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Protective Effect of *Moringa oleifera* on Haematological and Biochemical Parameters of Cattle from Industrial Fluoride Polluted Area

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ABSTRACT

In the present study forty cattle were divided into four groups of ten cattle in each. Group I and II served as healthy and diseased control, respectively. Group III was treated with *Moringa oleifera* fruit powder and group IV was treated with standard chemical antidote i.e. calcium and boron. Blood, urine and faeces were collected from the animals of all the groups on day 0, 30 and 60 of the experiment for estimation of fluoride and haemato-biochemical parameters to evaluate the efficacy of *Moringa oleifera* fruit powder. Fluoride affected cattle revealed significantly higher level of fluoride in serum as compared to normal cattle at different observation periods of the experiment. Significant reduction in fluoride concentration in serum was recorded in group III and group IV animals from day 30 onwards. Altered haemato-biochemical parameters were restored after the supplementation of *Moringa oleifera* fruit powder in fluorotic cows. It is concluded that, dried fruit powder of *Moringa oleifera* had ameliorative potential against industrial fluorosis in cattle and can be used as a fluoride alleviator for control of fluorosis.

Keywords: *Moringa oleifera*, fluorosis, haematology, serum biochemistry, cattle

Fluorosis in cattle can be clinically diagnosed by mottling, discolouration, hypocalcification, pitting of the enamel and excessive abrasion of the teeth. There will be osteofluorotic lesions as osteosclerosis, periosteal hyperosteosis, osteoporosis and osteophytosis (Swarup *et al.* 1998). Fluoride toxicity induces various biochemical changes like increase level of inorganic phosphorus and serum alkaline phosphatase (ALP) activity and decreased concentration of serum calcium (Maiti and Das 2004). Curative treatment is always futile once the bony lesions are prominent. Only the preventive measures become fruitful in known fluorotic areas. Therefore the exposed cattle with clinical manifestations need to be subjected to clinico-biochemical assessments to ascertain the degree of intoxication and suggestive remedial measures. A number of ameliorative agents have been tried, with varying degree of success (Maiti *et al.* 2004; Han-Bo *et al.* 2006). Of these calcium, aluminium, selenium, copper, and boron have shown potential to reduce fluoride toxicity. But some of them also have toxic effects when given in higher doses or for a longer duration of time.

Due to rich bio-diversity of India, a large number of plant species are available for treatment of various toxicities and musculoskeletal disorder. *Moringa oleifera* (Moringacea; English name Horse radish tree, Drumstick tree) is generally considered as vegetable and also used in Indian folk medicine for the treatment of various illnesses (Mishra *et al.* 2011). Extracts of *M. oleifera* seeds have been found to enhance the urinary fluoride excretion in short term trial and it lowered the serum fluoride concentration in rats (Stanley *et al.* 2002). The present study was carried out to assess the ameliorative potential of *M. oleifera* fruit powder in fluorotic cattle reared in periphery villages of aluminium smelter plants.

MATERIALS AND METHODS

Study site

The study site was selected on the basis of reported occurrence of fluorosis in bovine population in some villages around close proximity of aluminium smelter

plant in Odisha, India. Cows reared within 2 km radius of the smelter plant were selected for the present study.

Animals and experimental design

The animals were examined clinically for detection of cardinal signs and lesions suggesting of fluorosis. Among the all animal examined clinically, thirty adult fluorotic cattle (3-6 years) were divided in to three equal groups containing 10 animals in each. Another ten healthy adult cattle were selected from Bhubaneswar, free from fluoride pollution, served as healthy control group. Group I and group II served as healthy and disease control group, respectively. Group III was treated with medicinal herbs i.e. dried *M. oelifera* fruit powder @ 75 grams per animal (Ranjan *et al.* 2009). Group IV was treated with standard chemical antidote i.e. calcium in combination with boron @ 100 mg/kg body wt and 10 mg/kg body wt, respectively.

Plant Material

Unriped fruits of *M. oleifera* were procured from the local market, washed and dried at 40^{o}C in hot air oven for 96 hr and powdered using an electronic grinder. 75 grams of powder was packed into individual polythene packet. Each animals of group III were fed @1 packet daily for 60 days mixed with feed.

Collection of samples

Blood samples were collected on day 0, 30 and 60 of the experiment. About 10 ml of blood was collected from each animal from which 7 ml was kept for clotting and serum was separated for estimation of fluoride and biochemical parameters. Another 3 ml blood was stored in EDTA vial for haematological estimation.

Estimation of fluoride

The fluoride concentration in serum and urine was measured by ion specific potentiometry, using TISAB (Total Ionic Strength Adjustment Buffer) following the method adopted by Cernik *et al*. (1970) and in faeces by the method of Madhavan and Subramanian (2002) with modifications using a portable fluoride ion specific electrode (Orion Model 96-09) and ISE meter (Orion Model- 290A). The detection range of the instrument is in between 0.019 and 1900 ppm. Calibration of the instrument was made using five freshly prepared working standards. The accuracy and precision of the measurements were maintained by repeated analysis of the reference standard procured from Orion Research Incorporated Laboratory, USA.

Haemato-biochemical parameters

Haemoglobin (Hb), packed cell volume (PCV), total leukocyte count (TLC) and differential leukocyte count (DLC) were estimated as per Jain (1986). Calcium (Ca), phosphorus (P), total protein, urea, creatinine and alkaline phosphatise (ALP) activity were evaluated as per manufacturer's instructions using commercially available kits.

Statistical analysis

Data were analysed by one-way analysis of variance (ANOVA), with post hoc analysis by Duncan's multiple comparison tests using SPSS 22 software, and expressed as mean ± SE, with $P < 0.05$ considered statistically significant.

RESULTS AND DISCUSSION

The fluoride concentration in serum, urine and faeces of cattle of different experiment groups at different observation periods is presented in table 1. Fluoride affected cattle revealed significantly ($p < 0.05$) higher level of fluoride in serum as compared to normal cattle at different observation periods. The serum fluoride concentration was decreased significantly ($p < 0.05$) after supplementation of *M. oleifera* from day 30 onwards with respect to cattle reared in fluorotic zone. Significantly ($p < 0.05$) decrease in fluoride level was recorded in standard treatment group i.e. calcium along with boron on day 30 and 60 in relation to fluorotic cattle.

The fluoride affected cattle had significantly ($p < 0.05$) higher urinary fluoride level as compared to healthy cattle at different observation periods of the experiment. Significant ($p < 0.05$) decrease in urinary fluoride level was noted after supplementation of moringa and Ca with B on day 30 onwards as compared to fluorotic cattle received no treatment. Cattle from fluorotic area excreted higher

Table 1: Concentration of fluoride in serum, urine and faeces in cows of different treatment groups

Fluoride concent-ration	Group	Day 0	Day 30	Day 60
Serum (µg/ml)	I	0.12 ± 0.01	0.12 ± 0.01	0.12 ± 0.01
	II	0.59 ± 0.02*	0.61 ± 0.02*	0.63 ± 0.02*
	III	0.58 ± 0.03	0.33 ± 0.01†‡	0.24 ± 0.01†§
	IV	0.64 ± 0.05	0.32 ± 0.01†‡	0.24 ± 0.01†§
Urine (µg/ml)	I	2.75 ± 0.15	2.77 ± 0.14	2.72 ± 0.17
	II	14.83 ± 0.43*	14.77 ± 0.43*	15.41 ± 0.39*
	III	15.63 ± 0.44	9.63 ± 0.04†‡	9.27 ± 0.09†‡
	IV	15.72 ± 0.46	9.43 ± 0.11†‡	9.10 ± 0.28†‡
Faeces (µg/gm)	I	9.00 ± 0.32	9.07 ± 0.34	8.97 ± 0.35
	II	23.58 ± 0.47*	23.60 ± 0.50*	24.01 ± 0.38*
	III	23.91 ± 0.57	30.07 ± 0.99‡	31.13 ± 0.16‡
	IV	24.05 ± 0.40	31.10 ± 0.52‡	31.75 ± 0.34‡

Group I: Healthy control; Group II: Disease control; Group III: Moringa treated group, Group IV: Calcium with boron treated group. The values are expressed as mean ± S.E. (n = 10). *P < 0.05 compared with respective control cattle on different observation periods; †P < 0.05 compared with fluorotic cattle on different observation periods; ‡P < 0.05 compared with day 0 value; §P < 0.05 compared with day 0 and 30 values.

Table 2: Haematological parameters of cows of different treatment groups

Parameters	Group	Day 0	Day 30	Day 60
Hemoglobin concentration (gm %)	I	11.27 ± 0.26	11.18 ± 0.18	11.15 ± 0.15
	II	8.47 ± 0.13*	8.57 ± 0.10*	8.30 ± 0.09*
	III	8.57 ± 0.10	8.87 ± 0.07	9.20 ± 0.05†§
	IV	8.47 ± 0.13	8.77 ± 0.13	8.83 ± 0.12†
PCV value (%)	I	33.83 ± 0.79	33.00 ± 0.45	33.67 ± 0.67
	II	26.00 ± 0.58*	25.00 ± 1.03*	24.67 ± 0.56*
	III	24.00 ± 1.06	26.33 ± 0.92	30.17 ± 0.60†§
	IV	24.83 ± 1.07	26.17 ± 0.79	27.50 ± 0.43†§
Total Leucocyte Count per cubic mm	I	7266 ± 302	7200 ± 208	7233 ± 308
	II	5250 ± 212*	5133 ± 95*	5083 ± 174*
	III	5216 ± 101	5850 ± 174†‡	6333 ± 101†‡
	IV	5206 ± 171	5860 ± 260†‡	6416 ± 175†‡
Lymphocyte (%)	I	53.50 ± 1.64	54.33 ± 0.96	54.17 ± 1.47
	II	67.00 ± 0.58*	67.33 ± 0.42*	68.67 ± 0.21*
	III	68.33 ± 0.88	63.67 ± 0.49†‡	59.67 ± 0.56†§
	IV	66.50 ± 0.62	62.50 ± 0.99†‡	58.67 ± 0.76†§

Neutrophil (%)	I	39.50 ± 1.57	38.17 ± 1.08	38.17 ± 1.54
	II	17.67 ± 0.56*	19.17 ± 0.54*	17.17 ± 0.70*
	III	18.67 ± 0.62	23.67 ± 0.56†‡	29.83 ± 0.48†§
	IV	18.50 ± 1.41	25.50 ± 0.62†‡	32.33 ± 0.50†§
Eosinophil (%)	I	5.83 ± 0.31	5.83 ± 0.31	6.00 ± 0.26
	II	11.00 ± 0.37*	11.00 ± 0.37*	11.00 ± 0.52*
	III	11.00 ± 0.37	8.50 ± 0.43†‡	7.33 ± 0.21†§
	IV	11.17 ± 0.40	8.67 ± 0.20†‡	7.17 ± 0.31†§

Group I: Healthy control; Group II: Disease control; Group III: Moringa treated group, Group IV: Calcium with boron treated group. The values are expressed as mean ± S.E. (n = 10). *P < 0.05 compared with respective control cattle on different observation periods; †P < 0.05 compared with fluorotic cattle on different observation periods; ‡P < 0.05 compared with day 0 value; §P < 0.05 compared with day 0 and 30 values.

Table 3: Serum biochemical parameters of cows of different treatment groups

Parameters	Group	Day 0	Day 30	Day 60
Calcium (mg/dl)	I	10.14 ± 0.22	10.06 ± 0.30	10.35 ± 0.21
	II	5.98 ± 0.55*	6.02 ± 0.42*	6.06 ± 0.25*
	III	5.70 ± 0.17	6.02 ± 0.35	7.19 ± 0.28†§
	IV	6.07 ± 0.35	7.42 ± 0.27†‡	8.96 ± 0.24†§
Phosphorus (mg/dl)	I	5.17 ± 0.02	5.17 ± 0.02	5.17 ± 0.02
	II	7.60 ± 0.14*	7.55 ± 0.05*	7.49 ± 0.10*
	III	7.58 ± 0.03	6.90 ± 0.05†‡	5.88 ± 0.04†§
	IV	7.54 ± 0.03	6.14 ± 0.03†‡	5.48 ± 0.04†§
Total protein (gm/dl)	I	6.87 ± 0.18	6.81 ± 0.16	6.86 ± 0.21
	II	5.25 ± 0.20*	5.20 ± 0.20*	5.20 ± 0.20*
	III	5.20 ± 0.20	6.06 ± 0.11†	6.26 ± 0.17†‡
	IV	5.23 ± 0.19	5.74 ± 0.16	5.92 ± 0.13†‡
Urea (mg/dl)	I	24.12 ± 0.90	23.78 ± 0.74	24.63 ± 0.60
	II	63.30 ± 0.84*	63.67 ± 0.63*	63.43 ± 0.52*
	III	63.43 ± 0.74	61.48 ± 0.63	61.72 ± 0.43
	IV	63.15 ± 0.62	62.05 ± 0.74	61.65 ± 0.49
Creatinine (mg/dl)	I	1.57 ± 0.02	1.58 ± 0.02	1.59 ± 0.02
	II	3.83 ± 0.02*	3.83 ± 0.02*	3.84 ± 0.02*
	III	3.84 ± 0.01	3.82 ± 0.02	3.78 ± 0.01†§
	IV	3.83 ± 0.01	3.81 ± 0.01	3.81 ± 0.01

ALP (IU/L)	I	129.92 ± 3.16	131.69 ± 2.32	131.36 ± 3.13
	II	199.27 ± 6.02*	201.70 ± 3.64*	205.07 ± 5.77*
	III	203.63 ± 5.41	180.62 ± 4.74†‡	156.15 ± 1.72†§
	IV	199.96 ± 10.21	173.43 ± 7.16†‡	153.33 ± 5.09†§

Group I: Healthy control; Group II: Disease control; Group III: Moringa treated group, Group IV: Calcium with boron treated group. The values are expressed as mean ± S.E. (n = 10). $^{*}P < 0.05$ compared with respective control cattle on different observation periods; $^{\dagger}P < 0.05$ compared with fluorotic cattle on different observation periods; $^{\ddagger}P < 0.05$ compared with day 0 value; $^{\S}P < 0.05$ compared with day 0 and 30 values.

level of fluoride through faeces as compared to cattle from non-fluorotic area at different observation periods of the experiment. Significant ($p < 0.05$) increase in fluoride concentration in faeces was recorded at the end of the experiment in both moringa and Ca with B treatment group as compared to disease control group.

Haematological and serum biochemical parameters of different experiment groups at different observation periods are presented in table 2 and table 3, respectively. Altered haemato-biochemical parameters in fluorotic cattle at different observation periods were restored after supplementation of *M. oleifera* and Ca with boron.

Supplementation of *Moringa oleifera* fruit powder was able to reduce the serum fluoride level in affected cattle. Interference with fluoride absorption from the gut might have played a role in reducing serum fluoride concentrations. The lower molecular weight water soluble proteins in moringa seeds have strong positive charge that attracts highly electronegative fluoride ions resulting in formation of flocculants (Mangale *et al.* 2012). Also the presence of tannins, fibers and high concentration of minerals in moringa like calcium, aluminum, phosphorus, manganese, potassium, copper and iron are reported to form insoluble complexes with fluoride in gut (Kawo *et al.* 2009; Anjorin *et al.* 2010). This justifies the enhanced fluoride elimination in faeces leads to reduced absorption from intestine, thereby reduction in urinary and serum fluoride concentration.

Significant lower level of Hb, TLC and PCV were recorded in fluorotic cattle as compared to healthy animals. Fluoride induced altered haematological parameters were also reported in cattle (Dwivedi *et al.* 2000) buffaloes (Singh and Swarup, 1994), and goats (Kant *et al.* 2009) in previous studies. Fluoride exposure depresses bone marrow activity in cattle resulting in normocytic and normochromic anaemia due to reduced erythropoiesis (Dwivedi *et al.* 2000).

Increase in Hb, TLC and PCV value in both the treatment groups after 60 days of treatment might be due to prevention of oxidative damage to cell membrane of RBC (Bharti *et al.* 2007). Significant increase in lymphocyte, eosinophil and decrease in neutrophil percentage was recorded in fluorotic cattle as reported earlier (Dwivedi *et. al.* 2000). Significant restorations of above parameters were observed after supplementation of moringa and calcium with boron to the affected cattle.

Significant decrease in serum calcium and increase in serum phosphorus level was found in fluorotic cattle as compared to healthy animals. This was probably because of the decrease in absorption as well as enhanced excretion of calcium via urine (Bharti *et al.* 2007). These findings are in agreement with earlier workers (Maiti and Das, 2004; Bharti *et al.* 2007). However, supplementation of dried fruit powder of moringa in fluorotic cattle produced significant increase in calcium concentration which was also recorded by Ranjan *et al*. (2009) in fluoride exposed rabbits. The beneficial effect of moringa might be due to its richness in calcium (Kawo *et al.* 2009; Anjorin *et al.* 2010) and reduced absorption or an increase in elimination of fluoride from the body. But Ca and B supplementation is more efficient in increasing calcium concentration in blood which was also found by Maiti and Das (2004). This might be due to directly increased absorption and decreased urinary excretion of Ca due to high calcium intake and protective effect of B against the inhibitory effect of F on Ca ATPase in renal tissues, respectively.

Alkaline phosphatase activity was significantly higher in fluorotic cattle as compared to those from the non-fluorotic area. This finding is in agreement with many other observations reported in fluorotic animals (Maiti and

Das, 2004; Gupta *et al.* 2013). The increase in alkaline phosphatase is related to abnormal bone formation and stimulated osteoblastic activity (Radostits *et al.* 2000).

Supplementation of calcium and boron and dried fruit powder of moringa to the fluorotic cattle significantly reduced the activity of alkaline phosphatase. The beneficial effect of moringa on reduction of alkaline phosphatase activity might be due to the presence of high calcium content in the dried moringa fruit powder (Kawo *et al.* 2009). The significant lower level of serum total protein in fluorotic cattle is indicative of hepatic dysfunction (Maiti and Das 2004).

Supplementation of moringa significantly increased the serum total protein level in fluorotic cattle. It is reported that moringa contains monoterpenes, glycosides, organic acids, lipids, alkaloids, xanthenes, flavanoids (quercetin), β-carotenes and ascorbic acid which have hepatoprotective effect owed to anti-oxidant property (Aja *et al.* 2014).

Significant higher level of urea and creatinine in the fluorotic cattle was recorded than the cattle from non-fluorotic zone (Maiti and Das 2004). Kidneys play an important role in regulation of total body fluoride burden, and toxic doses of fluoride can result in renal dysfunction by inhibiting various enzyme systems in the kidneys. Consequent upon the treatment with moringa dried fruit powder, the creatinine level decreased significantly at 60 days of treatment and the urea level decreased non-significantly. This might be due to antioxidative property, reduced fluoride burden and higher Ca content of dried moringa fruit powder which protects renal and muscular damage (Ranjan *et al.* 2009). However, the protective effect of Ca and B might not be strong enough to alter the urea and creatinine level as compared to moringa.

The present study concludes that, dried fruit powder of *M. oleifera* have comparable ameliorative efficacy to standard chemical antidote i.e. Ca and B and can be used as a fluoride alleviator for control of fluorosis in fluorotic cattle.

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