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EXPERIMENTAL STUDIES ON VARIOUS LOHA PREPARATIONS W.S.R TO "MRITANI LOHANI RASI BHAVANTI"

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ABSTRACT

Ayurvedic system of medicine is the only one out of all traditional system of medicine where importance of metals for curing ailments was probably first recognized. Iron is the fourth common element and second most common metal in the earth crust and is a biologically essential component of every living organism. Despite the low requirement of iron in human body iron overload is rare and iron deficiency is common in certain parts of the world and in certain age groups6. Iron containing drugs used as hematinics are known to induce some adverse drug reactions -- gastrointestinal symptoms (nausea, vomiting, epigastric pain, colic pain, flatulence, constipation, black feces, and diarrhea. Iron containing compounds like *Loha Bhasma, Kasis Bhasma* and *Mandura Bahsma* are practiced since long and are indicated in a wide spectrum of diseases and can be a better alternative from Rasa shastra. Pharmacokinetics is proposed to study the absorption, the distribution, the bio transformations and the elimination of drugs in man and animals. A primary aim of pharmacokinetics analyses is to determine bioavailability. Evaluation of Pharmaco kinetics of *Loha bhasma*, *Kasis bhasma* and *Mandura bhasma* were carried out. Serum iron was estimated using AAS. Parameters like Cmax, Tmax etc. were calculated

Keywords: *Loha Bhasma, Kasis Bhasma*, *Mandura Bahsma*, Cmax, Tmax.

INTRODUCTION

Traditional medicines are used in the treatment of various chronic disorders and for the improvement of well-being of individuals. *Loha Bhasmas* have been prescribed by Ayurvedic physicians since long with rare mentions of toxicity. It is observed that herbomineral complexes are more stable and more interactive compared to plain herbs as these result in faster therapeutic action and have a longer shelf life[1]. In traditional methods of processing employed in Ayurveda, the metals are repeatedly subjected to *Shodana* (purification with naturally available ingredients), bhavana (trituration with herbal juice) and *Bhasmikarana* (calcination) to obtain the final product. These processes reduce the final product to nanometer size, which is believed to enhance its bioavailability and reduce its toxicity [2]. Pharmacokinetics which deals with the absorption, distribution, metabolism and excretion of the biomarkers or the new drug entity is the one of the regulatory requirements for an investigational new drug approval. Bioactive guided pharmacokinetic approach method is needed for Ayurvedic system of medicine to determine the pharmacokinetics of relevant markers in the formulation having number of markers[3]. The mentioned Verse is in the text like *Rasendra Mangal, Rasarnava, Ras Ratna Samuchchaya, Ras Ratnakar*, the verse signifies that the absorption and assimilation of different types of *Bhasma* of minerals / metals / sub metals & *Sindoora Kalpana* for internal administration in human body. In *Ayurvedic* formulations the concept of drug action and absorption is designed on the basis of the *Panchamahabhuta* theory, *Agni*, *Rasa*, *Guna*, *Virya*, *Vipaka* etc. of the drug. Pharmacokinetics studies supports the studies of preclinical toxicology in animals (toxicokinetics) because the drug levels in plasma or tissues are often more predictive than the dose to extrapolate the toxicity data to man. To go for the bio availability of the Ayurvedic formulations especially Herbo minerals is a daunting task but they provide strong evidence base to the safety and efficacy of herbo-mineral formulations. The present pharmacokinetic study was undertaken with a view to identify a few pharmacokinetic parameters for the iron containing preparations like *Loha, Kasis* and *Mandura bhasma.*

Aim and Objectives

- To carry out pharmacokinetic study of iron containing formulations like *Loha Bhasma, Kasis bhasma and Mandura bhasma* in rats.
- To determine the time dependent concentrations of administered drugs (*Loha, Kasis and Mandura bhasma*) in the collected serum of each animal
- To determine the Cmax, Tmax, etc. of the administered drugs.

To compare the pharmacokinetic parameters of the test drugs with that of the standard drug Ferrous sulphate.

Materials and Methods

- **Test Drugs**

1. *Loha bhasma*, 2. *Kasis bhasma*, 3. *Mandura bhasma* The samples were prepared in the Dept. of *Rasashastra* and *Bhaishajya Kalpana,* N.I.A. Jaipur.

- **Standard drug,** Ferrous sulphate
- **Selection of Animals and Animal Care**

The experiment was carried out in Biomedical and Industrial Lab, Jaipur. Ethical clearance was obtained from Institutional Animal Ethics Committee, before conducting the experiment (IAECAPPROVAL NO: ibir/iaec/2015/II/7**)**. The study was conducted on mature Sprague-Dawly rats, weighing 150 -200g. Animals were acclimatized for a period of seven days in laboratory conditions prior to the experiments. Rats were housed in poly propylene cages (six rats per cage), at an ambient temperature of 25 ± 2°C with 12 h light: 12 h dark cycle in the animal house of Biomedical and Industrial Lab. The animals were provided with standard pellet diet and water *ad libitum*. The Principles of Laboratory Animal Care were followed throughout the duration of the experiment.

- **Instruments used**: Weighing scale, needle, syringe, mono pan balance, rubber catheter, mortar & pestle, surgical instruments, cannula, sterilizer, pipette, glass slides, Beaker (40 ml, 100 ml, 250 ml), Measuring cylinder (100 ml, 10 ml, 50 ml), volumetric flask (100 ml), Sterile Blood Collection Vile etc.
- **Dose Calculation**: The dose was calculated by extrapolating the human dose to animal based on the

body surface area ratio by referring to the table of Paget and Barnes (1969).

- **Dose conversion:** Animal dose= Human Dose x 0.018x5/kg body weight, Considering the human dose of *Loha Bhasma, Kasis Bhasma and Mandura Bhasma* as 250mg, the dose to be administered in rats were calculated as 22.5mg/kg body weight.
- **Mode of administration:** Animals in each group were dosed as per the study design presented in table. Animals were kept fasting for 24 hours prior administration of drugs All the animals were weighed before dose administration and volume required for each animal was calculated according to weight. Dose formulations were prepared on the day of dosing. Sample of test drugs *Loha Bhasma, Kasis Bhasma and Mandura Bhasma* and the standard drug ferrous sulphate were taken 2g each in porcelain mortar and 2% CMC was added, the formed mixture was further ground for 5 minutes and the volume was made up with distilled water, so as to contain the required dose in solution.. The prepared suspension was administered orally with the help of feeding needle attached to a disposable 1ml syringe. Care was taken that no air bubble was passed at the time of dosing.

Table 1: Showing Grouping of animals

Group	No of rats	Intervention
Group A:	6	2% CMC Solution 5 ml/kg) per oral.
Group B:	6	*Kasis Bhasma* 22.5 mg/kg per oral, single dose
Group C:	6	*Loha Bhasma* 22.5 mg/kg per oral, single dose
Group D:	6	*Mandura Bhasma* 22.5 mg/kg per oral, single dose
Group E:	6	Ferrous Sulfate 22.5 mg/kg per oral, single dose

- **Procedure of blood withdrawal:** Rats were anesthetized by using ketamine and xylane mixture (90:10, IP, Dose volume:2ml/kg)[4]. A 2cm ventral cervical skin incision was made right of the midline. Underlying salivary and lymphatics were separated by means of blunt dissection to visualize the right common jugular vein. Jugular vein was then isolated from surrounding tissues and a pair of thread was passed below the blood vessel. Tunnel was made with the help of trocar to exteriorize the cannula towards neck. The exteriorized part was made secure in place with 3-0 lifeline sterile thread. Skin incision was closed, and the exteriorized cannula was filled with stock solution (100 IU/Ml of heparinized saline.)
- **Blood Sampling:** Blood samples were collected post dosage 0 min, 30 min, 60 min, 90 min, 120 min, 180 min and 240 min in microfuge tubes containing K2EDTA (20 µL/mL of blood, 200 mM) as anticoagulant. In jugular vein catheterized rats, after each sampling, equal volume of heparinized saline (10 IU/mL) was injected. Plasma was harvested from blood by centrifugation of samples at 2500 g for 10 min at 4°C and stored below -60°C until bio analysis[5].
- **Statistical analysis:** Exposure (Cmax) between different groups for oral dose administration were compared for statistical significance using a one-way Analysis of Variance (ANOVA) with Tukey's multiple comparisons set. A P value of less than 0.05 was considered significant. The statistical analysis was performed using Graph Pad instant software version 3.

Observations and Results

After administration of vehicle to the control group A the serum the blood samples were collected at 0, .5, 1, 1.5, 2, 3 and 4 hours respectively. However, the serum iron concentration was at non detectable level in all rats during the whole period.

Table 2: Showing serum iron concentration of Group B rats at various time interval post dosing of *Kasis Bhasma*.

Time Point (hours)	**Serum iron concentration mg/l of group B rats**							
	1	**2**	**3**	**4**	**5**	**6**	**Mean**	**SD**
0	ND	ND	ND	ND	ND	ND	-	-
0.5	0.85	0.86	0.87	0.84	0.83	0.85	0.85	0.0141
1	0.72	0.72	0.75	0.71	0.73	0.69	0.72	0.0200
1.5	0.68	0.71	0.69	0.65	0.69	0.67	0.616	0.6816
2	0.46	0.50	0.42	0.43	0.49	0.48	0.463	0.0326
3	ND	ND	ND	ND	ND	ND	-	-
4	ND	ND	ND	ND	ND	ND	-	-

As mentioned in table no.2 following administration of *Kasis Bhasma* serum iron concentration was non detectable assuming it to be zero at 0 hour followed by a mean plasma concentration of .85mg/ml at 5 hour. The concentration then decreased to .72 at 1 hour,0.616 at 1.5 hours, .463 at 1.5 hours and finally was non detectable at 3 and 4 hours post dosing. Maximum concentration Cmax was found to be .85 mg/l and Tmax. 5-hour post dosing as depicted in the graph 1.

Table 3: Showing serum iron concentration of Group C rats at various time interval post dosing of *Loha bhasma.*

Time Point (hours)	**Serum iron concentration mg/l of group C rats**							
	1	**2**	**3**	**4**	**5**	**6**	**Mean**	**SD**
0	ND	ND	ND	ND	ND	ND	-	-
0.5	1.28	1.22	1.34	1.25	1.31	1.27	1.278	0.042
1	0.58	0.61	0.63	0.55	0.53	0.57	0.577	0.034
1.5	0.45	0.43	0.41	0.49	0.47	0.46	0.452	0.026
2	0.25	0.29	0.31	0.19	0.21	0.26	0.252	0.041
3	0.12	0.15	0.13	ND	ND	0.11	0.124	0.016
4	ND	ND	ND	ND	ND	ND	-	-

Following administration of *Loha Bhasma* serum iron concentration was non detectable assuming it to be zero at 0 hour followed by a mean plasma concentration of 1.278mg/l at .5 hour. The concentration then decreased to .577 at 1 hour,0.452at 1.5 hours, .252 at 1.5 hours,.124 at 3 hours and finally was non detectable at 4 hours post dosing. Maximum concentration Cmax was found to be 1.278 mg/l and Tmax. 5-hour post dosing as depicted in the graph 2.

Table 4: Showing serum iron concentration of Group D rats at various time interval post dosing of *Mandura Bhasma.*

Time Point (hours)	Serum iron concentration mg/l of group D rats							
	1	**2**	**3**	**4**	**5**	**6**	**Mean**	**SD**
0	ND	ND	ND	ND	ND	ND	-	-
0.5	0.36	0.35	0.38	0.37	0.34	0.35	0.358	0.014
1	0.43	0.40	0.46	0.49	0.37	0.44	0.431	0.042
1.5	0.53	0.52	0.55	0.54	0.51	0.52	0.528	0.014
2	0.47	0.46	0.49	0.48	0.45	0.46	0.468	0.014
3	0.17	0.16	0.19	0.18	0.15	0.18	0.171	0.014
4	ND	ND	ND	ND	ND	ND	-	-

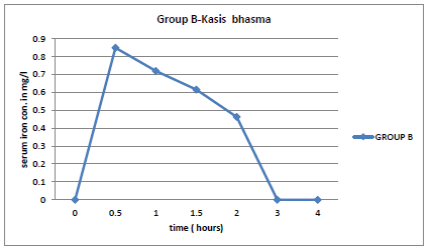
Following administration of *Madura bhasma* serum iron concentration was non detectable assuming it to be zero at 0 hour followed by a mean plasma concentration of .358mg/ml at .5 hour. At 1 hour it increased to .431 mg/l. The concentration then decreased to .528 at 1.5 hour,0.468 at 2 hours, .171 at 3 hours and finally was non detectable at 4 hours post dosing. Maximum concentration Cmax was found to be 0.528 mg/l and Tmax 1.5-hour post dosing as depicted in the graph 3.

Table 5: Showing serum iron concentration of Group E rats at various time interval post dosing of standard ferrous sulphate.

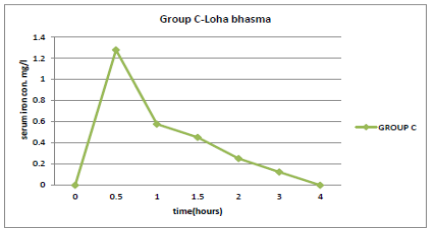
Time Point (hours)	Serum iron concentration mg/l of group E rats							
	1	2	3	4	5	6	Mean	SD
0	ND	ND	ND	ND	ND	ND	-	-
0.5	0.18	0.15	0.12	0.24	0.21	0.19	0.181	0.042
1	0.99	0.96	0.94	1.04	1.03	1.02	0.996	0.040
1.5	0.53	0.52	0.55	0.54	0.51	0.52	0.551	0.042
2	0.50	0.47	0.44	0.56	0.53	0.51	0.501	0.042
3	ND	ND	ND	0.11	ND	ND	0.11	-
4	ND	ND	ND	ND	ND	ND	-	-

Following administration of ferrous sulphate serum iron concentration was non detectable assuming it to be zero at 0 hour followed by a mean plasma concentration of .181 mg/l at .5 hour and increased to .996 at 1 hour. The concentration then decreased to .551 at 1.5 hours, .501 at 2 hours. At 3 hours no serum iron was detected all the blood samples except one which showed a value of .11. Serum iron became non detectable at 4 hours post dosing. Maximum concentration Cmax was found to be 0.996 mg/l and Tmax 1-hour post dosing as depicted in the graph 4.

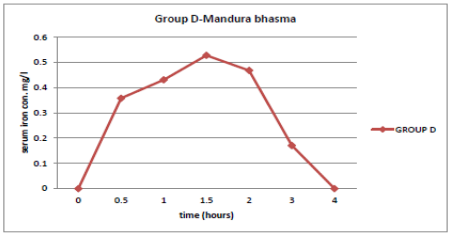
Graph 1. Serum iron concentration-time profile of *Kasis bhasma* following oral administration



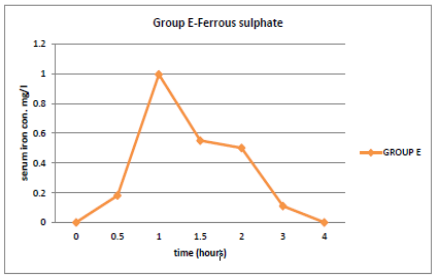
Graph 2. Serum iron concentration-time profile of *Loha bhasma* following oral administration



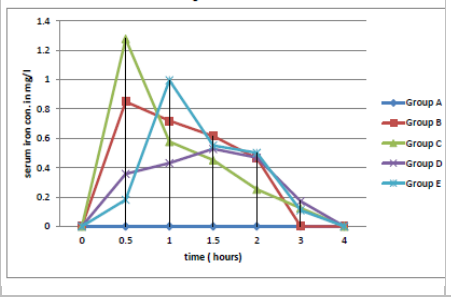
Graph 3. Serum iron concentration-time profile of *Mandura bhasma* following oral administration



Graph 4: Serum iron concentration-time profile of ferrous sulphate following oral administration



Graph 5. Serum iron concentration-time profile of test drugs and standard following oral administration



Graph 6: Showing Comparison of Cmax of various groups (group A excluded)

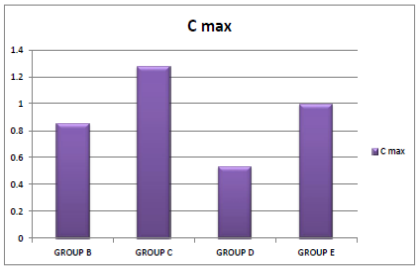


Table 6: showing Comparison of Cmax of various groups-One-way Analysis of Variance (ANOVA)

S. No	Comparison	Mean Difference	q value	P value	Significance
1.	Group B Cmax Vs Group C Cmax	-0.4283	33.775	P<0.001	***
2.	Group B Cmax Vs Group D Cmax	0.3217	25.364	P<0.001	***
3.	Group B Cmax Vs Group E Cmax	-0.1467	11.565	P<0.001	***
4.	Group C Cmax Vs Group D Cmax	0.7500	59.139	P<0.001	***
5.	Group C Cmax Vs Group E Cmax	-0.2817	22.210	P<0.001	***
6.	Group D Cmax Vs Group E Cmax	-0.4683	36.929	P<0.001	***

DISCUSSION

At 0.5-hour post administration of drug maximum concentration was reached by *Loha bhasma* which was 1.278mg/l followed by *Kasis bhasma* which showed a serum concentration of .85mg and *Mandura bhasma* .358mg/l. The least serum iron concentration was for the standard drug ferrous sulphate at .05 hour. But at 1-hour post administration ferrous sulphate showed the maximum concentration of .996mg/l followed by *Kasis Bhasma and Loha Bhasma*. Least serum iron concentration was for *Mandura*. At 1.5-hour post administration maximum concentration was seen in *Loha* administered group followed closely by standard group ferrous sulphate and *Mandura*. Least concentration was seen in iron administered group.

Cmax of group B, C, D and E were statistically analyzed using ANOVA. The comparison between the groups showed that the values were extremely significant. Thus, the study showed that the absorption and distribution of three *Loha* preparations differs according to the final *Bhasma* composition after interaction with various herbal complexes during its pharmaceutical processing.

The study showed that Cmax was higher for *Loha Bhasma* when compared to ferrous sulphate, *Kasis* and *Mandura*. Thus, the present work reveals that the absorption and plasma concentration of the three iron containing formulations are different even though the chemical composition is similar in the *Bhasma*. This can be attributed to the different processing of *Bhasma* and utilization of different herbs in their preparation which may have led to complex formation along with the major mineral part. The presence of different functional group was proved in analytical tests also. A higher concentration of *Loha Bhasma* can be attributed to the *Triphala* used in its preparation in a greater amount. Among the various ingredients *Triphala* which mainly consists of ascorbic acid (vitamin c), increases the bioavailability of iron by converting Fe3+ to Fe2+, while tannins and phenolics can reduce the iron by binding to it. In other words, this may also be taken as the various constituents of *Triphala* have antagonizing activity. The pharmacokinetics profiles of iron preparations thus provide useful information regarding the various aspect of drug activity of *Bhasma* preparations which can add to the evidence base of

Ayurveda which further help in the global acceptance of Ayurvedic system of medicine.

CONCLUSION

Maximum concentration Cmax of *Kasis bhasma* was found to be .85 mg/l, for *Loha bhasma* 1.278 mg/l, for *Mandura bhasma* .528 mg/l and for standard 0.996 mg/l. Tmax of *Kasis bhasma* was 30 minutes post dosing, for *Loha bhasma* 30 minutes, for *Mandura bhasma* 90 minutes and for standard 60 minutes. The study showed that Cmax was higher for *Loha bhasma* when compared to ferrous sulphate and other *Loha* preparations *Kasis* and *Mandura*. Although *Bhasmas* are complex materials, experimental analysis using modern techniques will be most attractive for the standardization of *Bhasma* medicines. This would definitely help in building confidence in use of such products for medication by ensuring genuinely, safety, efficacy, and batch to batch uniformity.

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