Bio-accessibility of Pre-synthesized Abhrak, Naga and Tamra Bhasma

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Abstract:

Ayurvedic bhasmas play an important role in ayurvedic medicines and are useful in the treatment of various diseases. Pharmacokinetic studies involving estimation of bio-accessibility of bhasma will be useful in prescribing proper dosage of bhasma. Present paper deals with bio-accessibility of Abhraka, Naga and Tamra Bhasmas which were synthesized in our laboratory. It is revealed that the bio-accessibility of elements viz. K, Ca, Al, Fe, Na and Si in abhraka bhasma is more in gastric digestion than in gastro-intestinal digestion. Bio-accessibility of lead was found to be more in gastric digestion than gastro-intestinal digestion in Naga bhasma prepared by pisti and putapaka method while in bhasma prepared by jarana method it is more in gastro-intestinal digestion. In case of Tamra bhasma, bio-accessibility of copper is more in Gastro-intestinal digestion and it is further enhanced with anupana process, especially using ghee.

Key Words: Abhrak Bhasma, Naga Bhasma, Tamra Bhasma, Bio-accessibility, Ayurvedic bhasma

1.Introduction:

Ayurvedic bhasmas which are organometallic preparations, form very significant part of ayurvedic medicines and are useful in the treatment of various diseases. They are rejuvenating and have specific pharmacological activity. Being prepared from metallic raw materials these bhasmas may not be absorbed completely for circulation and hence pharmacokinetic study of bhasmas will play important role in prescribing the proper dosage of bhasma. Bio-accessibility determination is one of the important parameters in these studies. It is defined as a fraction of a dose of bhasma that is absorbed from its site of administration and reaches the general circulation. It will depend on its transfer across the

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membrane lining of Gastrointestinal tract. It further depends upon several factors such as particle size, particle shape, chemical phase etc. Survey of the literature on bhasma reveals that there are several reports in the literature on synthesis of ayurvedic bhasmas[1-6] but relatively less reports are available on characterization[7-9] and bio-accessibility studies[10-12]. Previous reports from our laboratory dealt with synthesis and characterization of Abhraka [13], Naga [14], Tamra[15], Rajat [9], Mandur [8], Yasad [16] and Pittal[17] bhasmas using modern analytical techniques. Present paper deals with bio-accessibility of Abhraka, Naga and Tamra Bhasma which were synthesized in our laboratory.

In general, bio-accessibility is affected by the type, composition of food and by the simulated gastro-intestinal conditions which may affect the distribution of initial species. Many factors that affect the bio-accessibility of a compound may be divided into exogenous factors such as complexity of the food matrix, the chemical form of the compound of interest, structure and amount of co-ingested compounds[18] as well as endogenous factors including mucosal mass, intestinal transit time, rate of gastric emptying, metabolism and extent of conjugation and protein binding in blood tissues. There are two approaches to estimate bio-accessibility: in-vitro and in-vivo techniques.

In-vivo studies are both expensive and laborious, and possibility of measuring certain parameters during the experiments are often limited [19] In this technique, bio available amount of an element of interest is estimated as the difference in the concentration of elements in ingest and excreta, using radio tracers.

In-vitro methods are rapid and inexpensive [20].It involves the simulation of the gastric and Gastro-intestinal digestive conditions in laboratory as the experiments are carried out under simulated digestive conditions. The results obtained by in-vitro methods are based on the formation of the digestive products that are soluble or dialyzable. However, these methods are efficient to identify potential food products as nutrient supplements**[21].** In-vitro method is routinely used to estimate the bio-accessible concentrations of essential elements in the diet. It is shown that the bio-accessible values obtained by these methods can be well related with that of human subjects [22] and many animal models [23]

2. Experimental

2.1 Material for gastric and gastro -intestinal digestion.

For preparation of gastric and gastro -intestinal digestion, Hydrochloric Acid was procured from Merck Chemicals, NaCl, $NaHCO_3$ from Aquafine Injecta Pvt. Ltd. and Pepsin and Pancreatin Bile salt were procured from Otto chemicals.

2.2. Evaluation of Bio-accessibility of different bhasma by in vitro digestion method

Bio- accessibility of Abhrak, Naga and Tamra bhasmas was determined by in vitro gastric and gastro- intestinal digestion methods. For this purpose, gastric juice and intestinal juice were used.

2.2.1 Preparation of gastric juice

Freshly prepared solutions of digestive enzymes were used in all the experiments. All the solutions were prepared in deionized water. Solution of pepsin (1:3000) was obtained by dissolving 6 g of pepsin in 100 ml of 0.15 M NaCl. The pH of gastric juice was adjusted by adding drops of 6 M HCl.

2.2.2 Preparation of Intestinal juice

Pancreatic solution was prepared by mixing pancreatic juice 2% and bile salt 0.2 %. The mixture was shaken for 1 min and pH was adjusted to 6.8 by adding drops of saturated $NaHCO_3$.

The pancreatic juice (2%) and bile salt solution (0.2%) were prepared by dissolving 2 g of pancreatin and 200 mg of bile salt respectively in 100 ml of 0.15 M of NaCl.

The detailed procedure for estimation of Bio- accessibility is shown schematically in Fig.1

The % bio-accessibility (% B) of each element in the all the bhasma samples was calculated using the following equation

$$\% B = \{ ([GD] \text{ or } [GID]) / [T] \} x100 \qquad --1$$

Where,

GD = concentration of element in Gastric digestion

GID = Concentration of element in Gastro-intestinal digestion

T = Total concentration of element in the sample

2.3 Elemental analysis of Bhasma

Elemental analysis of bhasma was carried out using AAS /ICPOES technique. The step-wise procedure is shown in Fig. 2

3 Results and Discussion

Synthesis of bhasma mainly consists of three processes

Shodhana Process: This process is mainly used to remove physical and chemical impurities of the starting material and increase the bio-accessibility and brittleness. Material is treated with liquids such as- sesame oil, butter milk, cow urine, kanji, horse gram decoction, lemon juice.

Marana Process: In this process, the product obtained after Shodhana process is treated with -mercury, arsenic, sulphur or herbal products. The resulting product will show change in original structure, composition and colour. Original inorganic form changes to organometallic form and becomes bio-acceptable.

Amrutikaran Process: Toxic impurities in the obtained product are removed in this process and total purity of bhasma increases. For this purpose, bhasma is treated with cow ghee or aloe vera juice.

The present work deals with the bio-accessibility studies of Naga bhasma prepared by three methods viz. Jarana method, pisti (amalgam) method and putapaka (herbal media) method. The details of synthesis are described in our previous report[14]. The work also deals with the bio-accessibility studies of abhraka bhasma which was prepared by three different methods wherein shodhana process is carried out with cow milk (method 1), Cow urine(method 2) and triphala decoction(method 3) ; Marana process with Jaggery + Juice of Ricinus Communis(method(1), Cyperus rotundus (Musta) decoction+ Calotropis procera juice (method 2), turmeric powder decoction + Borax (Method 3) while dhanya abhrak process (with paddy) and Amritikaran process(with Cow ghee) are common for all the three methods. The details of synthesis are described in our previous paper[13]. The Tamra bhasma was prepared by traditional method and details are given in our previous report[15] The obtained results are discussed below.

3.1 Bio-accessibility of Abhrak bhasma

The Bio-accessibility of Abhrak bhasma prepared by three different methods was carried out using ICPOES method and is shown in Tables 1 and 2.

An examination of Tables 1 and 2 shows that bio-accessibility of elements viz. K, Ca, Al and Fe in abhraka bhasma was found to be more in gastric digestion than gastro-intestinal digestion. While in commercial samples, the bio-accessibility of these elements was found to

be more in gastro-intestinal digestion. K, Ca, Al and Fe require acidic medium for their absorption. Hence Abhrak bhasma prepared by method 1, 2 and 3 will be absorbed in better way than commercial samples. % bio-accessibility of sodium was found to be higher than 100% in all the samples as sodium was used for pH control

In Ayurveda, the Abhrak bhasmas prepared by different methods are used for treating different diseases. Abhrak bhasma prepared by method-1 is commonly used for treating anemia and tropical sprue. The above analysis shows that Abhrak bhasma prepared by method 1 shows greater bio-accessibility for Fe than other two methods, although other two methods contain greater elemental concentration than method 1. method-2 is commonly used for treating chronic cough while Abhrak bhasma prepared by method-3 is commonly used for treating asthma, the bio-accessibility of Mg is enough to treat chronic cough. From the study it appears that Abhrak bhasma prepared by method -1 is more bio-accessible than other two methods.

3.2 Bio-accessibility of Naga bhasma

The Bio-accessibility of Naga bhasma prepared by three different methods was studied using AAS technique and results are shown in Table 3.

As can be seen from Table 3 bhasma prepared by putapaka method shows more bio-accessibility than jarana and pisti method. It is also observed that bio-accessibility of lead is more in gastric digestion than intestinal digestion in putapaka and pisti method. In case of jarana method bio-accessibility of lead was found to be more in Gastro-intestinal digestion than gastric digestion. Commercial bhasma sample also shows same trend as that of bhasmas prepared by putapaka method.

Naga bhasma prepared by different methods are used for treating different diseases and have their own merits e.g. bhasma prepared by jarana method is commonly used for upper respiratory diseases, that prepared by pisti method is used for colitis and that prepared by putapaka method is used for treating diabetes mellitus.

Our earlier report[14] revealed that Bhasma prepared by jarana and pisti method show lead sulphide while putapaka method shows PbO i.e. lead oxide form. PbS form is relatively less toxic. The bio-accessibility of lead in putapaka method is more than other two methods.

3.3 Bio-accessibility of Tamra bhasma and in different anupana

In order to increase the bio-accessibility of bhasma, ayurvedic literature suggests the use of Anupana[24] process. Anupana is that material which is consumed along with the bhasma

which helps to increase the palatability of bhasma and improves the digestion and absorption. It acts as a vehicle which carries the bhasma particles to their target site. In this paper an attempt has been made to study bio-accessibility of tamra bhasma with different anupana.
The bio-accessibility of Tamra bhasma prepared in different anupanas was studied using AAS technique for elemental analysis and results are shown in Table 4.

As can be seen from Table 4, Tamra bhasma mixed with different anupana like honey and piper longum mixture, ghee and fermented juice of Aloe vera shows more gastric and gastro-intestinal bio-accessibility than used as alone. As per literature, anupana is used to enhance bio-accessibility of Tamra Bhasma. The above observation shows increased bio-accessibility in anupana than without anupana. It is found that Gastro-intestinal bio-accessibility is more than gastric digestion. The bio-accessibility is more in both gastric digestion and gastro-intestinal digestion by using ghee as a anupana.

Conclusions:

The bio-accessibility of elements: K, Ca, Al, Fe, Na and Si in abhraka bhasma was found to be more in gastric digestion than gastro-intestinal digestion as expected. Same trend is observed for lead in Naga bhasma prepared by pisti and putapaka methods while in bhasma prepared by jarana method, bio-accessibility of lead is more in gastro-intestinal digestion. The bio-accessibility of copper (Tamra) was found to be more in gastro-intestinal digestion than in gastric digestion. Further, it is enhanced in anupanas, ghee shows more bio-accessibility than other two anupanas.

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Figures:

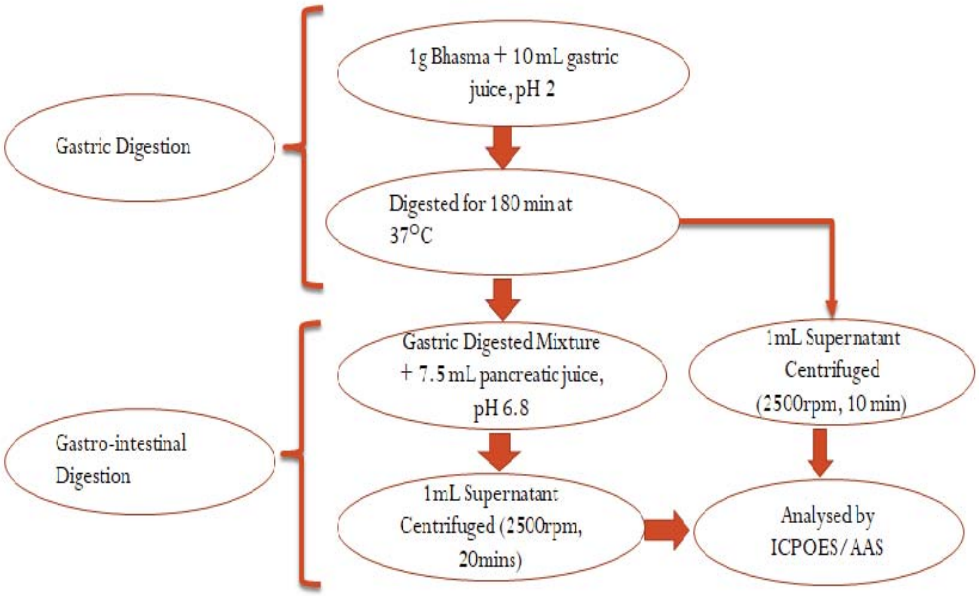

Fig.1 A Schematic diagram of Bio-accessibility estimation

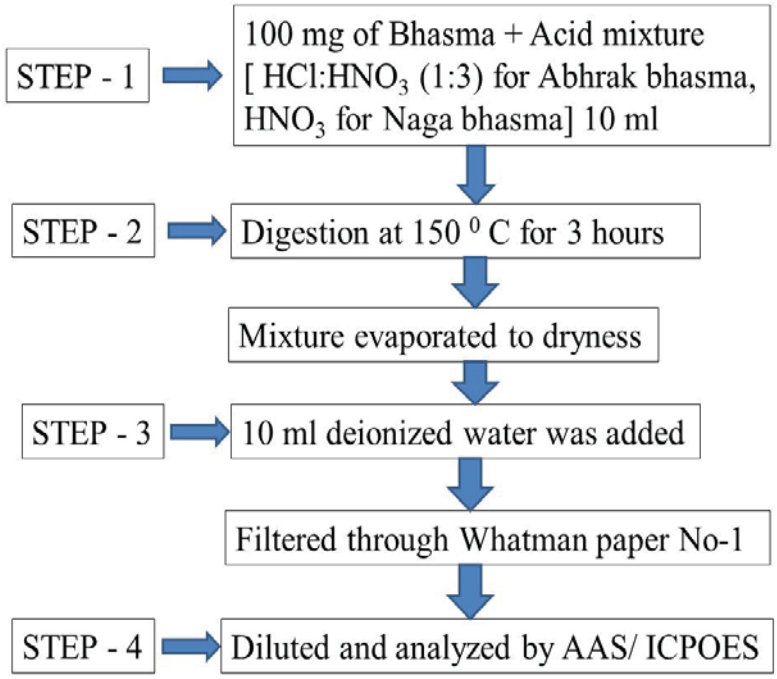

Fig.2. Schematic diagram for analysis by AAs/ICPOES

Tables:

Table 1: Bio-accessibility of elements in Abhrak bhasma

Method	Element	Concentration of Element / %	Bio-accessibility of Element / %	
			Gastric digestion	Gastro-intestinal digestion
Method -1	K	4.4	66.6	66.4
	Ca	6.2	67.1	52.7
	Al	2.64	44.3	0.7
	Mg	6.6	42.6	42.7
	Fe	7.4	12.9	0.4
Method-2	K	5.5	19.1	14.1
	Ca	7.3	26.2	9.0
	Al	7.2	23.7	0.02
	Mg	9.2	11.8	6.3
	Fe	11.4	1.84	0.01
Method -3	K	5.3	51.9	42.6
	Ca	5	20.2	8.9
	Al	4.5	36.4	0.03
	Mg	7.2	14.6	11.0
	Fe	14.3	0.2	0.02

Table 2: Bio-accessibility of elements in commercial Abhrak bhasma

	Elements	Concentration of Element / %	Bio-accessibility of Element / %	
			Gastric digestion	Gastro-intestinal digestion
Commercial -1	K	5.2	33.1	28.2
	Ca	5.5	35.3	38.7
	Al	5.3	22.3	17.4
	Mg	9.4	0.1	20.5
	Fe	11.8	3.5	1.4
Commercial -2	K	0.46	10.0	43.0
	Ca	11.4	0.1	26.5
	Al	0.01	10.0	16.5
	Mg	1.6	0.06	9.9
	Fe	37.5	0.002	0.05

Table 3: Bioaccessibility of Naga bhasma

Bhasma method		Concentration of lead / %	Bio-accessibility of lead / %	
			Gastric digestion	Gastro-intestinal digestion
Jarana method	with samanya shodhana	2.31	0.4	3.1
	without samanya shodhana	6.23	0.1	3.6
Pisti method	with samanya shodhana	5.56	5.6	1.6
	without samanya shodhana	2.69	3.1	3.7
Putapaka method	with samanya shodhana	2.28	8.7	2.1
	without samanya shodhana	2.90	8.9	8.7
Commercial -1		1.63	7.7	3.0
Commercial -2		1.45	7.3	4.8

Table 4 : Bioaccessibility of Tamra bhasma in different anupana

Anupana	Concentration of Copper / %	Bio-accessibility of Copper/ %	
		Gastric digestion	Gastro-intestinal digestion
Final Bhasma	59.8	0.4	1.0
Honey and Piper longum	59.8	1.4	4.1
Fermented juice of Aloe-vera	59.8	0.8	1.0
Ghee	59.8	1.0	1.4

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