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EFFICACY OF *LOHASAVA* AGAINST EXPERIMENTAL ANAEMIA

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ABSTRACT

By appropriate animal experiments *lohasava* has been shown to be an effective therapeutic against hypochromic anaemia attributable to iron deficiency.

INTRODUCTION

The usefulness of microbiological procedures for a general evaluation of *lohasava*, an Ayurvedic tonic preparation containing iron, has been the subject of a previous publication[5]. Evidence was presented therein to the effect that the microbiological method tried for assaying iron was rapid, easy and reliable to the extent to merit consideration for adoption as a standard test for the quality of the product. It has also been shown[4] that the bacterial species employed in the assay method can respond equally well to iron in all its combinations *viz.*, organic, inorganic, divalent or trivalent, cationic or otherwise. However, these tests did not allow evaluation of this tonic preparation for the availability therefrom of iron to the animal system ingesting it. In this paper are described the experiments designed, and results obtained, at determining the efficacy of *lohasava* as a therapeutic.

Iron indisputably is the most important constituent of *lohasava*. Iron may be administered in many forms in the animal system, but not all forms can be

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utilized by the body. Hence it was considered of utmost importance to ascertain if the iron available in the *lohasava* is in a form suitable for incorporation in the animal tissue. If so, it was argued, this could be measured by therapeutic evaluation of its haemopoietic activity in animals induced with experimental anaemia.

EXPERIMENTAL PROCEDURES AND RESULTS

Phenylhydrazine (hydrazinobenzene) has for long been used by several workers for inducing anaemia in animals. This chemical, in proper dosage, has

TABLE I

Record of haemoglobin in g. per 100 ml. of blood of the three groups of rats

Rat No.	Haemoglobin							
	Before injection	After injection (days) 2	4	6	10	14	16	18
Control group: (no iron)								
1 F	17.2	10.15	7.54	9.28	13.7	14.1	14.35	14.6
2 F	17.2	9.43	5.93	7.93	10.98	13.7	14.2	14.51
3 F	11.6	7.54	4.06	4.35	4.95	7.54	7.6	8.5
4 M	15.23	9.8	7.12	8.5	11.05	12.2	12.8	13.5
5 M	15.96	10.1	7.7	8.8	11.5	12.9	13.7	14.2
6 M	16.22	10.0	6.83	9.2	12.9	13.7	14.4	14.9
Standard group: (iron as salt)								
7 F	16.22	10.5	6.22	6.67	14.21	15.25	16.92	17.11
8 M	17.11	9.82	6.25	7.83	9.74	12.82	15.31	17.45
9 M	15.66	9.43	6.97	10.13	13.72	14.51	14.84	16.95
10 M	16.67	10.32	6.51	8.63	11.90	13.52	15.01	16.51
11 M	15.52	9.21	5.96	9.03	13.15	13.91	13.82	15.55
12 M	15.95	10.51	6.83	9.50	12.11	13.81	14.95	16.12
Lohasava group:								
13 F	17.11	9.86	6.81	8.74	12.32	15.32	16.95	17.31
14 F	17.9	8.76	6.51	9.57	14.79	15.52	17.04	17.69
15 F	15.95	7.25	4.93	6.96	12.23	13.35	14.55	15.66
16 M	17.42	10.51	6.58	8.64	10.42	14.56	16.12	17.35
17 M	16.67	10.32	6.72	9.11	12.23	14.91	15.22	16.53
18 M	15.95	9.81	6.54	9.87	13.11	13.8	15.02	16.14

When the results were treated statistically, it was observed that probability value for standard iron group was between 0.2 and 0.3. The result was, therefore, not significant upto 2 to 3% level. For *lohasava* the result was between 0.0 to 0.01 of probability; hence highly significant upto 0.1% level.

been shown to destroy specifically mature red blood corpuscles without affecting adversely other tissues in the animal body. Although Yeshoda[6], Elvehjem *et al.*,[3] Damodaran and Vijayaraghavan[2] had used phenylhydrazine at a level of 2 mg. per 100 g. body weight for their investigations, Chiplunkar and Sirsi[1] had reported that phenylhydrazine could be administered intraperitoneally upto even 12 mg. per 100 g. body weight without any fatal effect. From the preliminary experiments conducted, it was concluded that 6 mg. of phenylhydrazine were

TABLE II

Record of red blood corpuscles (Million cells per cmm.)

Rat No. Sex*	Red blood Corpuscles			
	Before injection	After injection (days) 4	10	18
Control group:				
1	8.7	2.97	3.81	5.3
2	8.34	2.01	2.23	3.5
3	3.86	0.91	1.33	2.3
4	5.64	3.1	3.99	5.45
5	6.84	4.0	4.2	5.1
6	8.66	3.65	3.85	5.6
Standard iron group:				
7	7.68	2.7	3.05	6.11
8	8.22	2.72	3.98	6.54
9	6.9	3.81	4.01	5.8
10	8.06	3.62	4.15	6.1
11	7.45	2.53	5.12	6.5
12	7.86	2.99	3.29	6.45
Lohasava group:				
13	8.68	2.0	3.51	5.95
14	8.14	2.35	2.97	6.86
15	7.68	1.7	3.24	4.95
16	8.4	3.8	4.18	5.98
17	8.11	3.65	4.5	5.65
18	8.03	2.8	3.1	5.53

* As in Table I

The results were statistically to find probability values for standard iron and *lohasava*. Probability value for standard iron group was between 0.2 and 0.3. The result was, therefore, not significant upto 2 to 3%. For *lohasava* the result was between 0.0 to 0.01 of probability; hence highly significant upto 0.1% level.

required to produce acute anaemia in the rats employed for the purpose and consequently all experiments were carried out at this level. The experimental anaemia thus produced, it was observed, was naturally overcome at the end of fifteen days.

For final experiments 18 adult albino rats weighing between 150 and 190 g. were chosen and divided into three groups of six rats each. Rats in each group were fed on different diets, made as follows, after three days of inducing experimental anaemia in them.

TABLE III

Record of body weights (g) of the rats

Rat No.	Sex*	Before injection	After injection (days)							
			2	4	6	8	10	12	14	16	18
Control group:											
1		177	171	158	164	172	172	171	170	170	171
2		172	161	156	161	162	162	162	162	162	164
3		172	172	157	157	159	160	162	162	163	165
4		162	161	158	164	166	166	168	170	171	172
5		158	156	158	164	168	169	169	171	172	172
6		180	177	168	175	177	177	178	178	180	181
Standard iron group:											
7		172	168	160	162	165	169	170	170	172	173
8		170	167	162	168	170	174	176	178	179	182
9		172	169	166	170	172	173	176	179	180	182
10		166	164	154	162	164	165	166	168	171	172
11		179	176	164	170	172	176	181	185	186	188
12		150	157	144	146	148	149	151	151	155	156
Lohasava group:											
13		184	175	165	169	170	173	175	178	179	181
14		174	170	160	162	162	162	162	162	164	166
15		149	145	139	143	143	144	145	145	147	148
16		109	109	108	124	125	128	130	133	134	136
17		188	186	182	185	185	185	186	186	188	188
18		187	177	185	186	186	187	188	190	191	193

* As in Table I

The results obtained were treated statistically to find if they are significant. The probability for standard iron was between 0.02 and 0.05. The results are, therefore, significant upto 0.5%. For *lohasava* the value of probability was beyond 0.5, therefore not significant upto 5 % level.

I. *Control group*:—These were given basal diet consisting of starch 55%, casein 15%, sucrose 10%, yeast 8%, fat 8%, and routine salt mixture 4%. 15 g. of the diet mixed with equal amount of water constituted the daily diet of each rat.

II. *Standard iron group*:—These six rats were given basal diet and in addition they were fed ferriammonium citrate at 50μg. of iron per 100 g. body weight. Iron was administered in the form of a solution by means of a stomach tube. This group furnished a positive control group.

III. *Lohasava group*;—All the animals in this group were administered *lohasava* (stomach tube feeding) at levels according to their body weights ensuring, however, that each animal received 50 μg. of iron per 100 g. body weight. In all other respects the animals were treated as those in Group I.

Weight individually of the rats were recorded every alternate day and the haemoglobin contents and R. B. C. counts determined at regular intervals. The results recorded are presented in Tables I, II and III. All the results were statistically treated to obtain the probability values.

Macroscopic prussian blue reaction:—At the end of 18 days the animals were killed and the various tissues were removed, cut into sections, and kept in prussian blue solution for 16 hours in a refrigerator. Thereafter, the tissue sections were washed successively in fresh supplies of water until no blue colour got washed away. The sections were then studied carefully for determining the distribution of iron in the various tissues. The results are presented in Table IV.

TABLE IV

Macroscopic prussian blue reaction on rat tissues

Tissues	Control group	Standard iron group	*Lohasava* group
Heart	Light blue	Light blue	Light blue
Liver	No stain	Blue	Blue
Spleen	No stain	Blue	Blue
Stomach	Bluish	Bluish	Bluish
Kidney	Light blue	Light blue	Light blue

DISCUSSION

It is clear from the results presented in Tables I and II that iron from *lohasava*, in whatever form it might have been, was available to the experimental animals for their early recuperation from anaemic conditions. Both the haemoglobin index and the red blood corpuscles counts registered a significant increase in the experimental group over the control as well as even the group administered with standard iron compound. In fact for *lohasava* the results were highly significant suggesting thereby the extraordinary high therapeutic value of the

preparation against hypochromic anaemias attributable to iron deficiency. It is, however, possible that the superior therapeutic effect of iron from *lohasava* over the iron in the ferriammonium citrate is due to presence in traces of other elements like cobalt, copper and so on in the iron used for its manufacture. Nonetheless the results of prussian blue tests tended to support the conclusion in favour of *lohasava* inasmuch as the liver and spleen (reservoirs for iron) of the animals receiving this preparation indicated an adequacy for the element. From the point of view of increase in body weight, *lohasava* did not appear to be of much value as compared to the standard iron group though both of these groups registered an increase in weight over the control; but there is every reason to expect that improvement in the preparation can be brought about by incorporating into it vitamins and amino acids in which it is deficient. But the foremost consideration for the manufacturer should be to ensure the level of its iron contents, as it has been shown that certain samples may contain as low as 28 mg. of iron per 100 ml. in contrast to others recording a level of over 300 mg. for the same volume[5]. It is in this context that the standardisation of this Ayurvedic medicinal preparation should be viewed and adequate measures taken to remedy the shortfalls. As a tonic preparation, *lohasava* is effective and has indeed stood the test of time.

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