Letter to the Editor

Copper Ameliorates Fluoride Toxicity in Fluoride and Molybdenum Fed Rabbits

Arjun L KHANDARE[1,#], Uday KUMAR[2], Priyanka SHANKAR[1], and Shanker RAO[1]

Hydrofluorosis is a major public health problem in India, which is aggravated by presence or absence of some minerals in water or food. Fluoride by itself or in combination with other elements like molybdenum (Mo) in diet or water changes excretion/retention of bone minerals. Sorghum and pearl millet grown and consumed in fluorosis endemic areas contained significantly higher amounts of Mo than that grown in non-fluorotic areas in India[1]. Epidemiological studies show that the prevalence of genu valgum in areas with fluorosis is significantly higher in subjects, whose staple is sorghum (4%) as compared to those whose staple is rice (1%)[2]. High molybdenum ingestion leads to secondary copper deficiency due to increased loss of copper through urine[3]. Copper plays an important role in collagen synthesis. In addition, a strong negative correlation exists between the concentration of copper in drinking water and the prevalence of genu valgum in areas endemic for fluorosis[4]. Endemic genu valgum has been identified as a manifestation of chronic fluoride toxicity in some parts of India[5]. This newer manifestation of skeletal fluorosis, seen mostly in adolescents with a marked predilection for males, is characterized by simultaneous occurrence of osteosclerosis of the spine and osteoporosis of the long bones[3]. The present study was undertaken to know the protective effect of copper supplementation on fluoride toxicity aggravated by Mo ingestion in terms of body composition, serum cholesterol and triglyceride, organ weight ratio as well as retention or mobilization of bone related elements [calcium (Ca), Magnesium (Mg), zine (Zn), and copper (Cu)] in hard tissue (ribs) in rabbits fed Fluoride (F), Fluoride+molybdenum (F+Mo) and Fluoride+molybdenum+Copper (F+Mo+Cu) for 6 months (*n*=6 each group).

Total body weight was significantly lower in F+Mo group as compared to control (Figure 1). Further, F+Mo

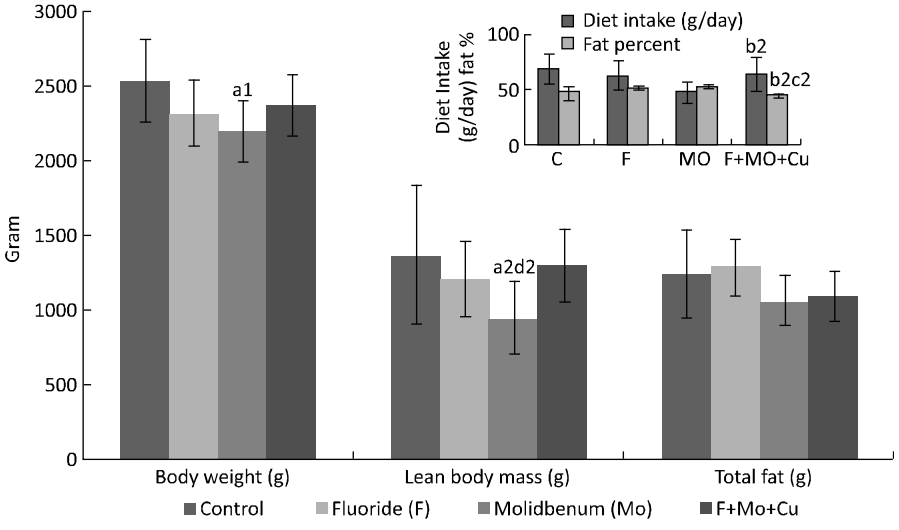

Figure 1. Body composition of control and different experimental groups after six months by TOBEC. Values are given as Mean±SD. a=Comparison with control group; b=Comparison with fluoride group; c=Comparison with F+Mo group; d=Comparison with F+Mo+Cu group; 1=*P*<0.01; 2=*P*<0.05.

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1. Food and Drug Toxicology Centre, National Institute of Nutrition, Indian Council of Medical Research, PO-Jama-I-Osmania, Hyderabad-500007, Andhra Pradesh, India; 2. Department of Pathology, National Institute of Nutrition, Hyderabad, India.

group showed significantly lower lean body mass as compared to control and F+Mo+Cu group (Figure 1). Increase in lean body mass in F+Mo+Cu group as compared to F+Mo group may be due to increase in muscle mass and body water variation due to supplementation of Cu to the former group. Total fat was comparable among groups (Figure 1). Serum triglycerides were signifi-cantly higher in F+Mo group as compared to control whereas the same was significantly lower in F+Mo+Cu group as compared to F+Mo group. Impaired lipid metabolism may be the reason for increase in serum triglyceride level in Mo fed group (group 3), as reported earlier also[6-7]. There was no significant difference in serum cholesterol levels among the groups (Table 1).

Urinary fluoride excretion, serum fluoride and rib fluoride deposition were significantly higher in experimental groups as compared to control (Tables 1 and 2). Further, serum fluoride as well as fluoride accumulation in ribs was significantly lower in F+Mo+Cu group as compared to F and F+Mo groups. This may be due to antagonistic effect of Cu on Mo as reported earlier[8-9]. Calcium deposition was significantly (P<0.05) higher in ribs of F+Mo+Cu group as compared to control group. On the other hands, phosphorus deposition was significantly higher in F+Mo+Cu than control (P<0.01) and F (P<0.05) groups. There was no significant difference in deposition of Mg, Zn, and Cu in ribs of rabbits among different groups (Table 2). Increased deposition of Mn in ribs of all experimental groups suggested production of cytotoxic free radicals and oxidative stress as reported earlier in goats[6].

Organ weight ratio (data not shown) of brain, adrenal gland and gallbladder were significantly higher in F+Mo group as compared to all other groups. The increase in gallbladder weight may be due to presence of gall stones found in two of the animals of F+Mo group. The reason for increase in weights of brain and adrenal glands was decrease in body weight without a decrease in brain weight. There was no significant difference in kidney and heart weight ratio among the groups.

Table 1. Urinary and Serum Parameters in Different Group of Rabbits after Feeding Fluoride, Fluoride +Molybdenum, Fluoride+Molybdenum+Copper for Six Months

Group	*n*	Urinary Fluoride (mg/24h)	Serum Fluoride (μg/mL)	Serum Cholesterol (mg/dL)	Serum Triglycerides (mg/dL)
Control	6	0.10±0.03	0.19±0.09	52.30±34.16	109.00±86.68
F	6	1.84±0.32 ^{a1}	0.86±0.20^{a1}	75.90± 18.25	179.30±73.70
F+MO	6	1.38±0.19 ^{a1}	1.04±0.35^{a1}	66.30±39.52	280.10±131.91^{a1}
F+Mo+Cu	6	1.63±0.78 ^{a1}	1.40±0.31^{a1b1c2}	54.30±39.39	150 .00±60.10^{c2}

***Note*.** Values are given as Mean±SD. a=Comparison with Control group, b=Comparison with Fluoride group, c=Comparison with F+Mo group. 1=P<0.01, 2=P<0.05.

Table 2. Percent Bone Ash and Mineral Content in Ribs in Different Group of Rabbits

Group	% Ash content	Ca (mg/g)	Mg (mg/g)	P (mg/g)	Zn (mg/g)
Control	0.41±0.016	359.9±91.02	6.2±2.09	376.7±11.73	255.9±65.57
F	0.39±0.009	398.0±11.31	7.6±1.36	391.1±50.89	278.6±36.67
F+Mo	0.38±0.006	385.8±10.12	7.4±1.21	395.6±16.15	294.1±21.11
F+Mo+Cu	0.61±0.010	424.7±32.70^{a2}	7.4±0.99	424.4±5.44^{a1,b2}	292.2±67.62
Group	**Mn (μg/g)**	**Cu (μg/g)**	**F (μg/g)**	**Ca /P (mg/g)**	
Control	10.2±3.03	32.6±17.55	44.6±32.02	0.955±0.07	
F	14.6±3.16^{a1}	25.7±7.02	1126.7±117.12^{a1}	1.00±0.2	
F+Mo	14.7±1.24^{a1}	74.7±62.94	946.5±401.74^{a1}	0.97±0.6	
F+Mo+Cu	16.2±3.18^{a1}	23.6±4.72	646.2±339.92^{a1,b1}	1.00±0.30	

***Note*.** Values are given as (Mean±SD). a=Comparison with control group, b=Comparison with fluoride group, c=Comparison with F+Mo group, 1=P<0.01, 2=P<0.05.

Histopathological changes (data not shown) in various organs including spinal cord calcification were consistently present in varying degrees in F group as compared to other groups. There were no such reports in animal study. However, there are degeneration and ossification of ligamenta flava cases from fluorotic area[10]. From the results, it was observed that there was significant difference in total nerve fibre counts (sciatic nerve) in both groups where Mo was involved as compared to control and only F groups (which were comparable). With respect to large fibre and small fibre distribution, the large fibre number was significantly more in all experimental groups as compared to control while small fibre number was significantly higher in F+Mo group only and decreased in Cu supplemented group (F+Mo+Cu). Within the experimental groups, it was seen that the large fibre content was more than small fibres and the greatest difference was in Cu supplement group (almost 50%). This would translate as increase in the response time of the animal to various sensory stimuli due to predominant large fibre content.

The present findings suggest that fluorosis per se does not affect total body fat or water. Additional burden of feeding molybdenum reduced food intake, body weight and lean body mass suggesting loss of muscle mass than body fat, which reduces working capacity. This anomaly was corrected by feeding Cu along with Mo. The limitation of the present study includes lack of estimation of HDL-cholesterol, LDL-Cholesterol and other markers of lipid metabolism like leptin, which might have provided a better understanding of the consequences of fluoride on lipid metabolism.

In conclusion, unregulated fat metabolism, organ toxicity and accumulation of various bone related elements in ribs were normalized on Cu supplementation. This study further confirms the interaction between Cu and Mo as well the beneficial role of copper in fluoride toxicity. However, the occurrence of histopathological changes observed in various organs of different groups need further investigation.

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Conflict of Interest Statement

The authors declare that there is no conflict of interest.

#Correspondence should be addressed to: Arjun L Khandare, E-mail: Alkhandare@yahoo.com

Biographical note of the first author: Dr. Arjun L. Khandare, born in 1959, M.Sc. Ph.D. in Biochemistry, majoring in the group leader of food and drug toxicology research center since 2005, working on fluoride and fluorosis since 2000.

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