

Estimation of Serum Copper and Iron in Albino Rats After Administering *Makshika* (Chalcopyrite) *Bhasma*

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ABSTRACT

Bhasmas, the *Ayurvedic* metallic medicine, heal the patients and prevents the onset of diseases as well as geriatric problems mainly by supplying the macro and micronutrients to the living system for maintenance of normal body physiology. Copper and iron are very essential elements for mammalian metabolism and physiology. In this research content it is illustrated that, *Makshika* (Chalcopyrite, CuFeS_2) *bhasma*, prepared by conventional *Ayurvedic* method containing copper and iron in form of different compounds, enhances the percentage of copper and iron, in Charles's foster Albino rats after 30 days of drug administration in therapeutic dose corresponding to human regimen. Iron concentration is increased from 0.8708 $\mu\text{g/ml}$ in control group to 1.2541 $\mu\text{g/ml}$ in treated group but statistically found insignificant. Whereas copper concentration is increased from 0.2345 $\mu\text{g/ml}$ to 0.4349 $\mu\text{g/ml}$ which is statistically significant. The percentage of copper and iron were determined by atomic absorption spectrophotometry. The animal experiments were conducted with the permission from the competent authority, Institute Animal Ethical Committee and following prescribed protocols.

Key words: AAS, Albino rats, *Makshika bhasma*, *Shodhana*, *Marana*

INTRODUCTION

Natural *Makshika Bhasma* and various compound formulations containing *Makshika Bhasma* as an important ingredient are in clinical practice for curing the diseases like *pandu* (anemia), *kshaya* (tubercular diseases), *apasmara* (epileptic syndrome), *anidra* (insomnia) and *hridaya roga* (cardiac diseases) etc. ^[1, 2] Also it is an important ingredient of different *Ayurvedic* compound formulations like *chandraprabha vati*. ^[3] Although the therapeutic efficacy of *Makshika bhasma* is well established clinically, ^[4] but in the direction of reverse pharmacology, determination of bioavailability of this peculiar Cu-Fe-S complex (*Makshika bhasma*) may open a new path way for its pharmacology and to provide a scientific database in support of its logical clinical practice. The determination of bioavailability is also an important principle of Pharmacokinetics; hence the study will also facilitate the research for developing the pharmacology of the said *bhasma* which is yet to develop. Study is conducted in Charles's foster

Albino rat and bioavailability in terms of its metallic constituents is determined by doing AAS (Atomic Absorption Spectrophotometry) of serum of treated animals and data is established by comparing it with control group.

MATERIALS AND METHODS

The *Makshika Bhasma* was prepared, following standard pharmaceutical techniques of *shodhana* (purificatory measure) and *marana* (incineration) described in *Rasa Shastra*. ^[5]

The study was conducted in Charles's foster strain, male albino rats weighing between 100 to 150 gm. All the animals were kept in colony cages at an ambient temperature of $25 \pm 2^\circ \text{C}$, with 45–55 % relative humidity and 10:14 h light and dark conditions. The animals were kept on standard rodent feed and water was allowed *ad libitum*. The experiments were conducted following Principles of Laboratory Animal Care and Use. ^[6] All the experiments were conducted after getting permission from institutional animal ethics committee, vide letter No. Dean/2009-10/700; dated 16-11-2009.

Grouping of Animals

The experimental animals were divided into following two groups, with six animals in each group-

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Group I (Control Group):

Only diluted honey (3ml honey + 4.5 ml de-ionized water) was administered to the animals.

Group II (Treated Group):

Makshika Bhasma was administered to the animals using rubber catheter in therapeutic dose, i.e. 4.5mg/200gm, [7] orally for 30 days with diluted honey (3ml honey + 4.5 ml de-ionized water). The actual amount administered was adjusted between 0.5ml to 1ml containing calculated dose of the drug for individual animal.

Procedure

Preparation of animal and drug administration

Animals were acclimatized for 7 days. The drug was administered orally as per the calculated dose, following extrapolation factor. [7] The calculated dose of trial drug was mixed with diluted honey, (3 ml Honey + 4.5 ml de-ionized water). Diluted honey was made maximum 0.5 -1ml and administered to the animal containing exact dose of the drug according to the weight of particular animal i.e. 4.5 mg/200 gm of animal. Oral route of administration was opted as mostly practiced in *Ayurvedic* system of treatment. In control group only diluted honey was given by feeding tube.

Assesment of effect

The trial drug *Makshika bhasma* contains copper (Cu) and iron (Fe) as major ingredient. Hence the bio-availability of Cu and Fe was tried to appraise by following the AAS of serum of both treated group (after 30days of trial drug administration) and control group.

After the completion of the experiment animals were sacrificed, [6] by using standard anesthetic agent (with chloroform) and blood was collected by bleeding from retro-orbital plexus. Serum was collected by centrifuging the blood samples.

Preparation of Sample for AAS: [8] Serum (0.5 ml) was taken in a test tube and mixed with 1 ml Conc. HNO₃. Then test tube was heated in a steam bath for about 30 min and the volume of the sample was made 25 ml with distilled water. The samples were subjected for AAS study. Reports for copper and iron were analyzed statistically and documented.

Statistical analysis

The data of present study was analyzed statistically (students paired ‘t’ test).

OBSERVATIONS AND RESULTS

Copper and iron are the elements essentially needed for the living system to maintain the normal physiology are considered as the basic parameters for the study. *Makshika bhasma* containing copper and iron as major metallic ingredients effectively used in clinical practice for such deficiency diseases. In this experimental study it is considered as trail drug and administered in therapeutic dose, [7] to albino rats following extrapolation factors for animal dose calculation with respect to human dose. During the experimental study all the animals of both the groups were observed for their

usual activities like food intake, drinking and social interactions among them in colony cages and found normal. After 30 days of trail drug administration the treated group is compared with the control group for assessing the change in copper and iron concentration in their serum through atomic absorption spectroscopy. The data obtained is statistically analyzed and compared. It is observed that all the rats in treated groups have showed the increasing of copper and iron level in their serum [Fig.1] while comparing with control group. Iron concentration is increased from 0.8708 µg/ml in control group to 1.2541 µg/ml in treated group but statistically found insignificant. Whereas copper concentration is increased from 0.2345 µg/ml to 0.4349 µg/ml which is statistically significant. The results are summarized in [Table 1] and graphically presented in [Fig. 1].

Table 1: The serum concentration of copper and iron with group comparison

S. No.	Elements	Group I (n = 6) (Mean ±SD)	Group II (n = 6) (Mean ±SD)	Group Comparison Group I- Group II
1	Iron (µg/ml)	0.8708 ±0.0909	1.2541 ±0.3834	t = 1.927 P > 0.05
2	Copper (µg/ml)	0.2345 ±0.0795	0.4349 ±0.1052	t = 3.412 P<0.01

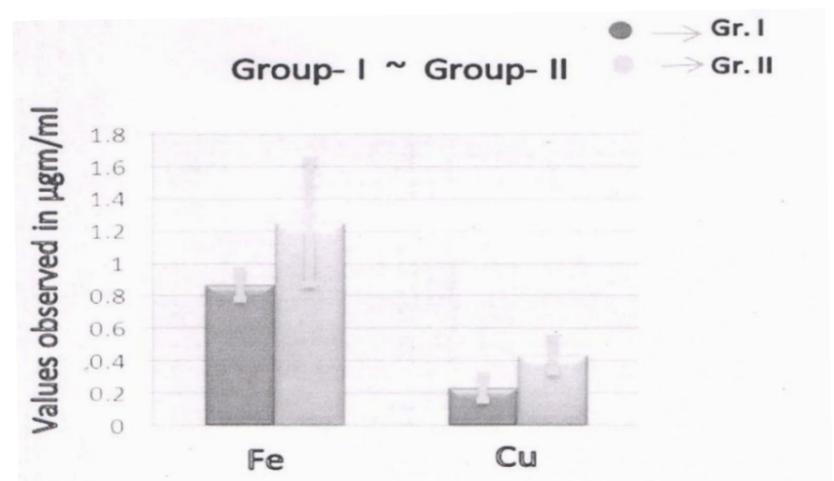


Fig. 1: The graph showing comparison of serum copper and iron levels between control and treated groups of experimental animals

DISCUSSION

Makshika Bhasma, an *Ayurvedic* medicine, contains different compounds of copper and iron as major components. [9] In the current study it is observed that following repeated administration of the said *bhasma* for 30 days in calculated therapeutic doses, the medicine went through metabolic process in animals similar to human body, with ensuing increase in serum levels of basic parameters of study i.e. iron and copper. The percentage of copper and iron, the major metallic constituents of the drug were found to

increase in serum of treated animals compared to the control group. Increase in serum iron in treated group, however, was found not to be significant (from 0.8708 µg/ml in control group to 1.2541 µg/ml in treated group), whereas there was a significant rise in level of serum copper in animals of treated group in comparison to the controls (from 0.2345 µg/ml to 0.4349 µg/ml).

As the trail drug is a mixture of multiple compounds, mostly of iron and copper, the serum concentrations of the same elements are tried to analyze. A sophisticated modern device, Atomic absorption spectroscopy usually used to detect trace metallic elements in the sample with optimum accuracy was employed to measure the concerned metallic elements in serum samples of all the animals. The said *bhasma* is massively used in *Ayurvedic* therapeutics as a constituent of a compound formulation or as a single drug in different types of diseases^[10] hence to provide a scientific data base of the *bhasma* at serum level, in terms of its metallic constituents, the study is designed, has reached at a conclusive end, that it significantly increases serum copper level. The insignificant increasing of the serum iron level may also prove significance with moderation of the dose.

CONCLUSIONS

Makshika bhasma is extensively used in *Ayurvedic* therapeutics,^[10] hence the current study will provide scientific data base of the *bhasma* at serum level, in terms of its metallic constituents. In the current study it is observed that following repeated administration of *Makshika bhasma* for 30 days in calculated therapeutic doses; ensures increase in serum levels of basic parameters of study i.e. iron and copper. Also, although there was a significant rise in level of serum copper in animals of treated group in comparison to the controls, the increase in serum iron was found not significant. The insignificant increasing of the serum iron level may also prove significance with moderation of the dose.

The current study justifies the rational clinical practice of the trail drug in copper deficiency diseases as well as in iron deficiency diseases with modification of the dose. Also the study enlightens the path for development of pharmacokinetics and pharmacodynamics of the said *bhasma* with more research in these directions.

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