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Discussion
Ayurveda is the most ancient system of medicine among the different traditional systems existing in the present world. In the Vedas there are many references about the disease Pandu. In Rigveda, Panduroga has been mentioned in the name of ‘Harima’ and in the Yajurveda, there is a reference about Pandu.\textsuperscript{1-2} Charaka and Susruta has elaborated this point in detail by stating that the moolasthana of raktavaha srotas lies with yakrit and pleeha and the predominant dosha associated with Pandu is pitta.\textsuperscript{3-4} Description about ‘Panduroga’ is also obtained in Garudapurana, Valmiki Ramayana and Agni purana. Mahabharata stated that the Hastinapura king had suffered from Pandu roga whose name itself is Pandu Raja.\textsuperscript{5} Description of this disease is also available in the classical Ayurvedic texts like Chakradutta, Vangasen, Yogaratnakara, Bhaishajyaratnavali, Basavarajeeyam, Madhavanidanam and Sarangadhara Samhita.\textsuperscript{6} Charak classified rogas according to Rupa (main complication), Varna (alteration of colour), Samuthana (according to etiology), Samsthana (according to clinical features) and Sthana (according to site or location). In this classification, the disease pandu felt in the group of varna. In panduroga there are some significant changes in the normal colour of the body.\textsuperscript{7} Monier William in his Sanskrit English dictionary has defined Pandu as “Pallor”. Most of the Acharyas expeted the disease under five varieties like Vataja, Pittaja, Kaphaja, Sannipataja and Mridbhakshanaja.\textsuperscript{8-11}

In Indian texts, Kapha has also an important role to play in the pathogenesis of panduroga. Excess intake of santarpana karaka regimen has been regarded as a causal factor for Pandu\textsuperscript{12}. Kaphaja vyadhi produces lakshanas like Panduta\textsuperscript{13}. Inspite of the Kapha being its prakrita avastha, the decreased vata displaces it along with pitta to all parts of the body cauing Pandu\textsuperscript{14}. Kapha in Twacha produces Shwetavabhasata, Kapha in Rakta produces Pandu\textsuperscript{15}. Vaya in Hridaya is responsible for the spread of the vitiated pitta via the dasha domains to the whole body.

The pathogenesis of kaphaja variety of Panduroga is not particularly specified in any of the Ayurvedic classics but pathogenesis for “Panduroga” in general has been mentioned. However, hypothesis of pathogenesis for the “Kaphaja Panduroga” can be made in accordance with the description of pathogenesis of Panduroga in general as mentioned in Caraka Samhita\textsuperscript{16}.

The knowledge of Pathya and Apathya is essential in treating the disease. All the nidanas mentioned in nidana aspect are to be strictly considered as aphathyas and are to be avoided. Those aharas and viharas, which are against the nidana as well as the disease, are called Pathyas. In contrary, the aggregating factors of the disease are called Apathyas. Pathya or food is capable of causing agnideepana should form as important principles of pathya-vyastha of pandu roga e.g. Madhura, tikta, kashaya rasa and Guru, rukshana, teekshna gunas\textsuperscript{17-19}. 
Fig 43: Mode of action of *Louha Bhasma* according to Ayurvedic text

*Charaka Samhita, Rasendra Sara Samgraha, Bhaishajya Ratnavali* etc. reviewed to gather information about *Louha Kalpas*. Critical analysis of these *Louha Kalpas* reveals that ancient seers administered iron in a better acceptable form\(^\text{20}\). *Astanga Sangraha* and *astanga Hridaya* mentions importance of *Louha Bahsma*. *Louha Bhasma* is used in *Āyurveda* since a long time but classically it was designed for therapeutic purpose from the reign of Nagarjua. It is categorized as *Shuddha Louha*\(^\text{21}\). Vagbhata said that *Louha Bahsma* is the superior drug for the treatment of *Pandu*\(^\text{22}\). *Charaka, Susruta, Vagbhata, Sarangadhara, Bhavamishra* and others mentioned many drug formulae which contains iron or iron compounds for the care of *Pandu*\(^\text{23}\). Due to its wide advocacy has *pandu hara dravya* in ayurvedic texts, *Louha Bahsma* has been selected for the present study on *Panduroga*, particularly in *Kaphaja Pandu Roga*, which is very much similar to iron deficiency anemia in human.

Now-a-days, iron deficiency is the most common and widespread nutritional disorder in the world including India\(^\text{24}\). There are no current global figures for iron deficiency, but using anaemia as an indirect indicator it can be estimated that most preschool children and pregnant...
women in non-industrialized countries, and at least 30-40% in industrialized countries, are iron deficient\textsuperscript{25-26}. Nearly half of the pregnant women in the world are estimated to be anaemic. Anemia was found to be significantly associated with educational and socioeconomic status, birth order, illiteracy, lower body mass index (BMI) and awareness regarding anemia but no association with age or menarchal age. It has been reported that iron deficiency adversely affects on cognitive performance and behavior, immune status, physical capacity and work performance, perinatal risks for mothers and neonates, impaired gastrointestinal functions, patterns of hormone production and metabolism\textsuperscript{27-28}.

Iron is an essential part of the haemoglobin and a valuable remedy in the treatment of anaemia. Sydenham has been introduced iron into clinical medicine for the treatment of anaemia\textsuperscript{29}. Until the last decade of nineteenth century, iron therapy of anaemia followed the principles enunciated by Sydenham and Bland and was comparable to modern practice. Iron preparations can be administered in three ways - orally, intravenously and intramuscularly. Oral therapy is the safest and best method of ferro-therapy and now generally accepted that oral iron is the treatment of choice in iron deficiency anaemia. Ferrous sulphate in tablets or capsules is still as efficient as any preparation. Eventually, ferrous sulfate replaces the iron stores found in hemoglobins in RBC, myoglobin and other heme enzymes and also allows the transportation of oxygen via hemoglobin. The mechanism of action of ferrous sulphate is in similar to the absorption in iron in gut from other sources like diet\textsuperscript{30}.

The calcined (putas) iron preparation in Ayurveda is known as louha bhasma\textsuperscript{22}. ‘Puta’ can be defined as a series of Ayurvedic procedure for preparation of metallic drug under specialized technique of ignition to achieve final end product. In the present study, Louha Bhasma was prepared based on the method described in Rasaratna Samuchaya with slight modification\textsuperscript{23}. Iron was used with fruits of Triphala (Terminalia chebula, Terminalia belerica, Emblica officinalis) and fresh raw cow’s urine in decoction and subjected to heat by Gajaputam. Three putam was prepared for the present studies: 50, 100 and 150. It is interesting to note that, iron content was gradually decreased with increment of number of puta of Louha Bhasma. The iron concentration was 35.7% in 150 puta of Louha Bhasma, 38.3% in 100 puta of Louha Bhasma, while, 39.5% in 50 puta of Louha Bhasma. But, carbon and sulphur content was reversed in this phenomenon. In 150 puta of Louha Bhasma, carbon content was 8.64% and sulphur content was 5.9%. Furthermore, acid insoluble portion was also high (27.1%) in 150 puta of Louha Bhasma than other 50 and 100 puta of Louha Bhasma.
Moreover, a comparative Atomic Absorption Spectrometral analysis of different putas of Louha Bhasma showed elemental concentrations varies with number of putapuk. 150 puta of Louha Bhasma showed maximum amount of Mg, Ca, Na, Al, K, Zn and Cu. In 50 puta of Louha Bhasma Mg concen was 1182 ppm, 100 puta of Louha Bhasma 5378 ppm and 8250 ppm in 150 puta of Louha Bhasma. Hence, 150 puta of Louha Bhasma contains 5.97 times higher than 50 puta of Louha Bhasma. Ca content was more than 3 times higher in 150 puta of Louha Bhasma than 50 puta. Furthermore, 150 puta of Louha Bhasma consisted with 15 important biologically relevant elements including Fe, K, Cl, Si, Na, S, Mg, Al, Ca, Mn, Zn etc. Most of these elements have the role in the formation heamoglobin and also maturation of red blood cells in bio-active functions. Hence, it may assumed that 150 puta of Louha Bhasma is better than iron oral supplement that contains only inorganic or organic iron. The energy dispersive x-ray microanalyses also confirmed the smaller articles (< 2.0 mm) content of iron. The larger particles i.e, for sodium, potassium, magnesium and chlorine were probably the major inorganic constituents of Triphala.

The therapeutic potency of Louha Bhasma increases with successive incineration under anaerobic condition (higher Putas) as a result of particle size disintegration as well as inclusion of more and more nutrients due to decoction with Triphala. It is very interesting to note that, from lower to higher Putas, the particle size gradually diminishes. The lowest particle size is reported in 150 puta of Louha Bhasma which is 5.477 µm. Lower particle size of Louha Bhasma not only helps to absorbed in the intestinal bed but also has to enhanced its possibilities for bioavailabilities. Furthermore, infra red (IR) spectral analysis confirmed the characteristic bands for polyphenols and lignins originated from the Triphala during decoction of iron with plants and cow’s urine. The presences of bound polyphenols have also been confirmed in Louha Bhasmas with chromatographic analyses.

Remarkable decreases were noted with HPTLC study in areas of polyphenols and/or gallotannoid iron chelates. Without putopak the content of bioavailable chelated/complexed iron was significantly lower than different putas of Louha Bhasma. The total area of the region tR 4-8.0 minutes was nearly 40% which is higher than without putopak and 150 putopak Louha Bhasma indicating graded chelation of Fe^{3+} ions with polyphenols of triphala with putopaks. This indicated graded chelation of Fe^{3+} ions with polyphenols of triphala as the number of putopakas was increased. Thus putopaka has improved the concentration of bioavailable iron complexes.

Distinct peaks of polyphenols and/or gallotannoid iron chelates were observed in Triphala, without putopak and different putas of Louha Bhasma (50,100 and 150). Presences of some
purine-gallic accomplexes were noted the \( t_R \approx 2.3 \) min. In the \( t_R \) regions 4.0 – 6.0 minutes several peaks of the polyphenols of *triphala* were observed, but PDA spectra indicated some shifting of UV spectra. These changes were probably contribution of *Louha bhasma* and organic matters of *Triphala* developed. Apart from the shifting of PDA spectra the formation of chelation/complexation was also evident from the increment in % area of chromatograms. Without *putopak* the total area of the region \( t_R 4 - 8.0 \) minutes was 22% and there was significant increment in this area (30 - 45%) as the number of *putopaks* has increased. This indicated graded chelation of Fe\(^{3+}\) ions with polyphenols of *Triphala* as the number of *putopaks* was increased. Thus *putopak* has improved the concentration of bioavailable iron complexes. The present studies confirmed that each of the *putas* of *Louha Bhasma* is not only differed from iron content but also in physical (size) and chemical nature (elemental composition). For this reasons, there are every possibilities to altere their bioavailabilities and metabolic conditions in biological systems.

In toxicological studies, different *putas* of *Louha Bhasma* show the LD\(_{50}\) was greater than 0.6 g/kg (p.o) in mice, which is equivalent to 7.38 mg/kg for human. Furthermore, 60 days continuous oral therapy of different *putas* of *louha bhasma* did not induced any adverse effects in rats. Hence, these observations conclusively supported the use of different *putas* of *louha bhasma* for human use without any serious adverse actions. Therefore, no observed adverse effect level (NOAEL) of different *putas* of *Louha Bhasma* was 600 mg/kg.

Iron is very important in maintaining many body functions, including the production of hemoglobin, the molecule in huma blood that carries oxygen. Iron deficiency is confirmed by a low serum ferritin, red cell microcytosis or hypochromia in the absence of chronic disease or haemoglobinopathies. Therefore, haemoglobin and red blood cells are the most important biomarkers in iron deficiency anemia (IDA). In the present preclinical animal trial, IDA drastically lowered the body weight in animals as also hemoglobin concentration, RBC numbers and other hematocrit values, like PCV, MCH and MCHC. But, different *putas* of *Louha Bhasma* therapies significantly improve the body weight gain and other hematocrit values related with anemia. The maximum improvement was noted in 150 *puta* of *Louha Bhasma* therapy even better than standard iron drug, Fefol.

Ferritin is a protein that stores iron in the body cells particularly in hepatocytes and reticuloendothelial cells that release iron in circulating blood in need. Transferrin is a protein that combines with ferritin to transport it to where new red blood cells are made. In the preclinical studies, iron deficiency anemia significantly reduced the serum ferritin concentration than normal control rats. But, treatment with different *putas* of *Louha Bhasma* drastically enhanced
the ferritin concentration in serum. Among all, 150 putas of Louha Bhasma has the maximum effect. Unlike ferritin, serum TIBC and serum iron have also been enhanced after Louha Bhasma treatment in IDA rats.

*Lauha Bhasma* is most commonly used preparations of incinerated iron. This is indicated for the same as well as different diseases like in anemia, edema, jaundice etc. and is also used as immunomodulators (*Rasayana*). There are several scientific studies regarding effect of iron deficiency anemia on general health and immunity. Srikantia et al (1976) studied on young children and indicated that both the cell mediated immune response and the bactericidal activities of leucocytes are impaired when levels of Hb fall to 10 g/dl or less. Strauss (1978) analysed the literature available till 1977 to define possible relationship between infections, immune function and state of iron imbalance and stated that inflammatory response is clearly diminished in iron deficiency. In the present studies, lymphocytes numbers in spleen and splenic plaques, and also peritoneal macrophages in anemic rats were significantly reduced, but, treatment with different putas of Lauha Bhasma enhanced not only the numbers of lymphocytes both in spleen and splenic plaques but also increased the population of peritoneal macrophages. Conversely, in IDA rats, granulopectic index has been reported to elevate than normal rats, as because less number of peritoneal macrophages present in IDA control rats, while, test drugs have the capabilities to diminished the granulopectic index than IDA rats, better than standard drug Fefol. Not only these, moreover, different putas of Lauha Bhasma therapies evidenced to stimulate haemagglutination reaction and delayed type hypersensitivity in rats. In comparison to other putas, 150 puta of Lauha Bhasma has the maximum immune potentiating actions.

Iron is a key player in hemoglobin synthesis an erythrocyte production. At the same time, it is a potent poison to mammalian cells and an indispensable nutrient for many disease causing germs and microbes. Therefore, its metabolism in mammalians is very complex and stringently controlled by many different genes and proteins. Transferrin is the main protein involved in iron transport in plasma. It proved to be a useful parameter for assessing both iron deficiency and iron overload. The saturation of transferrin is a strong indicator of iron overload. Dietary factors that influence iron absorption, such as phytate, polyphenols, calcium, ascorbic acid, and muscle tissue, have been shown repeatedly to influence iron absorption in single-meal isotope studies, whereas in multimeal studies with a varied diet and multiple inhibitors and enhancers, the effect of single components has been, as expected, more modest. The result of metabolic studies of different putas of Lauha Bhasma in iron deficiency anemic rats indicated that 150 putas of *louha bhasma* therapy significantly enhanced hemoglobin concentration 82%, serum iron level 54.8%, serum ferritin concentration 62.2% and TIBC level 15.43% within 28 days. The bioavailability
of 150 putas of Lauha Bhasma significantly improved the iron deficiency anemic concentration in rats that may be due to its particle size and presence of other nutritive elements in the test compound as supported evident by chemical analysis of Lauha Bhasma. On the basis of comparative studies of different putas of Lauha Bhasma it has been observed that 150 puta of Lauha Bhasma was most potential and effective in IDA. Hence, further clinical studies in human were conducted with 150 puta of Lauha Bhasma only.

Therefore, four principle strategies for correcting micronutrient efficiencies in populations are considered to use for correcting iron deficiency anemia, either alone or in combination. These are education combined with dietary modification, to improve iron intake and bioavailability; iron supplementation, iron fortification of foods and the new approach of biofortification. However, there are some difficulties in the application of some of these strategies when considering iron.

Finally, a prospective, randomized, parallel group, comparative, intervention, clinical study was conducted on 150 puta of Lauha Bhasma and standard drug Fefol at Institute of Post Graduate Ayurvedic Education and Research, Kolkata and J.B. Roy State Ayurvedic Medical College & Hospital, Kolkata in accordance with good clinical practice guidelines and was conform to the declaration of WHO-Helsinki, following approval by the Institutional Clinical Research Ethics committee (ISM&H/PS-5/1/04 dt. 18/05/2004).

From 434 subjects screened, only 130 participants showed iron deficient anemic patients, which were divided in two groups: 80 subjects for 150 puta Louha Bhasma and 50 subjects for standard drug Fefol® (Ferrous sulphate + Folic acid). Finally, 100 subjects were enrolled for groups (N=50 for each group) as they only gave their written consent. At the end of study, 39 subjects were completed in 150 puta of Louha Bhasma and only 16 subjects completed for Fefol, rest were dropped out. Patients were selected irrespective of age, sex, religion, occupation, income status, habitat, Prakriti, etc. Before starting the test drug trials, all subjects were received modern anti-amebic drug (Tinidazole: 600 mg + Norfloxacin: 400 mg) twice in a day for 7 days and anthelmintic (Albendazole: 400 mg) single dose. After that, the test drugs, 150 putas of Lauha Bhasma and standard drug, Fefol were given at the dose of 250 mg/day/orally for 60 days.

In this clinical trial, 55 completed cases were reviewed under which, 30 subjects were female, 67.28% subjects belonged to Hinduism, 67.28% subjects were coming from below poverty level, 49.1% from rural area and 38.19% were illiterate. The prevalence of iron deficiency varies greatly according to host factors: age, gender, physiological, pathological, environmental, and socioeconomic conditions. According to Ayurvedic classification of Prakriti, maximum subjects were identified as Kaphalvana-Pittaja (23.63%). The pallor of
anemia was associated with weakness and tiredness. Previously, it has been noted that iron deficiency anemia adversely affects the hematological and hematocrit values, iron metabolism and immune status. All the common subjective clinical sign and symptoms like, lassitude, fatigue, dizziness, palpitation, pallor, oedema, glossitis etc. were observed in IDA patients and that were cured in post treatment groups, particularly with 150 putas of Lauha Bhasma.

It has been observed that 150 putas of Lauha Bhasma treated patients have better improvement than fefol group in IDA associated with the improvements of Hb, RBC, PCV, MCV, MCH and MCHC. Louha bhasma treatment improved 60.86% hemoglobin, 26.19% RBC count, 38.1% PCV, 10.63% MCV, 27.02% MCH and 14.19% MCHC. In comparison with fefol, Louha bhasma (150 putas) potentiated more effective action in the treatment of anemia. Moreover, 150 putas of Lauha Bhasma enhanced the concentrations of serum iron, ferritin and TIBC in iron deficient anemic population which may indicate that Lauha Bhasma has the prominent iron supplement properties, particularly in IDA.

There are several scientific studies regarding effect of iron deficiency anemia on general health and immunity. The precise molecular defect remains undefined but the abnormality is detected by several assays measuring cell mediated immunity. Normal function is usually restored following iron repletion. Patterson et al (2000) studied 14,762 young and 14,072 mid-age women in Australia and reported that iron deficiency is associated with decreased general health and well being and increased fatigue. In 2001 they confirmed that treatment with either iron supplementation or high iron diet, both results in improved mental health and decreased fatigue. Grondin et al (2008) concluded that iron deficiency impairs the perceived general health in female students and suggested that further research should be conducted on this subject. In the present study significant increase of IgA (12.84%), IgG (6.29%) and IgM (20.67%) were observed with 150 putas of Lauha Bhasma, better than Fefol® treated group.

Finally, in metabolic and safety studies indicated that 150 puta Louha of Bhasma on the basis of parameters like Hb%, protein, serum albumin, GOT, GPT, ALP, serum urea and creatinine have the abilities to restrict iron deficient anemia and may be useful in therapeutic approaches of iron deficient anemia. Hence, it may conclude that 150 puta Louha of Bhasma of ancient Ayurvedic origin is an efficient iron preparation for management of iron deficiency anemia.
References

1. Rigveda 10-50-11-13


5. Garuda Purana Chapter 184/29


