EFFECT OF INGESTION OF COPPER *BHASM* ON RED CELL INDICES, IRON PARAMETERS AND ESSENTIAL ELEMENTS IN CHICKS

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ABSTRACT

In order to scientifically evaluate the effect of copper bhasm, it was orally administered in a dose of 2 mg/day for seven days to one month old male chicks. There was significant fall in total red cells, and rise in plasma iron, PCV and MCV indicating hemolysis, compensated by increased heme synthesis. A significant increase in plasma iron binding capacity points to beneficial effect of copper bhasm on liver as transferrin is synthesized in liver. Ferritin (storage iron) was unaltered in seven days treatment. The effect of copper bhasm on tissue distribution of essential elements was variable. Calcium, sodium and potassium were significantly increased in all the tissues. The last two may have been contributed by the bhasm itself. Magnesium behaviour was similar to calcium. Iron and zinc showed variable effects. Copper level in whole blood, plasma and erythrocytes increased significantly showing that copper bhasm was well absorbed and the effect observed can be ascribed to it. The bhasm was well tolerated and no growth retardation was seen.

KEY WORDS

Copper bhasm, Chicks, Tissues, Copper, Sodium, Potassium, Calcium, Magnesium, Zinc, Iron, Iron parameters, Hematological parameters.

INTRODUCTION

Copper is an essential trace element. It is a constituent of several enzymes, such as superoxide dismutase, lysyl oxidase, cytochrome oxidase, monoamine oxidase, ferroxidase I and tyrosinase (1) and has important role in absorption and transport of iron and hemoglobin synthesis (2).

Copper *bhasm* (ash), as such, and as an ingredient of several preparations has been widely used in Ayurveda (the indigenous system of medicine) for a very long time, viz, *Gulma kalanala rasa*, *Surya vartta rasa*, *Tamraparpati* and *Jalodarari rasa* (3). In addition, drinking of water kept in copper utensils is considered beneficial for health. However, the scientific study of copper *bhasm* is lacking except one report of Tripathi and Singh (4). These authors found that copper *bhasm* induce

hemolysis due to generation of superoxide radicals and lipid peroxidation in liver of rats. The aim of the present study was precisely to scientifically evaluate the effects of copper *bhasm*.

Copper is a member of 1st B of periodic table of elements and has been placed with group 1st A of alkali metals because the electronic structure of the elements of the periodic table of groups 1st A and 1st B both have one electron in the outer most orbit. Hence it exhibits two valencies, viz., one & two (5). It was, therefore, decided to see the effects of copper *bhasm* on tissue distribution of essential elements having valency +1 (sodium, potassium) and +2 (calcium, magnesium, iron and zinc).

The interaction of copper with iron has been under intense investigations (6). The effect of copper on iron metabolism, red cell morphology and hematological parameters has been investigated (7). Chronic ingestion of excess copper is known to cause microcytic anemia and hemolysis (8). Again, such an effect has never been investigated using copper *bhasm*. Hence it was planned to incorporate this aspect in the present study.

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MATERIALS AND METHODS

For the present study, the chick was used as an experimental animal because of its easy availability in the local market, ease of handling and larger quantity of blood available for analysis.

One month old, young male chicks of the same breed (*Gallus Serrugineus*) weight about 1 kg, were purchased from the local poultry. They were divided into two groups - Control and Experimental, consisting of 12 and 18 chicks respectively. The chicks were given normal "Finisher mash" diet as generally recommended by the breeders (9) and water *ad libitum*

The chicks of the experimental group received one pellet daily in the morning containing 2 mg of copper *bhasm*. This regimen was followed for seven days so the cumulative dose of copper *bhasm* of an individual chick was 14 mg.

The copper *bhasm* was personally prepared by Vaidya shri B. L. Gaur, director, National Institute of Ayurved, Jaipur. It is a jet black powder resembling charcoal. The pellets were prepared by homogeneously mixing wheat flour and the copper *bhasm* in the ratio of 1gm wheat flour for 2mg copper *bhasm* making dough and then pellets of about 1gm in weight. The pellets were dried at room temperature and then used for feeding.

After a week of oral ingestion, the chicks were fasted overnight and then sacrificed in the morning by cutting the jugular vein by a knife. Similarly, the controlled group chicks were also sacrificed. The blood was collected in a heparinized vial. It was used for making peripheral blood film (PBF) and estimating Hb, TRBC and PCV. The remaining blood was centrifuged to separate plasma from erythrocytes. The body of the chick was dissected, and liver, kidney and spleen were taken out and preserved in formalin.

The whole blood was used to estimate Hb, TRBC and PCV as described by Dacie and Lewis (10). MCV, MCH and MCHC were calculated as usual. Plasma iron and total iron binding capacity were measured in an auto analyzer by using kits employing ferene as a chromogen. Percent saturation of iron in the plasma was calculated from these values. Ferritin in plasma was determined by an ELISA employing kit provided by syntron bioresearch, USA.

The essential element (Na, K, Ca, Mg, Fe, Zn and Cu) were estimated in liver, spleen, kidney and erythrocytes. For this purpose, the organs were washed in normal saline immediately after removal from the animal and blotted dry. Exactly weighed

piece of these tissues were placed in silica crucibles, dry ashed in a muffle furnace at 700°C for 6 hours to completely decompose the organic matter. Similarly 1 ml of whole blood was also ashed. After dry ashing of tissue and blood the crucible was cooled and the ash dissolved in 1 ml of nitric acid, the extract was transferred to a volumetric flask and the volume made up to 10 ml using double distilled water. Sodium and potassium were estimated by flame photometric method (11). The other elements (Cu, Zn, Fe, Ca & Mg) were determined by atomic absorption spectrophotometer (12). Copper was estimated in plasma directly by AAS after 1:6 dilution.

Analysis of bhasm: Copper *bhasm* was checked for its safety by performing the tests based on density (pure *bhasm* floats on water) and development of color with curd (13). The mineral content of copper *bhasm* was determined by us as for tissue after dry ashing, dissolution in HNO₃ and dilution. This extract was assayed for all the seven elements. The estimation of carbon, hydrogen and nitrogen was done by Regional Sophisticated Instruments Center (RSIC) of Central Drug Research Institute (CDRI, Lucknow). Oxygen and sulphur could not be estimated.

Statistical Analysis: The data of chicks of control and experimental groups were calculated statistically by applying Student's 't' test (14).

RESULTS AND DISCUSSION

Although copper *bhasm* is widely used in Ayurvedic system of medicine, the scientific study of its effects are lacking. The present study is aimed to fill this void.

Table 1: Percent Composition of Copper Bhasma

Element	Percentage
Copper	41.18
Iron	1.37
Zinc	2.50
Calcium	0.37
Magnesium	0.48
Sodium	20.20
Potassium	13.50
Carbon	1.40
Hydrogen	0.91
Nitrogen	0.00
Others (O,S etc)	18.09
Total	100

Copper *Bhasm*: In Ayurved, the chemical composition of drugs are not described so we first determined the elemental composition of copper *bhasm*. This is shown in Table 1. It can be seen that copper accounts for more than 40%. While sodium and potassium content was also substantial, the rest of the minerals were present in negligible amounts.

The dose of *bhasm* for the chicks was arrived from recommended dose in humans which is 1/8 to 1 ratti (15). One ratti is equivalent to 120 mg and taking average weight of Indians as 60 Kg, the dose comes out to be 0.25 to 2 mg/kg body weight. For the chicks, we selected the higher side of the dose. The oral ingestion of copper *bhasm* for 7 days in this dose (2 mg/kg/day) did not cause any toxicity. The chicks appeared healthy, grew normally, no retardation in growth was seen and they accepted the drug very well.

In order to ascertain whether copper contained in the *bhasm* was absorbed, the plasma, erythrocyte and whole blood level of copper was measured. This is shown in Table 2. It can be seen that the average rise in copper in whole blood was 1.8 times while in plasma 2.7 times and in the erythrocytes it was 1.4 times. This conclusively establishes that copper of *bhasm* was absorbed and hence the effects produced can be ascribed to it.

Table 2: Copper Level in Whole Blood, Plasma and Erythrocyte ($\mu\text{g/ml}$)

	Control Group (12)	Experimental Group(18)
Whole Blood	1.0 ± 0.29	$1.8 \pm 0.53^*$
Plasma	1.5 ± 0.48	$4.04 \pm 1.19^*$
Erythrocyte	0.50 ± 0.24	$0.7 \pm 0.17^{**}$

All values are mean $\pm$ SD; *Statistically highly significant $P < 0.001$, ** $P < 0.025$

Effect of copper *bhasm* on hematological and iron parameters: Copper is known to have important role in iron metabolism and copper deficiency develops hypochromic microcytic anemia, similar to iron deficiency anemia (16). The effect of ingestion of copper *bhasm* on red cell parameters

was studied by us and is shown in Table 3. Although seven days is very short duration for observing effect on red cells, a statistically significant fall was noted in red cell numbers, while Hb and PCV remained unaltered. MCV and MCH also showed significant alteration as these parameters are calculated using TRBC. A significant fall in TRBC with significant rise in MCV and MCH shows hemolysis with compensatory increased synthesis of heme. This finding is consistent with the report that copper induces hemolysis of RBC's (8) and increases heme synthesis in avian nucleated erythrocytes (17).

No previous worker has described hematological parameters after feeding copper except Gipp et al (7). These authors reported significant decrease in TRBC in pigs by feeding copper. They also found decreased MCV, MCH and MCHC by giving high copper (250 ppm) diet to pigs.

Plasma iron is the indicator of transport iron or the availability of iron for Hb synthesis, while ferritin is the index of the storage iron. In this study a significant rise was observed in plasma iron as well as total iron binding capacity of plasma, but no change in percent saturation or ferritin level (Table 4). Understandably one week is too short a period to alter storage iron. However, increased plasma iron (together with decreased TRBC) is consistent with hemolytic effect of copper. In this study, total iron binding capacity of plasma also showed significant rise (Table 4). A significant rise in TIBC in copper treated chicks corroborates alleged beneficial effect of copper *bhasm* in the liver diseases because transferrin is synthesized in liver along with albumin and some other proteins (3, 4).

Effect of copper *bhasm* on tissue distribution of essential elements: Ayurvedic *bhasms* are preparations of minerals and their continued long term ingestion may bring about changes in tissue distribution of other elements which may have its own biological effects. We have examined the effect of copper *bhasm* ingestion on the content of essential elements – sodium, potassium, calcium, magnesium, iron and zinc, and of course, copper in the blood, liver, kidney and spleen of chicks. The tissue content of elements is presented in Table 5 to Table 8. In some elements the effect varied from one tissue

Table 3: Hematological Values of Chicks

	Hb (g%)	TRBC (10^6 /cu.mm)	PCV (%)	MCV (fl)	MCH (pg)	MCHC (g%)
Control group (12)	8.0 ± 0.87	2.59 ± 0.58	30.5 ± 3.0	117.7 ± 35.0	31.0 ± 7.5	26.3 ± 3.6
Experimental group (18)	8.3 ± 0.78	2.04 ± 0.39	31.4 ± 2.5	153.9 ± 34.7	40.9 ± 8.6	26.6 ± 2.8
	NS	$P < 0.01$	NS	$P < 0.01$	$P < 0.01$	NS

All values are mean $\pm$ SD; NS = Non significant

Table 4: Plasma Iron Parameters of Chicks

	PI (µg/dl)	TIBC (µg/dl)	PS (%)	Ferritin (ng/ml)
Control group(n=12)	83.9 ± 13.8	159.2 ± 40.4	55.4 ± 14.9	23.8 ± 2.8
Experimental group (n=18)	104.4 ± 25.4	208.3 ± 33.0	51.9 ± 16.7	24.0 ± 2.8
	P<0.05	P<0.01	NS	NS

All values are mean ± SD; NS = Non significant

Table 5: Essential Elements in Blood of Chicks (µg/ml)

	Cu	Fe	Zn	Ca	Mg	Na	K
C	1.0 ± 0.31	28.0 ± 5.11	7.6 ± 1.82	8.2 ± 4.12	50.4 ± 19.93	1116 ± 378	230 ± 59
E	1.8 ± 0.66	31.1 ± 6.31	6.7 ± 2.87	16.3 ± 5.77	58.0 ± 10.70	1466 ± 278	368 ± 63
	P<0.001	P<0.05	NS	P<0.001	NS	P<0.05	P<0.05

C = Control group (12); E = Experimental group (18); NS = Non significant

to another, while in others some consistent pattern was discernible. The following conclusions were derived from these observations:

1. As expected copper level was increased significantly in all the tissues; 2. The two monovalent elements – sodium and potassium, increased significantly in all the tissues examined. These may have come from the *bhasm* itself because the substantial weight of *bhasm* contained these two elements (Table 1); 3. A significant increase was noted in the calcium content of all the tissues. It could not have been contributed by the *bhasm* as its amount in it was extremely small; 4. Magnesium level increased in all the tissues but the rise in blood was not significant. Magnesium and calcium are chemically and metabolically similar hence the similarity in

the effect of copper on these two elements; 5. Iron was increased in all the tissues, but the increase in spleen and kidney was not significant. The increase in tissue iron is understandable in view of established role of copper in absorption, transport and mobilization of iron; 6. No regular pattern was seen in zinc status of tissue of chicks receiving copper *bhasm*.

No previous worker has seen the effect of copper on tissue level of elements except iron. A high intake of copper was reported to decrease iron in pig liver (17, 18) and plasma (18). Our findings are just the reverse. The reason may be differences in the animal species used or the dose and form of copper administered.

Table 6: Essential Elements in Liver of Chicks (µg/g)

	Cu	Fe	Zn	Ca	Mg	Na	K
C	2.8 ± 0.46	20.0 ± 6.0	18.4 ± 2.59	3.0 ± 0.96	106.1 ± 18.18	680 ± 268	1433 ± 274
E	3.4 ± 0.51	27.3 ± 5.9	20.6 ± 4.49	4.0 ± 1.66	123.6 ± 9.53	1438 ± 322	1794 ± 343
	P<0.001	P<0.01	NS	P<0.05	P<0.01	P<0.001	P<0.01

C = Control group (12); E = Experimental group (18); NS = Non significant

Table 7: Essential Elements in Spleen of Chicks (µg/g)

	Cu	Fe	Zn	Ca	Mg	Na	K
C	1.2 ± 0.29	21.1 ± 5.67	17.8 ± 2.22	1.9 ± 0.59	99.7 ± 14.09	1300 ± 202	1866 ± 451
E	1.5 ± 0.40	30.2 ± 9.31	20.0 ± 7.13	4.6 ± 1.82	128.6 ± 11.74	1891 ± 505	2416 ± 386
	P<0.05	NS	NS	P<0.001	P<0.001	P<0.001	P<0.01

C = Control group (12); E = Experimental group (18); NS = Non significant

Table 8: Essential Elements in Kidney of Chicks (µg/g)

	Cu	Fe	Zn	Ca	Mg	Na	K
C	2.5 ± 0.42	15.8 ± 5.81	18.3 ± 2.42	4.9 ± 1.78	106 ± 14.4	1416 ± 373	1091 ± 215
E	2.8 ± 0.45	18.5 ± 6.76	17.9 ± 4.37	6.3 ± 1.59	119 ± 6.4	2011 ± 437	1294 ± 329
	P<0.05	NS	NS	P<0.001	P<0.01	P<0.001	P<0.05

C = Control group (12); E = Experimental group(18); NS = Non significant

REFERENCES

- Sharma DC, Riyat M. Biochemistry for Dental Students, B.I Publications Pvt. Ltd, New Delhi 2007; pp 311-13.
- Sharma DC. Text book of Biochemistry. Paras Medical Publisher, Hyderabad 1999; pp 319.
- Nadkarni AK. Indian Materia Medica. Vol.2, 3rd ed, Popular Prakashan, Bombay 1976; pp 47-51.
- Tripathi YB, Singh VP. Role of *Tamra bhasm*, an ayurvedic preparation in the management of lipid peroxidation in liver of albino rats. Ind J Exp Biol 1996; 34: 66-70.
- Block JH, Roche EB, Soine TO, Wilson CO. Inorganic medicinal and pharmaceutical chemistry. Lea and Febiger, Philadelphia, 1986; pp 48-77 and 325-30.
- Seelig MS. Review: The relationships of copper and molybdenum to iron metabolism. Am J Clin Nutr 1972; 25: 1022-37.
- Gipp WF, Pond WG, Tasker J, Compen D, Vankrook L, Visek WJ. Influence of level of dietary copper on weight gain, hematology and liver copper and iron storage of young pig. J Nutr 1973; 103: 713-9.
- FernandesA, Mira ML, Azevedo MS, Manso C. Mechanisms of hemolysis induced by copper. Free Radic Res Commun 1988; 4: 291-8.
- North MO. Commercial chicken production manual. 3rd ed, Van Nostrand Reinhold, New York 1984; pp 485-95.
- Dacie JV, Lewis SM. Practical Hematology. 8th ed, Churchill Livingstone, Edinburg 1994; pp 44-82, 412-4.
- Sharma DC, Riyat M. Practical Medical Biochemistry, B.I Publications Pvt. Ltd, New Delhi 2007b; pp79-83.
- Sharma DC. Analysis of trace elements. Ind J Clin Biochem 1988; 3 (Suppl): 128-34.
- Jha CB. A textbook of rasa shastra. 2nd ed. Chaukhambha Surabharati Publishers, Varanasi 1998; pp 325-34.
- Mahajan BK. Methods in biostatistics for medical students and research workers Jaypee Brothers, New Delhi 1991; pp146,151.
- Danks DM. Copper deficiency in humans. Ann Rev Nutr 1988; 8: 235-57.
- Orten JM, Orten AU, Sardesai VM, Chang LC. The role of copper in the biosynthesis of heme. Monograph 1990; pp 643-65.
- Bradley BD, Graber G, Condon RJ, Frobish LT. Effect of graded levels of dietary copper in copper and iron concentration in swine tissue. J Anim Sci 1983; 56: 625- 30.
- Luo XG, Dove CR. Effect of dietary copper and fat on nutrient utilization, digestive enzyme activities and tissue mineral level in weanling pigs. J Anim Sci 1996; 74: 1888-96.