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Standardization And Safety Evaluation of Kasis and Swarnamakshik Bhasma: From Traditional Preparation to Cell Line Studies

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KEYWORDS

Ayurveda, Iron Deficiency Anemia, *Kasis*, MTT Assay. *Swarnamakshik*

ABSTRACT:

Introduction: *Kasis Bhasma* (ferrous sulfate) and *Swarnamakshik Bhasma* (copper iron sulfide) are important Ayurvedic formulations traditionally used for iron deficiency anemia (IDA). Standardization is essential to ensure safety, consistency, and global acceptance of these medicines.

Objectives: This study aimed to prepare and evaluate both Bhasmas using classical Ayurvedic methods, along with modern analytical tools, to establish their composition, nanoscale characteristics, safety, and therapeutic relevance in the management of IDA.

Methods: Formulations were prepared through *Shodhana* (purification) and *Marana* (calcination) and characterized using UV spectroscopy, ATR-FTIR, dynamic light scattering (DLS), X-ray diffraction (XRD), atomic absorption spectroscopy (AAS). MTT cytotoxicity assay was performed on Caco2- human intestinal epithelial cells.

Results: *Kasis Bhasma* showed high iron content (1,199.169 mg/kg), while *Swarnamakshik Bhasma* contained both iron and copper (52.13 mg/kg and 61.76 mg/kg, respectively). Particle size analysis confirmed nanoscale dimensions (123–199 nm; polydispersity index, PDI < 0.2), thereby enhancing bioavailability. XRD revealed crystalline structures, with toxic heavy metals below detection limits, and essential trace elements (Zn, Ca, Mg) were present. Cytotoxicity assays indicated >80% cell viability, confirming the safety of the preparations. *Kasis Bhasma* and *Swarnamakshik Bhasma* are traditionally administered orally to correct *Pandu Roga* (iron deficiency anaemia) and restore systemic vitality. Their cytocompatibility was validated on Caco-2 intestinal cells to ensure gastrointestinal safety and biological tolerance of these orally intended herbo-mineral preparations.

Conclusions: The present study has successfully helped in developing a standardized analytical profile of the *Kasis* and *Swarnamakshik Bhasma* and have also demonstrated the safety margin of these Bhasmas using cell line studies.

1. Introduction

Ayurveda employs mineral-based preparations called Bhasmas, processed through Shodhana (purification) and Marana (calcination) to ensure safety and therapeutic use. *Kasis Bhasma*, prepared from purified ferrous sulfate (green vitriol), and *Swarnamakshik Bhasma*, derived from chalcopyrite ($CuFeS_2$), are well-established Ayurvedic herbo-mineral formulations traditionally prescribed for iron deficiency anemia, hepatosplenomegaly, jaundice, chronic fever, digestive disorders, and general debility [1].

Iron is vital for haemoglobin synthesis and oxygen transport, while copper supports iron absorption and metabolism. Ayurvedic texts describe *Kasis Bhasma* as a hematinic and digestive stimulant, and *Swarnamakshik Bhasma*as rejuvenative and metabolic regulator [2]. These minerals together contribute to improving the hematological profiles and systemic availability. Although the bhasma has the potential to be used as an Active Pharmaceutical Ingredient, it lacks the validation through recent standardization techniques and safety testing. Thus, the modern scientific validation is

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essential to confirm their safety, efficacy, and elemental properties.

2. Objectives

The current study focuses on the pharmaceutical standardization and evaluation of kasis and *Swarnamakshik Bhasma* using both regular preliminary tests and modern analytical techniques. These includes UV-Visible spectroscopy, Attenuated Total Reflectance Fourier Transform Infrared spectroscopy (AT-FTIR), Particle size analysis, X-ray diffraction (XRD), atomic absorption spectroscopy (AAS), and Scanning electron microscopy – Energy dispersive X-ray spectroscopy. *In vitro* MTT assay on human intestinal epithelial (Caco2) cells to assess cytotoxicity [3]. By analysing the physicochemical characteristics and elemental composition, this study aims to bridge traditional knowledge with modern scientific validation for the effective management and use of Bhasma in treating Iron Deficiency anaemia.

3. Methods

Bhasma preparation

Selection of raw materials

The bhasmas were prepared according to the guidelines of Rasa Shastra, traditional values, and the opinions of experts [4].

Kasis: There are two variants of Kasis they are *Pushpa Kasis* and *Valuka Kasis*. *Pushpa Kasis* is a blue-green, crystalline form that is utilised for medical purposes and was thus used to prepare Bhasma [5].

Swarnamakshik: This selection was carried out in accordance with the guidelines prescribed in Rasa Tarangini, which ensures authenticity and suitability for Bhasma preparation [6].

Collection and authentication of raw materials

Both Kasis (ferrous sulphate) and Swarnamakshik (copper ferrous sulphate) were procured from the Astang Ayurveda and Unjha Pharma Nagpur, India. Other herbal components, including *Bhringaraja Swarasa* (Eclipta alba juice), *Nimbu Swarasa* (lemon juice), and materials like cow dung cakes, lime juice, and ghee, were freshly prepared and collected from standard sources [7].

Shodhana (Purification process)

Kasis Shodhana: For the Kasis shodana process, 1500 grams of raw Kasis were taken and subjected to swedana (Sudation) using Bhringaraja for 3 hours as per rasa shastra texts. The Swarasa was prepared using the swarasabhava method by boiling 8 times the water with bhringaraja powder and reducing it to one-fourth.

Swarnamakshik Shodhana: For the Swarnamakshik process, 180 grams of powdered Swarnamakshik was roasted with 200 ml of lime juice in an open pan for 48 minutes, following the method of Vagabhatacharya [7].

Sulphur Shodhana: (for Swarnamakshik): The 245g of Swarnamakshik was purified by using the cow's milk-based sublimation method. A ghee-smeared cloth containing sulphur was suspended over the cow's milk in a sealed earthen assembly. This setup was heated with cow dung cakes repeatedly for 3 times. The sublimated Swarnamakshik containing sulphur was collected and washed with warm water after each cycle [8].

Pellet Preparation

Kasis Bhasma: The 846 grams of Shuddha Kasis was titurated in khalva yantra with *Nimbu Swarasa* for 3 hours, until a thick consistency was obtained. This prepared mass was spread using a spatula into flat, round pellets of 2 cm diameter and 3 mm thickness, and then dried on plastic sheets under shade [9].

Swarnamakshik Bhasma: For pellet preparation, 200 grams of purified Swarnamakshik and Shuddha Gandhaka (Sulphur) were mixed with lime juice and titrated for 15 minutes. After water evaporation, uniform pellets of similar size were prepared and dried in the shade [9].

Marana (Incineration process)

Kasis Bhasma marana: The pellets were placed inside the clay disc assembly, sealed using mud-smeared cloth and dried. This assembly was subjected to puta using an electric muffle furnace at 750ºC for 2 hours. After 2 hours, allow the Bhasma to cool naturally. This cycle of incineration was repeated six times, with each cycle followed by grinding with *Nimbu Swarasa* and pellet reforming until the Kasis obtained [10].

Swarnamakshik Bhasma marana: The prepared *Swarnamakshik Bhasma* pellets were placed in a sealed

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earthen crucible and incinerated using a muffle furnace, replicating the varaha puta temperature curve. After each cycle, the obtained Bhasma was triturated with lime juice and resubjected to marana. This process was repeated for five cycles to obtain a pure Swarnamakshik [10].

Cow dung cake preparation: Cow dung cakes of different compositions were prepared using a combination of rice husk, cow dung, and sawdust in the ratios of 60:40, 82:18, and 76:24. The calorific values were determined using a bomb calorimeter to optimise the heat output and simulate the traditional varaha puta conditions for future standardisation references [11].

Preliminary characterization and spectrometric analysis

The preliminary physicochemical characterization of bhasmas was performed as per the guidelines of the Ayurvedic Pharmacopoeia. UV spectroscopic analysis was performed by using a UV-1900i Plus UV-Vis spectrophotometer for quantitative estimation and identifying the spectral properties of both Kasis and *Swarnamakshik Bhasma* [12]. The presence of functional groups like Hydroxyl (-OH), amine (-NH), and metal oxygen bonds in the Bhasma was analysed by pressing them against a high-refractive-index prism, diamond, using an ATR-FTIR instrument [12]. The X-ray diffraction patterns for identifying the crystalline nature of both Swarnamakshik and *Kasis Bhasma*s were performed on a Philips 1710 X-ray diffractometer with CuKα radiation operating at 30 kV and 20 mA [13].

Table 1. Summary of the findings from the preliminary characterization test

Test	***Kasis***	***Swarnamakshik***
Colour (Varna)	Reddish brown	Copperish brown
Odour	Metallic	Metallic
Floating test (Varitara)	Floats on water surface	Floats on water surface
Fineness test (Rekhapurnata)	Passed the test	Passed the test
Loss of metallic luster (Nischandratva)	No metallic luster seen	No metallic luster seen
Loss on ignition	23.121/23.1455×100 = 99.89% (positive loss on ignition)	16.1089/ 16.1216 ×100= 99.92% (Positive loss on ignition)

Bhasma particle characterization

The particle size polydispersity index (PDI) of raw kasis and swarnamakshik was analysed using a particle size analyser (Zetasizer, Anton Paar Litesizer 500) at 25 °C [14]. The nano-particle morphology and particle size of kasis and *Swarnamakshik Bhasma* samples were analysed using a ZEISS Ultra Plus Scanning Electron Microscope. A small portion of the sample was sprinkled onto a double-sided carbon tape and mounted on aluminium stubs to get an electron image for SEM.

Aflatoxin, microbial, nutritional and heavy metal testing

The detection of aflatoxins (B1, B2, G1, and G2) was evaluated using thin-layer chromatography (TLC). A mobile phase consisting of chloroform: acetone: 2-propanol in a 85:10:5 (v/v/v) ratio was employed, and the developed chromatograms under UV light at 365 nm were examined against a standard aflatoxin mixture. Microbial quality assessment was carried out following the standard procedures outlined in the WHO guidelines. The analysis included the determination of total bacterial and total fungal counts, as well as the detection of specific pathogens, namely Escherichia coli, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Salmonella spp*. Nutritional and elemental analysis of various essential minerals, such as calcium, magnesium, potassium, barium, and manganese, along with the major elements, such as iron, copper, zinc, and other trace elements present in the Bhasma, were analysed by preparing their acid digestion followed by subjecting them to an atomic absorption spectrophotometer (AAS). These tests were performed at the Standard Analytical Laboratory, Pvt. Ltd., ISO 9001–2008 certified lab located at 69, Functional Industrial Estate, Pat parganj,

Delhi, 110,092 [15].

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Table 2. Nutritional and heavy metal test on *Kasis* and *Swarnamakshik Bhasmas*

Test Parameters	Units	Results
Copper	mg/kg	61.76
Calcium	mg/kg	2222.33
Potassium	mg/kg	58.92
Phosphorous	ppm	46.77
Iron	mg/kg	1204.382
Zinc	mg/kg	136.690
Nickel	mg/kg	16.52
Manganese	mg/kg	208.41
Sodium	mg/kg	861.956
Magnesium	mg/kg	208.41
Lead	mg/kg	BLQ
Cadmium	mg/kg	BLQ
Arsenic	mg/kg	BLQ
Mercury	mg/kg	BLQ

Table 3. Aflatoxin and microbial analysis on *Kasis* and *Swarnamakshik Bhasmas*

Kasis			*FeSO4 7H2O*		
Culture Condition	**% cell viability**	**IC 50 concentration (µg)**	**Culture Condition**	**% cell viability**	**IC 50 concentration (µg)**
Untreated	100	NA	Untreated	100	NA
6.25 µg	99.28		6.25 µg	98.92	
12.5 µg	97.35		12.5 µg	98.62	
25 µg	95.82		25 µg	96.27	
50 µg	93.39		50 µg	92.37	
100 µg	84.61		100 µg	80.46	

Cell line toxicity assay (MTT Assay)

Maintenance of Cell line:

The Caco2 (Human colorectal or intestinal epithelial cell line) was purchased from NCCS, Pune, India. The cells were maintained in MEM (E) media supplemented with 2mM L- glutamine and Earle's BSS adjusted to contain 1.5g/L Sodium bicarbonate, 0.1mM NEAA and 1mM Sodium pyruvate and 20% FBS along with the 1% antibiotic-antimycotic solution in the atmosphere of 5% CO2, 18-20% O2 at 370C temperature in the CO2 incubator and sub-cultured for every 2 days. Passage number of Caco2 cells was P34 used for the present study. After reaching 80% of density, cells were detached by using 0.025% trypsin and 0.01% EDTA (in D-PBS) solution. Cell viability and count were performed using hemocytometer. The appropriate density of cells was seeded in T25 flasks and cultured until further use of the cells for cell-based assays.

Table 4. The % cell viability values of CaCo2 cells after 24 hours of *Kasis* exposure.

Test parameters	**Units**	**Results**	**Limit**	**Loq**
Aflatoxin B1	mg/kg	BLQ	NMT 0.5	0.001
Aflatoxin B2	mg/kg	BLQ	NMT 0.1	0.001
Aflatoxin G1	mg/kg	BLQ	NMT 0.5	0.001
Aflatoxin G2	mg/kg	BLQ	NMT 0.1	0.001
B1+B2+G1+G2	mg/kg	BLQ	NMT 5.0	0.001
Total bacterial count	Cfu/gm	735	NMT 10^5	-
Total fungal count	Cfu/gm	<10	NMT 10^3	-
E. Coli	Per gm	Absent	Absent	-
Salmonella	Per gm	Absent	Absent	-
P. aeruginosa	Per gm	Absent	Absent	-
S. aureus	Per gm	Absent	Absent	-

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Table 5. The % cell viability values of CaCo2 cells after 24 hours of *Swarnamakshik* exposure

Swarnamakshik			***CuSO4***		
Culture Condition	**% cell viability**	**IC 50 concentration (µg)**	**Culture Condition**	**% cell viability**	**IC 50 concentration (µg)**
Untreated	100	NA	Untreated	100	54.39
6.25 µg	91.22		6.25 µg	95.79	
12.5 µg	86.59		12.5 µg	87.56	
25 µg	85.39		25 µg	79.80	
50 µg	80.07		50 µg	53.17	
100 µg	***79.68***		***100 µg***	***7.24***	

Cell viability and proliferation studies

The cytotoxicity effect of the given molecules was analysed on Caco2 cells by performing the MTT assay. MTT is a tetrazolium salt that is converted into insoluble purple-colored formazan crystals by the action of lactate dehydrogenase enzyme released by mitochondria. Briefly, cells in 200 µL of suitable media and at a density of 15,000 were plated in a 96-well plate and incubated at 37°C for 48 hours to achieve the desired confluence. After 48 hours, the spent medium was replaced with various working concentrations of given molecules (mentioned in the Excel sheet) diluted in the Media. After the drug addition, cells were incubated for 24 hours at 37°C with a 5% CO2 atmosphere. Cells without any treatment were used as a control. After incubation, cells were treated with 100 µL of MTT (0.5 mg/mL) reagent and incubated for 3 hours at 37°C. DMSO (100µL) was used to dissolve the formazan crystals, and the purple color was measured at 570 nm using a microplate reader (xMark Microplate Spectrophotometer, BioRad). The viability of cells treated with MEM alone was considered as 100%. % cell viability is calculated using the following formula:

% cell viability = [OD of treated cells/OD of Untreated cells] * 100

The Biocompatibility of Kasis and *Swarnamakshik Bhasma* was analysed using the MTT assay on Human Intestinal epithelial Cells (Caco2). These cells were treated with varying concentrations of (6.25 to 100 µg/ml) for 24 hours. Post-incubation, MTT reagent was added, and the absorbance was measured at 570 nm. The percentage of cell viability was calculated, and morphological changes were observed [16].

Cell morphological assessment:

Morphological changes in Caco2 cells were assessed post-treatment with the given test compounds at various concentrations after 24hours at 37°C. Cells were observed and recorded by using an Inverted Biological Microscope (CKX-41, Olympus) with the help of a camera and recorded using MICAM software at 20x magnification. Scale: 100 µm.

4. Results

Preliminary characterization and spectrometric analysis

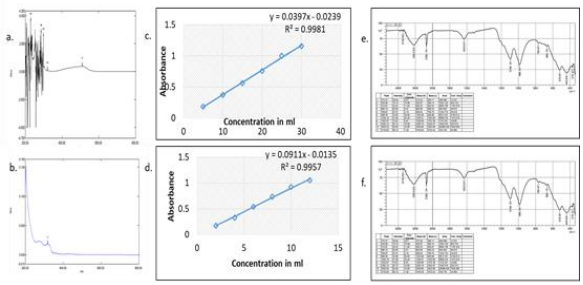

Figure 1. a. U.V Spectrum of *Kasis Bhasma*, b. U.V Spectrum of Swarnamakshik bhasma, c. Standard calibration curve of Kasis in distilled water, d. Standard calibration curve of swarnamakshik in distilled water, e. ATR Spectrum of *Kasis Bhasma*, f. ATR Spectrum of Swarnamakshik Bhasma

As shown in Table 1, preliminary tests revealed that Kasis and *Swarnamakshik Bhasma* exhibited reddish-brown and copperish-brown colors, with both displaying a metallic odor. Both samples float on the surface, indicating reduced density and proper lightness. The fineness test confirms that both samples are suitable for absorption, and the absence of metallic cluster shows that both Bhasmas have completely incinerated. The Loss on ignition was 99.89% for Kasis and Swarnamakshik, indicating high purity and minimal organic or volatile residue. These results indicate the successful

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transformation of raw material into bioacceptable therapeutic Bhasma form. The UV Colorimetry analysis was done to identify the presence of Iron by using 1,10-phenanthroline and ammonium acetate reagents, which formed a red-orange complex with ferrous ion. The absorbance of Kasis and *Swarnamakshik Bhasma* was measured at 510 nm and 485 nm, respectively. A linear calibration curve (R^2 = 0.9981 and R^2 = 0.9957) was obtained, confirming the reliability of quantification. This method enabled the accurate determination of iron content in Kasis and copper content in Swarnamakshik, which helps in validating the therapeutic relevance of Kasis and Swarnamakshik for anemia management. The UV-Visible spectroscopy study was performed in the range of 200 to 600 nm. For Kasis, the maximum absorption is observed at 510 nm, and for Swarnamakshik, it is observed at 485 nm. These maximum values indicated the ferrous and copper ion transitions, which confirm the elemental identity of the respective Bhasmas. The calibrated curves (Fig. 1 for Kasis and Swarnamakshik) showed linearity (R^2> 0.99), demonstrating the method's precision and specificity for active medicament detection. The ATR-FTIR spectra provided the molecular-level detection of bhasma composition. The Kasis showed (-OH) in 1430 $^{cm-1}$, (-CO) in 1283 $^{cm-1}$, and (-NH) in 1172 $^{cm-1}$ stretching, indicating the hydration and surface-bound organic ligand. The Swarnamakshik exhibited peaks at 3012 cm-1 (-OH), 1300 $^{cm-1}$ (-CO), and (C=C) and (-NH) bonds, reflecting its more complex sulphide metal structure. These profiles (Fig. 1) showed the successful transformation and stabilization of mineral content in Marana, as indicated by the ATR spectra of Kasis and Swarnamakshik. The X-ray diffraction analysis showed the crystalline nature of both Kasis and Swarnamakshik. However, the Kasis showed distinct peaks consistent with iron sulphate compounds (the obtained peaks matched the standard peaks), while Swarnamakshik aligned with chalcopyrite (CuFeS2). The presence of sharp and defined peaks, validated by successful mineral phase conversion and retention of crystalline integrity during the calcification process, confirmed the successful conversion. The obtained diffraction pattern for Kasis and Swarnamakshik (shown in Fig.2).

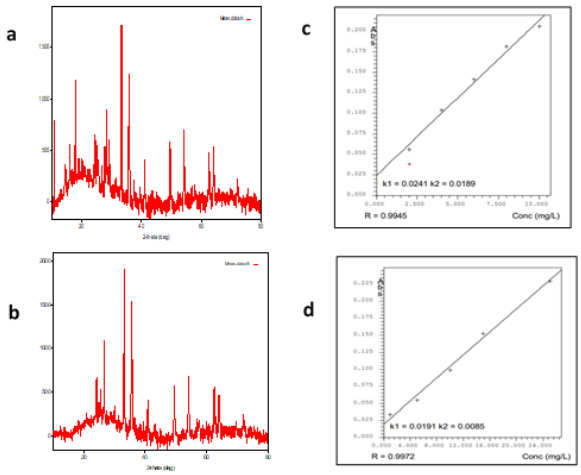

Figure 2. a. XRD spectra of *Kasis Bhasma*, b. XRD spectra of swarnamakshik Bhasma, c. The AAS calibration curve for *Kasis Bhasma*, d. The AAS calibration curve for Swarnamakshik Bhasma

Bhasma particle characterization

The Dynamic Light scattering (DLS) analysis method showed that *the Kasis particle size is 150.72 nm with a* polydispersity index (PDI) of 0.174. (Shown in Fig. 3). On the other hand, *Swarnamakshik* showed the consistent nanoparticle size between 123.08 nm with a low PDI value of 0.179 (Fig. 3), which indicates the Bhasma's nanosized particles with homogeneity and dispersibility. These nano-range particle sizes, enhanced dissolution, and potential for increased drug distribution and drug bioavailability.

Morphology and particle size of *kasis* and *Swarnamakshik Bhasma* were determined by SEM analysis (Figure 4). *Kasis Bhasma* appeared as irregular, amorphous porous particles with a size ranging between 0.5–2 µm. *Swarnamakshik Bhasma* exhibited an amorphous, flaky morphology with particle sizes ranging from ~1 to 3 µm. SEM images recorded at four different magnifications: 1µm, 10 µm, 20 µm, and 50 µm. Images show particle size in the range of 0.5 -3 µm. Smaller and more porous particles offer a greater surface area, thereby enhancing solubility and dissolution. This supports better bioavailability and permeation flux, particularly for minerals like iron and copper, which rely on transporter-mediated uptake.

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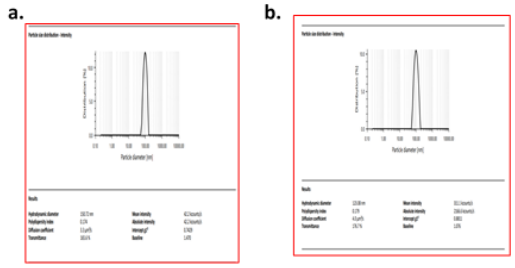

Figure 3. a. Graph of particle size analysis by zeta sizer of *Kasis Bhasma* showing a sharp peak with particle size of 150.72 nm and 0.172 PDI, b. Graph of particle size analysis by zeta sizer of *Swarnamakshik Bhasma* with particle size of 123.08 nm and 0.179 PDI.

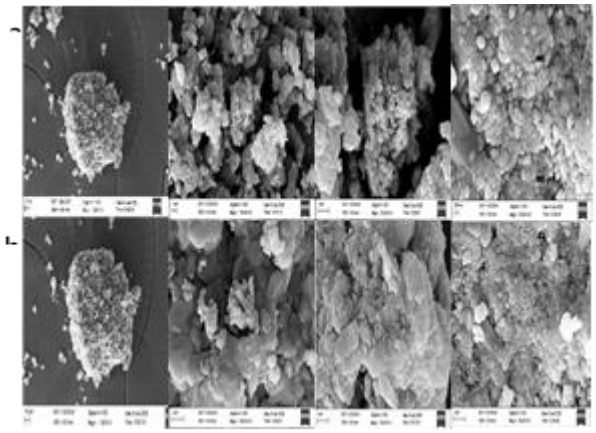

Figure 4. a. SEM micrographs of *Kasis Bhasma* showed amorphous, flaky morphology with particle size ~1–3 µm, b. SEM micrographs of *Swarnamakshik Bhasma*appeared as irregular, amorphous porous particles with size ranging between 0.5–2 µm. SEM images recorded at four different magnifications 1µm, 10 µm, 20 µm and 50 µm. Images shows particle size in range of 0.5 -3 µm.

Aflatoxin, microbial, nutritional and heavy metal testing

Aflatoxin analysis revealed that the natural mineral bhasmas did not contain any type of aflatoxins. The total bacterial and total fungal count were found to be 735 CFU/gm and <10 CFU/ gm, respectively, which were within the standard limit. Moreover, the results showed the absence of other bacteria. Heavy metals Lead, Arsenic, Mercury, Cadmium, and Cobalt were below detection limit. The pathogens such as *E. coli, S. aureus, P. aeruginosa, and Salmonella* were found to be absent. The Trace mineral profile showed the presence of essential micronutrients such as Iron, Copper, Zinc, Calcium, Magnesium, Sodium, Manganese, and Phosphorus, all of which were found to be present within therapeutic levels. As per AYUSH and WHO safety standards (tables 2 and 3).

The Bhasmas with bioavailable minerals and absence of toxic elements confirm their safety, nutritional value, and clinical treatment in the management of iron deficiency anemia and its related deficiencies. The Atomic absorption spectrophotometry study revealed that *Kasis* contained a highest concentration of elemental iron (approximately 1,199.169 mg/kg), (shown in table 2) on other *Swarnamakshik* exhibited the significant levels of both iron and copper (52.13 mg/kg and 61.76 mg/kg respectively) (shown in table 2) The Calibration curves using ferrous and copper sulphate standards displayed high correlation coefficients, which ensuring the analytical accuracy (Fig.3). These results confirm the role of bhasma as an iron supplement, and useful for the treatment of iron deficiency anemia

Cell line toxicity assay

The MTT cytotoxicity assay on CaCo-2 cells revealed that *Kasis Bhasma* maintained a cell viability of 80.46% at 100 µg/ml, which was comparable to its standard counterpart $FeSO_4$ (84.61%). Similarly, *Swarnamakshik Bhasma* exhibited 79.68% viability, whereas its standard salt $CuSO_4$ induced marked cytotoxicity, reducing viability drastically to 7.24% as shown in Tables 4 and 5. Overall, the observed results concluded that given formulations were biocompatible and determined the maximum safe dose and other supplements against the Caco2 cells after 24 hours of exposure for further studies, such as ROS expression by H2DCFDA staining and various cytokines measurement in treated and non-treated Caco2 cells, respectively (Figure 5). This stark contrast highlights the detoxification achieved through the classical Ayurvedic Shodhana and Marana processes, which not only mitigate the inherent toxicity of raw minerals but also enhance their biological compatibility. Statistical evaluation further substantiated these findings. Both Bhasmas showed no significant cytotoxic effect compared to untreated control cells ($p > 0.05$), indicating their safety at the tested concentration. In contrast, *Swarnamakshik Bhasma* vs $CuSO_4$ exhibited a highly significant difference ($p < 0.001$), while *Kasis Bhasma* showed a significant difference ($p < 0.01$). These results underscore that the Bhasma preparations, unlike their raw mineral salts, are biocompatible, less toxic, and

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suitable for therapeutic application in the management of iron deficiency anemia (Figure 6) .

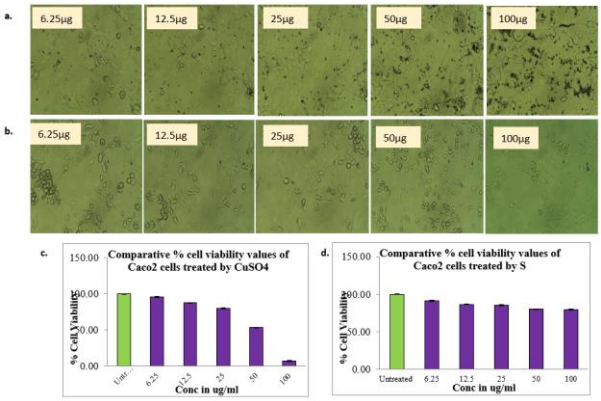

Figure 5. a. Microscopic images of cell viability of Caco-2 cell line with increasing concentration of standard $CuSO_4$, b. Microscopic images of cell viability of Caco-2 cells with increasing concentration of Swarnamakshik Bhasma, c. Bar graph representing the % cell viability of $CuSO_4$, d. Bar graph representing the % cell viability of Swarnamakshik Bhasma.

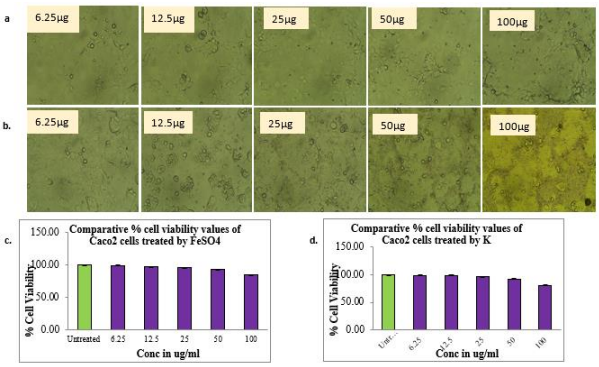

Figure 6. a. Microscopic images of cell viability of Caco-2 cell line with increasing concentration of standard $FeSO_4$, b. Microscopic images of cell viability of Caco-2 cells with increasing concentration of *Kasis Bhasma*, c. Bar graph representing the % cell viability of $FeSO_4$, d. Bar graph representing the %cell viability of *Kasis Bhasma*.

Discussion

The present study focused on a comprehensive evaluation of Kasis and *Swarnamakshik Bhasma* for the treatment of iron deficiency anemia, through traditional and modern scientific assessment techniques [17]. When compared with the previous findings [18], the current findings showed improved results in terms of standardization, particle size distribution, elemental consistency, and therapeutic bioavailability in the management of IDA [19].

The preliminary evaluation test, such as Color (*Varna*), Odor, Floating (*Varitara*), Fineness test (*Rekhapurnata*), Loss of metallic lustre (*Nischandratva*), was evaluated, and both *Kasis* and *Swarnamakshik* were found to be within the limit as per *Rasa Tarangini*. However, in earlier literature where variability in color and floatability occasionally occurred due to inconsistent puta conditions, the current batch of Bhasmas shows the uniformity in their physical characters, such as reddish brown, for Kasis and copperish brown for Swarnamakshik and consistent floating behavior and enhanced control over traditional processing techniques [20].

The Analytical validation of Bhasma reveals that Bhasma contains the active constituents, which were present within the limits. From the data point of view, UV Colorimetry and UV Visible Spectroscopy showed the elemental composition of Bhasma. The lambda max value of *Kasis* found to be 510 nm and for *Swarnamakshik* was 485 nm were consistent with the electronic transition of ferrous and copper ions, which in turn confirms the presence of these therapeutic metals in detectable and quantifiable concentrations [21]. These high correlation coefficient (R^2>0.99) of calibrated curves indicates a strong method for precision and linearity when compared with earlier investigations (R^2>0.95) [22].

The ATR FTIR analysis further confirmed the presence of characteristic functional groups such as (-OH), (-NH), and (-CO), suggesting that possible organometallic complexation or residual phytoconstituent interaction with the Bhasma surface. This enhances the biocompatibility and absorption efficiency, which are supported by Ayurvedic claims of improved assimilation and bioavailability, and overcomes possible side effects of poor absorption of allopathic ferrous and copper sulphate medicines [23].

The particle size analysis revealed that both Bhasma, such as *Swarnamakshik* and *Kasis,* showed a broader distribution, which includes submicron to micron-sized particles depending on the active constituent. The lower poly dispersity index (PDI<0.2) in the *Swarnamakshik* sample denoted the homogeneity and stability, which are crucial parameters influencing dissolution rate and

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bioavailability. X-ray diffraction analysis showed the presence of a crystalline phase in both Bhasma, which correlated with the intended mineral forms, iron sulphate in *Kasis* and chalcopyrite (CuFeS2) in *Swarnamakshik*. These finding confirmed the efficacy of traditional puta techniques in achieving consistent mineral phase transformation.

The atomic absorption spectroscopy further established the therapeutic metals (such as elemental iron) present in higher concentration (approximately 12043.82 mg/kg), which showed a strong hematinic property [24]. The *Swarnamakshik* also contains significant copper levels (61.76 mg/kg), which enhance iron metabolism through ferroxidase activity.

The nutritional and toxicological analysis study revealed the presence of essential trace elements such as zinc, calcium, magnesium, and manganese, while toxic metals (Lead, Cadmium, manganese, and arsenic) were below the detectable limits. This assures the formulation's safety for human consumption as per the WHO and AYUSH.

The MTT assay study revealed the biocompatibility of *Kasis* and *Swarnamakshik Bhasma* on human Caco2 intestinal epithelial cells. Both formulations maintained over 80% cell viability at therapeutic concentration (6.25 to 100 µg/ml), which indicated minimum toxicity. *Kasis* exhibits the higher safety margins, while *Swarnamakshik* showed a mild reduction in viability at higher doses, likely due to the presence of copper content. Caco-2 intestinal cells were selected to assess the cytotoxicity of *Kasis Bhasma* and Swarnamakshik Bhasma, as these formulations are orally administered for hematinic and rejuvenative benefits. The intestinal epithelium being the first site of exposure, this model reflects their interaction with human enterocytes. The results indicated acceptable viability, suggesting good intestinal compatibility. Thus, the study scientifically supports the oral safety of these traditional metallic preparations. These findings, however, support their safe gastrointestinal absorption and align with traditional ayurvedic claims of internal safety for oral therapeutic use [16, 25].

These findings further proved that the ayurvedic claim that Kasis and Swarnamakshik bhasma, when prepared using the traditional methods, are pharmaceutically standardized, validated, and therapeutically suitable for treating iron deficiency conditions when compared with the previous findings.[26] These results support their inclusion in integrative medicine practices and the bridging of their traditional wisdom with evidence-based healthcare.

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Conflict of interest statement

The authors report no conflict of interest.

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