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A PHARMACEUTICO-ANALYTICAL INVESTIGATION OF *RAJAT BHASMA* SYNTHESIZED THROUGH MODIFIED TECHNIQUES EMPLOYING MERCURIAL COMPOUNDS

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ABSTRACT :

Background: The quality of metal *Bhasma* and the number of *Puta* (specific quantum of heat) required for its preparation depends on the raw materials used. Mercury and its compounds are considered superior for producing metallic *Bhasma*, which can be applied in the form of an amalgam with metal or as a paste over metal foil. **Aim:** To evaluate the impact of equal quantities of mercury and sulfur in paste form on *Bhasma* preparation and to compare the results with those of previous studies. **Methodology:** *Rajat Bhasma* (RB) was prepared using equal quantity of *Shuddha Parad* (processed mercury) with *Shuddha Gandhak* (processed sulphur) in every *Puta* and in the paste form in the first *Puta*. *Rajat Bhasma* was analysed for ancient *Bhasma Praiksha* (tests), its physicochemical parameters, elemental analysis through XRF, crystal study through XRD, and particle size through dynamic light scattering (DLS). **Results:** Three *Puta* were required to prepare an RB that passed all ancient parameters. RB contained 17.7 % silver, 30.7 % mercury, and 50.8 % sulfur. The XRD study showed peaks of silver sulfide in the RB. RB contains particles in the range–300-400 nanometres. **Conclusion:** Adopting the use of *Kajjali* in the first *Puta* and an equal quantity of mercury and sulphur in the remaining *Puta's* reduces the number of *Puta's* required to three. This also resulted in variations in the particle size and percentage of silver, mercury, and sulfur in the final RB.

KEYWORDS: *Rajat Bhasma*, *Rajat Marana,* Silver *Bhasma* .

1. INTRODUCTION

Ayurveda is a scientific discipline that focuses on the therapeutic applications of metals, minerals, and plant-based preparations to treat various conditions. [1] One such application in Ayurveda is the use of *Bhasma*, which are herbo-metallic ashes that contain organometallic complexes formed by calcinating the metal along with herb-mineral ingredients. [2] The correct calcination process should result in the formation of *Bhasma* that does not contain free metal or free organic constituents. [3] *Bhasma* can be prepared using four distinct media, with the highest quality achieved through the utilization of *Parad* (mercury) and its compounds, followed by *Gandhak* (sulfur) and its compounds. When prepared solely using herbal media, the quality of *Bhasma* diminishes. It is inadvisable to employ *Ari-lauha* (enemy metal-metal, which destroys the metallic nature of other metals) in the preparation of metal *Bhasma*, as they may produce deleterious effects rather than therapeutic benefits. [4]

The quality of *Bhasma* depends on various factors such as the quality of the starting materials, processing ingredients, method of processing, application of heat (*Puta*), and repetition of that heat. The quality of a finished product can be influenced by alterations to any intermediate process, without modifying the raw material used. Ancient scholars have advocated the utilization of mercury and its compounds in the preparation of *Bhasma*; however, they did not provide specific details regarding the process of mixing with the metal. There is a divergence of opinion among scholars concerning the process of mixing mercury and its compounds with metal in the initial *Puta* (specific quantum of heat), as well as the quantity to be employed in further *Puta studies*. Some scholars have formulated an amalgam of mercury with metal, subsequently incorporating *Shuddha Gandhak* (processed sulfur) and transforming it into a homogeneous mixture. Certain scholars posit that the initial *Kajjali* (black powder obtained after triturating *Shuddha Parad* (processed mercury) with *Shuddha Gandhak*) should be prepared and subsequently combined with *Shodhita* metal, whereas others recommend applying a paste of *Kajjali* to thin foils of high-melting-point metals. Some scholars have employed half or one-fourth of the quantity of *Kajjali* following the first *Puta*, while others have proposed using an equivalent quantity. There is also a perspective that mercury and its compounds should not be incorporated after the third or fifth *Puta*. Therefore, investigating the variation in mercury mixing and its impact on *Bhasma* can provide insights into the number of *Putas* required, its physicochemical properties, and therapeutic benefits.

In Rasashastra, silver stands out among various metals as a noble element with significant therapeutic potential. The medicinal applications of *Rajat Bhasma* (RB) are diverse, addressing conditions such as *Madhumeha* (diabetes mellitus), *Jwara* (fever), *Bhrama* (vertigo), *Yakrit vikara* (liver disorders), and *Manasa rogas* (psychological disorders), among other ailments. [5] Research articles pertaining to the RB preparation using mercury and its compounds were identified through search engines such as Google and

PubMed using "*Rajat Bhasma,*" "Silver *Bhasma*" words. Only articles in which RB was prepared using mercury and its compounds were selected.

The research works of Durga et al., Naik et al., Rohit et al., and Diksha et al. used mercurial compounds to prepare RB. Durga et al. prepared RB using *Khalva Yantra* (mortar and pestle) with equal quantities of *Kajjali* and a sufficient quantity of *Kumari Swarasa* (aloe vera juice) as *Bhavana Dravya* (medium used for levigation). [6] In separate investigations, Naik et al. [7] and Rohit et al. [8] prepared RB by creating an amalgam of the *Shuddha Parad* with *Shuddha Rajat* (processed silver) foils. *Shuddha Gandhak* was subsequently added to the amalgam to prepare *Kajjali*, and pellets were formed after levigating *Kajjali* with aloe vera juice. As per Naik et al., *Kajjali* used up to the third *Puta* and aloe vera juice used for the remaining *Putas*. [7] According to Rohit et al., half of the quantity of *Kajjali* was used up to the third *Puta*. Then onwards half quantity of sulphur was used up to ninth *Puta*, and aloe vera juice was used for the remaining *Putas*. [8]

To date, no research has been conducted on the preparation of RB using *Kajjali* as paste in the first *Puta* and equal amounts of mercury and sulfur in the subsequent *Putas* until the desired *Bhasma* characteristics appear. Therefore, the present study was conducted to assess the effects of incorporating mercurial compounds (*Kajjali*) in paste form in the first *Puta* and adding equivalent quantities of the mercurial compound in subsequent *Putas* on the number of *Putas* and physicochemical and analytical parameters.

2. MATERIALS AND METHODS

Instruments:

Stainless steel veesel, iron *kadhai*, spatula, measuring flask, *Khalva Yantra* (stone-20inch long), Horizontal muffle furnace (12x12x18 inch inside area, 1000^{O}C maximum temperature, temperature regular with electronic display) etc. are used in the pharmaceutical procedures. Muffle furnace, Hot air oven, crucible, conical flask, pH meter, X-ray diffractometer (PW3040/60 X'pert PRO, Panalytical X'pert Powder from Netherland), Scanned electronic microscope (SEM-FEI Nova Nano FESEM 450,Line resolution = 1 nm), X-ray fluoresce (XRF-Rigaku NEX DE), Particle size analyzer (Anton Paar Lite Sizer 500) were used in the analytical study.

Collection and authentication of raw Materials:

Thin silver sheets, *Parada* (mercury), *Gandhaka* (Sulfur) were procured from local vendor. Silver sheets were subjected for XRF studies to assess the purity. *Kumari* and *Agasti Patra* (Sesbania Grandiflora Linn.) was procured form the gardens of our Institute and authenticated from Dravyaguna department of our Institute. *Til Taila* (seasame oil), *Kulattha* (Dolichos Biflorus Linn.) were procured from the local vendors. Gomutra (Cows Urine) were collected from Goshala. *Takra* (Butter Milk) was prepared in the department as per *Bhavprakash*. [9] *Kanji* (Sour gruel prepared from rice) was prepared in the department as per the *Rasayanasara*. [10]

Rajat Shodhana:

Purification of Silver was done by *Nirvapa* (quenching) method. Thin silver foils were heated to red hot stage and subsequently immersed 7 times in *Tila Taila*, *Takra*, *Gomutra*, *Kanji*, and *Kulattha Kwatha* (decoction of Dolichos biflorus

Linn.) consecutively for *Samanya Shodhana* (Simple Purification). [11] Similarly three times quenching was done in *Agasti Patra Swarasa* (juice of Sesbania Grandiflora Linn. leaves) for *Vishesha Shodhana* (Special Purification). [12] (Table 1)

Table 1 Observations made during *Samanya* and *Vishesha Shodhana* of *Rajat*

Sl. No.	Name of the Liquid	Quantity in ml before quenching (ml)	Quantity in ml after quenching (ml)	Gain/loss (ml)	pH before quenching	pH after quenching	Change in pH (Avg.)	Temperature of liquid before quenching (^{O}C)	Temperature of liquid before quenching (^{O}C)	Gain/loss (^{O}C)
01	*Tila taila*	100ml	87.90	12.10↓	5.53	5.07	0.46 ↓	27	38.47	11.47 ↑
02	*Takra*	100ml	88.42	11.58↓	4.67	4.58	0.09↓	30	35.33	5.33↑
03	*Gomutra*	100ml	88.80	11.20↓	6.84	7.00	0.16↑	29	31.99	2.99↑
04	*Kanji*	100ml	88.85	11.15↓	4.46	4.32	0.14↓	32	35.94	3.94↑
05	*Kulattha kwatha*	100ml	87.52	12.48↓	5.51	5.55	0.04↑	32	35.04	3.04↑
06	*Agasti patra swarasa*	100ml	90.33	9.67↓	5.68	5.62	0.06↓	28.	30.33	2.11↑

Note **:** ↓ **-** decreased, ↑- increased

***Gandhak Shodhana*:**

Purification of *Gandhak* was carried out by *Dhalana* (melting and pouring in other liquid) method. [13] Raw *Gandhak* was placed in a stainless-steel container containing 1/4th Ghee and melted over a mild flame. Four layers of muslin cloth were tied around the mouth of a container containing milk. Once the raw sulfur had melted, the mixture was poured into the container through a cotton cloth. This process was repeated twice. The sulfur obtained was washed with hot water and dried.

Parad Shodhana:

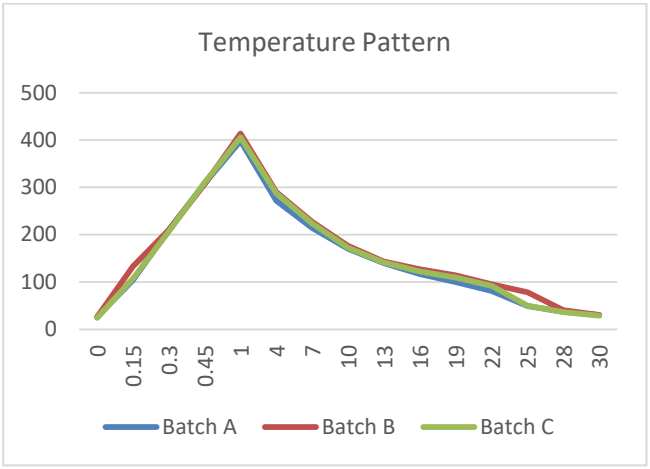
The Ashuddha Parad was triturated with an equal amount of *Rason* (Allium Sativum Linn.) paste and half the quantity of *Saindhav* (rock salt) for seven days. After seven days, *Parad* was washed with lukewarm water. [14]

Preparation of RB:

RB was prepared as per the method suggested in *Rasa Tarangini*. [15] *Shuddha Parad* and *Gandhak* were triturated for six days to prepare *Kajjali*. *Kajjali* was levigated with Aloe vera juice until a paste was formed. This paste was applied to both sides of the *Vishesh Shodhita Rajat* foils, and *Puta* was administered through EMF. [Figure 1] [Graph 1] After the first *Puta*, *Rajat* had transformed into a powder. In the following two *Puta's*, *Kajjali* was added twice to the powder obtained after the first and second *Puta* and triturated with aloe vera juice, resulting in pellet formation. The dried pellets were placed in an earthen saucer, and *Puta*

was administered through EMF. After the third *Puta,* the RB met all classical parameters. (Table 2)

Figure 1: Step of *Rajat Bhasma* a- Process of making silver foils red hot for quenching, b- quenching of red hot silver foils into *Agasti Patra Swarasa*, c- Application of paste of *Kajjali* and aloe vera on silver foils, d- *Sharava Samputa*, e- Levigation of *Rajat* powder with aloe vera juice during second *Puta*, f- Preparation of Pellets, g- powdering of pellets after *Puta*



Graph 1: Temperature pattern in the preparation of *Rajat Bhasma*

Table 2 Details of ingredients used in the preparation of *Rajat Bhasma*

Puta	**Batch**	***Vishesh Shodhita Rajat Patra* (g)**	***Kajjali* (g)**	**Aloe vera juice (ml)**
First *Puta*	Batch A	49	98	Quantity sufficient
	Batch B	48	96	Quantity sufficient
	Batch C	49	98	Quantity sufficient
Puta	**Batch**	**Material obtained after first *Puta* (g)**	***Kajjali* (g)**	**Aloe vera juice (ml)**
Second *Puta*	Batch A	66	132	Quantity sufficient
	Batch B	91	182	Quantity sufficient
	Batch C	98.5	197	Quantity sufficient
Puta	**Batch**	**Material obtained after second *Puta* (g)**	***Kajjali* (g)**	**Aloe vera juice (ml)**
Third *Puta*	Batch A	109	218	Quantity sufficient
	Batch B	133.5	267	Quantity sufficient
	Batch C	112.5	225	Quantity sufficient

Physical and Chemical Tests of RB Samples:

Several classical tests were performed on RB samples, including *Rekhapoornatva* (*Bhasma* entering creases of finger), *Varitaratva* (floats on water surface), *Nischaandratva* (devoid of shine), *Niswadu* (no metallic taste), *Apunarbhava* (test carried out using five substances to check inability to revert back into its metal form), *Niruttha* (test carried out using copper foil to check inability to revert back into its metal form). [16-21] Physicochemical tests, such as pH, ash value, acid insoluble ash, water soluble ash, and loss on

drying, were also conducted. [22-26] Additionally, sophisticated instrumental analyses were carried out using X-ray diffraction, scanning electron microscopy (SEM), and Particle size analysis was performed using dynamic light scattering (DLS) technique.

RESULTS

The results of traditional *Bhasma* test, physico-chemical parameters are given in the table 3, 4 and figure 2.

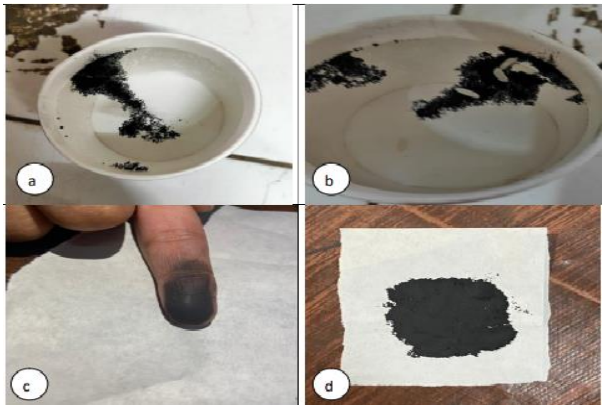


Figure 2: Demonstration of *Bhasma Pariksha* (test) on *Rajat Bhasma*. a- *Varitaratva*, b- *Unam*, c- *Rekhapurnatva*, d- *Nishchandratva*

The XRF was used to determine the elemental composition of raw silver and *Rajat Bhasma.* These findings are presented in table 5. The XRD patterns of RB are depicted in Figure 3 to 5 and Table 6, which show 2θ value of approximately 26.4867, 30.6617, 43.7998, predominantly consisting of cubic and slender structures of Ag_2S and Ag_2O. These findings indicate that *Rajat Bhasma* contains significant amounts of silver sulphides.

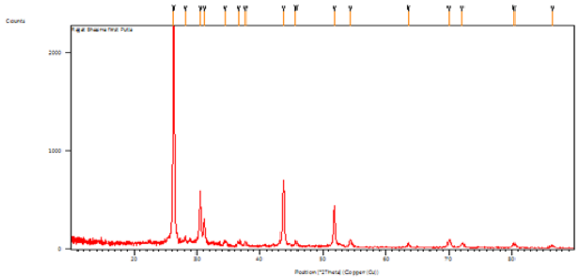


Figure 3 X-ray Diffraction Pattern of raw *Rajat* foils

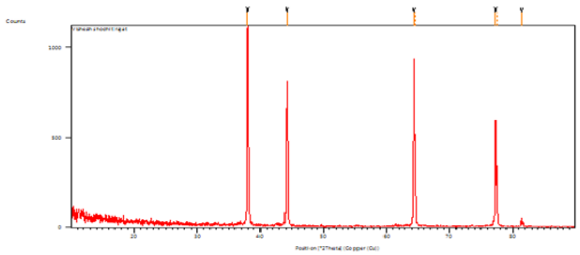


Figure 4 XRD analysis of *Vishesh Shodhita Rajat*

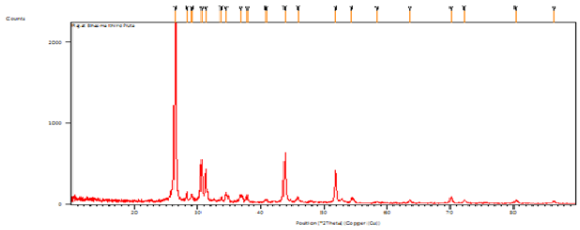


Figure 5 XRD analysis of *Rajat Bhasma* after 3rd *Puta*

Table 3 Results of Chief desired characteristics of *Rajat Bhasma* (1st to 3rd *Puta*)

***Pariksha* (Test)**		***Rekha-purnatva***	***Varitaratva***	***Unnama***	***Nirutthatva***	***Nishchandratva***
Batch A	**1st**	-	-	-	-	-
	2nd	+	Partially Present	Partially Present	-	-
	3rd	+	+	+	+	+
Batch B	**1st**	-	-	-	-	-
	2nd	+	80% Present	Partially Present	-	-
	3rd	+	+	+	+	+
Batch C	**1st**	-	-	-	-	-

	2nd	+	80% positive	Partially present	-	-
	3rd	+	+	+	+	+

Table 4 Physico-chemical analysis of *Rajat Bhasma*.

Test	**Raw Silver**	***Rajat Bhasma* after 3rd *Puta***
Color	White with luster	Black
Loss on drying (%)	Nil	0.18
Ash value (%)	99.98	35.49
Acid insoluble ash (%)	99.98	32.10
Water soluble ash (%)	0.02	6.89

Table 5 Results of XRF for Elemental analysis of Raw Silver and *Rajat Bhasma*

Element %	**Ag**	**S**	**Hg**	**Cl**	**N**	**Fe**	**As**	**C**	**Cu**
Raw silver	95.03	0.021	0.00	2.681	0.01	0.00	0.00	2.258	0.00
Rajat Bhasma	17.7	50.8	30.7	0.328	0.00	0.152	0.031	0.00	0.22

Table 6: X-ray diffraction of the *Vishesh Shodhita Rajat* and *Rajat Bhasma*

Vishesh Shodhita Rajat					
Compound Name	Obs. Max	Net Height	FWHM	d (Obs. Max)	Intensity
	2-Theta °	Cps	2-Theta °	Angstrom	%
Ag_2O	37.9742	1091.02	0.2066	2.36952	100.00
Ag_2O	44.1613	735.84	0.2362	2.05085	67.44
Ag_2O	64.3151	926.76	0.1440	1.44725	84.94
Ag_2O	77.2897	581.94	0.1800	1.23348	53.34
Rajat Bhasma					
Compound Name	Obs. Max	Net Height	FWHM	d (Obs. Max)	Intensity
	2-Theta °	Cps	2-Theta °	Angstrom	%
Ag_2S	26.4687	2184.93	0.1771	3.36750	100.00
Ag_2O	30.6617	457.94	0.2362	0.2362	20.96
Ag_2S	43.7998	611.99	0.1771	2.06693	28.01

The FEG-SEM was employed to analyze the surface of the samples at a high magnification, which enabled the examination of closely spaced features. This resulted in a three-dimensional representation of the samples. The raw *Rajat*, *Shodhita Rajat*, and *Rajat Bhasma* were analyzed using FEG-SEM, and the results are presented in table 7 and figure 6. The raw silver had a harder surface with uneven borders, while *Rajat Bhasma* showed a more uniform surface. The particle size

analysis of the samples was conducted using Dynamic Light Scattering (DLS), and the results are presented in table 8.

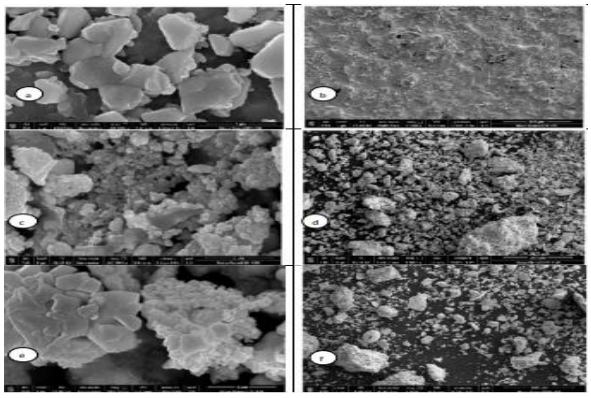


Figure 6: SEM images a- *Vishesh Shodhit Rajat* at 1µm magnifications, b- *Vishesh Shodhit Rajat* at 100 µm magnifications, c- *Rajat Bhasma* after 1st *Puta* at 10 µm magnifications, d- *Rajat Bhasma* after 1st *Puta* at 100 µm magnification, e- *Rajat Bhasma* after 3rd *Puta* at 10 µm magnification, f- *Rajat Bhasma* after 3rd *Puta* at 10 µm magnification

Table 7 Results of FEG-SEM of *Vishesh Shodhita Rajat* and *Rajat Bhasma*

Sample	**Surface**	**Border**
Raw silver	Granular to crystalline form	Halide and cubic in shape
Vishesh Shodhita Rajat	granular to massive incrustations crystalline form	Round to cubic in shape.
Rajat Bhasma	granular to massive incrustations crystalline form	Monoclinic slender to cubic in shape.

Table 8 Results of Dynamic Light Scattering for Particle size of *Rajat Bhasma*

Sl. No.	Sample name	Average Particle Size (nm)	Particle Size distribution (nm)
1	*Rajat Bhasma* 3rd *Puta*- Batch 1	481.8	438.8
2	*Rajat Bhasma* 3rd *Puta*- Batch 2	432.9	381.1
3	*Rajat Bhasma* 3rd *Puta*- Batch 3	445.8	430.4
	Average	453.5	

DISCUSSION

Bhasmikaran (Calcination) is a unique process that involves the complete conversion of a zero valent metal into an oxide or sulphide state. [27] This process is useful for removing toxic properties from metal and introducing therapeutic properties. [28] The quality and quantity of raw materials as well as the processes used have a significant impact on the quality of the final product. Mercury and its compounds are the preferred raw materials for preparing metal *Bhasma*. There is a difference in opinion regarding the quantity and process of mixing mercury or mercury compounds with metals when preparing *Bhasma*. In this study, *Kajjali* was used as a paste in the first *Puta* and mixed with metal in subsequent *Puta's*. Additionally, equal amounts of mercury and sulfur were used in the subsequent *Puta* to assess their effect on the number of *Puta* and various parameters of *Bhasma.*

Effect on the number of *Puta and Ancient Bhasma Test*

After the completion of the third *Putakarma*, the RB demonstrated the *Rekhapurnatva*, *Varitaratva*, and *Unam* tests, indicating decreased particle size and lightness. The *Nischandratva* test results for *Bhasma* revealed the absence of free mercury and metallic silver. The *Bhasma* samples successfully passed the *Apunarbhava* and *Niruttha* tests, indicating the absence of free metals in *Bhasma*. According to Rohit et al., *Rajat Bhasma* passed the *Rekhapoornatva* test after the fifth *Putakarma*, while *Varitaratva* and *Nichandratva* became positive after the 15th *Putakarma*. *Niswadu* was evident in the tenth *Putakarma*, and the *Apunabhava* and *Niruttha* tests were positive after the 17th *Putakarma*. Naik et al. required 20 *Putakarmas* to pass all ancient parameters. The *Rekhapoornatva* was observed after the 8th *Putakarma*, while 25 *Putakarmas* were needed to pass all the remaining ancient *Bhasma* tests in the study by Durga et al. This difference is due to the use of only eight cow dung cakes in the study by Durga et al. while Rohit et al. used 22-23 cow dung cakes (2.5 kg) in the *Laghu Putakarma*, which produced a temperature range of 596^{O}C to 625^{O}C after 8 h. Naik et al. used 2 kg of cow dung cakes for the *Laghu Putakarma* and achieved a temperature range of 500-550^{O}C. However, the temperature inside *Sharava* remains less by 100-150^{O}C than the temperature inside *Puta*. In the present study, a temperature of 400^{O}C was gradually reached after 60 min, after which the muffle furnace was switched off and allowed to cool on its own. Variations in the temperature inside the *Puta*, the temperature inside the *Sharava Samputa* (sealed earthen saucer-containing pellets), and the duration of maintenance of the highest temperature can impact the quality and number of *putakarmas* required to prepare RB.

When subjected to fire, RB emits fumes. This may be attributed to the presence of excess free sulfur in the final product, which could have resulted from the use of equal quantities of mercury and sulfur in the next *Puta*. However, no ancient scholar has employed the *Nirdhumatva* test, which involves the non-emission of fumes when kept on fire, to assess the RB. RB exhibits black coloration, and it is hypothesized that silver may have undergone conversion to silver sulfide (Ag_2S) or silver oxide (Ag_2O), both of which are black compounds. This transformation could have occurred during the *Samanya* and *Vishesh Shodhana* processes because of the interaction of atmospheric oxygen with heated silver or because of the reaction between silver and sulfur utilized during the *Marana* (incineration process of *Bhasma* preparation) process.

Effect on the yield percentage

In the present study, an average 138% increase in yield was observed, whereas 22% [8] and 95% [29] weight gains were reported in previous studies. According to stoichiometric calculations, 100 g of silver can combine with 14.82 grams of sulfur to form silver sulfide. [30] If the entirety of the raw silver reacted with sulfur to form silver sulfide, there would be an approximate 15% increase in yield. The increase in yield attributable to the organic matter from aloe vera-like herbs is likely to be negligible. If the total quantity of raw silver reacts with oxygen to form silver oxide, the yield

would increase by approximately 8%. The conversion of silver to silver sulfide increased the yield by up to 15%. The higher yield (>15%) was attributed to sulfur (50.8%) and mercury (30.7%) detected in RB. The elevated percentages of sulfur and mercury were due to the use of equal quantities of mercury and sulfur in the products obtained after each *Puta*. The higher percentage reported by Diksha et al.. is attributed to the use of mercury and sulfur up to 15 *Puta*. Hence, the use of mercury and sulfur in a greater number of *Puta* increases the yield of *Bhasma*. Rohit et al. reported a slightly higher yield than expected (15%). Their lower yield percentage is due to the use of *Kajjali* in up to three out of 17 *Puta* varieties.

Effects on the physicochemical parameters

The physicochemical values of RB were as follows: pH 4.34, indicating an acidic nature; ash value of 35.49, while and acid-insoluble ash value of 32.10. These values were lower than those reported in other studies. [6-8] The lower ash value and acid-insoluble ash content may be attributed to the presence of free or loosely bound sulfur in *Bhasma*, which evaporates during the application of heat at 550°C. Additionally, the higher sulfur content may also contribute to the lower pH value compared with other studies. The use of equal amounts of mercury and sulfur in the two subsequent *Puta* may have led to lower ash values and acid-insoluble ash. Moreover, RB had a low loss of 0.18% on drying, indicating that it had minimal moisture content and an unlikely chance of bacterial or fungal growth. RB was analyzed for its silver content, which was found to be 12.88% (AAS) and 17.7% (XRF). Previous research studies have reported varying percentages of silver in RB, ranging from 26.72%, 70.72%, and 81.77%. [6-8] The current study found 50.8% sulfur, while previous studies reported 20.34%, 10.34%, and 13.15% sulfur in RB. [6-8] Approximately 14% of sulfur is required to form silver sulfide, which suggests that RB contains approximately 36% more sulfur that is not bound to silver. The lower percentage of silver in RB may be due to the presence of free or loosely bound sulfur in *Bhasma,* resulting from the use of an equal quantity of mercury and sulfur in the subsequent two *Puta*. An XRF study found 30.7% mercury in the RB. The presence of mercury in RB was attributed to the equal quantities of mercury and sulfur used in all three *Puta*. Durga et al. found 45.51% Hg in RB was due to the use of *Kajjali* in all 25 *Puta* and application of less quantum of heat generated through eight cow dung cakes. [6] Durga et al. identified mercury sulfide peaks in their XRD study, which were not observed in the present study.

Effect on chemical compounds formed

The application of heat in an open environment during the *Shodhana* process may lead to the reaction between silver and oxygen. This may have led to the formation of silver oxide crystals during *Vishesh Shodhana*. After the third *puta*, silver oxide molecules were reduced in comparison with silver oxide molecules after *Vishesh Shodhana*. The complete decomposition of silver oxide to silver and oxygen gas occurred at 400°C. The application of heat (approximately 400°C) during *Puta* may lead to the breakdown of the silver oxide formed during *Shodhana*.. Silver molecules react with the

sulfur present in *Kajjali* to form silver sulfides. During the *Marana* procedure, the molecular structure of silver sulfides transforms into a cubic slender crystalline structure. The application of heat between 350 °C and 580°C led to the formation of silver sulfide with cubic body-centered crystals (argentite). The presence of silver oxides in *Shodhita Rajat* and silver sulphides in RB is consistent with previous research in which an amalgam of silver and mercury was prepared and subsequently formed into *Kajjali*. [6-8] However, silver sulphide exists in the form of orthorhombic crystals of Acanthite. [7] Durga et al. identified major peaks of HgS and Ag2S in their XRD study. The presence of HgS peaks is attributed to the use of *Kajjali* in each *Puta* and the application of a lower quantum of heat generated through eight cow dung cakes. [6]

Effect on Particle Size

The average size of the RB particles was determined to be 453.5 nm, with 96% of the particles falling within the range of 381.1 to 481.8 nm. The maximum particle size is relatively uniform and stable. However, the particle size did not fall within the nanoscale range (1-100 nm), despite passing all ancient tests after the third *Puta*. Some researchers have produced RB by creating an amalgam of *Rajat* with mercury and subsequently forming *Kajjali* using *Shuddha Gandhak*. It was found that the volumetric mean diameter size of RB after the 17th *Puta* was 17.28 µm, and the smallest particle size was 2.30 µm. [31] According to Naik et al., the mean particle size of RB was 54.16 nm, as calculated through SEM images. [6] Durga et al. reported that the mean particle size of RB particles is 2.7 nm. [5] An increased number of *Puta* and the use of mercury in consecutive *Puta* may lead to a decrease in the particle size of RB.

The utilization of *Kajjali* paste in the initial step, followed by equal quantities of mercury and sulfur in the subsequent two steps, enabled the transformation of *Shodhita Rajat* into quality RB (which meets all ancient *Bhasma* criteria) within the three *Puta*s. An increase in RB yield was also observed. This phenomenon is attributed to the presence of mercury and excess sulfur in RB, resulting from the use of equal quantities of mercury and sulfur in the two consecutive *Puta*. This was further evidenced by the lower percentage of silver. Raw silver is fully converted into cubic slender crystals of silver sulfide, which can also facilitate size reduction to 300-400 nanometers. The application of more than three *Puta* may reduce the particle size of silver sulfide to below 100 nm. The presence of excess sulfur may facilitate the movement of *Shakhagat Pitta* (*Pitta* accumulated in the body tissues) into *Kostha* (intestine) due to the *Pitta Sarak* (excretion of bile through the liver) property of sulfur. This may enhance the antibacterial properties of RB. If mercury is utilized in every *Puta* until the formation of quality *Bhasma*, as observed in the present study and Durga et al., then mercury is present in the final *Bhasma*. Consequently, it is advisable to avoid using equal quantities of mercury after a second or third *Puta*. However, there is a need to investigate the effect of 1/4 quantities of mercury in the second or third *Puta* on the number of *Puta* and the presence or absence of mercury in RB. The addition of sulfur to the next two consecutive *Puta*

may result in the presence of excess free sulfur in the RB. Further studies are required to explore the role of excess sulfur in augmenting *Pitta Sarak* and antimicrobial properties in experimental studies. Additional research is necessary to determine whether the presence of Hg and free sulfur in RB is detrimental.

CONCLUSION

The application of *Kajjali* paste in the initial *Puta,* equal amounts of mercury and sulfur in the subsequent *Puta's* resulted in a reduction in the number of *Puta's* required to produce *Rajat Bhasma.* The number of *Puta* decreased to three. This change led to variations in the particle size and percentage of silver, mercury, and sulfur in *Rajat Bhasma*. However, the silver compound in the final product remained unchanged, specifically the presence of silver sulfide in *Rajat Bhasma*. The higher quantity of sulfur present in *Rajat Bhasma* and the variation in the particle size warrant further investigation into its safety and effectiveness in animal studies. Thus, it can be concluded that alterations in raw materials, quantity, and processes affect the quality of the final product. Further elaborative studies are required to determine the exact method of mixing mercurial compounds and the quantity of mercurial compounds to be added as per the number of Puta to obtain the safe and therapeutically best *Rajat Bhasma*.

Abbreviations:

RB- *Rajat Bhasma*

XRD-X-ray diffraction

XRF- X-ray Florescence

SEM-Scanned electronic microscope

DLS-Dynamic Light Scattering

AAS-Atomic Absorption Spectroscope

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