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Pharmaceutical Standardization of an Arsenic based Ayurvedic formulation – *Haratala Bhasma*

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ABSTRACT: The application of toxic minerals and metals in the healing process was established since ages in Ayurveda. Orpiment *(Haratala)*, among them was a popular mineral whose intervention in purified and calcinated form was recommended in disorders of skin, epilepsy, syphilis, bronchial asthma, carcinoma etc. However, its toxic ability was not be undermined and given the due consideration. The pharmaceutical processing included the purification and calcination, described in Rasaratna Samucchaya and Rasa Tarangini which converted the toxic raw Orpiment *(Haratala)* into least toxic, therapeutically active calcinated form. In this study, standard manufacturing process of the calcination of Orpiment *(Haratala)* was established with reproducibility and feasibility. The Physico-chemical characterization of the final product as per the guidelines of Pharmacopeia Laboratory of Indian Medicine in addition to the classical confirmatory parameters of calcinated Orpiment *(Haratala)* were performed to evaluate the quality and safety of the drug. Further toxicity studies and clinical trials would provide highlight the efficacy and revalidate the traditional practices associated with the mineral Orpiment *(Haratala)*.

KEYWORDS: Calcination, Characterization, *Haratala Bhasma*, Orpiment *(Haratala)*, Pharmaceutical standardization, Standard Manufacturing Procedure

I. INTRODUCTION

The quote "All things are poisons and nothing is without poison, only the dosage makes a thing not poison" by Paracelsus (1493 A.D -1541 A.D), father of Toxicology (1), holds significance in the context of several questions raised by the scientific community towards the utilization of heavy metals (Mercury, Lead, etc.) and toxic minerals such as Realgar (As_4S_4) "*Manashila"* and Orpiment (As_2S_3) "*Haratala"* in the therapeutics (2). The 1000 years history of Rasashastra reveals the development of several formulations using the above mentioned minerals in innumerable diseases. Thus, unprejudiced scientific understanding of the pharmaceutical processing adopted in conversion of toxic minerals such as Orpiment *(Haratala)* is essential to understand the techniques adopted by the traditional scientists to convert toxic Arsenic into non-toxic therapeutically efficacious product (3).

In the present experiment, raw Orpiment *(Haratala)* was selected and its traditional calcination procedure was attempted to be standardized such that a Standard Manufacturing Procedure (SMP) was developed. The pharmacovigilance unit of Ayurveda was set up under the name of Pharmacopeia Laboratory of Indian Medicine (P.L.I.M.), Ghaziabad, India in 1970, whose guidelines were adopted for pharmaceutical standardization (4).

The pharmaceutical processing of Orpiment *(Haratala)* includes 3 stages (5):

- Purification (*Shodhana*) of Orpiment *(Haratala)*
- Levigation (*Bhavana*) of Orpiment *(Haratala)* in the presence of calcinated sea shell (*Shukti Bhasma*) and juice extract of Aloe *vera* Linn. (*Ghrita Kumari*)
- Calcination (*Marana*) of the pellets prepared after levigation using dried cow dung cakes (*Upala*).

Thus, each of these procedures requires standardization and accurate processing whose absence would prove to be toxic for administration. Physico-chemical characterization is also performed in the study as per the guidelines of P.L.I.M., which includes organoleptic characterization, confirmatory parameters of calcinated Orpiment *(Haratala)* (*Bhasma Siddhi Lakshana*), percentage of loss on drying, total ash value, acid- insoluble ash, and water- soluble ash (6). Standardization of all the parameters are attempted in the experiment by performing the entire experiment in three batches Batch I, Batch II and Batch III respectively. Thus, reproducibility and feasibility of the procedure is determined. The development of SMP for the preparation of calcinated Orpiment *(Haratala)* is essential with due consideration to its wide range of therapeutic utility. The therapeutic index of Orpiment *(Haratala)* included syphilis (*Phiranga),* disease involving "*Rakta Dhatu"* and

"*Vata Dosha" (Vatarakta),* acute manifestations of skin disorders (*Visarpa),* diseases involving dry scaling of skin *(Vipadika),* disorders of skin involving excessive discharges (*Vicharchika),* chronic ailments of skin *(Kusta),* hemorrhoids *(Arsha),* intermittent fever *(Vishama Jwara),* epilepsy *(Apasmara),* fistula in-ano (*Bhagandara),* subcutaneous ulcerations *(Vrana),* anal sinus *(Nadi Vrana),* blisters on skin (*Visphota),* other diseases related to "*Vata – Kapha Dosha"* and "*Rakta Dhatu"* (7). Thus, diverse diseases of infective, auto-immune, inflammatory pathology in both acute and chronic stages, the Orpiment *(Haratala)* in calcinated form is advised to be administered at a dose of 0.5 mg/kg – 1 mg/kg body weight to an adult of 60 kg body weight (8).

II. MATERIALS AND METHODS

Orpiment *(Haratala)* (As_2S_3), shells of Ostridae family (*Shukti*) were procured from the Ayurveda Pharmacy, Banaras Hindu University. *Benincasa hispida* Thunb. (*Kushmanda*) fruit, lime stone powder (calcium carbonate) were procured from the local market. Aloe *vera* Linn. was collected from the botanical garden of the institute. Earthen crucibles, earthen pot, dried cow dung cakes of approx. 200 g. were purchased from the local market, while, mortar and pestle (*Khalva Yantra*), cora cloth, iron spatula were taken from the departmental laboratory. Instrument specifications are given in Table 1 & 2. Following steps are required in the preparation of *Haratala Bhasma:*

1. Procurement of Orpiment *(Haratala)* which matches with the specifications as described in Rasaratna Samucchaya (9).

2. Purification process of raw materials – Orpiment *(Haratala),* sea shells.

3. Calcination process of Sea Shells.

4. Levigation of purified Orpiment *(Haratala),* calcinated sea shell and Aloe *vera* juice extract (10).

5. Calcination of the grinded mixture.

The ideal features of Orpiment *(Haratala)*: Although the mineral Orpiment *(Haratala)* was extracted from its ore, the raw material comprised the specific features described in Rasaratna Samucchaya (9) as explained in Table 3.

Purification of Orpiment (*Haratala Shodhana*): The raw Orpiment *(Haratala)* was converted into the coarse powder (Fig. 1) and placed at the center of a quadrilateral double layered cora cloth. The edges of the cloth were approximated and lifted upwards. A cotton thread was tied over the cora cloth just above the upper margin of Orpiment *(Haratala)*. This apparatus appeared to be in the form of a spherical ball (*Pottali*) (Fig. 2) which was suspended in an earthen pot filled with juice extract of *Benincasa hispida* Thunb. and boiled for 3 h (Fig. 3). This was followed by the repetition of boiling in lime water (*Churnodaka*) (11). The final product after drying under sunlight and weighed. The variation in pH of the liquid media used for boiling before and after the procedure, variation in weight of Orpiment *(Haratala)* were documented.

Purification of shells of Ostridea family (*Shukti Shodhana*): As described above, the sea shells (Fig. 4) were taken in a cora cloth and suspended in an earthen pot filled with supernatant liquid of the fermented rice gruel (*Kanji*) (12) and boiled for 3 h.

Calcination of shells of Ostridea family (*Shukti Marana*): The purified sea shells were subjected to intense heat at the temperature of 750°C in the Electric Muffle Furnace (EMF). It was preceded by levigation in the mortar and pestle using juice extract of Aloe *vera* Linn. The entire cycle was repeated for four times in order to achieve the confirmatory parameters of calcinated sea shell (*Bhasma Lakshana of Shukti)* (13). (Fig. 5)

Levigation of purified Orpiment *(Haratala)*, calcinated sea shell with *Aloe vera* juice extract (*Bhavana* procedure): Equal proportions of purified Orpiment *(Haratala)* and calcinated sea shells were converted into fine powder in a mortar. The juice extract of Aloe *vera* Linn. was added such that the entire mixture was converted into thin paste form. Trituration process was carried out until the thin paste changed into thicker consistency such that it could be made into small pellets of around 3 cm. diameter and 0.5 cm. thichness. The pellets were dried under sunlight and placed in earthen crucibles. These crucibles were calcinated using dry cow

dung cakes each possessing a weight of 200 g. (Fig. 6) After complete self-cooling was achieved, the crucible was opened and the weight of the pellets were measured. This entire procedure of levigation and calcination was carried out sequentially so as to fulfill all the confirmatory parameters of calcinated Orpiment *(Haratala)* (*Bhasma Lakshana)* defined by Rasa Tarangini and Rasaratna Samucchaya (14). (Fig. 7)

Physiochemical Characterization: The calcinated Orpiment *(Haratala)* was standardized based on all the analytical parameters as directed by the guidelines given in P.L.I.M., to assure the quality and safety of all raw drugs and the final formulation itself (6). The parameters include organoleptic characteristics, confirmatory parameters of calcinated Orpiment *(Haratala)* (*Bhasma Lakshanas)* defined by Rasa Tarangini and Rasaratna Samucchaya, the authoritative textbooks of Rasa Shastra mentioned in Drug & Cosmetic Act 1940, Schedule I, other parameters including the loss on drying, total ash value, acid insoluble ash, water soluble ash etc. (15).

- Determination of loss on drying: A petri dish was cleaned with distilled water and dried in an oven at 105^0 for 2 h. 1 g. drug sample was taken in the pre-weighed dried petri dish. It was dried in an oven at 105^0 until a constant weight was attained. The petri dish was taken out, self-cooled and weighed immediately. The weight loss i.e. loss on drying was calculated and expressed as % w/w (15).

- Determination of total ash value: 2 g. accurately weighed powdered samples were taken in tarred silica crucibles and incinerated at a temperature not exceeding 450^0 so as to attain carbon free ash. The samples were cooled and weighed (15).

- Determination of acid insoluble ash: The ash obtained from the above method (Total Ash Value) was transferred to a flask and added with 25 ml. of 6N HCL and boiled for 5 min. The solution was filtered through an ash free filter paper and insoluble matter was collected on the filter paper, washed with hot water and then it was taken in Gooch crucible and ignited to achieve constant weight (15).

- Determination of water soluble ash: The ash of each sample was washed for 5 min. with 25 ml. of water and insoluble matter was collected on an ash free filter paper. It was washed with hot water, and ignited to constant weight at 450^0. The weight of the insoluble matter was calculated by subtracted the weight of the water soluble ash from the total weight of the ash (15).

Water-soluble ash = weight of ash - water of insoluble ash

III. RESULTS

During the purification of Orpiment *(Haratala)* in the juice extract of *Benincasa hispida* Thunb. and lime water, characteristic pungent odor was observed. There was slight discoloration of the liquid media during the boiling process. The variation in weight of the Orpiment *(Haratala)* & pH before and after the experiment are mentioned in Table 4 and 5 respectively. The purification of sea shells resulted in enhancement of lustre and clearance from the physical impurities. Changes in the pH of the liquid media, variation of weight of sea shells were documented in Table 6. The confirmatory parameters of calcinated sea shells was attained after 4 cycles of levigation using juice extraction of Aloe *vera* Linn. and calcination in EMF at 750°C. The morphological changes, amount of juice extract utilized for levigation is described in Table 7. Table 8 describes the last of confirmatory parameters of calcination of sea shells.

The levigation of purified Orpiment *(Haratala)* and calcinated sea shells with the addition of juice extract of Aloe *vera* Linn. converted the yellow powder of Orpiment *(Haratala)* into dark greenish yellow thin paste. With continuous trituration, the finer and softer pellets were obtained. The pellets prepared were dark yellow in color. The amount of juice extract required for levigation, total weight of dry cow dung cakes used for each process of calcination, morphological changes in the pellets among the three batches are reported in the Table 9, 10 and 11 respectively. On 4 cycles of levigation and calcination, the parameters of calcination as described by Rasaratna Samucchaya were attained in all the three batches as depicted in Table 12, 13 & 14.The final product attained was greyish colored, soft and finer in consistency. The other organoleptic characteristics are explained in Table 15. The total percentage of loss on drying of all the 3 batches were 1.6%, 1.7% and 1.5% respectively. The total ash value of the 3 batches constituted 98.65%, 99.16%, 98.21% respectively. 3%, 2.8% and 3.15% of the total ash remained insoluble in hydrochloric acid while 5.7%, 6.91% and 5.1% of the total ash was soluble in water.

IV. DICUSSION

Importance of the pharmaceutical processing: The transformation of raw Orpiment *(Haratala)* into a calcinated form using traditional methodologies was essential in order to nullify the toxic effects of Orpiment *(Haratala)* whose predominant chemical compound As_2S_3 (Arsenic trisulphide) is a proven toxic material. Rasaratna Samucchaya also described the toxic effects of *Haratala* if consumed without purification (16). It included:

- Reduction in life span either qualitative or quantitative *(Ayughnam)*
- Renal disorders including complications of Diabetes Mellitus *(Prameha)*
- Increase in temperature of the body *(Tapa)*
- Development of blisters over the skin *(Sphota)*
- Development of contractures *(Anga Sankocha)*
- Irritant reaction on contact *(Kshobha)*
- Burning sensation throughout the body *(Analpa Daha)*
- Development of involuntary movements including tremors *(Kampa)*
- Pricking type of pain *(Toda)*
- Death *(Mrityu)*

Thus, the purification followed by calcination results in least toxic and therapeutically efficient product which can be used clinically in a stipulated dose. The boiling processing of Orpiment (*Haratala)* sequentially in juice extract of *Benincasa hispida* Thunb. and lime water for 3 hours each converted the toxic raw Orpiment into purified form which was non- toxic when administered in therapeutic dosage. Several preparations utilized Purified Orpiment as a part of their formulation such as *Smriti Sagara Rasa, Takaleshwara Rasa, Rasa Manikya, etc. Benincasa hispida* Thunb. was also a recommended drug used in the treatment of adverse effects produced by consumption of non-purified Orpiment (*Ashuddha Haratala Sevana Janya Vikaras)* (17). Thus, the *Benincasa hispida* Thunb. may act as an antidote for *Haratala* since it was used in the treatment of Orpiment (*Haratala)* poisoning. The Boiling of Orpiment (*Haratala*) in *Benincasa hispida* Thunb. would probably aid in

reducing its toxic properties. The boiling of Orpiment (*Haratala)* in lime water set forth for interaction of Arsenic trisulphide with Calcium. Calcium was a well-established chelating agent, which helped in reduced absorption of Arsenic from GI tract into the systemic circulation (18). Thus, the toxicity of further reduced by this procedure. Henceforth, the objective of purification was fulfilled when performed in the above mentioned liquid media.There was a loss of 1-3 g in each batch due to the entry of fine particles of Orpiment (*Haratala)* into the liquid media. Minor quantity also was dissolved in the lime water changing it into mild whitish- yellow transparent solution. The probable reason for this change was due to the formation of Calcium arsenate (19).
The initial pH of juice extract of *Benincasa hispida* Thunb. was observed to be about 6.3 – 6.8 which was acidic in nature (20). At the mid procedure of the boiling, the pH was observed to be 6.5 – 6.7 and at the terminal part of the procedure, the pH remained at 6.6 – 6.8. Thus, not much of changes were brought about due to its interaction with Orpiment (*Haratala).*

The pH of the lime water after 12 hours of its preparation was 11.8 – 12.2 (21). The pH at the mid procedure of boiling was 11.7 – 11.9. At the end of the experiment, the pH was slightly reduced to 11.4- 11.6. Since the pH of Arsenic trisulphide was slightly acidic, its interaction with lime water would have reduced the pH slightly (22).
After the purification process, the Orpiment *(Haratala)* was converted into fine powder. It was added with calcinated sea shell & juice extract of Aloe *vera* Linn. During the process of levigation, the particles got smoother and finer due to the constant pressure applied in the process of trituration. The particle size would be reduced with repeated levigation procedure (23). The particle-size reduction occurred by collision of particles with the surfaces of the equipment as well as with each other. The collision events involved compression, impact and attrition, and cutting or shear as the main mechanisms for particle-size reduction. This technique was termed as "Wet Milling" (24). The density of solids in the mill was an important parameter directly affecting breakage mechanisms. Density has a direct relationship with the number of particle–particle or particle–wall collisions as well as the force of the collisions. Feed rate to the mill and mill residence time also directly affected the solids' concentration in the mill. Thus, an understanding of the particle density in the mill was crucial since it can impact the milling rate and efficiency (25), both of which are important for scale-up purposes. For example, *Tangsathitkulchai et. al.* found that by increasing concentrations from 30 to 55% solids (by volume), the mill net power increased with increased powder filling, and then decreased after an optimum value was achieved (26).
Due to the nature of the milling process, the milled powders produced always exhibit a range of particle sizes or a characteristic particle-size distribution. In many cases, this range of particle sizes obeys a log normal

distribution; however, some processed powders can exhibit multimodal distributions (26).In this procedure, calcinated sea shells and juice extract of Aloe *vera* Linn. were levigated in the mortar. The levigation of Orpiment *(Haratala)* with the calcinated sea shells in a slightly acidic media of *Aloe vera* aided in the interaction of Arsenic with Calcium carbonate. Similar technique was adopted in co-precipitation of Arsenic using Calcite during the treatment of ground water as described by *G. Roman-Ross et. al.* (27). Due to calcination and the interaction with Calcium carbonate of the calcinated sea shells, free ionic Arsenic would be removed thus, the toxic effects of elemental Arsenic could be avoided.

Selection of quantity of heat to be given: In the experiment, the standardization of dry cow dung cakes in terms of the quantity of cow dung was measured and calcination procedure was performed. In the classics, for the purpose of calcination the dried cow dung collected from forest (*Vanopala*) were used. By definition, the *Vanopala* was the dried cow dung collected naturally from the animal habitat (28). Here the human manipulation of the shape and size was not done. Hence the approximate weight of each of such cow dung was around 50 g. The dry handmade cow dung cakes available in the present era were a mixture of dry husk mixed with the cow dung. The weight of these cow dung cakes were more than that of the naturally dried cow dung.

For the preparation of calcinated Orpiment *(Haratala)*, the calcination process described in Rasa Tarangini was- "*Laghu Puta". Laghu Puta* was a special variety, where fixed number of cow dung cakes were not mentioned in any of the treatises. Their quantity used were slightly more than that of "*Kukkuta Puta"* & less than the "*Gaja Puta"*. Hence, amount of cow dung cakes used would be approximately 5kg – 6 kg for the raw material of 1kg. The amount of raw material was ranging from 140g. – 200g. Hence approximately around 1kg±200g. of cow dung cakes were used based on the calculations described above.At the end of 1st cycle of calcination, the pellets were hard with rough edges and thus, the confirmatory parameters of calcinated Orpiment *(Haratala)* were not completely fulfilled. The percentage of loss seen was 29.28%., 25.11%, and 26.37% respectively in the three batches. The reason for the loss was the sublimation of Sulphur in major quantity and traces of Arsenic. At the end of 2nd cycle of calcination, the pellets were dark greyish with shades of yellow. The edges were rough, hard and difficult to break. None of the confirmatory parameters of calcinated Orpiment *(Haratala)* were positive especially the "*Nirdhuma Pariksha"*- Presence of Sulphur resulted in thick smoke when the pellet was incinerated. The percentage of loss after 2nd cycle of calcination were 16.15%, 16.07% and 13.42% respectively in the three batches. The reason for the loss was due to the sublimation of Sulphur and Arsenic. Hence, the pellets required further calcination.

At the end of 3rd cycle of calcination, the pellets were dark greyish with smoother edges, softer and finer on pounding. However, the "*Nirdhuma Pariksha"* was negative. This reflected the presence of Sulphur which produced pungent fumes on heating. The percentage of loss after 3rd cycle of calcination were 12.84%, 13.30% and 11.47% respectively in the three batches. The reason for the loss was due to the sublimation of Sulphur and Arsenic. Hence, the pellets required further calcination.At the end of 4th cycle of calcination, the pellets were very fine and soft with dark greyish color. All the batches tested positive for "*Nirdhuma Pariksha"* and other confirmatory parameters of calcinated Orpiment *(Haratala).* The percentage of loss after 4th cycle of calcination were 11.95%, 12.19%, 11.19% respectively in the three batches. The reason for the loss was due to the sublimation of Sulphur and Arsenic.

Organoleptic characterization: Different organoleptic parameters were described as general parameters such as taste, sound, touch etc. Any calcinated product (*Bhasma*) should be devoid of taste & any characteristic metallic sound. On touch, they must be very minute, smooth without having sharp edges. Odor & Color were individualistic to different materials calcinated. The calcinated Orpiment *(Haratala)* possessed a peculiar garlic like odor and greying color due to the presence of calcinated sea shell. Thus, all the organoleptic parameters of the three different batches are said to possess the same organoleptic characteristics.The confirmatory parameters of calcinated Orpiment *(Haratala)* were the classical standardization parameters used to confirm the accurate processing of calcination. (29). It included different physical and chemical parameters which again were classified into general parameters and specific parameters. General parameters included "*Varitara"* & "*Unama"* – depicting the specific gravity of the final product, lesser than that of the water. "*Laghutva"* also reflected the negligible mass/weight of the calcinated Orpiment *(Haratala). "Rekhapurnatva"* and "*Sukshmatva"* described the minimized particle size of the calcinated compound. *"Avami"* was synonymous with the lack of taste which contemplated the absence of metallic taste produced by the presence of free metal in the final product. *"Nishchandratva"* also demonstrated the visual test for the absence of free metals. Certain specific tests like "*Nirdhuma Pariksha"* was exclusively described for calcinated Orpiment *(Haratala),* where, the presence of smoke revealed the presence of free Sulphur while the "*Nirdhumatva"* was aimed at by giving repeated cycles of levigation and calcination (7).

The calcination process of Orpiment *(Haratala)* was completed after 4 cycles in all the batches thus, indicating the establishment of SMP for calcinated Orpiment *(Haratala)*. The positive result of all the classical parameters proved the quality & safety of "*Haratala Bhasma"* to be administered. By assessing the **Loss on Drying,** total moisture content in the calcinated Orpiment *(Haratala)* would be determined. Excess of moisture provided rich platform for the growth of fungi and other microbes. Hence, minimum moisture content was essential to maintain the purity of the drug. The Batches I, II & III contained 1.6% 1.7% & 1.7% of moisture respectively. Due to the hygroscopic property of the Calcium Carbonate, the final product would had absorbed the environmental moisture. Hence the storage in air tight containers was recommended for sustained purity of the drug (30).

Total Ash value revealed the purity of the drug. It was the process of mineralization for pre-concentration of trace substances before chemical analysis. Ash was the term given to all non-aqueous residue that remained after a sample was burnt, which consisted mostly of metal oxides. Ash was one of the criteria in the proximate analysis of biological materials, consisting mainly of salty, inorganic constituents and metal salts (4). The 3 batches of calcinated final product after heating at 450℃, consisted of 98.65%, 99.16% and 98.21% of ash respectively. This reflected the presence of inorganic components in highest proportion when compared to the organic salt or any metal salts. Out of this high proportion of total ash, majority of the ash particles easily dissolve in concentrated Hydrochloric Acid. Only 3%, 2.8% and 3.15% of the 3 batches of calcinated Orpiment *(Haratala)* respectively failed to dissolve in the acid. The reason for the non- dissolution was the presence of Silica in the final product. The presence of Silica could be due to its presence in traces in the raw material taken. The process of levigation also may lead to the erosion of the Mortar and pestle whose main component – the Silica, could add to the final product. The minimum percentage of **Acid Insoluble Ash** confirmed the minimum percentage of Silica in the drug and thus asserting the purity of the formulation.The test "*Varitaratva"* can be positive only when the drug sprinkled over the still water floats completely. For this to happen, the drug should neither dissolve in the water nor settle at the bottom due to the specific gravity. However, due to the dissolution property of calcinated sea shells, traces of the final product was found to be dissolved in water. The percentage of **Water Soluble Ash** were 5.7%, 6.91% and 5.1% respectively in the three batches.

Development of SMP of Ayurvedic pharmaceutical procedures in large is an essential part of pharmacovigilance. The calcination procedure of Orpiment *(Haratala)* performed in three different batches using traditional methodology required 4 cycles of levigation and calcination to fulfill all the parameters of calcination as per Rasaratna Samucchaya. The organoleptic characteristics and the parameters as per the P.L.I.M guidelines also were similar in the three batches with minimal diversions. Hence, this technique can be used as an SMP by all the pharmaceutical units in production of calcinated Orpiment *(Haratala)*. Several advancements are being observed in the last decade regarding development of nano-based drugs (31-37). Bhasma are the ancient nanomedicines,and Hartala Bhasma is one of these ancient nanodrug treasures (38-40).Quality control is a crucial process for traditional medicines (41-46). Standardization is said to be accurate when the therapeutic application of all the batches yield similar results. Hence, randomized clinical trials supported by the toxicity studies would provide definite information regarding the quality, safety and efficacy of calcinated Orpiment *(Haratala)*. For, further evaluation of the physical and chemical changes occurring during the pharmaceutical processing, sophisticated techniques such as X-ray Diffraction (X-RD), Scanning Electron Microscopy (SEM), Raman Spectroscopy, Inductive Coupled Plasma Mass Spectroscopy (ICP-MS) etc. could be used which will aid in detailed understanding of the traditional science in the language of contemporary science. Safety evaluation studies are crucial for these traditional medicines, therefore should be of utmost importance in establishing biological profile of such drugs (47-48).

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TABLE 1: INSTRUMENTAL SPECIFICATIONS USED FOR PURIFICATION PROCESS:

Sl. No.	Name of the Instrument	Dimension	Purpose
1	Earthen pot	Diameter – 15 cm. Volume- 3 l	Boiling or Orpiment *(Haratala)* and sea shells in liquid media (*Swedana*)
2	Cora cloth	l * b = 15 cm	For binding and suspending of Orpiment *(Haratala)* and sea shells during boiling
3	Other Instruments: Weighing machine, Wooden twig, gas stove		

TABLE 2: INSTRUMENTS USED FOR LEVIGATION PROCESS:

Sl. No	Name of the Instrument	Dimension	Purpose
1.	Mortar	Depth- 11.5 cm Length- 51 cm Breadth- 30.5 cm	For levigation
2.	Pestle	Length- 25 cm Diameter of inferior surface- 6 cm	For levigation
3.	Iron Spatula	Length- 20 cm	For levigation

TABLE 3: MORPHOLOGICAL FEATURES OF IDEAL RAW ORPIMENT *(HARATALA)* AS PER RASARATNA SAMUCHHAYA:

Identification features as per Rasaratna Samucchaya	Presence of the features in the raw material selected for experiment
Golden color (*Swarna Varna*)	Present
Denser (*Guru*)	Present
Unctuous on touch (*Snigdha*)	Present
Presentation of flakes/ layered appearance	Present
Shining appearance	Present

TABLE 4: VARIATION IN WEIGHT DURING PURIFICATION OF ORPIMENT *(HARATALA)* OF 3 BATCHES:

Batches	I.W.O (in g.)	V.B.H (in l.)	V.L.W (in l.)	F.W.O (in g.)	W.L.O (in g.)
1	100	1.9	2	100	-
2	100	2	1.8	99	1
3	100	2	2	100	-

TABLE 5: VARIATION IN pH DURING ORPIMENT *(HARATALA)* PURIFICATION OF 3 BATCHES:

Batches	pH of B.B.B	pH of B.A.B	pH of L.B.B	pH of L.A.B
1	6.7	6.6	11.7	11.5
2	6.5	6.4	11.6	11.4
3	6.8	6.5	11.7	11.6

TABLE 6: VARIATION IN WEIGHT OF SEA SHELLS AND pH OF FERMENTED RICE GRUEL DURING PURIFICATION OF SEA SHELLS*:*

Batches	**I.W.S (in g.)**	**V.F.R (in l.)**	**F.W.S (in g.)**	**W.L.S (in g.)**	**pH of F.R.B.B**	**pH of F.R.A.B**
1	150	1.75	147	3	2.9	4.2

TABLE 7: VARIATION IN WEIGHT & OBSERVATIONS DURING THE CALCINATION OF SEA SHELLS:

Calcination No.	**M.T in EMF**	**D.M.T (in min.)**	**I.W.S (in g.)**	**W.A (in g.)**	**W.S.A.L (in g.)**	**W.S.A.C (in g.)**	**W.L.S (in g.)**	**Observation**
1	700	60	145	-	-	135	10	Sea shells became softer and brittle. The color turned dark grey
2	650	30	135	45	143	129	14	Sea shells became finer and pellets were powered on slight powder
3	600	30	129	40	134	117	17	The color of sea shells turned whitish grey
4	650	60	117	40	125	114	9	The color of sea shells turned more whitish and finer

TABLE 8: RESULTS OF PARAMETERS OF CALCINATED SEA SHELLS (*BHASMA LAKSHANA)* AFTER EACH CALCINATION OF SEA SHELL:

Calcination No.	**Parameters of Calcinated sea shells (*Bhasma Lakshana*)**						
	Varitara	***Rekha Purnatva***	***Laghu***	***Unam***	***Sukshma***	***Sweta Varna***	***Slakshnatva***
1	-ve	-ve	-ve	-ve	-ve	-ve	-ve
2	-ve	+ve	+ve	+ve	+ve	-ve	+ve
3	-ve	+ve	+ve	+ve	+ve	+ve	+ve
4	Partially +ve	+ve	+ve	+ve	+ve	+ve	+ve

TABLE 9: VARIATION OF WEIGHT & OBSERVATIONS DURING CALCINATION OF ORPIMENT *(HARATALA)* BATCH I:

Calcination no.	**V.A (in ml.)**	**W.P.B.L *(in g.)***	**W.P.A.L *(in g.)***	**W.C. (in g.)**	**W.P.A.C *(in g.)***	**Observation**
1	75	180	198	1200	142	Pellets were dark greyish yellow with rough edges, hard
2	55	142	161	1100	135	Pellets were dark greyish with yellow tinge & rough edges, hard

3	55	135	148	900	129	Pellets were dark greyish with smooth edges, softer and finer
4	45	129	137	800	122	Pellets were dark greyish with smooth edges, softer and finer and lighter

TABLE 10: VARIATION OF WEIGHT & OBSERVATIONS DURING CALCINATION OF ORPIMENT *(HARATALA)* BATCH II:

Calcination no.	**V.A (in ml.)**	**W.P.B.L *(*in g.*)***	**W.P.A.L *(*in g.*)***	**W.C. (in g.)**	**W.P.A.C *(*in g.*)***	**Observation**
1	70	180	195	1200	148	Pellets were dark greyish yellow with rough edges, hard
2	50	148	164	1100	141	Pellets were dark greyish with yellow tinge & rough edges, hard
3	50	141	158	900	137	Pellets were dark greyish with smooth edges, softer and finer
4	40	137	149	800	131	Pellets were dark greyish with smooth edges, softer and finer and lighter

TABLE 11: VARIATION OF WEIGHT & OBSERVATIONS DURING CALCINATION OF ORPIMENT *(HARATALA)* BATCH III:

Calcination no.	**V.A (in ml.)**	**W.P.B.L *(*in g.*)***	**W.P.A.L *(*in g.*)***	**W.C. (in g.)**	**W.P.A.C *(*in g.*)***	**Observation**
1	70	180	201	1200	150	Pellets were dark greyish yellow with rough edges, hard
2	60	150	164	1100	142	Pellets were dark greyish with yellow tinge & rough edges, hard
3	45	142	157	900	139	Pellets were dark greyish with smooth edges, softer and finer
4	139	45	152	800	126	Pellets were light greyish with smooth edges, softer and finer and lighter

TABLE 12: RESULT OF CONFIRMATORY PARAMETERS OF CALCINATED ORPIMENT *(HARATALA)* AFTER EACH CYCLE OF CALCINATION - BATCH I:

Calcination No.	**Confirmatory parameters of calcinated Orpiment *(Haratala Bhasma Lakshana)***						
	Varitara	***Rekha Purnatva***	***Laghu***	***Nishchandratva***	***Nirdhuma***	***Sukshma***	**Y.P with HNO_3**
1	-ve	-ve	-ve	-ve	-ve	-ve	+ve
2	-ve	-ve	+ve	-ve	-ve	-ve	+ve
3	-ve	+ve	+ve	-ve	-ve	+ve	+ve
4	+ve	+ve	+ve	+ve	+ve	+ve	+ve

TABLE 13: RESULT OF CONFIRMATORY PARAMETERS OF CALCINATED ORPIMENT *(HARATALA)* AFTER EACH CYCLE OF CALCINATION - BATCH II:

Calcination No.	**Confirmatory parameters of calcinated Orpiment *(Haratala Bhasma Lakshana)***						
	Varitara	*Rekha Purnatva*	*Laghu*	*Nishchandratva*	*Nirdhuma*	*Sukshma*	Y.P with HNO_3
1	-ve	-ve	-ve	-ve	-ve	-ve	+ve
2	-ve	-ve	+ve	-ve	-ve	-ve	+ve
3	-ve	+ve	+ve	-ve	-ve	+ve	+ve
4	+ve	+ve	+ve	+ve	+ve	+ve	+ve

TABLE 14: RESULT OF CONFIRMATORY PARAMETERS OF CALCINATED ORPIMENT *(HARATALA)* AFTER EACH CYCLE OF CALCINATION - BATCH III:

Calcination No.	**Confirmatory parameters of calcinated Orpiment *(Haratala Bhasma Lakshana)***						
	Varitara	*Rekha Purnatva*	*Laghu*	*Nishchandratva*	*Nirdhuma*	*Sukshma*	Y.P with HNO_3
1	-ve	-ve	-ve	-ve	-ve	-ve	+ve
2	-ve	-ve	+ve	-ve	-ve	-ve	+ve
3	-ve	+ve	+ve	-ve	-ve	+ve	+ve
4	+ve	+ve	+ve	+ve	-ve	+ve	+ve
5	+ve	+ve	+ve	+ve	+ve	+ve	+ve

TABLE 15: ORGANOLEPTIC CHARACTERS OF THE 3 BATCHES OF CALCINATED ORPIMENT (*HARATALA*):

Parameters	**Batch-1**	**Batch-2**	**Batch-3**
Sound*(Shabda)*	No perceptible sound produced during chewing	No Perceptible sound produced during chewing	No perceptible sound produced during chewing
Tactile sensation *(Sparsha)*	Soft, Smooth.	Soft, Smooth	Soft, Smooth.
Color *(Varna)*	Greyish	Greyish	Greyish
Taste *(Rasa)*	Tasteless	Tasteless	Tasteless
Odor *(Gandha)*	Garlic odor	Garlic odor	Garlic odor

(Fig. 1): Raw Orpiment *(Haratala)*

(Fig. 2): Orpiment bound by cora cloth before purification (*Pottali*)

(Fig. 3): Purification of Orpiment (*Haratala Shodhana* using *Swedana Yantra*)

(Fig. 4): Raw Sea shell of Ostridea family (*Shukti*)

(Fig. 5): Calcinated pellets of Sea shells formed after 4th Calcination (*Shukti Bhasma*)

(Fig. 6): Traditional calcination process of Orpiment (*Haratala Marana*)

(Fig. 7): Greyish calcinated Orpiment after 4th Calcination (*Haratala Bhasma*)

Table and figure titles and legends:

TABLE 4: VARIATION IN WEIGHT DURING PURIFICATION OF ORPIMENT *(HARATALA)* OF 3 BATCHES:

I.W.O refers to Initial weight of raw Orpiment *(Haratala)*; V.B.H stands for Volume of juice extract of *Benincasa hispida;* V.L.W is Volume of Lime Water; F.W.O refers to Final Weight of purified Orpiment *(Haratala)*; W.L.O indicates Weight Loss of Orpiment *(Haratala)*.

TABLE 5: VARIATION IN pH DURING ORPIMENT *(HARATALA)* PURIFICATION OF 3 BATCHES:

B.B.B stands for juice extract of *Benincasa hispida* before boiling; B.A.B refers to juice extract of *Benincasa hispida* after boiling; L.B.B is Lime water before boiling; L.A.B indicates Lime water after boiling.

TABLE 6: VARIATION IN WEIGHT OF SEA SHELLS AND pH OF FERMENTED RICE *GRUEL* DURING PURIFICATION OF SEA SHELLS*:*

I.W.S indicates the Initial weight of Sea shells; V.F.R refers to Volume of Fermented Rice gruel; F.W.S is Final weight of Sea shells after purification; W.L.S stands for Weight Loss of Sea shells after purification; F.R.B.B refers to Fermented Rice gruel before boiling; F.R.A.B indicates the Fermented Rice gruel after boiling.

TABLE 7: VARIATION IN WEIGHT & OBSERVATIONS DURING THE CALCINATION OF SEA SHELLS:

M.T refers to Maximum temperature set at Electric Muffle Furnace; D.M.T indicates the Duration of Maximum temperature in EMF; I.W.S suggests the Initial Weight of Sea shells taken; W.A stands for the weight of Aloe *vera* pulp taken; W.S.A.L is the weight of sea shells after levigation; W.S.A.C indicates the Weight of sea shells after calcination; W.L.S refers to Weight Loss of Sea shell after calcination.

TABLE 9: VARIATION OF WEIGHT & OBSERVATIONS DURING CALCINATION OF ORPIMENT *(HARATALA)* BATCH I:

V.A suggests the volume of Aloe *vera,* W.P.B.L indicates weight of pellets before Levigation; W.P.A.L refers to weight of pellets after levigation; W.C is the weight of dry cow dung cakes; W.P.A.C indicates the weight of pellets after calcination.

TABLE 10: VARIATION OF WEIGHT & OBSERVATIONS DURING CALCINATION OF ORPIMENT *(HARATALA)* BATCH II:

V.A suggests the volume of Aloe *vera,* W.P.B.L indicates weight of pellets before Levigation; W.P.A.L refers to weight of pellets after levigation; W.C is the weight of dry cow dung cakes; W.P.A.C indicates the weight of pellets after calcination.

TABLE 11: VARIATION OF WEIGHT & OBSERVATIONS DURING CALCINATION OF ORPIMENT *(HARATALA)* BATCH III:

V.A suggests the volume of Aloe *vera,* W.P.B.L indicates weight of pellets before Levigation; W.P.A.L refers to weight of pellets after levigation; W.C is the weight of dry cow dung cakes; W.P.A.C indicates the weight of pellets after calcination.

TABLE 12: RESULT OF CONFIRMATORY PARAMETERS OF CALCINATED ORPIMENT *(HARATALA)* AFTER EACH CYCLE OF CALCINATION - BATCH I:

Y.P with HNO_3 refers to Yellow precipitate on heating with Nitric acid.

TABLE 13: RESULT OF CONFIRMATORY PARAMETERS OF CALCINATED ORPIMENT *(HARATALA)* AFTER EACH CYCLE OF CALCINATION - BATCH II:

Y.P with HNO_3 refers to Yellow precipitate on heating with Nitric acid.

TABLE 14: RESULT OF CONFIRMATORY PARAMETERS OF CALCINATED ORPIMENT *(HARATALA)* AFTER EACH CYCLE OF CALCINATION - BATCH III:

Y.P with HNO_3 refers to Yellow precipitate on heating with Nitric acid.

Fig. 1: Raw Orpiment *(Haratala)*

Fig. 2: Orpiment bound by cora cloth before purification (*Pottali*)

Fig. 3: Purification of Orpiment (*Haratala Shodhana* using *Swedana Yantra*)

Fig. 4: Raw Sea shell of Ostridea family (*Shukti*)

Fig. 5: Calcinated pellets of Sea shells formed after 4th Calcination (*Shukti Bhasma*)

Fig. 6: Traditional calcination process of Orpiment (*Haratala Marana*)

Fig. 7: Greyish calcinated Orpiment after 4th Calcination (*Haratala Bhasma*)