Original Research Article

Comparative Ameliorative Efficacy of Tamarind Pulp against Boron on Fluorotic Calves Reared in Periphery Villages of Aluminium Smelter Plant

Mirashree Pati¹, Ghana Syam Parida¹, Kruti Debnath Mandal¹,²*, Amit Raj Gupta¹,³ and Ramesh Chandra Patra¹

¹Department of Veterinary Clinical Medicine, Ethics And Jurisprudence, College of Veterinary Science and Animal Husbandry, Orissa University of Agriculture and Technology, Bhubaneswar-751003, Odisha, India
²Department of Veterinary Clinical Complex, Faculty of Veterinary and Animal Sciences, RGSC-BHU, Barkachha, Mirzapur, Uttarpradesh, India
³Department of Veterinary Medicine, Ethics and Jurisprudence, West Bengal university of Animal and Fishery Sciences, WB, India

*Corresponding author

Abstract

The aim of this investigation was to carry out the comparative therapeutic study on fluorotic calves reared in vicinity of an aluminium smelter plant. The F burden in the environmental samples (soil, fodder and water) collected from the vicinity of aluminium smelter was significantly higher than that of non-fluorotic zone. Significantly higher level of F was also recorded in plasma, urine and faecal samples which were associated with significant alterations in haematobiochemical parameters in the F-exposed calves compared to the calves reared in non-fluorotic area. Supplementation of tamarind pulp in the feed significantly altered the F level in plasma, urine and faeces in the exposed calves along with restoration of haematobiochemical parameters by day 60 in comparison to the routinely used F-binding agent (Boron) which proved its better efficacy against the development and/or progression of fluorosis.

Keywords
Tamarind, Fluorosis, Calf, Aluminium smelter plant

Introduction

Fluorosis is observed in areas with high levels of Fluoride (F) in ground water (Choubasia 1999) or in areas where industrial wastes contaminate water, soil and vegetation (Ergun et al., 1987). Smelting of aluminium ore is an important source of F emitted to the environment in gaseous and particulate form (Swarup et al., 1998). The disease is mainly characterized by brown discolouration, hypomineralisation, and increased attrition of teeth, bony exostosis, lameness and painful gait (Patra et al., 2000). Calves exhibited the highest prevalence of fluorosis as they are more sensitive and susceptible and less tolerant to fluoride (Shupe 1980) and also due to their rapidly growing skeleton. Curative treatment is always futile once the skeletal lesions are prominent. Management measures
aimed at preventing fluorosis in cattle reared in areas with endemically high environmental F levels are only fruitful during early stages of life (during calfhood). Aluminium (Al), calcium (Ca), boron (B), selenium (Se) and some hormones have been evaluated for mitigation of fluorosis with variable success rates (Kessabi et al., 1986; Han and Shi 2001; Maiti and Das 2004). Most are unfit for therapeutic purposes due to side effects upon prolonged use. Tamarindus indica L. (Tamarind) has been reported to exert a beneficial effect on F toxicity (Khandare et al., 2000). In the present study, the prophylactic efficacy of dried powder of tamarind pulp as an ameliorative agent in comparison to standard chemical antidotes is investigated in calves with natural fluorosis for the first time.

Materials and Methods

Study site

The study was conducted within the 5km radius of an aluminum smelter plant at Angul, Odisha, (latitude 20° 95’ N to 21° 10’ N and longitude 84° 55’ E to 85° 28 ’E) and 150 km away from Bhubaneswar, the state capital of Odisha, India.

Animals and experimental protocol

Eighteen fluorotic calves of 6-10 months old, born and reared within the 0-3 km radius from the smelter, having average of 81.2 kg body wt with visible signs of dental fluorosis such as deep yellow and brown discolorations, linear pigmented vertical streaks, loss of luster, attrition and motting of teeth with or without pitting, were selected randomly from different properties without any clustering and were divided into 3 equal groups with 6 animals each. The calves were mostly maintained on grazing with supplementation of locally harvested paddy straw (1 kg) as daily ration for 60 days. Among them, one group served as disease control without any treatment. The second group was supplemented by tamarind pulp powder (daily receiving orally 30 g/animal). The third group received daily 5 g/animal boric acid (Merck ltd, kolkata, India). Calves (6-10 months old with mean age of 8 months), living in Bhubaneswar (an area with low environmental F level) served as the negative control.

Collection of samples

Water samples, soil samples and fodder samples (dry and green) were collected from different sources from the polluted area following standard protocol. Blood, urine and faecal samples were collected from each animal between 6 - 8 A.M. on day 0, 30 and 60.

Estimation of fluoride

F concentrations were estimated by ion specific potentiometry using total ionic strength adjustment buffer and a portable F specific electrode (Orion model 94 09 BN, Thermo Fisher Scientific, Waltham, MA, USA), and meter (Orion Model-290A) with detection range between 0.019 and 1900 mg/L.

Haemato-biochemical parameters

Haemoglobin concentration (Hb) was determined by Sahli’s hemometer and packed cell volume (PCV) was determined by microhaematocrit method. Total leucocyte count (TLC) and differential leucocyte count (DLC) were determined by using the standard reference methods (Benzamin 1985). The activity of Alkaline phosphatase (ALP) and Aspartate transaminase (AST), and the level of urea, creatinine, total protein, Ca and phosphorus were estimated in plasma samples.
by semi auto analyser (Model no- Microlab 300, Merck, India) using commercial reagent kits supplied by Crest Biosystems, Goa, India.

Statistical analysis

The data obtained for each parameter were compiled and analyzed using SPSS 22 software for post hoc analysis by Duncan’s multiple comparison tests and least square deviation (LSD) to find out the mean, standard error, range and significant difference of mean values between the groups and the paired t-test was conducted for comparisons of the groups within the periods (Day 0, 30 and 60) with P<0.05 considered statistically significant.

Results and Discussion

The concentration of fluoride in environmental and plasma samples

The F concentrations in soil, water, fodder and plasma samples in relation to the distance from the plant are shown in table 1.

Effect of treatment on biological sample

The plasma, urine and faecal F concentrations in different groups are given in table 2. The mean F concentration in the plasma, urine and faecal samples of fluorotic animals at day 0 was significantly (P<0.05) higher than the corresponding negative control (Group I). The F concentrations in plasma and urine of the calves treated with B and tamarind, were significantly lower and the faecal F levels were significantly (P<0.05) higher by day 60 compared to day 0.

However, a greater effect was observed in tamarind-treated calves with a 66% reduction in plasma F levels, compared to a 51% reduction in the B-treated group (Table 3).

Haematological parameter

Table 4 depicts significantly lower level of haemoglobin, TLC and haematocrit value in fluorotic calves than calves from a low F area and the same increased significantly (P<0.05) after 60 days of the administration of either B or tamarind, though the rise was significantly higher upon treatment with tamarind than B. The above parameters increased significantly (P<0.05) towards the end of treatment periods, while during the 1st half both the treatments failed to show any significant improvement.

Plasma biochemical parameters

Calves from the high F area had significantly (P<0.05) lower Ca, total protein and albumin levels, and higher phosphorous, ALP, AST, urea and creatinine levels than calves from the low F areas. By day 60, treatment with tamarind or B had resulted in a significant (P<0.05) increase in Ca and a significant decrease in phosphorous, ALP and AST. Overall, tamarind was found more potent than B in increasing Ca and total protein level, and reducing AST, urea and creatinine level than B after the treatment period (Table 3).

Industrial fluorosis and its effect on animal-environment eco-system

Although, F from industrial sources is mostly ejected to the air, it may cause toxic contamination of soil, water and vegetation in the industrial vicinity (Patra et al., 2000). F in soil and water that is used for irrigation ultimately finds its way into forage and grains and it eventually enters into the food chain of human and animals which has deleterious impact on their health.
Table.1 Fluoride level (mean ± SE) in ppm in soil, water and feed samples of Fluoride

| Sample  | Fluoride polluted area | Unpolluted area |
|---------|------------------------|----------------|
| Soil    | 166.07±10.49<sup>b</sup> | 3.3±0.21<sup>a</sup> |
| Water   | 2.34±0.134<sup>bB</sup> | 0.11±0.02<sup>a</sup> |
| Ground  | 0.731±0.084<sup>bA</sup> | 0.19±0.04<sup>a</sup> |
| Fodder  | 186.83±28.96<sup>bY</sup> | 2.73±0.02<sup>a</sup> |
| Dry     | 139.75±5.41<sup>bX</sup> | 7.15±0.93<sup>aY</sup> |
| Green   | 0.13±0.007<sup>b</sup>  | 0.059±0.005<sup>a</sup> |

Polluted area and Unpolluted area
The values (Mean± S.E., n=6) with dissimilar superscript (small letter) within a row varies significantly at p< 0.05. The significant differences in the mean levels of F between ground water and surface water tested for different distance range and similar procedure has been done to test the differences in the means between green and dry fodder. Means bearing different superscripts (capital letters) differ significantly at 5% level (p<0.05)

Table.2 Concentrations of F (ppm) in bio samples of calves from both less exposed and fluorotic area at different observation periods

| Parameter | Group         | Day 0            | Day 30           | Day 60           |
|-----------|---------------|------------------|------------------|------------------|
|           | Group I       | 0.084±0.005<sup>A</sup> | 0.086±0.005<sup>A</sup> | 0.083±0.003<sup>A</sup> |
| Plasma F  | Group II      | 0.433±0.018<sup>B</sup> | 0.449±0.017<sup>D</sup> | 0.466±0.019<sup>D</sup> |
|           | Group III     | 0.483±0.020<sup>B</sup> | 0.321±0.013<sup>bC</sup> | 0.235±0.009<sup>aC</sup> |
|           | Group IV      | 0.442±0.017<sup>bB</sup> | 0.257±0.029<sup>bB</sup> | 0.150±0.016<sup>bB</sup> |
| Urine F   | Group I       | 2.383±0.230<sup>A</sup> | 2.342±0.220<sup>A</sup> | 2.35±0.213<sup>A</sup> |
|           | Group II      | 10.633±0.559<sup>B</sup> | 11.167±0.526<sup>D</sup> | 11.533±0.542<sup>C</sup> |
|           | Group III     | 11.717±0.248<sup>cB</sup> | 9.917±0.209<sup>bc</sup> | 8.4±0.259<sup>bC</sup> |
|           | Group IV      | 10.867±0.489<sup>cB</sup> | 8.716±0.631<sup>BB</sup> | 7.283±0.367<sup>ab</sup> |
| Faecal F  | Group I       | 6.471±0.530<sup>A</sup> | 6.622±0.476<sup>A</sup> | 6.573±0.194<sup>A</sup> |
|           | Group II      | 20.797±0.767<sup>B</sup> | 20.95±0.739<sup>B</sup> | 21.03±0.605<sup>B</sup> |
|           | Group III     | 21.155±0.676<sup>bB</sup> | 24.747±0.635<sup>bC</sup> | 26.100±0.245<sup>bC</sup> |
|           | Group IV      | 21.200±0.665<sup>ab</sup> | 24.783±0.510<sup>bc</sup> | 26.000±0.369<sup>bc</sup> |

The values (mean ± SE, n = 6) bearing no common superscript in small letters in a row and capital letters in a column differ significantly at P<0.05.
Group I: Negative control; Group II: Fluorotic calves; Group III: Fluorotic calves receiving boron; Group IV: Fluorotic calves receiving tamarind
Table 3: Comparative efficacy of boron and tamarind treatment on different parameters

| Parameters                         | Difference of values from day 0 to day 60 in Boron-treated calves | Difference of values from day 0 to day 60 in tamarind-treated calves |
|------------------------------------|------------------------------------------------------------------|---------------------------------------------------------------------|
| Plasma F (ppm)                     | (-) 0.25±0.01A                                                   | (-) 0.3±0.08B                                                       |
| Urine F (ppm)                      | (-) 3.31±0.34                                                   | (-) 3.58±0.23                                                       |
| Faecal F (ppm)                     | (+) 4.94±0.64                                                   | (+) 4.8±0.67                                                        |
| Haemoglobin (g%)                   | (+) 0.83±0.25A                                                  | (+) 1.7±0.17B                                                       |
| Total leucocyte count (10³/mm³)    | (+) 0.23±0.09A                                                  | (+) 0.7±0.16B                                                       |
| Packed cell volume (%)             | (+) 2.33±0.55A                                                  | (+) 4.83±0.47B                                                      |
| Calcium (mg/dl)                    | (+) 1.16±0.09A                                                  | (+) 1.47±0.08B                                                      |
| Phosphorus (mg/dl)                 | (-) 1.14±0.1                                                   | (-) 1.34±0.06                                                       |
| Alkaline phosphatase (IU/L)        | (-) 11.15±0.2                                                  | (-) 12.6±0.3                                                        |
| Aspartate transaminase (IU/L)      | (-) 16.08±0.37A                                                | (-) 20.72±0.43B                                                     |
| Total Protein (gm/dl)              | (+) 0.15±0.02A                                                 | (+) 0.36±0.04B                                                      |
| Albumin (gm/dl)                    | (+) 0.04±0.02                                                  | (+) 0.06±0.03                                                       |
| Urea (mg/dl)                       | (-) 2.93±0.17A                                                 | (-) 3.75±0.11B                                                      |
| Creatinine (mg/dl)                 | (-) 0.12±0.02A                                                 | (-) 0.25±0.05B                                                      |

The values (mean ± SE, n = 6) bearing dissimilar superscripts (capital letters in a row) differ significantly at P<0.05.

“+” sign indicates increment in value from day 0 to day 60 whereas “–” sign indicates reduction in value from day 0 to day 60

Table 4: Haematological parameters in calves from both less exposed and fluorotic area at different observation periods

| Parameter                     | Group              | Day 0            | Day 30           | Day 60           |
|-------------------------------|--------------------|------------------|------------------|------------------|
| Haemoglobin (g%)              | Group I            | 11.00±0.32B       | 11.13±0.25B       | 11.10±0.28D      |
|                               | Group II           | 7.86±0.23A       | 7.83±0.19A       | 7.58±0.16A       |
|                               | Group III          | 7.70±0.22AbA      | 7.97±0.18abA      | 8.53±0.07bB      |
|                               | Group IV           | 7.73±0.11aA      | 7.87±0.08aA       | 9.43±0.08bC      |
| Total leucocyte count (10³/mm³)| Group I            | 9.80±0.20B       | 9.53±0.31B       | 9.35±0.36C       |
|                               | Group II           | 6.95±0.14aA      | 6.67±0.3A        | 6.63±0.19A       |
|                               | Group III          | 6.86±0.18aA      | 6.98±0.19aA      | 7.10±0.20AB      |
|                               | Group IV           | 6.94±0.07aA      | 7.33±0.13abA     | 7.65±0.16bB      |
| Packed cell volume (%)        | Group I            | 32.83±0.98B       | 33.5±0.76C       | 33.3±0.88D       |
|                               | Group II           | 23.50±0.76A      | 23.5±0.56A       | 23.17±0.48A      |
|                               | Group III          | 23.17±0.54abAB   | 24±0.63abAB      | 25.5±0.22bB      |
|                               | Group IV           | 23.66±0.82abAB   | 23.67±0.82abAB   | 28.5±0.22bC      |

The values (mean ± SE, n = 6) bearing no common superscript in small letters in a row and capital letters in a column differ significantly at P<0.05.

Group I: Negative control; Group II: Fluorotic calves; Group III: Fluorotic calves receiving boron; Group IV: Fluorotic calves receiving tamarind
The surface water had higher F than ground water due to contamination by sewage, sludge, fumes and suspended particulate matters released from the smelter which were gradually deposited on water bodies. This is contrary to the picture of hydrofluorosis, where the F concentration of ground water was much higher as a result of geothermal

| Parameter                  | Group     | Day 0          | Day 30          | Day 60          |
|---------------------------|-----------|----------------|-----------------|-----------------|
| Calcium (mg/dl)           | I         | 10.856 ± 0.31^B | 10.722 ± 0.182^C | 10.761 ± 0.189^C |
|                           | II        | 6.855 ± 0.147^A | 6.766 ± 0.103^A  | 6.745 ± 0.134^A  |
|                           | III       | 7.037 ± 0.064^A  | 7.840 ± 0.058^BB | 8.197 ± 0.112^BB  |
|                           | IV        | 7.031 ± 0.058^AB | 8.039 ± 0.092^BB | 8.497 ± 0.059^BB  |
| Phosphorus (mg/dl)        | I         | 4.847 ± 0.198^A  | 4.834 ± 0.182^A  | 4.763 ± 0.16^A   |
|                           | II        | 7.053 ± 0.084^B  | 7.015 ± 0.033^C  | 7.042 ± 0.09^C   |
|                           | III       | 7.133 ± 0.092^BB | 6.265 ± 0.18^B   | 5.985 ± 0.091^AB  |
|                           | IV        | 7.126 ± 0.074^B  | 6.177 ± 0.141^B   | 5.783 ± 0.066^B  |
| Alkaline phosphatase (IU/L)| I         | 150.997±1.007^A | 147.12±1.051^A   | 144.447±0.903^A  |
|                           | II        | 185.983±0.622^B  | 190.329±1.300^C  | 193.39±1.170^C   |
|                           | III       | 185.35±0.940^B   | 179.224±0.860^BB | 174.21±0.890^BB  |
|                           | IV        | 185.639±1.410^B  | 178.673±2.020^BB | 173.089±1.930^AB  |
| Aspartate transaminase (IU/L)| I         | 51.790±1.415^A  | 52.074±1.401^A  | 52.349±1.432^A   |
|                           | II        | 87.271±0.621^B   | 87.498±0.619^B    | 87.584±0.590^D   |
|                           | III       | 86.375±0.552^B   | 79.216±0.405^BC  | 70.300±0.458^AC  |
|                           | IV        | 86.956±0.699^B   | 75.387±0.378^B    | 66.251±0.238^AB  |
| Total Protein (gm/dl)     | I         | 7.062±0.130^B    | 7.083±0.119^B    | 7.093±0.135^C    |
|                           | II        | 6.500±0.127^A    | 6.368±0.165^A    | 6.257±0.159^A    |
|                           | III       | 6.553±0.172^A    | 6.642±0.179^AB   | 6.705±0.166^ABC  |
|                           | IV        | 6.485±0.102^A    | 6.653±0.101^AB   | 6.848±0.114^B    |
| Albumin (gm/dl)           | I         | 3.662±0.035^B    | 3.576±0.038^B    | 3.514±0.063^B    |
|                           | II        | 3.130±0.082^A    | 3.081±0.096^A    | 3.025±0.105^A    |
|                           | III       | 2.972±0.078^A    | 2.993±0.084^A    | 3.016±0.091^A    |
|                           | IV        | 3.005±0.073^A    | 3.026±0.068^A    | 3.062±0.065^A    |
| Urea (mg/dl)              | I         | 27.983±0.879^A   | 28.166±0.742^A   | 27.573±0.526^A   |
|                           | II        | 36.272±1.833^B   | 37.813±1.931^C   | 37.775±1.106^C   |
|                           | III       | 36.437±0.974^B   | 34.688±1.094^B   | 33.505±1.070^B   |
|                           | IV        | 36.880±1.375^B   | 34.414±1.525^B   | 33.134±1.433^B   |
| Creatin (mg/dl)           | I         | 1.133±0.078^A    | 1.160±0.056^A    | 1.178±0.049^A    |
|                           | II        | 1.834±0.111^B    | 1.860±0.108^B    | 1.890±0.096^B    |
|                           | III       | 1.880±0.072^B    | 1.805±0.067^B    | 1.763±0.054^B    |
|                           | IV        | 1.958±0.045^BB   | 1.881±0.044^B    | 1.710±0.058^BB   |

The values (mean ± SE, n = 6) bearing no common superscript in a row and capital letters in a column differ significantly at P<0.05.

Group I: Negative control; Group II: Fluorotic calves; Group III: Fluorotic calves receiving boron; Group IV: Fluorotic calves receiving tamarin
activity rather than industrial contamination (Maiti et al., 2003). The green fodder contained less F than dry fodder due to low dry matter content and high moisture content. The dietary intake of animals and the concentration of F in diet largely affect the degree of fluorosis. However, the present study was conducted on farm-based animals (suffering from industrial fluorosis) which were mostly maintained on grazing with little supplementation dry fodder, hence the minor variations in diet could not be studied. The plasma F level of calves within a 3 km radial distance from the smelter plant was above the normal level of 0.2 ppm as stated by Radostits et al. (2009) and also much higher than the unpolluted area. The WHO (2006) has set the guideline value at 1.5 mg / L of F, but mild forms of dental fluorosis begin to occur at lower levels (Hussain et al., 2005).

**Efficacious treatment of tamarind for ameliorating fluorosis compared to boron**

In the present investigation, elevated levels of plasma F, urine F and faecal F were observed in fluorotic calves than calves from low F area. Significant reduction of plasma F following 60 days of B administration was due to formation of Boron-Fluoride complex (BF₄⁻) in GI tract followed by its elimination in faeces (Elsair et al., 1980), thereby increasing the faecal F level. The decreased urinary excretion of F following boron supplementation points to a reduction in F absorption from the gastrointestinal tract and resultant decreased excretion of F via kidney (Bharti et al., 2007).

Interference with F absorption from the gut due to administration of dried pulp of tamarind fruits might have played a role in reducing plasma F concentrations as indicated by increased faecal F concentrations. There was also significant reduction in plasma F by tamarind pulp supplementation compared to B. This property of tamarind can be attributed to high concentrations of minerals, like Ca and phosphorus, along with zinc, potassium and iron (Caluwe et al., 2010) which are reported to form many active insoluble complexes with F in gut. Tamarind fruit is also rich in tannin and fiber (Khandare et al., 2002) which would have helped in enhancing F elimination in faeces and in reducing absorption, resulting in lower bioavailability and concomitantly lower urinary loss. On the contrary enhanced urinary F excretion by the use of tamarind pulp extract was reported in dogs (Khandare et al., 2000), human (Khandare et al., 2002) and rats (Dey et al., 2011) though examination of faecal F levels were not included in these studies.

The presence of anemia in F-affected animals was also reported in calves (Bharti et al., 2007), buffaloes (Swarup and Singh 1989) and cows (Singh and Swarup 1994 and Mandal et al., 2015) whereas decrease in TLC was previously observed by Behera (1993) and Swarup and Singh (1989) in cattle. On the contrary, leucocytosis was observed in F-induced buffalo calves by Gill and Dumka (2013). F-induced disorders in hematopoietic organs in mice and in human hematopoietic progenitor cells have also been recorded (Machalinska et al., 2002). These facts might have resulted in anaemia and leucopenia in fluorotic calves in the present investigation. The lower Hb level may possibly be due to toxic effect of F on the serum level of iron and poor retention of iron (Hoogstratten et al., 1965). Significant PCV changes in the study were also due to toxic effects of F on the RBC cell membrane and subsequently shrinkage of cell. In the present investigation, the higher Hb and PCV values after 60 days of treatment might be due to prevention of oxidative damage to cell membrane of RBC by B (Bharti et al., 2007) in B supplementation group, though it failed to exert any significant effect on the TLC level. But more significant
increase in the above parameter along with TLC in tamarind supplementation group might be due to high iron and antioxidant like polyphenols and flavanoids, reported to be present in tamarind (Martinello et al., 2006). Similar reports of improved haematological parameters was also reported by Mandal et al., (2015) after feeding of Moringa oleifera fruit powder in fluorotic calves due to reduced fluoride load in plasma.

A significantly lower Ca in fluorotic calves as compared to negative control was probably because of the decrease in absorption in addition to enhanced excretion of Ca via urine (Bharti et al., 2007). Moreover, decrease in Ca-ATPase activity was responsible for increase in urinary Ca causing hypocalcaemia (Singh and Swarup 1999). However, supplementation of B to the fluorotic animals produced significantly higher Ca concentration at the end of experiment due to its protective effect against the inhibitory effect of F on Ca-ATPase in renal tissues. But, supplementation of dried fruit pulp of tamarind was more efficient in increasing Ca concentration in blood which might be due to its richness in Ca (Ishola et al., 1990) and increase in elimination of F from the body.

The average serum phosphorus content of fluorotic calves was found to be more than the normal value. This is because the circulatory parathormone level increases in F intoxication (Singh and Swarup 1999) which regulates metabolism of Ca and phosphorus causing hyperphosphatemia due to concurrent hypocalcaemia. However, contradictory hypophosphoraemia was observed in fluorotic cattle by Mehedintu et al., (2000).

Treatment with B and tamarind pulp significantly lowered the phosphorus level which is in accordance with Bharti et al., (2007) and Gupta et al., (2013), respectively.

ALP activity was significantly higher in fluorotic calves as compared to those from the low F area. It is almost a consistent finding in natural and experimental fluorosis (Jagadish et al. 1998; Maiti and Das 2004). Since F stimulates osteoblastic activity and causes lysis of osteocyte (Araya et al., 1990), the increase in ALP can be related to bone changes associated with increased F in the serum (Farley et al., 1987). The protective effect of B on bone and teeth deformation can be assumed from the lower serum activity in fluorotic calves supplied with B. Tamarind pulp also significantly lowered the ALP in the fluorotic calves. The beneficial effect of tamarind on alkaline phosphatase activity might be due to high Ca content in the dried pulp (Gopalan et al., 1982; Ishola et al., 1990).

Significantly higher plasma AST and lower total protein and albumin, indicative of hepatic dysfunction, might be due to hepatotoxic effect of F compounds (Jagadish et al., 1998; Singh et al., 2002). There was significantly lower AST level upon treatment with B which might be due to decreased plasma F burden, thereby reversing the hepatic metabolic status. The anti-oxidative property of B might also contribute this change. But the amount of B fed to the fluorotic calves might not be sufficient enough to elicit any significant alteration in plasma total protein and albumin level. Supplementation of tamarind pulp powder significantly decreased the AST level and increased the total protein in plasma. It has been reported that Tamarindus indica contains flavanoids, β-carotenes and ascorbic acid (Caluwe et al., 2010) which have hepatoprotective effect (Pimple et al., 2007).

There was a significantly higher level of urea and creatinine in the fluorotic calves than the calves from low F zone (Metwalli et al., 1995; Sharma et al., 2010), indicative of degenerative changes in the kidney (Singh and Swarup 1999). Kidneys play an important
role in regulation of total body F burden, and toxic doses of F can result in renal dysfunction by inhibiting various enzyme systems in the kidneys. This increased level might also be due to catabolism of protein because of partial starvation in affected animals (Swarup et al., 2001).

Consequently, both the treatments tended to decrease urea and creatinine levels, tamarind more so than B, but that only tamarind decreased creatinine levels significantly below day 0 levels. This might be due to antioxidative property of tamarind which protects renal and muscular damage. Tamarind also contains Ca and ascorbic acid which were reported to cause inhibition of phosphodiesterase, thereby augmenting c-AMP levels that are involved in the activation of several phosphokinase in liver and muscle (Chinoy et al., 1993). In the present study both tamarind and B supplementation exerted beneficial effect on F concentrations in biological samples and also on haematobiochemical parameters, but tamarind seemed to be more potent than that of B. But as the study was based on grazing animals which were also provided with supplementary feeding (other than treatments), there might be minor differences in F intake. So a meager possibility of biasness in result may not be excluded.

In conclusion, the present study revealed toxic levels of F in environmental samples within 3km radial distance of Al smelter plant, for which grazing of livestock in this area should be avoided as far as possible and provision of ground water(such as deep well or bore well) as drinking water should be practised. In this study, changes in F level in bio-samples and restoration of haematobiochemical parameters after supplementation with tamarind pulp to calves suggest that this compound may play a beneficial role in reducing F exposure and the risk of juvenile fluorosis and its progression.

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**Declaration of Interest**

The authors declare that there is no conflict of interest.

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