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Original Article

Physicochemical and pharmacological assessment of a traditional biomedicine: *Mukta shouktic bhasma*

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Abstract

Mukta shouktic bhasma (MSB) is a traditional Ayurvedic medicinal preparation. This biomedicine is synthesized through special calcination of mother of pearl as mentioned in the classical Ayurvedic text. Physicochemical characterization of MSB was carried out using modern techniques such as transmission electron microscopy, scanning electron microscopy, X-ray powder diffraction analysis, Fourier transform infra-red spectroscopy, inductively coupled plasma analysis, energy dispersive X-ray analysis, and thermogravimetric analysis. The study showed that the raw material *mukta shouktic* (mother of pearl) is an organo-mineral matrix containing calcium carbonate in aragonite form. The aragonite form of calcium carbonate transforms to a stable calcite form during the process of bhasma formation and forms the main crystalline component of MSB. The heat treatment does result in partial conversion of calcite to calcium oxide, which appears as calcium hydroxide (not more than 2% w/w) in the final product. The organic content of processed material degraded gradually. Physical evaluation revealed that MSB is a fine grayish white powder having a poor flow property with narrow particle size distribution of 1.22 to 22.52 μm having a mean particle size of 10.20±0.45 μm. A clearly identifiable fraction of MSB particles was below 50 nanometer. The presence of nanosized particles in MSB might impart the therapeutic property of this medicine. Trace element analysis of MSB revealed the presence of metals, like arsenic, lead, chromium, cadmium, mercury, and tin under regulatory acceptable limits at the prescribed dose of MSB. Energy dispersive X-ray analysis revealed calcium as the major element (40.22 wt %) in MSB. Microbial load for the formulation was found to be within limits. Animals were found to be safe up to a maximum dose of 2000 mg/kg body weight in acute toxicity studies. A significant (P<0.05) reduction in hyperpyrexia in rat was produced by MSB.

Key words: Ayurveda, calcium preparation, mineral preparation, mother of pearl, antipyretic

1. Introduction

Ayurveda is an ancient traditional medicine system, originated in India and evolved and practiced over thousands of years. *Bhasma* is the well known potent preparation of the traditional Ayurveda. *Bhasma* literally means 'ash'. *Bhasmas* are inorganic preparations produced by an alchemic process, which converts a metal or mineral into its compounds like carbonates, oxides, etc. *Bhasmas* of iron, calcium, copper, tin, silver, gold, lead, and zinc are commonly used. The advantages of these preparations over plant preparation is their stability, lower dose and easy availability (Anonymous, 1978; Sharma, 1985; Mishra, 2004). The lack of understanding of traditional methods resulted in a difficulty to reproduce authentic preparations.

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Very few reports are available where attempts have been made to understand the physico-chemical properties of *bhasma*. Dixit (1987) first gave the scientific basis for standardization of *bhasma*. Chemical and pharmacological evaluation of karpura shilajit bhasma (asphalt ash), swarna bhasma (Gold ash), tamra bhasma (copper ash), lauha bhasma (iron ash), godanti bhasma (gypsum ash), have been reported by Pandit *et al.* (1999), Mitra *et al.* (2002), Wadekar *et al.* (2005), Saleem *et al.* (2006) and Dubey *et al.* (2007), respectively. Nanoparticles have been reported in jasada bhasma (zinc oxide ash) and sawarna bhasma by Brown *et al.* (2007) and Bhowmick *et al.* (2008). The literature reveals the need of scientific methods for assessing and maintaining the quality of this ayurvedic preparation.

Mukta shouktic bhasma (MSB) is a calcium-containing *bhasma*. This biomedicine is synthesized through special calcination of the mother of pearl. MSB is used as an antacid, anti-pyretic, and as a source of calcium. It is also used in tuberculosis, cough, asthma, dysmenorrhea, arthritis, rheumatism, conjunctivitis (Sharma, 1985). Recent studies have shown that adding heated oyster shells to the diet of elderly patient increased the bone mineral density of the lumbar spine (Bailmain *et al.,* 1999). MSB is one third to one half as potent an anti-inflammatory as the amino salicylic acid (Chauhan *et al.,* 1998). Further, even as MSB is widely used for its antipyretic activity, there is no scientific reports on antipyretic activity of MSB. Considering all these facts, it was found worthwhile to carry out a systematic and scientific study of MSB with respect to physico-chemical properties, toxicity, and antipyretic activity.

2. Material and Methods

2.1 Raw materials

The mother of pearls (pearl mother shells, *mukta shouktic*) obtained from *Pinctada Margaritifera* belonging to genus *Pinctada Roding* were procured from a local market of Indore (Madhya Pradesh), India. *Nimbu swarus* is fresh lemon juice obtained from the fruits of *Citrus lemon* (Family: Rutaceae). The lemon juice was filtered using muslin cloth. *Aloe vera* gel is a colorless mucilaginous fillet of gel (parenchymatous cell mass) obtained by peeling off the outer layers of the leaves including the pericyclic cells of more than two-years-old *Aloe vera* (L.) Burm. f.

2.2 Chemicals

Paracetamol injection (Paracip, Cipla labs ltd., Goa, 150 mg/ml) was procured from a local market.Commercially available dried baker yeast (*Saccharomyces cerevisiae*, Saf do Brasil Produtos Alimentycios Ltd, Brazil) was suspended in pyrogen-free 0.9%w/v sodium chloride in a water bath at 37^{o}C for 5 min (50mg/ml). All chemicals used for the physico-chemical analyses were of analytical grade and procured from Merck (Mumbai, India).

2.3 Animals

Albino rats of Wistar strain of either sex weighing between 150-200 g were used. The study was approved by the institutional ethical committee (465/01/96/CPSCSEA), which follows the guidelines of CPSCEA (Committee for the Purpose of Control and Supervision of Experimentation on Animals), which complies with international norms of INSA (Indian National Science Academy).

2.4 Preparation of MSB

MSB was prepared under the guidance of an authentic traditional practitioner, whose family has been synthesizing *bhasmas* for a few generations as per the method described in Ayurvedic texts (Anonymous, 1978; Sharma, 1985). The process of synthesis of *bhasma* is divided broadly into three stages:

1) Cleaning (*shodhana*): The mother of pearl fragments was gently crushed to smaller fractions of <10 mm using an agate mortar and pestle. Pieces of mother of pearl were first cleaned with hot water to remove dirt material. The mother of pearl fragments were then immersed in lemon juice (*nimbu swarus*) and boiled for 90 min in a specially prepared hanging sealed earthen pot (*dola yantra*). This process is known as boiling (*swedana*). The solution was filtered off to get the cleaned mother of pearl fragments (*shodhit mukta shoutik*), which were subjected to first calcination. For calcination the cleaned mother of pearl fragments were placed in sealed earthen pot (*sarava samputta*) and subjected to ignition in a traditional furnace (*gaja-puta*) as described in Ayurvedic literature to obtain an intermediate. The stable intermediate can be stored in sealed earthen pot untill further use.

2) Trituration (*bhavana*): The intermediate obtained after the first calcination was then treated with *Aloe vera* gel and triturated using an automated mortar and pestle at 1000 rpm (Pharmaceutical machinery Ltd., Mumbai). The total time of trituration was 8 hrs. The mixture was pressed in the form of cakes (*chakrikas*) and dried in the shade for 48 hrs. These dried cakes were immediately subjected to further processing.

3) Calcination (*marana*): The cakes were calcinated to obtain the intermediate. The procedure was repeated two more times with *bhavana* untill the sample showed a positive response to all the traditional tests (Anonymous, 1978; Sharma, 1985) for bhasma (Table 1), to obtain the final product MSB.

Bhasma preparation and its physicochemical evaluation were performed in triplicate.

2.5 Physicochemical evaluation

All the samples were scanned on Phillips make X-pert powder diffractometer and 2θ scan was from 10^{o} to 100^{o} using Ni filter Cu K alpha radiation and NaI scintillator. The

Table 1. Traditional Tests for formation of *bhasma*.

S. No.	Test
1.	No metallic luster observed
2.	Product fills the finger lines when taken between index finger and thumb
3.	Sample floats on water
4.	Product did not regain luster on heating again at same temperature.
5.	No gain in weight of Ag metal piece (sample + Ag metal piece, ignite)

FTIR spectra were recorded on Thermonicolet IR-200 spectrophotometer with DTGS detector in the region of 400 to 4000 cm^{-1}. Each spectra is an average of 24 scans of 2 cm^{-1} resolution. The thermo gravimetric analysis was performed using a Perkin Elmer series TG analyzer up to 1000°C at a heating rate of 10°C per min in air atmosphere. Quantitative elemental composition was carried out by EDAX (EDAX Inc., Mahwah, NJ, USA). A Perkin Elmer ELAN 6000 ICP equipped with an As-91 was used for the determination of trace elements. The mean particle size and particle size distribution was studied using a Brookhaven-Zeta plus (Holtsville, NY, USA) instrument. A transmission electron microscope (TEM) (Tecnai-G2, FEI, Hillsboro, USA) was used to study the nanosized particles in bhasma. Surface analysis of particles was done using scanning electron microscope (SEM) (JEOL JSM 200).

2.6 Evaluation of powder properties

Bulk density and tapped density were determined using standard methods. These values were used to indirectly calculate the flow properties by deriving Carr's index. The static angle of repose was determined by the funnel method (Anonymous, 1996; Martin *et al.*, 1991).

The calcium carbonate and calcium hydroxide content in MSB was determined using titrimetric procedure. The acid base titration was used to determine the carbonate ion and complexometric titration was used to determine the calcium ion (Anonymous, 1996).

2.7 Microbial evaluations

Microbial evaluation of MSB was carried out according to Pharmacopoeia of India (Anonymous, 1996). The bhasma was tested for the presence of contaminating fungus (yeast and moulds), *Staphylococcus aureus*, *Salmonella* sp., *Escherichia coli*, and total aerobic microbial count.

2.8 Acute toxicity studies

Rats were fasted for 24 hrs prior to drug administration. A total of five animals were used. MSB uniformly dispersed in 2% sodium carboxymethylcellulose suspension (500 mg/ml) was administered as a single oral dose equivalent to 2000 mg/kg body weight. Food was withheld for a further 4 hrs. Animals were observed individually at least once during the first 30 min after dosing, and then periodically during the first 24 hrs (with special attention during first 4 hrs), and daily thereafter for a period of 14 days. Mortality, if any, was determined over a period of 2 weeks (OECD, 2001). LD_{50} was calculated as per OECD guidelines.

2.9 Antipyretic activity studies

MSB uniformly dispersed in 0.9%w/v sodium chloride solution (500 mg/ml) as a vehicle and was administered orally at a dose of 250 and 500mg/kg. Paracetamol (150mg/kg) is used as a standard antipyretic drug and is administered to the control group.

Induction of bakers yeast induced pyrexia:

The animals were transferred to the experimental room 2 hrs before the experiments for acclimation to the environment and were trained to remain quiet in a restraint cage. Four groups of animals, each group containing eight animals were housed, and the initial basal rectal temperature was measured. All animals were injected with baker yeast (135 mg/kg, i.p.). Rats were then returned to their housing cage. Temperature changes were recorded every hour up to 12 hrs using a digital thermometer (SK-1250MC, Sato Keiryoki Mfg. Co., Ltd., Japan), and expressed as the difference from the basal value. Animals received treatment at the fourth hour after yeast administration. Normal saline (5 ml/kg, body wt.) was administered orally to the first group (control group) of animals. The second group received paracetamol (120 mg/kg, i.p.). The third and fourth group of animals were administered with MSB 250 mg/kg and 500 mg/kg body weight (per oral) respectively (Tomazetti *et al.*, 2005; Hullati and Sharada, 2007).

2.10 Statistical analysis

The statistical analysis was carried out using GraphPad Prism, version 5.0. All *in-vivo* experimental results were expressed as mean ± standard error of the mean

3. Results

3.1 Preparation of *Mukta shouktic bhasma* (MSB)

The MSB was prepared following strictly the method mentioned in the ayurvedic text. The calcination was repeated till sample gave positive results to all tests for *bhasma* as mentioned in Ayurveda (Table 1).

3.2 Crystalline phase identification with X-ray diffraction (XRD)

The starting material in the preparation of MSB was

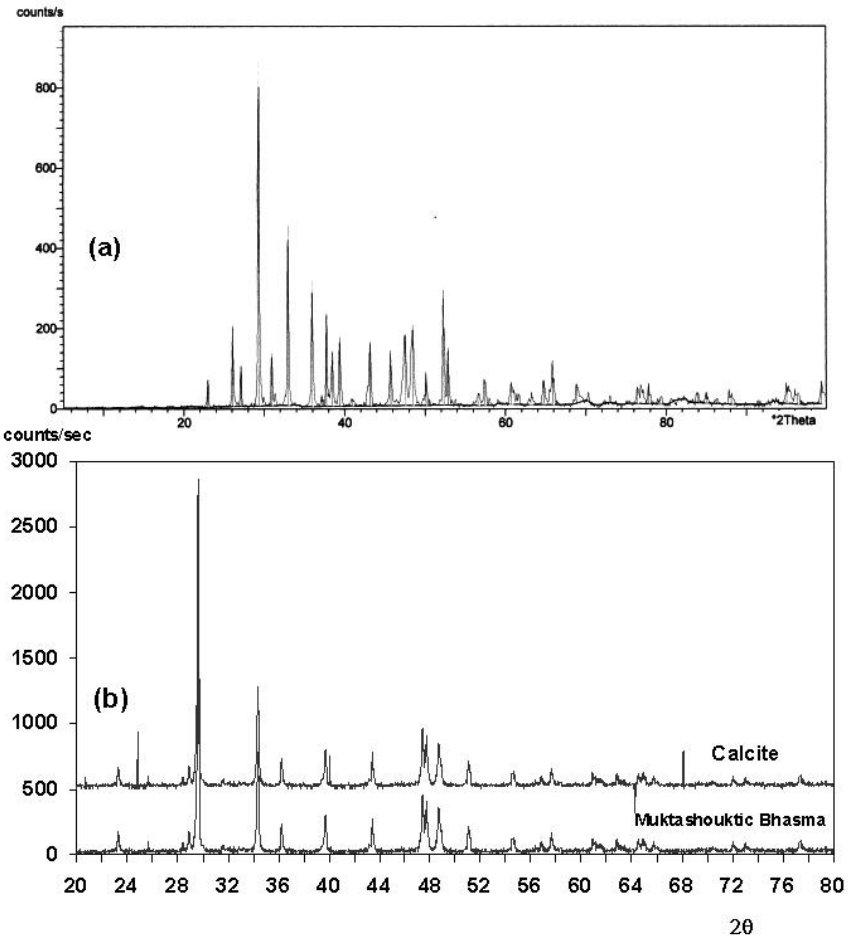

Figure 1. (a) XRPD graph of mother of pearl (b) XRPD graph of calcite and MSB.

powdered mother of pearl with a characteristic peak of aragonite (Figure 1(a)) appearing at d = 2.70 Å (2θ = 32.94), d = 2.49 Å (2θ = 35.97) d = 2.38Å (2θ = 37.72) and d = 1.74Å (2θ = 52.25). The observed results are in confirmation with reported composition of mother of pearl (Bailmain *et al.*, 1999; Marin and Luquet, 2005). XRD pattern of MSB was compared with standard calcite as shown in Figure 1(b). Sample identification was done by matching the d-spacing with the standard JCPDS database. The diffraction patterns indicate calcite as the major crystalline phase present in MSB. Peaks at d = 3.04 Å (2θ =29.28), d = 2.28 Å (2θ = 39.32), d = 1.91 Å (2θ = 47.41), d = 1.87 Å (2θ = 48.42), and d = 1.42 Å (2θ = 65.44) confirm the presence of calcite as the major crystalline phase in the sample. In the MSB sample the lines of aragonite disappeared and the product contains only the calcite form of calcium carbonate. Low intensity lines indicate the presence of calcium hydroxide, d= 2.628 Å (2θ = 34.00), 4.9 Å (2θ = 17.94), 1.927 Å (2θ = 47.41). This may be attributed to the hydrolysis of calcium oxide formed due to partial decomposition of calcite during calcination cycles (Engin *et al*., 2006). The mean crystal size of the MSB particles was calculated from the XRPD pattern (2θ for 100% intensity peaks) following the Scherer's Equation (Wadekar *et al.*, 2006). The mean crystal size of MSB was found to be 30.48 nm whereas the mean crystal size of standard calcite was 39.80 nm (Table 2). MSB particles have a smaller crystal size than standard calcite.

3.3 Fourier transform infra-red spectroscopic studies

Bailmain *et al.* (1999) and Bowen and Tang (1996) reported the IR spectrum of mother of pearl, in which they obtained the carbonate ions of the mineral with the internal vibration modes of CO_3^{2-} ν_4 mode at 713 cm^{-1} and 700 cm^{-1}, ν_2 mode at 864 cm^{-1} and 844 cm^{-1}, and ν_1 mode at 1090 cm^{-1} and 1490 cm^{-1}, respectively. The strong IR band at 1709 cm^{-1} attributed to C=0 groups of the carbonate ions. The splitting of ν_4 is a characteristic of the aragonite structure of calcium carbonate. A broad absorption around 3400 cm^{-1} indicates the stretching vibration of a structural water molecule. Absorption peaks for organic matter were observed between 3000 and 2500 cm^{-1}. The IR spectrum of powdered mother of pearl (Figure 2(a)) obtained during the study was in confirmation with reported data on mother of pearl and different crystal forms of calcium carbonate (Bailmain *et al.*, 1999; Dickinson and Macgrath, 2001). It showed all the character-

Table 2. Comparison of crystal size of standard calcite and MSB using XRPD.

Sample	2θ values at I/ I_0 = 100 (degree)	FWHM (radian)	d-Value (Å)	Crystallite size[(a)] (nm)
MSB	29.32	0.0047	3.04282	30.48
Standard calcium carbonate (calcite)	29.405	0.0036	3.03288	39.80

[(a)] Scherrer's equation, $d = 0.9\lambda/\beta\cos\theta$, where d is diameter of crystal, λ is X-ray wavelength of analysis, β is FWHM in radian, θ is theta in Braggs equation for X-ray diffraction.

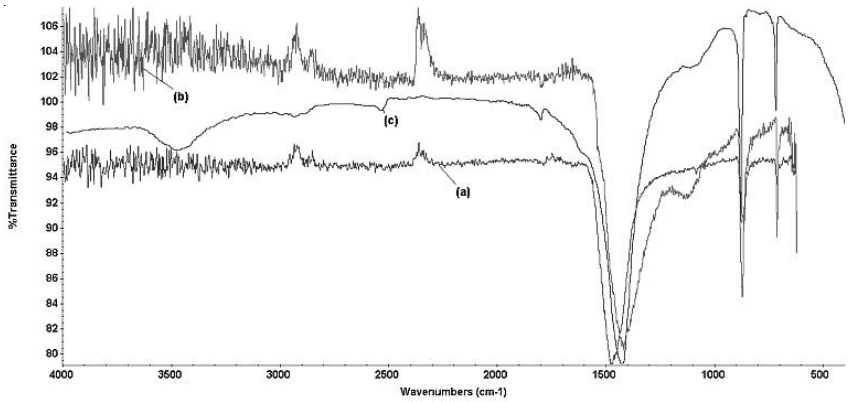

Figure 2. (a) FTIR spectrum of mother of pearl (b) FTIR spectrum of MSB (c) FTIR spectrum of calcite.

istic bands for natural aragonite in the region of 4000 to 400 cm^{-1}.The studies confirmed that powdered mother of pearl is an aragonite crystal of calcium carbonate embedded in an organic matrix.

Characteristic peaks of the calcite form of calcium carbonate (Figure 2(c)) with few modifications were evident in the IR spectra of MSB (Figure 2(b)). Absorption bands of CO_3^{2-} molecules shifted to higher energies (ν_3 mode to 1470 cm^{-1}, ν_2 mode to 1040 cm^{-1}, ν_4 mode to 820 cm^{-1}) in the final product MSB as compared to standard calcite. The vibrations of the CO_3^{2-} ions in a crystalline structure of MSB may have been considerably affected by its environment. The absorption band of water molecules decreases with increasing temperature. The absorption bands, attributed to organic matter, were found to decrease slowly with increasing calcination temperature and number of calcination cycles. Stretching bands in the region of 3650 cm^{-1} to 3600 cm^{-1} appeared in the final product MSB indicating the formation of calcium hydroxide (Bailmain *et al.*, 1999; Engin *et al*., 2006).The results of FTIR studies indicating that the presence of calcium hydroxide were in agreement with the findings of XRD studies as mentioned above.

3.4 Thermo-gravimetric studies

A thermograph (Figure 3 (b)) of MSB showed a small weight loss up to 600°C, which may be attributed to the loss of moisture content of the crystal. A gradual weight loss up to 43% w/w was also observed between 800 and 900°C due to gradual conversion of calcium carbonate to calcium oxide. The raw material (powdered mother of pearl) showed (Figure 3 (a)) a weight loss of slightly higher percentage up to 600°C and above, when compared to MSB; which may be attributed to the loss of organic material.

3.5 Particle size analysis with Dynamic light scattering (DLS)

The particle size of MSB ranges between 1.22 and 10.20 μm having a mean particle size of 22.52±0.45 μm. 6% of the particles were also found to have a particle size less than 50 nm.

3.6 Transmission electron microscopy (TEM)

A photomicrograph of the bulk particles shows that the particles are irregular rod shaped. The TEM photomicrograph of MSB shows the appearance of 15-50 nm particles in the sample. This result is in good agreement with the DLS result and confirms the presence of a significant amount of nanometer size particles in the sample.

3.7 Scanning electron microscopy

SEM images of standard calcite and MSB samples after different cycles of calcinations are shown in Figure 4.

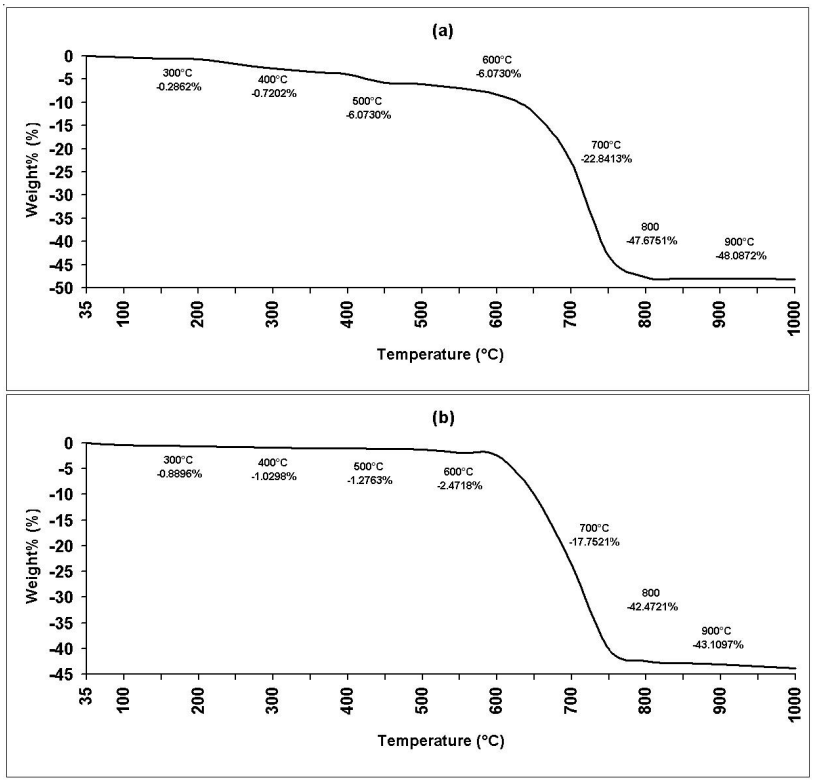

Figure 3. (a) TGA curves of mother of pearl (b) TGA curves of MSB.

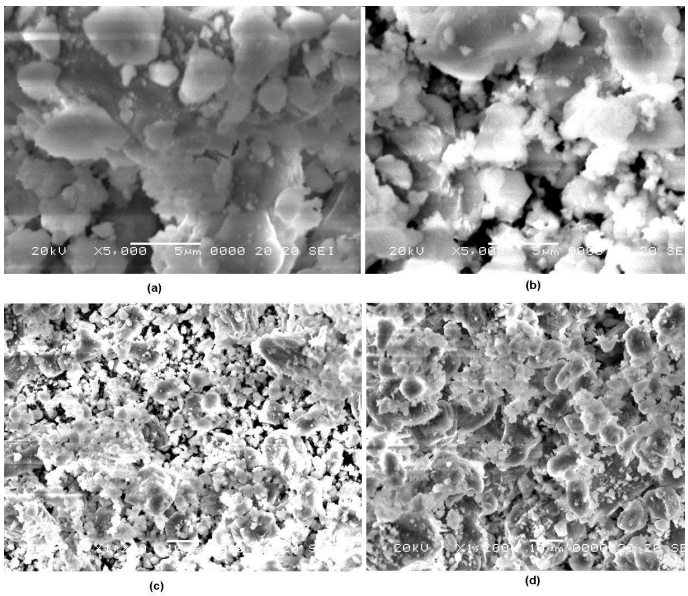

Figure 4. (a) SEM of standard calcite. (b) SEM of MSB after 1st calcination cycle. (c) SEM of MSB after 2nd calcination cycle. (d) SEM of MSB after 3rd calcination cycle.

It was found that: (i) samples at different calcination cycles showed a higher degree of agglomeration than those shown by standard calcite sample. (ii) The morphology of MSB samples was remarkably different from that of standard calcite. (iii) Spongy and relatively compact microcrystalline aggregates of calcite were observed after the first calcination cycle, which were covered by small dusty crystallites. (iv) Second calcination cycles resulted into a spongy nature of the crystallites with increased agglomeration as indicated by the increased particle size. (v) A distinct change in the morphology was also observed with last calcination cycles as several well-defined rod-shaped particles were seen in the

SEM of MSB. This simply means that repeated calcination cycles are necessary to stabilize the particles to a minimum particle size.

3.8 Chemical composition analysis by ICP and EDAX

An average elemental content of triplicate batches of MSB using EDAX and ICP analysis are shown in Table 3. The EDAX analysis revealed calcium as the major element (40.22 wt %) in MSB. The element analysis revealed the presence of heavy metals like chromium, lead, and cadmium in MSB. Other heavy metals like arsenic, mercury, and tin were below the detection limit of the ICP analysis. The detection limit in the ICP analysis is much lower than the acceptable concentration of heavy metals as per standard regulation given in Table 3 (Anonymous, 2006).

3.9 Powder properties evaluation

MSB is a non-lustrous white powder having a bulk density of 0.908±0.031 g/cm^3, a tapped density of 1.218± 0.047 g/cm^3 and an angle of repose of 36.171.28^o. The value of Carr's index was found to be 46.21±0.5. The calcium carbonate and calcium hydroxide content of MSB determined by titrimetric methods was found to be 98.6±1.53 and 1.2±0.42% (w/w), respectively. The difference in the assay value of the calcium carbonate obtained by acid-base titration and complexometric titration gives the amount of calcium hydroxide.

3.10 Microbial evaluations

Microbial load of the MSB was found negative for the presence of *Escherichia coli*, *Salmonella* spp. and *Staphylococcus aureus*. The total aerobic count was <1x10^5 CFU/g (Table 4).

3.11 Acute toxicity studies and dose determination

The LD_{50} of MSB as per OECD guideline falls under class four with no signs of acute toxicity with up to a maximum dose of 2000 mg/kg. Any changes in normal behavioral pattern or signs and symptoms of toxicity and mortality were not observed up to this dose level.

3.12 Antipyretic activity studies

The result for antipyretic studies showed that MSB produced significant antipyretic activity when compared to the control group (Figure 5).

Table 3. (a) Comparison of elemental composition of mother of pearl and MSB detected by EDAX analysis.

Particulars	Element	EDAX (wt%) (Mean± S.D.)*
Mother of pearl (mukta shouktic)	Ca	43.26±0.07
	C	16.09±1.12
	O	40.65±0.08
Mukta shouktic bhasma	Ca	40.22±0.05
	C	16.42±1.4
	O	42.80±0.09

*values are shown as mean ± S.D.(n=3)

Table 3. (b) Comparison of elemental composition of mother of pearl and MSB detected by ICP analysis.

Element	Acceptance limit of elemental concentration of product(µg/mg)	Concentration of elements of mother of pearl(µg/mg ± SD)†	Concentration of elements in MSB.(µg/mg ± SD)†
As	0.0196	0.0392±0.0083	BDL*
Cr	1	0.010989±0.0011	0.0026993±0.0008
Cd	0.0126	0.0012389±0.0002	0.0021423±0.0003
Pb	0.0406	0.10509±0.0028	0.0010604±0.0004
Hg	0.0406	BDL*	BDL*
Sn	40	BDL*	BDL*

† values are shown as mean ± S.D.(n=3)
* Below detection limits of the instrument.

Table 4. Quality assessment of MSB.

Test parameter	Test	Inference	
Identity	Macroscopic	Non lustrous, grayish white, fine powder.	
	Physical properties†	Bulk density	0.908±0.031 g/cm^3
		Tapped density	1.218±0.047 g/cm^3
		Particle size range	1.22-10.20 µm
		Angle of repose	36.17±1.28^o
		Carr's index	46.21± 0.5
		Loss on drying at 110 °C	< 0.5 % w/w
		loss on ignition	< 0.05% w/w
	XRPD	Characteristic d-spacing value at 3.04 Å, 2.28 Å, 1.91 Å, 1.87 Å, 1.42 Å. (calcite form of calcium carbonate)	
	TGA	43% weight loss at 800^{o}C	
	IR spectroscopy	1040 cm^{-1}, 820 cm^{-1} (calcite form of calcium carbonate)	
Purity	Contaminating fungus (Yeast and mould)	<1x10^4 CFU/g	
	Total Aerobic Count	< 1x10^5 CFU/g	
	Escherichia coli	Absent	
	Salmonella spp.	Absent	
	Staphylococcus aureus	< 1 X 10^2 CFU/g	
	Arsenic	< 0.14 mg/Kg body weight/day	
	Cadmium	< 0.09 mg/Kg body weight/day	
	Lead	< 0.29 mg/Kg body weight/day	
	Total mercury	< 0.29 mg/Kg body weight/day	
Quantity	Quantitative test†	Calcium content: 40.22±0.05%	
		Calcium carbonate: 98.6±1.53 % w/w	

† values are shown as mean ± S.D.(n=3)

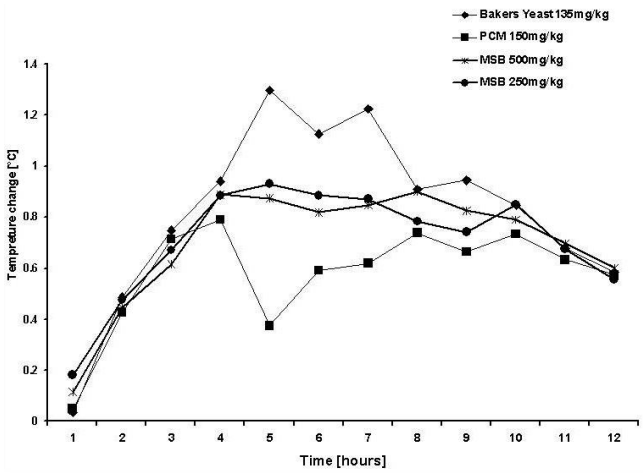

Figure 5. Antipyretic activity of MSB.

4. Discussion

Preparation of bhasma is a very complex procedure. However, this process has been followed strictly untill today for maintaining the quality and efficacy of the product. Cleaning of mother of pearl by lemon juice for ninety minutes resulted in a porous surface with complete loss of luster. Cleaning thus helps to remove dirt and any other organic layers at the surface. The time of treatment with lemon juice should not be more than 90 min, as longer treatment will result in excessive loss of calcium in the mother of pearl as calcium citrate.

Marana, the process of calcination of an intermediate sealed in earthen pots was carried out in a traditional furnace (*gaja-puta*). During the calcination the lines of aragonite disappeared and product obtained contained only the calcite form of calcium carbonate (Bailmain *et al.*, 1999; Huang and Li, 2009). The temperature inside the sealed earthen pots placed in the gajaputa varies between 600 and 900^{o}C; these observations were in agreement with the studies of Ketkar *et al.* (2003). Such a high temperature supports the conversion of calcium carbonate into calcium oxide, which should result into a high concentration of calcium oxide in the final product (Engin *et al*., 2006); however, MSB contains mainly calcium carbonate in calcite form along with less intense peaks for calcium hydroxide. This phenomenon may be attributed to the bhavana with *Aloe vera* gel. Incineration of *Aloe vera* gel might have helped in maintaining carbon and oxygen atmosphere inside the sealed earthen pots, which prevents decarbonation of calcium carbonate to calcium oxide (Ketkar *et al.*, 2003). The bands for organic matrix were modified at different stages of the process. The partial

destruction of the organic matrix was indicated by weight loss during the synthesis with decreased band intensities. Thus, MSB is chemically a mixture of calcium carbonate in calcite form with not more than 2% w/w of calcium hydroxide.

It is evident from the XRD studies (Table 2) that repeated trituration and calcination decreased the crystal size of the MSB to a value less than that of standard calcite. Further, DLS and TEM results show the presence of a significant amount of nanoparticles in the MSB (Figure 4). Nanosized particles can attach with the cell surface and can diffuse readily inside the cells. Thus, the size of the particle is able to influence the efficacy (Wadekar *et al.*, 2006; Gao *et al.*, 2008). Due to the extremely small sizes these particles may impart the contribution of the biological effect of MSB. The scanning electron micrograph (Figure 5) revealed size stabilization of particles on repeated calcination. From all the above observations, it can be concluded that repeated trituration and calcination cycles definitely impart specific physicochemical characters to MSB, which might be responsible for the potent therapeutic activity of this unique class of medicine.

The ICP analysis (Table 3) revealed heavy metals like chromium, lead, and cadmium in MSB. It was also observed that all the elements were quite below regulatory permissible limits for trace elements in a natural drug product (Anonymous, 2006). The powder properties of MSB clearly indicated that MSB is a fine powder with poor flowability that needs to be granulated for improving the flow characteristics. The microbial load of the preparation was under permissible limits for drug products as per Indian Pharmacopoeia (Anonymous, 1996).

The results of antipyretic activity showed that activity of MSB was comparable to that of paracetamol (standard drug). Yeast-induced fever is called pathogenic fever. Its etiology includes production of prostaglandins, which set the thermoregulatory center at a higher temperature. Inhibition of prostaglandin synthesis could be the possible mechanism of antipyretic action. Also, there are several mediators or multi-processes underlining the pathogenesis of fever. Inhibition of any of these mediators may bring the antipyretic effect, which requires further investigation (Hajare *et al.*, 2000; Reanmongkol *et al.*, 2003). It is interesting to note that MSB at 250 mg/kg dose showed a comparable activity to the 500 mg/kg dose as the difference was not significant ($P > 0.05$) in the paired t-test. Therefore it appears that the antipyretic activity of MSB was not dose-dependent.

4. Conclusions

Although *bhasmas* are complex materials, physicochemical analysis (Table 4) using modern techniques will be most attractive for the standardization of herbomineral medicines. This would definitely help in building confidence in use of such products for medication by ensuring safety, efficacy, and batch to batch uniformity.

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