Research Paper

Comparative Physicochemical Evaluation of a Marketed Herbomineral Formulation: *Naga Bhasma*

M. GARG*, S. DAS AND G. SINGH
Department of Pharmaceutical Sciences, Maharshi Dayanand University, Rohtak-124 001, India

In the practice of Ayurveda, where herbomineral formulations are said to be made biocompatible through specific processes like *Shodhana* and *Marana*, the western medical science on the contrary has raised the safety concerns of these formulations in the recent past. In the present study, comparative physico-chemical analysis of *Naga bhasma*, a herbo-mineral preparation having a reputation of miraculous drug commonly used to treat several health disorders, was carried out using five marketed formulations through analytical methods like differential scanning calorimetry, X-ray difraction, thermogravimetric analysis, Fourier Transform infrared spectroscopy and also subjected for particle size analysis and estimation of trace and heavy metals to access the safety of these formulation. The results revealed variable observations regarding particle size, metal form and content of lead. The presence of free lead in five different formulations indicated towards the possible risk of severe side effects to the consumer. Present findings certainly put doubt over the safety of this formulation but at the same time, variation in the results with all five formulations also indicated that these formulations were not prepared as per the mentioned Ayurvedic text. Hence, enforcement of strict regulatory guidelines is strongly warranted before launching into the market. Further, a series of biological studies need to be conducted before taking any final verdict on the safety of this formulation.

Key words: Herbo-mineral preparations, *Naga bhasma*, physico-chemical evaluation, quality control

As per *Charak Samhita*, "Only that which can bring about a cure is a medicine and it is only he, who can relieve his patients of their ailments who is the best physician". The human physiology is unable to produce essential minerals as it manufactures certain vitamins, proteins and enzymes. So our ancient saints introduced metallic medicine into mainstream. Importance of metals and minerals in medicine is mentioned in *Charak Samhita* (500 BC), but their more scientific, systematic and extensive use was initiated by Nagarjuna and others (8-12th AD). These metalomineral preparations constitute a separate branch of Ayurveda called *Rasashastra*. Alchemy (*rasavidya*) prospered in India during the 9-18th century in the hands of the *rasavadins* (alchemists)[1]. It came to be in full vigour during the period 10-14th century. The Indian alchemical texts (the *rasashastras*) of even the 11-12th century refer to many compositions to be used as medicine for treating specific ailments. The *Bhasmas* date as far back as the 8th century for which Nagarjuna is credited as the foremost authority of these preparations. All of this is well before the times of Lavoisier in the 1770s, he perfected the art of calcination of metals to subvert the grand old ideas of chemistry and revolutionized it. Curiously, metals and minerals have been part of European pharmacology since the Renaissance (14-17th century). Paracelsus (1490-1541) in his alchemical training introduced the use of metals like mercury, arsenic, tin, lead and antimony - all toxic, in the medical practice which were believed to give perfect health and cure all diseases[2].

The herbomineral preparations essentially contain minerals and metals as integral part of the formulations but these metals are not present in elemental form. They are in the compound form and their fate in the body will not be the same as it is for the elemental form of heavy metals. The sophisticated manufacturing process of *Shodhana* and *Marana* ensure that deep changes are taking place in these minerals. The finished form after reaction with several organic and inorganic material of herbal origin is finally responsible for action, changing the properties of the toxic metal, making it therapeutically effective and provide safety of very high grade[3]. The

***Address for correspondence**
E-mail: mgarg2006@gmail.com

traditional physicians believe that the 'soul' of the plant/herbal juices is incorporated into the 'body' of metal during the ashing process[4-7].

To maintain the quality of Indian Systems of Medicine (ISM), Government of India has laid down Good Manufacturing Practices (GMP) norms for the manufacturing of Ayurvedic formulations. The Drugs and Cosmetics Act 1970 (section 33A to 33 N of Chapter IV A) and Drugs and Cosmetics Rules 1945 (Rule 150 to Rule 170) exclusively guide about manufacturing, sale and distribution of Ayurvedic drugs. Recently, significant analytical tests for standard and quality of Ayurvedic medicines (Rule 160), additional rules to regulate Ayurvedic drugs (Rule 169) and to cope regulatory demands of export of Ayurvedic medicines have been amended. Additionally, Department of AYUSH, Govt. of India has notified regulations for shelf life period of Ayurvedic formulations under rule 161B[8]. It is obviuos that Govt. of India is well aware of the quality control of Ayurvedic drugs but at the same time it is also true that no clear cut guidelines have been issued for the manufacturing and quality control of herbomineral drugs and formulations.

Naga bhasma is a herbomineral preparation which has a status of miraculous drug in the ISM and mainly consists of purified lead metal, *nirgundi* (*Vitex negundo* Linn.) leaf, turmeric (*Curcuma longa* Linn.) powder, *chichiri* (*Plectranthus coesta* L Her.) herb, *neem* (*Azadirachta indica* A Juss.) and *vaasa* (*Adhatoda vasica* Nees.) leaf[9-10]. It has traditionally been used to treat spleen enlargement, urinary disorders, hemorrhoids, bone wound, goitre, indigestion, inflammation of intestine, osteomalacia, diarrhea, dysentery, hyperacidity, diabetes, leukorrhea, blood disorders, infertility, chronic paralysis, oligospermia, regenerate germinal epithelium of testes and as potent revitalizer[11]. Since, *Naga bhasma* is used since ages for several purposes, almost negligible attention has been made by the scientific community for the scientific validation of this formulation for biological efficacy and quality control aspects. Believing that standardization is a measurement for ensuring the quality and is used to describe all measures, which are taken during the manufacturing process and quality control leading to a reproducible quality[12], the present study was planned to investigate five different marketed formulations of *Naga bhasma* for physicochemical parameters through modern analytical procedures like X-ray diffraction (XRD), Fourier transformed infrared spectroscopy (FTIR), thermogravimetric analysis (TGA), differential scanning colorimetry (DSC), particle size and atomic absorption spectroscopy (AAS) and some other parameters for safety and efficacy.

MATERIALS AMD METHODS

Five marketed formulations of *Naga bhasma* were procured from the New Delhi market as per the details mentioned in Table 1.

X-ray diffraction studies:

XRD studies were carried out for the detection of phase of lead present and to detect compound type whether crystalline/amorphous in nature. The XRD patterns of the solid samples were recorded on RIGAKU model X-ray diffractometer using CuKα radiation (λ=1.5418 A°) filtered by nickel foil over the range of diffraction angle 5.0 to 65.0°. The instrument was operated at 40 kV and 30 mA and XRD patterns were recorded at a scanning rate of 2θ/min.

TGA and DSC studies:

TGA was used to determine total weight change in the sample formulations during thermal treatments. An SDT Q600 TGA/DSC of TA instrument was used to record the weight loss of the samples between 0 and 800°. Dry nitrogen gas was used as a purge and the scan speed was 10 °/min. An isothermal period at 25° was run for 10 min prior to the measurements and any changes in weight during that period were monitored. In all measurements between 5 mg and 10 mg of sample was used. The thermograms obtained from the TGA results were analyzed using TA universal analysis NT software[13]. A performance matched platinum/platinum-rhodium thermocouple

TABLE 1: LIST OF DRUGS AND INGREDIENTS USED IN ANALYSIS

Name of product	Manufacturer	Batch no.	Mfd. lic. no.	Code given
Nag Bhasam peet	Bhartiya ayurvedic pharmacy, Delhi	612	553-15 M (HR)	A
Nag Bhasma	Shree baidyanath ayurved bhawan, Jhansi	10	A-1800/89	B
Nag Bhasam (Seesa)	Dindayal aushadhi, Gwalior	400	25-D/22/92	D
Nag Bhasma	Gurukul kangdi pharmacy, Haridwar	171	A-54/77	G
Nag Bhasma	Unjha ayurvedic pharmacy, Gujrat	415	GA/418	U

embedded in each beam ensures accurate and precision transition and differential temperature measurements (TA Instruments Manual).

The DSC was perofrmed using same instrument and the specifications mentioned as above. In all measurements, samples used between 5 mg and 10 mg. Samples were placed in open platinum pans and these pans were placed on sample holders. The thermograms obtained for the DSC results were analysed using TA universal analysis NT software.

Particle size analysis:
Particle size of different formulations were determined by using Microtrac S3500 particle size analyzer after suspending a small amount of formulation in aqueous dispersing phase and stirring for 5 min on a magnetic stirrer at room temperature.

FTIR studies:
FTIR spectral analysis was used to ascertain the presence of organic matter in the finally prepared drug. IR spectra of all the samples selected for comparative purpose were recorded in KBr pellets in the region 4000-400 cm^{-1} on Perkin-Elmer FTIR spectrophotometer model 1600.

Atomic absorption spectroscopy analysis:
Concentrations of different elements i.e., trace elements and heavy metals present in all formulations were determined using atomic absorption spectrophotometer being one of the most reliable methods of estimation in this regard. In this study, atomic absorption spectrophotometer model AAS4141, Electronics Corporation of India Limited, Hyderabad was used for determination of trace elements. The hollow-cathode lamps of Al, Cu, Mg, Zn (ECIL) and Ca (Photron) were employed as radiation source. The fuel used for Ca, Mg, Cu and Zn was air/acetylene and for Al was N_2O/acetylene/air. Samples were digested by wet digestion technique and quantitative analysis was carried out using calibration curve technique.

RESULTS AND DISCUSSION

XRD spectra details represented in Table 2 shows the presence of PbO phase in all five formulations in litharge and massicot form whereas three out of five formulations were also found to contain other phases like Pb_3O_4, Pb_2O_2Cl, $PbSO_4$, $Pb_3Sb_2S_6$ and $Pb_3(CO_3)_2(OH)_2$. Maximum peak was found to be of 2θ width which denoted that particles are of micron size. Maximum peak width is 3-5θ which denotes that all *Naga bhasma* formulations have amorphous particles.

TGA plots of five sample formulations showed variable pattern of weight change at different temperatures but significantly between 600º-700º. Weight change was observed in all formulations from -27.5 to +3.5% w/w. Exceptional weight increase was observed in formulation D. Details of percentage change in weight are represented in Table 3.

Variation in endotherms in DSC plots of all formulations reveals that different phases of lead melt or show phase change at different temperatures. A reconstructive litharge (PbOL) to massicot (PbOM) phase transformation occurs at temperature range of 525-575º. All formulations of *Naga bhasma* show various endotherms at different temperatures which suggest presence of lead in different phases.

The particle size of the five sample formulations were estimated by automatic particle size analyzer to confirm the presence of micro-size particles observed in XRD studies. All formulations contained particles ranging in size 1.6-248.9 μm. The particle size distribution pattern shows a sigmoid curve except in formulation G. Formulations A and D have particle size within range of 2-52 μm and other three have bigger particles than 52 μm. Particle size distribution pattern of all five sample formulations are represented in Table 4.

TABLE 2: PHASES OF LEAD PRESENT AS INTERPRETED BY XRD

Formulation	Phases of Lead (Pb) present
A	PbO (litharge and massicot form)
B	PbO (litharge and massicot form), Pb_3O_4, Pb_2O_2Cl
D	PbO (litharge and massicot form)
G	PbO (litharge and massicot form), Pb_3O_4, $PbSO_4$, $Pb_3Sb_2S_6$
U	PbO (litharge and massicot form), Pb_3O_4, $Pb_3(CO_3)_2(OH)_2$

TABLE 3: TOTAL PERCENTAGE WEIGHT LOSS/GAIN AS INTERPRETED BY THERMOGRAMS

Formulation code	Percentage weight change
A	−27.9
B	−2.53
D	+3.66
G	−3.20
U	−7.99

The FTIR spectrum denotes the presence of different organic functional groups. All formulations contain tertiary alcohol, dialkyl ketones, secondary amides and 2-substituted pyridines in general. Other functional groups present in some are halogens in B, D, G, U; aldehydes in A, D, G, U; carboxylic acids in A and G; and alkyl amine in formulation U. Details are represented in Table 5.

Calibration curves were plotted using standard procedure for the estimation of trace elements and heavy metals. Estimated concentrations of trace elements and heavy metals have been given in Tables 6 and 7, respectively.

Naga bhasma is a reputed herbomineral preparation in the Indian system of medicines and used for various disorders in our body since ages. To scientifically validate this formulation, the present attempt was made to screen five different marketed *Naga bhasma* formulations for their physicochemical properties. The X-ray diffraction studies conducted on these five sample formulations reveal that all formulations of *Naga bhasma* contain lead in PbO (plumbous oxide) phase, present in litharge and massicot forms and also other phases viz. Pb_3O_4, Pb_2O_2Cl, $PbSO_4$, $Pb_3Sb_2S_6$ and $Pb_3(CO_3)_2(OH)_2$ in three formulations. Pb_3O_4 form is found present in formulations B, G and U. As per scientific information available, Pb_3O_4 is a compound ($2PbO.PbO_2$) and cannot be used for internal human use as it harms foetus and also significantly impair the fertility system[12]. On that basis it can be said that these formulations should be discouraged for human use. Moreover, PbO in litharge form is not recommended for internal human use as it causes reproductive disorders and is harmful if inhaled, ingested or come in contact with skin[12]. Since the litharge form is found present in all the five sample formulations, it puts a certain question mark over the safety of this formulation. But at the same time the other form massicot was also present in all five samples which is an acceptable form for internal use so this needs to be checked once again whether this formulation was prepared as per the Ayurveda text strictly or not. Moreover, maximum peak width is of 2θ in XRD spectra which denote that particles are of micron size and not nano size as claimed by previous research papers. If sample contains nano size particles then peaks width should be greater than 20θ. The presence of micron size particles were further confirmed by automatic particle size analyzer. All formulations contain particles ranging in size 1.6-248.9 μm. All samples have micron size particles which get entrapped in the crevices of skin and are smooth in touch except some particles of formulations G and U. These analyses are in contrary to previous studies[9] which have reported that after preparing *Naga bhasma* in the laboratories of Department of *Rasa Shastra*, the particles were obtained as small

TABLE 4: PARTICLE SIZE RANGE AS INTERPRETED BY PARTICLE SIZE ANALYSIS

Formulation	Particle size range (μm)
A	2.750-52.33
B	2.312-104.7
D	2.750-52.33
G	2.312-248.9
U	1.635-176.0

TABLE 5: FTIR SPECTRA IN ALL FIVE SAMPLE FORMULATIONS

Functional groups	A (ν cm^{-1})	B (ν cm^{-1})	D (ν cm^{-1})	G (ν cm^{-1})	U (ν cm^{-1})
2- substituted pyridines	417.68	415.82	412.70	417.60, 411.39, 405.63	418.99, 405.87
Halogens	-	612.42	748.11	616.06, 685.34	712.93, 685.74
Aldehydes	866.54	-	867.13	844.48	874.43
Carboxylic acid	930.52, 1456.38	-	-	987.04, 910.18	-
Alkyl amine	-	-	-	-	1128.26
Tertiary alcohol	1412.78	1384.45	1457.11	1446.84	1421.04
Secondary amides	1636.62, 3448.37	1637.94, 3415.70	1638.08, 3448.54	1632.06, 3445.65	1637.73, 3448.92
Dialkyl ketone	2843.59, 2917.14	2847.31, 2924.96	2925.31	2921.23	2917.14

TABLE 6: TRACE ELEMENTS CONCENTRATION ESTIMATED BY ATOMIC ABSORPTION SPECTROSCOPY

Formulation code	Ca Mean±SD	Cu Mean±SD	Mg Mean±SD	Zn Mean±SD	Al Mean±SD
A	70.66±8.1	6.83±1.24	993.33±60	13±0.8	320±16.1
B	901.1±21.3	340.35±20.13	753.3±35.1	614.4±7.18	537.7±21.3
D	41.76±9.16	38.13±1.12	293.3±11.54	21.66±0.28	363.5±18
G	6776.6±136.1	40.8±0.98	2653.3±40.41	412.3±2.15	1300±70.2
U	57876.6±1685	195.3±2.45	1363.3±57.7	426.8±3.55	1365±71.7

SD=Standard deviation, Values are expressed as mean±SD (*n*=3); values are given in ppm

TABLE 7: HEAVY ELEMENTS CONCENTRATION ESTIMATED BY ATOMIC ABSORPTION SPECTROSCOPY

Formulation code	As Mean±SD	Hg Mean±SD	Cd Mean±SD	Pb Mean±SD
A	7.37±0.078	3.745±0.006	2.5±0.00	14750±435
B	5.762±0.067	1.12±0.001	2.63±0.00	30803±591
D	2.01±0.011	BDL	2.5±0.00	20666±248
G	10.72±0.055	2.47±0.004	2.5±0.00	47010±433
U	BDL	1.498±0.002	30.96±0.00	56590±874

BDL=Below detectable limit, SD=Standard deviation, Values are expressed as mean±SD (*n* = 3); values are given in ppm

as 60 nm. Formulations A and D contain particles within range of 2-52.5 μm which is in alignment to estimation reported earlier[14] whereas, formulations B, G and U contain particles as big as 104.7 μm, 248.9 μm and 176.0 μm.

TGA plots of all formulations show change in weight at different temperatures but show exact change in weight from range 600º to 700º. As per scientific knowledge if pure PbO is heated in nitrogen atmosphere up to 600º, it contains substantially more massicot than those heated in air which is more acceptable form for preparing this formulation[15]. However, it was not clear how *Naga bhasma* is prepared by different manufacturers that have shown significant weight changes among themselves i.e., from -27.5 to +3.5% w/w. Exceptional weight increase was observed in formulation D. So method of preparation of all these formulations cannot be the same.

FTIR studies conducted on these five sample formulations revealed the presence of various organic functional groups *viz.* tertiary alcohol, dialkyl ketones, secondary amides and 2-substituted pyridines common in all samples whereas some other variable functional groups were also present in some formulations like halogens, aldehydes, carboxylic acids and alkyl amine. It is important to mention here that presence or absence of these different functional groups affect the quality and efficacy of a particular formulation.

Variation in endotherms in DSC plots of all formulation reveals that different phases of lead melt or show phase change at different temperatures. A reconstructive litharge (PbO_L) to massicot (PbO_M) phase transformation occurs at temperature range of 525-575°. PbO_L has tetragonal crystalline dark red color particles and PbO_M has orthorhombic crystalline bright yellow color particles[15,16]. This shows that all formulations contain PbO in litharge form in very high ratio which can be potentially dangerous for human health. The presence of PbO_L and PbO_M was also confirmed by *Ishtavarnattwa* (color) test which show that all samples have different colors ranging from buff color-yellow-dark orange.

Based on the AAS results, it was observed that all marketed *Naga bhasma* sample formulations are rich source of essential elements Mg, Ca, Zn, Cu and Al. Presence of these essential elements may play an important role in maintenance of health and as true revitalizer and energizing formulation. Also, the difference in the presence and absence of arsenic and mercury in different formulations clearly show that these *Naga bhasma* are prepared by different methods. Percentage of lead in formulations A, B, D, G and U are 1.5, 3.0, 2.0, 4.6 and 5.5% w/w, respectively which are quite variable and sufficient to affect the quality of a drug. However, previous studies suggest that *Naga bhasma* contains approximately 5% w/w of lead (Pb) when prepared in laboratory[9,14].

Although, present findings risesuncertainity over the safety of this formulation yet variation in the results with all five formulations also indicate that these preparations were not prepared as per the mentioned Ayurvedic text. Moreover, these Ayurvedic formulations have been practised since ancient times, therefore it is suggested and concluded that the manufacturing of these formulations should be regulated strictly before launching them into the market and more scientific studies need to be conducted to establish the final verdict on the safety of this and several other similar formulations.

REFERENCES

1. Mukherji B. The wealth of Indian alchemy and its medicinal uses. Delhi: Shri Sat Guru Publications; 1998.
2. Kapoor RC**.** Some observations on the metal-based preparations in the Indian Systems of Medicine. Indian J Tradit Knowl 2010;9:562-75.
3. Vohora SB, Athar M. Mineral drugs used in Ayurveda and Unani medicine. New Delhi: Narosa Publishing House; 2008.
4. Saper RB, Kales SN, Paquin J, Burns MJ. Heavy metal content of ayurvedic herbal medicine products. J Am Med Assoc 2004;292:2868-73.
5. Kumar A, Nair AG, Reddy AR, Garg AN. Bhasmas: Unique ayurvedic metallic-herbal preparations, chemical characterization. Biol Trace Elem Res 2006;109:231-54.
6. Kales SN, Christophi CA, Saper RB. Hematopoietic toxicity from lead containing Ayurvedic medications. Med Sci Monit 2007;13:295-8.
7. Saper RB, Phillips RS, Sehgal A, Khouri N, Davis RB, Paquin J, *et al*. Lead, mercury and arsenic in US and Indian manufactured ayurvedic medicines sold. Int J Am Med Assoc 2008;300:915-23.
8. Chaudhary A, Singh N. Herbo-mineral formulations (Rasaoushadhies)

of Ayurveda-an amazing inheritance of ayurvedic pharmacies. Anc Sci Life 2010;30:18-26.
9. Singh SK, Gautam DN, Kumar M, Rai SB. Synthesis, characterization and histopathological study of a lead based Indian traditional drug: *Naga bhasma*. Indian J Pharm Sci 2010;72:24-30.
10. Sudha A, Murty VS, Chandra TS. Standardization of metal-based herbal medicines. Am J Infect Dis 2009;5:193-9.
11. Joshi D. Rasa Amritam. Varanasi, India: Chaukambha Sanskrit Bhawan; 1998. p. 127-9.
12. Rasheed A, Marri A, Naik MM. Standardization of Bhasma importance and prospects. J Pharm Res 2011;4:1931-3.
13. Abdennour B, Jonathan CB, Neil RC, Richard CT, Philip AJ, Philip MW, *et al*. An assessment of beclomethasone dipropionate clathrate formation in a model suspension metered dose inhaler. Int J Pharm 2010;391:98-106.
14. Tate PM, Patgiri BJ, Prajapati PK. Pharmaceutical Standardization of *Naga bhasma*. Ayurveda 2009;30:300-9.
15. Munson MJ, Riman RE. Observed phase transformations of oxalate-derived lead monoxide powder. J Therm Anal 1991;37:2555-66.
16. Wadekarv M, Gogte V, Khandagale P, Prabhune A. Comparative study of some commercial samples of *Naga bhasma*. Anc Sci Life 2004;23:1-9.

Accepted 15 December 2012
Revised 13 December 2012
Received 4 March 2012
Indian J. Pharm. Sci., 2012, 74 (6): 535-540