MAGNESIUM HYDROXIDE FOR PROTECTION AGAINST FLUORIDE TOXICITY IN RABBITS

Arjun Khandare,[a] Uday Kumar,[b] Shanker Rao[c]
Hyderabad, India.

SUMMARY: The present study was undertaken to assess the potential of magnesium hydroxide, ($Mg(OH)_2$), to alleviate fluoride (F) toxicity in rabbits. Eighteen 2.5-month-old New Zealand white rabbits of both sexes were divided equally into three groups: 1 (control), 2 (F group), and 3 (F + Mg group). F was administered orally (10 mg F ion/kg bw/day) to the group 2 animals while group 3 received 20 mg $Mg(OH)_2$/kg bw/day plus the foregoing amount of F. After 7 months, 24-hr urine, blood, and fecal samples were collected for three consecutive days. As expected, serum F and total alkaline phosphatase (ALP) in group 2 became significantly higher ($p<0.01$). Dietary and water intake and weight gain in this group were lower compared to the other two groups, but fecal and urinary F excretion was significantly higher ($p<0.01$). Moreover, except for Zn in group 3, urinary and serum Ca, Mg, Zn, Cu, ALP, osteocalcin, and 25-hydroxy vitamin D in groups 2 and 3 were not significantly different from the control group. We conclude that administration of $Mg(OH)_2$ reduces F toxicity by increasing fecal and urinary F excretion without inducing appreciable toxic effects.

Keywords**:** Fluoride toxicity alleviation; Magnesium hydroxide; Rabbit serum; Urinary excretion.

INTRODUCTION

Excessive fluoride (F) concentration in a community water supply can cause dental and skeletal fluorosis. The latter has even been associated with endemic genu valgum in parts of India where it apparently affects mainly adolescents and children.[1] Medical intervention without significantly reducing F intake has not been very effective in reversing early stages of skeletal fluorosis; hence alternative strategies are needed to treat this malady. Calcium supplementation has been found to interfere with F absorption,[2,3] Boron[4] and aluminium sulphate[5] have been used for this purpose in earlier studies, but with limited success. Some years ago, Marier,[6] Guminska,[7] and later Machoy and co-workers[8,9] drew attention to the significance of magnesium (Mg) in biological interactions with F. The toxic effect of F ion plays a key role in acute Mg deficiency. F and Mg affect the activity of acid phosphatases responsible for catalyzing reactions leading to the formation of apatite crystals. In general, F interactions with Mg most frequently decrease enzymatic activity.[10] Mg influences the process of amelogenesis,[11] and Marier's further studies showed that F and Mg intake also has some systemic effects.[12] A lower content of Mg and F in the external layer of enamel may lead to enamel erosion.[13] The bioavailability of Mg and F depends on their mutual ratio in the diet,[14] and interactions most frequently decrease enzymatic activity.[15] The greatest practical significance of Mg-F interactions, however, seems to be in processes of bone and tooth mineralization, and in the formation of uroliths.[7,15] There are also reports on the protective effect of intravenous/oral administration of magnesium salts ($MgCl_2$, MgO, $Mg(OH)_2$ and serpentine) against F toxicity.[16] On the other

[a]For correspondence: Arjun Khandare, Assistant Director, Food and Drug Toxicology Research Center, National Institute of Nutrition, Hyderabad, India. E-mail: alkhandare@yahoo.com; [b]Department of Pathology, National Institute of Nutrition, Hyderabad, India; [c]Food and Drug Toxicity Research Center, National Institute of Nutrition, Hyderabad, India.

hand, an excessively high daily dose of 200 mg of $Mg(OH)_2$ administered as enteric-coated tablets for 70 days to rabbits given 22 mg F/day caused weakened bones, diarrhoea, and malnutrition.[17] However, none of these Mg compounds are currently in use as public health measures owing to insufficient data on their anti-F efficacy and toxicity.

The present study was therefore undertaken to assess possible alleviation of F intoxication by $Mg(OH)_2$ administered orally to rabbits under controlled laboratory conditions, along with F, and to search for any potential toxic effects of this extra Mg on various haematological and biochemical parameters.

MATERIALS AND METHODS

Eighteen New Zealand white rabbits of both sexes (2.5 months old with an average body weight of 1.4 kg) were obtained from the National Centre for Laboratory Animal Sciences, Hyderabad India. After ethical clearance for the design and protocols of this research was obtained from Institute's Animal Ethics Committee, the animals were randomly distributed into 3 groups of 6 animals: group 1 (Control) received the standard laboratory rabbit diet and water; group 2 (F group) received 10 mg F/kg bw/day [from 10 mg F ion/mL, prepared by dissolving 22.21 g of NaF in 1.0 L of distilled water and administered orally according to their weight]; group 3 (F + Mg group) received 20 mg $Mg(OH)_2$/kg bw/day [from milk of magnesia containing 8% w/w $Mg(OH)_2$, 80 mg/mL] given orally along with the same amount of F as in group 2. All the animals received the same diet *ad libitum* for 7 mo. Water and diet intake along with body weight were recorded every fortnight. After 7 mo, 24-hr urine and feces samples were collected for three consecutive days at the end of the experiment, during which time fasting blood samples were also collected for biochemical and haematological analysis.

Biochemical investigations: Urinary and fecal F samples collected over three 24-hr periods were analysed with a F ion selective electrode (EA 940, Boston MA). Serum Ca, and serum and urinary Mg, Zn, and Cu were analysed using an Atomic Absorption Spectrophotometer. Total and bone specific alkaline phosphatase were analysed on same day by the heat inactivation methods of Walter and Moss, respectively.[18,19] Serum 25-hydroxy-vitamin D_3 and osteocalcin were analyzed by the chemiluminescence kit method.

Haematology: Total RBC, WBC, and differential counts, haemoglobin, packed cell volume, and peripheral smear examination were undertaken in all animals.

Statistical Analysis: Mean and SD values were calculated for all the parameters. Mean values were compared by analysis of variance, "F test" with Post Hoc Test. Level of significance was considered as 0.05, and SPSS package version 15.0 was used for statistical analysis.

RESULTS

Food intake and body weight: Diet and water intake are shown in Table 1.

Table 1. Effect of $Mg(OH)_2$ on diet and water intake of the control and F group rabbits. Values are mean±SD.

Group	N	After 3 mo		After 7 mo	
		Diet (g)	Water (mL)	Diet (g)	Water (mL)
Control	6	94.4±7.70	135.9±32.16	104.2±21.96	130.7± 23.51
Fluoride	6	88.2±14.07	91.8±15.89	68.7±21.89$^{a\dagger b*}$	88.5 ±12.83^{a†}
F + Mg	6	88.6 ± 8.14	107.5±16	96.9±10.89	102.2±19.14^{a*}

Values bearing superscripts ^{a} = significantly different from Control, ^{b} = significantly different from F + Mg; level of significance *p=<0.05, †p<0.01.

Weights of the F group 2 animals decreased significantly ($p<0.05$) compared to the control group 1 and the F+Mg group 3 rabbits (Figure 1).

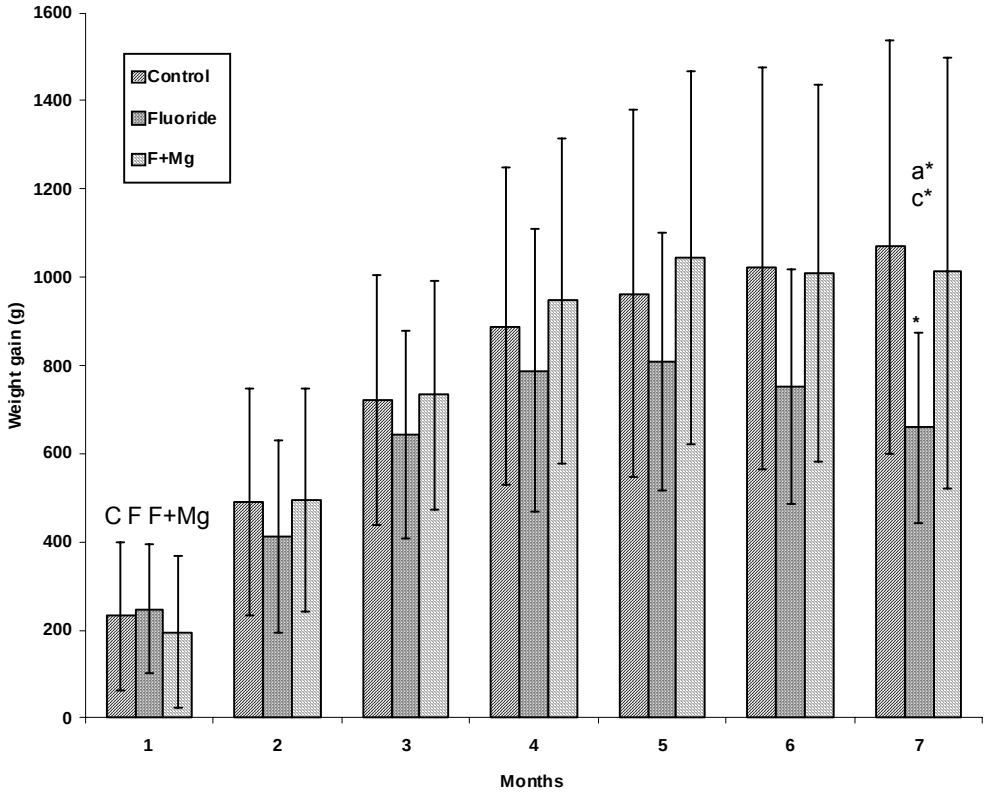

Figure 1. Body weight gains in different groups. Values bearing superscripts: a = significantly different from Control, c = significantly different from F+Mg. *Significant at p<0.5. The order of the columns for each month, from left to right, is control group, fluoride group, and fluoride+magnesium group.

Serum biochemical parameters: Serum total alkaline phosphatase, bone specific alkaline phosphatase, F, and calcium were significantly different among the different groups (Table 2). There were no significant differences in the other parameters of osteocalcin, 25-hydroxy vitamin D_3, Mg, Zn, and Cu.

Table 2. Effect of Mg(OH)2 on serum parameters in the control and F group rabbits. Values are mean±SD.

Group	N	Serum								
		TALP (IU/L)	BALP (IU/L)	Ca (mg/dL)	Mg (mg/dL)	Zn (mg/dL)	Cu (mg/dL)	Osteocalcin (ng/mL)	Vit D (ng/mL)	Fluoride (µg/mL)
Control	6	37.8 ±13.66	28.0 ±25.95	12.4 ±1.32	3.0 ±0.34	0.24 ±0.04	0.17 ± 0.057	19.0 ±10.44	14.2 ±3.20	0.26 ± 0.073
Fluoride	6	96.0± 24.87$^{a\dagger c*}$	50.7 ±21.86^{a*}	9.8 ±1.53$^{a\dagger c*}$	3.3 ±3.13	0.16 ±0.04	0.12 ± 0.012	23.9 ±9.35	13.2 ±2.49	1.54 ± 0.229$^{a\dagger c\dagger}$
F+ Mg	5	51.5 ±24.21	41.6 ±26.56	12.6 ±0.80	3.1 ±0.45	0.19 ±0.02	0.13 ±0.03	19.8 ±8.26	12.2 ±2.13	0.89 ± 0.083$^{a\dagger b*}$

Values bearing superscripts [a] = significantly different from Control, [b] = significantly different from Fluoride, [c] = significantly different from F + Mg; level of significance *p<0.05, †p<0.01.

Haematology: The various parameters studied did not show any significant differences between the groups.

Urinary and faecal parameters: Twenty-four hr urinary and faecal F showed significantly higher levels in groups 2 and 3 compared to group 1; however, it was also significantly higher in group 3 as compared to group 2 (Figure 2).

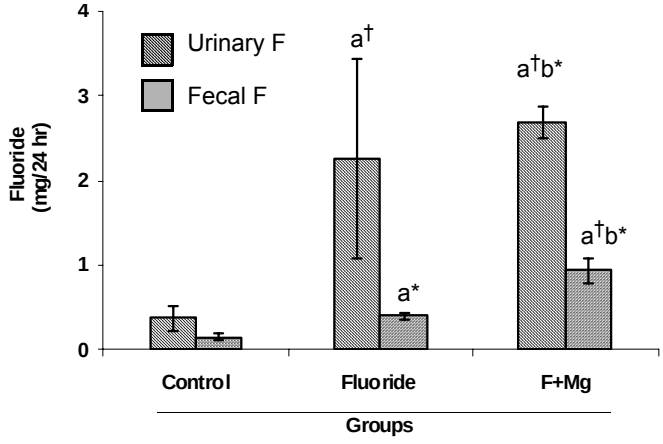

Figure 2. Urinary and fecal 24-hr F after 7 months of treatment of the rabbits with F and F+ Mg. Values bearing superscripts: a = significantly different from Control, b = significantly different from Fluoride. Level of significance: * p<0.05, †p<0.01.

There were no significant differences in urinary volume, pH, creatinine, calcium, Mg, Zn, and Cu levels among the groups (Table 3).

Table 3. Effect of $Mg(OH)_2$ on urinary parameters in the control and F group rabbits. Values are means±S D; none of the values differed statistically by p<0.05.

Group	N	Urinary parameter						
		Volume (mL)	pH	Creatinine (mg/24 hr)	Ca (mg/24hr)	Zn (µg/24hr)	Mg (mg/24 hr)	Cu (µg/24hr)
Control	6	33.3 ±15.21	7.6 ±0.79	55.6 ±30.41	48.6 ±37.23	83.3 ±49.25	19.1 ±16.60	5.0 ±1.89
Fluoride	6	29.9 ±13.64	7.3 ±0.94	38.5 ±26.62	32.5 ±22.25	95.9 ±62.62	11.8 ±5.94	3.6 ±0.96
F+Mg	6	35.7 ±5.79	7.1 ±0.79	45.0 ±26.38	41.8 ±24.07	100.0 ±50.99	18.7 ±14.41	3.8 ±0.73

DISCUSSION

Increased faecal fluoride in group 3 can be explained by the ability of Mg to form an insoluble complex with F in the intestinal tract.[13] In the present study, there was no change in serum Mg levels in group 3, which is in agreement with an earlier study.[20] However, in another study,[21] there was a change in serum Mg levels in which the maximum benefit occurred at 100 ppm F whereas 200 ppm showed marked toxicity when Mg was deficient. Mg deficiency in animals reduces production of energy relevant to the Mg-ATP system,[12] and this may be the reason that, in the present study, we found that weight loss in the F group 2 was significantly higher than in the F+Mg group 3. The important carbohydrate metabolism enzymes are inhibited by F through competitive inhibition with Mg.[22] This competitive inhibition further reduces Mg availability. Fluoride interacts with Mg on the enzyme in a reversible reaction, causing a 90% decrease of the catalytic activity. Simultaneous feeding of Mg with F would therefore be expected to help overcome this situation and reduce the adverse effect of F.

The degree of fluorosis is known to differ considerably in high F areas of India and the USA. In India, crippling bone disease occurs with greater frequency in endemic areas of fluorosis,[23-25] even though the F content in some of the drinking water may be similar in the two countries. A greater intake of water containing large amounts of F in India, in addition to different geo-climate conditions and type of diet consumed that may be deficient in Mg, Ca, and P, may also help explain this difference in F effects in India and the USA. A significant reduction in fluorosis in Mg-fed animals, possibly due to a decrease in the intestinal absorption of F, has also been observed in earlier studies.[20,26]

The increase in serum alkaline phosphatase in the F+Mg group 2 was significant compared to group 3, showing that Mg likewise has a beneficial effect on bone metabolism.

In conclusion, as evident by increased urinary and faecal F excretion, simultaneous administration of a Mg compound (milk of magnesia) along with F reduces F absorption. Thus it can be reasonably suggested that administration of

Mg in a F-intoxicated background could be beneficial with little or no adverse effect on biochemical and haematological parameters in the body.

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