CONTENTS

Haematinic Evaluation of *Lauha Bhasma* and *Mandura Bhasma* on HgCl₂-Induced Anemia in Rats

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Sarkar, *et al.*: *Lauha Bhasma* and *Mandura Bhasma* on HgCl₂-Induced Anemia in Rats

The present study was carried out to evaluate the haematinic effect of two ayurvedic preparations of iron on mercuric chloride-induced anemia in rats. *Lauha bhasma* and *Mandura bhasma*, two well-known ayurvedic iron preparations, are commonly used to treat anemia. In Charles Foster strain rats of either sex, anemia was induced by administering mercuric chloride (9 mg/kg). *Lauha bhasma* and *Mandura bhasma* (11 mg/kg) were evaluated for their haematinic activity. The observed results suggest that *Lauha bhasma* and *Mandura bhasma* possess significant (P<0.05) haematinic and cytoprotective activity.

Key words: *Lauha Bhasma*, *Mandura Bhasma*, iron, mercuric chloride, anemia, haematinic

Mercury and its compounds have had a long history in medicine. While not as important in modern medicine today, certain mercury salts are still used widely in ayurvedic system of medicine. Metallic mercury is relatively non-toxic. The mercurous (Hg⁺) and mercuric (Hg⁺⁺) cations are toxic[1]. Mercury vapor, however, is toxic. Mercury poisoning from inhaling mercury vapour is believed to have occurred in scientists working with mercury, in industrial situations and in people living near industrial plants emitting mercury vapour in the air[2,3]. The classic example used to illustrate mercury poisoning is that of the fishermen and their families living around Minamata Bay in Japan[4]. Mercury induced haematological effect among occupationally or accidentally exposed human beings are well established[5] and its effects on experimental animals are also well documented[6].

The empirical use of different preparations of iron in the treatment of anaemia dates from ancient times[7]. The incinerated iron preparations of ayurveda are known as *Lauha bhasma, Mandura bhasma, Abhraka bhasma* and so on. The preparation of these *bhasmas* are followed according to unique methods described by ancient scholars includes s*hodhana* (purification), *marana* (incineration) and several grades of incineration under the term *puta*. Incineration of iron is done not only for making it finer but also for increasing its quality[8], so that it can be specifically effective for the eradication of different ailments.

Lauha bhasma and *Mandura bhasma* are the two most commonly used preparations of incinerated iron. These are indicated for the same as well as different diseases. So many studies have so far been carried out on individual preparation. Pandit *et al*, investigated *Lauha bhasma* for haematinic activity and haemoglobin regeneration efficacy on agar gel diet and phlebotomy induced iron deficiency anemia in rats and reported significant haematinic and haemoglobin regeneration efficiency in comparison to control and standard ferrous sulphate containing drug[9]. Kanase *et al*. studied curative effects of *Mandura bhasma* on liver and kidney of rats and noticed total recovery in two weeks[10]. But comparative scientific study of these two *bhasma* preparations at pharmacological level is very less. So the present programme was undertaken to evaluate the haematinic activity of these two *bhasma* preparations on mercuric chloride-induced anemia in Charles Foster rats. The present study confirms to the guidelines laid by the Institutional Animals Ethical Committee.

In this study, anemia was induced in rats by administering mercuric chloride solution. Accumulation

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of mercury in the blood of mice has already been proved using inorganic radio mercury[11]. Mercury induced anemia was also reported in mice exposed to 0.1 mg and 0.5 mg of $HgCl_2$ for 100 d and 30 d respectively via drinking water[12].

MATERIALS AND METHODS

Charles Foster rats of either sex weighing between 180-250 g were used for this study. They were obtained from the animal house attached to the pharmacology laboratory. They were housed in breeding cases at an ambient temperature with a natural day and night cycles. The animals had free access of Amrut brand rat pellet feed supplied by Pranav Agro Industries and tap water.

Lauha bhasma and *Mandura bhasma* were prepared in practical laboratory of Department of Rasashastra and Bhaishajya Kalpana, I.P.G.T. and R.A., Gujarat Ayurved University. The therapeutic dose of *Lauha bhasma* and *Mandura bhasma* is 65 mg to 250 mg/d[8]. In the present study, human dose of both the *bhasma* preparations has been decided to be 125 mg/d. The dose for experimental study of the drugs was calculated by extrapolating the human dose to animal dose based on the body surface area ratio. Drug suspension was prepared in 3% gum acacia solution (1 ml in 50 ml distilled water) and solution of $HgCl_2$ was prepared by dissolving it in absolute alcohol (50 mg in 1 ml) and adding distilled water.

Experimental design:
In the present study, mercuric chloride ($HgCl_2$) was used to induce anemia in rats. Solution of $HgCl_2$ was administered in 9 mg/kg dose through oral route by No.3 simple rubber catheter for 30 d. This protocol was arrived at after carrying out initial pilot studies. In the treated groups, suspension of test *bhasmas* (*Lauha Mandura bhasma*) was given in 11 mg/kg dose along with mercuric chloride solution (9 mg/kg). A control group was also kept to observe the significant occurrence of anemia.

Study protocol:
A total of 24 Charles Foster rats of either sex weighing between 180-250 g were taken and divided randomly into 4 groups, each containing 6 animals, 3 male and 3 females each. The treatment schedule was as follows, group I comprised of vehicle (50 ml distilled water with 1 ml of absolute alcohol) treated

control animals, group II animals received 9 mg/kg solution of $HgCl_2$, group III animals were treated with 9 mg/kg solution of $HgCl_2$ and 11 mg/kg suspension of *Lauha Bhasma*, and group IV animals were treated with 9 mg/kg solution of $HgCl_2$ and 11 mg/kg suspension of *Mandura Bhasma*. Suspensions of test drugs were prepared in 2.2 mg/ml, concentration and solution of $HgCl_2$ was prepared in 1.8 mg/ml, concentration.

The treatment schedule was continued for 30 d with daily doses of test drugs and vehicle. Gross behaviour was observed throughout the study period. On 31st day rats were sacrificed by stunning, blood was collected from jugular vein for haematological and biochemical tests, spleens were collected for the histopathological study.

Statistical analysis:
All the values were expressed as mean±SEM (standard error of mean). The data were analysed by student's 't' test and ANOVA (Dunnet's 't' test). A level of $P<0.05$ was considered as statistically significant. Level of significance was noted and interpreted accordingly.

RESULTS AND DISCUSSION

The mean body weight of the albino rats in different treatment groups was recorded before and after drug treatment. The data pertaining to the effect of test drugs on body weight changes have been presented in Table 1. A significant decrease ($P<0.05$) in body weight was found in $HgCl_2$-treated group. A slight but not significant decrease in body weight was obtained in *Lauha bhasma* with $HgCl_2$ treated group. An apparent increase in body weight was observed in both *Mandura bhasma* with $HgCl_2$-treated and control groups.

Loss of body weight is a common clinical feature of anemia. In *Lauha bhasma* with $HgCl_2$-treated

TABLE 1: CHANGES IN BODY WEIGHT IN CONTROL AND TREATED RATS

Group	Dose (mg/kg)	Body weight (g)		
		Initial	Final	Change in %
Control	-	214.0±09.0	221.0±16.0	2.7±3.7
$HgCl_2$	09	215.3±07.7	201.0±11.5	6.9±2.5*
Lauha bhasma+ $HgCl_2$	11+09	209.6±09.3	204.8±06.4	1.3±5.9
Mandura bhasma+ $HgCl_2$	11+09	203.6±10.0	209.2±10.4	3.2±5.4

Figures in this parenthesis indicate changes in body weight after 30 days of treatment. *$P<0.05$ by the student's 't' test

group the decrease in body weight was marginal and it was increased in *Mandura bhasma* with $HgCl_2$-treated group. This reversal of body weight decrease by *Mandura bhasma* could be considered as a significant effect. It indicates reversal of the toxicant induced tissue degenerative changes. In this respect, *Mandura bhasma* is better in comparison to *Lauha bhasma*. Body weight change is the sum of the effects occurring in different parts of the body and reversal of the toxicant induced decrease is an index of good tissue or cytoprotective activity of the test drugs.

Post treated haematological parameters in vehicle control, $HgCl_2$ control, *Lauha bhasma* and $HgCl_2$ and *Mandura bhasma* and $HgCl_2$-treated groups were estimated and have been presented in Table 2. A statistically significant ($P<0.05$) decrease in mean haemoglobin level, total RBC count and haematocrit value was obtained in $HgCl_2$ control group in comparison to vehicle control group. A statistically significant ($P<0.05$) increase in mean haemoglobin level, total RBC count and haematocrit value was observed in *Lauha bhasma* with $HgCl_2$-treated group in comparison to $HgCl_2$ control group. A statistically significant increase ($P<0.05$) in mean haemoglobin level and haematocrit value and a statistically significant ($P<0.05$) increase in total RBC count was observed in *Mandura bhasma* with $HgCl_2$-treated group in comparison to $HgCl_2$ control group. Here Dunnet's 't' test to determine effect of test drugs against $HgCl_2$ control group shows *Mandura bhasma* has more significant effect than *Lauha bhasma* on haemoglobin level, total RBC count and haematocrit value. Other haematological findings do not show

significant adverse sign except marginal to moderate changes in platelet indices in $HgCl_2$-treated group and most of the changes were found to be reversed by test drugs administration.

$HgCl_2$ is a severe corrosive and leads to irritability, stomatitis and colitis. It is toxic to liver and kidney leading to uremia[13]. Reduction in the serum erythropoietin due to liver and kidney damage and uremia cause hemolysis and bone marrow depression leads to decrease in haematocrit, RBC count and haemoglobin percentage[14]. The blood picture in the present study supports this feature. Here haemoglobin content of blood, haematocrit and total RBC content were decreased in $HgCl_2$ treated group. And these were increased in the *Lauha bhasma* with $HgCl_2$ and *Mandura bhasma* with $HgCl_2$ administered groups, suggestive of haematinic effect of *Lauha bhasma* and *Mandura bhasma*. However the exact mechanism of alleviation of the $HgCl_2$-induced anemia is not known from the available data. It would be interesting to ascertain whether the increase is due to increased availability of iron in the usable form or is due to cytoprotective effect of the preparations considering the fact that they are not simply iron preparations but a cocktail of many trace elements[15].

Observed biochemical parameters after treatment have been presented in Table 3. A statistically significant increase in blood sugar and blood urea and significant decrease in serum alkaline phosphatase and SGOT level was observed in $HgCl_2$-treated group. Other parameters were not affected to significant extent. Both the drugs showed tendency towards reversal of

TABLE 2: HAEMATOLOGICAL OBSERVATIONS AFTER TREATMENT IN CONTROL AND TREATED RATS

Parameters	Groups (Dose mg/kg)			
	Control	HgCl$_2$ (9)	*Lauha bhasma* + HgCl$_2$ (11+9)	*Mandura bhasma* + HgCl$_2$ (11+9)
Hb g %	15.18±0.42	12.60±0.34*	14.10±0.43*#	14.54±0.37*#
RBC 10^{12}/l	8.58±0.32	7.19±0.20*	8.20±0.26*#	8.44±0.39*#
Haematocrit %	49.50±1.3	41.95±0.8*	45.92±1.4*#	48.08±1.0*#
RDW %	14.6±0.86	10.6±2.13	13.9±0.69	13.6±1.10
MCV fl	57.85±0.81	58.45±0.91	56.06±0.85	57.24±1.67
MCH pg	17.73±0.30	17.57±0.18	17.22±0.41	17.30±0.56
MCHC g/dl	30.67±0.31	30.07±0.52	30.72±0.49	30.22±0.28
TLC 10^9/l	7.1±1.4	7.0±1.7	8.2±0.8	5.2±0.7
Polymorph %	33.7±5.1	35.5±6.6	30.2±6.0	23.8±1.4
Lymphocyte %	65.2±5.4	63.3±6.7	67.0±5.2	76.2±1.4
Platelet 10^9/l	1096.3±137.5	847.7±142.4	1168.0±196.1	1119.4±114.0
Plateletcrit %	11.1±0.95	08.5±1.27	09.2±2.07	07.3±0.89
PDW %	09.42±0.22	11.03±0.72	08.88±0.49*	08.52±0.23*
MPV fl	7.82±0.13	6.92±0.23*	7.34±0.32	7.26±0.18

The parameters were measured 30 days after treatment. Control group was given vehicle only. $HgCl_2$ and test drugs were administered daily. Test drugs were given after 15 min of $HgCl_2$ treatment. Statistical significance at *$P<0.05$; by the student's #$P<0.05$; by the Dunnet's 't' test. $HgCl_2$ treated group is compared with control group; test drug treated groups are compared with $HgCl_2$ treated group

TABLE 3: BIOCHEMICAL OBSERVATIONS AFTER TREATMENT IN CONTROL AND TREATED RATS

Parameters	Groups (Dose mg/kg)			
	Control	HgCl$_2$ (9)	*Lauha bhasma* + HgCl$_2$ (11+9)	*Mandura bhasma* + HgCl$_2$ (11+9)
Blood sugar (mg/dl)	082.5±04.55	128.3±11.85*	106.3±06.46	082.1±02.50*
Blood urea (mg/dl)	36.82±1.78	44.42±3.03*	29.64±1.72*	31.46±0.98*
S Creatinine (mg/dl)	1.02±0.04	0.96±0.02	0.90±0.03	0.83±0.03*
S Cholesterol (mg/dl)	56.98±4.9	68.06±6.3	43.04±4.3*	43.06±4.4*
S. Triglyceride (mg/dl)	85.8±6.99	80.4±8.79	56.06±0.85	57.24±1.67
S Total protein (g/dl)	7.3±0.14	7.9±0.27*	7.1±0.33	7.3±0.34
SGOT (IU/l)	482.5±19.2	294.2±52.2*	366.8±57.0	368.4±32.6
S Alkaline phos. (IU/l)	36.35±5.6	13.13±3.2*	38.32±4.5*	43.98±2.9*

The pattern of changes in biochemical constituents after treatment for 30 days. Statistical significance at *$P<0.05$; by the student's 't' test. HgCl$_2$ treated group is compared with control group; test drug treated groups are compared with HgCl$_2$ treated group
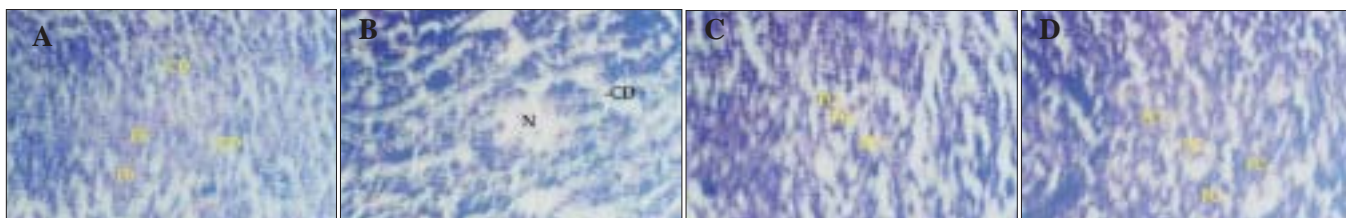
Fig. 1: Photomicrographs (×400) of representative section of spleen of rat from treated groups
Cell depletion (CD), fibrosis (Fb), and necrosis (N) observed in HgCl$_2$ treated group (A and B) compared to cell depletion (CD) and fatty changes (FC) in *Lauha bhasma*+HgCl$_2$ treated group (C) and moderate cellularity and mild fatty changes (FC) in *Mandura bhasma*+HgCl$_2$ treated group (D). A: Control group, B: HgCl$_2$, C: *Lauha bhasma*+HgCl$_2$ and D: *Mandura bhasma*+HgCl$_2$

these toxicant induced changes. The changes observed after HgCl$_2$ administration can be mainly attributed to the toxicant induced liver damage. Reversal of most of these changes by test drugs administration indicates that they do have some element of cytoprotective activity.

Microscopic examination of sections of spleen from different groups was observed under microscope at different magnifications. Normal cytoarchitecture of spleen was observed in control group, whereas cell depletion, fibrosis and necrosis were observed in the cytoarchitecture of spleen in HgCl$_2$ treated group. In the *Lauha Bhasma* with HgCl$_2$ treated group mild fatty changes and cell depletion was observed. In *Mandura Bhasma* with HgCl$_2$ treated group only mild fatty changes with moderate cellularity in the cytoarchitecture of spleen was observed. Photographs from representative sections of spleen from different groups have been presented in fig. 1.

Spleen is the store house of dead RBC and breakdown of hemoglobin also occurs in the spleen. Hemolytic anaemia leads to accelerated breakdown of hemoglobin causing larger iron deposition in spleen[16]. This is likely to be the cause of fibrosis and necrosis observed in the spleen in HgCl$_2$ treated group. This disturbance in the cytoarchitecture was significantly reversed by

test drugs administration. In this respect *Mandura bhasma* was comparatively better because in addition to attenuating the fibrosis, it restored cellularity to moderate level thus inhibiting the toxicant induced cell depletion. *Lauha bhasma* also produced significant protective effect against necrosis and fibrosis but it did not reverse the cell depletion.

Analysis of the data of the haematinic study reveals both *Lauha bhasma* and *Mandura bhasma* have significant haematinic activity. Both are equiactive in this respect. However taking into consideration, changes in all the parameters it can be suggested that though both the drugs are effective as haematinic agents, *Mandura bhasma* has the better activity profile especially in the light of changes observed in ponderal and histopathological parameters. It seems to possess significant haematinic and cytoprotective activities.

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