International Journal of Pharmacy and Pharmaceutical Science

ISSN Print: 2664-7222
ISSN Online: 2664-7230
IJPPS 2024; 6(2): 36-43
www.pharmacyjournal.org
Received: 17-05-2024
Accepted: 20-06-2024

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Characterization of iron-based ayurvedic medicine Abhrak Bhasma (Shataputi) produced by various manufacturers and its pharmacokinetic profiling in Wistar rats

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DOI: https://doi.org/10.33545/26647222.2024.v6.i2a.123

Abstract
Aim & Objective: The main aim of the study was to characterize 5 commercial Abhrak Bhasma 100puti preparations from 5 different manufacturers by using modern scientific techniques and to study their bioavailability in Wistar rat.
Materials and Methods: Abhrak Bhasma (AB) Shataputi was characterize by X-ray diffraction (XRD), Scanning electron microscope (SEM), Energy Dispersive X-ray analysis (EDAX), Nanoparticle tracking analyzer (NTA), inductively coupled plasma optical emission spectroscopy (ICP-OES). Bioavailability of Abhrak Bhasma 100 puti was studies using non compartmental rat model with daily dose of 11.25 mg/kg according to their body weight.
Results: The chemical phase and particle size was significantly different for all 5 AB preparations. Bioavailability study confirms difference in various pharmacokinetic parameters such as Cmax, Tmax, half-life ($t_{1/2}$), AUC etc. The maximum absorption (Cmax) was observed in sample A compared with other samples.
Conclusion: The morphology as well as chemical phase of the five Abhrak Bhasma studied were different from each other, which might be responsible for different pharmacokinetic profiles in Wistar rats.

Keywords: Abhrak Bhasma, ayurveda, bioavailability, wistar rat, herbo-mineral

1. Introduction
In India, several complementary medicinal formulations have demonstrated their therapeutic usefulness in the management of a variety of disorders [1]. The awareness of and ongoing usage of herbal remedies from the Indian systems of medicine known as Ayurveda, Siddha and Unani are highly developed from ancient times [2]. Ayurveda is a traditional system of medicine that has existed for thousands of years in the Indian subcontinent [3]. Origin of plants, animals and metal/minerals are used to prepare ayurvedic medicines [4]. One of the ayurvedic branches known as Rasashastra deals with metals and minerals known as 'Rasadravyas' [5-7]. In Ayurveda, the process of turning metals and minerals into oxide at a higher oxidation state is known as bhasma. The unique herbo-metallic ayurveda technique known as "Bhasma" include repeatedly igniting metals and minerals with herbal extracts and decoctions to eliminate their harmful effects. It is advised to take it with a variety of Anupanas (vehicle), including milk, honey and ghee [5, 8]. Mineral mica (Biotite) [K (Mg, Fe) 3(AlSiO10) (OH) 2] from Abhrak Bhasma is produced through repeated calcination. Minerals become edible when they undergo repeated calcination or combustion (puta), which changes the metallic state into an oxide form at a particular temperature. According to the classical Rasashastra literature, the distinctive characteristics of Abhrak (Mica) are Shataputi (100 cycles) and Sahastraputi (1000 cycles) Abhrak Bhasma [9-12]. Bhasma is superior to herbal products, so physicians preferably use bhasma in their clinical practices. Improper preparation of bhasma leads to severe toxicity such as heavy metal poisoning and sometime may cause mortality. The use of herbal plant extracts in metal-based drugs eliminates the toxic effects of metals [13, 14].

The manufacture of bhasma is given significant priority in the Rasashastra book with several methods to maximise its medical usage and efficacy [15]. Diverse bhasma preparation techniques can produce varying kinds of bhasma with different colours and altered physicochemical qualities [16]. Abhrak bhasma is used in the treatment of anaemia, chronic cough, bronchitis, asthma [17, 18]. It exhibits therapeutic qualities in a variety of malignancies and hepatitis, and it also aids in kidney and liver function and has diuretic properties [19-22]. Shataputi Abhrak bhasma shows Immunomodulatory activity [23]. Different manufacturing routes have been used by different manufacturer to produce Abhrak bhasma 100puti according to various Ayurvedic text references (Table 1). Different textual references confirm different morphology and chemical moiety. Not only the morphology but also its chemical moiety can be responsible for its bioavailability. Bioavailability is an important pharmacokinetic activity of the therapeutic drug that provide details of what fraction of the administered drug reaches the systemic circulation. After oral administration, Bhasma could be available in the circulatory system and various tissues. Many factors affect the bioavailability of bhasma such as particle size, shape, chemical phase, etc. In this study we determine the concentration profile of Iron (Fe) in blood with time in wistar rats and also characterize 5 different Abhrak bhasma of different ayurvedic text references. For this purpose, XRD (X-Ray Diffraction), SEM-EDAX (Scanning Electron Microscopy-Energy Dispersive Spectroscopy), NTA (Nanoparticle tracking analysis) were used.

2. Materials and Methods

2.1 Chemicals

Abhrak Bhasma Shataputi (AB-A) was manufactured by Shree Dhootapapeshwar Ltd. Panvel, India. The different Abhrak bhasma 100puti samples used for the study was procured from Ayurvedic chemist shop.

2.2 Characterization of Abhrak Bhasma 100 puti

Abhrak Bhasma 100 puti was characterized to understand its physicochemical properties. The Structural and phase details of Abhrak bhasma was analysed by powder X-ray Diffractometer (XRD, MiniFlex 600, Rigaku, Japan) and peaks were compared with ICDD PDF-2 2021 (International Centre for Diffraction Data). Scanning electron microscope with EDAX (SEM) (JEOL, Japan) was used to observe morphology and elemental composition. Iron (Fe) concentration in blood was analyzed by inductively coupled plasma optical emission spectroscopy (ICP-OES, Avio-200 Perkin Elmer, USA). Particle size of Abhrak bhasma 100puti was determined by Nanoparticle Tracking Analyzer (NTA, NS300, Malvern Panalytical, UK).

2.3 Animals

Thirty healthy Female Wistar rats (150-250 g) from the in-house Animal Facility of Shree Dhootapapeshwar Ayurvedic Research Foundation (SDARF) was used for the study. The weight variation of animals did not exceed±20% of the mean weight. Animals were provided with standard diet and water *ad libitum*. Animals were housed in plastic cages below standard conditions, temperature 22±2 °C and humidity 30-70%, with 12 h dark/light cycle. The animals were acclimatized for a minimum period of seven days prior to study.

2.4 Animal ethics

The experimental protocol was approved by the Institutional Animal Ethics Committee of SDARF (Protocol number: SDARF/2021/01). The experiments were conducted according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India.

2.5 Experimental Design

The animals were randomly divided into group of five, each group contains six animals (Table 2). Group I received Abhrak Bhasma Shataputi of SDL mixed with honey and lukewarm water at dose of 11.25 mg/kg according to the body weight. Remaining all four groups receives Abhrak Bhasma Shataputi of different competitors mixed with honey and lukewarm water at dose of 11.25 mg/kg according to the body weight.

2.6 Bioavailability study

All animals were fasted for 4-6 hours prior to dosing (Feed of animals withheld. Animals were allowed free access of water). Initially blood was collected at zero hour from all animals. All animals were orally gavaged with Abhrak Bhasma mixed with honey and lukewarm water. After drug administration, blood was collected at interval of 1, 2, 3, 4, 6, 8, 12, 24, 48 & 72 hours. 1 ml of blood was collected at each time. The Blood was analyzed for the Iron content by using ICP-OES (Inductively Coupled Plasma Optical Emission Spectrometry).

2.7 Estimation of Iron content in Blood

The collected blood was kept on Bunsen burner at 100^0C to form carbon free Ash. 10 ml Hydrochloric acid (HCL) was added to the ash sample. This mixture was kept for digestion, followed by filtration after complete digestion. The filtered sample was further analyzed for Iron (Fe) content on ICP-OES. [24]

2.8 Statistical Analysis

PK Solver software was used to calculate various PK parameters such as time of peak concentration (Tmax), maximum concentration (Cmax) terminal elimination slope (λ_z), area under the zero and first moment curves from 0 to last time t (AUC_{0-t}, $AUMC_{0-t}$), half-life ($t_{1/2}$), mean residence time (MRT) and apparent volume of distribution based on the terminal slope (V_z/F).

3. Results

3.1 XRD Analysis

Different XRD peaks of Abhrak Bhasma A, B, C, D and E was observed as shown in Figure 5. Data obtained from XRD analysis such as crystallite size, phase present, DB card number and crystal system of AB was presented in Table 3. XRD of sample AB-A shows presence of Kalsilite [K Al Si O4], Phlogopite [K (Mg2.17 Fe0.83) (Si4 O10) (O0.81 (O H) 1.19] and small amount of Alluaudite [(Na1.254 Li0.375) Mn1.478 Fe1.5 (P O4)3]. XRD of AB-B confirms presence of Augite [Ca (Mg,Fe) Si2 O6], Kalsilite [K Al Si O4], Manganiceladonite [K Mn +3 Mg Si4 O10 (O H)2] and Talc [Mg3 Si4 O10 (O H)2]. AB-C sample shows presence of Glauconite [(K, Na) (Fe, Al, Mg)2 (Si,Al)4 O10 (O H)2] and Hematite [Fe2O3]. AB-D confirms presence of Clinoenstatite, calcium magnesium catena-disilicate [Ca0.15 Mg0.85) Mg (Si2 O6), Magnesium diiron (III) oxide, Mangesioferrite [(Mg0.198 Fe0.802) (Mg0.802 Fe1.198)

O4] and Glauconite [(K, Na) (Fe, Al, Mg)2 (Si,Al)4 O10 (O H)2]. XRD of AB-E shows presence of Diopside, Fe+2 bearing [Ca (Mg0.69 Fe0.31) (Si2 O6)], K2 Mg Si3 O8, Edenite [Na Ca2 Mg5 Al Si7 O22 (O H) 2] and Phlogopite (K Mg3 (Si Al) O10 (O H) 2]. The crystallites size calculated using Debye-Scherrer formula and found to be maximum for AB-C (57.7 nm), whereas the other four samples had crystallites size in-between 28 to 55nm.

3.2 SEM-EDAX analysis

SEM-EDAX analysis was showed in Table 4 and Figure 3. Irregular and agglomerated shape particles was observed in AB-A sample on SEM analysis and EDAX shows presence of Fe, Mg, Al, Si, O as major element and Na, Ca in trace amount. Irregular and agglomerated shape particles was observed in AB-B sample and EDAX shows presence of Fe, Mg, Al, Si, O, Ca and Na. agglomerated spherical shape particles with presence of Fe, Mg, Al, Si, and O was observed in AB-C sample. Irregular and agglomerated spherical structure of particles with presence of Fe, Mg, Al, Si, O, Ca, and Na was observed in AB-D sample. AB-E sample consist of agglomerated spherical structural morphology with presence of Fe, Mg, Al, Si, O, Ca and Na.

3.3 NTA

Nanoparticle tracking analysis (NTA) presented in Table 5 and Figure 4. AB-A sample confirms particle size varies from 10 nm to 100 nm and average particle size was found to be 42 nm. 50% of particles are below 29 nm in size. In NTA graph of AB-B sample, particle size varies from 15 nm to 180 nm and mean particle size was found to be 59 nm. Particle size for AB-C sample varies from 3nm to 100 nm and average particle size was found to be 45nm. AB-D sample particle size varies from 5nm to 180 nm and mean particle size was found to be 53 nm. Particle size for AB-E sample varies from 5nm to 160nm and average particle size was found to be 45 nm.

3.4 Bioavailability study

The concentration of Abhrak Bhasma 100puti in blood at different time intervals after oral administration in Wistar rats were shown in Table 6 and Figure 2. After oral administration, maximum drug absorbed was observed in sample AB-A compared with other competitor samples. The maximum concentration i.e., Cmax of sample AB-A in blood was found to be 654.50 ppm. The time required to absorb maximum concentration of drug i.e., Tmax in sample AB-A, AB-B, AB-C was found at 6 hours.

4. Discussions

Many ayurvedic drug manufacturers do not follow all steps mentioned in ayurvedic text references due to huge demand. This negligence may lead to toxicity instead of benefits and also shows variations in physicochemical parameters and different chemical moiety than expected. Therapeutic activity and pharmacokinetic availability of any drug is depend on their physicochemical properties such as shape, size, composition and chemical moiety. The physicochemical characterization is the most important parameter to prove quality and efficacy of ayurvedic herbo-mineral formulations. This study found variations in physicochemical properties in marketed AB samples prepared using different Ayurvedic textual references.

The morphology and chemical composition of all 5 AB preparations was different. Control on particle size and shape during bhasma preparation is quite challenging after many typical ayurvedic process. NTA graph shows variation in particle size in all 5 bhasma preparations. Irregular particle shape such as flake, spherical, agglomerated spherical was observed on SEM-EDAX analysis in 5 different preparations of bhasma using different ayurvedic text references.

Bioavailability of Iron was investigated to know the efficacy in terms of pharmacokinetic (Pk) properties. Morphology and chemical moiety of particle can affect absorption of bhasma.

In bioavailability study, we observed that pharmacokinetic parameters of all 5 AB preparation are different with respect to the maximum assimilation of Iron in blood (Cmax). The difference in Cmax might be due to physicochemical properties of Abhrak Bhasma. After oral administration, the maximum concentration was found at 6 hours. The AB-A sample shows maximum absorption of drug in the systemic circulation as compared with other samples. The observed variation in bioavailability could be due to variation in physicochemical parameters such as particle size, shape and chemical moiety present.

Table 1: AB 100puti prepared using different textual references

Abhrak Bhasma (Sample code)	Date of manufacturing	Textual manufacturing reference as per label	Batch No.
AB-A	Nov-20	*Ayurved Prakash 2*	P201100046
AB-B	Jan-18	*Rasendra Sar Sangrah*	171030001
AB-C	Oct-19	*Ras Tarangini / Dashamastarang*	SB00039
AB-D	Nov-20	*R.T.S. 160*	009
AB-E	Jun-20	Rastantra-saar & Siddha-prayog Sangraha (Part 1st)	01/02

Table 2: Grouping of wistar rats and dose level of Abhrak Bhasma 100puti for different competitors.

Group No.	Groups	Dose (mg/kg)	No. of Animals	Blood Collection Time
I	AB-A	11.25	6	G1A-0, 2, 4, 8, 24, 72hrs.
				G1B-1, 3, 6, 12, 48hrs.
II	AB-B	11.25	6	G2A-0, 2, 4, 8, 24, 72hrs
				G2B-1, 3, 6, 12, 48hrs.
III	AB-C	11.25	6	G3A-0, 2, 4, 8, 24, 72hrs.
				G3B-1, 3, 6, 12, 48hrs.
IV	AB-D	11.25	6	G4A-0, 2, 4, 8, 24, 72hrs.
				G4B-1, 3, 6, 12, 48hrs.
V	AB-E	11.25	6	G5A-0, 2, 4, 8, 24, 72hrs.
				G5B-1, 3, 6, 12, 48hrs.

Table 3: XRD data for AB 100puti prepared using different text reference.

Sample	Crystallite size (nm)	Phase	DB Card number	Crystal system
AB-A	28.5	Phlogopite [K (Mg2.17 Fe0.83) (Si4 O10) (O0.81 (O H)1.19]	01-083-3044	Monoclinic
		Kalsilite [K Al Si O4]	00-066-0070	Hexagonal
		Alluaudite [(Na1.254 Li0.375) Mn1.478 Fe1.5 (P O4)3]	01-074-7143	Monoclinic
AB-B	54.7	Augite [Ca (Mg,Fe) Si2 O6]	00-024-0203	Monoclinic
		Manganiceladonite [K Mn +3 Mg Si4 O10 (O H)2]	00-069-0188	Monoclinic
		Talc [Mg3 Si4 O10 (O H)2]	00-029-1493	Monoclinic
		Kalsilite [K Al Si O4]	00-011-0579	Hexagonal
AB-C	57.7	Glauconite [(K, Na) (Fe,Al,Mg)2 (Si,Al)4 O10 (O H)2]	00-058-2023	Monoclinic
		Hematite [Fe2O3]	01-089-0599	Trigonal
AB-D	28.5	Magnesium diiron (III) oxide, Mangesioferrite [(Mg0.198 Fe0.802) (Mg0.802 Fe1.198) O4]	01-076-2850	Cubic
		Glauconite [(K, Na) (Fe,Al,Mg)2 (Si,Al)4 O10 (O H)2]	00-058-2023	Monoclinic
		Clinoenstatite, calcium magnesium catena-disilicate [Ca0.15 Mg0.85) Mg (Si2 O6)	01-070-8553	Monoclinic
		Phlogopite (K Mg3 (Si Al) O10 (O H)2]	00-010-0495	Monoclinic
		Diopside, Fe+2 bearing [Ca (Mg0.69 Fe0.31) (Si2 O6)]	01-071-6477	Monoclinic
		K2 Mg Si3 O8	00-019-0973	Trigonal
		Edenite [Na Ca2 Mg5 Al Si7 O22 (O H)2]	00-023-1405	Monoclinic

Table 4: EDAX analysis results for AB 100puti prepared with different textual references.

Element (Atom %)	AB-A	AB-B	AB-C	AB-D	AB-E
Oxygen (O)	60.47±0.68	60.55±0.63	61.95±0.72	64.50±0.62	59.36±0.71
Sodium (Na)	0.79±0.10	2.26±0.12	ND	1.89±0.11	1.40±0.11
Magnesium (Mg)	3.66±0.16	8.78±0.20	3.34±0.17	6.56±0.18	8.85±0.22
Iron (Fe)	6.72±0.26	3.85±0.17	10.66±0.35	5.27±0.20	4.75±0.20
Aluminium (Al)	7.09±0.21	4.73±0.15	8.38±0.24	4.89±0.15	3.44±0.14
Silicon (Si)	21.00±0.34	16.59±0.26	15.67±0.33	14.07±0.24	16.26±0.28
Calcium (Ca)	0.27±0.08	3.25±0.13	ND	2.83±0.12	5.93±0.18
Total	100	100	100	100	100

Table 5: Particle size analysis results for AB's prepared using different text references.

Sample	Particle size			
	Mean (nm)	D10 (nm)	D50 (nm)	D90 (nm)
AB-A	42	10	29	84
AB-B	59	18	52	97
AB-C	45	2	23	108
AB-D	53	9	38	108
AB-E	45	11	31	90

Table 6: Pharmacokinetic Parameters of 5 Different AB 100puti Preparations.

Parameters	AB-A	AB-B	AB-C	AB-D	AB-E
λ_z (1/h)	0.00817	0.00876	0.00822	0.0079	0.004039
$t_{1/2}$ (h)	84.8752	79.078568	84.3482	87.7547	171.5972
T_{max}(h)	6	6	6	8	8
C_{max} (ppm)	654.5	608.98	623.59	636.51	564.46
AUC $_{0\text{-}t}$ (ppm*h)	33045.1	32392.885	32895.1	33111.1	29572.58
AUC $_{0\text{-}inf\_obs}$ (ppm*h)	74670.4	70201.071	75584.7	81905.3	114035.9
AUMC $_{0\text{-}inf\_obs}$ (ppm*h^2)	9152742	8107677.6	9350170	1079860	27973025
MRT $_{0\text{-}inf\_obs}$ (h)	122.575	115.49222	123.704	131.843	245.3001
Vz/Fobs ((mg)/(ppm)/h)	0.01845	0.0182828	0.01811	0.01739	0.024423

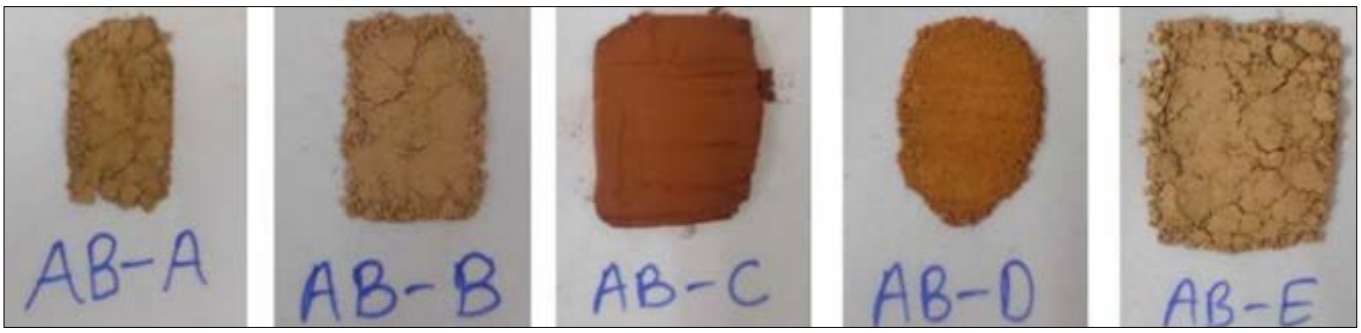


Fig 1: Variation in colours of Abhrak Bhasma 100puti

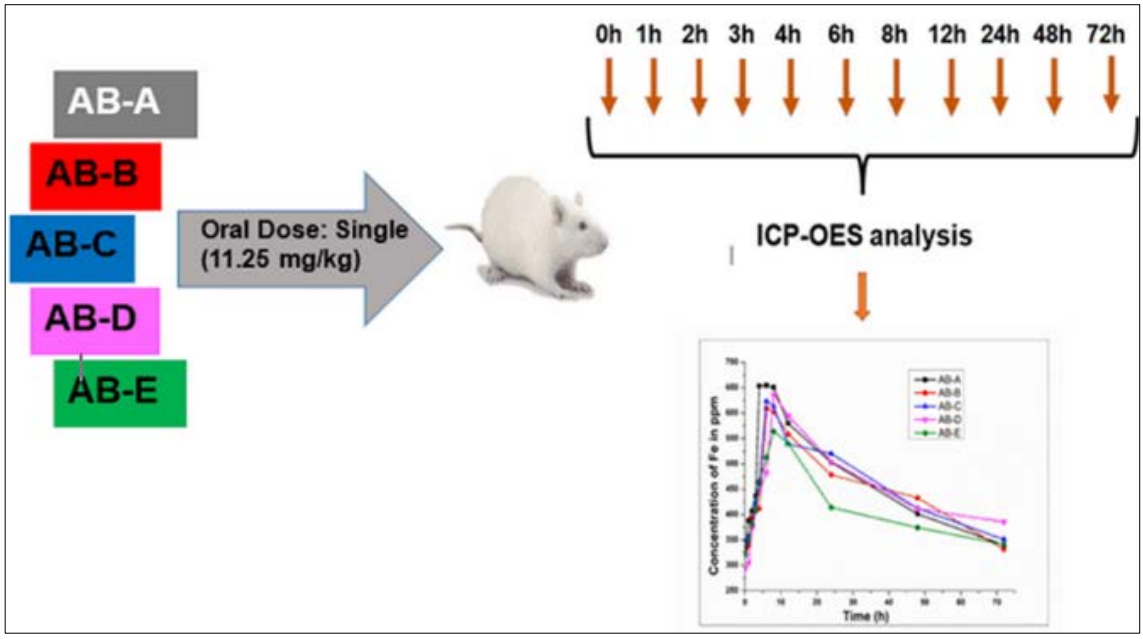
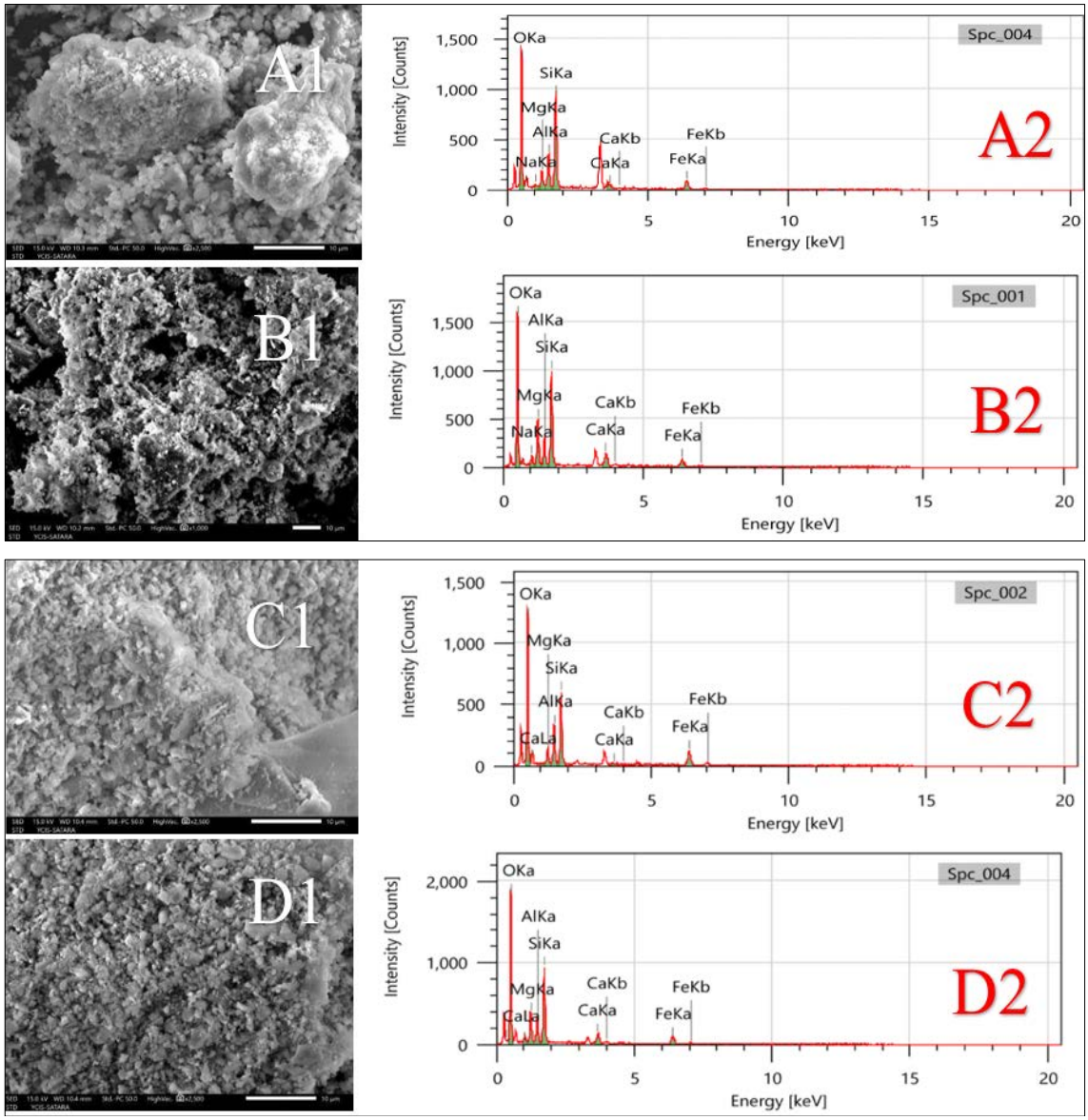


Fig 2: Graphical representation of Iron (Fe) content in Blood



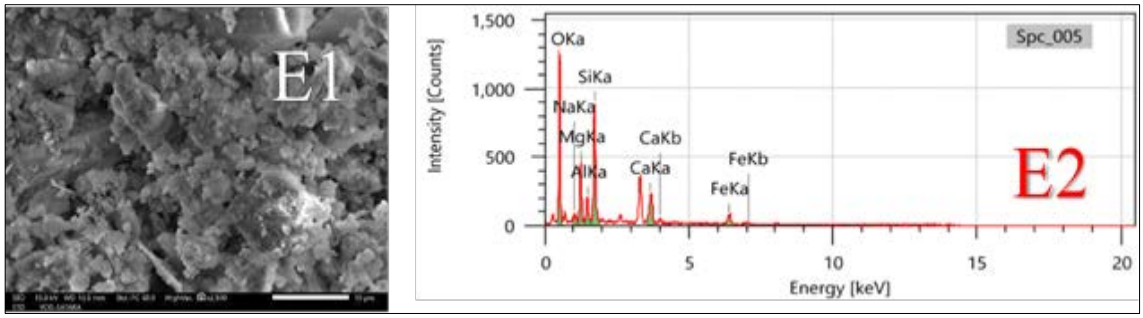


Fig 3: SEM (AB-A: A1, AB-B: B1, AB-C: C1, AB-D: D1, AB-E: E1) and EDAX (AB-A: A2, AB-B: B2, AB-C: C2, AB-D: D2, AB-E: E2) for AB 100puti prepared with different textual references.

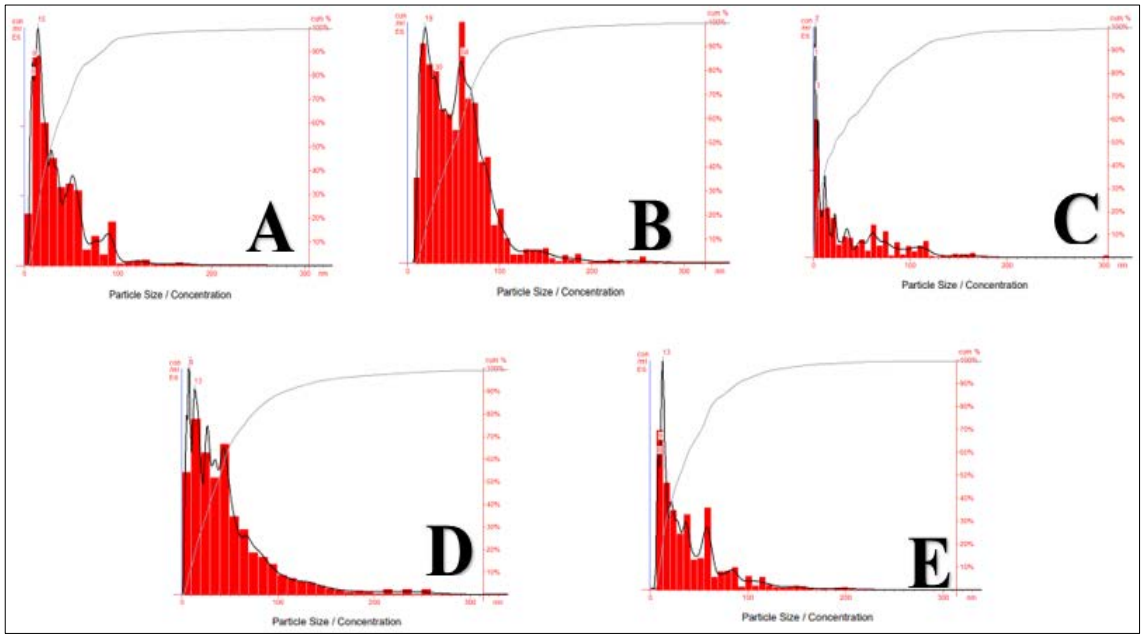
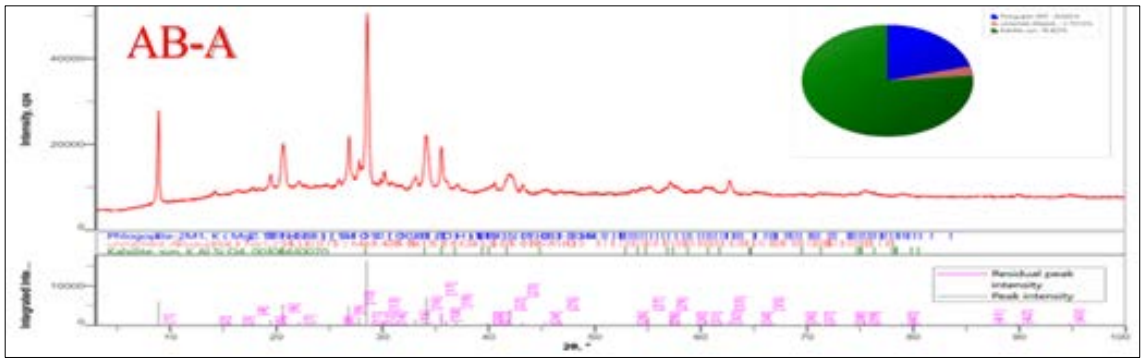
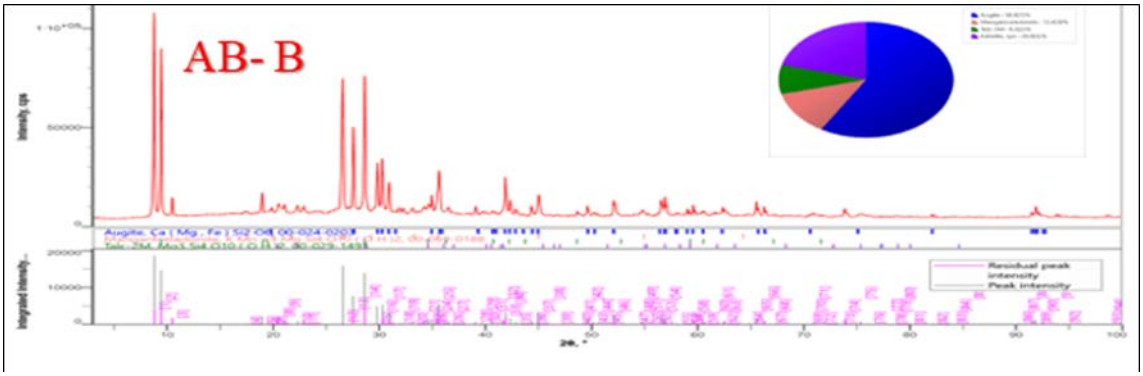


Fig 4: Particle size analysis (NTA) for AB 100puti prepared using different text references, A: AB-A, B: AB-B, C: AB-C, D: AB-D, E: AB-E (Inside each figure contains graph of relative intensity versus particle size)





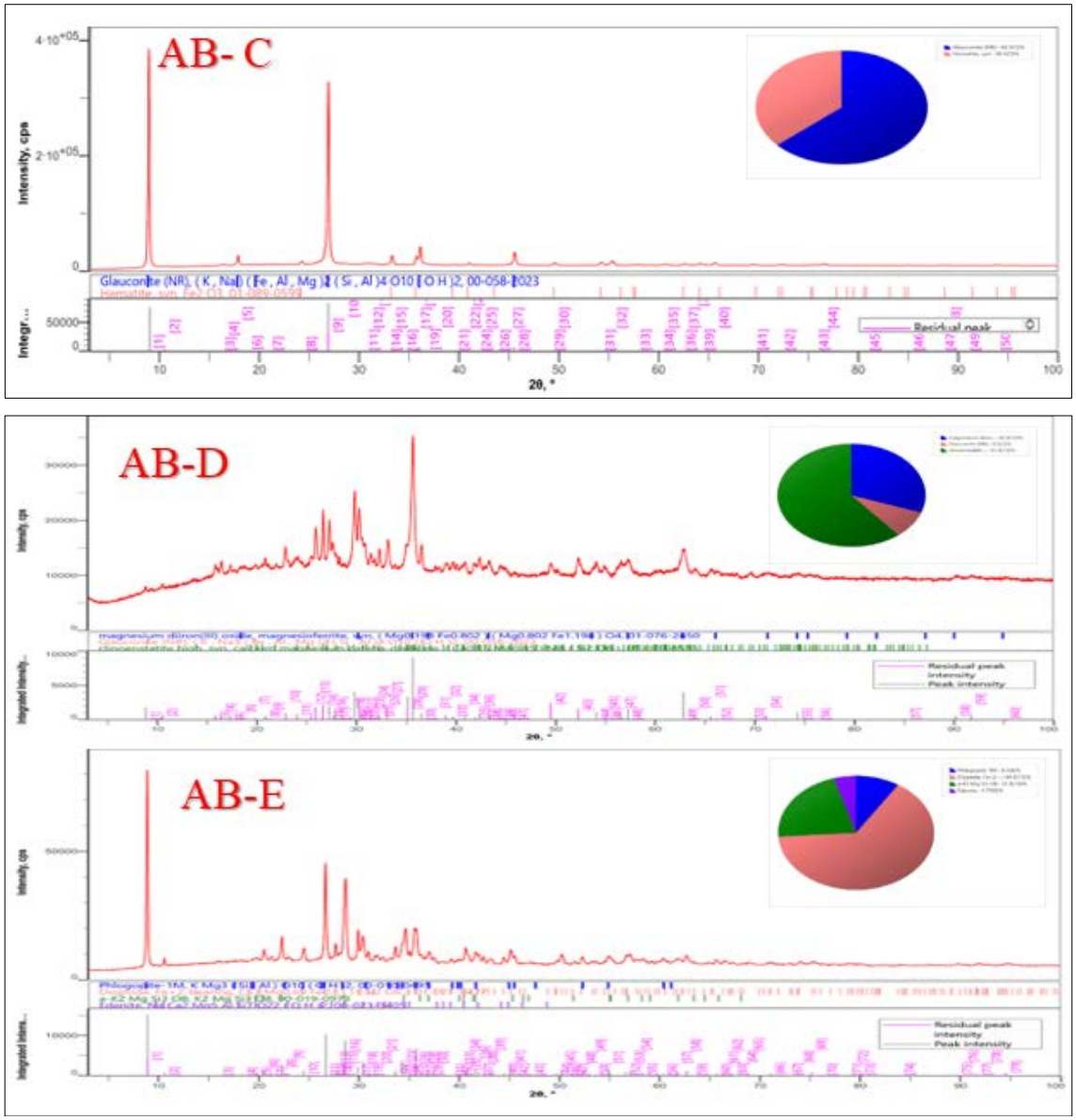


Fig 5: XRD pattern for AB 100puti prepared with different textual references

5. Conclusions

Iron is one of an active element in Abhrak Bhasma for its desired therapeutic efficacy. The current study determines the difference in Abhrak Bhasma prepared by 5 different manufacturers using different ayurvedic text references. We demonstrate that the morphology and chemical phase of 5 different AB preparation were different from each other, which might be responsible for variation in pharmacokinetic profile in wistar rats. Hence, this study shows that the preparation of Abhrak bhasma using different text reference shows variation in efficacy.

6. Conflict of interest

The authors declare that there are no conflicts of interest.

7. Acknowledgement

Author would like to thank Dr. Rajesh Raut, Botany Department, The Institute of Science, Mumbai for providing NTA facility.

8. References

1. Dwivedi V, Anandan EM, Mony RS, Muraleedharan TS, Valiathan MS, Mutsuddi M, *et al*. *In vivo* effects of traditional Ayurvedic formulations in Drosophila melanogaster model relate with therapeutic applications. PLOS One; c2012, 7.
2. Gopinath H, Shivashankar M. Herbo-metallic Indian nano-medicine Abhrak Bhasma (MICA): A periodical review. Res J Pharm Biol Chem Sci. 2016;7:2373-81.
3. Ilangovan SS, Sen S. Nano-Bhasmas for chronic non-communicable diseases. Res J Pharm Biol Chem Sci. 2016;7:925-31.
4. Agnivesha, Editor. Charaka Samhita: Chapter 1, Verse 69. 2nd Ed. Varanasi: Chaukhamba Sanskrita Sansthana; c2000. p. 12.
5. Tripathi YB, Sharma GMK, Singh VP, Sinha RK, Singh D. X-ray diffraction and microscopic analysis of tamra bhasma: An Ayurvedic metallic preparation. Indian J Tradit Knowl. 2003;2:107-17.
6. Wadekar MP, Patel RK. Preparation and characterization of a copper-based Indian traditional drug: tamra bhasma. J Pharm Biomed Anal. 2005;39(2):951-5.
7. Pritam D, Vaity C, Shah ND, Ninad D, Sathe S, Ashwini D, et al. Exploring rajatadi loha in the light of

research work: A review. Int Ayurveda Publ. 2016;3(2):838-43.
8. Hareshwar S, Mayuri D, Raman B. Preparation of abhrak bhasma and its evaluation on modern parameters. Int. J Ayurveda Pharma Res. 2017;5(2):30-6.
9. Kapoor RC. Some observations on the metal-based preparations in the Indian Systems of Medicine. Indian J Tradit Knowl. 2010;9:562-75.
10. Bhatia P, Purushottam GK. Analytical evaluation of an Ayurvedic formulation-Abhraka Bhasma. Int. J Pharm Sci Rev Res. 2013;23:17-23.
11. Tamhankar YL, Gharote AP. Effect of Puta on *in vitro* anticancer activity of Shataputi Abhrak Bhasma on lung, leukemia, and prostate cancer cell lines. J Ayurveda Integr Med. 2020;11(2):118-23. https://Doi.org/10.1016/j.jaim.2017.07.007
12. Kumar A, Nair GC, Reddy VR, Garg N. Bhasmas: Unique Ayurvedic metallic-herbal preparations, chemical characterization. Biol Trace Elem Res. 2005;3:231-54.
13. Joaquin P. An investigation on the activity pattern of alchemical transmutations. J Sci. Explor. 2002;16:593-602.
14. Das B, Achintya M, Jayaram H. Management of madhumeha (Diabetes mellitus) with current evidence and intervention with Ayurvedic rasausadhies. Indian J Tradit Knowl. 2011;10(4):624-8.
15. Rajput D, Mesram P, Patgiri BJ. Critical review on pharmaceutical prospects of nagabhasma (Incinerated lead). Int J Pharm Biol Arch. 2014;5(5):46-53.
16. Rasheed A, Marri A, Madhu Naik M. Standardization of bhasma-importance and prospects. J Pharm Res. 2011;4(6):1931-3.
17. Kantak S, Rajurkar N, Adhyapak P. Synthesis and characterization of Abhraka (Mica) bhasma by two different methods. J Ayurveda Integr Med. 2020;11:236-42. DOI: 10.1016/j.jaim.2018.11.003
18. Subedi RP, Vartak RR, Kale PG. Study of general properties of Abhrak Bhasma: A nanomedicine. Int. J Pharm Sci Rev Res. 2017;44(2):238-42. https://globalresearchonline.net/journalcontents/v44-2/47.pdf
19. Tamhankar YL, Gharote AP. Effect of Puta on *in vitro* anticancer activity of Shataputi Abhrak Bhasma on lung, leukemia, and prostate cancer cell lines. J Ayurveda Integr Med. 2020;11(2):118-23. DOI: 10.1016/j.jaim.2017.07.007
20. Buwa S, Patil S, Kulkarni PH, Kanase A. Hepatoprotective action of abhrak bhasma, an Ayurvedic drug in albino rats against hepatitis induced by CCl4. Indian J Exp Biol. 2001;39(10):1022-7.
21. Teli P, Chougule P, Jadhav J, Kanase A. Abhrak bhasma mediated alterations in liver and kidney functions in male albino rats during carbon tetrachloride induced toxicity. Int. J Res Ayurveda Pharm. 2013;4(5):696-700. DOI: 10.7897/2277-4343.04514
22. Kumar RCS, Ilango K, Kumar RM, Vasanth K, Kumar RVM, Mitti JG. Evaluation of physicochemical properties and diuretic activity of biotite calx (Abhraka bhasma) processed with *Tribulus terrestris* L.: An experimental study. Int. J Res Ayurveda Pharm. 2015;6(3):303-9. DOI: 10.7897/2277-4343.06361
23. Laxman TY, Deodatta BD, Tryambaklal MM, Nilesh S, Ekta T. Screening of immunomodulatory effect of Abhrak Bhasma-Ayurveda's Rasayan. Int. J Ayurveda Pharma Res; c2015. Available from: https://ijapr.in/index.php/ijapr/article/view/4
24. AOAC Official Methods of Analysis, 18th Edition. (AOAC-Association of Official Analytical Chemist)