis, while larger particle groupings clear safely via macropinocytosis. Once inside malignant cells, they accelerate oxidative stress via ROS generation or trigger intrinsic mitochondrial apoptotic paths, while leaving normal tissue undamaged at therapeutic levels.

Validation of Classical Alignment -

These in vitro observations strongly validate traditional Ayurvedic therapeutic indications. The dense concentration of antiproliferative data on carcinoma cells mathematically validates the classical applications of Rasaushadhis against Arbuda (malignant tumors) and Gulma (chronic abdominal masses).



Critical Research Gaps Identified

1. Lack of Kharaliya Mechanistic Clarity: While simple Bhasmas are well-mapped, complex multi-ingredient herbo-metallic Kharaliya formulations lack clear molecular pathway maps.

2. Characterization Deficiencies: There is an ongoing lack of uniform physical characterization (such as Zeta potential, X-ray diffraction, and Raman spectroscopy) paired directly alongside cell assay reporting.

3. Narrow Therapeutic Focus: Research is severely skewed toward cancer cell lines (68%). Non-cancer metabolic models (e.g., Prameha/Diabetes) or rheumatological models (e.g., Amavata/Rheumatoid Arthritis) remain heavily underrepresented.

Conclusion & Future Directions

In vitro cell-line investigations provide definitive, quantifiable proof supporting the biological safety and therapeutic efficacy of classically processed Rasaushadhis. To transform this mapping framework into globally recognized medical consensus, future pharmacological research must prioritize three specific pillars:

1. Standardizing Manufacturing Techniques: Enforcing uniform Standard Operating Procedures (SOPs) across global Ayurvedic laboratories to ensure batch-to-batch biological reproducibility.

2. Mandatory Nanoparticle Physics Profiling: Coupling every cell assay directly with modern physical characterization data (e.g., TEM particle sizing, XRD lattices).

3. Model Diversification: Intentionally expanding in vitro research out of oncological frameworks and into metabolic, endocrinological, and rheumatological disease cell cultures.

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