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Original Research Article

Estimation of mercury levels in blood by GFAAS after administration of two variants of Shwasakuthara Rasa in human volunteers

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ABSTRACT

Introduction: Mercury is in use as medicine since thousands of years in India. It is the most important raw material of medicines belonging to Rasashastra, a branch of Ayurveda. It is need of time to establish data of pharmacokinetics of mercurial medicines. This is a preliminary study to know the absorption of Shwakakuthara Rasa, a herbo-mineral formulation.

Aim: The study drug viz Shwasakuthara Rasa is a complex triturated drug having herbal and mineral ingredients. In this study, bioavailability of mercury from two samples Shwaskuthar Rasa was assessed.

Materials and Methods: Shwaskuthar Rasa samples [S 1 and S 2] were manufactured by two formulas from Ayurvedic literature. Ingredients of the formulation are detoxified mercury, detoxified sulfur, detoxified realgar, detoxified borax, detoxified Aconitum ferox Piper nigrum, Piper longum, and Zingiber officinalis. Detoxification of said ingredients was done by specific processes mentioned in Ayurvedic literature. Per cent amount of detoxified mercury is 7.14 % and 11.1 % in S1 and S2 respectively and amount of Piper nigrum is 60% and 22.2 %, respectively. After administration of 125 mg dose of the S1 and S2 to 8 normal volunteers, blood samples were collected at 0, 1, 1.5 and 2 hours. The blood mercury levels were assessed by Graphite Furnace Atomic Absorption Spectroscopy (GFAAS).

Results: It is evident that mercury gets absorbed from both formulations. There is difference in absorption pattern. After 2 hours of administration of formulation; mercury level in blood from S1 is significantly higher than S2.

Conclusions: The increased absorbance of mercury from S1 can be attributed to much higher levels of P. nigrum in it than S2.

1. Introduction

The branch of Ayurveda called Rasashastra deals with the science of pharmaceutics and pharmacology of mercurial preparations. [In Sanskrit language, 'Rasa' means mercury and 'Shastra' means science.] Mercury, known as Parad in Sanskrit is an important ingredient of most formulations in Rasashastra. Its bioavailability from formulations is not reported in human beings. It is used in medicine after carrying out meticulous processing using various herbal and /or animal products. The formulation thus prepared is effective and safe. There are thousands of formulations in Rasashastra where the base drug is kajjali, triturated product of mercury and sulfur. It is believed that kajjali may be acting as a vehicle or catalyst in the formulation.

The formulations of mercury are in use since thousands of years in India. As there are no apparent adverse effects when properly processed as per guidelines in Ayurvedic literature, they are in practice for so many years here. The herbo-mineral formulations prepared by formulae in Ayurvedic texts like Rasaratnasamucchay, Yogaratnakara etc are approved India and are manufactured and sold by pharmaceutical companies in India.

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Nonetheless, it is necessary to have evidence of bioavailability of mercury as a part of the various formulations of Rasashastra. It is essential to understand the logic and pharmacology of the wonderful combinations of mercury. This is necessary for global acceptance.

Black pepper [Piper nigrum L.] is another important herbal drug which is an ingredient of about 3500 Ayurveda medicines.[1,2] Long pepper [P. longum] also is a commonly used drug in Ayurvedic formulations. The phyto-constituent piperine from these peppers is a proven bioavailability enhancer for many drugs.[3,4]

The formulation Shwaskuthar Rasa is a complex triturated formulation containing detoxified mercury, detoxified sulfur, detoxified borax, detoxified Realgar, detoxified Zingiber officinale, Piper longum, Piper nigrum and detoxified Aconitum ferox. There are 11 different formulas of this particular drug in various ancient texts in Ayurveda, having same ingredients and variation in proportion. Especially there is huge difference in proportion of peppers.[5,6]

This study aimed to assess the comparative bioavailability of Mercury, from two samples of the drug 'Shwaskuthara Rasa' having difference in proportion of ingredients. This formulation is available in Indian market and it is being prescribed commonly by Ayurveda Physicians in India.

2. Materials and Methods

2.1. Ingredients of the formulations

Mercury, sulfur, realgar, borax, Aconitum ferox, Piper nigrum, Piper longum and Zingiber officinalis are the basic raw ingredients of the formulation. Drugs required for detoxification of the raw drugs are Allium ceppa, rock salt, Zingiber officinalis [fresh], cows ghee, cow's milk.

All raw materials were procured from authentic source from local markets in Pune city,in India. The raw drugs were analyzed in Quality Control Laboratory to confirm that their compliance with standards in Ayurvedic Pharmacopoeia of India. The quality control testing was done in Quality Control Laboratory of BVDU College of Ayurveda, Pune 411043.

In Rasashastra, mineral raw drugs are used commonly. Detoxification of mineral ingredients is carried out by various processes. The intension is to render them into body friendly harmless form for medicinal use or for further processing before being used as a medicine. This is known as 'Shodhan' process.

Five of its ingredients of Shwasakuthara Rasa viz. mercury, sulfur, borax, realgar, A. ferox need to undergo detoxification process before further process. Detoxification of mercury was done by triturating it with rock salt and fresh garlic [Allium ceppa] paste for 56 hours followed by washing with hot water to separate out mercury.[7] Detoxification of sulfur was done by melting it with cow's ghee and pouring in cow's milk for 7 times. Each time, fresh boiled milk was used.[8] After that sulfur was washed, dried and finely powdered. Detoxification of realgar was done by triturating it with fresh ginger juice for 7 days.[9] After that it was dried. Detoxification of roots of Aconitum ferox was done by boiling its small pieces with Cow's milk for three hours,[10] followed by drying and powdering. Detoxification of borax was done by heating in a pan it till it loses crystalline form[9] followed by powdering. Dry fruits of P. nigrum and P. longum and dry rhizome of Zingiber officinalis were powdered. The powdering was carried out using grinders. The powdered materials were passed through 60 mesh sieve. Detoxified mercury and detoxified sulfur were triturated in mortar and pestle until black, smooth and non-shiny powder is formed. This powder is known as Kajjali. Fine Powders of detoxified realgar, borax, A. ferox, P. longum, p. nigrum and Z. officinale are mixed with kajjali and triturated for three hours in a mechanized end runner. Greyish black powder thus obtained is known as 'Shwasakuthara rasa.

Table 1: S1 sample ofShwasakuthara Rasa

S1	
Piper nigrum	59.49%
Zingiber officinalis	2.38%
Piper longum	2.38%
Aconitum ferox [detoxified]	7.14%
Mercury [detoxified]	7.14%
Sulfur [detoxified]	7.14%
Realgar [detoxified]	7.14%
Borax [detoxified]	7.14%

Table 2: S2 sample ofShwasakuthara Rasa

S2	
Piper nigrum	22.20%
Zingiber officinalis	11.10%
Piper longum	11.10%
Aconitum ferox [detoxified]	11.10%
Mercury [detoxified]	11.10%
Sulfur [detoxified]	11.10%
Realgar [detoxified]	11.10%
Borax [detoxified]	11.10%

The percentage proportion of each ingredient as per Tables 1 and 2 . The percentage proportion was calculated as per formulas in Rasashastra texts Yogaratnakara[5] and Bhaishajya Ratnawali.[6]. In these texts the proportion of ingredients is mentioned in ancient Ayurvedic system of measures which was converted in percentage proportion form using guidelines in Ayurvedic pharmacopoeia of India.

Two samples of Shwaskuthar Rasa were manufactured by the formulas given in Tables 1 and 2. The samples were coded as S1 [Yogaratnakara text],[5] S2 [Bhaishajya

Ratnawali text].[6]

2.2. Methodology of bioavailability study

Permission of BVDU College of Ayurved's Institutional Human Ethics committee [IHEC] was obtained to conduct bioavailability study. This study was carried out using Guidelines in Helsinki declaration. Informed written consent was obtained from all volunteers. ICMR guidelines were followed.[11] As Shwasakuthara rasa is not a new drug, the present investigation was first step of exploration of its pharmacology in terms of modern science. The drug Shawaskuthara Rasa is in market in India and is prescribed commonly all over the country. It is prescribed in a dose of 125-250 mg twice or thrice a day for chronic bronchial asthma, cough. Eight normal individuals were selected as volunteers for the study. Age group was between 20-40 years. These subjects were healthy and on no medications for last 1 year. Informed written consent was obtained from the subjects. They were kept NBM for 8 hours. S1 was administered in 125 mg dose to 4 volunteers with 200 ml of water. S2 was administered in remaining 4 individuals in same manner. Shimadzu Digital balance was used to measure accurate dose. Blood samples were collected at 0, 1, 1 $\frac{1}{2}$, 2 hrs after administration of the dose. To hypothesize this time range, reference of a research on a Traditional Chinese pill containing cinnabar [HgS] was taken showing absorption peak of mercury at one hour[12] after administration. The blood was stored in heparinized vacutainers before analysis. Immediately thereafter the samples were analyzed by Atomic Absorption Spectroscopy with hydride generation technique, for mercury content. The blood samples were digested with nitric acid before subjecting to AAS testing. Internal standard used was mercury chloride.

3. Observations

Table 3: Data of absorption of Hg from S1 and S2 in blood.

	Blood mercury levels in micrograms/litre [ppb]			
	0 hr	1 hr	1.5 hr	2 hr
S1V1	30.01	37.171	369.968	209.755
S1V2	67.827	76.572	312.77	168.01
S1V3	64.125	73.168	309.612	171.621
S1V4	50.671	58.788	345.786	170.2
Mean S1	53.15825	61.42475	334.534	179.8965
S2V5	78.521	379.41	461.44	79.029
S2V6	66.49	345.96	372.48	95.071
S2V7	64.96	355.012	380.613	94.381
S2V8	36.09	316.56	366.458	46.67
Mean S2	61.51525	349.2355	395.2478	78.78775

Table 4: Data ofHg levels in blood after administration of S1 and S2. S=sample, V=volunteer

	Blood mercury levels in micrograms/litre [ppb]			
	0 hr	1 hr	1.5 hr	2 hr
meanS1	53.15825	61.42475	334.534	179.8965
meanS2	61.51525	349.2355	395.2478	78.78775

Table 5: Mean absorbance of Hg

	Blood Hg concentration at 1.5 hr	Blood Hg concentration at 2 hr
t Cal	2.646654	7.733072
t table	5.840848	5.840848
P value	0.038607	0.002248
	P non significant	P highly significant

It was evident in this study that even the 0 hour blood samples showed traces of mercury; which means it was present in the blood before administration of study drug. Hence; the 0 hr blood Hg value was subtracted from the readings and the actual absorbance of Hg from two types of Shwasakuthara Rasa was calculated.

Table 6: Levels of mercury in blood from S1 and S2 [coming from the study drug] [S=sample, V=volunteer]

	Mercury levels in blood in microgm/liter assuming zero mercury before Dosing of S1 and S2			
	0 hr	1 hr	1.5 hr	2 hr
S1V1	0	7.161	339.958	179.745
S1V2	0	8.745	244.943	100.183
S1V3	0	9.043	245.487	107.496
S1V4	0	8.117	295.115	119.529
	0 hr	1 hr	1.5 hr	2 hr
S2V5	0	300.889	382.919	0.508
S2V6	0	279.47	305.99	28.581
S2V7	0	290.052	315.653	29.421
S2V8	0	280.47	330.368	10.58

Table 7: Mean absorbance of Hg from S1 and S2

	Blood mercury concentration in micrograms/litre [ppb]			
	0 hr	1 hr	1.5 hr	2 hr
mean S1	0	8.2665	281.3758	126.7383
mean S2	0	287.7203	333.7325	17.2725

4. Results

The results of GFAAS show that mercury gets absorbed in traces from the single oral dose of S1 and S2 samples of Shwaskuthar Rasa. There is difference in bioavailability of mercury in blood from single oral doses of S1 and

Table 8: Studentst test to above stated assumption data

	1.5 hr	2 hr
t Cal	2.119522	6.499574
t table	5.840848	5.840848
P value	0.062119	0.003698
	Diff. not significant	Diff. highly significant

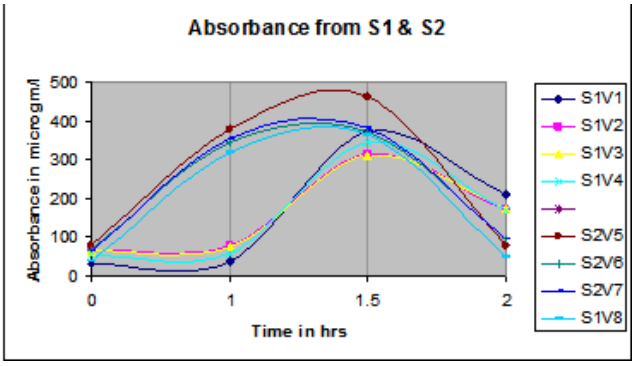

Chart 1: Bloodmercury concentration in micrograms/litre from S1 and S2 in 8 volunteers after single oral dose[125mg]

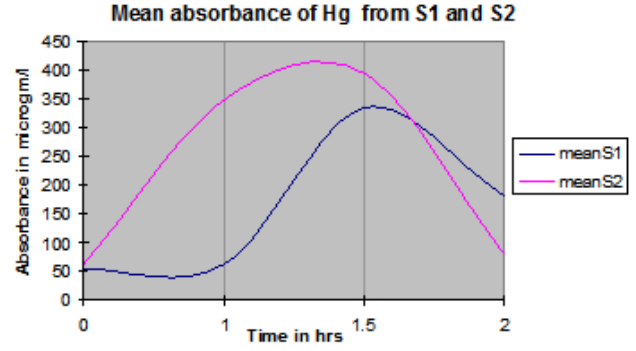

Chart 2: Mean concentration in blood of Hg from Shwaskuthar Rasa S1 and S2

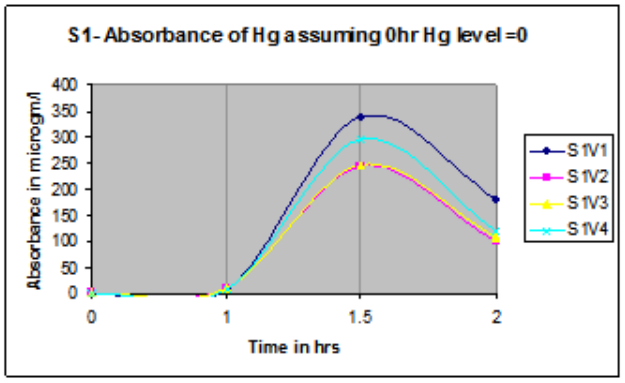

Chart 3: Blood mercury concentration in micrograms/litre [ppb] from S1 assuming that Hg level in blood at 0 hr were 0 microgm/l

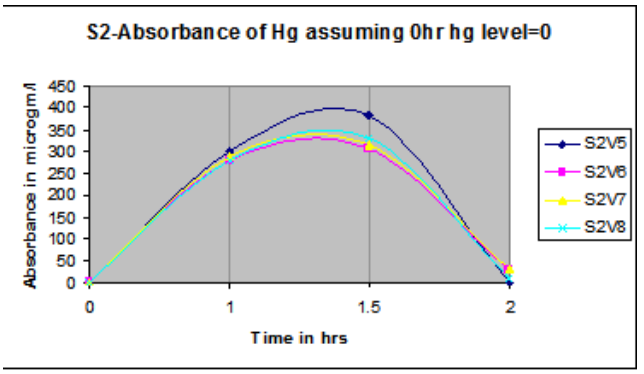

Chart 4: Blood mercury concentration in micrograms/litre [ppb] from S2 assuming that Hg level in blood at 0 hr were 0 Microgm/l

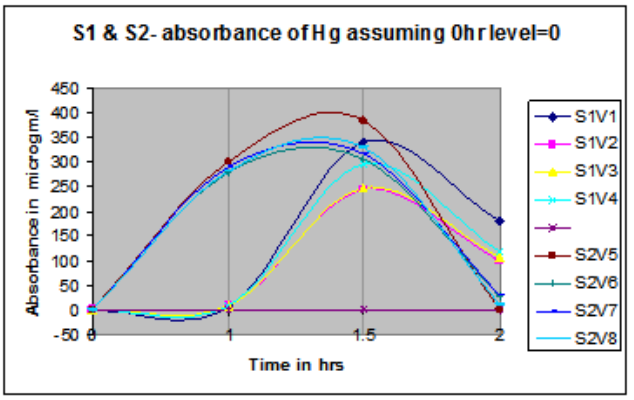

Chart 5: Blood mercurycon centration in micrograms/litre [ppb]g from S1 and S2 assuming that Hg level in blood at 0 hr were 0 Microgm/litre

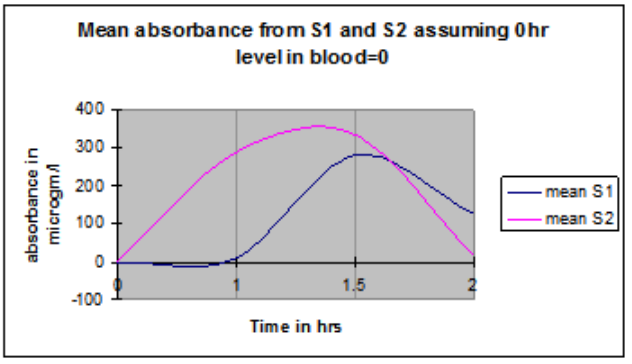

Chart 6: Mean blood mercurycon centration in micrograms/litre [ppb] from S1 and S2 assuming that Hg level in blood at 0 hr were 0 Microgm/l

S2, in human volunteers in 125 mg dose. In both cases, the absorption in blood is in traces ie ppb levels [in microgram/litre]

In case of S2, the Hg level in blood starts to rise up immediately after administration and reaches at peak just before 1 $\frac{1}{2}$ hrs after dosing. However, in case of S1, the blood Hg level remains at baseline until one hour after dosing. It starts to rise after 1 hr and attains peak few minutes before 1 $\frac{1}{2}$ hours. There is a sharp increment in absorption, taking place in a span of $\frac{1}{2}$ hour. By statistical student's t test the difference in absorbance at 1.5 hours is non-significant [P=0.038607] at 0.01 level of significance.

In case of S2, the blood mercury level falls sharply and attains 78.7 microgm/l at 2 hrs after dosing. However, in case of S1, the level reaches 179.8 microgm/l at 2 hrs after dosing. Student t test shows there is highly significant difference in the absorbance level at 2 hours (P=0.002248), at 0.01 level of significance.

The mean residential time of S1 and S2 is 81.53 min and 70.03 min respectively. The increased absorption and increased MRT in case of S1 can be attributed to more amount of peppers in it than S2.

5. Discussion

Comparative bioavailability of mercury from two different samples of Shwaskuthara Rasa was studied. It was assessed by measurement of blood mercury levels after administration of single oral dose in healthy human volunteers.

The bioavailability study was conducted as elaborated in methodology. The results of GFAAS showed that mercury gets absorbed from single oral dose of Shwasakuthara Rasa. Mercury bioavailability in human healthy volunteers varies from single oral dose of S1 and S2. In both cases, the absorbance is in microgm/litre levels, ie in traces.

According to student t test the difference in absorbance of Hg from S1 and S2 at 1.5 hours is non-significant [P=0. 038607]. Student t test shows there is highly significant difference at 0.01 level of significance in the absorbance of mercury from S1 and S2 at 2 hours [P= 0.002248].

Considering the fact that in S1, the amount of peppers is almost double and that of mercury is half as compared to S2, the differences in absorbance can be attributed to quantity of peppers (P. nigrum and P. longum). Hence, it is concluded that peppers are mainly responsible for the increased bioavailability of mercury in S1.

Though the amount of Hg is much less in S1, its absorption peak is almost near to that of S2 at 1.5 hrs with non-significant difference by t test (P=0.038607). It can be noted that the rate of fall of blood mercury level is slower in case of S1 than S2. This also can be attributed to difference in proportion of ingredients, especially of black pepper [P. nigrum] and long pepper [P. longum]

The traces of Hg in patients' blood at 0 hr are attributed to non-medicinal sources, like water, food etc. If it is assumed that the bloods Hg level were zero microgm at zero hour, actual absorbance from Shwasakuthara Rasa is obtained. It is seen that the difference in absorbance of Hg from S1 and S2 is not significant at 1.5 hrs [P=0.062119] and highly significant at 2 hrs [P=0.003698].

The mean residential time [MRT] of S1 [81.53 min] is more than that of S2 [70.03 min], as calculated by trapezoidal rule. MRT denotes the average amount of time spent by the drug in the body before being eliminated. It represents the time for 63.2 % of the intravenous bolus to be eliminated.

Hence, it is concluded that the peppers have a role in delaying the biotransformation of mercury and may be other constituents of the formulation.

Detoxification of mercury was done using garlic and rock salt. Recent studies show that the sulfur containing constituents in Garlic including allicin (dially disulphide-oxide), Aillin (S-ally cysteine sulphoxide), and Diallydisulphide are quite capable of binding to and eliminating mercury as a normal part of their physiological actionchelation.[13] As the Shuddha ie detoxified mercury is used to formulate the medicine, mercury absorbed from the formulation may be well excreted from the body without causing any harm to the body physiology. The history of safe use of these formulations since centuries ascertains this. It is important to note that the processes of detoxification and specific drug combinations as described in the texts of Ayurved are of immense importance in converting the minerals into consumable, safe and effective medicine.

6. Conclusion

It is evident that higher amount of piperine containing ingredients [P. nigrum and P. longum] are responsible for increased bioavailability of mercury from Shwasakuthara Rasa from single oral dose in human volunteers. Though the percentage of mercury is more in S2, the mean residential time of Hg in S2 is less than that of S1. Hence, it is concluded that peppers have a role in increasing bioavailability and delaying the biotransformation of mercury. Considering the fact that P. nigrum is a part of more than 3500 formulations in Ayurveda, which includes pure herbal and herbal -mineral complex medicines, this study yields important evidence.

7. Acknowledgements

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8. Conflicts of Interest

All contributing authors declare no conflicts of interest.

9. Source of Funding

None.

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