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Effect of *Tamarindus indica* seed powder to ameliorate fluoride toxicity in cattle

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Abstract
Present study confirmed the presence of fluoride (0.7 to 2.6µg/ml)) to toxic levels in drinking water of some villages of Durg district of Chhattisgarh State. Significant increased concentration of fluoride was also recorded in blood and urine samples of cattle showing clinical signs of flurosis in affected locations. Present study also revealed the status of oxidative stress due to increase fluoride level in blood and antioxidant potential of *Tamarindus indica* L seed powder (TISP) on fluoride induced oxidative stress. The correlation between fluoride body burden and lipid peroxidation was found positive whereas negative correlation exist with blood fluoride levels and Reduced Glutathione level (GSH), antioxidant enzymes like Super oxide dismutase (SOD) and Catalase (CAT). *Tamarindus indica* L seed powder (TISP) was found effective to increase excretion of fluoride in urine and significant (p<0.05) alleviate the toxic effects of fluoride and overcome fluoride toxicity in animals from the affected area.

Keywords: Cattle, fluoride, toxicity, tamarind, seed, antioxidant

1. Introduction
Fluoride is a highly reactive halogen element that binds with almost all cations to form stable fluoride complexes. Soluble fluoride is distributed over the earth's surface and atmosphere as a result of natural processes such as erosion, hydraulic leaching, volcanic activity, and to a lesser extent by mining and manufacturing processes. Animals when grazing over soluble fluoride rich soil can ingest toxic doses of fluoride, especially if the pasture is overgrazed and animals are grazing small plants close to the soil [1]. Drinking water fluoride concentration more than 1.5 may result in clinical symptoms of fluorosis [2]. Both acute and chronic forms of toxicity may occur due to excess fluoride in animals. However, the chronic form of the disease, termed as fluorosis, is a condition in which characteristic toxic effects result from prolonged ingestion of elevated level of fluoride in food and water [3].
Chronic exposure to fluoride induces an array of deleterious impacts in livestock animals, experimental animals, as well as in humans leading to a progressive degenerative disease, dental mottling and several types of skeletal dysfunctions [4, 5]. Fluoride also affects non-skeletal organs such as the liver, kidney, heart and brain [4]. Main mechanism of these deformities, after exposure of F is mainly the generation of different types of ROS production [6]. Chronic exposure of fluoride could induce the loss of structure and function of skeletal muscles, brain and spinal cord [7]. Free radical generation, LPO, and altered antioxidant systems are considered to play a vital role in posing toxic effects of F. Increased oxidative stress has also been directly linked to oxidation of macromolecules of the cell that may cause injury to the brain or induce a variety of adverse cellular responses [8].
Excess fluoride (F) intake in mammals exerts toxic health effects in many ways, including inhibition of enzymes, production of free radicals and lipid peroxidation (LPO) and deposition in various tissues, especially bones and teeth [9]. Recently, certain medicinal herbs have been found to enhance urinary fluoride excretion and reduce oxidative stress in fluoride intoxication [10].
Therefore, the present study was undertaken to study the fluoride concentration in affected locations responsible for fluorosis in cattles, oxidative stress induced due to fluoride exposure and ameliorative potential of *Tamarindus indica* seed powder in fluoride toxicity

2. Materials and Methods
2.1 Study area: A survey was conducted in some villages of Durg district of Chhattisgarh state with the complaint of deep-brownish staining teeth, swollening joints, lameness, debility,

mortality in livestock population. Drinking water samples of underground water was collected to determine the fluoride concentration and cattle of the affected region were examined for various signs and symptoms of fluorosis. The villages with water fluoride concentration more than 1.5 ppm and cattle showing signs of dental lesions or lameness were considered fluorotic.

2.2 Preparation of tamarind seed powder: *Tamarindus indica* fruit was procured from the local market. After proper authentication, seeds were separated from pods, washed properly using distilled water. Thereafter seeds were dried in hot air oven at 80 ^{0}C and powdered finely with the help of grinder and stored in air tight jar for further use.

2.3 Experimental design: Twenty cattle with clinical signs of fluorosis, in between age group of 3- 6 years belonging to either sex were randomly selected and further divided into 2 groups (Group –II and III) with each group comprising of 10 animals. The animals of Group-I were selected from non-fluorotic area (College of veterinary science & A.H., Anjora dairy farm) and served as a healthy control group. Group II animals of affected area were not subjected to any treatment while the animals of Group-III were treated by oral administration of dried powder of *Tamarindus indica* L. seed @ 100 grams/day for six weeks.

Collection of samples: Blood and urine samples in desirable volume were collected 0 and 45th day of the trial and processed to determine the body fluoride burden. Serum sample (1 ml) was taken in a clean plastic beaker to which 25 ml distilled water was added followed by addition of TISAB II in the ratio of 1:1 with water. About 100 ml of urine was collected from each animal in a clean polypropylene bottle for fluoride and various other biochemical analyses. Urine samples were acidified with acetic acid (@ 1ml acetic acid /100 ml urine) and maintain at -4 ^{o}C till used**.** The fluoride concentration was measured by ion specific potentiometry, using a portable fluoride ion specific electrode. The fluoride responsive electrode was then calibrated with a standard stock solution of fluoride and the concentration of fluoride in the sample was determined by using microprocessor ionalyzer (Model 1901).

Blood samples were also processed for the measurement of Lipid peroxidation and Antioxidant status of the body.

Lipid peroxide level in blood was determined following the method of Placer *et al.* [11]. The nmol of MDA (malonaldehyde) per ml of RBC haemolysate was calculated using 1.56×10^5 as the extinction coefficient [12]. Lipid peroxide level in the erythrocyte was expressed as nmol MDA/mg of haemoglobin. Haemoglobin was estimated in blood by cyanomethaemoglobin method and the results were expressed in gm%. The non enzymatic antioxidant glutathione (GSH) is estimated by dithio-bis-2-nitro benzoic acid (DTNB) method as per the procedure of Prins and Loos [13]. SOD activity was measured as per the method of Marklund and Marklund [14] was expressed in unit/mg of haemoglobin. Catalase enzyme activity was estimated spectrophotometrically in RBC hemolysate after appropriate dilution following the method of Cohen *et al.* [15] was expressed in unit/mg of haemoglobin

2.4 Statistical analysis: Analysis of Variance (ANOVA) and Duncan's multiple range test (DMRT) were used to compare the means at different time intervals among different groups. Paired't' test was used to compare the mean at different intervals with respective base values in each group [16].

3. Results and Discussion

3.1 Fluoride concentration: It was found that the level of F in ground water samples of studied area ranges from 0.7 to 2.6 (µg/ml) which is quiet higher than the maximum permissible limit of WHO. As the F percolates from the soil to ground water through leaching process the concentration of fluoride was observed more in ground water as compared to surface water [17].

Fluoride concentration was significantly (P<0.05) increased in blood and urine samples of group II and III animals as compared to control healthy group (Table 1). At the end of 6 weeks, animals treated with *Tamarindus indica* fruit powder (TIFP) showed significant reduction in fluoride level in blood samples whereas increased concentration in urine, confirm the excretion of fluoride and thereby reduced body burden. Higher concentrations of fluoride in serum and urine in animals from fluorosis endemic areas have been reported by various authors [18].

Table 1: Effects of *Tamarindus indica* L. on fluoride concentration in biological fluids

Groups	Blood (µg/ml)	Urine (µg/ml)
Healthy control	0.04±0.02	0.76±0.04
Fluoride Intoxicated	3.78±0.22*	5.47±0.38*
Fluoride + TISP	1.45±0.41^a	8.33±0.23^a

Values are mean ± SE; n = 10; * (p<0.05) Fluoride intoxicated compared to Healthy control and fluoride intoxicated untreated compared with fluoride exposed and TISP treated.

The beneficial effect of tamarind might be due to tannin and high fiber contents in dried pulp. In addition, high contents of calcium, copper and other minerals amino acids and vitamin in the dried pulp of the tamarind fruit might have some synergistic role in fluoride removal from the body and other associated beneficial effects [19].

3.2 Oxidative stress and antioxidants

Oxidative stress produced with an increased production of ROS and decreased a level of antioxidants in the body. LPO induces disturbance in the structure of cellular membrane, causes alteration in the integrity, permeability and functional disturbances, which modifies low density lipoprotein (LDL) and generates potentially toxic products [20]. Further, LPO products have been shown to be mutagenic and carcinogenic. MDA has been widely used as a biomarker for lipid peroxidation of essential fatty acids and provide a good measure of peroxidation, which is the chief mechanism of cell damage [21]. Present study confirms the significant (P<0.05) elevation of MDA level in animals of fluorotic area as compared to the healthy controls. The results of the present findings corroborates with the finding by Cetin *et al.* [22]. But significant (P<0.05) decline in lipid peroxidation was observed in animals of group III receiving treatment of Tamarindus indica fruit extract (Table 2). Ekambaram *et al.* [23] also observed free radical scavenging effect of tamarind pulp is authenticated by the decreased MDA content in fluoride toxicity studies.

Table: 2. Effects of *Tamarindus indica* L. on fluoride induced oxidative stress markers

Groups	LPO (nmol/g Hb)	GSH (µg/ml)	CAT (µmol H_2O_2 decomposed/min/mg Hb)	Superoxide Dismutase (IU/mg Hb)
Healthy control	12.81±0.28	15.29±0.55	142.53±2.12	4.42±0.34
Fluoride Intoxicated	28.67±0.34*	10.40±0.60*	113.37±1.38*	2.26±0.45*
Fluoride + TISP	21.06±0.72[a]	12.36±0.17[a]	124.21±1.82[a]	3.38±0.24[a]

Values are mean ± SE; n = 10; * (p<0.05) Fluoride intoxicated compared to Healthy control and fluoride intoxicated untreated compared with fluoride exposed and TISP treated.

Reduced glutathione emphasize the importance of maintaining the redox state of the cell [24]. Over production of reactive oxygen species at the mitochondrial level results in decrease of reduced Glutathione (GSH) and damage of cellular components [25]. The present study revealed a significant (P<0.05) decrease in GSH concentration in group II animals which may be due to conversion of reduced form to oxidized form (GSSH) by excessive production of reactive oxygen species. Present study supported the findings of Lim *et al*. [24]. Tamarind pulp has phenolic compounds with antioxidants properties results in amelioration of ROS generated to fluoride toxicity and responsible for increased GSH level in treatment group.

The role and effectiveness of the first line defense antioxidants which basically include superoxide dismutase (SOD), catalase (CAT). These enzymes respectively dismutate superoxide radical, breakdown hydrogen peroxides and hydroperoxides to harmless molecules [26]. Present study reported that the significant decrease activity of SOD and CAT enzymes in animals showing signs of fluorosis as compared to healthy control animals. Fluoride is an electronegative halogen and has affinity toward micro minerals like Cu and Zn and decreases their absorption results in increase in an urinary fecal excretion responsible for its decrease status in the body. A downward trend of Zn, Cu effects arising from oxidative stress induced by fluorosis [27]. These metals are important components of the enzyme structure and have affected the antioxidant capacity of the enzyme system [28]. Total antioxidant activity *Tamarindus indica* L. seeds are due to high flavonoid, tannin, polyphenol, anthocyanin and oligomeric proanthocyanidin content [29]. *Tamarindus indica* fruit extract treatment was found effective to reduce lipid peroxidation and restore the normal antioxidant status in group III animals. A similar observation was reported by [30].

Conclusion

Thus, it can be concluded that drinking ground water in some villages of Durg district of Chhattisgarh state is contaminated with fluoride and is responsible for dental and skeletal deformities. The present results also corroborate that the F induces the oxidative stress by increasing pro-oxidant LPO and decreasing the antioxidants like glutathione. Decreased activity of SOD and catalase was observed which concomitantly may affect the health status of animal and responsible for the clinical signs exhibiting by them. The administration of *Tamarindus indica L* seed powder in intoxicated animals was found to be effective in excretion of fluoride burden of body and reversing the oxidative stress.

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