BENEFICIAL EFFECT OF TAMARIND INGESTION ON FLUORIDE TOXICITY IN DOGS

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SUMMARY: The effect of tamarind ingestion on fluoride retention was studied in three groups of dogs – control (Group I), fluoride-supplemented (Group II), and fluoride and tamarind-supplemented diets (Group III). The urinary excretion of fluoride was significantly higher in Group II (2.45 ± 0.39 mg F/day) and Group III (3.78 ± 0.35 mg F/day) as compared to Group I (0.205 ± 0.21 mgF/day). The difference between Group II and III was also significant. The femur fluoride content of Group II animals (4.0 ± 0.67mg F/gm ash) was significantly higher as compared to that of Group III (2.0 ± 0.52 mg F/gm ash) suggesting a beneficial effect of tamarind ingestion on fluoride retention and toxicity.

There were no significant differences in hematological parameters between the groups, although hemoglobin levels and packed cell volume tended to be lower in Group III as compared to Group I. Histopathological study showed that except in kidneys where congestion and tubular changes were greater in experimental groups as compared to control, all other organs were comparable between the groups.

Keywords: Bone fluoride, Femur fluoride, Fluoride toxicity, Tamarind ingestion, Urinary fluoride.

INTRODUCTION

Fluorosis is an important public health problem all over the world. However, drugs are not available for the treatment of skeletal fluorosis, making medical intervention impossible. It is reported that serpentine is of therapeutic value but opinion is divided about its efficacy and toxicological evaluation is not available.[1] Calcium supplementation interferes with fluoride absorption in animals[2,3] and humans, presumably due to the strong affinity between Ca^{++} and fluoride and the low solubility of CaF_2. Calcium chloride and calcium gluconate have been used in acute fluoride poisoning.[4] Intravenous administration of magnesium compounds (MgO or $Mg(OH)_2$) is been reported to increase excretion of fluoride in urine and faeces[5,6] and decrease the amount retained in bones. Aluminium sulphate and boron have also been tried for this purpose.[7,8]

Sriramachari[9] and Maruthamuthu *et al*[10] reported the binding of fluoride by tamarind *in vitro*. However, the effect of tamarind ingestion on fluoride metabolism has not been studied. The present study in dogs was initiated to assess the effect of tamarind ingestion on the urinary excretion of fluoride, the mineral composition (F, Ca, Mg, and P content) of bones, and histopathological and hematological parameters.

MATERIALS AND METHODS

A total of 18 mongrels, 2.5 to 3.0 months old and weighing 3.5 to 4 kg were used for the study. The animals were acclimatized to laboratory conditions for

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three months and then were randomly distributed into three groups: Group I (Control) was on a stock diet of cooked rice and pulses; Group II was fed a stock diet + sodium fluoride (10 mg/dog/day); and, Group III was fed a stock diet + sodium fluoride (10 mg/dog/day) + tamarind paste.

The dogs were housed individually with free access to diet and water for a further period of 3 months. Fluoride was administered to the dogs in pieces of bread. Fluoride solution (10 mL containing 10 mg NaF) was layered on a piece of bread and administered without wastage. Bread without fluoride was also fed to control dogs. Ten grams of Tamarind paste, a ready-to-eat commercial product made of whole ripened tamarind pulp after removal of pericarp and seeds and containing 60% moisture, were mixed with cooked rice + red gram (2:1) and served to Group III dogs.

During the last week of the experiment, animals were transferred into metabolic cages. Then, 24-hour urine samples (with few drops of toluene) and faeces were collected for four days. Blood samples were collected (with EDTA as anticoagulant) and hemoglobin, PCV, total leukocyte count estimations, and peripheral smear determinations, were made. All animals were then given thiopental (Pentothal 50 mg/kg body weight) by i/v. Death was instantaneous. Their lungs, liver, kidney, pancreas, and intestine were removed, fixed in 10% neutral buffered formalin and subjected to routine histopathological examination. Out of the original total of 18 dogs, two in Group I and one each in Groups II and III died early in the initial stages of the study and hence were not included in the results. Also, one animal each of Group II and III died one and two days, respectively, before the conclusion of the experiment for unknown reasons. In these two animals, blood and urine samples could not be collected, but the bones and other organs were included for analysis.

Fluoride content of urine and bone ash[11] was determined with a fluoride ion-selective electrode (Orion Expandable Ion Analyzer EA 940, Boston, MA). Calcium and magnesium in bone ash were determined by atomic absorption spectrophotometry. Phosphorus was estimated by the method of Chen *et al*.[13]

RESULTS

Food intake and weight gains were similar in all groups. The average water consumption was significantly less in experimental groups than in controls (312 mL, 63 mL, and 70 mL in Groups I, II, and III, respectively). The volume of urine excreted by experimental Groups II and III was also significantly less ($P < 0.05$) than that of controls (Table 1). There was no significant difference in urine pH among the groups (Table 1).

Biochemical study: Dogs fed the diet containing tamarind (Group III) had significantly higher urinary fluoride excretion ($P < 0.01$) than Group II (Table 1). These results show that the retention of fluoride in Group III was less than in Group II. Also, estimation of fluoride in femur bone indicated that accumulation of fluoride in that bone was significantly less ($P < 0.01$) in Group III than in

Group II (Table 2). There was no significant difference in femur calcium, magnesium, and phosphorus content between groups (Table 2).

Table 1. Final body weight and 24-hr urine (last 4-days)

Group (No. of Dogs)	Body weight (kg)	Urine volume (mL)	Urine pH	Urine fluoride (mg/24hr)
I Control (4)	5.98 ± 0.19	308.12 ± 77.33	8.40 ± 0.22	0.205 ± 0.031
II Fluoride (4)	5.58 ± 0.23	*123.83 ± 28.66	8.29 ± 0.29	†2.35 ± 0.39
III Fluoride + Tamarind (4)	5.24 ± 0.39	*130.36 ± 18.12	7.85 ± 0.18	†3.68 ± 0.35

Values are Mean ± S.E.
*Denotes pairs of groups significantly different at 0.05 level ($P<0.05$).
†Denotes pairs of groups significantly different at 0.01 level ($P<0.01$).

Table 2. Terminal bone femur analyses
(Ash weight, fluoride, calcium, magnesium and phosphorus)

Group (No. of dogs)	Ash weight (gm)	Fluoride (mg)	Calcium (gm)	Magnesium (mg)	Phosphorus (mg)
I Control (4)	3.28 ± 0.65	1.53 ± 0.42	1.87 ± 0.28	34.42 ± 5.32	942 ± 296
II Fluoride (5)	3.35 ± 0.93	*11.38 ± 0.79	1.79 ± 0.56	32.93 ± 10.14	1095 ± 266
III Fluoride+Tamarind (5)	3.40 ± 0.46	*6.80 ± 1.48	1.64 ± 0.27	29.40 ± 5.73	1081 ± 186

Values are Mean ± S.E.
*Denotes pairs of groups significantly different at 0.01 level ($P<0.01$).

Hematological study: The hematological parameters of the various groups were not significantly different, although a trend could be seen in Group III suggesting lower Hb% and PCV values as compared to Group I (Table 3). The mean neutrophil and lymphocyte counts were different between Group I and Group II. However they were not statistically significant.

Histopatholgical study: The lungs, liver, kidney, pancreas, and intestines of all animals were examined in order to look for any adverse effects on Group II and Group III as compared to Group I. No gross abnormalities were detected in the organs examined.

Microscopically, the pancreas and intestines showed no changes in all the groups while lungs showed grades I and II pneumonic changes in all groups. Foreign body giant cell reaction, edema, and other changes in lungs were greater in the experimental groups. Liver changes were almost comparable in all groups. More glomerular and tubular changes were seen in the experimental groups (Table 4) as compared to controls.

Table 3. Terminal hematology parameters (mean ± S.E.)

Hemoglobin (gm %)	Packed cell volume (PCV)	Leukocyte count (thousands)	Neutrophils (%)	Lymphocytes (%)	Eosinophils (%)	Monocytes (%)
Group I Control						
12.01 ± 1.05	36.00 ± 2.67	10 ± 4.97	65.75 ± 6.43	27.00 ± 5.25	3.00 ± 1.29	4.25 ± 0.62
Group II Fluoride						
11.90 ± 0.73	34.66 ± 2.02	8.00 ± 3.60	61.00 ± 13.05	33.66 ± 13.42	1.33 ± 0.88	4.00 ± 1.73
Group III Fluoride + Tamarind						
9.84 ± 0.75	28.00 ± 2.51	15.66 ± 9.66	63.00 ± 9.84	31.33 ± 8.51	0.33 ± 0.33	5.33 ± 1.76

No of dogs per group = 4. Platelets: Adequate; RBCs: N/N, few Echinocytes.
*Total leukocyte counts, diff. counts and platelets based on peripheral smear.

Table 4. Terminal histopathology details

Organ/Change	Control (n=4)				Fluoride (n=5)					Fluoride+tamarind (n=5)				
Lungs														
Normal	-	+	-	-	+	-	-	+	-	-	-	-	-	-
Interstitial pneumonitis														
Grade I	-	-	+	+	-	-	-	-	-	-	+	+	-	+
Grade II	+	-	-	-	-	+	-	-	-	-	-	-	-	-
Grade III	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Fibrosis/Hemorrhage/Parasites	-	-	+(F)	-	-	-	-	-	-	+(H)	-	-	+(P)	-
Edema/others	-	-	-	-	-	-	+(E)	-	+FBGC	-	-	-	+(E)	-
Kidneys														
Normal	+	+	+	+	-	+	-	+	+	+	+	+	-	+
Glomerular vascular congestion	-	-	-	-	+	-	+	-	-	-	-	-	+	-
Tubules cloudy swelling	-	-	-	-	+	-	+	-	-	-	-	-	+	-
Liver														
Normal	-	+	-	-	-	+	-	+	-	+	+	+	-	+
Focal areas of necrosis	+	-	+	-	+	-	-	-	+	-	-	-	+	-
Congestion	+	-	+	+	-	-	+	-	-	-	-	-	+	-
Parasites	-	-	-	-	+	-	-	-	-	-	-	-	-	-

DISCUSSION

It is known that high protein intake increases fluoride excretion and decreases fluoride retention in bones of rats,[14] and similar results were observed in the present study. Chinoy and Mehta[15] described the beneficial effects of glycine and glutamine on testis of fluoride treated mice. Though bones were not studied, the authors suggest that a protein-supplemented diet may ameliorate the toxic effects of fluoride in endemic areas. Some reports[14,16] indicate that increased gastric acidity enhances fluoride absorption due to formation of non-ionic hydrogen fluoride. It remains to be seen whether tamarind exerts such an effect. It is known that an acidic urine increases fluoride excretion, but in the present study, the urine samples of all the groups did not show any significant differences in pH.

Tamarind and fluoride had no adverse effects on hematological parameters studied, although in another investigation fluoride was shown to reduce hemoglobin and hematocrit levels in rabbits.[17] Lungs, liver, and kidney did not show any gross abnormalities. However, the exact mechanism by which tamarind ingestion decreases fluoride retention in bones is not known. Increased urinary excretion is one contributing factor. Tamarind contains considerable amounts of tartaric acid. Tartrate-resistant acid phosphatases are known to be present in bone cells,[18] but the effect of tartrate-sensitive phosphatases, if any, in bone is worth looking into.

Although the therapeutic value of tamarind in the management of urolithiasis has received attention in recent years,[19] its potential usefulness for fluorotic patients as suggested by the present study requires further investigation, which is now in progress.

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