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# Comparative studies on plasma mineral status of cattle in fluoride toxic brackish water zone of Punjab, India

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## ABSTRACT

**Objective:** Chronic fluoride intoxication or fluorosis is a worldwide health problem in humans and animals. The present research work was aimed to assess the status of copper, zinc, cobalt, manganese, magnesium, calcium and phosphorus in blood of fluorotic cattle in brackish water zone of Punjab. **Methods:** The present study was conducted in villages of district Muktsar, a brackish water zone, of Punjab state. Cattle ( $n=103$ ) showing signs of dental lesions or lameness, from the villages with water fluoride concentration more than 1 ppm, were selected for the study whereas cattle ( $n=98$ ) from villages with water fluoride concentration less than 1 ppm and with no clinical signs served as control. Blood samples were collected from both the groups and were analysed for minerals. **Results:** Significantly ( $P<0.05$ ) higher plasma F concentrations were observed in animals of fluorotic region in comparison to healthy control animals. Concentrations of plasma Ca, Mg, Cu and Zn were significantly lower in cattle of hydrofluorotic region. Plasma phosphorus, iron and iodine concentrations were higher in animals of hydrofluorotic region whereas Mo and Mn did not differ between the two groups. **Conclusions:** Present study indicated decrease in certain essential minerals in animals of fluorotic region and such changes may contribute to the toxic effects associated with exposure to excess fluoride and salinity.

## 1. Introduction

Chronic fluoride intoxication or fluorosis is a worldwide health problem and is endemic in those areas where the fluoride content in drinking water is relatively high. Its primary manifestations in human and mammals are mottling of teeth and osteosclerosis of the skeleton. Cattle reared in fluoride enzootic areas of India for a long time show clinical signs of osteo- and dental-fluorosis [2, 9]. Mechanisms of chronic toxicity are not clearly understood but enzyme inhibition and ability of this highly electronegative and reactive halogen element to substitute for hydroxide ions seems to be one of the possible mechanisms of toxic effects [6]. Excess intake of fluoride through contaminated water and fodder, intake of rock phosphates, dust and fumes emanating from superphosphate fertilizer factory contribute to the development of fluorosis in the livestock population

[7]. Many studies on fluoride intoxication in experimental and naturally affected animals have revealed alternation in micro and macro minerals status in blood and other organs or tissue [7, 15] but there seems to have paucity of reports on the status of micro and macro minerals in blood of cattle naturally affected with chronic fluoride toxicity in brackish water zone. Brackish water is water that has more salinity than fresh water, but not as much as seawater. Salinity of water and water logging are common problems especially in southwestern districts of Punjab [8]. Due to waterlogging and subsequent salinization, the fertile productive land is gradually becoming unproductive. Fluoride concentrations were reported to increase with salinity [14]. The present research work was aimed to assess the status of copper, zinc, cobalt, manganese, magnesium, calcium and phosphorus in blood of fluorotic cattle in brackish water zone of Punjab.

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## Material and Methods

The study was conducted in villages of district Muktsar, a

brackish water zone, of Punjab state. Fluoride concentration of drinking water of different villages was estimated and cattle of the region were examined for various signs of fluoride toxicity. The villages with water fluoride concentration more than 1 ppm and cattle showing signs of dental lesions or lameness were considered enzootic for fluorosis whereas villages with water fluoride concentration less than 1 ppm and cattle with no clinical signs were considered fluorosis free.

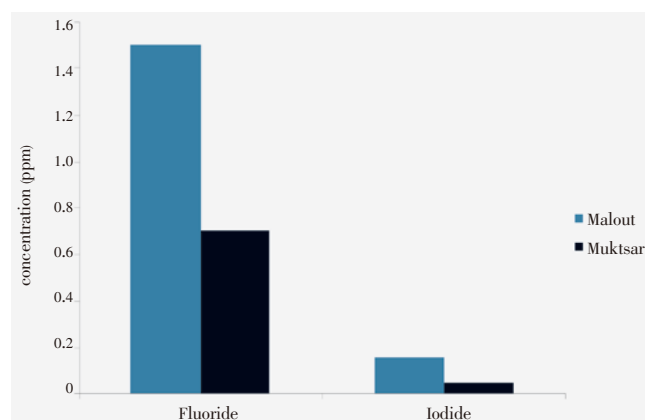
For the estimation of plasma minerals, whole blood (15 to 20 ml) was collected from lactating (milk yield 8–18 liters per day) animals ( $n=103$ ), showing signs of fluorosis, in heparinised mineral free glass vials. Similarly, blood samples were collected from apparently healthy cattle ( $n=98$ ) reared in villages free from fluorosis, with no signs of fluorosis, to serve as control. Plasma was harvested for mineral estimation by centrifugation (3000 rpm for 15 minutes) and was stored at  $-10^{\circ}\text{C}$  temperature in deep freezer for subsequent analysis.

Two milliliter of plasma sample was digested on hot plate in one cycle of 10 ml double glass distilled concentrated nitric acid (E. Merck India Ltd., Mumbai) and then in one cycle of 2.0 ml hydrogen peroxide (E. Merck India Ltd., Mumbai). The volume of the resultant digestate was made 10.0 ml with double glass distilled water. Concentrations of various minerals viz., Ca, Mg, Cu, Zn, Mn, Fe and Mo were measured by Inductively Coupled Argon Plasma (ICAP) spectrometry. Plasma Pi was estimated by method of Taussky and Shorr [13]. F and I level in plasma and water samples were estimated by using Digital Ion-analyzer (Orion 4 Star pH. ISE Benchtop) equipped with fluoride and iodide specific electrodes. The statistical significance of the differences among mean was compared by using SPSS for Windows (version 16.0; Microsoft).

## Results

In the present study, high fluoride concentration was recorded in water samples of Malout block ( $1.5 \pm 0.03$  ppm) of the district whereas lowest concentration ( $0.7 \pm 0.004$  ppm) was recorded in Muktsar block (Fig. 1). Intake of fluoride from water led to high fluoride in cattle of the region. Significantly higher concentrations were observed in animals ( $0.183 \pm 0.01$  ppm) of fluorotic region in comparison to healthy control animals ( $0.139 \pm 0.009$  ppm).

Concentrations of plasma Ca, Mg, Cu and Zn were significantly lower in cattle of hydrofluorotic region in comparison to healthy control animals (Table 1) whereas Pi and Fe concentrations were significantly higher in the former group. Plasma iron and iodine concentrations were higher in animals of hydrofluorotic region whereas Mo and Mn did not differ between the two groups.



**Fig.1.** Water fluoride and iodide levels (in ppm) in fluorotic and non-fluorotic region

**Table 1**

Plasma mineral concentrations in cattle (Mean  $\pm$  S. E)

Parameter	Non Hydrofluorotic(N= 98)	Hydrofluorotic(N= 103)
Ca (mg/dl)	9.76 $\pm$ 0.18	9.23 $\pm$ 0.18 *
Pi (mg/dl)	5.08 $\pm$ 0.12	6.28 $\pm$ 0.20 *
Ca: P ratio	1.92	1.46
Mg (ppm)	2.91 $\pm$ 0.08	2.48 $\pm$ 0.05 **
Fe ( $\mu$ mol/l)	99.05 $\pm$ 7.37	131.68 $\pm$ 11.69 *
Cu ( $\mu$ mol/l)	10.67 $\pm$ 0.35	8.41 $\pm$ 0.38 **
Zn ( $\mu$ mol/l)	26.96 $\pm$ 1.44	20.96 $\pm$ 0.92 **
Mn ( $\mu$ mol/l)	1.03 $\pm$ 0.04	1.31 $\pm$ 0.27
Mo (ppm)	0.153 $\pm$ 0.04	0.149 $\pm$ 0.01
F (ppm)	0.139 $\pm$ 0.009	0.183 $\pm$ 0.01*
I (ppm)	2.07 $\pm$ 0.06	2.45 $\pm$ 0.06 **

\* Significant difference ( $P<0.05$ )

\*\* Significant difference ( $P<0.01$ )

## Discussion

South-western region of Punjab in India is known for its brackish water and high fluoride content in underground water [11]. Fluoride content of water varied within the district and for such variations; there could be various causes such as use of phosphate fertilizers, different geo-chemical conditions. Moreover, fluoride concentrations were also reported to increase with salinity [14].

Serum fluorine reflects the fluorine status of current diet rather than cumulative effect of fluorine exposure, but on the other hand, clinical signs, as were observed in the affected animals, may not appear for many weeks and months in animals ingesting moderate amounts of fluoride [12].

Concentrations of plasma Ca, Mg, Cu and Zn were significantly lower in cattle of hydrofluorotic region in comparison to healthy control group (Table 1) in the present study. In gastrointestinal tract, fluoride being highly electronegative halogen forms complexes with cations like Mg and Ca and thereby reduces their absorption [14].

Hypocalcaemia observed in cases of chronic F intoxication is attributed to chemical interaction between calcium and F, lowering the bioavailability of ingested [5]. Moreover, salinity of the region may also contribute to decrease these minerals as NaCl increase concentrations of  $\text{Na}^+$  and  $\text{Cl}^-$  and reduce concentrations of  $\text{Ca}^{2+}$ ,  $\text{K}^+$  and  $\text{Mg}^{2+}$  in many plant species used as fodder [1].

Comparatively lower levels of Cu and Zn in fluorotic animals may be attributed to their reduced absorption from gastro-intestinal tract. Increase in urinary and fecal excretion of various minerals may be another factor responsible for their decreased status in the body. Increased fecal and urinary excretion of zinc after fluoride exposure has been reported in humans [4]. Moreover, zinc (Zn) and copper (Cu) are essential for certain body functions and may also be utilized to mitigate ill effects arising from oxidative stress induced by fluorosis. A downward trend of Zn, Cu, Co, and Mn in soft tissue levels of rabbits has been recorded during F intoxication [10]. Similarly to the effect on the uptake of macro elements, salt stress of the region can also exert stimulatory and inhibitory influence on the uptake of some trace elements [3].

Comparatively higher concentrations of iodide in water of the region (Fig. 1) could be attributed to increased plasma iodine concentrations in animals of fluorotic region.

Thus, it can be concluded that alteration in level of different micro and macro minerals occur in fluorosis and such changes may also contribute to the toxic effects associated with exposure to excess fluoride and salinity. Supplementation of the minerals, plasma levels of which were significantly decreased in fluorotic animals, may help to alleviate the effects of fluorosis in these animals.

### Conflict of interest statement

We declare that we have no conflict of interest.

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