Ameliorative potential of *Tamarindus indica* leaves in fluorotic calves and its socioeconomic impact assessment

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A Thesis submitted to
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in
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By
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CERTIFICATE-I

This is to certify that the thesis entitled **"Ameliorative Potential of *Tamarindus indica* Leaves in Fluorotic Calves and Its Socioeconomic Impact Assessment"** submitted in partial fulfilment of the requirements for the award of the degree of **Master of Veterinary Science (Department of Clinical Veterinary Medicine, Ethics and Jurisprudence)** to the Orissa University of Agriculture and Technology is a faithful record of bonafide and original research work carried out by **Dr. Saumya Ranjan Sahoo** under my guidance and supervision. No part of this thesis has been submitted for any other degree or diploma.

It is further certified that the assistance and help received by him from various sources during the course of investigation has been duly acknowledged.

CHAIRMAN
ADVISORY COMMITTEE

CERTIFICATE-II

This is to certify that the thesis entitled **"Ameliorative Potential of *Tamarindus indica* Leaves in Fluorotic Calves and Its Socioeconomic Impact Assessment"** submitted by **Dr. Saumya Ranjan Sahoo** to the Orissa University of Agriculture and Technology, Bhubaneswar, in partial fulfilment of the requirements for the degree of **Master of Veterinary Science** (**Department of Veterinary Clinical Medicine, Ethics and Jurisprudence)** has been approved/disapproved by the student's Advisory Committee and the external examiner.

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ABSTRACT

The present study was aimed at assessing the impact of fluoride pollution on fluorotic calves reared in emerging fluoride-rich industrialized areas with particular reference to evaluate the impact of industrial fluorosis on socioeconomic conditions of rural farmers and to evaluate the functional effects including haemato-biochemical parameters, and the ameliorative efficacy of tamarind leaves in fluorotic calves**.** The investigation was carried out in the vicinity of the aluminium smelter plant, NALCO, Angul, Odisha. The socioeconomic study was conducted among 30 farmers belonging to five adjacent villages of the plant, whose animals have suffered with fluorine contaminants. Forty fluorotic calves having clinical signs of dental fluorosis were selected and screened for fluoride levels in their bio-samples. The selected calves were divided equally into 4 groups for the present research work. Among them, one group served as disease control. Tamarind leaves powder of 5g and 10g were supplemented to two groups respectively orally on daily basis for 60 days and the standard chemical antidote i.e. Calcium was also supplemented for the same period to other group. Blood, urine and faeces were collected from the calves of all the groups on day 0, 30 and 60 of the experiment for estimation of fluoride and hemato-biochemical parameters to evaluate the efficacy of *Tamrindus indica* leaf powder.

Socioeconomic status of the farmers residing in the vicinity of the aluminium smelter is affected owed to following factors (1) The life span of animals gradually decreases in fluorotic affected animal (2) Animals having skeletal form of fluorosis drought power decreases gradually due to development of lameness (3) Milk production decreases gradually (4) Growth rate of the animal decreases gradually (5) The animals can not be sold due to poor growth, and loss of productivity so the socio economic status of the farmer not so developed (6) Dental fluorosis affected animals having gradually development of lesion on teeth followed by total eruption of teeth, so that anorexia developed and body condition gradually decreases (7) Cost of treatment gradually increases.

Fluoride affected cattle revealed significantly higher level of fluoride in plasma, urine and faeces as compared to normal cattle at different observation periods. Significant reduction in fluoride concentration in plasma and urine and increased fluoride excretion through faeces was recorded in group III, group IV and group V animals from day 30 onwards. Altered haemato-biochemical parameters were restored after the supplementation of *Tamrindus indica* leaf powder in fluorotic calves. It is concluded that, dried powder of *Tamrindus indica* leaf had ameliorative potential against industrial fluorosis in calves and can be used as a fluoride alleviator for control of fluorosis.

CONTENTS

CHAPTER-I

INTRODUCTION

Fluorosis is a chronic disease caused by continuous ingestion of small but toxic amounts of fluorine in the diet or drinking water over a long duration. Death losses are rare and restricted largely due to acute poisoning. But the major losses are due to the un-thriftiness caused due to chronic fluorosis. It poses a great threat to human as well as livestock health. Fluorine toxicity was first identified in the 1930's in the cattle and subsequently being reported time to time by various researchers all around world. Minor teeth lesion occurs at 5 ppm, while the level exceeds of 10 ppm the excessive wear and tear of tooth occurs. Systemic effects occur when the water contains 30 ppm of fluorine (Ulemale *et al*., 2010). Chronic intoxication occurs when bore water contains 12 – 19 ppm fluorine.

Fluorosis is both endemic and global, spanning several continents. Natural geological sources and more recently the advent of increased industrialization and attendant environmental pollution have contributed greatly to the increasing incidence of fluoride-related human health issues. High levels of fluoride in drinking water have become a potential health hazard all over the world, with approximately 66.62 million victims in India alone. Depending upon the source of fluoride contamination, there are two types of pollution: artificial and natural. When fluoride contamination is caused by anthropological activities and/or industrial effluents, it is referred to as artificial fluoride pollution while significant presence of the chemical in water, soil and air is referred to as natural**.** Fluorosis in livestock is mainly attributable to industrial fluorosis.

In India main source of fluoride for animals are fluoride rich effluents, dust and smoke from aluminium smelters, super phosphate fertilizers plants and brick kilns (Patra *et al*., 2000). It can precipitate over a considerable distance on vegetation, soil and water bodies, thus causing industrial fluorosis (Patra *et al*., 2000; Singh and Swarup, 1994). These industrial operations release F in both gaseous and particulate forms. Hydrogen fluoride, silicon tetrafluoride and flurosilic acid are the common gaseous forms. Particulate fluorides include sodium fluoride, fluorspar, cryolite and aluminium fluoride (Robinson, 1978). Fluoride in soil and water used for irrigation ultimately finds its way into forage and grains. Contamination of the surrounding

area, particularly in the direction of the prevailing wind, may extend 8 – 9 km. Fluorosis is a wide spread entity in 25 countries across the globe. Fluorine toxicity was first identified in the 1930's and has subsequently been described from time to time in the literature. Fluorine is present in phosphate rock or its derivatives that may be used in mineral supplements for farm animals and can be a by-product of industrial processes which may contaminate animal feeds and water supplies.

Exposure to fluoride delays and alters mineralisation by replacing hydroxyapatite in the crystalline structure of bone and mostly affects the matrices supporting formation of enamel, dentine, cementum and bone. Teeth are affected during development due to damage to ameloblasts and odontoblasts which fail to accept minerals normally. Skeletal fluorosis interferes with formation of adequate matrix and mineralization by osteoblasts resulting in a dysfunction of normal sequences of osteogenesis, acceleration of bone remodelling, production of abnormal bone (exostosis, sclerosis) and in some cases, accelerated resorption. Abnormal and excessive bone formation leads to sub-periosteal hyperostosis with thickened and irregular surfaces of long bones.

Several factors may interact to change the toxicity of fluoride in individual animal herds' e.g. daily dosage or dietary concentration of fluoride, total exposure time, availability of fluoride in the source ingested, age and species of animal exposed and nutritional factors. Fluoride toxicity is enhanced by poor nutrition and alleviated somewhat by high dietary intakes of calcium and aluminium. Lesions occur first on the medial surface of the proximal third of the metatarsal and later on the mandible, metacarpals and ribs. The pelvis, vertebrae and other bones of the distal limbs are also affected.

Calves exhibited the highest prevalence of fluorosis and this may be, because calves are more sensitive and susceptible and less tolerant to fluoride (Shupe, 1980) and also ingest more fluorinated milk and water, so having a greater chance of exposure to fluoride. Curative treatment is always futile once the bone lesions are prominent. Only the preventive manage-mental measures from the younger stage i.e. during calf hood, reared in endemic fluorotic area becomes fruitful.

Defluoridation of water is the only available option to reduce its fluoride content, but the techniques are unaffordable and beyond the reach of underprivileged communities in fluoride endemic areas across the globe. Several natural adsorbents

such as red soil, charcoal, brick, fly-ash, serpentine and alum have been used to reduce the fluoride content in water (Chidambaram *et al*., 2003). Apart from defluoridation, reports also indicate the utility of plant products, for example tamarind fruit pulp, seeds of *Moringa oleifera*, bark extracts of *Terminalia arjuna* and black berry juice as possible therapeutic agents for amelioration of fluoride toxicity (Sinha *et al*., 2007; Ranjan *et al*., 2009; Hassan *et al*., 2009). Additionally, plant metabolites such as a 43-kDa protein isolated from *Cajanus indicus*, quercetin and curcumin have been shown to ameliorate fluoride-induced oxidative stress and improve the functions of liver, kidney and erythrocytes (Manna *et al*., 2007; Nabavi *et al*., 2011a, 2011b; Nabavi *et al*., 2012). Recent reports indicated the beneficial effects of seasonally available edible fruits (*Limonia acidissima, Mangifera indica, Emblica officinalis*) as dietary supplements/adjuncts and high-protein diets on various metabolic parameters associated with fluoride-induced alterations in body metabolism (Rupal and Narasimhacharya, 2011).

Tamarindus indica L. (Fabaceae; common name: tamarind) is a multipurpose tropical tree: the leaves, flowers, fruits and seeds are used to make curries, salads, stews and soups in many countries. The tender leaves of *T. indica* are traditionally used with lentils in Southern India, replacing the tamarind fruit. Earlier studies reported the ameliorative potential of tamarind on toxic effect of fluoride by reducing serum and bone fluoride concentration (Ranjan *et al*., 2009; Dey *et al*., 2011, Gupta *et al*., 2013). As there are no reports concerning the utility of tamarind leaves against sodium fluoride-induced toxicity in calves reared in fluorotic endemic area, we evaluated the efficacy of tamarind leaves in mitigating fluoride-induced haemato-biochemical parameters.

Therefore the present investigation was envisaged on the following aspects:

OBJECTIVES

1. To estimate the fluoride concentrations in biological and environmental samples of the selected areas.
2. To assess the impact of fluorosis on socioeconomic status of the farmers in the affected areas.
3. To assess the haematological and biochemical alterations of fluorotic calves.
4. To assess the ameliorative potential of tamarind leaf in calves exposed to industrial fluorosis.

CHAPTER-II

REVIEW OF LITRATURE

2.1 Prevalence

2.1.1 World

Ehrlich (1954) reported chronic fluorosis poisoning in cattle reared around aluminium work in France.

Mussill (1954) reported the occurrence of industrial fluorosis in cattle farms near aluminium work in Italy. The prevalence of fluorosis was estimated to be 41% and 78% respectively in two different farms.

Murray (1967) recorded fluorosis in 40 animals out of 450 cattle in a dairy herd near Nairobi as a result of high fluoride content of drinking water.

Tatarov*et al.* (1968) recorded fluorosis in cattle near a cement industry for the first time in Bulgaria.

Balazova and Hluchan (1969) reported an outbreak of fluorosis in cattle reared in the vicinity of an aluminium factory in Czechoslovakia.

Grunder (1972) studied the damage to cattle by fluoride in the vicinity of the aluminium factory in Germany and suggested practical prophylactic measures.

Ewy*et al.* (1976) reported the incidence of fluorosis in cattle near an industry in Poland which emitted fluoride. The incidence of fluorosis was recorded 55% and 48% at a distance of 5 and 10km from the plant, respectively.

Riet-Correa *et al.* (1986) studied fluoride intoxication in cattle due to industrial pollution caused by rock phosphate processing plant in Brazil.

Dental fluorosis was found in 279 numbers of cattle of 19 farms located within 4.5-17.5 km of four factories. Severity of the lesion was directly related to the distance from the plants.

Kessabi*et al.* (1986) observed occurrence of fluorosis in cattle, sheep and goat around the industrial town of Safi, Central Morocco.

Shupe*et al.* (1987) examined approximately 99,000 animals in 322 herds and 988 heads were fond with dental fluorosis were investigated in Utah State University, Logan, USA.

Jubb*et al.* (1993) observed chronic fluoride toxicosis in an extensive beef cattle herd in northern Australia. Upto 15% of the herd was lame and diseased.

Vandermissen*et al.* (1993) studied on the impact of fluorine pollution on the health of bovine and their production level in industrial regions of Wallonia, Belgium. He concluded that the most recognized sources of fluorine were phosphate plants, aluminium factories, coal fired power station, brick, glass and ceramic making industries.

Araya *et al.* (1993) observed osteofluorosis in cattle in Lonquimay Valley in Southern Andes in Chile after volcanic eruption in 1988-89.

Botha *et al.* (1993) investigated two outbreaks of fluorosis in cattle and sheep in the Northern Transvaal, South Africa. Twenty two cows in a herd of 33 exhibited severe dental lesions and lameness.

Raghib*et al.* (1994) studied the effect of fluorine industrial waste product of Hammadi Aluminium Factory, Upper Egypt, on the health of Friesian Cows and found dental fluorosis in 184 exposed cows.

Milhaud *et al.* (1997) studied prevention of industrially induced fluorosis in cattle and sheep in an industrial area at Vlissingen, Netherland where two industrial plants were emitting fluorides, one produced phosphorus from natural phosphates and the other produced aluminium through electrolysis.

Mehedintu*et al.* (2000) studied industrial fluorosis of ruminants in Romania and found 21% cattle with fluorosis in the polluted area.

2.1.2 India

Kerur (1971) reported fluorosis in cattle in 9 villages of Lathitaluka of Amrel district of Gujarat due to high fluoride concentration in drinking water.

Cheeran*et al.* (1987) reported the incidence of fluorosis in cattle in Eloor industrial area of Kerala. The prevalence of fluorosis was 100% in cattle.

Swarup and Singh (1989) recorded 60 cases of fluorosis out of 265 cattle and buffaloes during their investigation, near a brick kiln congested zone of Ghaziabad district in U.P.

Singh and Swarup (1994) reported the occurrence of fluorosis in buffaloes in several villages of Ghaziabad district of U.P. Out of 750 buffaloes surveyed, 65 exhibited fluorotic lesions. It was presumed that dust and smoke generated by large number of brick kilns might be source of excessive fluoride in animals.

Dwivedi*et al.* (1997) recorded hydrofluorosis in water buffaloes in the Unnao district of Uttar Pradesh along with toxic level of fluoride in drinking water.

Sharma *et al.* (1997) reported occurrence of hydrofluorosis in cows and buffaloes with an overall prevalence of 7.71% in the villages of 4 districts of Punjab. The disease was more common in buffaloes (97.5%) than cows (2.5%).

Swarup*et al.* (1998) conducted a clinical survey in the vicinity of an aluminium smelter plant to record the occurrence of fluoride intoxication in cattle. They observed the overall incidence of fluorosis was 42.31% and the highest incidence (58.27%) was within 3 km distance from the smelter plant.

Choubisa (1999) reported Chronic fluoride toxicity in the form of osteo-dental fluorosis in cattle, buffaloes, sheep and goat population from 21 villages of Banswara, Durgapur and Udaipur districts of Southern Rajasthan where fluoride content in drinking water varied from 1.5-4 ppm with more prevalence in the calves of <1 year

age. At fluorine content of 4 ppm in water, 100% calves, 65.6% buffaloesmand 61.0% cattle were affected due to dental fluorosis of varying degree.

Muralidhara*et al.* (2000) studied fluorosis in bovines, in Karnataka, India. They have shown that the plasma F level of cattle was higher in Chitradurga area.

Patra*et al.* (2000) investigated the occurrence of fluorosis in cattle and buffaloes around superphosphate fertilizer plant in Udaipur district of Rajasthan. Out of 166 animals examined, the prevalence was 17.4% in calves below 1 year, 37.2% in cattle between 1 and 3 years, 61.3% in cattle above 3 years and 72% in buffaloes above 1 year of age. They concluded that the consumption of fodder and water contaminated by the fumes and dusts emitting from superphosphate fertilizer plants resulted in the development of chronic fluorotic lesions in the cattle and buffaloes.

Dwivedi*et al.* (2007) identified a 40.34% prevalence of fluorosis in buffalo from the Unnao district of India .Dental lesions were present invariably in all affected animals whereas lameness, painful bony exostosis and emaciation were recorded in 28.17%, 8.45% and 76.00% of the animals.

Upadhyay*et al.* (2007) screened a total 1261 bovines (728 cattle and 533 buffaloes) to study the prevalence of bovine fluorosis in 8 districts in Central East India (CEI) such as Durg, Raipur, Bilaspur, Bastar and Korba from Chhattisgarh and Jabalpur, Mandla and Dhindori from Madhya Pradesh. The overall prevalence of bovine fluorosis was 3.96%, and the disease was almost equally distributed among cattle and buffaloes. The prevalence of fluorosis was higher in animals aged 3-5 years followed by those aged 5-7 years, >7 years and 0-3 years. The prevalence was greater in local or non-descript breeds of cattle and buffaloes than in crossbreds and others. Both male and female bovines were equally affected.

Choubisa (2008) conducted surveys in the Baroda, Kheda and Manpur villages in Udaipur, Rajasthan, India, where water fluoride level was below 1.0 ppm. It was shown that among the 75 calves, 172 adult cattle and 116 adult buffaloes, 32 (42.66%), 53 (30.81%) and 40 (34.48%), respectively, had varying degrees of enamel mottling.

Trangadia and Kaul (2008) studied prevalence of fluorosis in 15 villages of Kunkavav, Lathi and Liliyatalukas of Amreli district of Gujarat. The majority of animals having dental lesions were above 3 years of age.

Choubisa (2010) studied the incidence of fluorosis in domestic ruminants in Rajasthan, India .Out of 229 sheep and goats and 112 cattle and buffaloes examined, 43 (19.0%) sheep and goats and 78 (70.0%) cattle and buffaloes had fluorosis. Young cattle and buffaloes also had a higher incidence of dental fluorosis compared to older animals. Immature goats and sheep did not have dental or skeletal fluorosis.

Choubisa*et al.* (2011) observed toxic effects of chronic fluoride (F) exposure in the form of osteo-dental fluorosis in domestic ruminants including cattle (*Bostaurus*), buffaloes (*Bubalusbubalis*), camels (*Camelusdromedarius*), sheep (*Ovisaries*), and goats (*Capra hircus*) in the Dungarpur district of Rajasthan, India. Among immature ruminants, osteo-dental fluorosis was not observed among 34 goats, 28 sheep, and 12 camels. However, among 43 immature cattle and 37 buffalo calves, the dental and skeletal fluorosis rates were 51.1% and 18.6% and 62.2% and 21.6%, respectively.

2.1.3 Odisha

Samal and Naik (1992) carried out an epidemiological survey on fluorosis in sheep in the vicinity of an aluminium factory at Hirakud, Orissa. Of the 83 sheep examined, 67.5% (above 1 year age) had mild to severe degree of dental lesions.

Mahapatra (1997) recorded dental fluorosis in cattle in the vicinity of brick kilns near Bhubaneswar in Orissa, which was found to be 19.33% and 9.63% in Balianta and Balakati, respectively.

Maiti*et al.* (2003) observed chronic fluoride toxicity in cattle in the form of dental fluorosis in 9 villages of Nayagarh District of Orissa. In all affected villages prevalence of dental fluorosis was 18.09%, the prevalence in calve (< 1year age) was greater than adults. Fluoride intake through the water especially ground water contributed to the development of fluorosis in cattle.

Maiti and Das (2004) studied endemic dental fluorosis in cattle in villages of 3 blocks of Nayagarh and Khurda district in Orissa and found 21.32% cattle affected with different degree of dental lesions.

Mishra *et al.* (2009) recorded the prevalence of dental fluorosis in cattle population in three areas of western Orissa as 60.5% in Hirakud, 39.5% in Kuchinda and 25.5% in Deogarh.

2.1.4 Angul district

Behera (1993) studied the incidence of fluorosis in cattle around the aluminium factory at Angul. Out of 727 cattle examined, 399 (54.9%) were observed to have been affected with fluorosis.

Singh (1994) while investigating on impact of industrial fluorosis on sheep and goats near aluminium smelter plant at Angul, Orissa, recorded fluorosis in 36.03% and 39.2% in sheep and goats, respectively. Sheep and goats below 1 year did not exhibit any noticeable clinical signs.

Singh *et al.* (2002) conducted clinical survey near the aluminium smelter plant at Angul in Orissa, India. Out of 933 goats, 319 animals showed signs of dental fluorosis.

Sahoo e*t al.* (2003) observed industrial fluorosis in sheep near an aluminium smelter plant in Angul, Orissa, India. Out of 1271 sheep examined, 458 (36.03%) animals showed signs of dental fluorosis.

Kanungo (2005) observed industrial fluorosis in cattle within a radial distance of 0-6 Km. from aluminium smelter plant of NALCO, Angul, Orissa. Out of 920 cattle examined, 330 animals (35.87%) were found to be fluorotic.

2.2 Clinical signs

Chronic fluoride toxicosis, more popularly known as fluorosis in animals develops gradually and insidiously. Months or even years may pass between the beginning of excess fluoride ingestion and manifestation of readily observable clinical

signs (Shupe, 1967). Basing on the clinical manifestations, fluorosis can be divided chiefly into three categories- dental fluorosis, osteofluorosis and non-skeletal fluorosis.

2.2.1 Dental fluorosis

Rand and Schmidt (1952) recorded signs of dental fluorosis in cattle with mottling, staining and excessive wearing of permanent teeth as the first indications of fluoride absorption.

Mortenson*et al.* (1964) studied the fluorotic signs in cattle in 24 affected herds and recorded decreased enamel percentage of teeth. They classified the teeth lesions into six categories (0-5) basing on the severity of the fluorine effects.

Tatarov*et al.* (1968) recorded severe dental lesions in cattle and sheep suffering from industrial fluorosis. Teeth lesions were the most severe in cattle upto 3-4 years and percentage of animals showed dental lesions was lower in 7-16 years of age.

Shearer *et al.* (1978) noted the clinical signs of dental fluorosis in cattle which received high fluoride diet for five years. The lesions were incisor defects such as hypomineralized outer enamel, coronal cementum hyperplasia, disrupted subsurface pigment band, hypoplastic pits, puckered incremental lines, periodic radiolucent regions, positive protein staining, and decrease in micro-hardness of the outer enamel.

Krook*et al.* (1983) described five expressions of dental fluorosis in cattle exposed to industrial fluoride pollution: (1) hypercementosis with tooth ankylosis, cementum necrosis and cyst formation; (2) delayed eruption of permanent incisor teeth; (3) necrosis of alveolar bone with recession of bone and gingiva; (4) oblique eruption of permanent teeth, hypoplasia of teeth with diastemata and (5) rapid progression of dental lesions.

Singh and Swarup (1994) observed dental lesions only in 19 (29%) buffaloes out of 59 in brick kiln area. Lesions varied from slight to definite mottling of incisors and excessive rate of attrition in few cases.

Metwalli*et al.* (1995) noticed reduced appetite, dental lesions in the form of staining, mottling and blackish discolouration especially the incisor teeth in fluorotic cattle of Fowa villages (Kafr EL-Sheikh Governorate in Egypt).

Choubisa*et al.* (1996) concluded that mottled enamel was the first and earliest visible signs of excessive fluoride intake during the period of teeth eruption. A distinct brown or black stain of enamel of incisors was observed frequently in affected cattle and buffaloes of 2 to 9 years of age. Dental changes like brownish discoloration, shortened incisors due to abnormal wearing and roughened anterior surface were observed in most of the adult animals where, mean fluoride level of water was more than 3 ppm.

Mahapatra (1997) recorded the teeth abnormalities comprising of discoloration, loss of lusture, appearance of pigmentation, mottling, erosion of teeth with pitting, brittleness of enamel and gingivitis as signs of dental fluorosis in cattle near brick kilns.

Patra*et al.* (2000) recorded dental lesions such as mottling, brownish discoloration and deformity of teeth in all fluorotic buffaloes and calves around a superphosphate fertilizer plant. However, 45% of fluorotic cattle in the age group of 1-3 years exhibited dental lesions. Involvement of teeth was least in cattle over 3 years of age.

Sahoo*et al.* (2003) examined 1271 nos. of sheep near an aluminium smelter plant at Angul district of Orissa and observed the clinical signs of dental fluorosis in 36.03% cases.

Maiti*et al.* (2004) while conducting an epidemiological investigation in Orissa observed the prevalence rate of dental fluorosis was higher in the cattle <1year nand was lowest in the group of cattle >2 years . Affected cattle had brown discolouration of various degrees, loss of luster, mottling, attrition or uneven wearing with or without pitting.

Kanungo (2005) observed clinical signs of brown pigmentation, chalk light dull white discolouration of incisors, wearing, mottling, pitting of enamel, abrasion of enamel and defective dental development in fluorotic cattle near an aluminium smelter plant.

Choubisa (2008) found that the relatively higher percentage of dental fluorosis in calves was related to greater sensitivity and less tolerance to fluoride, while excessive abrasion of the teeth in adult animals may be due to long exposure to fluoride.

Choubisa (2010) studied the signs of dental fluorosis in camels. He found that front (incisors) and upper cheek teeth were light brown to deep yellow in colour. Irregular wearing of teeth was found, however, only with severe dental fluorosis.

2.2.2 Skeletal fluorosis

Rand and Schmidt (1952) opined that lameness was the first symptom in cattle affected with osteofluorosis which was accompanied by exostoses of long bones, jaw bones and ribs. Stiff gait was also observed in addition to bilateral lameness.

Mussill (1954) also recorded exostoses on ribs and leg bones in fluorotic cattle. One third of the affected animals were lame.

Reinhard (1959) reported exostoses of bones especially on the ribs in all affected cattle around aluminium factory.

Kerur (1971) reported lameness and un-thriftiness as first signs of fluorosis.

Other observed signs were stiffness, painful gait, dragging of fore or hind limbs and bony exostoses of mandible, metacarpal and metatarsal bones in some animals.

Jones (1972) noticed arthritis of hind limbs as the most prominent feature of osteofluorosis which resulted stiff, short strides and compensatory hypertrophy of shoulder muscles. Enlarged hooves and bony exostoses on radius were also observed in some cases.

Shupe (1980) studied the effects of fluorosis in cattle in areas of industrial pollution. Poor dentition, stiffness of leg and lameness were the manifestations of fluorotic cattle.

Mayline*t al.* (1987) noticed brown mottling of bone, severe retardation of cartilage cell, atrophy of osteoblasts, osteopenia, atrophy of bone marrow cells, serous atrophy of bone marrow fat and severely stunted growth in fluorotic dairy cattle.

Swarup and Singh (1989) observed the severely affected animals were emaciated and some of them moved in 'knee-posture' due to severe lameness. Palpation of affected limbs revealed painful protuberances particularly on medial and lateral aspects of metatarsal, metacarpal and facial bones.

Araya *et al.* (1990) during an investigation on outbreak of fluorosis in bovine following volcanic eruption recorded kyphosis and lameness especially of fore limbs in all affected animals. Several affected animals stood on their carpuses. Palpation or pressing revealed bony exostoses and pain in metatarsus.

Singh and Swarup (1994) observed the manifestations of osteofluorosis in buffaloes were lameness involving more commonly forelimbs, painful restricted movements, knee posture, boneyexostoses and kyphosis, deformity of hooves, swelling of fetlock, hock and other joints were also found in some animals.

Sahoo (1995) reported single or multiple exostoses of ribs, metatarsus, metacarpus and mandible as signs of osteofluorosis in affected cows around an aluminium smelter plant.

Choubisa*et al*. (1996) recorded prevalence of skeletal fluorosis in 35.8% cattle and 37.5% buffaloes. The highest prevalence (62.6%) of skeletal fluorosis was observed at 6ppm fluoride concentration of water. For skeletal fluorosis only clinical evidence such as dental fluorosis, stunted growth, low milk yield, emaciation, intermittent lameness, snapping sound in feet during walking and diarrhoea were considered.

Swarup*et al.* (1998) reported osteofluorotlic symptoms in cattle during a clinical survey in the vicinity of aluminium smelter. The recorded signs were lameness due to involvement of either one or both hip joints, palpable bony exostoses and hoof deformity.

Choubisa (1999) observed hind limb lameness, stiffness and bony exostoses in older animals more than 7 years of age. None of the calves affected with skeletal fluorosis. The prevalence and severity of skeletal fluorosis increased with increasing fluoride concentration of water and age.

Patra*et al.* (2000) reported bony exostoses, rib abnormalities and inability to walk as signs of osteofluorosis in cattle above 3 years age group around superphosphate fertilizer plant. Out of 18 affected buffaloes only one had bony exostoses.

Swarup*et al.* (2001) observed clinical signs of lameness, bony exostoses and dental lesions varying degree in fluorotic cows of Udaiapur district of Rajasthan. Debility, inability to walk and defective mastication were also noticed in severely affected animals. Extostoses of metatarsal, metacarpal bones were the most frequently observed clinical signs in the first year where as exostoses of ribs seen in subsequent year.

Kanungo (2005) observed clinical signs of osteophytosis of ribs, mandible, metacarpal, metatarsal, pelvic vertebrates and lachrymal bones crossed leg posture crawling of knees, lifting of fore leg from ground, arched back, parrot mouth an deformed overgrown hooves in fluorotic cattle near aluminium smelter.

2.2.3 Non-skeletal fluorosis

Reinhard (1959) found emaciation, reduced milk yield, apathy and reproductive disorders as general signs of fluorosis in cattle.

Murray (1967) in a herd of 450 cattle near Nairobi 40 animals were culled in three years because of loss of condition and fall in milk yield as a result of fluorosis.

Jones (1972) recorded reduced milk yield and poor body condition in dairy cattle in addition to osteodental lesions. Appetite remained good except in advanced cases.

Hillman *et al.* (1979) reported that cattle with fluorosis developed hypothyroidism, anaemia, and eosinophilia of leukocytes.

Swarup and Singh (1989) noted poor body condition with wasting of shoulder and hind quarter muscle, loss of hair with rough skin coat, reduced appetite and poor performance in fluorotic animals in brick kiln congested zone.

Raghib*et al.* (1994) noticed general emaciation, stunted growth, rough hair, respiratory and digestive disorders in affected cows around an aluminium factory in Egypt.

Singh and Swarup (1994) noted generalized emaciation, cud-dropping, poor appetite, rough body coat, constipation with intermittent diarrhoea and reduced milk yield as general clinical signs in fluorotic buffaloes in a brick kiln area.

Metwalli*et al.* (1995) observed reduced appetite, general loss of body condition, lameness and dental lesions in chronic bovine fluorosis affected cases in brick kiln established areas in Egypt.

Sahu (1995) observed unthriftiness, partial anorexia and loss of milk yield as common symptoms of cows with fluorosis.

Sharma *et al.* (1997) reported occurrence of hydrofluorosis in cows and buffaloes in 4 districts of Punjab. Dental mottling, lameness and higher respiration and heart rates were common symptoms. Pallor of mucosae was noticed in severely affected animals. Reproductive disorders of prolonged postpartum anoestrus and increased incidence of vaginal prolapse were also recorded.

Jagadish*et al.* (1998) recorded cud-dropping, unthriftiness, lameness, anaemia, reduction in milk yield, poor appetite and diarrhoea as general signs of fluorosis in cattle which received mineral mixture containing excessive fluoride.

Patra*et al.* (2000) observed debility, inability to walk and decreased milk yield in cattle and buffaloes reared around a superphosphate fertilizer plant in Rajasthan.

Sahoo*et al.* (2003) recorded loss of glossiness of skin, general unthriftiness, loss of appetite, alopecia and occasional diarrhoea in sheep around an aluminium factory of NALCO at Angul, Orissa.

Maiti*et al.* (2003) observed anaemia, unthriftiness, rough skin coat, loss of glossiness of skin and inappetance in affected cattle in endemic area of Nayagarh and Khurda district of Orissa.

Kanungo (2005) observed anorexia, rough body coat, emaciation, improper mastication, agalactia, impaired infertility, painful gait unthriftiness and intermittent diarrhoea in flurotic cattle near aluminium smelter of NALCO, Angul, Orissa.

Chaurasia*et al.* (2007) assessed the genotoxic effects of non-permissible high concentration of fluoride salts in ground water, in Swiss albino mice. Fluoride salts present in the ground water might have interfered the phagocytosis and produce oxygen free reactive radicals that attack the nucleophilic sites of the DNA leading to the loss of important gene segments responsible for cell growth and the ageing.

Liu *et al.* (2008) concluded that fluoride can induce colloidal and nodular goiter, and cause abnormal changes of thyroid metabolism function.

Wu *et al.* (2009) examined that fluorosis can cause myocardial cell damage and changes in myocardial structure and ECG in rats.

Ranjan*et al.* (2009) revealed emaciation, hypogalactia, repeat breeding and anoestrus in cattle population reared in fluoride endemic areas of district Nawada (Bihar) and Udaipur (Rajasthan), India.

Shashi*et al.* (2010) carried out the histopathological examination of pancreas of fluoridated rats which exhibited structural alterations in the exocrine glands. The acini revealed hypertrophy, pyknotic nucleus, necrosis and uremic alterations. Acini became lobulated and reveal increased pigmentation.

2.3 Fluoride concentration

2.3.1 Fluoride concentration in water

Kerur (1971) analysed fluoride level of water samples from 3 of 9 affected villages of Amreli district of Gujrat and it varied from 4 to 14 ppm.

The WHO has set the guideline value at 1.5 mg / l of fluoride, but mild forms of dental fluorosis begin to occur at lower levels. Above 1.5 mg / l, prolonged intake of fluoride can cause dental fluorosis, 3 – 6 mg / l, skeletal fluorosis, more than 6 mg / l, crippling skeletal fluorosis (WHO, 1984b; Brouwer*et al*., 1988).

Singh (1994) found average fluoride concentration of water samples collected from well, ponds and canal of 0-3 km., 3-6 km and above 6 km distance from an aluminium plant were 3.11, 2.01 and 1.39 ppm respectively.

Choubisa (1996) recorded fluoride concentration of drinking water 1.52 to 4.0 ppm in some villages of Banswara district of Rajasthan. The highest fluoride level (5.5 ppm) was observed in an open well and that was lower in the surface water.

Dwivedi*et al.* (1997) found the mean fluoride content of water in fluoride endemic villages of Nalgonda (A.P.) and Unnao (U.P.) districts which were 0.65 + 0.087 ppm, and 2.01 + 0.51 ppm, respectively.

Sharma *et al.* (1997) reported occurrence of hydrofluorosis in cows and buffaloes in 4 districts of Punjab. Fluorosis was associated with toxic fluoride level (3.14+or-0.42 ppm) in underground water.

Swarup*et al.* (1998) examined water samples in the vicinity of aluminium smelter that contained higher concentration of fluoride which decreased with distance from the smelter. Ground water of nearest zone (0-3 km) had maximum level of fluoride (1.59 + 0.20 ppm) and lowest in the distant zone (> 6 km). The fluoride level of surface water varied from 1.12 + 0.17 ppm (nearest zone) to 0.47 + 0.2 ppm (distant zone).

Choubisa (1998) during his investigation in ten villages of Udaipur district of Rajasthan observed the drinking water contained 0.3 to 0.7 mg/l fluoride resulting in chronic fluorosis in cattle.

Muralidhar*et al.* (2000) recorded fluoride concentration of water ranged from 0.89 ppm to 9.49 ppm in endemic fluorosis area of Karnataka.

Patra*et al.* (2000) observed the mean value fluoride content of pond water was 1.186 + 0.286 ppm as compared to that of tube wells 0.479 + 0.351 ppm around superphosphate fertilizer plants that revealed contamination of surface water with industrial fumes and particles and caused fluorosis in cattle.

Swarup*et al.* (2001) examined the fluoride concentrations in the environmental sample collected from four villages around (<3 km) superphosphate fertilizer units in Udaipur district (India) during the month of July for two consecutive years (1998 and 1999.High fluoride level in surface water (0.782+or-0.249 mg/l in 1998 and 1.186+or-0.286 mg/l in 1999) contaminated from fertilizer manufacturing units was suggested as the possible source of excess fluoride ingestion by the animals.

Maiti*et al.* (2003) recorded Fluoride levels (mean) of ground water and surface water in two blocks of Nayagarh district of Orissa, were 1.30+or-0.16 ppm, 0.66+or-0.08 ppm and 1.12+or-0.19 ppm, 0.48+or-0.05 ppm respectively.

Maiti*et al.* (2004) recorded mean fluoride level as low as 0.62 + 0.18 ppm and as high as 4.75 + 3.61 ppm in drinking water (ground water) in 12 randomly selected villages of Khurda and Nayagarh district of Orissa where there was prevalence of endemic dental fluorosis in cattle. There was a highly positive correlation (+ 0.664) between prevalence of dental fluorosis and fluoride content of drinking water.

Kanungo (2005) examined water samples in the vicinity of aluminium smelter plant of NALCO at Angul, Orissa and found the fluoride content of ground water which ranged from 1.57 ppm to 2.70 ppm with a mean value of 2.11 + 0.15 ppm. Similarly the fluoride concentration of surface water ranged from 2.94 ppm to 6.32 ppm with a mean value of 4.55 + 0.43. The fluoride levels of ground water and surface water from the control area were recorded to be 0.15 ppm and 0.18 ppm,

respectively. Thus fluoride concentration of surface water of the affected area was found significantly higher than the control area.

Srikanth*et al.* (2008) surveyed chronic fluoride intoxication in the form of dental and skeletal fluorosis in five villages of the Palamau district, Jharkhand, India. Out of 238 sources of drinking water, mainly from groundwater, the majority had elevated fluoride concentrations. In one water source even a concentration of 12 mg F/L was observed.

Hussain*et al.* (2013) determined Fluoride concentrations in groundwater of 121 habitations of Bhilwara tehsil of Bhilwara district of Rajasthan to examine the potential F-induced toxicity in rural locations. Fluoride concentrations in the tehsil ranged from 0.5 to 5.8 mg/l. In the tehsil, 69 villages (57%) were found to have F concentration beyond the maximum desirable limit recommended in Bureau of Indian Standards (BIS).

Jha*et al.* (2013) observed that occurrence of fluoride in groundwater is due to weathering and leaching of fluoride-bearing minerals from rocks and sediments. Fluoride when ingested in small quantities (<0.5 mg/L) is beneficial in promoting dental health by reducing dental caries, whereas higher concentrations (>1.5mg/L) may cause fluorosis.

2.3.2 Fluoride concentration in fodder

Ehrlich (1954) reported that grass of the pasture adjoining the aluminium works contained 30-50 times more fluoride than normal.

Tatarov*et al.* (1968) estimated fluorine level of the herbage upto 5 km from industrial works which ranged from 3.91 to 18.4 ppm. Jones (1972) recorded 6.5 to 13.1 ppm fluorine in herbage contaminated with fluorine from industrial fallout during the outbreak of fluorosis in a dairy herd.

Araya *et al.* (1990) reported high fluoride level from 240-315 ppm in the hay and grass samples following volcanic activity and suggested that fluoride contaminated forage had been the main cause of fluoride intoxication in cattle.

Samal and Naik (1992) analysed 16 forage samples (legumes) commonly grazed by local sheep near aluminium factory for fluoride content. Fluoride value ranged from 24.0 to 360.0 ppm.

Wang *et al.* (1992) determined the fluoride content of grass samples at each month collected from the industrially polluted areas. There was considerable variation in the fluoride content of grass sample in each month.

Singh and Swarup (1994) recorded higher fluoride level in wheat straw (200.8 + 0.70 ppm) and barseem (98.64 + 0.23 ppm) collected from brick kiln belt than that (8.53 + 0.23 ppm) of unaffected areas. It was concluded that fluoride contaminated forage was the main cause of the fluorosis.

Dwivedi*et al.* (1997) recorded hydrofluorosis in water buffalo in the Unnao district of Uttar Pradesh. The forage samples contained 22.50 ±0.82 mg/kg fluoride.

According to Mahapatra (1997), the mean fluoride content of paddy straw and pasture grass of Balianta and Balakati were 174.4 and 47.66 ppm in composite pasture grass and 30.0 and 10.8 ppm in paddy straw respectively whereas that of Bhubaneswar were 8.8 and 2.4 ppm, respectively.

Patra*et al.* (2000) analysed fodder samples collected from different places around superphosphate factory and found the value ranged from 401 to 875.0 ppm. It was concluded that fluoride intake through the contaminated fodder contributed to the development of fluorosis in livestock.

Swarup*et al.* (2001) examined the fluoride concentrations in the environmental sample collected from four villages around (<3 km) superphosphate fertilizer units in Udaipur district (India) during the month of July for two consecutive years (1998 and 1999.High fluoride level in fodder (534.4±74.9 mg/kg) contaminated from fertilizer manufacturing units was suggested as the possible source of excess fluoride ingestion by the animals.

Kanugo (2005) analyzed fluoride level in paddy straw and green grass from fluorosis affected villages near aluminium smelter plant of NALCO at Angul and

found 36.42 + 2.72 ppm and 16.22 + 0.81 ppm respectively whereas the fluoride content of paddy straw and green grass of control area was found to be 1.84 ppm and 1.15 ppm respectively. From the assessed values it was obvious that very high concentration of fluoride was recorded in paddy straw (36.42 ppm) as compared to green grass (16.22 ppm).

2.3.3 Fluoride concentration in urine

Reinhard (1959) found that urinary fluoride level in affected cattle was usually over 2 mg / litre and sometimes it exceeded 10 mg/litre. Murray (1967) opined when fluoride content of urine was more than 10 ppm then cattle showed severe symptoms of fluorosis.

Rao and Pal (1978) recorded urinary fluoride level in moderate and severe fluorosis in cattle, the values varied from 8.54 to 20.96 ppm and 14.71 to 30.09ppm, respectively.

Hillman *et al.* (1979) detected urinary fluoride level in fluorotic cows and found it to range from 1.04 to 15.7 ppm with an average of 5.13 ppm.

Araya *et al.* (1990) recorded a high level of fluoride in urine, varying from 69 to 122 ppm in fluorotic cows following a volcanic eruption.

Behera (1993) recorded the mean fluoride content of urine 31.0 ppm, 17.7 ppm and 13.3 ppm in fluorotic cattle of 0-3 km, 3-6km and above 6 km distance respectively from aluminium smelter plant.

Ray *et al.* (1994) compared the fluoride level of urine samples collected from cattle reared in fluorotic and non-fluorotic area and found it to be 29.21 + 3.15 ppm and 1.00 + 0.07 ppm respectively.

Singh and Swarup (1994) detected higher concentration of fluoride (27.89 + 1.32 ppm) in urine of fluorotic buffaloes of brick kiln areas than of healthy animals (7.92 + 0.30 ppm).

Osheim and Rasmusson (1995) described a selective ion electrode method for measuring fluoride in bovine urine at concentrations of 2 to 40 mg/litre. Urine was buffered and measured against known fluoride (F) standards. Typical normal urine contains less than F 10 mg/litre and levels greater than 10 mg/litre support a clinical diagnosis of fluorosis in cattle.

Sahu (1995) also recorded urinary fluoride level of cows in industrial fluorotic area which varied from 3.42 to 52.12 ppm.

Singh *et al.* (1995) observed fluorosis in cows due to mineral mixture supplement and revealed higher level of urinary fluoride (33.71 + 2.22 ppm) than that of healthy controls.

Dwivedi*et al.* (1997) estimated urinary fluoride in hydrofluorotic animals in Nalgonda (A.P.) and Unnao (U.P.) districts. The values were 8.46 + 2.12 ppm and 10.64 + 1.23 ppm in Nalgonda and Unnao respectively.

Mahapatra (1997) found higher level of urinary fluoride in affected cattle in Balianta (17.603 + 1.963 ppm) and Balakati (10.034 + 0.853 ppm) than healthy controls (3.882 + 1.744 ppm).

Jagadish*et al.* (1998) observed the fluoride levels of urine in moderately and severely affected cows were 17.47 + 0.61 ppm and 24.47 + 0.38 ppm respectively. Both the values were significantly higher as compared to 3.18 + 0.37 ppm in healthy animals.

Swarup*et al.* (1998) also recorded high fluoride content in the urine of affected cattle around an aluminium smelter. The value varied from 14.56 ppm to 26.45 ppm whereas in unpolluted area urine fluoride level was 8.05 + 0.05 ppm.

Patra*et al.* (2000) reported high urinary fluoride in affected animals reared around superphosphate fertilizer plant. The mean fluoride values of urine were 26.4 + 6.17 ppm in calves below 1 years of age, 26.2 + 3.86 ppm in cattle of 1-3 years, 27.5 + 4.63 ppm in cattle above 3 years and 28.6 + 4.73 ppm in buffaloes over 1 year.

According to Radostits*et al*. (2000), normal cattle have urine levels of up to 2 – 6 mg / kg in urine and cattle on fluorine intakes sufficient to cause intoxication may have urine levels of 16 – 68 mg / kg when the specific gravity of urine corrected to 1.040.

Swarup*et al.* (2001) revealed the occurrence of fluorosis through clinical examination of cattle population in four villages around (<3 km) superphosphate fertilizer units in Udaipur district (India) during the month of July for two consecutive years (1998 and 1999). Affected animals had higher concentrations of fluoride in urine (range 5.10-89.10 mg/l in 1998 and 7.64-51.10 mg/l in 1999).

Maiti*et al.* (2004) recorded 3.68 + 0.257 ppm fluoride in urine of cattle affected with endemic dental fluorosis whereas 0.629 + 0.028 ppm in healthy control.

Kanungo (2005) recorded mean value for urine fluoride as 11.764 + 0.211 ppm in fluorotic cattle as against normal value of 0.937 + 0.057 ppm.

Upadhyay*et al*. (2007) screened a total 1261 bovines (728 cattle and 533 buffaloes) to study the prevalence of bovine fluorosis in 8 districts in Central East India (CEI) such as Durg, Raipur, Bilaspur, Bastar and Korba from Chhattisgarh and Jabalpur, Mandla and Dhindori from Madhya Pradesh, The mean fluoride contents in urine of cattle and buffaloes were 3.61±0.27 and 2.37±0.83 ppm, respectively.

2.3.4 Plasma fluoride concentration

Shupe (1980) reported that fluoride level of blood upto 0.30 ppm had no adverse effect in the body whereas 0.50 ppm fluoride produced adverse effects.

Araya *et al.* (1990) measured fluoride concentration in serum in cattle with fluorosis. Fluoride level varied from 0.91 to 1.87 ppm with an average of 1.30 ppm in serum.

Behera (1993) reported that serum fluoride concentration of cattle reared within 0-3km, 3-6 km and above 6 kms distance from the aluminium smelter plant ranged from 0.9 to 1.3 ppm, 0.5 to 0.7 ppm and 0.3 to 0.4 ppm respectively.

Ray *et al.* (1994) reported average fluoride content in the serum of fluorotic cattle to be 0.86 + 0.05 ppm as against 0.11 + 0.03 ppm in control.

Singh (1994) recorded high serum fluoride concentration ranging from 0.22 to 1.16 ppm with an average of 0.618 ppm in fluorotic cattle than that of healthy animals (0.094 + 0.021 ppm).

Singh and Swarup (1994) recorded higher concentration of fluoride (1.39 + 0.061 ppm) found in serum of fluorotic buffaloes in brick kiln belt compared with the healthy animals (0.37 + 0.20 ppm).

Sahoo (1995) recorded an increased serum fluoride level in lactating cows with fluorotic symptoms near aluminium plant. The maximum and minimum values were 2.37 and 0.18 ppm respectively.

Singh *et al.* (1995) reported significantly high fluoride concentration in serum (1.22 + 0.052 ppm) collected from cows which had fluorosis after ingesting mineral mixture supplement for successive 3 years.

Dwivedi*et al.* (1997) detected mean serum fluoride level of 0.30 + 0.30 ppm and 0.57 + 0.05 ppm respectively in the animals in fluoride endemic villages of Nalgonda (A.P.) and Unnao (U.P.) district.

Mahapatra (1997) recorded fluoride concentration in blood samples of the cattle with dental fluorosis as 0.651 and 0.435 ppm in Balianta and Balakati block of Orissa, respectively and that of control animals was 0.147 ppm only.

Jagadish*et al.* (1998) estimated serum fluoride level during their investigation on fluoride toxicosis in a bovine herd. The average serum fluoride level in moderately and severely affected animals was 0.59 + 0.02 ppm and 0.73 + 0.02 ppm, respectively and that of healthy animals was 0.18 + 0.01 ppm.

Swarup*et al.* (1998) analysed fluoride level of serum of affected animals maintained in three zones viz., nearest zone (0-3km), middle zone (3-6km) and distant zone (> 6 km) around an aluminium smelter. The serum fluoride levels of affected

animals of three zones were 1.03 + 0.08 ppm, 0.80 + 0.80 ppm and 0.63 + 0.05 ppm, respectively and that of unaffected animals (unpolluted area) was 0.27 + 0.01 ppm. They further observed that the plasma fluoride level of bovine in dental fluorosis in some areas of Karnataka were as follows : Chitradura 1.37 + 0.07 ppm, Ramnagar 0.47 + 0.02 ppm, Kolar 0.48 + 0.02 ppm and Magadi 0.5 + 0.05 ppm.

Patra*et al.* (2000) recorded serum fluoride level of fluorotic cattle and buffaloes around superphosphate fertilizer plant. Mean fluoride concentration in serum was 1.53 + 1.27 ppm in calves below 1 year of age, 0.56 + 0.17 ppm in cattle of 1-3 years, 0.49 + 1.13 ppm in cattle above 3 years and 0.60 + 0.07 ppm in buffaloes over 1 year respectively. These values were significantly higher than those of control animals (0.046 + 0.001 ppm; range 0.043 – 0.048 ppm).

According to Radostits*et al*. (2000) the normal cattle have blood levels of upto 0.2 mg/dl of blood and cattle on fluorine intakes sufficient to cause intoxication may have blood levels of 0.6 mg/dl.

Swarup*et al.* (2001) revealed the occurrence of fluorosis through clinical examination of cattle population in four villages around (<3 km) superphosphate fertilizer units in Udaipur district (India) during the month of July for two consecutive years (1998 and 1999). Affected animals had higher concentrations of fluoride in serum (range 0.31-1.07 mg/l in 1998 and 0.13-2.02 mg/l in 1999).

Maiti*et al.* (2004) while conducting a study on biochemical changes in endemic dental fluorosis in cattle recorded high fluoride level in the body 0.45 + 0.078 ppm. Whereas 0.055 + 0.002 ppm in healthy control.

Kanungo (2005) recorded fluoride content in serum of affected cattle near aluminium smelter of NALCO, Angul, and Orissa which ranged from 0.794 ppm to 1.360 ppm. The mean value for serum fluoride of the affected cattle was recorded to be 1.059 + 0.038 ppm as against the normal mean value of 0.321 + 0.019 ppm.

Upadhyay*et al.* (2007) screened a total 1261 bovines (728 cattle and 533 buffaloes) to study the prevalence of bovine fluorosis in 8 districts in Central East India (CEI) such as Durg, Raipur, Bilaspur, Bastar and Korba from Chhattisgarh and

Jabalpur, Mandla and Dhindori from Madhya Pradesh, The mean fluoride contents in the plasma of cattle and buffaloes were 0.06±0.00 and 0.08±0.00 ppm, respectively.

2.4 Haematological alterations

Swarup and Singh (1989) recorded significantly low Hb (9.48 +or-0.16 g/dl) and total leukocyte counts (6.8+or-0.32 x 103/micro lit) in buffaloes near a brick kiln congested zone of Ghaziabad district in U.P. .

Karram and Ibrahim (1992) reported increased mean corpuscular volume (MCV) in Blood samples from dromedaries (7-12 years old), kept within 4 km of a superphosphate plant (Assiut, Egypt), Decreases in total erythrocytes (RBC), haemoglobin (Hb), packed cell volume (PCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) levels were associated with the increased MCV values. Lymphocytosis, neutropenia and monocytopenia were present in animals from affected areas. The results showed that anaemia may be associated with chronic fluorosis in camels.

Hamid *et al.* (1994) recorded elevated erythrocyte counts, packed cell volume and haemoglobin level in blood of fluorotic hen reared near a superphosphate fertilizer producing factory. Total leukocyte counts were decreased.

Metwalli*et al.* (1995) reported significant decreases in haemoglobin and haematocrit in fluorosis affected cows in Fowa villages where many brick kilns were established.

Jagadish*et al.* (1998) revealed mild microcytic hypochromic anaemia and mild eosinophilia in severely affected fluorotic cattle.

Singh *et al.* (2002) recorded a significant decrease in Hb, total erythrocyte count and total leukocyte count, and an increase in the percentage of eosinophils in the goats with fluorosis, kept near an aluminium smelting plant in Orissa, India.

Maiti*et al.* (2003) observed anaemia, decrease in TEC and Hb level in affected flurotic cattle in endemic area of Nayagarh and Khurda district of Orissa.

Upadhyay*et al.* (2005) studied the haematology of fluoride affected cattle and buffalo which revealed mild anaemia with few exceptions of mild leukopenia and eosinophilia.

Han-Bo *et al.* (2006) indicated that erythrocytes, haemoglobin, packed cell volume values and Na±K+ ATPase activity from affected cattle on the high fluoride site of Qingtongxia Ningxia, China, were significantly reduced as compared with the corresponding samples of normal control cattle from non-fluoridated area. A great number of echinocytes were present in the peripheral blood, and subsequent anaemia was observed.

Trangadia and Kaul (2008) studied prevalence of fluorosis in 15 villages of Kunkavav, Lathi and Liliyatalukas of Amreli district of Gujarat. Haematological examination of affected animals revealed significant decrease in haemoglobin concentration and increased percentage of eosinophils as compared to control healthy animals.

Vinaykant*et al.* (2009) studied that exposure of sodium fluoride to goat produced significant reduction in haemoglobin (Hb), packed cell volume (PCV), total leucocytes count (TLC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) and increase in blood clotting time. However, values of total erythrocyte count (TEC) and differential leucocytes count (DLC) were not significantly changed.

Sharma *et al.* (2010) reported that the high fluoride level in drinking water was responsible for the significant reduction of blood components such as haemoglobin, packed cell volume, and total erythrocyte count in the blood of the fluorotic buffaloes from different areas in Punjab.

Agha *et al.* (2012) stated that oral administration of sodium fluoride to albino rats caused significant decrease in RBC, HCT, MCV, MCH, MCHC and PLT and increase in WBC, lymphocytes and granulocytes.

2.5 Biochemical alterations

Gentile *et al*. (1975) found moderately raised serum alkaline phosphatase values in fluorotic cattle.

Kessabi*et al.* (1983) evaluated the serum sample collected from 100 sheep (endemic fluorosis) of the Darmous area of Morocco. Urea, creatinine, alkaline phosphatase and sorbitol dehydrogenaze were significantly increased whereas glucose, cholesterol, total protein and albumin decreased as compared to healthy animals.

Wheeler and Fell (1983) have related the activity of alkaline phosphatase in fluorosis to abnormal formation of bone and osseous tissue.

Zhai*et al.* (1985) reported increased activities of serum alkaline phosphatase in sheep, experimentally infected with fluorosis.

Swarup and Singh (1989) recorded elevated serum alkaline phosphatase (57.40 u/dl) values in severely affected buffaloes near a brick kiln congested zone of Ghaziabad district in U.P as compared with healthy buffaloes from unpolluted areas.

Jundang (1992) studied biochemical changes in 40 native goats pastured for 18 months in an area severely polluted by industrial fluoride. Affected goats showed increased serum alkaline phophatase activities of 40.76 + 20.13 U/l as compared to 20.32 + 14.31 U/ l in control animals.

Botha *et al.* (1993) reported outbreak of fluorosis in sheep in South Africa. Blood of the affected sheep showed inversion of albumin globulin ratio and elevation of alkaline phosphatase activity.

Hamid *et al.* (1994) recorded reduced Albumin/globulin ratio, total proteins in fluorotic hen.

Singh and Swarup (1994) reported the occurrence of fluorosis in buffaloes in several villages of Ghaziabad district of U.P. Affected animals had higher urea and creatinine concentrations and alkaline phosphatase activities than healthy buffaloes.

Metwalli*et al.* (1995) reported chronic clinical bovine fluorosis in Fowa villages where many brick kilns were established. Serum total protein was significantly decreased, whereas serum alkaline phosphatase activity, fluoride content, urea, and creatinine were significantly increased, compared with control animals.

Jagadish*et al.* (1998) revealed significantly higher alkaline phosphatase, aspartate aminotransferase, and fluorine level in plasma of affected cattle those fed with the concentrate having very high fluoride content (186 mg/kg).

Singh and Swarup (1999) investigated biochemical changes in serum and urine of fluorotic cattle and buffalo and higher levels of fluoride were recorded in serum and urine of affected animals. The affected animals showed significantly elevated values of serum urea, nitrogen, creatinine, alkaline phosphatase. Changes in urine included increased level of alkaline phosphatase in fluorotic animals.

According to Radostits*et al.* (2000), there is a significant correlation between the amount of fluoride fed and the concentration of alkaline phosphatase in the serum. The increase in phosphatase activity is probably related to the abnormal formation of bone. The increased SAP activity may be three to seven times the normal level.

Muralidhar*et al.* (2000) recorded alkaline phosphatase enzyme activity in plasma of clinical bovine fluorosis cases which was higher in Chitradurga (109.0+0.17 U/l) whereas the activity was low (36.85 + 0.27 U/l) in Magadi. In plasma sample of the slaughtered animals the activity of enzyme was considerably high (131.5 + 0.43 U/l). In this study the activity of alkaline phosphatase was comparatively higher than the normal values (11-21 U/l) in bovines which indicated damage to bone / osseous tissue. He concluded that in clinical cases of fluorosis apart from the plasma F, ALP activity will aid in confirming diagnosis.

Singh *et al.* (2002) recorded a significant decrease in total protein, albumin, glucose and cholesterol in affected goats near an aluminium smelter plant in Orissa, compared with healthy goats; whereas, creatinine, alkaline phosphatase, aspartate aminotransferase and alanine aminotransferase values were significantly increased.

Sahoo*et al.* (2003) recorded alkaline phosphatase activity in serum of healthy control sheep (27.89 + 0.91 IU/l) and the ALP activity was 55.29 + 1.43 IU/l in fluorotic sheep. Total protein, albumin, glucose and cholesterol values of the fluorotic animals were significantly less than the corresponding values of the healthy animals.

Maiti and Das (2004) reported higher activity of serum alkaline phosphatase in affected cattle (39.61 + 1.06) in comparison to healthy control (10.05 + 0.81 IU/ l).

Upadhyay*et al.* (2005) studied plasma and urinary biochemical profiles of cattle and buffaloes with a plasma fluoride level of >0.3 ppm. Higher levels of plasma fluoride, plasma urea nitrogen, and ALP and AST activities whereas the concentrations of total cholesterol, total protein, albumin and globulin decreased in the affected animals. The levels of plasma creatinine and A/G rati remained unchanged. The affected animals had higher ALP activity in urine.

Xiu*et al.* (2006) reported significantly decreased alkaline phosphatase activity in serum, significantly increased urea nitrogen and elevated creatinine and decreased Na^+, in young pigs of about 50 days old those received the basal diet with additionally supplemented NaF, it shows that chronic excessive fluoride exposure is deleterious to kidney structure and function of pigs.

Trangadia and Kaul (2008) studied prevalence of fluorosis in 15 villages of Kunkavav, Lathi and Liliyatalukas of Amreli district of Gujarat. Serum biochemical studies showed significant decrease in total protein level whereas serum alkaline phosphatase activities were significantly increased in affected animals.

Ranjan*et al.* (2009) revealed increase in alkaline phosphatase activity in cattle population reared in fluoride endemic areas of district Nawada (Bihar) and Udaipur (Rajasthan), India.

Sharma *et al.* (2010) reported that the high fluoride level in drinking water was responsible for the significant increase in ALP, ALT, AST, UN, creatinine, and glucose in the blood of the fluorotic buffaloes from different areas in Punjab.

2.6 Ameliorative effect of different agents used in fluorosis

2.6.1 Aluminium

Becker *et al.* (1950) indicated that aluminium sulphate or chloride diminished the absorption of fluorine from the intestinal tract.

Grunder (1972) conducted an experiment on 24 milk cows in a polluted area near an aluminium factory, and found that administration of a low fluoride food mixture containing 3% aluminium sulphate and chloride in equal parts reduced fluoride uptake and absorption per kilogram dry food by about 15%.

Said *et al.* (1975) conducted the experiment to find out the alleviating effect of aluminium chloride in sheep administered with water containing 30 ppm of fluoride. Aluminium chloride @ 3.3gm/day was given daily orally to each sheep for two years. No substantial impacts on sheep were recorded except decreased fluorine content of bones.

Gentile *et al.* (1975) treated 28 cattle with fluorosis of different severity with phosphorous and aluminium sulphate which did not alter the serum Mg, Ca, P and alkaline phosphatase value.

Said *et al.* (1977) studied Aluminium chloride as a possible alleviator of fluorosis in sheep maintained in pens and found that more F was excreted in the faeces when $AlCl_3$ was added to the diet but no alleviation of symptoms of fluorosis (mostly dental aberrations) was noted. The accepted safe level in the form of NaF(70-100 ppm F) was regarded as being too high, certainly in case of Wither sheep.

Mehra (1981) experimented ameliorative measures of calcium chloride, magnesium chloride and aluminium chloride against fluorosis in cattle by feeding 50 –75 gm daily for 6 months. Cattle given chloride had no clinical sign of chronic fluorosis but 2 of the 3 controls certainly and the 3rd probably had exostoses in the metacarpal and metatarsal bones.

Kessabi*et al.* (1986) studied the alleviating effect of aluminium sulphate in male sheep affected with experimental fluorosis. Disturbance in general health status and osteodental symptoms were alleviated by administration of aluminium sulphate. Most disturbances were dose-related and alleviated by simultaneous administration of aluminium sulphate (13.5mg Al/kg), mainly for the lower F dose (1.9mg F/kg. b. wt.). Aluminium sulphate protection seemed to be very effective in sheep fed with fluoride @ 1.9 mg / kg b.wt. But it was comparatively less effective when fed @ 4.7 mg / kg. b.wt.

Kessabi*et al.* (1988) studied experimental fluorosis in sheep and alleviation effect of Aluminium sulphate and found that Aluminium sulphate decreased the digestive absorption of fluoride (about 33 to 45 %) and reduced the fluoride in serum, urine, bones and teeth.

Singh (1994) made therapeutic trial by using aluminium sulphate (100mg / kg. b. wt.) orally in fluorotic sheep. A significant decrease in serum fluoride content (from mean 0.654 mg / l to 0.56 mg / l) and urine fluoride content (from mean 9.03 mg / l to 7.88 mg / l) were observed. Also trial was made by using aluminium chloride at the same dose rate and serum fluoride content decreased from mean 0.60 + 0.9 mg / l to 0.53 + 0.08 mg / l and urine fluoride content decreased from 10.22 to 8.92 mg / l. Both the aluminium salts failed to alleviate the existing teeth abnormalities though the serum and urine fluoride content decreased significantly in fluorotic sheep.

Milhaud *et al.* (1997) prevented industrially induced fluorosis in cattle and sheep beyond 3 km area around the plant by distribution of rations containing 6% aluminium sulphate and replacing contaminated food with fluoride free food.

According to Radostits*et al.* (2000), aluminium salts act as neutralizers of the hydrofluoric acid produced in the stomach and because of their insolubility they are safe even in large quantities (30 gm of Aluminium sulphate daily for prevention, more for treatment). These salts are the principal substances used to detoxicate food and water. They are relatively ineffective, reducing the accumulation of fluorine in bone by only 20-30% and are thus referred to as “alleviators”.

Attia*et al.* (2004) reported that dietary Al minimized the effect of high fluoride toxicity and reduced the fluoride level in plasma, muscles and kidney of laying hens.

Tao *et al.* (2004) studied the effects of Mg-Al mixed oxides (MAO) on fluorosis in growing-finishing pigs. The MAO markedly increased fluorine excretion in the feces) and urine and significantly decreased fluorine levels in the serum, liver, kidney, and longissmusdorsi muscle. Moreover, the added MAO also reduced the toxic effects of excess fluoride on various serum biochemical indexes. Thus can effectively alleviate fluorosis in growing and finishing pigs.

Vinay-Kant *et al.* (2009) indicated that concurrent administration of aluminium sulphate failed to show ameliorative action on the enzymatic alterations induced during subacute intoxication by NaF in goat.

2.6.2 Calcium and Boron

Seffner *et al.* (1983) fed 5mg / kg NaF to pigs for 1 year, cortices of long bones became thicker and cancellous bone developed osteopenia. Simultaneous feeding of high doses of borax (upto 3 mg / kg) reduced NaF effect on bone cortices.

Wang *et al.* (1995) studied preventive effects of feed supplementation on industrial fluorosis in goats for 6 months and found that the heights of the 1st pair of incisors increased when feed mixture contained maize, wheat bran, $CaCO_3$ and $CuSO_4$ or soya bean meal when compared with goats fed on pasture. Feed supplementation significantly increased blood haemoglobin, total protein and albumin.

Vasistha*et al.* (1998) reported that the supplementation of boron in high fluoride diet reduced the absorption of fluoride in the body as revealed by normal alkaline phosphatase activity in treated lambs.

Chinoy*et al.* (1993) experimented with ascorbic acid and calcium and their combination as therapeutic effects in experimental fluoride toxicosis in rats.

Simultaneous administration of ascorbic acid, calcium and sodium fluoride to rats resulted marked recovery in all the parameters.

Wang *et al.* (1995) found that relocation of goats to low fluoride areas was beneficial only if done before permanent teeth have developed. Grass cultivation and dry season nutrient supplementation were both beneficial in increasing the life span of the animals. Tooth trimming only provided temporary support in improving mastication. Feed supplementation with Ca, Mg, B, Al and Se did not provide any protection against industrial fluorosis.

Ekambaram*et al.* (2002) studied modulation of fluoride toxicity in rats by calcium carbonate (50 mg / kg b.wt. orally) and prevented toxicity of fluoride by maintaining serum fluoride at a less toxic level. Further the toxic effects of fluoride are reversible if its exposure is withdrawn for two months.

Maiti*et al.* (2004) viewed treatment with boron (10mg / kg. b.wt.) and calcium (100mg/kg b.wt.) in combination for 90 days resulted in quantitative and gradual reduction of fluorosis in serum and urine and alkaline phosphatase level. The boron and calcium in combination at higher doses may have therapeutic value for management of endemic fluorosis in cattle.

Kanungo (2005) reported selenium, boron and calcium as antioxidant minerals which were found effective to bring amelioration of oxidative damages in fluorotic cattle. Selenium was found to be more efficacious in compare to calcium and boron.

Bharti*et al.* (2008) evaluated the protective role of boron on the serum profile of buffalo calves fed a high fluoride ration. Dietary F caused a significant depressing effect on serum Ca and Zn which was improved with B supplementation. However, serum Fe and Cu did not show any significant change on F or F+B supplementation. The serum ALP and phosphorus level were increased significantly on F feeding but declined significantly when B was fed. The findings suggested beneficial effect of boron on serum minerals and ALP in buffalo calves fed high fluoride ration.

2.6.3 Others

Xue*et al.* (2000) studied antagonistic effect of selenium and zinc on the renal impairment induced by fluoride in rats through their antioxidation. The cooperative effect of Na_2SeO_3 and $ZnSO_4$ were more powerful than either Na_2SeO_3 or $ZnSO_4$ alone.

Han Bo *et al.* (2001) reported that the dietary supplementation of Se, Cu and Mg in endemic fluorosis had beneficial effects on the mineral status and blood biochemical constituents after 83 days. It was concluded that Se, Cu and Mg can effectively alleviate bovine endemic fluorosis under natural conditions.

Liu and Kang (2003) reported that selenium can protect the functional disorder of thyroid caused by induced fluorosis in roman chicks using 4 mg/kg of sodium selenate and 1000 mg/kg of fluorine as sodium fluoride in feed.

Han Bo *et al.* (2004) studied the effect of selenium, copper and magnesium on antioxidant enzymes and lipid peroxidation in bovine fluorosis. They reported that fluorosis is associated with decreased serum level of selenium and copper. So it was found to be beneficial for high fluorotic cattle to supplement with proper selenium, copper and magnesium to increase fluoride excretion and obtain the protective activity of oxidative enzymes and to decrease lipid peroxidation and free radical contents.

Han Bo *et al.* (2006) suggested that fluoride exposure could cause erythrocyte damage, whereas selenium supplementation could antagonize fluoride-induced generation of free radicals and cumulative effects of lipid peroxidation in erythrocytes. Selenium supplementation might help in alleviating the possible hazards associated with bovine endemic fluorosis.

2.6.4 Tamarind

Gopalan*et al.* (1982) reported nutritive value of tamarind pump (*Tamarindusindica*) as 100 gm of pulp contains moisture 20.9 gm, protein 3.1 gm, fat 0.1 gm,mineral 2.9gm, fibre 5.6 gm, carbohydrate 67.4gm, energy 283 kcal, Ca 170

mg, P100 mg, I 10.9 mg, carotene 60 µg, riboflavin 0.07 mg, niacin 0.7 mg and vitamin C 3mg.

Bhattacharjee (1999) estimated that *Tamarindusindica*ripe fruit contains 55% tamarind pulp, 33% seed and 12% fibre. The pulp contains tartaric acid, citric acid malic acid, sugar, protein minerals, carbohydrate, fibre, vitamin A and niacin.

Khandare*et al.* (2000) reported that *Tamarindusindica*fruit pulp enhanced urinary fluoride excretion in dogs.

Khandare*et al.* (2002) opined that tamarind intake is likely to help in delaying progression of fluorosis by enhancing urinary excretion of fluoride in human. It also reduces excretion of magnesium and zinc.

Ranjan (2007) made experimental study on evaluation of ameliorative potential of *Tamarindusindica*(Imali) in fluoride intoxicated rabbits, and found useful in amelioration of fluorosis. Experiment was done in New Zealand white rabbits by treating with aqueous extract of *T. indica*at a dose rate of 100mg/kg. b.wt. orally for 90 days. The efficacy was compared with that of Aluminium sulphate @ 20mg / kg. b.wt. orally for the same period and it was found that *T. indica*extract had more efficacy in ameliorating fluorosis.

Ranjan*et al*. (2009) found the beneficial effect of aqueous extract of Moringa seeds and tamarind pulps in mitigation of fluoride toxicity in rabbit. Changes in plasma biochemistry revealed less hepatic and renal damage receiving plant extract and fluorinated water compared to animals receiving only fluorinated water.

Ekambaram*et al.* (2010) concluded that tamarind pulp may be useful to prevent the oxidative damage caused by consumption of excessive amounts of fluorine.

Dey*et al* (2011) suggested that concomitant use of tamarind fruit pulp extract can reduce fluoride concentration in blood and bone and enhanced urinary excretion in rat, indicating the ameliorative potential of fruits of tamarind in fluoride toxicity.

According to Gupta *et al.* (2013) supplementation of Tamarind fruit pulp powder in diet significantly reduced the fluoride concentration in serum of cattle. Interference of fluoride absorption from the gut due to administration of tamarind fruits might have played a role in reducing serum fluoride concentration.h

CHAPTER-III

MATERIALS AND METHODS

The present study entitled "A**meliorative potential of *Tamarindus indica* leaves in fluorotic calves and its socioeconomic impact assessment**" was carried out in the Department of Veterinary Medicine, College of Veterinary Science and Animal Husbandry, Orissa University of Agriculture and Technology, Bhubaneswar from November 2014 to June 2015. The investigation was carried out in the vicinity of the aluminium smelter plant, NALCO, Angul, Odisha.

3.1 Area of study

Talcher-Angul Industrial complex of Odisha is situated at latitude 20°95 'N to 21° 10' N and longitude 84° 55' E to 85° 28 'E, 139m above sea level (ASL), and 150 km away from Bhubaneswar, the state capital of Odisha. For the present investigation, 6 villages namely Bonda, Tulasipal, Gadasantri, Santri and Languliabeda villages of Angul district were selected, that are located within 3 km radius of aluminium smelter plant of National Aluminium Company (NALCO). The major pollutants from its smelter plant include fluoride, sulphur dioxide and suspended particulates among which fluoride has been emerged as a chief pollutant in the air, soil and water of the locality. Domesticated calves became fluorotic as they principally thrived on grazing of free pastures, besides being supplied with small quantity of cow milk, fodder and concentrate. Surface water (pond) was the main source of drinking water to livestock where as ground water, NALCO supplied water were also used.

3.2 Screening of animals

To derive estimates of the prevalence of fluorosis in calves, house-to-house surveys were made in the early morning and late evening when the animals are generally available and in herds in fields in the day-time. Only native calves that had been in the areas from birth were considered. Calves of either sex from 2 different age groups (6 months to 1 year and 1 year to 1.5 years) were picked up randomly from the

affected villages near the smelter plant and the teeth of the animals were carefully examined for signs of fluorosis. A total of 113calves were examined for characteristic dental fluorosis lesions and skeletal signs. Forty fluorotic calves having clinical signs of dental fluorosis were selected and screened for fluoride levels in their bio-samples. The selected calves were divided equally into 4 groups for the present research work. Among them, one group served as disease control. The selected herbal preparation i.e. the tamarind leaves powder of 5g and 10g were supplemented to two groups respectively orally on daily basis for 60 days and the standard chemical antidote i.e. Calcium (in form of calcium carbonate @ 15g/animal) was also supplemented for the same period to the rest group. Laboratory reagent grade calcium carbonate ($CaCo_3$) was manufactured by Merck Private limited.

For the healthy control study, ten animals from the unpolluted area of Bhubaneswar were selected. Fluoride concentrations were estimated in water and fodder as well as in the bio-samples of the selected animals in that area to rule out any possibility of fluoride pollution. The bio samples from the selected animals of the five different groups were collected on day 0, 30 and 60 of the experimental period.

3.3 Collection of environmental samples

3.3.1 Water

Water samples were collected from different sources such as ponds, tube well and dug well in the five selected villages and stored in pre-cleaned and sterilized polythene bottles of one-litre capacity following standard protocols. The water samples were immediately refrigerated after collection.

3.3.2 Fodder

Feed and fodder samples (cultivated and native plants, straw, bran and concentrate mixtures) were collected, then dried at 80°C for 48 hours in the oven, then ground with a mortar and pestle, lastly stored in vials in order to determine the Fluoride content.

3.4 Collection of bio-samples

3.4.1 Blood

Blood samples, around 15 ml from each animal, were collected aseptically from the jugular vein with sterilized syringe and needle using heparin as anticoagulant. Half of the blood sample was centrifuged at 2000 rpm for 10 minutes to separate plasma for biochemical analysis and estimation of fluoride, calcium, phosphorus and other minerals. The plasma samples were stored in a deep freezer at -40^{o}C temperature. Remaining whole blood was used for haematological analysis. The haematological and enzymatic parameters were carried out within 24 hours of collection of the blood sample. The other non-enzymatic biochemical parameters and the minerals including fluoride estimation were performed within next 24 hours.

3.4.2 Urine

About 20 ml urine from each animal (n=40) was collected directly in a 50 ml polyethylene bottle early in the morning hour and were transported to the laboratory in an ice box and were stored in refrigerator for analysis of fluoride.

3.4.3 Faeces

Faecal sample was collected from the screened animals (n=40), directly from the rectum in pre-labelled plastic specimen bottle in morning time. Pre-labeled specimen bottles were given to the cattle owner with the strict instructions to collect the faeces of cattle in to the bottle in the morning hours of above schedule days.

3.5 Preparation of herbal antidote

Tender leaves of *Tamarindus indica* were procured from the local village. The leaves were air dried ground to powder with the help of electronic grinder and stored air tight container. 5 grams and 10 grams of dried *Tamarindus indica* leaf powder was poured into separate zip polythene packet and a total of 1200 packets were made (5 gms- 600 packets and 10 gms-600 packets). Group III and IV animals were fed with one packet of 5 gms and 10 gms respectively daily for 60 days to each animal.

3.6 Estimation of fluoride

Portable fluoride ion specific electrode (Orion model 94 09 BN) and ISE meter (Orion Model-290A, Thermo Fisher Scientific Inc.) was used for the estimation of fluoride. Before analyzing sample, the instrument was calibrated with 0.1 ppm, 1 ppm and 10 ppm F standard solution. Fluoride concentration in plasma, urine and water was measured by the method of Cernik *et al*. (1970). Samples were diluted to suitable concentrations and mixed with total ionic strength adjustment buffer II (TISAB II). Fluoride concentration in fodder and faecal sample were estimated by the method of AOAC (1995). The fodder was dried at 80 degree C and grounded to fine powder. The powered sample was dissolved in 10% hydrochloric acid and kept 48 hr. After several times centrifugation of the solution and dilution of supernatant with 10% HCl the fluoride concentration in this solution was estimated using ion selective electrode after mixing with 15% sodium acetate. The fluoride concentration was expressed in ppm after multiplying with dilution factor.

3.7 Haematological parameters

Haemoglobin (grams/dl) was estimated by Sahli's method as recommended by Benjamin (1978). Packed cell volume (PCV) was determined using Wintrobe's method as described by Coles (1986). Total leukocyte count (TLC) was done according to the method described by Benjamin (1978). Differential leukocyte count (DLC) was made on blood films stained by Giemsa staining method (Schalm *et al.,* 1986).

3.8 Biochemical parameters

Liver and kidney function tests were evaluated using commercially available kits as per manufacturer's instruction. Activity of plasma Alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN) were estimated by Auto-analyser (TURBO CHEM100 © 2013 CPC Diagnostics) using commercial reagent kits.

3.8.1 Alanine aminotransferase (ALT)

The activity of serum Alanine aminotransferase (ALT) was estimated by LDH-NADH and Kinetic UV method, using the reagent kit supplied by Jeev Diagnostics Pvt. Ltd, Chennai-602101, Tamilnadu, India.

Principle: ALT catalyses the reversible transfer of amino group between L-Alanine and α- Ketoglutarate to form Pyruvate and Glutamate. The Pyruvate formed reacts with NADH in the presence of Lactate dehydrogenase to form NAD. The rate of oxidation of NADH to NAD is measured as a decrease in absorbance which is proportional to the ALT activity of the sample at wave length of 340 nm.

$$\text{L-alanine+ α- Ketoglutarate} \xrightarrow{\text{ALT}} \text{Pyruvate + L-Glutamate}$$

$$\text{Pyruvate + NADH + } H^{+} \xrightarrow{\text{LDH}} \text{Lactate + } NAD^{+}$$

Calculations

SGPT (ALT) Activity in U/L 370C = ΔA/min.x 1768

3.8.2 Aspartate amino transferees (AST)

The activity of serum Aspartate aminotransferase **(**AST) was estimated by MDH-NADH, Kinetic UV method as supplied by LAB KIT Jeev Diagnostics Pvt. Ltd, Chennai-602101, Tamilnadu, India.

Principle: AST catalyses the transfer of amino group between L-aspartate and α-ketoglutarate to form oxaloacetate and glutamate. The oxaloacetate formed with NADH in the presence of malate dehydrogenase to form NAD. The rate of oxidation of NADH to NAD is measured as a decrease in absorbance which is proportional to the SGOT (AST) activity in the sample at wave length of 340 nm.

$$\text{L- Aspartate + α Ketoglutarate} \xrightarrow{\text{AST}} \text{Oxaloacetate +L – glutamate}$$

$$\text{Oxaloacetate +NADH +} H^{+} \xrightarrow{\text{MDH}} \text{Malate +} NAD^{+}$$

Calculations

SGOT (AST) activity in U/L 37°c = ΔA/ min. × 1768

3.8.3 Creatinine

The serum creatinine was estimated by Alkaline Picrate method, using the reagent kit supplied by Jeev Diagnostics Pvt. Ltd, Chennai-602101,Tamil Nadu,India.

Principle: Picric acid in an alkaline medium reacts with creatinine to form an orange coloured complex with the alkaline picrate. Intensity of the colour formed was directly proportional to the amount of creatinine present in the sample at wave length 520 nm.

Creatinine + Alkaline Picrate $\rightarrow$Orange Coloured Complex

Calculations

Creatinine in mg % = Abs.T / Abs. S × 2.0

3.8.4 Urea

The serum urea was estimated by Modified Berthelot method, using the reagent kit supplied by Jeev Diagnostics Pvt. Ltd, Chennai-602101,Tamilnadu, India.

Principle: Urease hydrolyses urea to ammonia and CO_2. The ammonia formed, further reacts with a phenolic chromogen and hypochlorite to form a green coloured complex. Intensity of the colour formed is directly proportional to the amount of urea present in the sample at wave length of 570 nm.

$$\text{Urea} + H_2O \xrightarrow{\text{UREASE}} \text{Ammonia} + CO_2$$

Ammonia +Phenolic chromogen + Hypochlorite $\rightarrow$Green coloured complex

Calculations

Urea in mg/dl = Abs. T / Abs. S × 40

3.9 Estimation of minerals

3.9.1 Calcium

Serum calcium was estimated by direct calorimetric method, using calcium Kit manufactured by Crest Bio-system, a division of Coral clinical systems, Goa.

Principle: Calcium with Arsenazo III (1,8-Dihydroxy-3,6-disulpho-2,7-napthalene-bis azo-dibenzenearsenoic acid) at natural pH, yield a blue colour complex. The intensity of colour formed is proportional to calcium concentration in the samples at 650 nm.

Calculations

Calcium (mg/dl) = Abs. T / Abs. S × 10

3.9.2 Phosphorus

Inorganic phosphorus in serum was determined by using phosphorus Kit manufactured by Crest Bio-system, A division of Coral clinical systems, Goa.

Principle: Inorganic phosphate reacts in acid medium with ammonium molybdate to form a phosphomolybdate complex with yellow colour. The intensity of the colour formed is proportional to inorganic phosphate concentration in the sample at wavelength of 340 nm.

Inorganic phosphorus + Ammonium molybdate→Phospho-Molybdate complex

Phosphorous Molybdate complex + Metol→Molybdenum blue complex

Calculations

Phosphorus (mg/dl) = Abs. T / Abs.S × 5

3.10 Operationalization of socioeconomic variables

3.10.1 Education

It refers to the number of formal years of schooling or college study of the respondents. The respondents were classified on basis of education as;

Sl. No.	Variables	Score
1	Functionally literate	1
2	Primary	2
3	High School & Above	3

3.10.2 Occupation

It refers to the source of livelihood of the respondents.Occupation was categorized as farming and government job. All these were ancillary to dairy business. The scoring is as below:

Sl. No.	Variables	Score
1	Farming	1
2	Government Job	2

3.10.3 Family type

Family type was operationalized on the basis of whether the members were jointly staying & sharing a common kitchen. The scoring is done as follows

Sl. No.	Variables	Score
1	Nuclear	1
2	Joint	2

3.10.4 Income

It refers to the income of the respondents. Income of the respondent is classified as follows

Sl. No.	Variables	Score
1	BPL	1
2	Lower Income Group	2
3	Higher Income Group	3

3.10.5 No of animals kept by the respondents

It refers to the number of animals kept by the respondents. It is categorized as follows

Sl. No.	Variables	Score
1	Up to Two	1
2	Three to Five	2
3	Above Five	3

3.10.6 Type of animal reared

Respondents having type of animals is classified as below

Sl. No.	Variables	Score
1	Draught	1
2	Both Milk and Draught	2
3	Milk	3

3.10.7 Avg. age of animals

It refers to the age of animals kept by the respondents and categorized as follows

Sl. No.	Variables	Score
1	Up to Two Year	1
2	Three to Five Year	2
3	Above Five Year	3

3.10.8 Sources of water used

It refers to the type of water used by the respondents for their animals and it is classified as follows

Sl.No.	Variables	Score
1	Well water	1
2	Tube well water	2
3	Both Well and Tube well Water	3
4	Nalco Supplied water	4

3.10.9 Type of feed fed to the animals

Respondents supplemented type of feed to their animals are categorized as follows

Sl. No.	Variables	Score
1	Straw	1
2	Rice bran	2
3	Both	3

3.10.10 Means of feeding

It refers to the type of grazing to the animals by the respondents and it is classified as below

Sl. No.	Variables	Score
1	Grazing	1
2	Semi Intensive	2
3	Stall fed	3

3.10.11 Clinical signs observed

Clinical signs in affected calves observed during study are classified as follows

Sl. No.	Variables	Score
1	Non Skeletal Fluorosis	1
2	Dental Fluorosis	2
3	Skeletal	3
4	Both skeletal and dental fluorosis	4

3.10.12 Selling of affected animals

It refers to frequency of selling of the affected animals by the respondents and it is categorized as below

Sl. No.	Variables	Score
1	As usual	1
2	Frequently	2

3.10.13 Market price of affected animals

Respondents getting the price of the affected animals are classified as follows

Sl. No.	Variables	Score
1	lower	1
2	Usual	2
3	Higher	3

3.11 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 22 software, and expressed as mean ± SE, with $P<0.05$ considered statistically significant.

Figure 1: Evaluation of socioeconomic status of farmers

Figure 2: Calf showing sign of dental fluorosis

Figure 3: Blood sample collection of fluorotic calf

Figure 4: Fluoride estimation

Figure 5: Auto analyser for biochemical estimation

Figure 6: Biochemical analysis

CHAPTER-IV

RESULTS

4.1 Study of Socioeconomic profile of the respondents

Animal health has direct impact on socioeconomic livelihood of the livestock farmers. Better is the animal health more is the production. Hence, it is desired that there should be better health condition of the animals in the farmer's house. Many times it is seen that there are external factors which affect animal health causing less production of animals. Such factors may be manmade or natural factors. Though human beings do not have control on natural factors but can have control on manmade factors. As such, the area where study was conducted happens to be an industrial area where Nalco Smelter Plant is located. The animals as well the people living around the Smelter Plant are severely affected by the emissions coming out from the plant.

In India main source of fluoride for animals are fluoride rich effluents, dust and smoke from aluminium smelters, super phosphate fertilizers plants and brick kilns. It can precipitate over a considerable distance on vegetation, soil and water bodies, thus causing industrial fluorosis. These industrial operations release F in both gaseous and particulate forms. Hydrogen fluoride, silicon tetrafluoride and flurosilic acid are the common gaseous forms. Particulate fluorides include sodium fluoride, fluorspar, cryolite and aluminium fluoride. Fluoride in soil and water used for irrigation ultimately finds its way into forage and grains. Contamination of the surrounding area, particularly in the direction of the prevailing wind, may extend 8 – 9 km. It had a severe hazardous on the animal health causing the deterioration of health and decrease in production, productivity, draught ability of the domestic animals in particular and all the animals in general. In order to access the impact of such poison contaminants on the animal health, attempt was made to collect the data from the livestock owners whose animals have been affected with fluorine contaminants. Blood samples were collected from the affected animals of the respondents for clinical investigation.

Table 1: Approximate distance of the villages under study from the Nalco smelter plant

Name of the villages under study	Approximate air Distance (km) from the Nalco smelter plant
Bonda	0-0.5
Tulasipal	0-0.5
Languliabeda	0.5-1
Santri	1-1.5
Gadasantri	1.5-2

Table 2: Fluoride concentration (ppm) in water and fodder samples from experimental site

Name of the villages under study	Water	Fodder
Bonda	1.894	198.5
Tulasipal	1.808	186.5
Languliabeda	1.055	130.3
Santri	1.313	195.2
Gadasantri	0.423	122.0

Table 3: Fluoride concentration in plasma, urine and faeces of fluorotic calves reared in vicinity of aluminium smelter plant

Sample	Healthy	Disease Control
Plasma	0.083 ± 0.005^{a}	0.965 ± 0.031^{b}
Urine	2.56 ± 0.31^{a}	11.33 ± 0.16^{b}
faeces	9.85 ± 0.97^{a}	24.46 ± 0.56^{b}

The respondents were selected within 3 km radius of the location of the smelter plant, as because the animals are directly contaminated with the poisonous emission of the plant and there is more severity of the disease on the animals. The socioeconomic study was conducted among 30 farmers belonging to five adjacent villages of the plant, whose animals have suffered with fluorine contaminants.

Socioeconomic variable of respondents such as education, occupation, family type, income, possession of number of animals, possession of type of animals, age category of animals, sources of water used, type of feed fed to the animals, methods of feeding to the animals, type of clinical signs exhibited by the animals and economic status of the farmers of affected animals were the parameters taken for the study.

4.1.1 Education profile

Education of the livestock farmers has a great impact on the quality of management of animals. It has direct bearing on production of animals. Some earlier studies have revealed that, education of livestock farmers have significantly influenced on the production of animals. It is because educations of the livestock farmers help to manage scientifically animal health, animal shed, feeding, breeding and marketing etc. of animals. Hence, in order to know the impact of education on managing fluorosis affected animals reared by them, data were collected and presented in the Table no. 4.

4.1.2 Occupation profile

The people residing around the smelter plant are mostly farmers and depending upon agriculture for their livelihood. They had bullocks and cows as the assets of the livestock. Under this study the researchers attempted to know the occupation profile of the owners of fluorosis affected animals around the smelter plant. Data were collected and presented in the Table no. 5.

4.1.3 Family type

The family type is one of the factors which determine the success of livestock farming. More are the family members in a family, more are the opportunities to utilise the family member for better return from the livestock farm. In order to know the type of family having fluorosis affected animals in the study area researchers collected the data and presented in the Table no. 6.

Table 4: Distribution of respondents as per education

Sl. No.	Education	Frequency	Per cent (%)
1	Illiterate	8	26.66
2	Primary	10	33.34
3	High School & above	12	40

Table 5: Distribution of respondents as per occupation

Sl. No.	Occupation	Frequency	Per cent (%)
1	Farming	28	93.34
2	Government Job	2	6.66

Table 6: Distribution of respondents as per family type

Sl. No.	Family type	Frequency	Per cent (%)
1	Nuclear	17	56.66
2	Joint	13	43.34

4.1.4 Income

Income of the respondents is a yardstick to measure the standard of living of them. As such it is understood that more is the income of the people better will be the status, higher will be the expenditure, improved quality of life, capable of saving and higher purchasing power. In fluorosis affected area where animals are severely affected due to fluoride contamination, there is loss of production of animals due to deterioration of animal health. This affects the income of the people and hence directly affects their standard of living. In order to access the income category of respondents of fluorosis affected animals, the data were collected and presented in the Table no. 7.

4.1.5 Possession of the animals by the respondents

In order to know the number of animals by the respondents, the data were collected with the help of structural interview schedule and presented in the Table no. 8.

4.1.6 Possession of type of animals by the respondents

In rural Odisha the farmers keep different type of domestic animals, cows, bullocks, buffaloes, sheep, goats etc. as an alternative source of income next to agriculture. The researchers in order to know the type of animals are being reared by inhabitants by the surrounding area of the smelter plant, even if knowing disasters effect of fluoride contamination, the data were collected and analysed as per the Table no. 9.

4.1.7 Possession of animals of different age

Usually farmers keep different age group of animals starting from younger ones to older. The middle age group animals particularly bullocks are used for draught purpose and cows are meant for milk. In order to know the age category of fluorosis affected animals reared by respondents the data were collected and analysed as per the Table no.10.

Table 7: Distribution of respondents as per income

Sl. No.	Income	Frequency	Per cent (%)
1	BPL	11	36.66
2	Lower Income Group	13	43.34
3	Higher Income Group	6	20

Table 8: Distribution of respondents as per number of animals

Sl. No.	No of Animals	Frequency	Per cent (%)
1	Up to Two	10	33.33
2	Three to Five	10	33.33
3	Above five	10	33.34

Table 9: Distribution of respondents as per type of animals

Sl. No.	Type of Animal	Frequency	Per cent (%)
1	Milk	22	73.33
2	Draught	4	13.33
3	Both Milk and Draught	4	13.34

Table 10: Distribution of respondents as per avg. age of animals

Sl. No.	Avg. age of animal	Frequency	Per cent (%)
1	Up to Two Year	8	26.66
2	Three to Five Year	17	56.67
3	Above five Year	5	16.67

4.1.8 Source of water used for animal

The emissions of the smelter plant contaminate soil, water and air of surrounding area. Severity of contamination is expressed to animals' health in the area closer to the plant. The animals are mainly affected through contaminated feed and water, even though the traces of such pollutants are found in soil and air. In order to know the sources of water used for animals, data were collected and analysed as per the Table no. 11.

4.1.9 Type of feed fed to the animals

The area where study was conducted happens to be a rural area. The farmers usually give the conventional feed like straw, rice bran to the animals in the shed and let loose the animals to graze outside during day time. In order to know the type of feed used by the respondents to fed the animals, data were collected and presented in the Table no. 12.

4.1.10 Means of feeding to animals

It is a usual practice among the farmers that they allow the animals to graze outside during daytime and fed the animal in shed during evening and morning time. In order to know the feeding practice followed by the respondents to feed their animals, data were collected and analysed as per the Table no.13.

4.1.11 Clinical signs exhibited by fluoride affected animals

Studies conducted earlier on fluoride contamination revealed that different parts of the body are affected at different degree. As such two important parts of the body such as teeth and bone are mostly affected due to fluoride contamination. However, in order to know the degree of affection of fluoride contaminant on different parts of the body of animals in the study area, the data were collected and presented in the Table no. 14.

Table 11: Distribution of respondents as per sources of water

Sl. No.	Sources of water	Frequency	Per cent (%)
1	Well water	15	50
2	Tue well water	4	13.33
3	Both Well and Tue well Water	8	26.67
4	Nalco Supplied water	3	10

Table 12: Distribution of respondents as per type of feed

Sl. No.	Type of Feed	Frequency	Per cent(%)
1	Straw	3	10
2	Rice bran	7	23.34
3	Both	20	66.66

Table 13: Distribution of respondents as per feeding habit

Sl. No.	Feeding habit	Frequency	Per cent (%)
1	Grazing	11	36.66
2	Stall fed	18	60
3	Semi Intensive	1	3.34

Table 14: Distribution of respondents as per clinical signs

Sl. No.	Clinical signs	Frequency	Per cent (%)
1	Dental Fluorosis	8	26.66
2	Skeletal	1	3.34
3	Both Dental and Skeletal Fluorosis	20	66.66
4	Non Skeletal Fluorosis	1	3.34

4.1.12 Selling fluoride affected animals

Farmers keep the animals when animals are useful to them. When they feel that animals are less productive, unable to plough and liable to them, they usually try to dispose the animals. As such the fluoride affected animals lose the ability to work and the productivity decreases day by day based on the severity of the disease. In order to know the selling behaviour of the respondents of affected animals, attempt was made to document the data from the respondents of the animals affected from fluoride contamination and given in the Table no. 15.

4.1.13 Market price of fluoride affected animals

People keep the livestock animals to fetch profit in terms of selling milk, meat, manure and using the animals for draft purpose etc. However, in many instances the farmers sell the live animals for instant money in the nearby market. They fetch good margin of profit when the animals are having sound health and good look. But the animals affected with fluoride contaminants are less productive and deformed. In order to know the market price of the affected animals, attempt was made to collect data and presented in the Table no.16.

4.2 Haematological parameters

4.2.1 Haemoglobin concentration

The haemoglobin concentration in different experimental groups at different observation periods is presented in Table no. 17. Significant ($p<0.05$) decrease in haemoglobin concentration was recorded in fluorotic calves on day 0 (7.65±0.22 gm%), day 30 (7.83±0.18 gm%) and day 60 (7.90±0.24 gm%) as compared to healthy calves (11.10±0.19 gm%, 11.38±0.18 gm% and 11.08±0.18 gm%, respectively). Haemoglobin concentration in calves supplemented with tamarind (5gm) was significantly ($p<0.05$) increased on day 30 (8.58±0.13 gm%) and on day 60 (9.18±0.22 gm%) as compared to day 0 valve (7.68±0.18 gm%).

Table 15: Distribution of respondents as per selling of animals

Sl. No.	Selling of animals	Frequency	Per cent (%)
1	Frequently	28	93.34
2	As usual	2	6.66

Table 16: Distribution of respondents as per market price of affected animals

Sl. No.	Market price of affected animals	Frequency	Per cent(%)
1	Usual	2	6.66
2	Higher	0	0
3	lower	28	93.34

Table 17: Haemoglobin concentration in calves from fluorotic area along with normal calves

Group (n=10)	Day 0	Day 30	Day 60
I	11.10 ± 0.19^{B}	11.38 ± 0.108^{E}	11.08 ± 0.18^{D}
II	7.65 ± 0.22^{A}	7.83 ± 0.18^{A}	7.90 ± 0.24^{A}
III	7.68 ± 0.18^{aA}	8.58 ± 0.13^{bB}	9.18 ± 0.22^{cB}
IV	7.48 ± 0.12^{aA}	9.13 ± 0.11^{bC}	9.95 ± 0.06^{cC}
V	7.85 ± 0.14^{aA}	9.80 ± 0.11^{bD}	10.03 ± 0.09^{bC}

Group I: Healthy control, Group II: Disease control, Group III: Tamarind 5g treatment, Group IV: tamarind 10g treatment, Group V: $CaCO_3$ 10g treatment

Values (mean±S.E.) having no common superscript (small letter in a row and Capital letter in a column) differ significantly at $p<0.05$.

Significantly ($p<0.05$) increased in haemoglobin concentration was recorded in fluorotic calves received tamarind leaves @ 10gm on day 30 (9.13±0.11 gm%) and on day 60 (9.95±0.06 gm%) as compared to non-treated fluorotic calves (7.83±0.18 gm% and 7.90±0.24 gm%). Significantly ($p<0.05$) increased in haemoglobin concentration was observed after supplementation of $CaCO_3$ on day 30 (9.8±0.11 gm%) and day 60 (10.03±0.09 gm%) as compared to non-treated fluorotic calves (7.83±0.18 gm% and 7.90±0.24 gm%).

4.2.2 PCV percentage

The PCV percentage in different experimental groups at different observation periods is presented in Table no. 18. Significant ($p<0.05$) decrease in PCV percentage was recorded in fluorotic calves on day 0 (23.75±0.85%), day 30 (24.75±1.03%) and day 60 (24.00±0.71%) as compared to healthy calves (33.75±0.85%, 33.75±0.85%, 33.75±1.03%, respectively). PCV percentage in calves supplemented with tamarind (5gm) was significantly ($p<0.05$) increased on day 60 (28.50±0.65%) as compared to non-treated fluorotic calves (24.00±0.71%).

Tamarind supplementation @ 10gm to fluorotic calves significantly ($p<0.05$) increased PCV percentage on day 30 (31.50±0.65%) and day 60 (31.50±0.65%) as compared to non-treated fluorotic calves (24.75±1.03% and 24.00±0.71%). Significantly ($p<0.05$) increased in PCV percentage was observed after supplementation of $CaCO_3$ on day 30 (29.75±0.85%) and day 60 (30.00±0.41%) as compared to non-treated fluorotic calves (24.75±1.03% and 24.00±0.71%).

4.2.3 Total leucocyte count (cu.mm)

The total leucocyte count in different experimental groups at different observation periods is presented in Table no. 19. Significant ($p<0.05$) decrease in total leucocyte count was recorded in fluorotic calves on day 0 (5412.50±380.99 mm^3), day 30 (5250.00±170.78 mm^3) and day 60 (5350.00±216.98 mm^3) as compared to healthy calves (8950.00±367.99 mm^3, 8562.50±353.18 mm^3 and 8662.50±326.83 mm^3, respectively). Total leucocyte count in calves supplemented with tamarind (5gm) was significantly ($p<0.05$) increased on day 60 (6525.00±131.48 mm^3) as compared to non-treated fluorotic calves (5350.00±216.97 mm^3).

Table 18: PCV percentage in calves from fluorotic area along with normal calves

Group (n=10)	Day 0	Day 30	Day 60
I	33.75 ± 0.85^{B}	33.75 ± 0.85^{C}	33.75 ± 1.03^{D}
II	23.75 ± 0.85^{A}	24.75 ± 1.03^{A}	24.00 ± 0.71^{A}
III	24.75 ± 0.48^{aA}	24.50 ± 0.65^{aA}	28.50 ± 0.65^{bB}
IV	25.00 ± 0.41^{aA}	31.50 ± 0.65^{bBC}	31.50 ± 0.65^{bCD}
V	25.50 ± 0.65^{aA}	29.75 ± 0.85^{bB}	30.00 ± 0.41^{bBC}

Table 19: Total leucocyte count (cu.mm) in calves from fluorotic area along with normal calves

Group (n=10)	Day 0	Day 30	Day 60
I	8950.00 ± 367.99^{B}	8562.50 ± 353.18^{D}	8662.50 ± 326.83^{E}
II	5412.50 ± 380.99^{A}	5250.00 ± 170.78^{A}	5350.00 ± 216.98^{A}
III	5325.00 ± 303.79^{aA}	5550.00 ± 95.74^{aAB}	6525.00 ± 131.49^{bB}
IV	5325.00 ± 179.69^{aA}	6275.00 ± 335.09^{bBC}	7675.00 ± 110.86^{cD}
V	5375.00 ± 205.64^{aA}	6325.00 ± 179.69^{bC}	7025.00 ± 205.64^{bCD}

Group I: Healthy control, Group II: Disease control, Group III: Tamarind 5g treatment, Group IV: tamarind 10g treatment, Group V: $CaCO_3$ 10g treatment

Values (mean±S.E.) having no common superscript (small letter in a row and Capital letter in a column) differ significantly at $p<0.05$.

Tamarind supplementation @ 10gm to fluorotic calves significantly ($p<0.05$) increased total leucocyte count on day 30 (6325.00±179.69 mm^3) and day 60 (7025.00±205.64 mm^3) as compared to non-treated fluorotic calves (5250.00±170.78 mm^3 and 5350.00±216.987 mm^3). Significantly ($p<0.05$) increased in total leucocyte count was observed after supplementation of $CaCO_3$ on day 30 (6325.00±179.69 mm^3) and day 60 (7025±205.64 mm^3) as compared to non-treated fluorotic calves (5250.00±170.78 mm^3 and 5350.00±216.98 mm^3).

4.2.4 Neutrophil count (%)

The neutrophil percentage in different experimental groups at different observation periods is presented in Figure no. 7. Significant ($p<0.05$) decrease in neutrophil percentage was recorded in fluorotic calves on day 0 (22.25±0.75%), day 30 (21.75±1.25%) and day 60 (22.50±2.90%) as compared to healthy calves (38.75±0.25%, 38.75±1.50% and 38.50±0.65%, respectively). Neutrophil percentage in calves supplemented with tamarind (5gm) was non-significant as compared to non-treated fluorotic calves.

Tamarind supplementation @ 10gm to fluorotic calves significantly ($p<0.05$) increased neutrophil percentage on day 60 (32.00±1.22%) as compared to non-treated fluorotic calves (22.50±2.90%). Significantly ($p<0.05$) increased in neutrophil percentage was observed after supplementation of $CaCO_3$ on day 60 (32.00±1.08%) as compared to non-treated fluorotic calves (22.50±2.90%).

4.2.5 Eosinophil count (%)

The eosinophil percentage in different experimental groups at different observation period is presented in Figure no. 8. Significant ($p<0.05$) increase in eosinophil percentage was recorded in fluorotic calves on day 0 (10.00±1.08%), day 30 (9.00±0.40%) and day 60 (9.00±0.71%) as compared to healthy calves (2.25±0.25%, 2.73±0.63% and 2.50±0.29%, respectively). Significant decrease in eosinophil percentage was observed in calves supplemented with tamarind (5gm) on day 60 (5.25±0.48%) as compared to non-treated fluorotic calves (9.00±0.71%).

Figure 7: Neutrophil count (%)

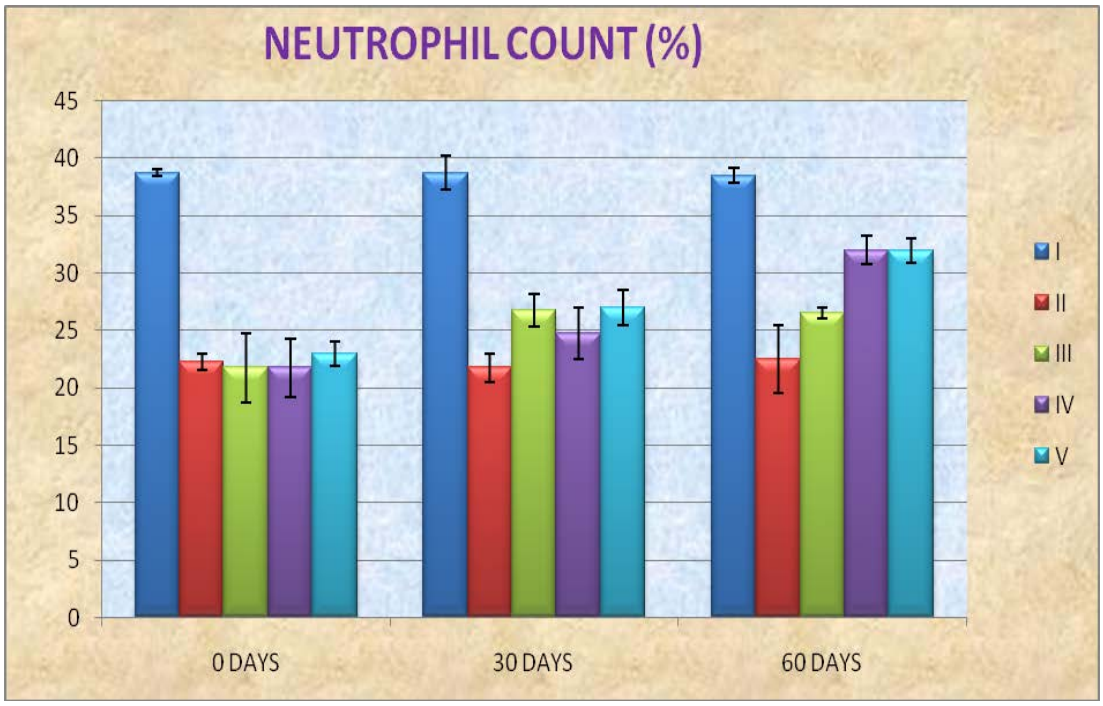
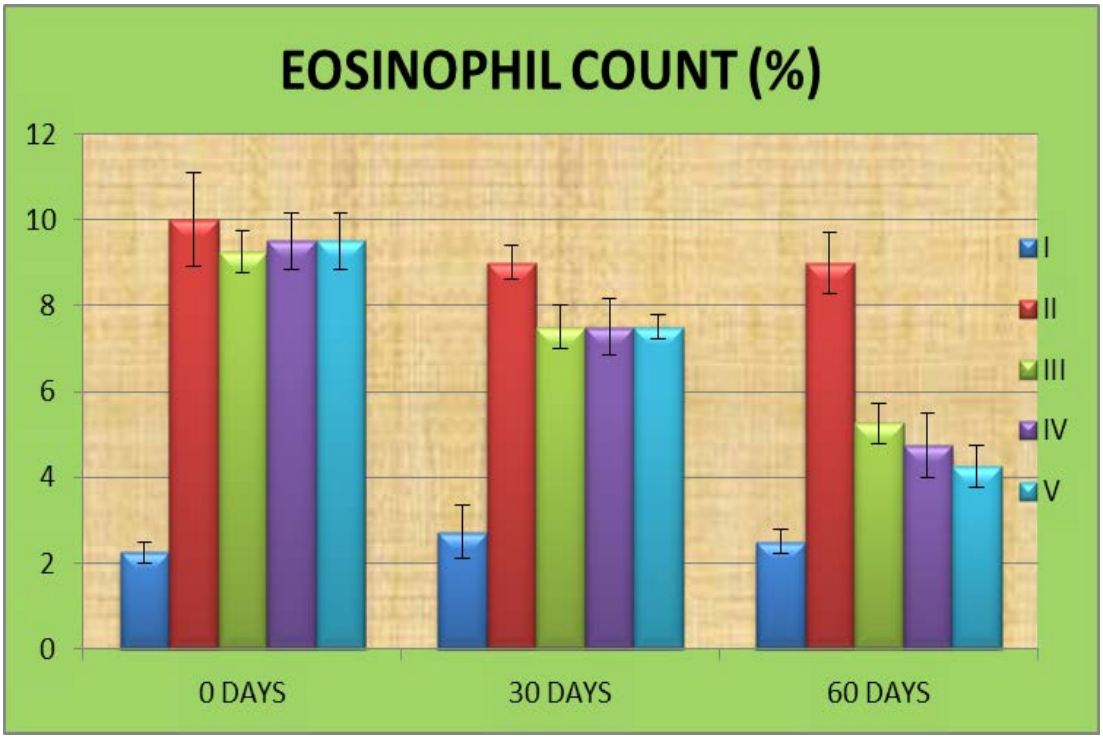


Figure 8: Eosinophil count (%)



Tamarind supplementation @ 10gm to fluorotic calves significantly ($p<0.05$) increased the eosinophil percentage on day 60 (4.75±0.75%) as compared to non-treated fluorotic calves (9.00±0.71%). Significantly ($p<0.05$) increased in eosinophil percentage was observed after supplementation of $CaCO_3$ on day 60 (4.25±0.48%) as compared to non-treated fluorotic calves (9.00±0.71%).

4.2.6 Lymphocyte (%)

The lymphocyte percentage in different experimental groups at different observation periods is presented in Table no. 20. Significant ($p<0.05$) increase in lymphocyte percentage was recorded in fluorotic calves on day 0 (66.50±1.66%), day 30 (67.25±1.55%) and day 60 (66.00±2.08%) as compared to healthy calves (57.75±0.48%, 57.50±0.96% and 57.75±0.48%, respectively). Lymphocyte percentage in calves supplemented with tamarind and $CaCO_3$ was non-significant as compared to non-treated fluorotic calves.

4.2.7 Monocyte count (%)

The monocyte percentage in different experimental groups at different observation periods is presented in Table no. 21. Non-significant increase in monocyte percentage in fluorotic calves was recorded on day 30 (2.00±0.58%) and day 60 (2.25±0.48%) as compared to healthy calves (1.00±0.00% and 1.25±0.25%). Significant decrease in monocyte percentage was recorded after supplementation of tamarind (5 gm and 10 gm) to fluorotic calves on day 60 (1.00±0.00% and 1.00±0.00%) as compared to non-treated fluorotic calves (2.00±0.58% and 2.25±0.48%).

4.3 Fluoride concentration in bio sample

4.3.1 Plasma fluoride level in calves

The plasma fluoride level in different experimental groups at different observation periods is presented in Figure no. 9. Significant ($p<0.05$) increase in

Table 20: Lymphocyte (%) in calves from fluorotic area along with normal calves

Group (n=10)	Day 0	Day 30	Day 60
I	57.75 ± 0.48^{A}	57.50 ± 0.96^{A}	57.75 ± 0.48^{A}
II	66.50 ± 1.66^{B}	67.25 ± 1.55^{B}	66.00 ± 2.08^{B}
III	67.75 ± 2.84^{B}	64.50 ± 0.87^{B}	66.50 ± 0.87^{B}
IV	67.75 ± 2.84^{bB}	66.50 ± 1.71^{abB}	62.25 ± 0.63^{AB}
V	66.50 ± 0.87^{B}	63.50 ± 0.87^{B}	61.75 ± 0.83^{AB}

Table 21: Monocyte count (%) in calves from fluorotic area along with normal calves

Group (n=10)	Day 0	Day 30	Day 60
I	1.25±0.25	1.00±0.00	1.25 ± 0.25^{AB}
II	1.25±0.25	2.00±0.58	2.25 ± 0.48^{B}
III	1.25±0.25	1.25±0.25	1.00 ± 0.00^{A}
IV	1.00±0.00	1.25±0.25	1.00 ± 0.00^{A}
V	1.00±0.00	2.00±0.58	2.00 ± 0.58^{AB}

Group I: Healthy control, Group II: Disease control, Group III: Tamarind 5g treatment, Group IV: tamarind 10g treatment, Group V: $CaCO_3$ 10g treatment

Values (mean±S.E.) having no common superscript (small letter in a row and Capital letter in a column) differ significantly at $p<0.05$

plasma fluoride level in fluorotic calves was recorded at day 0 (0.965±0.031 ppm), day 30 (0.958±0.035 ppm) and day 60 (1.003±0.025 ppm) as compared to healthy calves (0.083±0.005 ppm, 0.079±0.005 ppm and 0.076±0.004 ppm, respectively). Plasma fluoride level in calves supplemented with tamarind (5gm) was significantly ($p<0.05$) reduced on day 60 (0.326±0.014 ppm) as compared to non-treated group (1.003±0.025 ppm).

Significant ($p<0.05$) reduction in plasma fluoride level was observed after supplementation of tamarind @ 10gm on day 30 (0.600±0.035 ppm) and non-significant reduction on day 60 (0.535±0.083 ppm) as compared to non-treated group (0.958±0.035 ppm and 1.003±0.025 ppm). Significantly ($p<0.05$) lowered in plasma fluoride level was observed after supplementation of $CaCO_3$ on day 30 (0.655±0.0542 ppm) and non-significant on day 60 (0.605±0.021 ppm) as compared to fluorotic calves (0.958±0.035 ppm and 1.003±0.025 ppm).

4.3.2 Fluoride in urine sample in calves

The urinary fluoride level in different experimental groups at different observation periods is presented in Figure no. 10. Significant ($p<0.05$) increase in urine fluoride level in fluorotic calves was recorded at different observation periods of the experiment as compared to healthy calves. Urine fluoride level in calves supplemented with tamarind (5gm) was significantly ($p<0.05$) reduced on day 30 (10.87±0.27 ppm) and day 60 (10.33±0.26 ppm) as compared to non-treatment group (11.70±0.10 ppm and 11.75±0.08 ppm).

Significantly ($p<0.05$) lowered urine fluoride level was recorded in calves received tamarind leaves with feed @ 10gm on day 30 (10.33±0.22 ppm) and day 60 (9.82±0.19 ppm) as compared to non-treatment group (11.70±0.10 ppm and 11.75±0.08 ppm). Significantly ($p<0.05$) lowered in urine fluoride level was observed after supplementation of $CaCO_3$to fluorotic calves on day 30 (9.81±0.24 ppm) and day 60 (9.01±0.30 ppm) as compared to non-treated fluorotic calves (11.70±0.10 ppm and 11.75±0.08 ppm).

Figure 9: Plasma fluoride concentration (ppm)

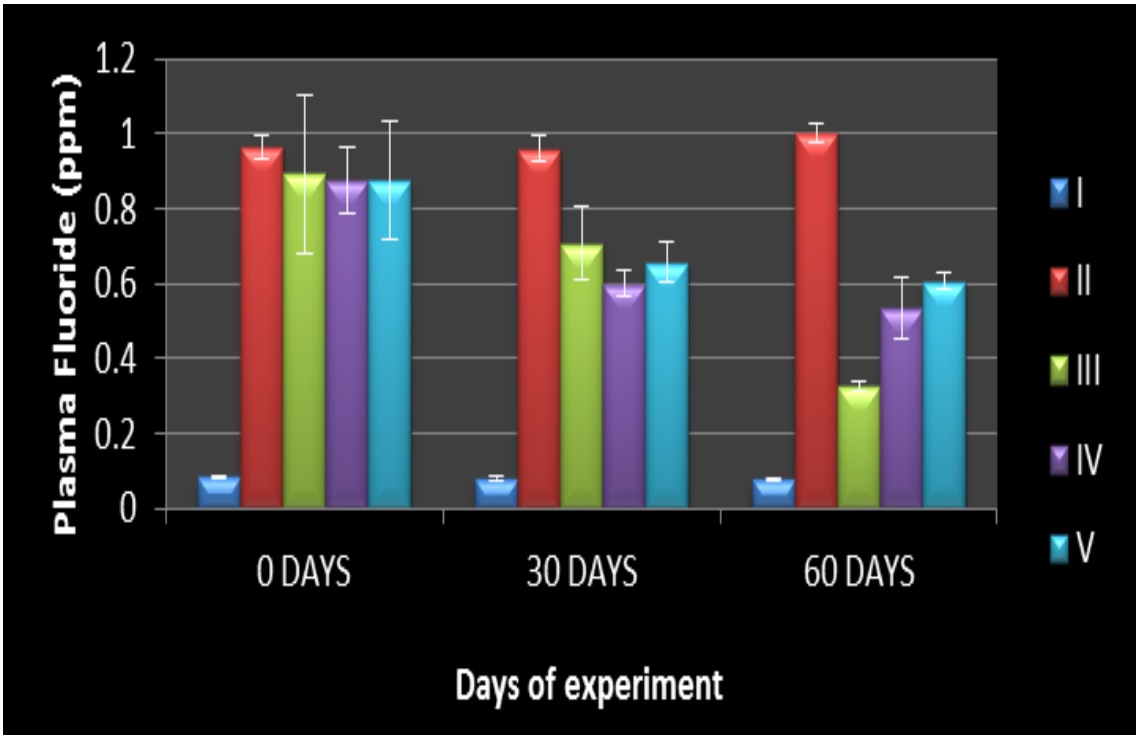
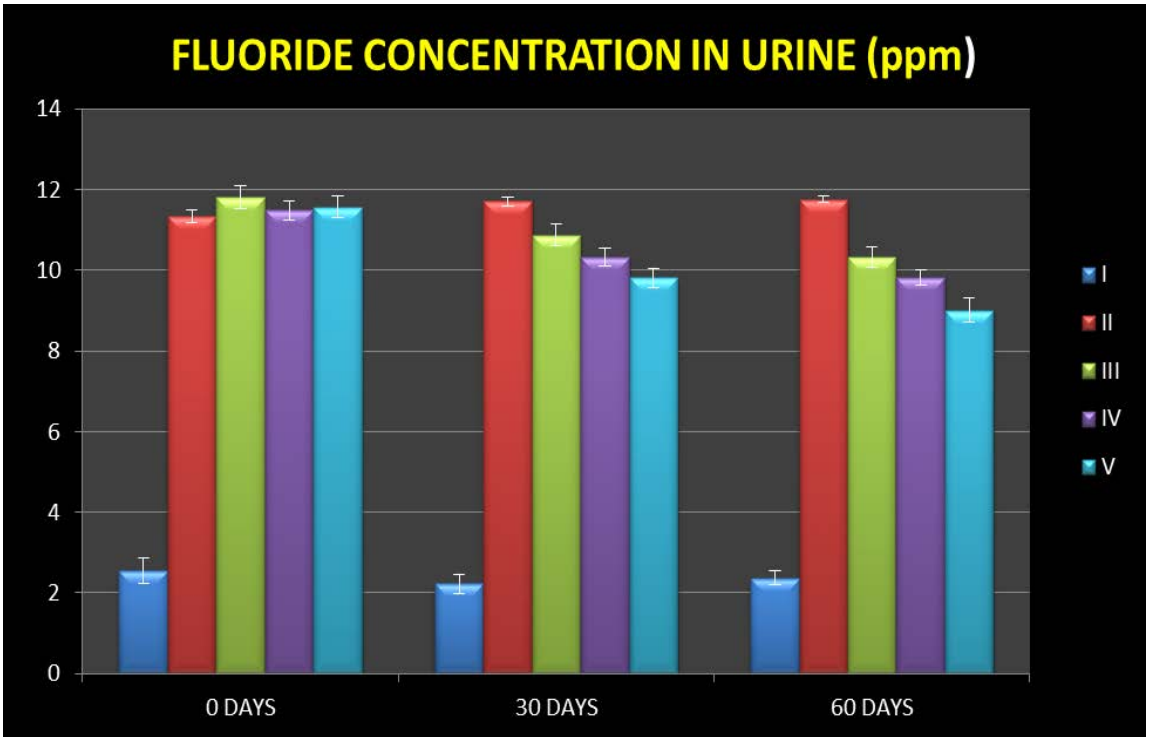


Figure 10: Fluoride concentration in urine (ppm)



4.3.3 Fluoride in faecal sample

The faecal fluoride level in different experimental groups at different observation periods is presented in Table no. 22. Significant ($p<0.05$) increase in faeces fluoride level was recorded in fluorotic calves on day 0 (24.46±0.56 ppm), day 30 (24.57±0.57 ppm) and day 60 (25.06±0.57 ppm) as compared to healthy calves (9.85±0.97 ppm, 9.53±0.27 ppm and 9.38±0.32 ppm, respectively). Faecal fluoride level in calves supplemented with tamarind (5gm) was significantly ($p<0.05$) increased on day 30 (25.58±0.53 ppm) and day 60 (29.06±2.11 ppm) as compared to day 0 value (22.87±0.49 ppm).

Tamarind supplementation @ 10gm to fluorotic calves significantly ($p<0.05$) increased thefaecal fluoride excretion on day 30 (27.45±0.61 ppm) and day 60 (28.76±0.67 ppm) as compared to non-treated fluorotic calves (24.57±0.57 ppm and 25.06±0.57 ppm). Significantly ($p<0.05$) increased in faecal fluoride level was observed after supplementation of $CaCO_3$ on day 30 (30.10±0.74 ppm) and day 60 (31.89±0.78 ppm) as compared to non-treated fluorotic calves (24.57±0.57 ppm and 25.06±0.57 ppm).

4.4 Biochemical parameters

4.4.1 The activity of ALT (U/L) in calves

The ALT activity in different experimental groups at different observation periods is presented in Table no. 23. Significant ($p<0.05$) increase in ALT activity was recorded in fluorotic calves on day 0 (20.84±1.16 U/L), day 30 (19.95±1.30 U/L) and day 60 (21.44±1.06 U/L) as compared to healthy calves (16.56±1.07 U/L, 16.26±0.59 U/L and 15.90±0.87 U/L, respectively). ALT activity in calves supplemented with tamarind irrespective of doses i.e 5 gm and 10 gm were non-significant as compared to non-treated fluorotic calves at different observation periods of the experiment. Significantly ($p<0.05$) decreased in ALT activity was observed after supplementation of $CaCO_3$ on day 60 (18.33±0.68 U/L) as compared to non-treated fluorotic calves (21.44±1.06 U/L).

Table 22: Fluoride in faecal sample in calves from fluorotic area along with normal calves

Group (n=10)	Day 0	Day 30	Day 60
I	9.85 ± 0.97^{A}	9.53 ± 0.27^{A}	9.38 ± 0.32^{A}
II	24.46 ± 0.56^{B}	24.57 ± 0.57^{B}	25.06 ± 0.57^{B}
III	22.87 ± 0.49^{aB}	25.58 ± 0.53^{bBC}	29.06 ± 2.11^{cC}
IV	23.92 ± 0.56^{aB}	27.45 ± 0.61^{bC}	28.76 ± 0.67^{bC}
V	23.83 ± 0.57^{aB}	30.10 ± 0.74^{bD}	31.89 ± 0.78^{bD}

Table 23: The activity of ALT (U/L) in calves from fluorotic area along with normal calves

Group (n=10)	Day 0	Day 30	Day 60
I	16.56 ± 1.07^{A}	16.26 ± 0.59^{A}	15.90 ± 0.87^{A}
II	20.84 ± 1.16^{B}	19.95 ± 1.30^{B}	21.44 ± 1.06^{C}
III	22.16 ± 1.13^{B}	23.05 ± 0.79^{B}	20.04 ± 0.80^{C}
IV	21.60 ± 0.62^{B}	21.70 ± 0.79^{B}	20.64 ± 1.18^{BC}
V	22.18 ± 1.00^{bB}	20.25 ± 0.86^{abB}	18.33 ± 0.68^{aAB}

Group I: Healthy control, Group II: Disease control, Group III: Tamarind 5g treatment, Group IV: tamarind 10g treatment, Group V: $CaCO_3$ 10g treatment

Values (mean±S.E.) having no common superscript (small letter in a row and Capital letter in a column) differ significantly at $p<0.05$

4.4.2 AST activity (U/L) in calves

The AST activity in different experimental groups at different observation periods is presented in Table no. 24. Significant ($p<0.05$) increase in AST activity was recorded in fluorotic calves on day 0 (86.80±0.66 U/L), day 30 (87.99±0.88 U/L) and day 60 (87.13±0.87 U/L) as compared to healthy calves (52.68±1.70 U/L, 53.16±1.66 U/L and 50.08±0.96 U/L, respectively). AST activity in calves supplemented with tamarind (5gm) was significantly ($p<0.05$) lowered on day 30 (78.53±1.03 U/L) and day 60 (77.09±0.97 U/L) as compared to non-treated fluorotic calves (87.99±0.88 U/L and 87.13±0.87 U/L).

Significantly ($p<0.05$) decreased AST activity was recorded in fluorotic calves supplemented with tamarind @ 10gm on day 30 (74.23±1.60 U/L) and day 60 (68.72±1.15 U/L) as compared to non-treated fluorotic calves (87.99±0.88 U/L and 87.13±0.87 U/L). Significantly ($p<0.05$) increased in AST activity was observed after supplementation of $CaCO_3$ on day 30 (78.88±0.53 U/L) and day 60 (69.70±0.32 U/L) as compared to non-treated fluorotic calves (87.99±0.88 U/L and 87.13±0.87 U/L).

4.4.3 Urea (mg/dL) in calves

The urea concentration in different experimental groups at different observation periods is presented in Table no. 25. Significant ($p<0.05$) increase in urea was recorded in fluorotic calves on day 0 (36.42±1.30 mg/dL), day 30 (35.52±1.22 mg/dL) and day 60 (35.64±1.30 mg/dL) as compared to healthy calves (27.98±0.62 mg/dL, 26.72±1.03 mg/dL and 26.07±0.72 mg/dL, respectively). The urea concentration in calves supplemented with tamarind and $CaCO_3$ were non-significant as compared to non-treated fluorotic calves.

4.4.4 Creatinine concentration (mg/dL) in calves

The creatinine concentration in different experimental groups at different observation periods is presented in Table no. 26. Significant ($p<0.05$) increase in creatinine concentration was recorded in fluorotic calves on day 0 (3.83±0.17 mg/dL), day 30 (3.83±0.10 mg/dL) and day 60 (3.68±0.12 mg/dL) as compared to healthy calves (1.20±0.14 mg/dL, 1.10±0.05 mg/dL and 1.20±0.08 mg/dL, respectively). Creatinine concentration in calves supplemented with tamarind (5gm) was significantly ($p<0.05$) decreased on day 60 (3.23±0.07 mg/dL) as compared to non-treated fluorotic calves (3.68±0.12 mg/dL).

Table 24: AST in (U/L) calves from fluorotic area along with normal calves

Group (n=10)	Day 0	Day 30	Day 60
I	52.68 ± 1.696^{A}	53.16 ± 1.663^{A}	50.08 ± 0.956^{A}
II	86.80 ± 0.662^{B}	87.99 ± 0.879^{D}	87.13 ± 0.869^{D}
III	87.16 ± 0.731^{bB}	78.53 ± 1.026^{aC}	77.09 ± 0.967^{aC}
IV	85.72 ± 0.620^{cB}	74.23 ± 1.599^{bB}	68.72 ± 1.149^{aB}
V	87.67 ± 1.090^{cB}	78.88 ± 0.527^{bC}	69.70 ± 0.322^{aB}

Table 25: Urea (mg/dL) in calves from fluorotic area along with normal calves

Group (n=10)	Day 0	Day 30	Day 60
I	27.97 ± 0.62^{A}	26.72 ± 1.03^{A}	26.07 ± 0.71^{A}
II	36.42 ± 1.30^{B}	35.52 ± 1.22^{B}	35.64 ± 1.30^{B}
III	36.12 ± 1.05^{B}	36.47 ± 0.62^{B}	34.88 ± 0.49^{B}
IV	36.72 ± 0.98^{B}	35.13 ± 0.65^{B}	36.33 ± 1.19^{B}
V	36.62 ± 0.96^{B}	36.48 ± 0.53^{B}	34.88 ± 0.82^{B}

Table 26: Creatinine concentration (mg/dL) in calves from fluorotic area along with normal calves

Group (n=10)	Day 0	Day 30	Day 60
I	1.20 ± 0.14^{A}	1.10 ± 0.05^{A}	1.20 ± 0.08^{A}
II	3.83 ± 0.17^{B}	3.83 ± 0.10^{C}	3.68 ± 0.12^{D}
III	3.76 ± 0.10^{bB}	3.58 ± 0.14^{bBC}	3.23 ± 0.07^{aBC}
V	3.96 ± 0.03^{bB}	3.25 ± 0.11^{aB}	2.94 ± 0.03^{aB}
V	3.68 ± 0.09^{bB}	3.49 ± 0.10^{abB}	3.30 ± 0.14^{aC}

Group I: Healthy control, Group II: Disease control, Group III: Tamarind 5g treatment, Group IV: tamarind 10g treatment, Group V: $CaCO_3$ 10g treatment

Values (mean±S.E.) having no common superscript (small letter in a row and Capital letter in a column) differ significantly at $p<0.05$

Tamarind supplementation @ 10gm to fluorotic calves significantly ($p<0.05$) decreased the creatinine concentration on day 30 (3.25±0.11 mg/dL) and day 60 (2.94±0.03 mg/dL) as compared to non-treated fluorotic calves (3.83±0.10 mg/dL and 3.68±0.12 mg/dL). Significantly ($p<0.05$) decreased in creatinine concentration was observed after supplementation of $CaCO_3$ on day 30 (3.49±0.10 mg/dL) and day 60 (3.30±0.14 mg/dL) as compared to non-treated fluorotic calves (3.83±0.10 mg/dL and 3.68±0.12 mg/dL).

4.4.5 Calcium concentration (mg/dL) in calves

The calcium concentration in different experimental groups at different observation periods is presented in Figure no. 11. Significant ($p<0.05$) decrease in calcium concentration was recorded in fluorotic calves on day 0 (7.11±0.28 mg/dL), day 30 (7.10±0.07 mg/dL) and day 60 (6.88±0.15 mg/dL) as compared to healthy calves (10.72±0.47 mg/dL, 10.79±0.24 mg/dL and 10.84±0.20 mg/dL, respectively). Calcium concentration in calves supplemented with tamarind (5gm) was significantly ($p<0.05$) increased on day 60 (7.88±0.11 mg/dL) as compared to non-treated fluorotic calves (6.88±0.15 mg/dL).

Tamarind supplementation @ 10gm to fluorotic calves significantly ($p<0.05$) increased the calcium concentration on day 30 (8.37±0.13 mg/dL) and day 60 (9.00±0.02 mg/dL) as compared to non-treated fluorotic calves (7.10±0.07 mg/dL and 6.88±0.15 mg/dL). Significantly ($p<0.05$) increased in calcium concentration was observed after supplementation of $CaCO_3$ on day 30 (7.89±0.13 mg/dL) and day 60 (8.63±0.14 mg/dL) as compared to non-treated fluorotic calves (7.10±0.07 mg/dL and 6.88±0.15 mg/dL).

4.4.6 Phosphorus concentration (mg/dL) in calves

The phosphorus concentration in different experimental groups at different observation periods is presented in Figure no. 12. Significant ($p<0.05$) increase in phosphorus concentration was recorded in fluorotic calves on day 0 (7.06±0.10 mg/dL), day 30 (6.95±0.05 mg/dL) and day 60 (7.08±0.04 mg/dL) as compared to healthy calves (4.64±0.23 mg/dL, 4.60±0.16 mg/dL and 4.82±0.18 mg/dL, respectively).

Figure 11 : Plasma calcium concentration (mg/dl)

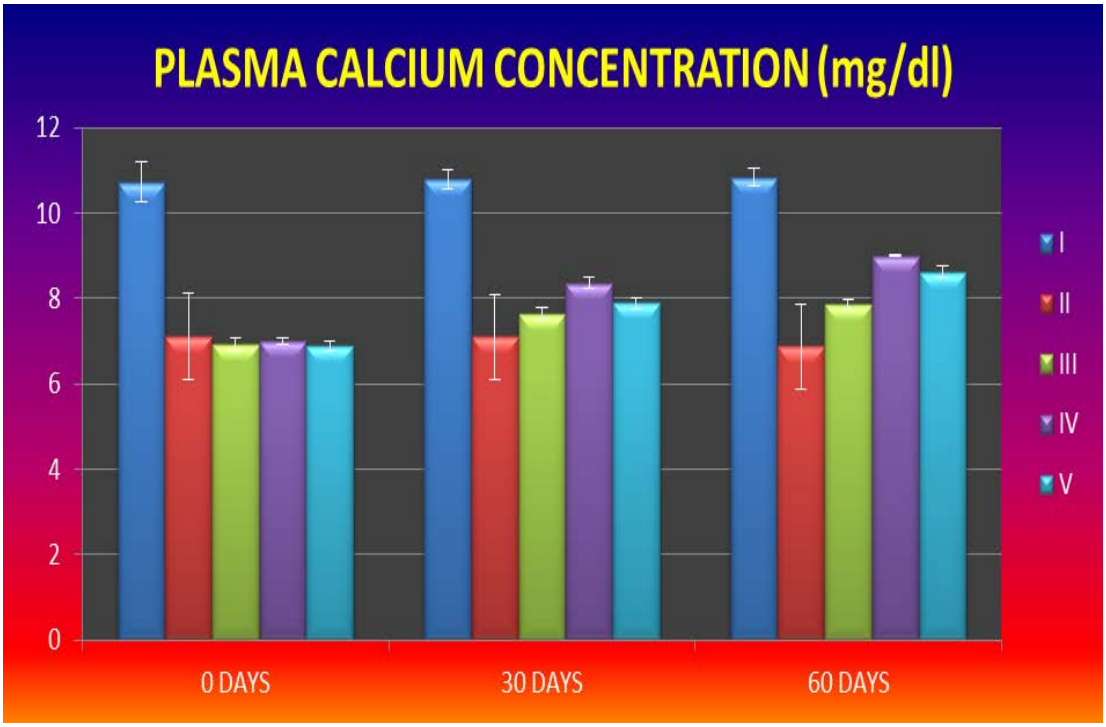
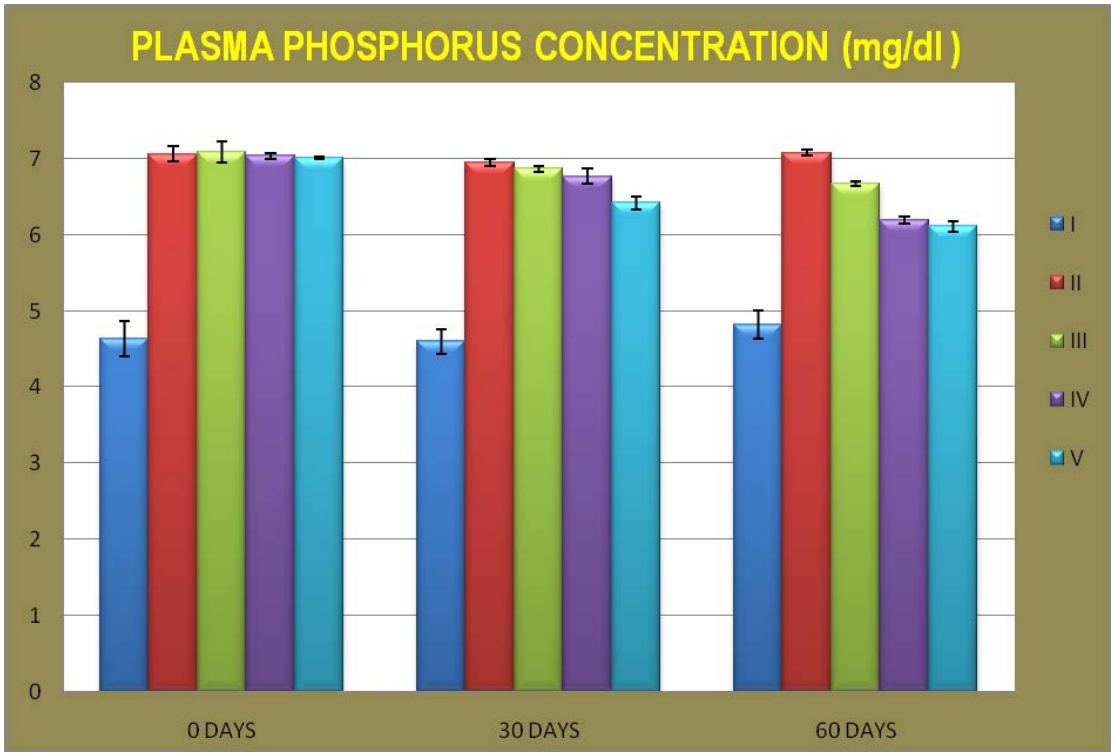


Figure 12 : Plasma phosphorus concentration (mg/dl)



Phosphorus concentration in calves supplemented with tamarind (5gm) was significantly ($p<0.05$) decreased on day 60 (6.67±0.03 mg/dL) as compared to non-treated fluorotic calves (7.08±0.04 mg/dL).

Tamarind supplementation @ 10gm to fluorotic calves significantly ($p<0.05$) decreased the phosphorus concentration on day 60 (6.19±0.05 mg/dL) as compared to non-treated fluorotic calves (7.08±0.04 mg/dL). Significantly ($p<0.05$) decreased in phosphorus concentration was observed after supplementation of $CaCO_3$ on day 30 (6.42±0.08 mg/dL) and day 60 (6.11±0.07 mg/dL) as compared to non-treated fluorotic calves (6.95±0.05 mg/dL and 7.08±0.04 mg/dL).

CHAPTER-V

DISCUSSION

Chronic fluoride intoxication or fluorosis is a worldwide health problem in man and animals. In India, fluorosis is endemic in more than 23 states, and about 62 million people are estimated to suffer from toxic effects of fluoride. Animals reared in fluorosis endemic areas are also affected and show varying degree of clinical symptoms and pathological changes. Industrial pollution (Swarup and Singh, 1989; Sahoo *et al*., 2004), high fluoride content in drinking water (Dwivedi *et al*., 1997; Maiti *et al*., 2004) and feed supplements containing high concentration of fluoride have been incriminated as major sources of excess fluoride to livestock in our country. Regular surveillance and monitoring of livestock health status in fluoride endemic area is required to check the devastating effects of fluoride by taking proper preventive and therapeutic measure.

At present, there are no suitable user-friendly non-toxic formulations for long term use to prevent fluorosis in animals. Thus, the present study was aimed at assessing the impact of fluoride pollution on fluorotic calves reared in emerging fluoride-rich industrialized areas with particular reference to evaluate the impact of industrial fluorosis on socioeconomic conditions of rural farmers and to evaluate the functional effects including haemato-biochemical parameters, and the ameliorative efficacy of tamarind leaves in fluorotic calves**.**

5.1 Socioeconomic status of farmers

5.1.1 Education profile

The present study revealed that maximum number of respondents (40 %) have passed high school certificate followed by 33.34 per cent have cleared primary education and 26.66 per cent respondents were illiterate. The analysis revealed that maximum number of respondents were cleared high school and kept the animals and they were well aware of toxic effects of fluorine on the animal health. The reason of keeping milch cows by highly educated people may be attributed to get milk for their

own consumption at their home and sell out the left out milk in the nearby market for getting extra income.

5.1.2 Occupation profile

Maximum percentage of the respondent (93.34%) belong to the farming category, followed by 6.66 per cent were having government job as their occupation. Hence, the data inferred that the reason of having maximum no. of farmers belonging to farming category because the area was predominantly inhabited by farmers depending on the agriculture as their livelihood.

5.1.3 Family type

Data revealed that 56.66 per cent of the respondents belonged to the nuclear family as against 43.34 per cent belonged to joint family. As such the earlier study agreed to the present finding in livestock rearing activities, most of the families were nuclear type.

5.1.4 Income

The present study revealed that 43.34 per cent of the respondents belonged to lower income group, who were deprived of getting benefits from the government at par with BPL groups. However, 36.66 per cent of respondents belonged to BPL category as against 20 per cent who belonged to higher income group. This study inferred that as a whole 80 per cent of the owners of the fluorosis affected animals were belong BPL category, lower income group, respectively.

5.1.5 Possession of the animals by the respondents

It is seen from the results that equal per cent i.e. 33.33 per cent of the respondents were having two animals, 3-5 animals and above five animals, respectively. As such it is inferred that 33.34 per cent of farmers were having animals more than 5 and had understood benefits of keeping animals in their home, even if they knew disasters effect of fluorosis disease to their animals.

5.1.6 Possession of type of animals by the respondents

It was found that maximum numbers of respondents i.e. 73.33 per cent were having cows followed by 13.33 per cent draught animals. However, the study revealed that 13.33 per cent of respondents were having both milch and draught animals. The study inferred that majority of the respondents were keeping cows for milk purpose and few numbers of respondents kept bullocks for ploughing or agriculture purpose. As such it is understood that the people were interested for keeping cows for getting milk for consumption or sell of milk in the surrounding villages for fetching profit, even if there was impact of deleterious effect of poison emission from the smelter plant.

5.1.7 Possession of animals of different age

The study revealed that 56.67 per cent respondents had animals whose age ranges from 3-5 years, followed by 26.66 per cent had animals of 2 years age and 16.67 per cent of respondents had animals more than 5 years age. The reason being of having maximum no. of animals of age within 3-5 years reared by the respondents was that this age group of animals are more productive in comparison to younger and older ones, despite of having knowledge on the threat of fluoride contamination. The respondents reared this age group of animals to meet the requirement of their draft as well as milk consumption in their family. The study concluded that even though there was loss of the farmer due to fluoride contamination to their animals still then the farmers were interested to rear the animals to meet the basic need of their food grains as well as requirement of milk and meat etc.

5.1.8 Source of water used for animal

The present report revealed that 50 per cent of respondents used well water for animal consumption, followed by 26.67 per cent respondents used both well and tub-well water, 13.33 per cent respondents used only tube well water. The interesting finding of the data revealed that though NALCO supplies fluoride free water for consumption purpose for both animals and human beings, only 10 per cent respondents used NALCO supplied water source for animal consumption. Hence the

study inferred that there is need to make people aware of about unsafe use of well water as maximum numbers of respondents i.e. 50 per cent used well water source for consumption of their animals.

5.1.9 Type of feed fed to the animals

The data of above table revealed that maximum percentage of respondents i.e. 66.66 per cent used straw and rice bran both to feed their animals followed by 23.34 per cent who fed only rice bran and 10 Per cent fed only straw to their animals. From this data it is inferred that people were habituated to feed the animals straw and rice bran in combination as they belong to agrarian family whose main sustenance is on agriculture.

5.1.10 Means of feeding to animals

The data from the present study revealed that 60 per cent of the respondents of the study area fed their animal in their stall, followed by 36.66 per cent allowed their animals to graze outside and only 3.34 per cent of respondents, they practice both grazing and stall feeding during day and night, respectively. Hence the above data revealed that farmers preferred to fed their animals in their stall rather than letting loose the animals outside for grazing. The reason may be due to the fact that the respondents were well aware of the contamination of water, soil and grass surrounding area due to the emission of the smelter plants, thus preferred to feed their animals in the stall.

5.1.11 Clinical signs exhibited by fluoride affected animals

The data revealed that animals of 66.66 per cent of respondents were suffered from both dental and skeletal fluorosis followed by animals of 26.66 per cent and of respondents suffered from dental fluorosis alone. Similarly animals of 3.34 per cent respondents were suffered from skeletal fluorosis alone. Animals of 3.34 per cent of respondents were suffered from non-skeletal fluorosis. From the above data it is inferred that the major attack of fluoride occurs on both teeth and bone simultaneously. Hence, farmers should be well educated on prevention of fluoride

contamination to their animals and as well as taking care of their animals after occurrence of the disease.

5.1.12 Selling fluoride affected animals

The present study revealed that 93.34 per cent of the respondents sold the animal frequently, when they found the sign of fluoride contamination of the animals. This is followed by 6.66 per cent of the respondent who were selling the animals as usual as before. From the analysis it is inferred that people were not interested to keep the fluoride affected animals for a long time in their shed, as these animals were less productive and not profitable.

5.1.13 Market price of fluoride affected animals

The data revealed that 93.33 per cent respondents opined that they sold the animals at lower price in the nearby market followed by 6.66 per cent of respondents agreed that they sold the animals as usual price as before. However, none of the respondent agreed that they sold the affected animals at higher price. Thus, the studies inferred that the animals suffered from fluorosis cost less value and are being sold at low price in the market causing a loss to the farmers.

5.2 Ameliorative study

Although the fluoride toxicity in man and animals is a worldwide problem, effective and safe treatment still remains elusive. A number of ameliorative agents including different metals like Al, B, Ca etc., and their compounds vitamins, hormones and other agents have been tried with varying degree of success. However, most of them have limited practical utility because of chronic nature of the problem and associated side effects with their prolonged uses.

India is having rich biodiversity with more than 45,000 plant species growing across the country. Several plant species, number reaching to thousands, have been found to possess medicinal properties. They have been used for treatment of various diseases in man and animals since times immemorial. There are many advantages of using herbal drugs, like low cost, easy availability and less/no potential toxicity or

side effects even after prolonged use. Few studies conducted in the past have also suggested that herbal drugs may be used for short-term management of fluorosis in animals (Khandare *et al.*, 2000; Stanley *et al.*, 2002).

In the present study, ameliorative potential of *Tamarindus indica* leaves powder was evaluated on fluorotic calves reared in vicinity of aluminium smelter plants. Calcium carbonate @100 mg/ kg body weight was given as standard therapy. The efficacy was evaluated in terms of their potential to reduce plasma fluoride concentrations and restoration of altered haemato-biochemical parameters in fluorotic calves.

Higher concentrations of fluoride in plasma and urine in animals from fluorosis endemic area have been reported by various authors (Patra *et al*., 2000; Muralidhara *et al*., 2000; Maiti and Das, 2004; Ranjan, 2009; Gupta *et al*., 2013). In the present study also, the concentration of fluoride in plasma of fluorotic calves was significantly high as compared to healthy control cattle. Supplementation of tamrind leaf powder with diet significantly reduced the fluoride concentration in plasma. Interference with fluoride absorption from gut due to administration of dried powder of tamarind leaf might have played roles in reducing plasma fluoride concentrations. Beneficial effect of tamarind might be due to tannin and high fibre contents in dried leaf (Ishola *et al*., 1990; El-Siddig *et al*., 2006). In addition, high content of calcium, copper and other minerals, amino acids and vitamin (Ishola *et al*., 1990; Almeida *et al*., 2009) in dried powder of tamarind leaf might have some additive roles in body fluoride removal or other associated beneficial effects.

Significant reduction in Hb, TLC and PCV in fluorotic calves than healthy ones was recorded in the present study. The presence of anaemia in fluoride affected animal was also reported in calves and other animals (Bharti *et al.*, 2007; Gill and Dumka, 2013; Swarup and Singh, 1989; Singh and Swarup, 1994; Vinay kant *et al*., 2009). Decrease in TLC was also observed by Behera (1993) and Swarup and Singh (1989) in cattle, Vinay-kant *et al*. (2009) in goats and Rao and Vidyunmala (2010) in mice. It is known that F intoxication depresses bone marrow activity in cattle (Radostis *et al.,* 2000) and F-induced disorders in hematopoietic organs in mice and in human hematopoietic progenitor cells are on record (Machalinska *et al*., 2002).

Decrease in Hb may be also possibly, 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 might be due to toxic effects of F on the RBC cell membrane and subsequently shrinkage of cell. Earlier studies also reported the haematological alterations in fluorotic animals (Maiti *et al*., 2003; Singh *et al*., 2002; Vinay kant *et al*., 2009).

In the present investigation, there was increase in Hb, TLC and PCV value in the treatment groups after 60 days of treatment. This might be due to prevention of oxidative damage to cell membrane of RBC by calcium (Bharti *et al.,* 2007) in $CaCO_3$ supplementation group. But the more significant increase in tamarind supplementation group might be due to high Fe and antioxidant like polyphenols and flavanoids, present in tamarind (Khandare *et al*., 2002; Martinello *et al*., 2006; Ranjan *et al*., 2009; Caluwe *et al*., 2010 and Dey *et al*., 2011).

A significantly lower concentration of calcium was noted in fluorotic calves 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 (WHO, 2002). Moreover, decrease in calcium ATPase activity was responsible for increase in urinary calcium causing hypocalcaemia (Singh and Swarup, 1999). These findings are in agreement with those of earlier workers (Maiti and Das, 2004; Bharti *et al*., 2007; Gupta *et al*., 2013) who also reported a decline in serum calcium in fluorotic animals. However, supplementations of tamarind leaf powder to the fluorotic calves produced significant increase in calcium concentration which was also found by Ranjan *et al*., (2009) and Gupta *et al*., (2013). The beneficial effect of tamarind might be due to its richness in calcium (Gopalan *et al*., 1982 and Ishola *et al*., 1990) and reduced absorption or an increase in elemination of fluoride from the body.

The average serum phosphorus content of fluorotic calves was higher as compared to healthy calves reared in fluoride free area. The present study supports the previous reports (Singh and Swarup, 1999; Maiti and Das, 2004; Upadhyay *et al*.,

2005; Ranjan *et al*., 2009). This is because circulatory parathormone level increases in fluoride intoxication (Singh and Swarup, 1999) which regulates metabolism of calcium and phosphorus causing hyperphosphatemia due to concurrent hypocalcemia. But treatment with tamarind leaves significantly decreased the P level which is in accordance with Maiti and Das (2004), Ranjan *et al.,* (2009) and Gupta *et al*., (2013).

Alkaline phosphatase level has diagnostic importance and indicates the damage of bone and affection of 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 calves as compared to calves from non fluorotic area. This finding was in agreement with many other observations reported in fluorotic animals (Maiti and Das, 2004; Bouaziz *et al*., 2004; Upadhyaya *et al*., 2005). Since fluoride stimulates osteoblastic acitivity (Arya *et al*., 1990), the increase in ALP can probably be related to the abnormal bone formation and stimulated osteoblastic activity with increased F concentration in serum (Farley *et al*., 1987). Supplementation of dried powder of tamarind leaves to the fluorotic calves significantly reduced the activity of alkaline phosphatase. The beneficial effect of tamarind on reduction of alkaline phosphatase activity might be due to presence of high calcium content in the dried leaf (Ishola *et al*., 1990; El-Siddig *et al*., 2006). Since supplementation of calcium has been reported to decrease the serum alkaline phosphatase activity in laboratory animals (Wang *et al*., 2008; He *et al*., 2008) and fluorotic cattle (Maiti *et al*., 2004) by various authors, the decrease in ALP activity could be due to its high content of calcium in dried leaf of tamarind as reported earlier.

The significant enhancement of plasma AST and ALT, indicative of hepatic dysfunction, might be due to hepato-toxic effect of F compounds which were also reported by Jagadish *et al.* (1998), Singh *et al.* (2002) and Maiti and Das (2004). Supplementation of tamarind leaf powder significantly decreased the AST and ALT level. It has been reported that *Tamarindus indica* contains flavanoids, β-carotenes and ascorbic acid (Martinello *et al.,* 2006 and Caluwe *et al.,* 2010) which have hepato-protective effect due to their anti-oxidant property (Pimple *et al.,* 2007; Ranjan *et al*., 2009 and Dey *et al*., 2011).

Significant rise in the level of urea and creatinine in the fluorotic calves than the calves from non fluorotic zone was recorded in the present study, which was also reported by Metwalli *et al.*(1995), Singh and Swarup, (1999), Swarup *et al*. (2001), Maiti and Das (2004) and Sharma *et al.* (2010). 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. High levels of serum urea and creatinine in the affected cows and buffaloes are therefore indicative of degenerative changes in the kidney (Singh and Swarup, 1999). This increased level might also be due to catabolism of protein because of partial starvation in affected animals (Swarup *et al*., 2001).

Consequent upon the treatment with tamarind leaf powder, the creatinine level decreased significantly and the urea level decreased non-significantly. This might be due to antioxidative property of tamarind which protects renal and muscular damage.

CHAPTER-VI

SUMMARY AND CONCLUSIONS

Chronic fluoride intoxication or fluorosis is a worldwide health problem in man and animals. In India, fluorosis is endemic in more than 23 states, and about 62 million people are estimated to suffer from toxic effects of fluoride (Susheela, 1999). Animals reared in fluorosis endemic areas are also affected and show varying degree of clinical symptoms and pathological changes. Industrial pollution (Swarup and Singh, 1989; Sahoo *et al*., 2004), high fluoride content in drinking water (Dwivedi *et al*., 1997; Maiti *et al*., 2004) and feed supplements containing high concentration of fluoride have been incriminated as major sources of excess fluoride to livestock in our country. Regular surveillance and monitoring of livestock health status in fluoride endemic area is required to check the devastating effects of fluoride by taking proper preventive and therapeutic measure.

At present, there are no suitable user-friendly non-toxic formulations for long term use to prevent fluorosis in animals. Thus, the present study was aimed at assessing the impact of fluoride pollution on fluorotic calves reared in emerging fluoride-rich industrialized areas with particular reference to evaluate the impact of industrial fluorosis on socioeconomic conditions of rural farmers and to evaluate the functional effects including haemato-biochemical parameters, and the ameliorative efficacy of tamarind leaves in fluorotic calves**.**

The respondents were selected within 3 km radius of the location of the smelter plant, as because the animals are directly contaminated with the poisonous emission of the plant and there is more severity of the disease on the animals. The socioeconomic study was conducted among 30 farmers belonging to five adjacent villages of the plant, whose animals have suffered with fluorine contaminants.

Socioeconomic variable of respondents such as education, occupation, family type, income, possession of number of animals, possession of type of animals, age category of animals, sources of water used, type of feed fed to the animals, methods of feeding to the animals, type of clinical signs exhibited by the animals and

economic status of the farmers of affected animals were the parameters taken for the study.

Significant decrease in haemoglobin concentration was recorded in fluorotic calves at different observation periods of the experiment as compared to healthy calves. Haemoglobin concentration in calves supplemented with tamarind and $CaCO_3$ was significantly ($p<0.05$) increased from day 30 onwards as compared to non-treated fluorotic calves.

Significant ($p<0.05$) decrease in PCV percentage was recorded in fluorotic calves at different observation periods of the experiment as compared to healthy calves. PCV percentage in calves supplemented with tamarind and $CaCO_3$ was significantly ($p<0.05$) increased from day 30 onwards as compared to non-treated fluorotic calves.

Significant ($p<0.05$) decrease in total leucocyte count was recorded in fluorotic calves at different observation periods of the experiment as compared to healthy calves. Total leucocyte count in calves supplemented with tamarind and $CaCO_3$ was significantly ($p<0.05$) increased as compared to non-treated fluorotic calves.

Significant ($p<0.05$) decrease in neutrophil percentage was recorded in fluorotic calves on day 0, day 30 and day 60 as compared to healthy calves. Neutrophil percentage in calves supplemented with tamarind (5gm) was non-significant as compared to non-treated fluorotic calves. Tamarind supplementation @ 10gm to fluorotic calves significantly ($p<0.05$) increased neutrophil percentage on day 60 as compared to non-treated fluorotic calves. Significantly ($p<0.05$) increased in neutrophil percentage was observed after supplementation of $CaCO_3$ on day 60 as compared to non-treated fluorotic calves.

Significant ($p<0.05$) increase in eosinophil percentage was recorded in fluorotic calves on day 0, day 30 and day 60 as compared to healthy calves. Significant decrease in eosinophil percentage was observed in calves supplemented with tamarind (5gm) on day 60 as compared to non-treated fluorotic calves. Tamarind

supplementation @ 10gm to fluorotic calves significantly ($p<0.05$) increased the eosinophil percentage on day 60 as compared to non-treated fluorotic calves. Significantly ($p<0.05$) increased in eosinophil percentage was observed after supplementation of $CaCO_3$ on day 60 as compared to non-treated fluorotic calves.

Significant ($p<0.05$) increase in lymphocyte percentage was recorded in fluorotic calves on day 0, day 30 and day 60 as compared to healthy calves. Lymphocyte percentage in calves supplemented with tamarind and $CaCO_3$ was non-significant as compared to non-treated fluorotic calves.

Non-significant increase in monocyte percentage in fluorotic calves was recorded on day 30 and day 60 as compared to healthy calves. Significant decrease in monocyte percentage was recorded after supplementation of tamarind (5 gm and 10 gm) to fluorotic calves on day 60 as compared to non-treated fluorotic calves.

Significant ($p<0.05$) increase in plasma fluoride level in fluorotic calves was recorded at day 0, day 30 and day 60 as compared to healthy calves. Plasma fluoride level in calves supplemented with tamarind (5gm) was significantly ($p<0.05$) reduced on day 60 as compared to non-treated group. Significant ($p<0.05$) reduction in plasma fluoride level was observed after supplementation of tamarind @ 10gm on day 30 and non-significant reduction on day 60 as compared to non-treated group. Significantly ($p<0.05$) lowered in plasma fluoride level was observed after supplementation of $CaCO_3$ on day 30 and non-significant on day 60 as compared to fluorotic calves.

Significant ($p<0.05$) increase in urine fluoride level in fluorotic calves was recorded at different observation periods of the experiment as compared to healthy calves. Urine fluoride level in calves supplemented with tamarind (5gm) was significantly ($p<0.05$) reduced on day 30 and day 60 as compared to non-treatment group. Significantly ($p<0.05$) lowered urine fluoride level was recorded in calves received tamarind leaves with feed @ 10gm on day 30 and day 60 as compared to non-treatment group. Significantly ($p<0.05$) lowered in urine fluoride level was observed after supplementation of $CaCO_3$to fluorotic calves on day 30 and day 60 as compared to non-treated fluorotic calves.

Significant ($p<0.05$) increase in faeces fluoride level was recorded in fluorotic calves on day 0, day 30 and day 60 as compared to healthy calves. Faecal fluoride level in calves supplemented with tamarind (5gm) was significantly ($p<0.05$) increased on day 30 and day 60 as compared to day 0 value.

Tamarind supplementation @ 10gm to fluorotic calves significantly ($p<0.05$) increased the faecal fluoride excretion on day 30 and day 60 as compared to non-treated fluorotic calves. Significantly ($p<0.05$) increased in faecal fluoride level was observed after supplementation of $CaCO_3$ on day 30 and day 60 as compared to non-treated fluorotic calves.

Significant ($p<0.05$) increase in ALT activity was recorded in fluorotic calves from day 0 onwards as compared to healthy calves. ALT activity in calves supplemented with tamarind irrespective of doses i.e 5 gm and 10 gm were non-significant as compared to non-treated fluorotic calves at different observation periods of the experiment. Significantly ($p<0.05$) decreased in ALT activity was observed after supplementation of $CaCO_3$ on day 60 as compared to non-treated fluorotic calves.

Significant ($p<0.05$) increase in AST activity was recorded in fluorotic calves on day 0, day 30 and day 60 as compared to healthy calves. AST activity in calves supplemented with tamarind (5gm) was significantly ($p<0.05$) lowered on day 30 and day 60 as compared to non-treated fluorotic calves. Significantly ($p<0.05$) decreased AST activity was recorded in fluorotic calves supplemented with tamarind @ 10gm on day 30 and day 60 as compared to non-treated fluorotic calves. Significantly ($p<0.05$) increased in AST activity was observed after supplementation of $CaCO_3$ on day 30 and day 60 as compared to non-treated fluorotic calves.

Significant ($p<0.05$) increase in urea was recorded in fluorotic calves from day 0 onwards as compared to healthy calves. The urea concentration in calves supplemented with tamarind and $CaCO_3$ were non-significant as compared to non-treated fluorotic calves.

Significant ($p<0.05$) increase in creatinine concentration was recorded in fluorotic calves on day 0, day 30 and day 60 as compared to healthy calves. Creatinine concentration in calves supplemented with tamarind (5gm) was significantly ($p<0.05$) decreased on day 60 as compared to non-treated fluorotic calves. Tamarind supplementation @ 10gm and $CaCO_3$ to fluorotic calves significantly ($p<0.05$) decreased the creatinine concentration from day 30 onwards as compared to non-treated fluorotic.

Significant ($p<0.05$) decrease in calcium concentration was recorded in fluorotic calves from day 0 onwards as compared to healthy. Calcium concentration in calves supplemented with tamarind (5gm) was significantly ($p<0.05$) increased on day 60 as compared to non-treated fluorotic calves. Tamarind supplementation @ 10gm and $CaCO_3$ to fluorotic calves significantly ($p<0.05$) increased the calcium concentration from day 30 onwards as compared to non-treated fluorotic calves.

Significant ($p<0.05$) increase in phosphorus concentration was recorded in fluorotic calves on day 0, day 30 and day 60 as compared to healthy calves. Phosphorus concentration in calves supplemented with tamarind (5gm) was significantly ($p<0.05$) decreased on day 60 as compared to non-treated fluorotic calves. Tamarind supplementation @ 10gm to fluorotic calves significantly ($p<0.05$) decreased the phosphorus concentration on day 60 as compared to non-treated fluorotic calves. Significantly ($p<0.05$) decreased in phosphorus concentration was observed after supplementation of $CaCO_3$ from day 30 onwards as compared to non-treated fluorotic calves.

CONCLUSIONS

Socioeconomic status of the farmers residing in the vicinity of the aluminium smelter is affected owed to below factors:

- The life span of animals gradually decreases in fluorotic affected animal.
- Animals having skeletal form of fluorosis drought power decreases gradually due to development of lameness.
- Milk production decreases gradually.

- Growth rate of the animal decreases gradually.
- The animals can not be sold due to poor growth, and loss of productivity so the socio economic status of the farmer not so developed.
- Dental fluorosis affected animals having gradually development of lesion on teeth followed by total eruption of teeth. So that anorexia developed and body condition gradually decreases.
- Cost of treatment gradually increases.
- Dental fluorosis was more prevalent than skeletal fluorosis in calves reared in fluoride affected area.
- Significant increase in plasma, urine and faecal fluoride concentration and alteration in haemato-biochemical parameters were observed in fluorotic calves.
- Supplementation of tamarind leaf to fluorotic calves decreases the plasma fluoride concentration and restored the altered haemato-biochemical parameters.

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