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Formulation and Evaluation of Anti-Arthritic Activity of Newly Prepared Rajata Bhasma

Afrin Alam[1*], Ashish Sarkar[1]

[1]School of Pharmacy YBN University, Ranchi, Jharkhand, India.

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Corresponding Author: Afrin Alam. School of Pharmacy YBN University, Ranchi, Jharkhand, India.

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Abstract

Bhasma is a powder of a substance obtained by calcination is called Bhasma. One of the herbs, metallic concoctions used in Ayurveda, an ancient Indian medical system, to treat a variety of illnesses is Rajata Bhasma. It needs to undergo various classical procedures like Shodhana and Marana to make it assimilable for the body. A number of steps in the pharmaceutical process are used to prepare a formulation. The preparation of different minerals, herbomineral, metallic, herbs-metallic, and marine-herbal compositions is the focus of the Ayurvedic field of rasa shastra. Since Rajat (silver) cannot be admitted in metal form, it is recommended that it be transformed into Bhasma form using several processes. The entire process of making Bhasma is considered part of its pharmaceutical preparation then after Rajat Bhasma is evaluated for Anti-Arthritic activity suitable methods are used for in vivo estimation of pharmacological activity.

Keywords: Rajat Bhasma, Anti-Arthritic, Nanoparticles, Immunomodulators, Inflammation.

Introduction

Rheumatoid arthritis (RA) is an inflammatory disease that causes severe disability and is typified by synovial inflammation and irreversible joint degeneration. Approximately 1% of people worldwide have been impacted, with a male to female. This disease's cause is currently unknown. This condition is caused by a variety of pro-inflammatory chemicals, such as reactive oxygen species, prostaglandins, leukotrienes, and cytokines generated by macrophages. The suppression of enzymes like COX and LOX for the metabolic control of arachidonic acid and the regulatory checks of these mediators released by immune cells may be the prospective targets for the treatment of chronic inflammatory diseases.**[1-4]** Given all of these facts, it is imperative to determine the analytical profile of Ayurvedic medicine in order to determine the nature of the final product. The current work was conducted to establish the analytical profile of Rajata Bhasma by evaluating physicochemical characterization and using advanced modern tools and techniques. Rajata Bhasma is a very popular organo-metallic preparation that is indicated in Sosha, Dhatukshaya, Prameha, Madatyaya, Visham Jwara, Pittaroga, Pleeharoga, Buddhimandya, Garbhashaya, Dosha, and Apasmara.**[5]** Bhasmas are a unique dosage form of Rasa Shastra as they have great therapeutic value. They get absorbed easily in the body even in very small doses and are quite effective. Nowadays, nanotechnology is an evolving topic of research and the scientific world. Here is a detailed description of the methods used to prepare Rajata Bhasma, including the specifics of the tools utilized in the pharmaceutical investigation.[6-7]. The present study aims to formulate the best method of preparation for Rajat Bhasma followed by physicochemical evaluation and study of the Anti-Arthritic activity of Rajat Bhasma.

Materials and Methods

Collection of Raw Materials

The best variety of Rajata was procured from the local jewelers' market of Ranchi and Parada, Gandhaka, Hingula, Tila taila & Kulattha procured from the local medicinal drug market, Nimbus was collected from the local vegetable shopkeeper and Ghritakumari collected from herbal garden of School of Pharmacy YBN University, Ranchi, Gomutra was procured from School of Agriculture Sciences, YBNU, Ranchi. The subject experts in the concerned department of the institute authenticated all the samples. Steel jar, Khalwa yantra (mortar and pestle), Sharava (earthen saucer), and Upala (cow dung cakes) are used in the procedure.

Preparation of Rajata Bhasma: The following steps involved in the Preparation of Rajata Bhasma

1. Raw material purification, i.e. Gandhaka, Rajata.
2. Parada extraction.
3. Rajata Bhasma (RB1) and Rajata Bhasma (RB2) are prepared using the puta technique.

Shodhana of Rajat - Nirvapa was used to purify the silver in Rajat Shodhan.

Samanya Shodhana of Rajata

Requirements- Rajata- 245 g, Tila Taila (Sesamum indicum Linn.)- 1500 ml, Takra- 1500 ml, Gomutra-1500 ml, Kanji-1500 ml, Kullatha Kwatha (Dolichos biflorus Linn.)-1500 ml.

Methodology- For Samanya Shodhana, thin silver foils were heated to red hot and dipped three times in each of Takra (Buttermilk), Tila Taila (Sesame oil), Gomutra (Cow's urine), Kulattha kwacha (decoction of Dolichos biflorus Linn.) & Aranala (Sour gruel made from rice).**[8-9]**

Vishesha Shodhana of Rajata

Requirements-Samanya Shodhita Rajata, Nimbu Swarasa (Citrus medica-3500 ml)

Methodology-Samanya Shodhita Rajata was reheated till red hot and then quenched seven times in Nimbu Swarasa. After each dipping, the juice was replaced. Finally, Shuddha Rajata was carefully collected.**[10]**

Gandhaka Shodhana- The dhalana technique was used to purify the Gandhaka.

Methodology- Raw Gandhaka was placed in a stainless steel container with 1/4th Ghee and melted over low heat. A four-layer muslin cloth was wrapped over the lip of the milk jug. The mixture was poured into the container using cotton fabric as soon as the Gandhaka melted. The procedure was then performed two more times. Gandhara was washed with hot water and dried after that. **[11-12]**

Parada - The Nadayantra technique was used to extract Parada from Hingula.

Procedure- Hingula was levigated with Nimbu swarasa and dried before being ground into a fine powder. On a cotton fabric equivalent to the weight of Hingula, uniform layers of powder were created, fastened with thread, and lit. To make the collecting of Parada easier, an earthen pot was inverted while it was being ignited. Parada was carefully gathered and filtered through four layers of folded cotton cloth **[13]**

Preparation of Rajat Bhasma

Rajat Bhasma was prepared in two distinct ways:

RB1 was made using 9 puta and RB2 was made with 17 puta. 12 techniques. In a mortar with a parade, amalgam was created when Rajat foil was broken up into little bits. Purified Gandhara was added to the amalgam and triturated till a suitable Kajjali was formed. Following that, impregnation with kumara swarasa is used to prepare Chakrikas (Pellets). In a sharvana, dried chakrikas were arranged, and laghu puta was offered. Rajata was fully powdered after the first puta. Half of the kajjali was added to the next two putas, which were then triturated with kumara swarasa and served. In place of kajjali, half of a gandhaka was added from 4 to 9 puta (Considered as an RB1). The other putas were completed without Kajjali or Gandhaka. Rajat Bhasma, 17 puta were supplied, which passed all classical parameters (Considered as a RB2).

Result and Discussion

Organoleptic and Physicochemical analysis of the prepared sample of Rajata Bhasma was carried out by classical parameters such as sound, touch, color, taste), Gandha (odor), and Niruttha test. Physicochemical Analysis was also carried out by following the standard methods for estimation of loss on drying, ash value, and acid-insoluble ash. In addition to this SEM was also carried out. **[13-15]**

Studied Parameters	Observations (% w/w)	
	RB1	RB2
Loss on drying	0.02±0.32	0.02±0.18
Total ash value	27.13±0.64	27.32±0.24
Acid insoluble ash value	12.33±0.25	17.21±0.33
Water soluble ash value	1.12±0.23	1.54±0.22

Particle size Analysis: The particle size of both the sample RB1 and RB2 were decreased extensively, which assisted in the assimilation and absorption of the drug into the body. The particle sizes of RB1 and RB2 were in the range of 3.48 – 57.11 µm, and 2.52 – 47.33 µm respectively.

SEM micrographs of RB1 and RB2 affirm the formation of micrometer (µm) samples due to a cluster of nanocrystallites. SEM also suggests a polycrystalline form of Bhasma. There may be the presence of several crystallographic grain boundaries in a single particle, which might be due to mixing with different herbs or due to heat treatment multiple times.

Anti-Arthritis Activity

According to experimental models, autoimmune arthritis may be brought on by mycobacterial infections, mostly through T-cell-mediated reactions. Rats were given injections of dead mycobacteria in liquid paraffin to cause arthritis. When compared to the standard and fraction-treated rats, the rat paw volume of the FCA-injected control rats is much larger. Rat paw edema volume was significantly reduced by isolated chemical therapy at a dose of 20 mg/kg when compared to the control group. The impact of RB1 and RB2 on arthritis produced by Freund's adjuvant model. It was discovered after 28 days that RB1 and RB2 significantly exhibit dose-dependent suppression of paw thickness, meaning that the chronic inflammation brought on by the adjuvant results in a reduction in paw thickness. Following the treatment of RB1 and RB2, the paw thickness dropped by 0.72±0.44 and 0.66±0.44 ml, respectively. Following the induction of Freund's adjuvant, the paw thickness, which was 0.24±0.92 ml, was dramatically reduced by the standard medication indomethacin. When compared to RB2, the RB1 was shown to be the most effective. In comparison to control animals, the injection of RB1 and RB2 into animals with Freund's adjuvant-induced arthritis increased their RBC and hemoglobin levels. The WBC count and ESR were significantly reduced after the administration of RB1 and RB2 compared to control animals.

Experimental Design

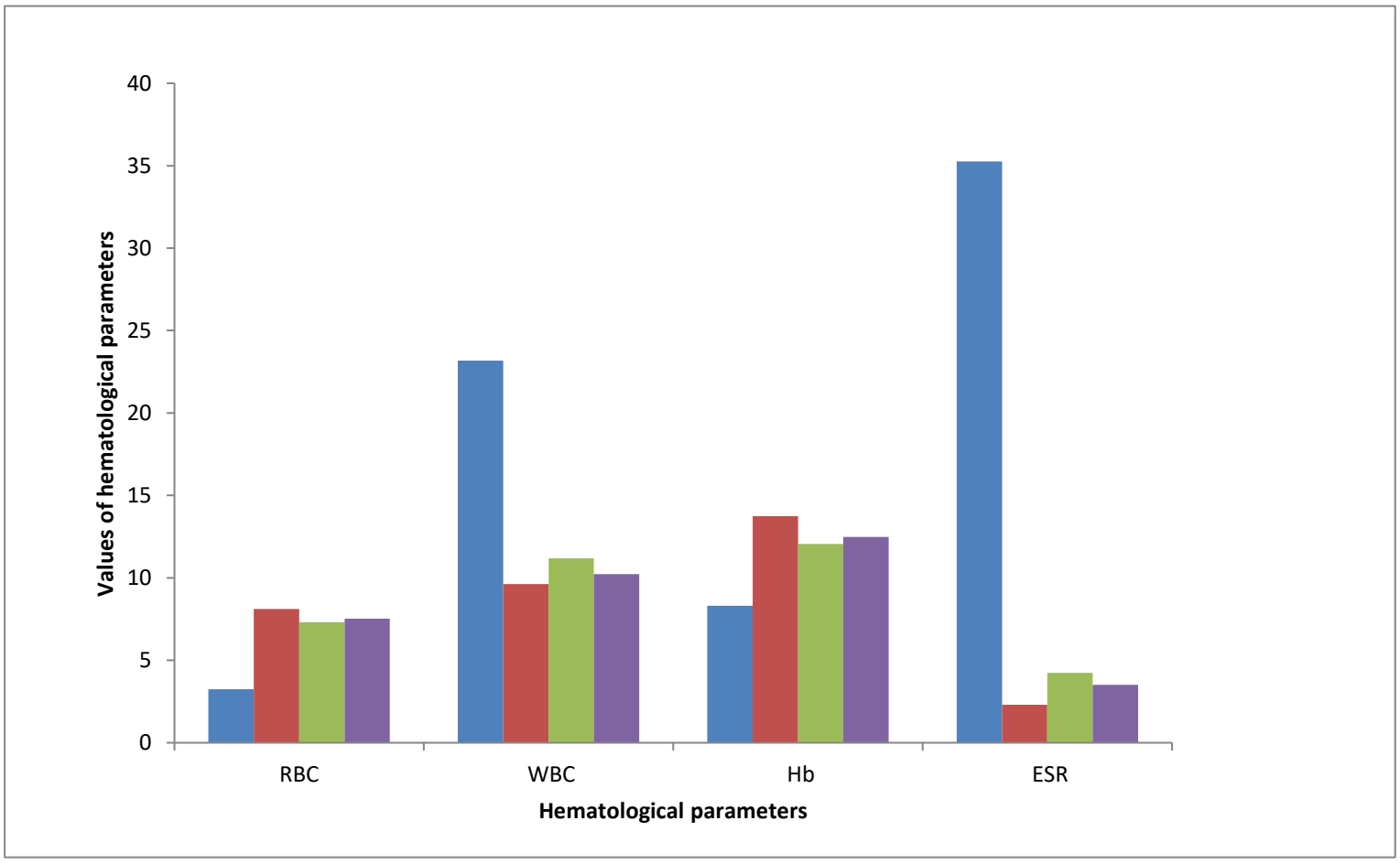
Either Sex Wistar rats (150-200 gm), were used and kept in quarantine for 10 days under standard husbandry conditions (27.3 oC, Relative humidity 65 ±10%) for 12 hrs in dark and light cycles respectively and were given standard food and water *ad. libitum*. All experiments were approved by the institutional ethical committee and were carried out according to the animal ethics committee guidelines.

Five groups of six rats each were randomly selected from among the rats one week before the experiment's start. Rats were given 0.1 milliliters of Freund's complete adjuvant (FCA) in the subplantar (s.p.) area of their left hind paw on day 0. A final concentrate of 0.6 mg/mL is obtained by extensively crushing Mycobacterium butyricum suspended in heavy paraffin oil using a pestle and mortar. The following day, the standard medication and test substances were administered, and this process lasted for 28 days. Four

groups of six experimental rats each were randomly selected, and they received the following treatment:

- Group I (control group) – Arthritic rats treated with distilled water.
- Group II – Arthritic rats treated with the standard drug Indomethacin at 10 mg/kg body weight.
- Group III – Arthritic rats treated with RB1 at 25 mg/kg body weight.
- Group IV – Arthritic rats treated with RB2 at 25 mg/kg body weight.

	Paw Volume After Induction				
	Day 0	**Day 7**	**Day 14**	**Day 21**	**Day 28**
Control	0.21±0.32	0.73±0.47	0.98±0.96	1.12±0.25	0.98±0.42
Indomethacin (10 mg/kg)	0.27±0.54	0.53±0.83*	0.45±0.63*	0.32±0.59*	0.23±0.91*
RB1 (25 mg/kg)	0.22±0.14	0.63±0.35	0.51±0.47*	0.40±0.47*	0.72±0.44 *
RB2 (25mg/kg)	0.26±0.28	0.59±0.62	0.48±0.41*	0.37±0.91*	0.66±0.44 *



The only Ayurvedic metal-based preparation is Bhasma, which is created by combining plants and transforming them into the proper form using intricate pharmacological procedures. Since the seventh century BC, they have been used as a preventive and supplemental alternative medicine on the Indian subcontinent, and they are frequently suggested for the treatment of a range of chronic illnesses. Autoimmune disease i.e. Arthritis cannot usually be successfully controlled by conventional modern therapy. Rajata Bhasma can be a good alternative to treat Arthritis. One of the elements influencing a drug's absorption and dissolution is particle size. Surface area and particle size are inversely proportionate to one another, with surface area increasing as particle size decreases. This causes the medicine to dissolve more readily and absorb more quickly. In contrast to the raw material, the particle cluster in the finished Bhasma is once more regular and homogenous. In this case, the reduction in particle size may be caused by the ensuing heat treatment in the form of Puta and wet grinding in the form of Bhavana. One way to describe the particle size is as the intended Bhasma standard. Following 17puta, Rajatabhasma was deemed the ideal bhasma for internal use after meeting the requirements of bhasmapariksha. The usage of several medications and puta was used to illustrate how Rajatabhasma was prepared. Because the bhasma made from Rasa is of the highest caliber, Shudha Gandhaka and Shudha Parda were used in this instance. To improve the medicinal qualities, various pharmaceutical process steps are employed. The pharmaceutical preparation of Rajata Bhasma by eliminating different contaminants in accordance with classical texts. As a byproduct of burning Rajata, Bhasma was also analyzed using both traditional and contemporary analytical methods to determine its physiochemical, microscopic, and chemical composition. Solid colloidal particles with sizes between 1 and 100 nm are called nanoparticles. Nanoparticles overcome barriers because of their small particle size. by the body's physiological barriers and readily pass through the blood vessels' stomach epithelium, cell walls, and brain barriers. Polymetric nanoparticles are the perfect medication delivery vehicle for antibiotics, vaccinations, and Immunomodulators as well as other treatments.

Conclusion

In the current investigation, arthritic control rats exhibited elevated erythrocyte sedimentation rate (ESR), decreased Hb levels, and decreased RBC count. Anemia is indicated by all of these symptoms. Significant recovery from the induced anemia was demonstrated by the RB1 and RB2 treated groups. The adjuvant-induced arthritic rats' markedly elevated leukocyte count might be the result of the immune system being stimulated to fight off invasive antigens, while the RB1 and RB2 treated groups' markedly decreased leukocyte counts demonstrated the immunomodulatory

impact. This demonstrates Rajat Bhasma's anti-arthritic properties. According to this study, Rajat Bhasma nanoparticles are what give it its anti-arthritic properties, and the active ingredient is what causes the results that are seen. Additional research is being done to determine the potential mechanism and responsible for biological activity. This study confirmed that nanoparticles obtained from Rajat Bhasma are responsible for its anti-arthritis activity and the effects observed are attributable to the presence of active constituent. Further studies are carried out for the possible mechanism and the identification of the active component responsible for anti-arthritis activity.

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