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STANDARDIZATION AND BIOAVAILABILITY OF AYURVEDIC DRUG LAUHA BHASMA-PART –II COMPARATIVE BIOAVAILABILITY STUDIES

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ABSTRACT: *After analyzing the Lauha Bhasma samples qualitatively and quantitatively, in-vivo studies on healthy adult male rabbits were carried out. Randomly selected anaemic rabbits were divided into different groups and three variants of Lauha Bhasma and ferrous sulphate sample were administered to each group. The effect of each formulation was monitored by measuring the haemoglobin (Hb) content spectrophotometrically (cyanomethaemoglobin method). Increase in the haemoglobin content was found to be significant in case of the ayurvedic formulations as compared to the ferrous sulphate sample.*

INTRODUCTION

On qualitative and quantitative analysis the samples of Lauha Bhasma were found to contain both the ferric and the ferrous iron, with greater proportion of ferric oxide than ferrous oxide (Verma et al., 1995).

Normally in the allopathic system of medication the ferrous form of iron is given whereas in the ayurvedic system it is the ferric form. It is said that the ferrous form of iron is better absorbed than the ferric form in the gastrointestinal tract (Goodman and Gilman, 1975; Crossland, 1980). The ayurvedic system of medication claims that the iron preparation Lauha Bhasma is better absorbed gastroin testinally, hence biologically available, and is devoid of the usual side effects associated with the oral/parenteral iron preparations of the allopathic system of medication, Hence, it be comes of interest to compare the marketed ayurvedic and allopathic iron preparations on the basis of the increase in haemoglobin (Hb) content and the resulting bioavailabilities.

METHODOLOGY

Our of the number of methods available for determining the haemoglobin content, the spectrophotometric method (cyanomethae moglobin method) was selected, as it was both accurate and reproducible, Blood, diluted suitably with cynanide-ferrocuamode reagent. Was measured at 540 nm (Varely, 1980; Oser, 1971). In view of the slow reaction, the diluted blood was made to stand for 10 minutes to ensure complete conversion of the haemoglobin. The haemoglobin content was caluculated was follows:

$$\text{Haemoglobin (g.dl)} = \frac{\text{A540 HiCN} \times 64{,}500 \times \text{Diluting factor}}{44.00 \times d \times 1000 \times 10}$$

where,
A540 HiCN = absorbence of the solution at 540 nm 64,500 = molecular weight of haemoglobin Dilution factor = 201 (when 20 µl of blood diluted to 4 ml)

44.0 = millimolar extinction coefficient
d=Layer thickness in cm
1000= Conversion factor (mg to gm)
10 = Conversion factor (g/L to g/dl)

Induction of iron deficiency anaemia

Healthy, adult, male rabbits were selected, having the weight range between 1.2 to 1.6 kg. Thenormal Hb values of the healthy male rabbits were determined, which was found to be 10-14 g/dl. The rabbits were put separately, in individual numbered cages. In order to induce iron deficiency anaemia, the rabbits were fed on a synthetic diet (Jha et al., 1968) and allowed to drink only double distilled iron free water. The diet was given in the quantity sufficient to give 200 calories /day/kg body weight till the haemoglobin content came down to the level 6-9 g/dl.

Comparative efficacy studies of Lauha Bhasmas with ferrous sulphate

Dried ferrous sulphate was assayed and found to contain 82% of ferrous sulphate, therefore, dose of 20 mg/day of the dried ferrous sulphate contained an equivalent of 6 mg/day of the elemental iron. Upon assay Lauha Bhasma was found to contain 81 - 85% of the total iron (Verma et al., 1995). Thus the calculated equivalent dose of 10 mg/day of Bhasma, was likely to contain 6 mg/day of the elemental iron.

The iron deficient anaemic rabbits were randomly selected and made into four groups each consisting of six rabbits, the animals were individually housed in numbered cages, along with the controlled synthetic diet, each group of animals was administered dried ferrous sulphate (20mg/day), HA Bhasma ordinary (10 mg/day), Lauha Bhasma Satputti (10 mg/day) and Lauha Bhasma Sahsatraputti (10 mg/day) respectively.

The required dose was administered to seven days, and the blood samples collected subsequently on the 2^{nd}, 4^{th}, 6^{th}, and the 8^{th} day were analysed. The data obtained are present in Table 1.

The total increase in the haemoglobin content after administration of the drug was considered for the statistical evaluation.

F-ratio test (Multiple comparison procedure)
Analysis of Variance

Sources of Variation	Degree of Freedom	Sum of Square	Mean Square	F-ratio
Among regiments	t-1=3	24.727083	8.1560322	
Within regimens	Σni-1	24.468097	S2=1.2234049	6.66666

Test for significance

The F-ratio is 4.94, as per F-Table (Osol, 1980) with F1=t-1=3 and F2 = Σ (ni-1) =20 degree of freedom. We find that the calculated F-ratio value 6.66666 is larger than the tabulated value 4.94 for P=0.01.

Therefore, probability of these four samples being drawn from the same population was less than 0.01. Hence it was concluded that the four data obtained differ from each other.

t-Test

The t-test (Osol, 1980) was successfully employed to find if the difference in data was significant (Table 2).

Results

The comparative bioavailability studies revealed that the ayurvedic iron preparation, Lauha Bhasma Sahastraputti and Lauha Bhasma Satputti, significantly increased the haemoglobin content of blood in the iron deficient anaetnic

Table -1

HAEMOGLOBIN VALUES AFTER ADMINISTRATION OF FERROUS SULPHATE AND LAUHA BHASMA

	AVERAGE HAEMOGLOBIN CONTENT (g/dl)						Increased Hb Content (g/dl)
	Before synthetic diet	After synthetic diet	After administration of drug				
			2nd day	4th day	6th day	8th day	
Ferrous Sulphate	10.57	6.60	7.80	8.53	9.06	10.45	3.85
	10.86	6.27	7.81	8.55	8.74	9.41	3.14
	13.92	8.36	8.79	8.56	9.62	9.73	1.37
	12.19	8.15	8.27	8.86	9.40	10.15	2.00
	10.75	8.27	8376	9.41	9.65	10.29	2.02
	13.12	8.71	8.95	9.29	9.74	10.40	1.69
Lauha Bhasmas	10.39	7.30	8.93	9.36	10.08	11.92	4.62
	10.60	8.61	9.21	9.57	10.21	10.97	2.36
	10.47	8.06	8.21	8.90	9.62	10.42	2.36
	11.22	7.39	7.98	8.53	9.03	9.80	2.41
	11.89	6.90	7.51	8.26	9.77	10.11	3.21
	12.39	7.96	7.96	8.59	9.80	10.50	3.30
Satputti	10.23	7.21	7.27	7.99	8.51	9.53	2.02
	13.21	8.47	9.41	10.59	11.89	12.31	3.84
	11.86	6.83	9.60	10.80	11.78	12.81	5.35
	10.59	7.62	8.41	9.80	10.59	11.51	3.89
	12.81	6.21	7.38	8.21	9.60	10.42	4.21
	11.22	8.59	9.80	10.59	11.51	12.91	4.32
Sahastra Putti	10.59	7.32	8.62	9.62	11.39	11.87	4.55
	11.22	8.56	11.50	12.64	13.16	13.63	5.07
	13.49	6.61	10.28	12.25	13.40	14.39	7.78
	11.53	7.52	8.27	9.20	10.48	11.24	3.72
	12.96	8.96	9.59	10.80	12.39	13.19	4.23
	10.23	6.95	8.26	9.65	10.50	11.94	4.99

Rabbits, in comparison to the ferrous sulphate sample. The increase in the haemoglobin content due to ordinary Lauha Bhasma was not significant when compared to the ferrous sulphate sample. Also, amongst the three variants of Lauha

Bhasma, the sahastraputti Bhasma was found to increase the haemoglobin content most significantly.

Table -2
STATISTICAL EVALUATION OF FERROUS SULPHATE AND THREE VARIANTS OF LAUHA BHASMA

Case Number	Sample No	S^2	S	t_{cal}	Two tail P=0.05 t =2.228 (as per t-table)	One tail P=0.05 t = 1.812 (as per t-table)
1	1 Vs 4	1.46492	1.2103421	3.30613	Highly Significant	1>4
2	2 Vs 4	1.04056	1.0200801	2.7054	Significant	2>4
3	3 Vs 4	0.87198	0.9337986	1.29529	Not Significant	-
4	2 Vs 3	0.98188	0.990899	1.5644	Not Significant	-
5	1 Vs 3	1.40624	1.1858527	2.94066	Significant	1>3
6	1 Vs 2	1.60522	1.266972	1.52885	Not Significant	

Key: Sample 1 Sahastraputti; Sample 2 Satputti; Sample 3 Ordinary; Sample 4 Ferrous Sulphate

DISCUSSION AND CONCLUSION

The mucosal block hypothesis and the active transport hypothesis (Soine, 1967) Profess that iron is intestinally absorbed only in the ferrousform. Even the ferric form gets converted to the ferrous form before being absorbed.)

According to the Iron Chelation Hy-pothesis (Saltman, 1965) either form of iron (Ferrous or ferric) is absorbed form the intestine. Therein the absorption of iron is exerted by the exorgenous or the endogenous ligands or chelating agents, these can bind either state of iron, to form low molecular weight complexes, capable of passively diffusing through the mucosal cell membrance of the intestine.

Martin (1981) have reported that both the ionic forms of iron are highly insoluble at neutral pH. The ferric iron reacts with low molecular weight compounds such as fructose, ascorbic acid, citric acid and amino acids toform complexes that allow ferric iron to remin soluble at the neutral pH of the intestinal fluid.

The capacity to increase the haemoglobin content was more in Sahastraputti Bhasma, although the elemental iron content was same in all the samples taken, On analyzing the PSD data of Bhasma, the sahastraputti Bhasma was found to contain finer particles than the ordinary and the satputti Bhasma. As the rate of dissolution of smaller particles is usually faster than of the larger ones and is dependent on the specific surface area in contact with the liquid medium (Lackman

and Lieberman, 1976), in our study it was observed that the specific surface area of sahastraputti was 4 times greater than that of the ordinary Lauha Bhasma (Verma et al., 1995). Hence a higher rate of dissolution and a higher bioavailability may be expected form this sample. This is in conformity with the fact that the reduced iron is effective provided that the material employed has smaller particles (Elwood, 1968; Cook et al., 1973). Satputti Lauha Bhasma was found to increase the haemoglobin content more significantly than the ferroussulphate sample. Although satputti Lauha Bhasma was not found stastistically superior to the ferrous sulphate sample, probably due to the present of coarser particles in the sample, This holds good with the finding of Cook (1973) That iron having larger particles has much lower bioavailability.

Conclusively, the ayurvedic iron preparation Sahastraputti Lauha Bhasma and satiputti Lauha Bhasma may be considered to be better drugs that the allopathic iron preparation Ferrous sulphate, on the basis of the bioavailability resulting due to increase in the haemoglobin content.

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