



## Research article

**Experimental intervention to prevent fluoride exposure**

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**ABSTRACT**

This study is attempted to develop a preventive measure against fluoride exposure in the endemic areas of India and other countries. This Experimental approach involves prevention of fluorosis with Calcium lactate and Ascorbic acid. The water samples were collected from endemic areas of Nalgonda district and its fluoride levels were estimated; and considered as fluoride control. The animal study on prevention of fluorosis was performed on 24 Wister albino rats which were divided into four groups. It was found that calcium lactate and ascorbic acid binds to fluoride ions in the GIT and forms an insoluble complex which significantly ( $P < 0.001$ ) decreases the fluoride absorption. It shows that diet supplemented with high calcium dose and ascorbic acid are significantly effective ( $P < 0.001$ ) in therapies of fluorosis. Calcium lactate and Ascorbic acid has shown potential in prevention of fluorosis. It can be a promising therapy against fluorosis.

**Keywords:** Fluorosis, skeletal fluorosis, Pre-clinical, Water.

**INTRODUCTION**

Fluoride is 13<sup>th</sup> most abundant element on our planet and is widely dispersed in nature. The World Health Organization and Indian Council of Medical Research have made the 1.5 mg/l as drinking water quality guideline value for fluoride. The prevalence of high fluoride level is reported from 22 states in India, affecting more than 40 million people. Fluorosis is a slow and progressive, which affects almost all parts in the body and results in health complaints having overlapping manifestations with several other diseases. The adverse effects associated are dental and skeletal fluorosis. It also affects the foetal cerebral function and neurotransmitters <sup>[1]</sup>. The global prevalence of fluorosis is reported to be about 32%.

In India, today most states, villages getting affected and becoming endemic and have been steadily increasing ever since the disease was discovered in India during 1930s. Most of the people in the villages of Nalgonda district of Andhra Pradesh, India have severe dental and skeletal fluorosis. Till now most of the techniques

developed are based on defluoridation of fluorine from water, but still the problem remains unresolved as of today. And few invasive treatments are developed only in case of dental fluorosis. Few studies indicated that calcium salts interfere with fluoride absorption. This Experimental Intervention effect on prevention of fluorosis through Animal experimentation will be an effort to provide low cost easily available prevention technique. After evaluating the data, this study suggests that there is an instant need to take ameliorative steps to prevent the population from risk of fluorosis. The main objective of this study is, to study the effect calcium lactate and ascorbic acid on reversal of skeletal fluorosis <sup>[2]</sup>.

**MATERIALS AND METHODS****Collection of water sample**

The Selection of control sample is based on criteria that it should be free from fluoride in any of its form in that drinking water (fluoride level should be within 1 mg/l). As per these criteria, distilled water is selected as control sample for animal studies. The fluoride

water sample selected for study is from Madannapur village of Nalgonda district of Andhra Pradesh, India as nearly 65% of the villagers were affected by different forms of fluorosis. The collection procedure of fluoride water sample were followed as: Three drinking water samples were collected from ground water sources of Madannapur village of the Nalgonda District and stored in precleaned, high density polyethylene bottles at 4 °C before being analyzed. Fluoride level was measured by using fluoride ion selective electrode Orion 9609 (Fluoride electrode manual, 1991) [3].

#### Animal Experimentation

Animals: Wister albino rats (150–200 g) procured from Sri Krupa Institute of Pharmaceutical Sciences (Siddipet, A.P India) was used in all experiments. Animals were housed in polypropylene cages in standard laboratory conditions of relative humidity 50% ±10%, temperature 22°C ±2°C, and 12/12 hour light–dark cycle for 7 days prior to experiments. Access of water and standard pellet food (Hindustan Unilever, Mumbai, India) supply was provided every day, ad libitum. All animal experiments were conducted as per the approval and guidelines of the institutional animal ethical committee (IAEC) 1230/a/08/ CPCSEA. Control water and fluoride sample were administered orally through water bottles. Calcium lactate and ascorbic acid were given either dissolved or dispersed in drinking water bottles at 37 °C.

#### Experimentation Design and Methodology

The 24 Wister albino rats were selected and divided into four groups having six animals in each group. The GROUP 1(normal control): Fed with normal diet and purified water for 30 days; the GROUP 2(control): Fed with normal diet and fluoride water (7.4 mg/l) for 30 days; the GROUP 3: Fed with calcium lactate 3.187mg/day along with fluoride water and with normal diet for 30 days, the GROUP 4: Fed with calcium lactate and ascorbic acid 1.75 mg/day dissolved in fluoride water along with normal diet for 30 days.

#### Determination of blood fluoride level

The blood samples were collected at the end of 30<sup>th</sup> day of study period via retro orbital puncture method and the blood samples were analyzed using “fluoride ion selective electrode attached” to Orion meter. Fluoride level was measured by using fluoride ion selective electrode Orion 9609 with expandable ion analyzer EA 940 (Fluoride electrode manual, 1991) [4].

#### Determination of bone fluoride level

At the end of 30<sup>th</sup> day of study period, Femur bones from thigh of rats were collected from all the six animals in each group. The samples were carefully packed in polyethylene papers and kept in a freezer below –10 °C. Aliquots of fat free, cleaned bones were accurately weighed and then ashed for 3 h at 600 °C in a muffle furnace. Fluoride level was measured by using fluoride ion selective electrode (Fluoride electrode manual, 1991).

#### Statistical analysis

The range of water fluoride level was tabulated for samples from each village. Mean and standard deviation of samples from each village and also from each block was calculated. Multiple comparison tests (Tukey-Kramer) were performed between the different groups with significance level ( $P \leq 0.001$ ) using SPSS 16.0 software.

## RESULTS

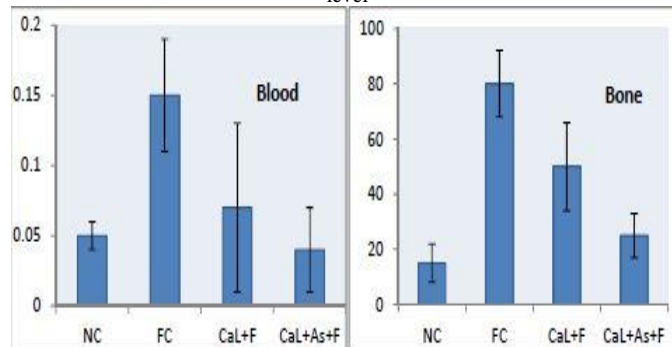
### Collection of water sample and analysis

The fluoride water sample for this experimental intervention was taken from Maddanapur village of Nalgonda District. The three samples of water were collected in the same village and mean fluoride level calculated was 7.4 mg/l. This fluoride sample was taken as positive control for the study.

### Effect of Calcium lactate and ascorbic acid on blood and bone fluoride levels

The result reported indicates that, the use of calcium lactate and ascorbic acid has shown a marked decrease in fluoride absorption when compared to the control group as in Table 1 and Table 2. The blood samples shown a decrease of blood fluoride level by 53.34% between Group3 (CaL+F) and Group2 (FC) ( $P < 0.001$ ); along with decrease of 73.34 % between group4 (CaL+As+F) and Group2 (FC) ( $P < 0.001$ ). The bone samples shown a decrease of bone fluoride level by 37.5% between Group3 (CaL+F) and Group2 (FC) ( $P < 0.001$ ); along with decrease of 68.75% between group4 (CaL+As+F) and Group2 (FC) ( $P < 0.001$ ) as shown in Figure 1 [5].

**Figure 1:** Graphical representation of Blood fluoride levels and Bone fluoride level



## DISCUSSION

Effect of fluoride level in drinking water and other dietary sources on fluorosis prevalence.

The drinking water fluoride level has significant influence on fluorosis prevalence even though it contributes only 41% of the total fluoride intake per day, excluding the water used for cooking and food processing. This may be due to maximum bioavailability of soluble fluoride in drinking water and it gets absorbed readily; this ultimately enhances the fluoride intake through food and beverages.

Effect of fluoride ion concentration in drinking water on bone and blood fluoride level.

The chronic intake of fluoride-rich water in endemic areas leads to a significant increase of bone and blood fluoride level that enhances the risk of skeletal fluorosis. The blood fluoride level can be

responsible for disturbance in foetal cerebral function, neurotransmitters and reduced intelligence. The threshold level of fluoride ingestion needed to cause skeletal fluorosis varies depending on water intake, water quality, and other dietary factors. Normally the fluoride exposure dose level from drinking water decreases with increase of age. Approximately 50% of the absorbed fluoride in each day becomes associated with calcified tissues within 24 h and the remainder is excreted in the urine but, the uptake of fluoride by the skeleton and blood is most efficient in children and decreases with age and this process may discontinue after the age of 55. In order to maintain the safe fluoride level in the bone and blood; and to avoid further fluorosis risk due to the excess fluoride accumulation in the bone and blood through fluoride absorption from water and food stuffs, the drinking water fluoride level should be maintained below 1.0 mg/l as shown in Table-1 and Table-2 [6].

**Table 1:** Detection of blood fluoride level (Mean  $\pm$  SD) (mg/l) in rats

Group1(N C)	Group2(FC)* **	Group3(CaL+F)* **	Group4(Cal+As+F) ***
0.0647	0.172	0.0174	0.0281
0.0482	0.138	0.0532	0.0613
0.0527	0.165	0.0827	0.0132
0.0455	0.129	0.1273	0.0692
0.0473	0.142	0.0673	0.0429
0.0416	0.154	0.0721	0.0253
0.05 $\pm$ 0.008	0.15 $\pm$ 0.016	0.07 $\pm$ 0.036	0.04 $\pm$ 0.022

**Table 2:** Detection of bone fluoride level (Mean  $\pm$  SD) (mg/kg) in rats

Group1(N C)	Group2(FC)* **	Group3(CaL+F)* **	Group4(Cal+As+F) ***
13.87	73.19	53.29	27.15
21.35	79.27	33.72	25.80
11.23	87.33	51.63	23.27
13.62	91.75	64.19	32.87
12.74	79.03	39.83	21.32
17.19	69.43	57.34	19.59
15 $\pm$ 3.67	80 $\pm$ 8.38	50 $\pm$ 11.28	25 $\pm$ 4.75

Effect of Calcium lactate and ascorbic acid on blood and bone fluoride level.

The drinking water fluoride level has significant influence on fluorosis prevalence even though it contributes only 41% of the total fluoride intake per day, excluding the water used for cooking and food processing. This may due to nearly 100% bioavailability of soluble fluoride in drinking water and it get absorbed readily through the gastrointestinal tract. In this study, it is clearly reported that, the use of calcium lactate and ascorbic acid has shown a marked decrease in fluoride absorption when compared to the control group as given in Table 1 and Table 2. The rationale behind decreased fluoride level is, calcium ion will bind with fluoride ions and form calcium salt which when interact with ascorbic acid forms a complex. So, the calcium lactate and ascorbic acid has shown the tendency to form an insoluble complex with fluoride in gastrointestinal tract and this insoluble complex decreases its absorption. Even it is expected that in Infants as well as in children, the level of bone calcium level which is decreased due to high fluoride exposure, can be reversed by using calcium rich

diet and ascorbic acid. This clearly shows that calcium lactate and ascorbic acid is an effective measure for reversal of fluorosis [7].

## CONCLUSION

The high exposure of fluoride level to people is an important concern. An immediate public awareness measure is required from both government and NGOs to educate the fluoride effected population. The experimentation revealed that calcium lactate and ascorbic acid were effective in the reversal of skeletal fluorosis. This awareness must be created in the fluoride endemic areas. The calcium rich diet and Vitamin-C are suggested in their daily diet for reversal of fluorosis.

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