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RESEARCH ARTICLE

EXPERIMENTAL STUDIES ON *HARAGOURIRASA* W.S.R *TO "MRITANI LOHANI RASI BHAVANTI"*

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Abstract

Ayurveda, one of the ancient systems of medicine, holds a vast domain of traditional knowledge within himself. It is considered as the science of life and has been practiced in India since times immemorial. A very systematic and elaborate step-wise procedure known as '*Bhasmikarana'* converts the metal from its zero valent state to a form with higher oxidation state, which is crucial from the point of view that during this process the toxic nature of the metal and its oxide is fully destroyed while rendering the metal oxide with high medicinal value. The mentioned Verse is in the text like *Rasendra mangal, Rasarnava, Ras Ratna Samuchchaya, Ras Ratnakar*, The verse signifies that the absorption &assimilation of different types of *Bhasma* of minerals / metals / sub metals & *Sindoora kalpana* for internal administration in human body. Reduction in the particle size is the motto of using in the form of *Bhasma* or Nano medicine. The finer the particle size of *bhasma* allows better rate of assimilation and absorption. This is a critical attempt to understand and apply the basis of *Ayurvedic* concept of *Bhasma* and the concept of Pharmacokinetics. Pharmacokinetics 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.

Introduction:-

Metal-based drugs prepared according to *Rasa Shastra* guidelines are considered to be safe and efficacious at minute doses without any adverse effects. 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[i]. However, the past decade has witnessed concerns in Western countries over the use of metals in *Ayurvedic* formulations and several reports have emerged linking their presence in formulations to toxic effects.[ii,iii]

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In *Rasa shastra* the metals and minerals are also termed as *Dhatus* and *Updhatus* because of their specific role in biological system that this can sustain body tissue by supplementing some of the essential elements to the tissues, whose deficiency causes many disease in the body . 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. There is an urgent need of standardization and pharmacokinetics of the *Ayurvedic* formulations in order to achieve the uphold position in the global market. Pharmacokinetics which deals with the absorption, distribution, metabolism and excretion of the biomarkers or the new drug entity is the one of the regulatory requirement 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. Also non compartmental analysis method should be applied for the analysis of pharmacokinetics of biomarkers from *Ayurvedic* formulations for successful pharmacokinetic evaluation.

As far as *Ayurvedic* formulations are concerned 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 *haragourirasa*.

Material and Methods:-

Test Drug

The sample of *Haragourirasa* was prepared in the Dept. of Rasashastra and Bhaishajya Kalpana, N.I.A. Jaipur.

Experimental design and animal management

The experiment was carried out in DATTA MEGHE INSTITUTE OF MEDICAL SCIENCES SAWANGI WARDHA (Maharashtra). Ethical clearance was obtained from Institutional Animal Ethics Committee, before conducting the experiment (IAEC APPROVAL NO:DMIMS(DU)/IAEC/2018-19/08). The study was conducted on mature Albino rabbits, weighing 1.50 -2.00kg. Animals were acclimatized for a period of seven days in laboratory conditions prior to the experiments. Rabbits are kept at an ambient temperature of 25 ± 2°C with 12 h light: 12 h dark cycle in the animal house of DATTA MEGHE INSTITUTE OF MEDICAL SCIENCES SAWANGI WARDHA (Maharashtra). 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 *haragourirasa* as 250 mg, the dose to be administered in rabbits was 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. 10% gum acaia solution was used as vehicle for drug administration. To prepare it 90 ml RO water was taken in a clean 250 ml borosil beaker then 10 g gum acacia powder was added in it after that it was put on a magnetic stirrer till it completely dissolved in water. It took about 10 minutes.

1. Then a calculated amount of drug according to body weight of rabbits was dissolved in this gum acacia solution.
2. It is convenient for *Rasaushadhi*, because it remains in suspended form in it.
3. Then calculated dose of this suspension were given orally to rabbits by 18 No. gavanging needle and 10 ml syringe.

Procedure:
For the study 10 healthy young adult albino rabbits weighing 1.5-2kg were employed for the study and grouped in the following manner:

Table No.1:- Showing the grouping of the animals.

GROUP A	(N=5)	Single dose given
GROUP B	(N=5)	Dose given for 7 days

The animals were randomly selected and marked in a specific manner to permit individual identification i.e.:

Table no.2:- Showing the marking of the animals.

Rabbit no.	Marking area
R1	No marking
R2	Head
R3	Body
R4	Tail
R5	Head and body

Same pattern of marking was followed in the both group. The animals were kept in their cages for 7 days prior to dosing to allow for acclimatization to the laboratory conditions The Test drug (Haragourirasa) was administrated to a group of experimental animals, by its oral route at one defined dose. A solution of 10% Gum acacia will be prepared and used as vehicle to administrate the drug.

Blood Collection Technique: Marginal Ear Vein
1. Rabbit was placed in a rabbit restrainer.
2. Using clippers, hair was removed from the ear.
3. Finger was placed, loosely fitting paper clip, or other form of light tourniquet at the base of the ear.
4. A 25-gauge, 5/8-inch, 1-inch needle was used.

Precautions:
1. Small quantities can be collected from the hub of a 25-gauge needle directly into a microhematocrit tube.
2. The larger needle is attached to a syringe and blood is collected slowly into the syringe to avoid collapsing the vein.
3. If blood does not flow readily, there may be a clot formation in the needle or too much negative pressure applied to the syringe. Release the negative pressure and slowly rotate the needle.
4. Repeat these steps as needed until the desired quantity of blood has been collected.

Blood Sampling
Blood samples were collected post dosage 0 hr, 1 hr, 2 hr, 4 hr, 8hr, 10 hr and 12 hr in microfuge tubes containing K2EDTA (20 µL/mL of blood, 200 mM) as anticoagulant. , 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 -20°C until bio analysis.

Observations And Results:-
After administration of vehicle to the control group A the serum the blood samples were collected at 0,1,2,4,8,10,12 hours respectively. However the serum mercury concentration was at non detectable level in all rabbits during the whole period.

Table no.3:- Showing serum mercury concentration of Group A rabbits at various time interval post dosing of *Haragourirasa*.

Time Point(hours)	Serum mercury concentration µg/l (PPB) of group A rabbits						
	1	2	3	4	5	Mean	SD
0	0	0	0	0	0	0	0
1	6.64	8.12	6.56	6.75	6.95	7.004	0.6407
2	8.33	10.56	9.42	6.77	10.61	9.138	1.6217
4	8.51	11.59	12.67	13.21	11.04	11.404	1.8536
8	26.03	25.71	18.49	21.88	18.44	22.110	3.7054
10	21.88	15.59	12.04	11.76	38.79	20.012	11.2614
12	9.99	11.02	8.33	8.12	15.02	10.496	2.7937

Graph Showing serum mercury concentration of Group A rabbits at various time interval post dosing of Haragourirasa.

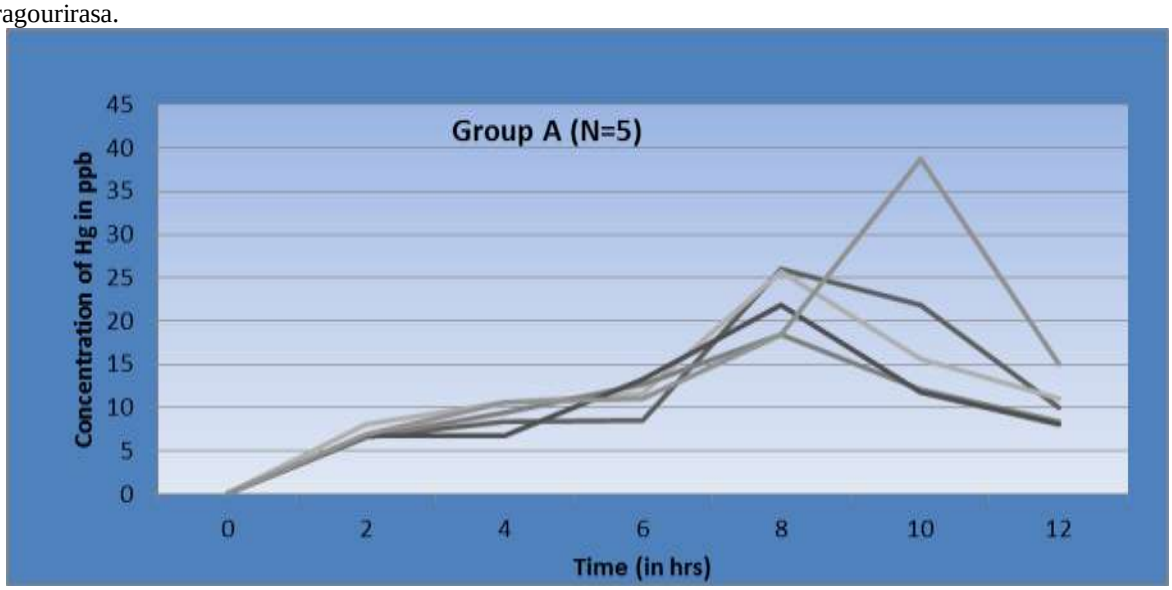

Table no.4:- showing serum mercury concentration of Group B rabbits after 7day time interval post dosing of *Haragourirasa.*

Time point (after 7 days)	**Serum mercury concentration µg/l (PPB) of group B rabbits**						
Animal	**1**	**2**	**3**	**4**	**5**	**Mean**	**SD**
Time Con. At 8th Hrs	**28.23**	**34.19**	**33.08**	**31.39**	**22.38**	**29.854**	**4.7397**

Discussion:-

Pharmacokinetics has emerged as an integral part of drug development especially when identifying a drug's biological properties. The term there by implies the time course and fate of drug in the body. Bio availability captures two essential features, namely how fast does the drug enter the systemic circulation (rate of absorption) and how much of the nominal strength enters the body (extend of absorption).Given that the therapeutic effect is a function of the drug concentration, the above properties of non-intravenous dosage forms are there for important in identifying the response to a drug dose.

As far as Ayurvedic formulations are concerned 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[iv].

In the present study Albino Rabbits were selected. Typically, in Pharmacokinetic studies, following administration of the compound, serial blood samples (usually 9-10 time points) are preferred, as they give a more robust concentration versus time profile within the same animal. These samples are analyzed for the parent or metabolite using a suitable analytical technique. The objective is to determine the rate of appearance and/or disappearance of the compound in the blood/plasma.

There are various factors which can affect the quality of Pharmacokinetic data such as stress during animal handling, blood loss with serial sampling, feed, age etc. Another important factor to consider is blood sampling site like Marginal Ear Vein, Central Ear Artery, Lateral Saphenous Vein, Cephalic Vein, Jugular Vein, Anterior Vena Cava, all of which have their own inherent advantages and disadvantage.

Following administration of *Haragourirasa* serum mercury concentration was non detectable assuming it to be zero at 0 hour followed by mean plasma concentration of 7.004 µg/L at 1 hour .The concentration then increased to 9.138 at 2 hour,11.404 at 4 hour and 22.11 at 8 hour. The concentration then decreased to 20.012 at 10 hour and to 10.496 at 12 hour. Post dosing as depicted in the graph.

The mean serum mercury concentration after administration of *Haragourirasa* for 7 days is 29.854. Results of the repeat-dose studies indicated that there was no accumulation of drug when given for 7 days and mercury is rapidly eliminated after discontinuation of dosing .Plasma drug levels at 8^{th} hour post dosage on 7^{th} day were similar to those obtained during the single- dose study.

The pharmacokinetics profiles of *Haragourirasa* thus provide useful information regarding the various aspect of drug activity of mercury preparations which can add to the evidence based study of *Ayurveda* which further help in the global acceptance of *Ayurvedic system* of medicine[v].

Conclusion:-

The mean serum mercury concentration after administration of Haragourirasa for 7 days is 27.576 µg/l. Results of the repeat-dose studies indicated that there was no accumulation of drug when given for 7 days and mercury is rapidly eliminated after discontinuation of dosing .Plasma drug levels at 8 hour post dosage on 7th day were similar to those obtained during the single- dose study. 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.

References:-

1. [i] Sarkar PK, Chaudhary AK. Ayurvedic Bhasma: the most ancient application of nano medicine. J Sci Ind Res. 2010; 69: 901-905
2. [ii] Saper RB, Kales SN, Paquin J, Burns MJ, Eisenberg DM, Davis RB, Philips RS. Heavy metal content of Ayurvedic herbal medicine products. J Am Med Assoc. 2004; 292(23): 2868-2873.
3. [iii]Borgese L, Zacco A, Bontempi E, Pellegatta M, Vigna L, Patrini L, Riboldi L, Rubino FM, Depero LE. Use of total reflection X-ray fluorescence (TXRF) for the evaluation of heavy metal poisoning due to the improper use of a traditional Ayurvedic drug. J Pharm Biomed Anal. 2010; 52(5): 787-790
4. [iv] Liu, Jie et al. "Mercury in Traditional Medicines: Is Cinnabar Toxicologically Similar to Common Mercurials?" *Experimental biology and medicine (Maywood, N.J.)* 233.7 (2008): 810–817. *PMC*. Web. 22 sept. 2018
5. [v] Liu, Jie et al. "Mercury in Traditional Medicines: Is Cinnabar Toxicologically Similar to Common Mercurials?" *Experimental biology and medicine (Maywood, N.J.)* 233.7 (2008): 810–817. *PMC*. Web. 22 sept. 2018.