

# Evaluation of Ayurvedic preparation lauha bhasma as a potential haematinic agent

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## Abstract

Anaemia is one of the most prevalent blood related disorder. The present study was carried out to study the haematinic effect of ayurvedic iron preparations lauha bhasma in treating anaemia in wistar rats of either sex. Anaemia was induced by intra-peritoneal administration of phenyl hydrazine. Three different doses of lauha bhasma were evaluated for the haematinic activity and compared with that of Ferrous sulphate. The results suggest that lauha bhasmas shows significant haematinic activity.

**Keywords:** Haematinic activity, haemolytic anaemia, phenyl hydrazine Lauha bhasma

## 1. Introduction

Anaemia is a condition characterised by decrease in the total amount of red blood cells (RBCs) or haemoglobin in the blood below the normal range for the given age and sex of the patient[1]. This generally results in lowered ability of the blood to carry oxygen [2]. The normal range of haemoglobin in females is  $14\pm 2.5$  g/dl, in males is  $15.5\pm 2.5$ g/dl and in infants is  $16.5\pm 3$  g/dl.[3] It can be caused by any condition resulting in a significant decrease in the total body erythrocyte mass. It is becoming one of the most common disorders of modern era.

Amongst various types of anaemia, iron deficiency anaemia is most prevalent [4,5]. Inadequate iron supply may retard erythropoiesis for weeks or months before these characteristic abnormalities of the red cells are recognizable.[6] The reduced cell size is associated with a number of other conditions in which haemoglobin synthesis is also depressed, including thalassemia, lead poisoning and pyridoxine responsive anemia.[7]

The presence of hypochromia and microcytosis of the circulating red cells generally has been considered essential for the diagnosis of iron deficiency anemia. Prescribing iron supplements is one of the major treatment approach [7]. Lauha Bhasma is the most frequently used Ayurvedic preparation of metallic iron. It is one of the ancient preparations for iron supplements and can be prepared by classical as well as mechanical methods.

The present study evaluates efficacy of Lauha bhasma (LB) for treating anaemia in wistar rats in comparison to ferrous sulphate, a standard iron supplement. The anaemia was induced by peritoneal administration of phenylhydrazine. The main evaluating parameter used was blood hemoglobin level in the animal groups along with other haematological parameters [8-10].

## 2. Materials and Methods

### 2.1. Preparation of doses

Marketed preparations of Lauha bhasma was purchased from local ayurvedic medical market. Phenyl hydrazine and ferrous sulphate were purchased from SD Fine Chemicals, Mumbai, India. All other chemicals used were of AR grade and were used as received.

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 1% gum acacia solution in water and solution of phenyl hydrazine was prepared by dissolving it in absolute alcohol (50mg in 1ml) and adding in acacia suspension[8,9].

All other chemicals used for study were procured from reputed Indian suppliers and were of AR grade.

### 2.2. Animals

The albino rats (Wistar strain) of either sex weighing 150-200 g, were selected for this study. The animals were housed in polypropylene cages at a temperature of  $25 \pm 2^\circ\text{C}$  with relative humidity of 40-60 % and 12 hrs light dark cycle, with

maximum of 5 animals housed per cage. Animals were fed with a standard rodent feed and water *ad libitum* during the complete study period. The study was approved by the Institutional Animal Ethical Committee (Registration No. 535/02/a/CPCSEA/Jan2002) of Institute of Pharmaceutical Education and Research, Wardha.

### 2.3 Acute oral Toxicity Study

Acute Toxicity studies were performed as per OECD-423 guidelines<sup>12</sup> to determine the safety doses. Acute Toxicity studies of the Lauha bhasma were carried out on wistar strain albino rats. Rats were fasted over night and weight of each animal was recorded just before starting study. Animals were divided into seven groups. They were fed orally with the Lauha bhasma in increasing dose levels of 500, 1000, 1500, 2000, 3000, 4000 and 5000 mg/kg body weight through oral feeding needle [11]. The animals were observed continuously for changes in signs and symptoms and mortality. Table 2 depicts acute oral toxicity studies for Lauha bhasma in rats.

### 2.4. Experimental Design

Six rats were kept as normal control group (Group 1), while 30 rats were made anaemic by intra-peritoneal of phenylhydrazine (80 mg/kg body weight) and kept for 24 hrs before starting study protocol. Phenyl hydrazine treated rats were randomly divided into five groups with six rats per group (Group 2 to 5) and treated as follows:

**Group 1: Normal Control Group** consisting of rats without anaemia which was administered with vehicle i.e. 1% acacia paste containing 1ml of absolute alcohol per 50 ml.

**Group 2: Anaemic Control Group** with rats having induced anaemia also received 1% acacia paste containing 1ml of absolute alcohol per 50 ml.

**Group 3: Standard Group** received oral single dose (1ml) of 20mg/kg body weight/day ferrous sulphate,

**Group 4: Test Group 1** received oral single dose (1 ml) of the 10 mg/kg body weight/day of lauha bhasma,

**Group 5: Test Group 2** received oral single dose (1 ml) of the 15 mg/kg body weight/day of lauha bhasm,

**Group 6: Test Group 3** received oral single dose (1ml) of 20 mg/kg body weight of lauha bhasm.

The animal grouping is summarized in Table no.1

**Table 1: Experimental Animal Groups**

Group No.	Group Name	No. of Animals	Doses mg/kg/day
1	Normal Control group	6	1ml 1% acacia paste containing 1ml of absolute alcohol per 50 ml.
2	Anaemic control group	6	1ml 1% acacia paste containing 1ml of absolute alcohol per 50 ml.
3	Standard group	6	1ml of 20mg/kg daily.
4	Test 1	6	1ml of 10mg/kg daily
5	Test 2	6	1ml of 15mg/kg daily
6	Test 3	6	1ml of 20mg/kg daily

### 2.5. Sampling Schedule

The study was carried out for 21 days. The blood (1-2ml) was withdrawn in EDTA from the retro-orbital plexus, under slight chloroform anaesthesia, on day 0 before phenyl hydrazine administration, and on days 1, 7, 14 and 21, days after phenyl hydrazine administration i.e. induction of anaemia.

### 2.6. Evaluation of haematological parameters

The haematonic activity was evaluated by assessing various blood parameters like red blood cell (RBC) count, haemoglobin (HB) concentration and packed cell volume (PCV). The mean cell volumes (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC) and white blood cells (WBC) count [9-11]. The parameters were determined by using Auto cell counter 920<sup>E</sup>, (Swelab Ltd).

### 2.7. Statistical analysis:

The data were statistically analyzed using a one-way ANOVA followed by Dunnett test. Statistical significance was determined at a level of  $p < 0.05$ . Statistic Results are expressed as the mean  $\pm$  SEM, (n=6)

## 3. Results

### 3.1. Acute toxicity study

Traditionally lauha bhasma is used in treatment of anaemia. Acute Oral Toxicity Study for Lauha bhasma in wistar Rats was carried out to evaluate the maximum safe dose of Lauha bhasm[12]. The acute toxicity testing revealed no death up to doses of 5000 mg/kg. The LD<sub>50</sub> was found to be more than 5g/kg body weight. The results are given in Table 2.

Table 2: Acute Toxicity study of Lauha Bhasma

Group	Dose (mg/kg)	Route	Death/N	Death%
1	500	Oral	00/6	0
2	1000	Oral	00/6	0
3	1500	Oral	00/6	0
4	2000	Oral	00/6	0
5	3000	Oral	00/6	0
6	4000	Oral	00/6	0
7	5000	Oral	00/6	0

3.2: Evaluation of Weight loss/gain

One of the symptoms of anaemia is loss in body weight. All the groups were evaluated in weight loss or gain after inducing anaemia and stating drug treatment [13]. From the results it can be observed that lauha bhasma produced significant increase ( $p < 0.05$ ) in body weight when compared with anaemic control. The results also indicate that the weight gain was not dose dependent. Though three different doses of lauha bhasma were administered the weight gain was comparable with standard drug. The results are depicted in Figure 1.

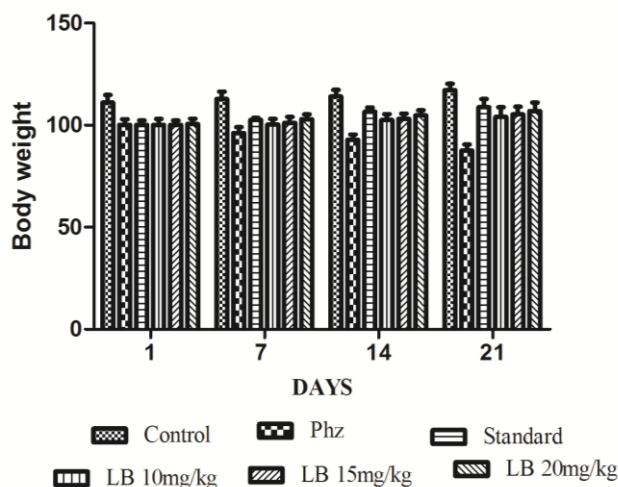


Figure 1: Weight gain/loss in different animal study groups

3.3: Evaluation of Haemoglobin content:

Anaemia is mainly diagnosed from haemoglobin content of blood [14]. A good haematinic should be able to prevent decrease in haemoglobin level in blood. It is reduced below the control rats phenyl hydrazine induced significant ( $p < 0.5$ ) decrease in Hb concentration. Thus it was observed that on 21 day LB at the dose of 10mg/kg it shows  $9.96 \pm 0.33$ , also on 15 mg/kg it shows  $11.98 \pm 0.52$  also on 20 mg/kg it shows  $12.55 \pm 0.8$  which shows significant result. The standard Ferrous sulphate showed haemoglobin level of  $14.56 \pm 0.64$ . The increase in haemoglobin was dose dependant.

The maximum dose of lauha bhasm, showing significant improvement in Hb content after 20 days. As anaemic rats show marked decrease in Hb content. Indicating good anti anemic property of Lauha Bhasma.

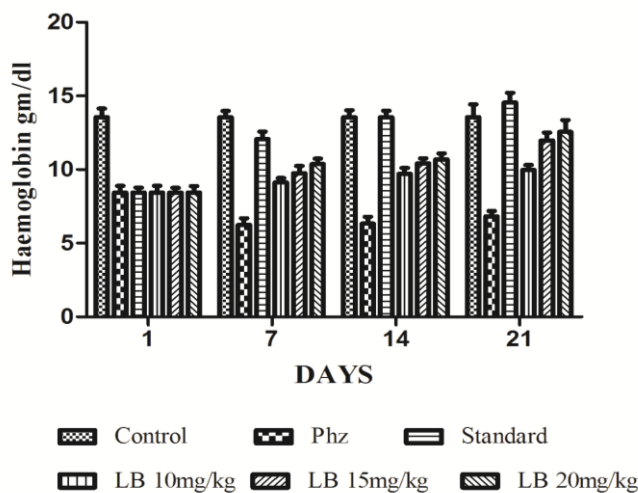


Figure 2: Haemoglobin content in different animal study groups

### 3.4: Evaluation of RBC content

Phenyl hydrazine induced significant decrease in RBC concentration (76.1%). The administration of the lauha bhasma evoked a significant increase in the RBC count as compared to untreated group [15]. The Results show slight decrease in RBC levels in all groups after 7 days may be due to effect of phenyl hydrazine. The RBC content gradually increases after 14 days and 21 days for treated rats. Lauha bhasma was able to reverse and retain RBC content. The effect was dose dependant for initial time periods but dose dependency was not significant at the end of treatment. The effects of the bhasma were comparable to those of standard ferrous sulphate.

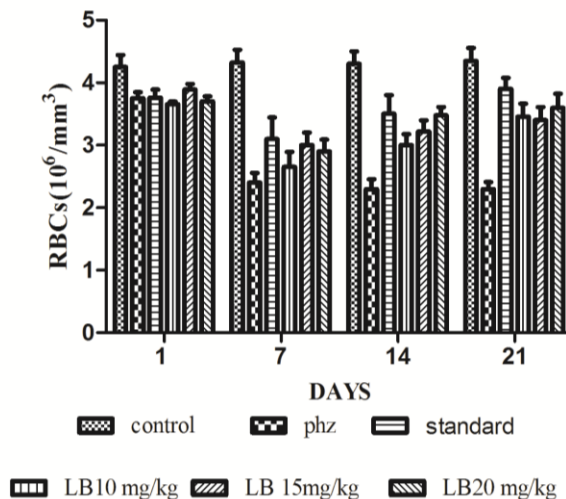


Figure 3: RBC content in different animal study groups

### 3.5: Evaluation of PCV

Decrease in Packed cell volume (PCV) can be observed and is significant diagnostic factor in anaemic patients<sup>16</sup>. In the rats treated with phenyl hydrazine induced significant (p<0.5) decrease in packed cell volume (PCV), the administration of the lauha bhasma resulted in a significant (p<0.5) increase in the PCV parameter as compared to non treated animals. The PCV was increased with increasing dose of lauha bhasm. The effect of bhasma was comparable to those of the ferrous sulphate especially at the dose of 20 mg/kg.

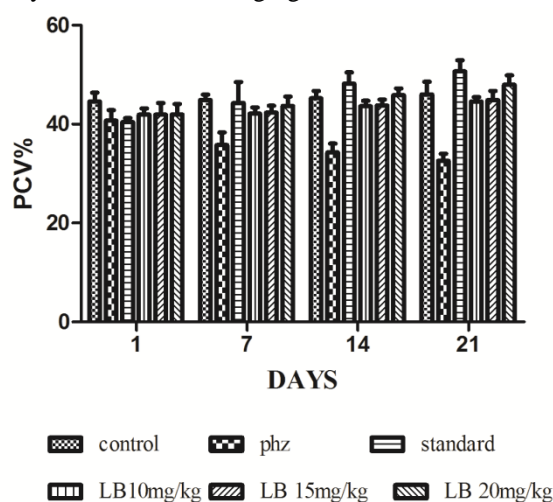


Figure 4: PCV evaluation in different animal study groups

### 3.6: Evaluation of MCHC:

In anaemic patients increase in MCHC is observed. The phenyl hydrazine treated anaemic rats showed significant (p<0.5) increase in MCHC concentration. The administration of the lauha bhasma evoked a significant (p<0.5) decrease in the MCHC depending on dose after 14 days. The positive effect of lauha bhasma on MCHC found to be at par with that of standard ferrous sulphate at minimum dose of 10 mg/kg.the results are presented in Figure 5.

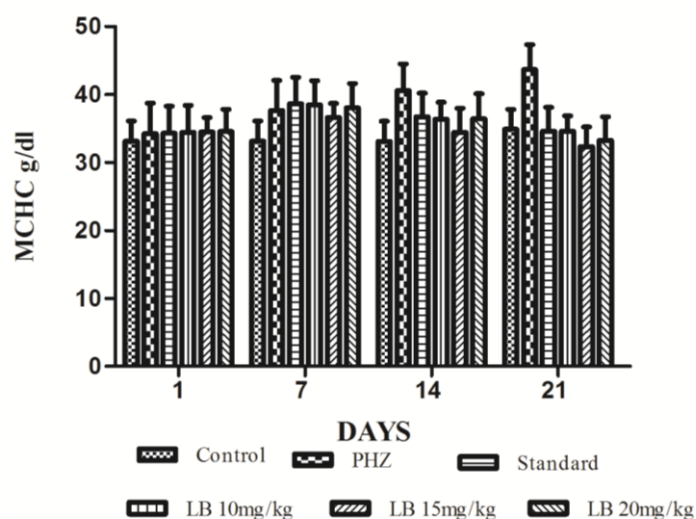


Figure 5: MCHC evaluation in different animal study groups

### 3.7: Evaluation of MCV

Like MCHC there is increase in MCV levels in anaemic conditions [17]. In the phenyl hydrazine induced anaemic rats significant ( $p < 0.5$ ) increase in MCV concentration was observed. The administration of the lauha bhasma evoked a significant ( $p < 0.5$ ) decrease in the MCV parameter as shown in Figure 6. The effect was dose dependant and was comparable to standard ferrous sulphate.

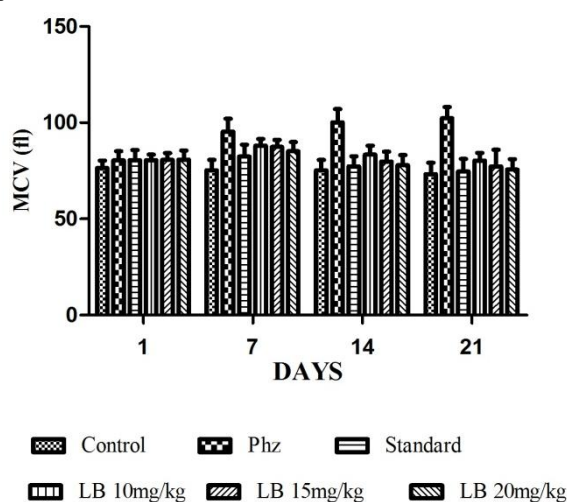


Figure 6: MCV evaluation in different animal study groups

## 4. Conclusion

The results of the present study indicate that Lauha bhasma was able to reverse anaemia induced by phenyl hydrazine in wistar rats. Also it is a safe drug as its  $LD_{50}$  is very high. Thus it can be concluded that lauha bhasma is very effective ayurvedic haematinic agent and can be useful in treatment of anaemia.

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