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Ameliorative effect of *Tamarindus indica* L. on biochemical parameters of serum and urine in cattle from fluoride endemic area

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ABSTRACT

The present study was undertaken to evaluate the ameliorative effect of tamarind (*Tamarindus indica* L.) in endemic fluorosis in cattle. Eighteen cows (3-6 years) were divided into three groups (Group I, II and III) of six animals in each. Groups I and II served as healthy and disease controls, respectively. Dried powder of tamarind fruit pulp was given for 90 days to Group III (treatment group) at the dose rate of 100 grams per animal. Calcium, phosphorus, alkaline phosphatase and fluoride concentration in serum and collagen degradation marker i.e. hydroxyproline in urine were considered for evaluating the efficacy of tamarind fruit pulp. A significant increase in calcium level was observed in cows treated with tamarind (6.49 ± 0.17 mg/dL) as compared to the disease control (5.80 ± 0.41 mg/dL). Increased activity of serum alkaline phosphatase (118.38 ± 2.93 units/L) and the higher concentration of hydroxyproline (27.88 ± 1.01 µg/mL) in cows of fluoride endemic area were decreased significantly ($P<0.05$) after supplementation of tamarind (95.24 ± 2.76 units/L and 20.17 ± 1.56 µg/mL, respectively). In conclusion the present study found that dried powder of *Tamarindus indica* fruit pulp has ameliorative potential on management of fluorosis in cattle.

Key words: amelioration, tamarind, fluorosis, hydroxyproline

Introduction

Environmental contamination due to various chemicals and its subsequent deleterious health effects, in overt or subtle forms in human and animal, merits serious attention

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globally. Fluorosis is a major health problem in both man and animals all over the world (MEHEDINTU et al., 2000; LOGANATHAN et al., 2003; CINAR and SELCUK, 2005). Currently, the human population in at least seventeen states of India and animals from over nine states are suffering from fluoride toxicity related problems in various forms or manifestations (SUSHEELA, 1999). In India, the main source of fluoride for animals are fluoride rich effluents, dust and smoke from aluminium smelters, superphosphate fertilizer plants and brick kilns (SWARUP et al., 1998; PATRA et al., 2000). Other sources include fluoride rich drinking water, feed supplements and mineral mixtures (MURALIDHARA et al., 2000). In addition, steel production plants, ceramic factories, coal burning power plants, glassworks and oil refineries also release fluoride rich effluents.

Fluorine, mostly found in the form of fluorides, is a highly reactive halogen element that binds with almost all cations to form stable fluoride complexes. 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 more common and widespread in as many as 23 countries over Asia, Africa, America and Australia (ANONYM., 2002). Management of fluoride toxicity in animals is a difficult task, as the effect of fluorosis is irreversible. A number of ameliorative agents have been tried, with varying degree of success. Of these, calcium (JOWSEY and RIGGS, 1978), aluminium (KESSABI et al., 1986), selenium (HAN-BO et al., 2001), copper (KHANDARE et al., 2005) and boron (MAITI et al., 2004) have shown potential to reduce fluoride toxicity. But some of them also have toxic effect when given in higher doses or for a longer duration of time e.g. toxic effects of aluminium are enhanced in the presence of fluoride ions (VAN-DER-VOET et al., 1999).

Herbal medicines are plant derived preparations with potential health benefits and their active ingredients are components of herbal medicines with definite therapeutic or prophylactic activities (ANONYM., 1993). The rich biodiversity of our country has gifted more than 1000 plant species for ethno-veterinary use (JAIN, 2000). Recently, certain medicinal herbs have been found to enhance urinary fluoride excretion and reduce oxidative stress in fluoride intoxication in laboratory animals (KHANDARE et al., 2000; STANLEY et al., 2002). *Tamarindus indica* L., (Leguminosae; English name: Tamarind, Hindi: Imli or Ambli) is a tree native to Africa, also cultivated in Sudan, Indonesia, Pakistan, Philippines, Java, Spain and Mexico (MARTINELLO et al., 2006), and has been shown to exert a beneficial effect on fluoride toxicity (KHANDARE et al., 2000; KHANDARE et al., 2010). Earlier studies from our laboratory suggested the beneficial effect of tamarind ingestion on fluoride toxicity by way of increased urinary excretion and decreased retention in the bones of experimental animals (RANJAN et al., 2009; DEY et al., 2011). However, the efficacy of tamarind in ruminants, especially in natural fluorosis, has never been assessed. Therefore, the present study was conducted to evaluate the ameliorative effect of dried powder of tamarind pulp in endemic fluorosis in cattle.

Materials and methods

Plant material. Pods of tamarind were procured from the local market and fruit pulp was separated manually. The fruit pulp was dried in an oven at 40^{o}C and powdered using an electronic grinder. The plant material was duly authenticated by the Central National Herbarium, Botanical Survey of India, Government of India (Botanical Garden, Howrah-103) and voucher specimen no. CNH/I-I (174)/2007/Tech-II/104 was assigned to it.

Study site. The study site is located 20 km from Chittorgarh city in Rajasthan at latitude 24.88^{o}N and longitude 74.63^{o}E with a recent emergence or persisting problem of fluorosis in the human and/or animal population. There are several superphosphate fertilizer plants located within 4 km radial distance from the study site. Cows (3-6 years) living in the Bareilly district, and free from fluoride pollution, served as control.

Animals and experimental design. Animals in the fluoride endemic area were examined clinically for detection of cardinal signs and lesions of fluorosis, i.e. lesions in the teeth, bony exostosis in metacarpal, metatarsal, ribs and frontal bones and lameness. Eighteen cows (3-6 years age group) were divided in three equal groups of six animals in each. Group I and II served as healthy and disease control, respectively. Group III (treatment group) animals received dried powder of tamarind fruit pulp with feed for 90 days at the dose rate of 100 grams per animal.

Collection of blood and urine samples. Blood samples were collected by jugular venepuncture on day 0, 30, 60 and 90 of experiment from all the groups and serum was harvested after clotting of blood. Urine samples were collected freshly in polypropylene tubes with toluene for estimation of hydroxyproline.

Biochemical parameters in serum and urine. The fluoride concentration in serum was measured by ion specific potentiometry, using TISAB (total ionic strength adjustment buffer) and following the method of CERNIK et al. (1970) 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 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.

Calcium, phosphorus concentrations and activity of alkaline phosphatase were determined in serum samples using commercially available kits and the concentration of hydroxyproline in the urine was estimated by the method of KIVIRIKKO et al. (1967).

Statistical analysis. Each sample was run in duplicate. Data were analyzed by one-way analysis of variance (ANOVA), with post hoc analysis by Duncan's multiple comparison tests using SPSS 11.5 software, and expressed as mean ± SE, with $P<0.05$ considered statistically significant.

Results

Fluoride concentrations in the serum of different groups is shown in Fig. 1. The mean fluoride concentration in the serum of fluorotic animals (Group II) was significantly ($P<0.05$) higher than the corresponding healthy control (Group I) values at different observation periods. The fluoride concentration in serum reduced significantly ($P<0.05$) from day 30 onwards in cows receiving treatment (Group III) and the concentration on day 90 was 41.79% lower than the untreated fluorotic animals.

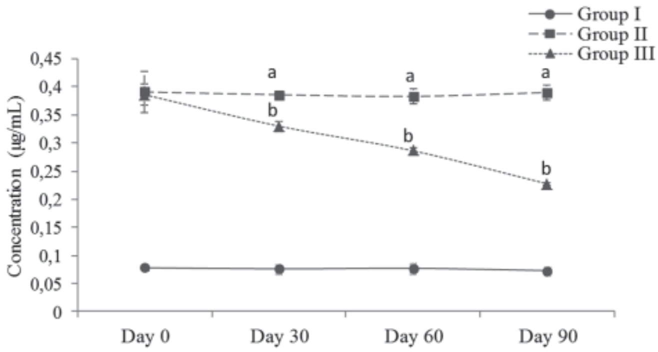

Fig. 1. Fluoride concentration in serum of cows from the fluoride polluted area before and after treatment: "a" indicates the significant difference between the healthy control and cows from fluoride endemic area; "b" indicates the significant difference between fluorotic and tamarind treated cows, ($^{a}P<0.05$, $^{b}P<0.05$).

Table 1. Calcium and phosphorus concentration (mg/dL) and activity of alkaline phosphatase (units/L) in serum of cows from fluoride polluted area before and after treatment

Parameter	Group	Day 0	Day 30	Day 60	Day 90
Calcium	I	10.75 ± 0.63^{B}	10.84 ± 0.38^{C}	10.69 ± 0.25^{C}	10.85 ± 0.64^{B}
	II	5.67 ± 0.25^{A}	5.70 ± 0.56^{A}	5.84 ± 0.17^{A}	5.80 ± 0.41^{A}
	III	6.04 ± 0.28^{A}	7.33 ± 0.29^{B}	7.07 ± 0.53^{B}	6.49 ± 0.17^{A}
Phosphorus	I	4.68 ± 0.29	4.67 ± 0.06	4.71 ± 0.48	4.69 ± 0.28
	II	5.3 ± 0.21	5.29 ± 0.15	5.28 ± 0.19	5.29 ± 0.21
	III	5.25 ± 0.22	5.18 ± 0.33	5.05 ± 0.23	4.99 ± 0.11
Alkaline phosphatase	I	36.41 ± 2.02^{A}	35.63 ± 2.93^{A}	36.93 ± 1.00^{A}	36.02 ± 0.97^{A}
	II	120.66 ± 3.15^{B}	119.97 ± 2.56^{B}	125.54 ± 3.19^{C}	118.38 ± 2.93^{C}
	III	119.34 ± 8.00^{cB}	114.20 ± 1.89^{bcB}	105.23 ± 1.05^{bB}	95.24 ± 2.76^{aB}

The mean (mean ± SE, n = 6) values bearing no common superscript in small letters in a row and capital letters in a column differ significantly at $P<0.05$. Group I: Healthy control; Group II: Fluorotic cows; Group III: Fluorotic cows receiving treatment.

Table 2. Hydroxyproline concentration (µg/mL) in urine of cows from fluoride polluted area before and after treatment

Group	Day 0	Day 30	Day 60	Day 90
I	13.75 ± 0.47^{A}	13.64 ± 0.47^{A}	13.87 ± 0.096^{A}	13.79 ± 0.35^{A}
II	27.55 ± 2.47^{B}	29.02 ± 3.63^{B}	28.55 ± 0.87^{C}	27.88 ± 1.01^{C}
III	27.55 ± 1.89^{cB}	25.65 ± 0.56^{bcB}	22.24 ± 2.13^{abB}	20.17 ± 1.56^{aB}

The mean (mean ± S.E., n = 6) values bearing no common superscript in small letters in a row and capital letters in a column differ significantly at P<0.05. Group I: Healthy control; Group II: Fluorotic cows; Group III: Fluorotic cows receiving treatment.

The mean calcium concentration in serum was significantly (P<0.05) lower in cows from the fluorotic area (group II) at different observation periods as compared to control animals (Group I) (Table 1). After giving the treatment, the calcium concentration in serum increased significantly (P<0.05) from day 30 onwards as compared to fluorotic animals. The phosphorus concentration in fluorotic animals (Group II) was slightly higher compared to healthy control animals (Group I) but they were non significant, and the treatment had no significant effect on the concentration of phosphorus in serum (Table 1).

Serum alkaline phosphatase activity was significantly (P<0.05) higher in fluorotic animals (Group II) as compared to healthy control animals (Group I) at different observation periods (Table 1). Animals receiving the treatment (Group III), showed a significant (P<0.05) decrease in alkaline phosphatase activity on day 60 (105.23 ± 1.05 vs. 125.54 ± 3.19 units/L) and 90 (95.24 ± 2.76 vs. 118.38 ± 2.93 units/L) as compared to fluorotic animals (Group II).

Table 2 depicts the concentration of hydroxyproline in urine. The hydroxyproline concentration was found to be significantly (P<0.05) higher in cows from the fluorotic area (Group II) as compared to animals living in the fluoride free area (group I). After receiving the treatment (Group III), the concentration of hydroxyproline in urine was significantly reduced (P<0.05) from day 30 onwards and the concentration was 11.61%, 22.10% and 27.65% lower than the untreated fluorotic animals (Group II) on day 30, 60 and 90 respectively.

Discussion

Higher concentrations of fluoride in serum and urine in animals from fluorosis endemic areas have been reported by various authors (PATRA et al., 2000; MURALIDHARA et al., 2000; MAITI and DAS, 2004). In the present study, the concentration of fluoride in the serum of fluorotic cows was significantly higher compared to healthy control cows. Supplementation of tamarind fruit pulp powder in the diet significantly reduced the fluoride concentration in the serum. Interference with fluoride absorption from the gut due to administration of dried pulp of tamarind fruits might have played a role in reducing serum fluoride concentrations. The beneficial effect of tamarind might be due to tannin and high fibre contents in dried pulp (ISHOLA et al., 1990; EL-SIDDIG et al., 2006). In addition, high contents of calcium, copper and other minerals, amino acids and vitamin (ISHOLA et al., 1990; ALMEIDA et al., 2009) in the dried pulp of the tamarind fruit might have some synergistic role in fluoride removal from the body or other associated beneficial effects.

A significantly lower concentration of calcium was noted in fluorotic cows 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). As fluoride is a highly electronegative element with a strong affinity towards electropositive elements, in the gastrointestinal tract, fluoride binds with calcium, thereby reduces their absorption (ANONYM., 2002) and causes hypocalcaemia (McIVOR, 1990). These findings are in agreement with those of earlier workers (MAITI and DAS, 2004; BHARTI et al., 2007) who also reported a decline in serum calcium in fluorotic animals. However, supplementations of dried fruit pulp of tamarind to the fluorotic animals produced a significant increase in calcium concentration. The beneficial effect of tamarind might be due to reduced absorption or an increase in excretion of fluoride from the body. KHANDARE et al. (2000) reported an increase in fluoride excretion in dogs given tamarind fruit pulp paste orally, along with fluoride.

The alkaline phosphatase level has diagnostic importance and indicates the damage to the bone and the affect on developing skeleton tissue of animals suffering from fluorosis, as phosphatase is associated with normal bone formation. Alkaline phosphatase activity was significantly ($P<0.05$) higher in fluorotic cows as compared to those from the non fluorotic area. This finding was in agreement with many other observations reported in fluorotic animals (MAITI and DAS, 2004; UPADHYAYA et al., 2005). Since fluoride stimulates osteoblastic activity (ARAYA et al., 1990), the increase in alkaline phosphatase can probably be related to abnormal bone formation and stimulated osteoblastic activity with increased fluoride concentration in the serum (FARLEY et al., 1987). Supplementation of dried pulp of tamarind fruits to the fluorotic cows significantly reduced the activity of alkaline phosphatase. The beneficial effect of tamarind on reduction of alkaline phosphatase activity might be due to the presence of high calcium content in the dried pulp (ISHOLA et al., 1990; EL-SIDDIG et al., 2006). Since supplementation of calcium has been reported by various authors to decrease the serum alkaline phosphatase activity in laboratory animals (HE et al., 2008) and fluorotic cattle (MAITI et al., 2004), the decrease in alkaline phosphatase activity could be due to its high content of calcium in the dried pulp of tamarind as reported earlier.

Hydroxyprolinuria has been observed in laboratory animals after fluoride intoxication (KHANDARE et al., 2005) and in human beings from a fluoride endemic area (ANASUYA and RAO, 1974). The present study has shown the higher concentration of hydroxyproline in urine from cows from the fluorotic endemic area as compared to cows from the non fluorotic area. Enhanced hydroxyproline in urine is due to collagen breakdown as a result of disturbances in the formation of cross-links in bone collagen (CURREY et al., 1996). Supplementation of dried pulp of tamarind fruit to the fluorotic cows significantly reduced the concentration of hydroxyproline in urine as compared to other untreated cows from this area. The ameliorative effect of dried pulp of tamarind fruit on reducing the concentration of hydroxyproline in urine might be due to the presence of copper in dried pulp of tamarind (ISHOLA et al., 1990; ALMEIDA et al., 2009). Copper is a cofactor required for enzyme lysyl oxidase, which helps in the formation of the collagen cross-links (SIEGEL and MARTIN, 1970), thus reducing the collagen breakdown. The beneficial effect of copper has been reported by KHANDARE et al. (2005) on fluoride intoxicated rabbits. They observed the decrease in urinary hydroxyproline after supplementation of copper with fluoride.

The results of the present study suggested that dried powder of *Tamarindus indica* fruit pulp has ameliorative potential on the management of fluorosis in cattle. However, further study is warranted aimed at the fractionation and identification of the active ingredients of tamarind fruit pulp responsible for amelioration of fluorosis.

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SAŽETAK

Istraživanje je imalo cilj procijeniti pozitivan učinak tamarindovca (*Tamarindus indica L.*) na endemsku fluorozu goveda. Osamnaest krava (u dobi 3 - 6 godina) bilo je podijeljeno u tri skupine (I,II i III) po šest životinja u svakoj skupini. Skupine I i II poslužile su kao kontrola zdravih i bolesnih životinja. Trećoj (pokusnoj) skupini davan je suhi prašak pulpe tamarindova ploda , tijekom 90 dana, u dozi od 100 grama po životinji. Učinkovitost praška procijenjivana je na temelju koncentracije kalcija, fosfora i alkalne fosfataze u serumu te hidroksiprolina u mokraći kao biljega za razgradnju kolagena. Statistički značajno viša razina kalcija bila je utvrđena u krava kojima je bio primijenjen tamarindovac (6,49 ± 0,17 mg/dL) u usporedbi s kontrolnom skupinom bolesnih krava (5,80 ± 0,41 mg/dL). Povišena aktivnost serumske alkalne fosfataze (118,38 ± 2,93 jedinica/L) u krava iz endemskih područja fluoroze bila je statistički značajno (P<0,05) snižena (95,24 ± 2,76 jedinica/L) nakon dodavanja tamarindovca. Isto je utvrđeno i za povišenu koncentraciju hidroksiprolina (27,88 ± 1,01 µg/mL) koja je nakon dodavanja tamarindovca bila statistički značajno (P<0,05) snižena na razinu od 20,17 ± 1,56 µg/mL. Ovo istraživanje potvrđuje da suhi prašak dobiven iz srži ploda biljke *Tamarindus indica* ima potencijal za poboljšanje fluoroze u goveda.

Ključne riječi: poboljšanje, tamarindovac, fluoroza, hidroksiprolin