

Original Research Article

<https://doi.org/10.20546/ijcmas.2020.907.144>

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

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### ABSTRACT

#### Keywords

Tamarind,  
Fluorosis, Calf,  
Aluminium smelter  
plant

#### Article Info

##### Accepted:

11 June 2020

##### Available Online:

10 July 2020

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 haemato-biochemical 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 haemato-biochemical 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.

### 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 calthood). 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 mottling 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 1<sup>st</sup> 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
<b>Soil</b>	166.07±10.49 <sup>b</sup>	3.3±0.21 <sup>a</sup>
<b>Water</b>		
Surface	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>
<b>Fodder</b>		
Dry	186.83±28.96 <sup>bY</sup>	2.73± 0.02 <sup>a</sup>
Green	139.75±5.41 <sup>bX</sup>	7.15±0.93 <sup>aY</sup>
<b>Milk</b>	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
<b>Plasma F</b>	Group I	0.084±0.005 <sup>A</sup>	0.086±0.005 <sup>A</sup>	0.083±0.003 <sup>A</sup>
	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>cB</sup>	0.321±0.013 <sup>bC</sup>	0.235±0.009 <sup>aC</sup>
	Group IV	0.442±0.017 <sup>cB</sup>	0.257±0.029 <sup>bB</sup>	0.150±0.016 <sup>aB</sup>
<b>Urine F</b>	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>aB</sup>
	Group IV	10.867±0.489 <sup>cB</sup>	8.716±0.631 <sup>bB</sup>	7.283±0.367 <sup>aB</sup>
<b>Faecal F</b>	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>aB</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±0.01 <sup>A</sup>	(-) 0.3±0.08 <sup>B</sup>
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±0.25 <sup>A</sup>	(+) 1.7±0.17 <sup>B</sup>
Total leucocyte count (10 <sup>3</sup> /mm <sup>3</sup> )	(+) 0.23±0.09 <sup>A</sup>	(+) 0.7±0.16 <sup>B</sup>
Packed cell volume (%)	(+) 2.33±0.55 <sup>A</sup>	(+) 4.83±0.47 <sup>B</sup>
Calcium (mg/dl)	(+) 1.16±0.09 <sup>A</sup>	(+) 1.47±0.08 <sup>B</sup>
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±0.37 <sup>A</sup>	(-) 20.72±0.43 <sup>B</sup>
Total Protein (gm/dl)	(+) 0.15±0.02 <sup>A</sup>	(+) 0.36±0.04 <sup>B</sup>
Albumin (gm/dl)	(+) 0.04±0.02	(+) 0.06±0.03
Urea (mg/dl)	(-) 2.93±0.17 <sup>A</sup>	(-) 3.75±0.11 <sup>B</sup>
Creatinine (mg/dl)	(-) 0.12±0.02 <sup>A</sup>	(-) 0.25±0.05 <sup>B</sup>

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±0.32 <sup>B</sup>	11.13±0.25 <sup>B</sup>	11.10±0.28 <sup>D</sup>
	Group II	7.86 ±0.22 <sup>A</sup>	7.83 ±0.19 <sup>A</sup>	7.58 ±0.16 <sup>A</sup>
	Group III	7.70±0.22 <sup>aA</sup>	7.97±0.18 <sup>aA</sup>	8.53 ±0.07 <sup>bB</sup>
	Group IV	7.73 ± 0.11 <sup>aA</sup>	7.87 ± 0.08 <sup>aA</sup>	9.43±0.08 <sup>bC</sup>
Total leucocyte count (10 <sup>3</sup> /mm <sup>3</sup> )	Group I	9.80±0.20 <sup>B</sup>	9.53±0.31 <sup>B</sup>	9.35±0.36 <sup>C</sup>
	Group II	6.95±0.14 <sup>A</sup>	6.67±0.3 <sup>A</sup>	6.63±0.19 <sup>A</sup>
	Group III	6.86±0.18 <sup>A</sup>	6.98±0.19 <sup>A</sup>	7.10±0.20 <sup>AB</sup>
	Group IV	6.94±0.07 <sup>aA</sup>	7.33±0.13 <sup>abA</sup>	7.65±0.16 <sup>bB</sup>
Packed cell volume (%)	Group I	32.83±0.98 <sup>B</sup>	33.5±0.76 <sup>C</sup>	33.3±0.88 <sup>D</sup>
	Group II	23.50±0.76 <sup>A</sup>	23.5±0.56 <sup>A</sup>	23.17±0.48 <sup>A</sup>
	Group III	23.17±0.54 <sup>aA</sup>	24±0.63 <sup>abAB</sup>	25.5±0.22 <sup>bB</sup>
	Group IV	23.66±0.82 <sup>aA</sup>	23.67±0.82 <sup>aAB</sup>	28.5±0.22 <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: Negativecontrol; Group II: Fluorotic calves; Group III: Fluorotic calves receiving boron; Group IV: Fluorotic calves receiving tamarind

**Table.5** Biochemical parameters in calves from both less exposed and fluorotic area at different observation periods

Parameter	Group	Day 0	Day 30	Day 60
<b>Calcium (mg/dl)</b>	I	10.856 ± 0.31 <sup>B</sup>	10.722 ± 0.182 <sup>C</sup>	10.761 ± 0.189 <sup>C</sup>
	II	6.855 ± 0.147 <sup>A</sup>	6.766 ± 0.103 <sup>A</sup>	6.745 ± 0.134 <sup>A</sup>
	III	7.037 ± 0.064 <sup>aA</sup>	7.840 ± 0.058 <sup>bB</sup>	8.197 ± 0.112 <sup>bB</sup>
	IV	7.031 ± 0.058 <sup>aA</sup>	8.039 ± 0.092 <sup>bB</sup>	8.497 ± 0.059 <sup>cB</sup>
<b>Phosphorus (mg/dl)</b>	I	4.847 ± 0.198 <sup>A</sup>	4.834 ± 0.182 <sup>A</sup>	4.763 ± 0.16 <sup>A</sup>
	II	7.053 ± 0.084 <sup>B</sup>	7.015 ± 0.033 <sup>C</sup>	7.042 ± 0.09 <sup>C</sup>
	III	7.133 ± 0.092 <sup>bB</sup>	6.265 ± 0.18 <sup>aB</sup>	5.985 ± 0.091 <sup>aB</sup>
	IV	7.126 ± 0.074 <sup>cB</sup>	6.177 ± 0.141 <sup>bB</sup>	5.783 ± 0.066 <sup>aB</sup>
<b>Alkaline phosphatase (IU/L)</b>	I	150.997 ± 1.007 <sup>A</sup>	147.12 ± 1.051 <sup>A</sup>	144.447 ± 0.903 <sup>A</sup>
	II	185.983 ± 0.622 <sup>B</sup>	190.329 ± 1.300 <sup>C</sup>	193.39 ± 1.170 <sup>C</sup>
	III	185.35 ± 0.940 <sup>cB</sup>	179.224 ± 0.860 <sup>bB</sup>	174.21 ± 0.890 <sup>aB</sup>
	IV	185.639 ± 1.410 <sup>cB</sup>	178.673 ± 2.020 <sup>bB</sup>	173.089 ± 1.930 <sup>aB</sup>
<b>Aspartate transaminase (IU/L)</b>	I	51.790 ± 1.415 <sup>A</sup>	52.074 ± 1.401 <sup>A</sup>	52.349 ± 1.432 <sup>A</sup>
	II	87.271 ± 0.621 <sup>B</sup>	87.498 ± 0.619 <sup>D</sup>	87.584 ± 0.590 <sup>D</sup>
	III	86.375 ± 0.552 <sup>cB</sup>	79.216 ± 0.405 <sup>bC</sup>	70.300 ± 0.458 <sup>aC</sup>
	IV	86.956 ± 0.69 <sup>cB</sup>	75.387 ± 0.378 <sup>bB</sup>	66.251 ± 0.238 <sup>aB</sup>
<b>Total Protein (gm/dl)</b>	I	7.062 ± 0.130 <sup>B</sup>	7.083 ± 0.119 <sup>B</sup>	7.093 ± 0.135 <sup>C</sup>
	II	6.500 ± 0.127 <sup>A</sup>	6.368 ± 0.165 <sup>A</sup>	6.257 ± 0.159 <sup>A</sup>
	III	6.553 ± 0.172 <sup>A</sup>	6.642 ± 0.179 <sup>AB</sup>	6.705 ± 0.166 <sup>ABC</sup>
	IV	6.485 ± 0.102 <sup>aA</sup>	6.653 ± 0.101 <sup>abAB</sup>	6.848 ± 0.114 <sup>bBC</sup>
<b>Albumin (gm/dl)</b>	I	3.662 ± 0.035 <sup>B</sup>	3.576 ± 0.038 <sup>B</sup>	3.514 ± 0.063 <sup>B</sup>
	II	3.130 ± 0.082 <sup>A</sup>	3.081 ± 0.096 <sup>A</sup>	3.025 ± 0.105 <sup>A</sup>
	III	2.972 ± 0.078 <sup>A</sup>	2.993 ± 0.084 <sup>A</sup>	3.016 ± 0.091 <sup>A</sup>
	IV	3.005 ± 0.073 <sup>A</sup>	3.026 ± 0.068 <sup>A</sup>	3.062 ± 0.065 <sup>A</sup>
<b>Urea (mg/dl)</b>	I	27.983 ± 0.879 <sup>A</sup>	28.166 ± 0.742 <sup>A</sup>	27.573 ± 0.526 <sup>A</sup>
	II	36.272 ± 1.833 <sup>B</sup>	37.813 ± 1.931 <sup>C</sup>	37.775 ± 1.106 <sup>C</sup>
	III	36.437 ± 0.974 <sup>B</sup>	34.688 ± 1.094 <sup>B</sup>	33.505 ± 1.070 <sup>B</sup>
	IV	36.880 ± 1.375 <sup>B</sup>	34.414 ± 1.525 <sup>B</sup>	33.134 ± 1.433 <sup>B</sup>
<b>Creatinine (mg/dl)</b>	I	1.133 ± 0.078 <sup>A</sup>	1.160 ± 0.056 <sup>A</sup>	1.178 ± 0.049 <sup>A</sup>
	II	1.834 ± 0.111 <sup>B</sup>	1.860 ± 0.108 <sup>B</sup>	1.890 ± 0.096 <sup>B</sup>
	III	1.880 ± 0.072 <sup>B</sup>	1.805 ± 0.067 <sup>B</sup>	1.763 ± 0.054 <sup>B</sup>
	IV	1.958 ± 0.045 <sup>bB</sup>	1.881 ± 0.044 <sup>abB</sup>	1.710 ± 0.058 <sup>aB</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

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

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 (BF<sub>4</sub>) 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 hypocalcemia. 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,  $\beta$ -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 haemato-biochemical 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 haemato-biochemical 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.

### **Acknowledgment**

Financial support provided by University Grant Commission in terms of Major Research Project to the Dr R. C. Patra, Principal Investigator is thankfully acknowledged.

### **Declaration of Interest**

The authors declare that there is no conflict of interest.

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#### How to cite this article:

Mirashree Pati, Ghana Syam Parida, Kruti Debnath Mandal, Amit Raj Gupta and Ramesh Chandra Patra. 2020. Comparative Ameliorative Efficacy of Tamarind Pulp against Boron on Fluorotic Calves Reared in Periphery Villages of Aluminium Smelter Plant *Int.J.Curr.Microbiol.App.Sci*. 9(07): 1254-1264. doi: <https://doi.org/10.20546/ijcmas.2020.907.144>