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Conclusion
‘Kaphaja Pandu Roga’ described in traditional Ayurvedic texts are resemble to iron deficiency anaemia in modern, one of the most severe and important nutritional deficiencies, affecting more than 200 million persons in the world today. The disease and treatment of Kaphaja Pandu Roga (iron deficiency anemia) with of louha bhasma in combination with medicinal plants was first described in Charaka Samhita. In this research programme, Louha Bhasma of 50, 100 and 150 varieties of putas were prepared in accordance with classical Ayurvedic methods with slight modification to established its bioavailability through chemical, pharmacological and clinical investigations.

Chemical analyses of different putas of Louha Bhasma elaborated some unique information, which are as follows:

AAS studies of louha bhasma not only established the presence of essential elements like, Fe, Na, Mg, Al, Mn, Cu, Zn, K, Ca & C etc but it also reported that Fe content was gradually decreased while, other elemental concentrations were gradually increased on the increment of putas from 50 to 150 variety. EDX studies analyses that particle size of louha bhasma was gradually decreased from lower (10 puta, 194.4 µm) to higher (150 puta, 5.477µm) putas and the size of iron was lower than 2.0 nm. XRF studies manifested the presence of useful nutritive elements like, Na, Mg, Al, Si, P, S, Cl, Mn, Fe, Ni, Cu, Zn, Br, Ba, K and Ca in maximum amount in 150 puta. HPLC & HPTLC studies predicted that 150 puta formed a chelation of Fe$^{n+}$ ions-complex with several bioactive constituents like, polyphenols, gallatonoids, purine – gallic acid complex. IR study confirmed the presence of specific bands for polyphenols and lignins in 50, 100, 150 putas of louha bhasma.

Pharmacological trial of Louha Bhasma (50, 100 & 150 putas) were subjected in toxicological and Iron deficiency aneamic models in rats. Summary of these results are given below:

Toxicological studies reported that, in acute toxicity testing, Louha Bhasma (50, 100 & 150 putas) showed no mortality up to the dose of 400 mg/kg, p.o., which was approximately 20 times higher than the animal therapeutic dose (21.4 mg/kg b.w.). The results of sub-chronic toxicity studies (4 week) of all putas revealed that there were no significant changes in hematological and biochemical parameters in blood and tissues when compared to control animals.

Iron deficiency nutritional diet induced anaemic models reported that, there was a marked change in body weight and blood heamoglobin concentration after the treatment of different
putas of Louha Bhasma (50, 100, 150 puta) and reference drug Fefol®. Other haematocrit values like, PCV, RBC, MCV, MCH were also significantly improved in all treated and Fefol groups than control anaemic rats and the efficacy was maximum in 150 puta treated groups. Further, serum ferritin, TIBC, serum iron (marker of iron absorption and transport) were markedly changed in test drug treated and reference drug animals than anaemic control. Highest change noted in 150 puta treated animals, i.e., it enhanced 62% serum ferritin level, 20% lowered TIBC concentration and 119% increased serum iron level than anaemic control.

It was also noted that Louha Bhasma modified several immunological regulators/modulators non-specifically and improved the immune responses in anaemic conditions. The louha bhasma therapy significantly enhanced the splenic lymphocytes count, the number and phagocytic activities of peritoneal macrophages, antigen-antibody related hemeagglutination or humoral response, delayed hypersensitivity reaction etc. The maximum efficacy was noted on 150 puta of louha bhasma. It enhanced 16% splenic lymphocytes, 21% plaque forming cells/spleen and 59% peritoneal macrophages, while it reduced 33% granulopoetic index and delayed hypersensitivity.

Metabolic studies revealed that blood haemoglobin, serum iron and serum ferritin were gradually increased, while TIBC concentration was lowered markedly. Haemoglobin per cent increased 82%, serum iron enhanced 55% serum ferritin improved 40%, while, TIBC reduced to 15% within 28 days treatment of 150 puta, that clearly evident its maximum bioavailability.

As chemical, pharmacological and toxicological investigations suggested 150 puta of Louha Bhasma was maximum efficacious then this evidence encourage undertaking clinical trial of 150 puta of louha bhasma as lead in the patients of iron deficiency anemia.

Clinical trial of the selected test compound, 150 puta of Louha Bhasma and reference drug Fefol® was performed according to good medical practice. In this trail 55 subjects was finally selected, maximum age 36-45yrs, un-employee 25, rural 27, primary/secondary 23, BPL 37, Kaphalvana pittaja 13, Hindu 37 and female 30 in number. The treatment of 150 putas louha bhasma and Fefol® depicted the following information:

Sign and symptoms of anemia were decreased significantly both 150 putas Louha Bhasma and fefol® treated groups. The per cent inhibition of clinical features of iron deficiency anemia with louha bhasma treated group were Pandu Varna 59%, Tandra 59%, Sranti 63%, Hridaspandan 70%, Bhranti 66%, Swas 65%, Karnanada 63%, Shirashula 64%, Dristialpata 65%. In Fefol® treated group there were decrease of Pandu Varna 52%, Tandra 57%, Sranti 60%, Hridaspandan 54%, Bhranti 57%, Swas 59%, Karnanad 57%, Shirashul 57%, Dristialpata 55%, which indicated potential improvement of subjective features.
The treatment of 150 puta improved haematological indices within 30 days. Haemoglobin concentration enhanced 61%, RBC increased 26%, PCV enhanced 38%, MCV improved 11%, MCH enhanced 27% and MCHC increased 14%. Some of the results were quite better than to Fefol® treated group, like, TLC, neutrophil, lymphocyte and platelets were significantly increased while, ESR and eosinophil count were significantly decreased. Average increase of lymphocytes with louha bhasma treatment may emphasise the idea of its role on immunomodulation.

The serum feritin and serum iron level were significantly improved in 150 putas louha bhasma and fefol® treated group. Enhancement of serum ferritin was 136%, serum iron 88% and inhibition of TIBC 17% with 150 putas of louha basma. The result indicates that the louha basma therapy has potent capacity to correct anaemia by replacing body storage iron. The immunoglobulin level increased significantly with 150 puta variety of louha bhasma like, IgA (13%), IgG (6%) and IgM (21%). This result indicates that 150 putas of louha basma increased both humoral as well as cell mediated immunity in human. Regarding haematological variables of 150 putas of louha bhasma, Hb%, serum iron, serum ferritin level were gradually increased from day 14 to day 28 while, TIBC level decreased. It is suggested that even short term therapy of 150 putas of louha bhasma can correct iron deficiency anaemia and may rebuilt iron store in patient with mild to moderate iron deficiency anaemia. There was no evidence on haematological, renal or hepatic toxicity after the therapy with 150 puta Louha Bhasma.

Therefore, finally it may concluded that the Ayurvedic preparation of Louha Bhasma, particularly 150 puta, comprises unique chemical composition of iron-phytochemical combination that increasing its bio-availability, acquiring potential pharmacological activity leading to significant clinical efficacy in Kaphja Pandu Roga Visa-a-Vis iron deficiency anaemia. Besides, it also has potential immunomodulatory (Rasayan) effect as also safe, well tolerated to use for therapeutic purpose.
Bird’s Eye view of the proposed plan of work for the Ph.D thesis

PHASE I

Chemical composition of different putas

- Conventional analytical method
- EDX
- AAS
- IR
- HPLC
- HPTLC
- XRF
- Particle size

Pre – Clinical Studies

PHASE II

Results

- Anaemia induced by phlebotomy with nutritional deficiency
- Hematological & biochemical studies
- Metabolic studies
- Immunological studies
- Toxicological studies

Clinical Studies

PHASE III

Subjective parameters

- Patients selection
- Objective parameters

Results

- Hematological & biochemical studies
- Metabolic studies
- Immunological studies

Final Documentation