AMELIORATIVE EFFICACY OF MEDICINAL HERBS IN CALVES EXPOSED TO INDUSTRIAL FLUOROSIS

THESIS SUBMITTED TO
THE ORISSA UNIVERSITY OF AGRICULTURE AND TECHNOLOGY
BHUBANESWAR
IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR THE DEGREE OF

MASTER OF VETERINARY SCIENCE
IN
VETERINARY CLINICAL MEDICINE, ETHICS AND JURISPRUDENCE

BY

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B.V. Sc. & A.H.

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Ethics and Jurisprudence

Bhubaneswar
Dated……

CERTIFICATE – I

This is to certify that the thesis entitled **"AMELIORATIVE EFFICACY OF MEDICINAL HERBS IN CALVES EXPOSED TO INDUSTRIAL FLUOROSIS"** submitted in partial fulfillment of the requirements for the award of the degree of **MASTER OF VETERINARY SCIENCE** (**Veterinary Clinical Medicine, Ethics and Jurisprudence**) to the Orissa University of Agriculture and Technology is an authentic record of *bonafide* research work carried out by ***Kruti Debnath Mandal*** 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 evidence and help obtained by him from various sources during the course of investigation has been duly acknowledged.

(M.R. Das)
CHAIRMAIN
ADVISORY COMMITTEE

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Bhubaneswar ***(Kruti Debnath Mandal)***

Dated:

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ABBREVIATION

%	:	Percentage
@	:	At the rate
µl	:	Microliter
A/G	:	Albumin/globulin ratio
ALP	:	Alkaline phosphatase
ALT	:	Alanine transaminase
AOAC	:	Association of official analytical chemist
AST	:	Aspartate transaminase
B	:	Basophill
B	:	Boron
BUN	:	Blood Urea Nitrogen
Ca	:	Calcium
$CaCO_3$	:	Calcium Carbonate
Co	:	Cobalt
dL	:	Decilitre (s)
DLC	:	Differential leucocyte count
E	:	Eosinophill
F	:	Fluoride
Fe	:	Iron
Fig	:	Figure
g	:	Gram
Hb	:	Haemoglobin
H_3BO3	:	Boric acid
i.e.	:	That is

Km	:	Kilometer
L	:	Lymphocyte
M	:	Monocyte
MCH	:	Mean corpuscular haemoglobin
MCHC	:	Mean corpuscular haemoglobin concentration
MCV	:	Mean corpuscular volume
mg	:	Milligram (s)
Mg	:	Magnesium
mm	:	milimeter
Mn	:	Manganese
N	:	Neutrophil
n	:	Number of animals
NALCO	:	National Aluminium Company
OUAT	:	Orissa University of Agriculture and Technology
P	:	Phosphorus
PCV	:	Packed cell volume
ppm	:	parts per million
S.E.	:	Standard Error
TEC	:	Total erythrocyte count
TLC	:	Total leucocyte count
TP	:	Total protein
U/L	:	Unit per liter
WHO	:	World Health Organisation
Zn	:	Zinc

FIG. NO. 1: PREVALENCE OF DENTAL AND SKELETAL FLUOROSIS

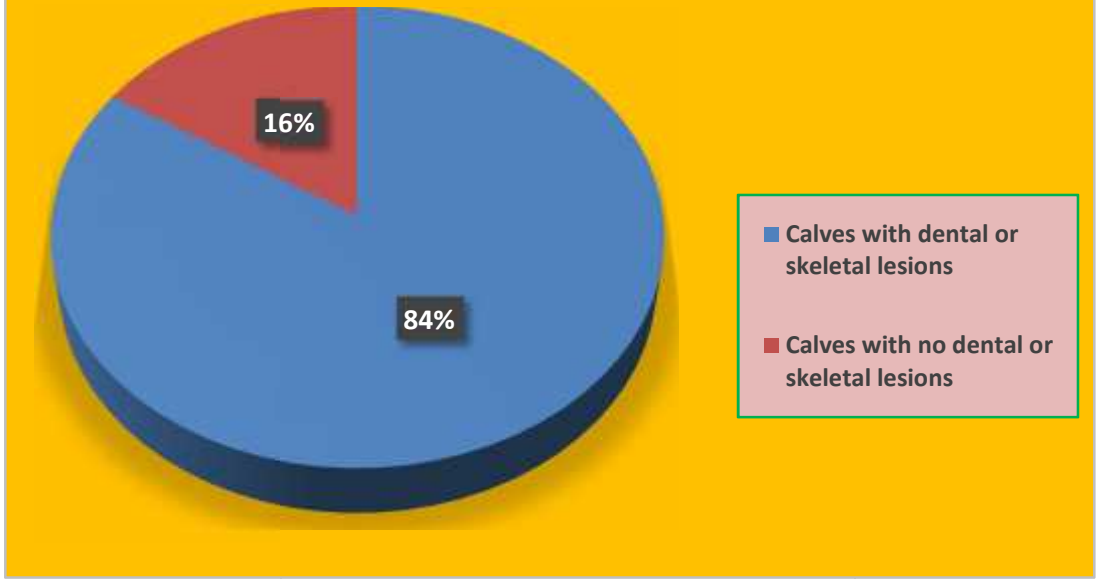
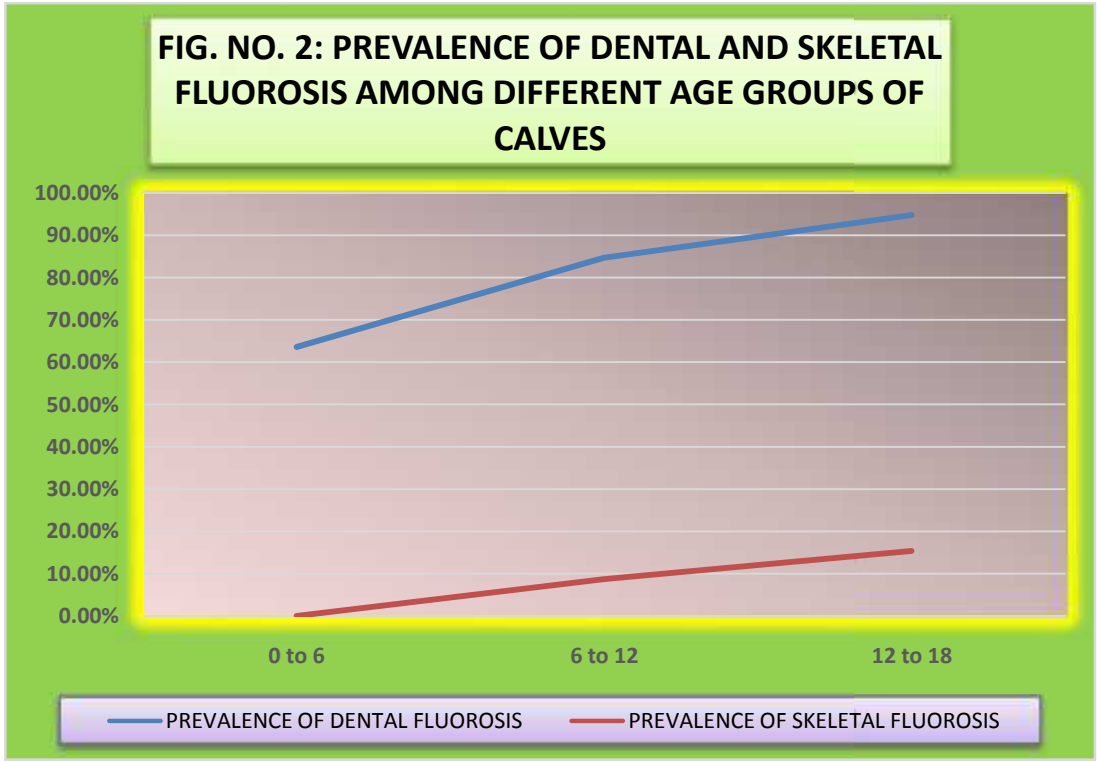


FIG. NO. 2: PREVALENCE OF DENTAL AND SKELETAL FLUOROSIS AMONG DIFFERENT AGE GROUPS OF CALVES



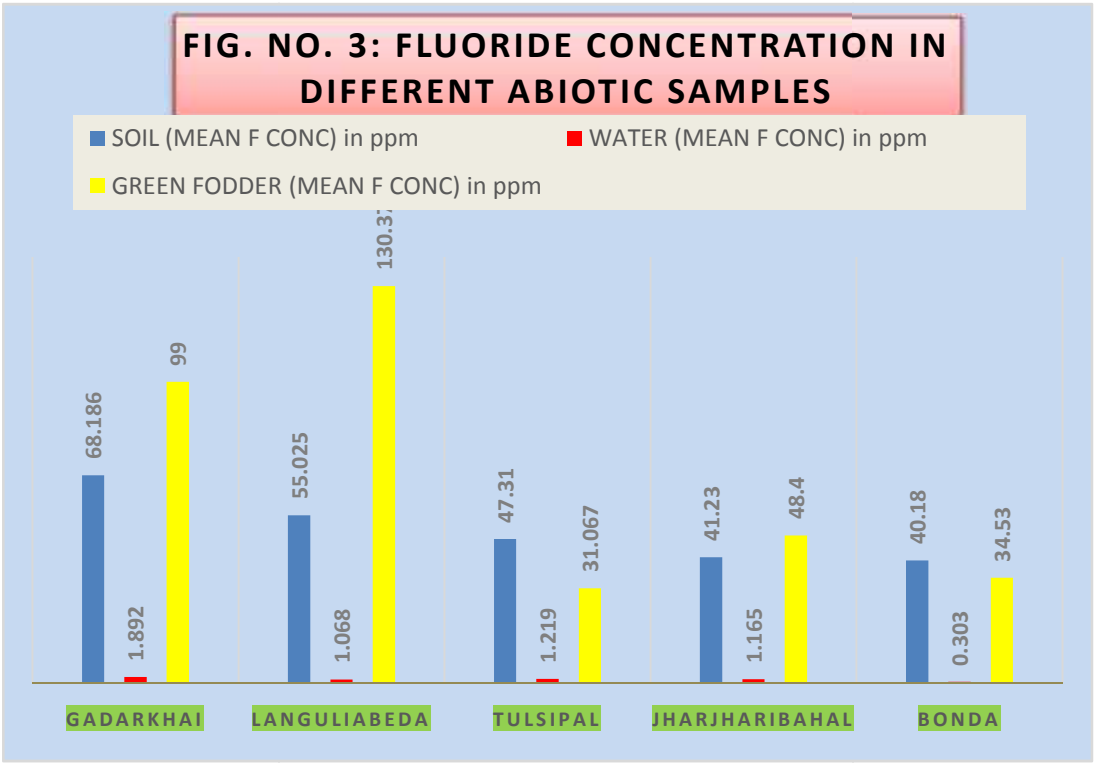


FIG. NO. 3: FLUORIDE CONCENTRATION IN DIFFERENT ABIOTIC SAMPLES
SOIL (MEAN F CONC) in ppm
WATER (MEAN F CONC) in ppm
GREEN FODDER (MEAN F CONC) in ppm
68.186
1.892
99
55.025
1.068
47.31
1.219
31.067
41.23
1.165
48.4
40.18
0.303
34.53
GADARKHAI
LANGULIABEDA
TULSIPAL
JHARJHARIBAHAL
BONDA

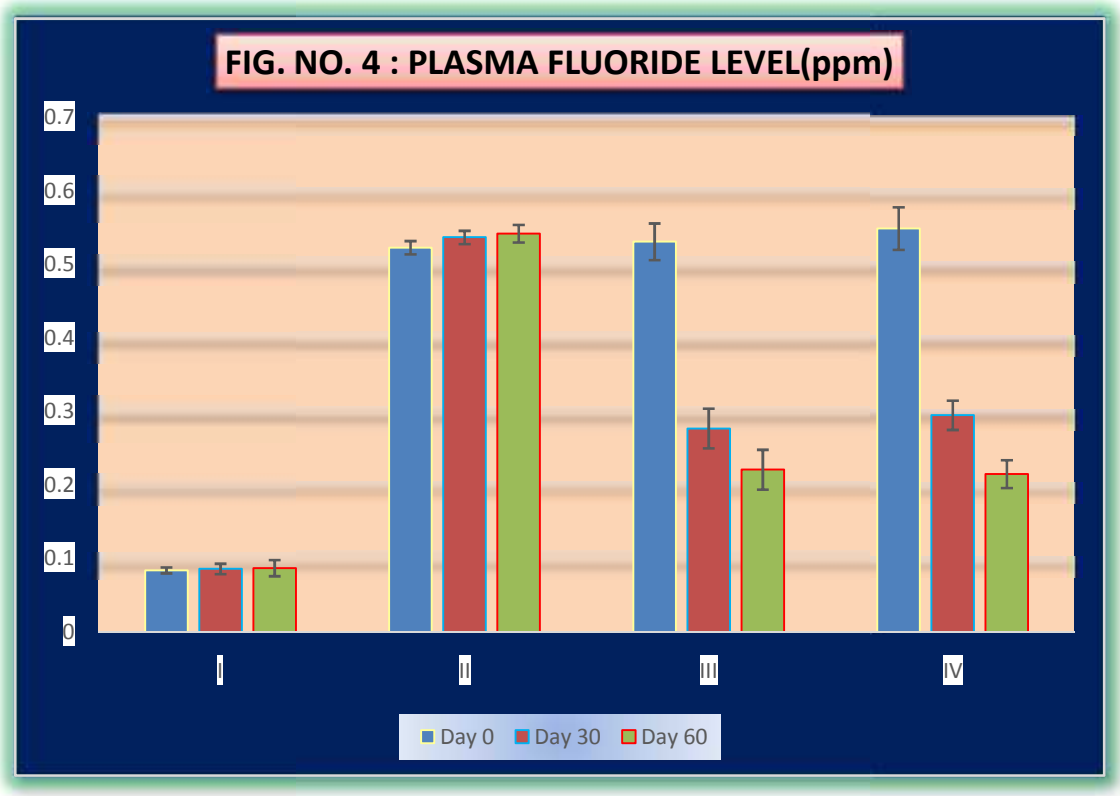


FIG. NO. 4 : PLASMA FLUORIDE LEVEL(ppm)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
I
II
III
IV
Day 0
Day 30
Day 60

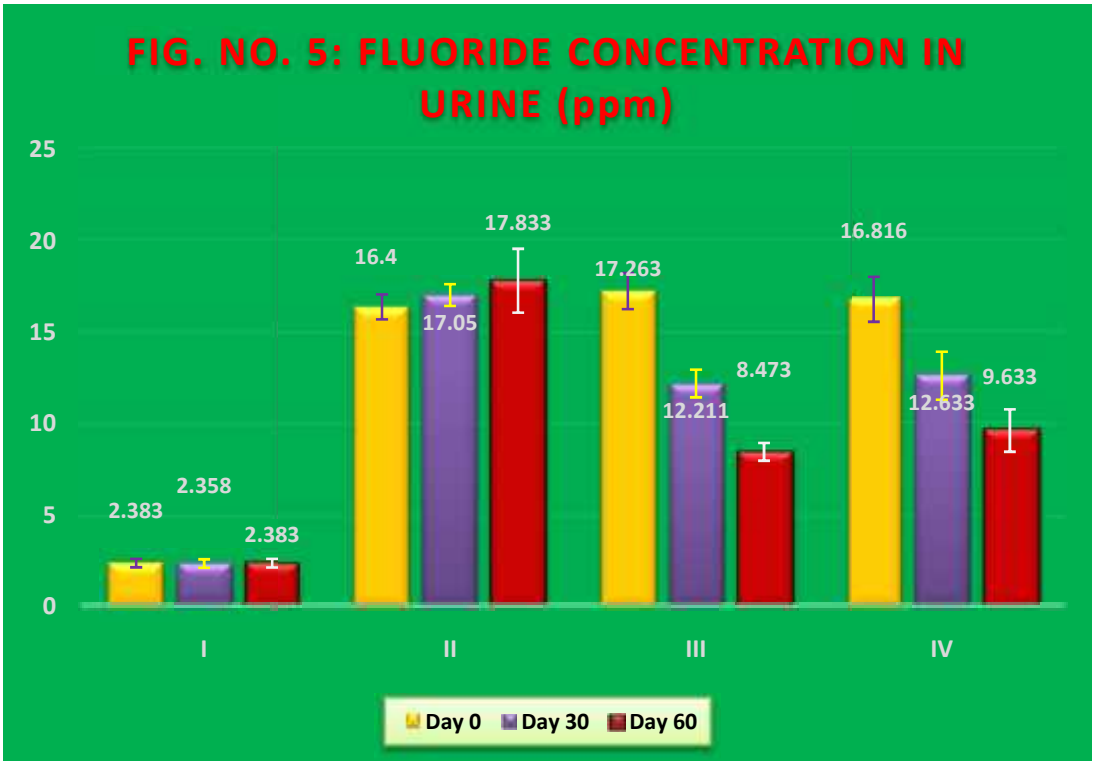


FIG. NO. 5: FLUORIDE CONCENTRATION IN URINE (ppm)
25
20
15
10
5
0
2.383
2.358
2.383
16.4
17.05
17.833
17.263
12.211
8.473
16.816
12.633
9.633
I
II
III
IV
Day 0
Day 30
Day 60

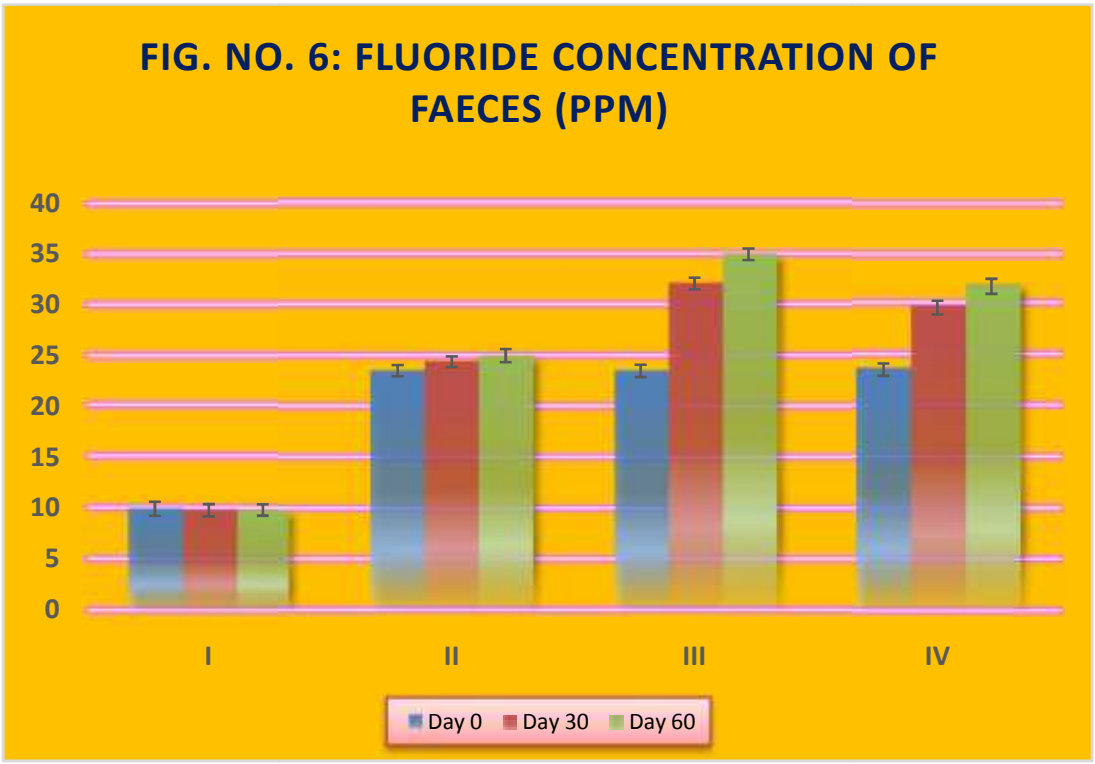


FIG. NO. 6: FLUORIDE CONCENTRATION OF FAECES (PPM)
40
35
30
25
20
15
10
5
0
I
II
III
IV
Day 0
Day 30
Day 60

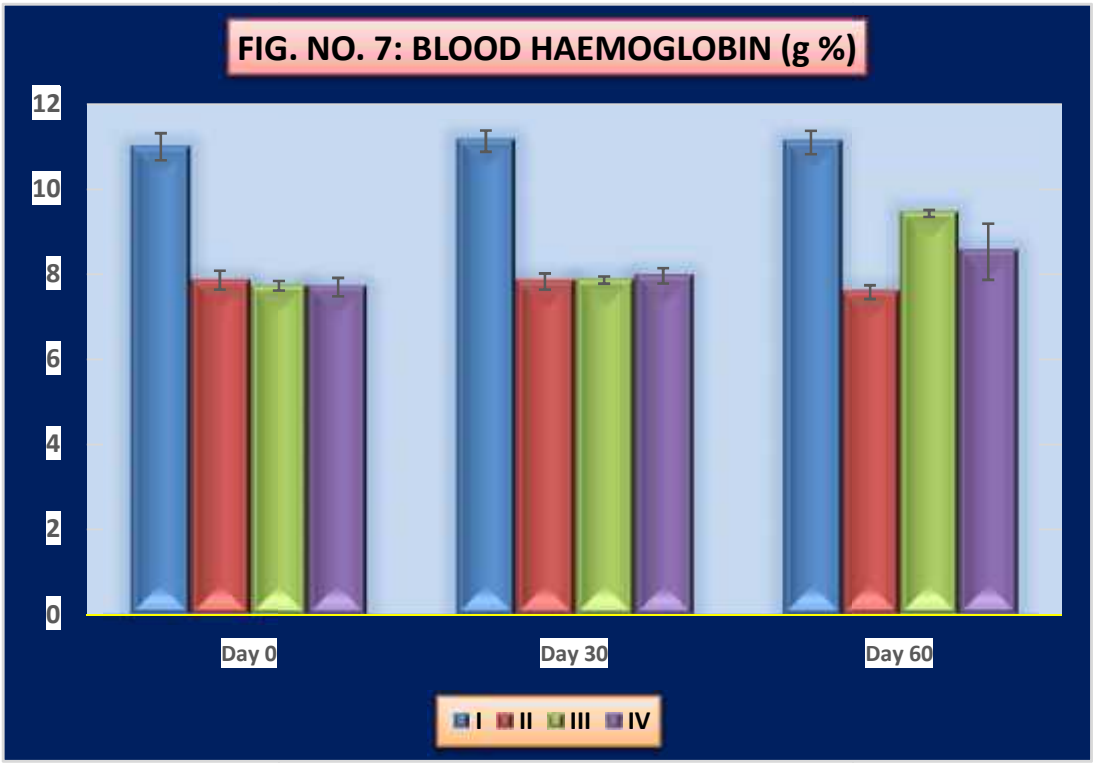


FIG. NO. 7: BLOOD HAEMOGLOBIN (g %)
12
10
8
6
4
2
0
Day 0
Day 30
Day 60
I
II
III
IV

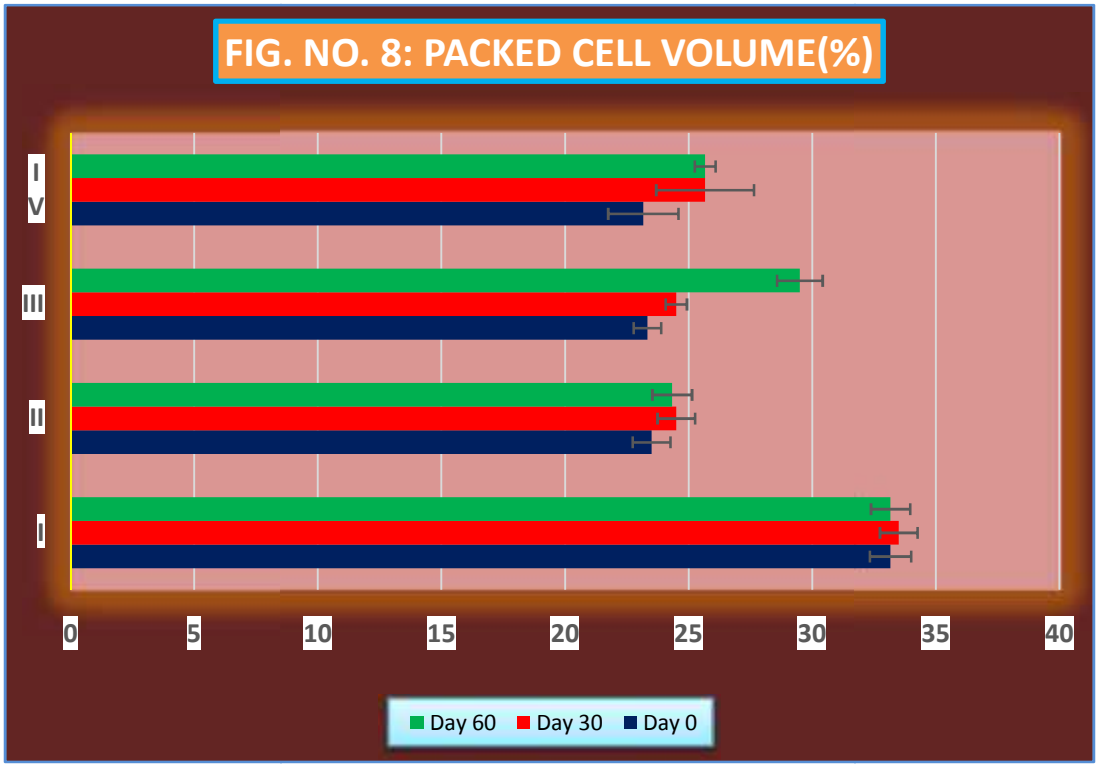


FIG. NO. 8: PACKED CELL VOLUME(%)
IV
III
II
I
0
5
10
15
20
25
30
35
40
Day 60
Day 30
Day 0

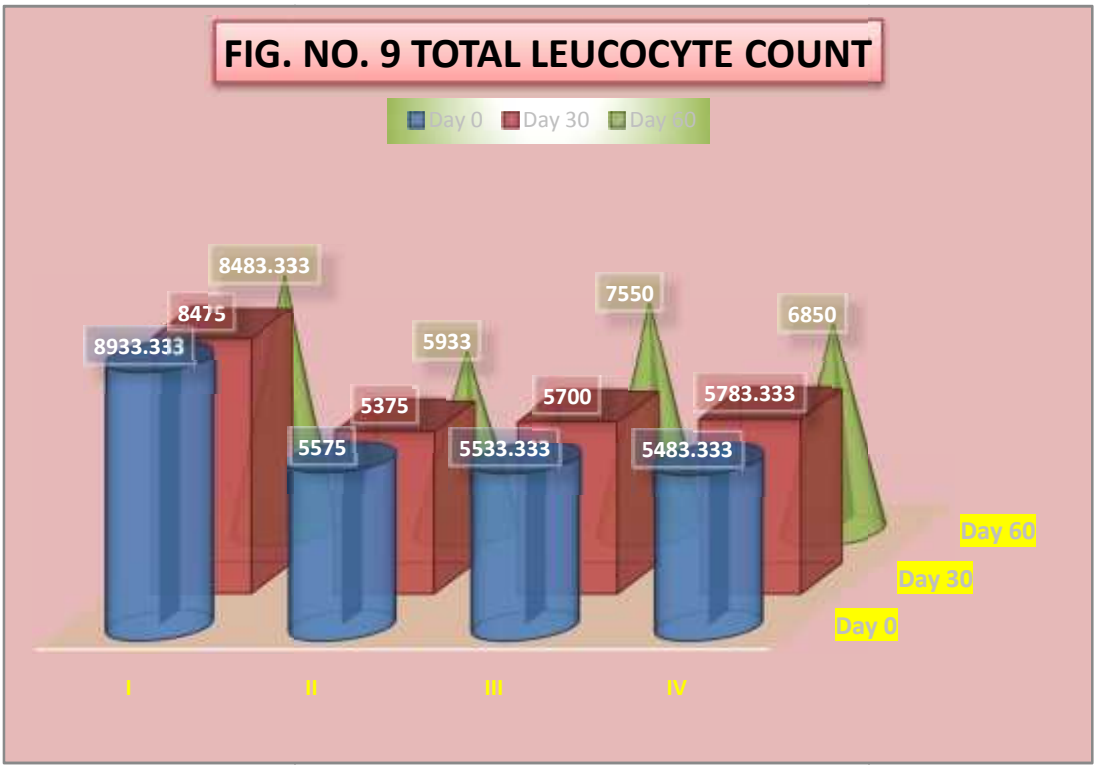


FIG. NO. 9 TOTAL LEUCOCYTE COUNT
Day 0
Day 30
Day 60
8933.333
8475
8483.333
5575
5375
5933
5533.333
5700
7550
5483.333
5783.333
6850
Day 60
Day 30
Day 0
I
II
III
IV

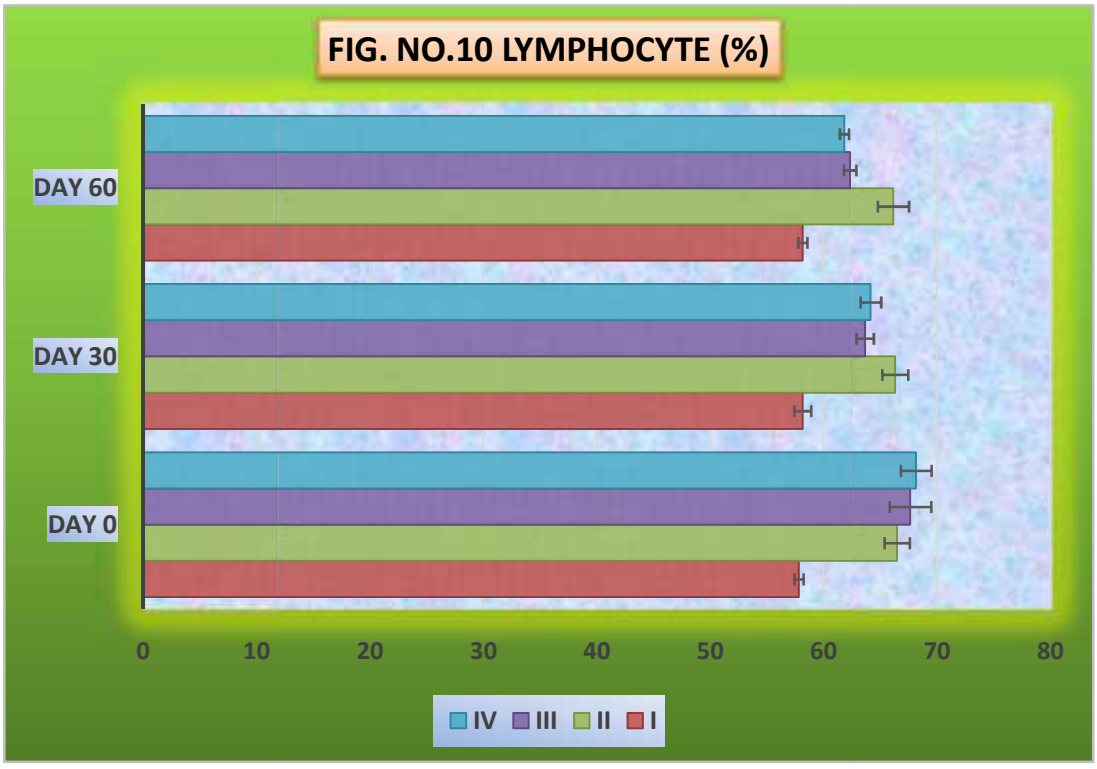


FIG. NO.10 LYMPHOCYTE (%)
DAY 60
DAY 30
DAY 0
0
10
20
30
40
50
60
70
80
IV
III
II
I

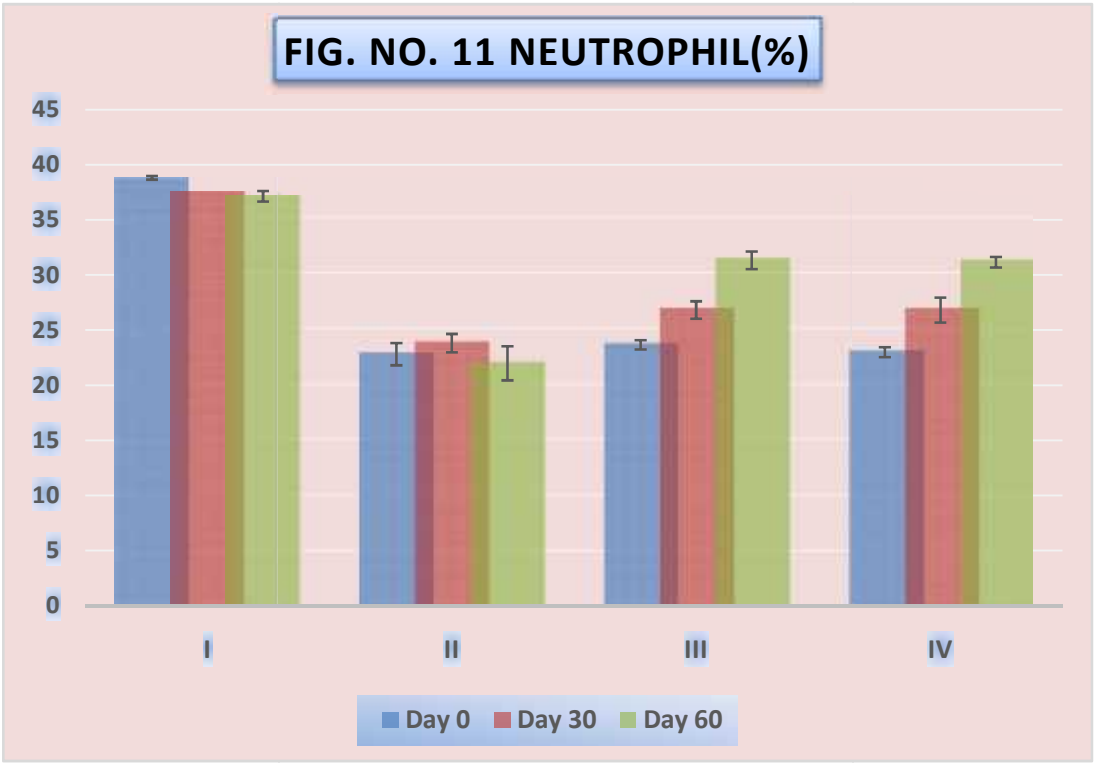


FIG. NO. 11 NEUTROPHIL(%)
45
40
35
30
25
20
15
10
5
0
I
II
III
IV
Day 0
Day 30
Day 60

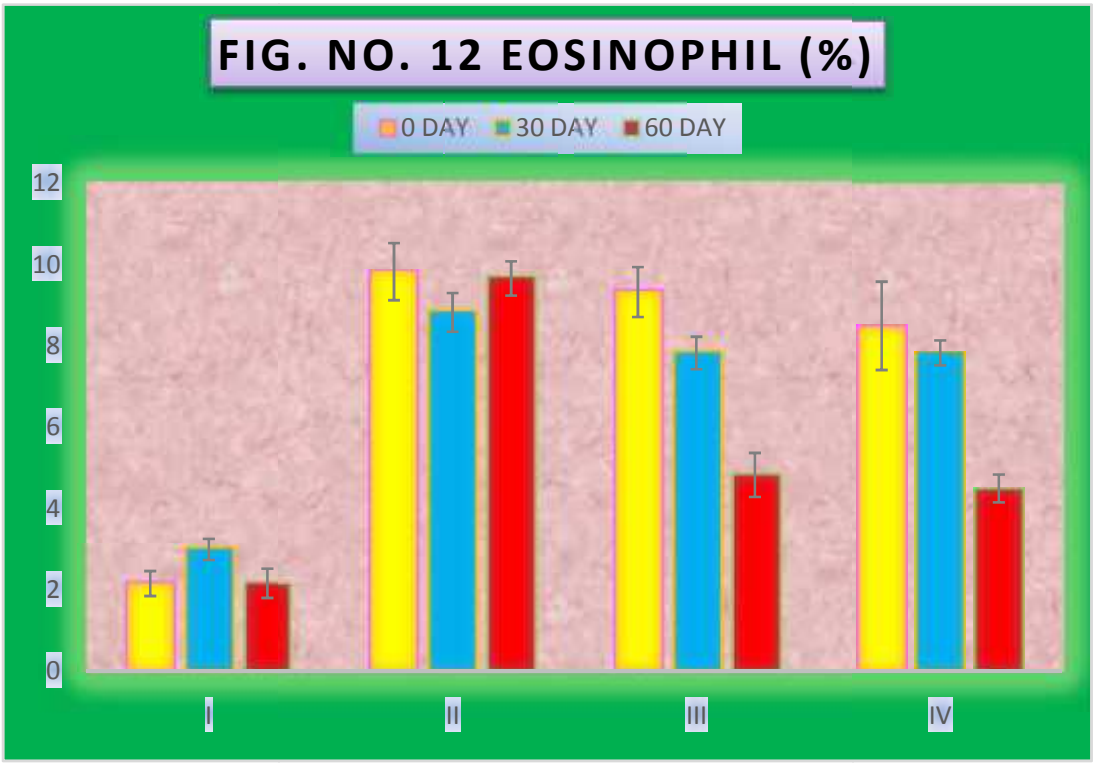
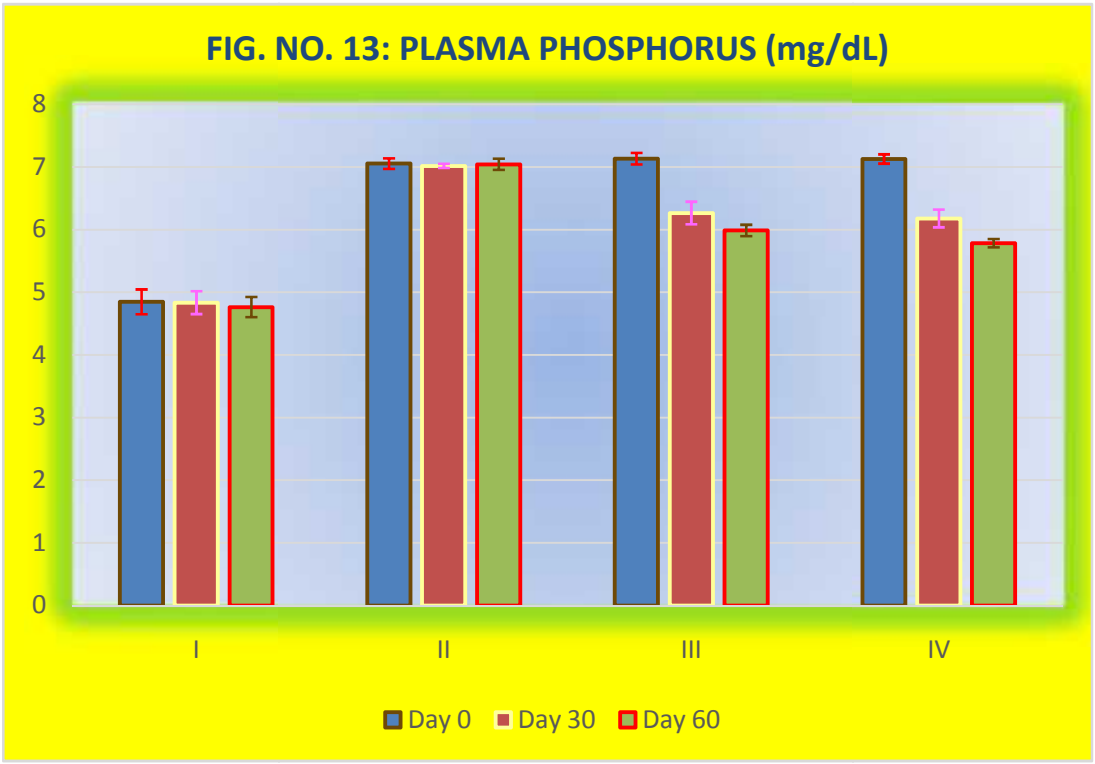


FIG. NO. 12 EOSINOPHIL (%)
0 DAY
30 DAY
60 DAY
12
10
8
6
4
2
0
I
II
III
IV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8
7
6
5
4
3
2
1
0
I
II
III
IV
Day 0
Day 30
Day 60

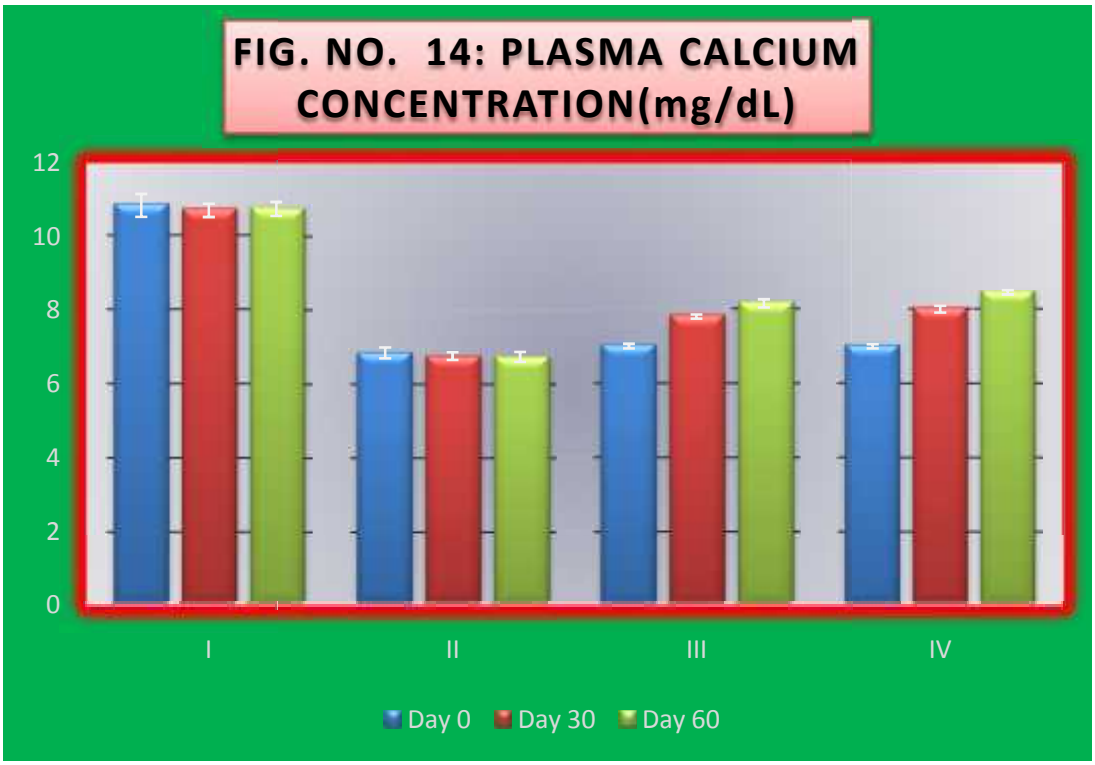
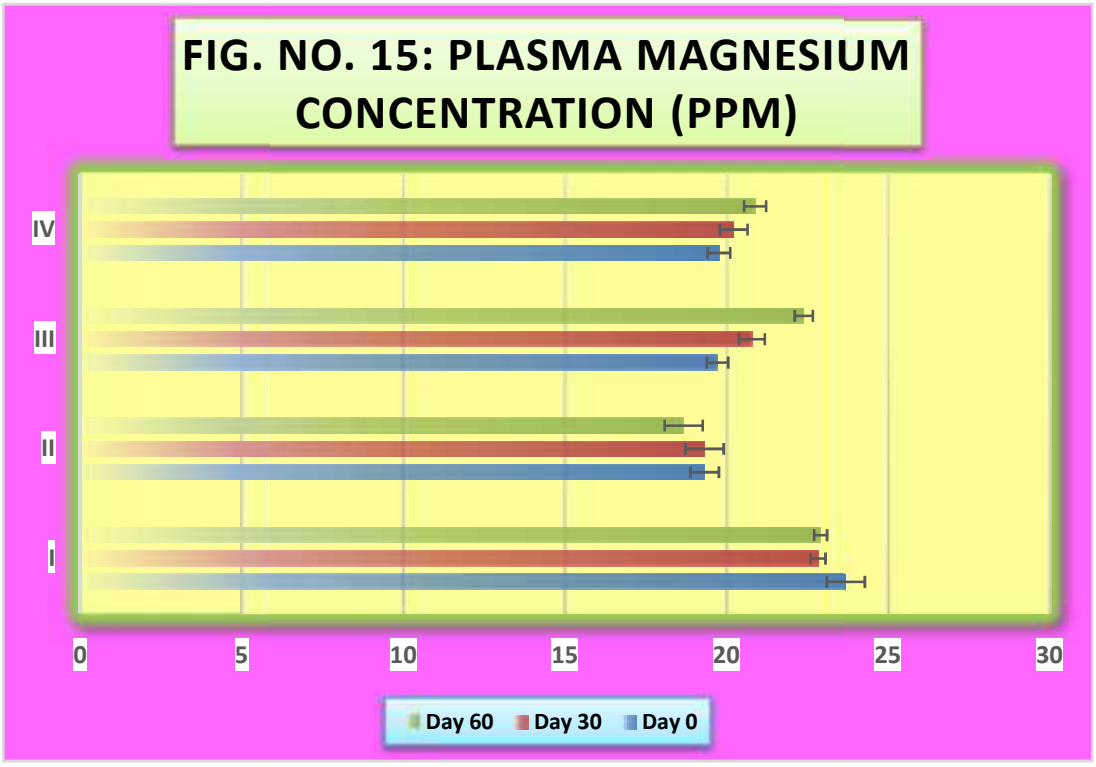


FIG. NO. 14: PLASMA CALCIUM CONCENTRATION(mg/dL)
12
10
8
6
4
2
0
I
II
III
IV
Day 0
Day 30
Day 60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IV
III
II
I
0
5
10
15
20
25
30
Day 60
Day 30
Day 0

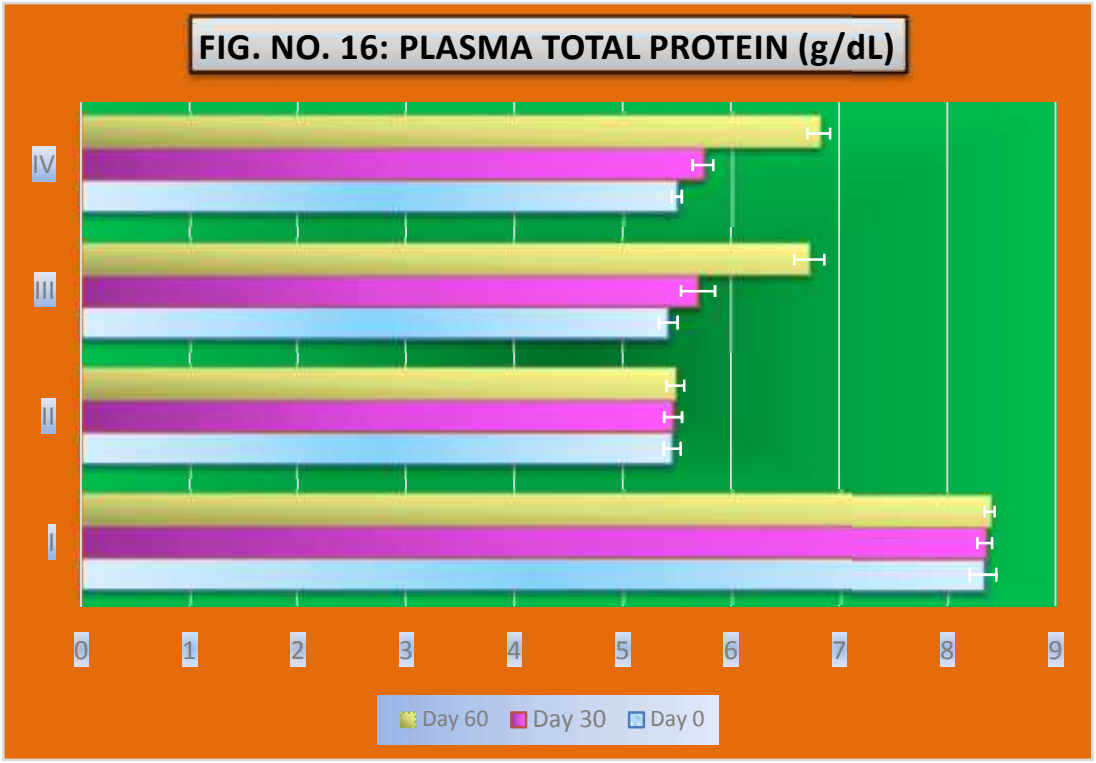
FIG. NO. 16: PLASMA TOTAL PROTEIN (g/dL)
IV
III
II
I
0
1
2
3
4
5
6
7
8
9
Day 60
Day 30
Day 0

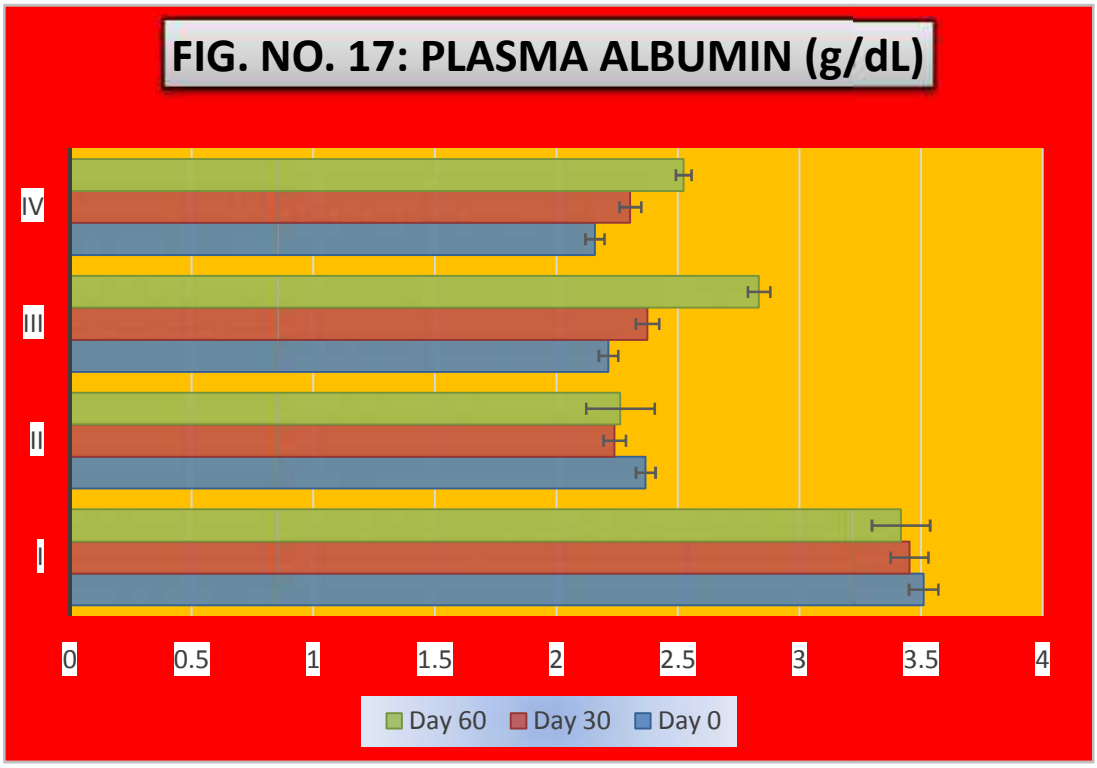
FIG. NO. 17: PLASMA ALBUMIN (g/dL)
IV
III
II
I
0
0.5
1
1.5
2
2.5
3
3.5
4
Day 60
Day 30
Day 0

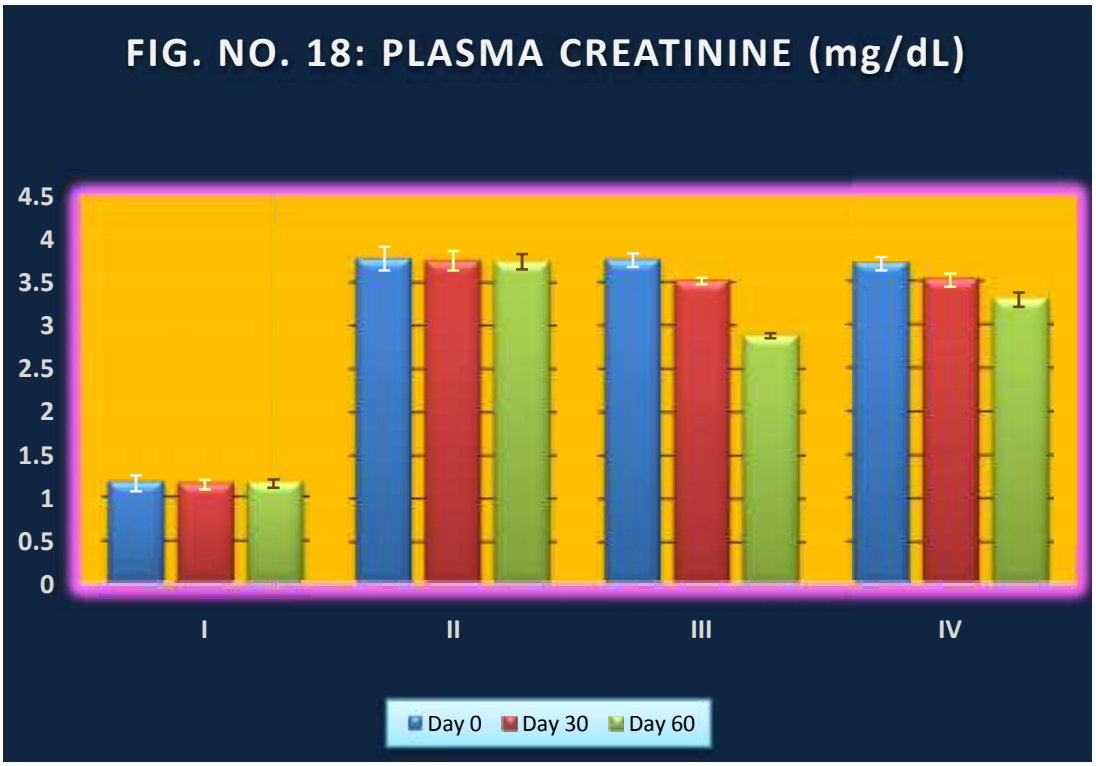
FIG. NO. 18: PLASMA CREATININE (mg/dL)
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
I
II
III
IV
Day 0
Day 30
Day 60

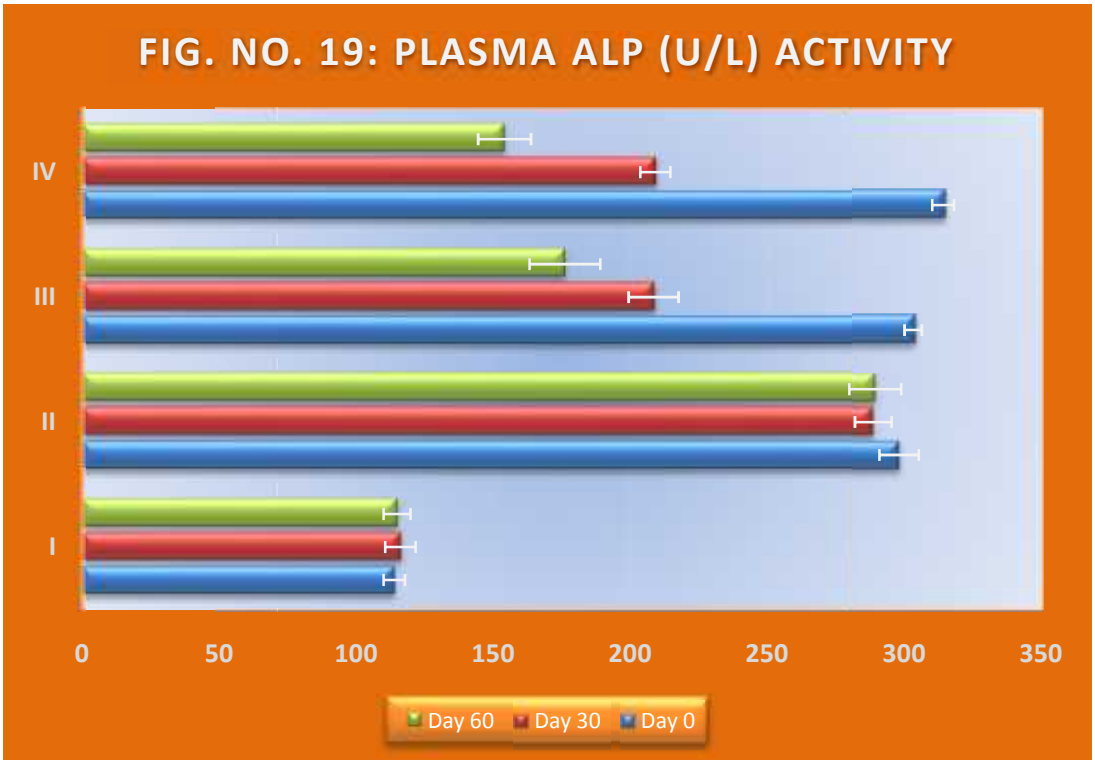
FIG. NO. 19: PLASMA ALP (U/L) ACTIVITY
IV
III
II
I
0
50
100
150
200
250
300
350
Day 60
Day 30
Day 0

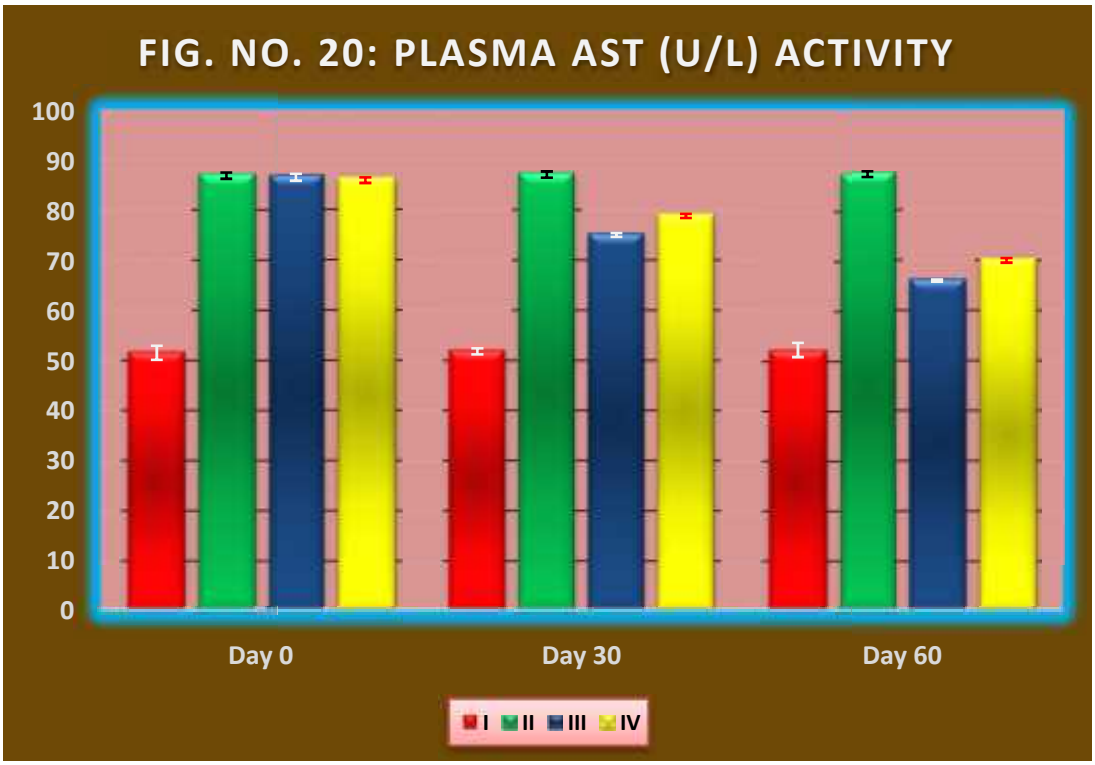
FIG. NO. 20: PLASMA AST (U/L) ACTIVITY
100
90
80
70
60
50
40
30
20
10
0
Day 0
Day 30
Day 60
I
II
III
IV

INTRODUCTION

Rapid industrialization plays an important role in anthropogenic pollution exerting hazardous impact on the environment. Health hazards in both man and animal results from such sources of life threatening pollutants. Cattle being the grazing animal suffer within a short period of exposure to these pollutants and remain at a state of high morbidity. Angul -Talcher areas attracted large industries because of huge deposits of coal and high flow of water in river Brahmani. Heavy industrialization in Angul-Talcher industrial complex has become the principal cause of environmental pollution. Deleterious effects of the pollution are reflected upon the health status of both human being and livestock inviting the intervention in the management of the physical components of environments like soil, water and air.

Fluoride acts as a double edged knife. At low concentration (<1 ppm), fluoride helps to prevent formation of dental carries but cumulative ingestion of small but toxic amount results in dental as well as skeletal lesions, commonly called as fluorosis (Warren and Levy, 2003). During last decades, numerous reports of fluorosis in animals and humans from India (Choubisa *et al*., 2012), China (Cao *et al*., 1996), U.S.A. (Crissman *et al*., 1980), Ausrtalia (Suttie *et al*., 1982) and other countries were observed. In India, common sources of fluoride intoxications are water, fodder, fluoride rich effluents, dust and smoke from aluminium smelters plants, copper, glass, enamel, iron, super phosphate fertilizers plants and brick kilns areas (Patra *et al*., 2000).

Fluorosis can be classified as natural and artificial depending upon the source of toxicity. Pollution due to human activities like release of industrial effluent without proper treatment into water bodies, application of fertilizers, insecticides, continuous release of fluoride rich gases into the environment, mineral mixture, tooth paste, etc are all attributed to artificial fluorosis. Whereas, presence of fluoride in water (ground and surface water), soil and air leads to natural or endemic fluorosis. Depending on the lesions of affection, again fluorosis is classified as dental, skeletal and non-skeletal fluorosis. Dental fluorosis is clinically manifested as mild to severe dental

mottling, bilateral striation horizontally (light to deep yellowish colour) and light to deep brown discoloration of the anterior teeth particularly in calves. In severe forms of dental fluorosis, irregular wearing of teeth with recession and swelling of gingiva, exposed cementum of incisor roots (Choubisa *et al*., 2012) leading to impaired mastication resulting in poor utilization of feed and unthriftiness (Shupe *et al*., 1987). The clinical manifestations of skeletal fluorosis are expressed as intermittent lameness and snapping sound of legs, wasting of body muscles and excessive periosteal exostoses of the mandibles, ribs, metacarpus, metatarsus regions (Choubisa *et al*., 2012), phalanges become short, thicker, broader, porous, brittle, hence are prone to fracture. Periosteal hyperosteosis or exostosis at joint or places of attachment of ligaments and tendons cause shifting pain and lameness (Radostits *et al*., 2000). The mechanism of fluoride-mediated injuries in non-skeletal tissues is that fluoride results in damage of tissue and cell biological membrane structure and the metabolism of biological molecule, resulting abnormal structure and function of the non-skeletal tissues. High intake of fluoride injures the ultrastructure of thyroid, kidney and brain (Shu *et al*., 2003). In all the tissues and organs, the fluoride content increases in a dose-dependent and a time-dependent manner (Inkielewicz, 2002). Affected animals exhibit major symptoms of colic, intermittent diarrhoea, excessive urination, irregular reproductive cycles, repeated abortions, and stillbirths (Choubisa *et al*., 2012).

The bovine calves are found to be more sensitive and highly susceptible to fluoride toxicosis revealing the maximum prevalence (92.2%) of dental fluorosis. Therefore, it is considered as a bio-indicators for fluoridated water as well as for endemicity of osteo-dental fluorosis (Choubisa, 2014). The major clinical manifestations of congenital fluorosis in calves of fluoride affected cows are brown discolouration of enamel as well as hypoplasia, brown mottling of bone and severely stunted body growth were observed by Maylin *et al*., (1987). The maximum susceptibility to fluoride toxicosis was found in bovines followed by equines, flocks (goats and sheep), and camelids (Choubisa, 2014). Among them, calves were found to be more susceptible to dental fluorosis than skeletal fluorosis (Modasiya, 2014, Choubisa *et al*., 2011, Shupe, 1987, Maiti *et al*., 2003).

Though fluorosis is irreversible, preventive measures are of prime importance. Supplementation with calcium carbonate (Bronckers *et al*., 2006,), aluminium salts (Kessabi *et al*., 1986), selenium (Han-bo *et al*., 2001), copper (Khandare *et al*., 2005) or boron (Bharti *et al*., 2007) will reduce absorption or facilitate excretion of excess fluoride.

India is rich in heritage of medicinal plants with traditional Ayurveda therapeutic knowledge giving us an opportunity to use them for ethno-veterinary medical practice. Alcoholic extracts of flowers (Rukmani *et al*., 1998) and roots (Rao and Mishra,1998) of *Moringa oleifera* have been reported to possess hepato-protective effect. Further, Ranjan *et al*. (2009) found the beneficial effect of aqueous extract of *Moringa* seeds and tamarind pulps to mitigate fluoride toxicity in cattle. With *Moringa oleifera* seeds extract, prevention of fluoride absorption from G.I. tract seems to be more important mechanism of action which contains cationic polyelectrolyte that has been proved efficient in water treatment as a substitute to aluminium sulfate as flocculent (Ndabigengeser and Narasiah, 1998). Thus, beneficial effect of aqueous seed extract can be attributed for the prevention of fluoride absorption by its flocculent action (Ranjan *et al*., 2009).

Therefore, keeping in view the above facts, the present research work **"Ameliorative efficacy of medicinal herbs in calves exposed to industrial fluorosis"** was undertaken with the following objectives.

- To estimate the fluoride concentrations in environmental samples of the selected areas.
- To estimate the fluoride level of blood and urine of calves from the endemic areas.
- To assess the haemato-biochemical alterations in calves of fluorosis endemic areas.
- To assess the ameliorative potential of medicinal herbs in calves exposed to industrial fluorosis.

REVIEW LITERATURE

2.1 Prevalence

2.1.1 Angul district

Mukherjee *et al*. (2003) conducted a study on suspended particulate matters, SO_2, NO_x and fluorides (gaseous and Particulate) in ambient air around aluminium smelter during February and August 1996. Gaseous fluoride in village air were varied between, 1.66 - 7.64 mg/m3 in February and 1.11 - 22.75 mg/m3 in August, whereas particulate fluoride ranged between, 0.054 - 19.61 mg/m3. Water sources of the villages near the smelter showed fluoride values above permissible limit. The study indicated higher fluoride pollution in air and water of the surrounding villages.

Sahoo *et al.* (2003) studied the fluorosis in sheep around an aluminium factory. They reported an increased concentration of fluoride in the urine, teeth and bones and pathognomonic clinical signs of fluorosis in sheep.

Panda and Kumar (2006) studied on industrial pollution and social security in Angul-Talcher belt of Orissa and reported the higher fluoride content in air and water in vicinity of smelter plants.

Nanda *et al.* (2011) examined ground water samples from 16 different villages during pre- and post- monsoon sesson-2009. The result of chemical analysis indicated the occurrence of high fluoride content in the ground water of the study area without any definite pattern. However the concentration of fluoride ion in the water samples of villages close to NALCO (National Aluminium Company) smelter plant touched the maximum permissible limit.

Patil (2011) accessed an environmental health impact of NALCO plant pollution around the vicinity villages on 2005-06. His finding showed the adverse effect of fluoride released from aluminium smelter plant on human health, animals and ecology. Human health was compromised by dental and

skeletal fluorosis, animals suffered with skeletal fluorosis and ecological effect was due to destruction of paddy field and crop yield.

Moharana *et al.* (2013) studied the eutrofication due to industrialization in Angul-Talcher industrial complex. His study on the water resources of Angul-Talcher industrial complex showed higher fluoride content than the standard water quality that was toxic to human beings, animals and plants (causes old age to set in early). Water of rivers like Brahmani and Nandira were found polluted with fluoride and other industrial effluent from NALCO plants as well as from other industries and coal mines.

Reza and Singh (2013) also researched on the ground water quality with respect to fluoride content in Angul district of Orissa, India by taking eighteen groundwater samples from different locations covering open wells as well as tube wells for determining various parameters (pH, TDS, F, Cl-, Na+, Ca2+ and Mg2+). Their study reflected the seasonal variation along with hydro-geochemical activities. Hydro-geochemical condition was the main source for fluoride contamination whereas the run-off and atmospheric deposition was responsible for additional F– concentration during post monsoon season.

2.1.2 Odisha

Maiti *et al.* (2003) indicated the fluoride toxicity in the form of dental fluorosis in cattle from nine villages under two blocks of Nayagarh district of Orissa. Out of 1117 cattle, 221 (18.09%) showed the signs of dental fluorosis. Fluoride levels (mean) of ground water and surface water in two blocks were 1.30 +/- 0.16 ppm, 0. 66 +/- 0.08 ppm and 1.12 +/- 0.19 ppm, 0. 48 +/- 0.05 ppm respectively.

Survey Report of Sahabhagi Vikash Abhiyan (2006-07) aroused concerned about ground water quality in Nuapada district as it showed presence of higher Fluoride level (more than 1 ppm) in 907 sources out of 4920 samples tested. Those 907 tube wells and wells were spread over 214 villages in all the five blocks of Nuapada district.

Mishra *et al.* (2009) investigated fluoride status of Hirakud environment revealed that the fluoride content varied from a minimum of 0.5 to a maximum of 0.65 (ppm) in pond water, 0.4 - 0.60 mg/L in ground water, 88.30 during 2005 – 2006.

Das *et al.* (2013) surveyed the Patripal Panchayat, Balasore, Odisha, India and reportedthe high concentration of fluoride in the water resources in Nuagan, Chakulia, Baharda, Kuanrpur of Patripal Panchaya. The value ranged from 0.6 mg/L to 5.83 mg/L in drinking water resources.

Routroy *et al.* (2013) reported seasonal variation of ground water in Nayagarh district, Odisha and also determined fluoride load by analysing both pre and post monsoon water samples. The percentage of total high fluoride containing water samples was found nearly double in pre-monsoon than in post-monsoon.

2.1.3 India

Patra *et al.* (2000) examined signs of dental discolouration, difficulty in mastication, bony lesions, lameness, debility and mortality in domesticated animals, reared around superphosphate fertiliser plants located approximately 15 km north of Udaipur, Rajasthan. Out of 166 animals they examined clinically, the prevalence rate was 17.4% (4/23) in calves below 1 year of age, 37.2% (16/43) in cattle between 1 and 3 years, 61.3% (46/75) in cattle above 3 years and 72% (18/25) in buffalo above 1 year.

Vaish and Vaish (2000) monitored 800 defluoridation units working with both techniques of activated alumina and the Nalgonda technique in villages of Dungarpur district of Rajasthan. People were daily using the units for last four years and they felt significant relief in non-skeletal fluorosis manifestations.

Bhargavi *et al.* (2004) analyzed the fluoride, calcium, magnesium, zinc, copper and phosphorus content in potable water and food samples from endemic and nonendemic villages for fluorosis. It was found that the F content

in water was significantly higher in endemic villages (4.20+/-1.6 ppm) whereas the Ca, Cu, and Mg contents were found to be significantly lower. The prevalence of dental fluorosis was positively correlated ($r=0.125$, $p<0.01$) to fluoride and negatively correlated to Ca and Cu content in drinking water in endemic villages.

Sabal and Khan (2008) investigated, determination of fluoride (F) in drinking water in (200 samples of) 40 villages of Phulera tehsil having fluoride content more than permissible limits. They found that water samples were alkaline with pH ranging from 7.05 to 10.16 and fluoride from 1.20 to 18 mg/l.

Kant *et al.* (2009) studied the efficacy of aluminiumsulfate as an ameliorative agentby using sodium fluoride alone and with aluminiumsulfate (ameliorative agent) orally daily for 30 days in healthy goats of group 1 and 2 respectively to access the radiographic changes in the forelimb. The radiographic changes that were observed in both the groups after 30 days exposure include an increase in overall density of the bony cortex.

Pandey and Pandey (2011) studied chronic fluoride intoxication in 10 villages of Udaipur receiving F emissions from phosphate fertilizer factories. According to them Khemli appeared to be the most affected village (with >48% cases) where, about 93% of 2 h air samples contained fluoride above 2.0 µg m and crops and vegetable F ranged from 27.5 to 143.4 µg. The study indicated that air-borne fluoride was the major factor for higher prevalence of fluorosis in these rural areas.

Sivasankar and Ramachandramoorthy (2011) conducted a study on the fluoride content in 419 groundwater samples from different sources in Rameswaram area, Southern India was determined in addition to the other physico-chemical parameters. They reported that about 15% of the groundwater samples were found to contain the F content greater than the WHO recommended limit.

Choubisa *et al.* (2012) accessed the chronic toxic effects of fluoride (F) in the form of osteo-dental and nonskeletal fluorosis in 99 domesticated cattle

(*Bostaurus*) reared in Chani village, Bikaner district of Rajasthan, India, nearby Thar desert. F in drinking water sources (bore wells) of this village varies between 1.5 and 2.5 ppm (mean 2.0 ppm).Out of 24 calves (<2 years age) and 75 cows (>3 years age),10 (41.7%) and 28 (37.3%), respectively, exhibited mild to severe dental mottling.

Narwaria and Saksena (2012) investigated in Karera block of Shivpuri district is facing the problem of ground water fluoride contamination. They determined fluoride in drinking water in the water sources of 10 villages and incidence of dental fluorosis in domestic animals. The fluoride concentration ranged from 1.65±0.0047mg in Hazinagar to 3.91±0.0072 mg in Dumduma village.

Sadat (2012) conducted a study to assess the fluoride concentration in the river (Godavari) and groundwater of the Nanded City, in Maharashtra. In the city, the fluoride was found to be in the range of 0.43 mg/l to 2.0 mg/l, while in river water it was found to be in the range of 0.23-1.76 mg/l. It was thereby established that the river, groundwater and even municipal tap water in Nanded, is found to cross the permissible limits of fluoride contamination in different seasons.

Arif *et al*. (2013) assessed water quality in Ladnu block of Nagaur district in Rajasthan with special reference to fluoride. They found that fluoride concentration of groundwater samples of thirty one villages were found to have a fluoride concentration above 1.5 mg/l out of forty villages with maximum fluoride concentration (7.1 mg/l) at Roja and minimum (0.5 mg/l) in Hudas.

Jha *et al*. (2013) used geo-statistical technique to study the spatial variability of fluoride (F) in the ground water in Unnao District, Uttar Pradesh, India. They found fluoride concentrations in both shallow and deep handpumps spatially distributed. It was also revealed that the shallow handpumps are highly contaminated with F as compared to deep handpumps.

Pandey *et al.* (2013) reported the ground water quality deterioration due to fluoride in the parts of Chhattisgarh state. Their report revealed that high contamination of fluoride content up to 7.00 ppm was found in Kolam, Muragaon and Saraitola villages and in the range of 1.0 to 1.2 ppm in Basanpalli, Bhalumuda, Dolesara, Penkapara, Kunjhemura villages, out of selected 60 sites.

Malvania *et al.* (2014) assessed the prevalence of dental caries and treatment needs among 12-year-old school going children in Vadodara City, Gujarat, India. They concluded that prevalence of dental caries was found to be of 17.15% among children.

Sharma and Sharma (2014) analysed the physicochemical properties of 20 water samples in Kherisadh Village, Haryana and reported that hardness of ground water and higher fluoride content might be the cause of dental and skeletal fluorosis.

2.1.4 World

Krook and Maylin (1979) reported occurrence of fluorosis in areas of Cornwall Island. They recorded stunted growth and severe dental fluorosis which interfered with drinking and mastication as the effect of chronic fluorine poisoning in cattle, caused by emission from an aluminium plant.

Crissman *et al.* (1980) observed that ambient air fluoride virtually never exceeded New York State standards. In a New York State dairy farm, downwind from the plant about 40% of the time and with the fields within 1300-2800 m of the plant, fluoride contamination of vegetation ranged from 13 to 25 ppm, well below the 40 ppm which is the 'tolerance' level by National Academy of Sciences. Still, New York State and U.S. Federal fluoride pollution standards do not protect cattle health.

Suttie (1982) indicated the adverse effects of fluoride ingestion in dairy cattle, as reduced productivity, dental and skeletal fluorosis in Ayrshire dairy herd located near a primary aluminium smelter.

Cao *et al*. (1996) found a new type of fluorosis in China called "brick tea type fluorosis" in Tibetan residents who regularly consumed brick tea containing high Fluoride levels.

Maldea *et al.* (1997) investigated fluoride content in food sample of areas like Awassa and Zwai, Ethiopia; Mwanza and Dar es Salaam, Tanzania; and Bujumbura, Burundi. They reported that prevalence of dental fluorosis in east Africa may not solely due to drinking water rather food habits could also contribute to some extent.

Kloos and Haimanot (1999) reviewed fluoride test data for 270 water sources in 126 communities and examination of the literature of fluorosis distribution in Ethiopia showed that fluorosis extends beyond the Rift Valley into some highland communities. Fluoride concentrations above 5.0 mg/l in the Rift Valley were found mostly in hot springs (100% of all sources), lakes (78%), shallow wells (54%) and boreholes (35%) and the lowest concentrations (below 1.5 mg/l) in springs and rivers.

Alarcon-Herrera *et al*. (2001) reported higher fluoride content in well water, dental fluorosis, and bone fractures in the Guadiana Valley of Mexico.

Gracea *et al*. (2003) reported the effect of ingestion of soil fluorine on grazing young sheep of Newzeland due to continuous use of phosphate fertilizers in crop fields.

Flueck (2013) reported that the Puyehue-Cordon Caulle volcanic eruption deposited large amounts of tephra (ashes) on about 36 million ha of Argentina in June of 2011.These tephracaused dental fluorosis, with bone fluoride levels in animals reaching up to 3,253 ppm.

2.2 Risk factors

The risk for developing skeletal fluorosis, and the course the disease will take, is not solely dependent on the dose of fluoride ingested. Indeed, people exposed to similar doses of fluoride may experience markedly different effect. The following are some of the factors that are believed to play a role:

2.2.1 Impaired kidney function

Pak (1989) indicated that the skeletal complication of fluoride was more common in renal disease. Because of the impairment in renal excretion of fluoride, high circulating concentrations of fluoride may be achieved in renal disease.

Ayoob and Gupta (2006) concluded that persons with renal failure could have a four times increased in skeletal fluoride content, ware at more risk of spontaneous bone fractures and akin to skeletal fluorosis even at 1.0 ppm fluoride in drinking water.

Bansal and Tiwari (2006) reportedthat the individuals with kidney disease had decreased ability to excrete fluoride in urine and were at risk of developing fluorosis even at normal recommended limit of 0.7 to 1.2 mg/l.

Schiffl (2008) indicated that patients with chronic renal insufficiency ware at an increased risk of chronic fluoride toxicity. Patients with reduced glomerular filtration rates had a decreased ability to excrete fluoride in the urine. Those patients may developed skeletal fluorosis even at 1 ppm fluoride in the drinking water.

2.2.2 Poverty/poor nutrition

Fisher *et al*. (1981) reasoned thatas the kidney is the main pathway of fluoride excretion, patients with chronic renal failure are especially vulnerable to osseous accumulation of ingested fluoride and to potentially deleterious effect.

Mithal *et al*. (1993) findings was also evident that the osteopenic radiological picture (fluorosis) was more commonly found in the poorer and undernourished population of the village.

Teotia *et al*. (1998) reported in calcium-deficient children the toxic effects of fluoride manifest even at marginally high (>2.5 mg/d) exposures to fluoride.

Littleton (1999) concluded that undernourished individuals appear to be more prone to develop dental and skeletal fluorosis.

He (2008) findings indicated that adequate amount of supplementary ingestion of dietary factors such as protein and calcium may significantly reduce some toxic effects of fluoride on bone development.

2.2.3 Genetic

Anand and Roberts (1990) suggested that predisposition to fluorosis (chronic toxicity) was biochemically mediated and genetically determined.

Polzik *et al*. (1994) helped to establish the existence of genetic predisposition to fluorosis and developed some criteria for estimating it and to prove that predisposition to fluorosis was associated with the same dermatographic features among the workers of industrial sectors.

Lavryashina *et al*. (2003) analysed the phenotype frequency distributions of several classical blood genetic markers and dermatographic characters among workers of Siberian aluminium plants those had been suffering occupational fluorosis and compromised between healthy workers which revealed a significant differences in frequencies of several (genetic) markers.

Vieira *et al*. (2005) conducted an epidemiological study on mice and human beings, and found that severity of dental fluorosis couldnot be explained simply by the amount of fluoride present in the tooth structure, indicated that genetics (susceptibility to fluoride) might played an important role on the severity dental fluorosis severity.

Mousny *et al.* (2006) assessed the effects of increasing fluoride doses (0 ppm, 25 ppm, 50 ppm, 100 ppm) on the bone properties in 3 inbred mouse strains that demonstrate different susceptibilities for developing enamel fluorosis (A/J a "susceptible" strain, 129P3/J a "resistant" strain and SWR/J an "intermediate" strain).Concordant with increasing fluoride doses were significantly increases of fluoride concentration in femoral and vertebral

bodies from all 3 strains. Fluoride treatment had little effect on the bone mineral densities (BMD) in 3 strains. Mechanical testing showed significant alterations in "bone quality" in the A/J strain, whereas moderate alterations in "bone quality" in the SWR/J strain and no effects in the 129P3/J strain were observed.The results suggested that genetic factors may contribute to the variation in bone response to fluoride exposure and that fluoride might affect bone properties without altering BMD.

2.2.4 Gastric acidity

Franke (1975) studied the relationship of gastric acid among 150 aluminium plant workers to the degree of severity of fluorosis. Results showed the gradualincreasing severity of fluorosis particularly in hyperacid personsand decreasing severity of hypo- or an-acid persons. These findings proved that fluoride resumption was obviously diminished by a deficiency of gastric acid and that it was enhanced by hyperacidity.

Krishnan *et al*. (2012) studied the absorption rate of fluoride from the stomach which was dependent on the pH of the gastric contents and chronic metabolic acidosis induced reduction in the renal clearance of fluoride. This again could resulted in major disturbances in enamel with mineralization defects resembling fluorosis due to high concentrations of fluoride in bone and enamel associated with the acidic state. The use of fluoride supplements was therefore contraindicated in RTA associated with enamel hypoplasia.

2.2.5 Repetitive/physical stress

Siddiqui (1955) indicated that physical strain was also found responsible as greater the strain, the more pronounced were the changes of fluorosis observed. Pain and stiffness were more severe in the joints shown mostly by the individuals – for example, the wrists, shoulders, and neck in the females, who were mostly engaged in household worksand the lumbar spine and the joints of the lower limbs in males working in the fields.

Singh *et al*. (1961)reported that physical strain may be also contributed to skeletal fluorosis, because the disease was found predominantly in manual workers, those showed involvement of cervical spine and skull.

Shupe and Olson (1971) observed the fluorotic changes first at the sites of greatest metabolic activity and stress within a given bone and in bones that were under the greatest stress from weight bearing and locomotion.

Anand and Roberts (1990) found that the spine was the most common part of the skeleton to be early affected with fluorosis and also severely though it is required to sustain the erect posture in men and has stresses and strains.

Prasad *et al*. (1994) indicated that the spine is the most common part of the skeleton to be first affected with fluorosis and also severely so because it is required to sustain the erect posture and has stresses and strains.

Littleton (1999) observed that the initial development of new fluorotic bone occurred where the sites most subjected to strain and minor trauma.

2.2.6 Age

Johnson (1965) observed that mottling was the result of the action of fluoride on osteoblasts during bone formation. Young bones undergoing extensive remodeling showed extensive mottling, while old bones with scant remodeling showed little mottling.

Turner *et al*. (1995) conducted a studyon fluoride effects on bone in young and healthy experimental animals. They found that failure to take stress may decreased by much as 29% after receiving 50 ppm of fluoride in older rats. Such dramatic losses of bone strength only have been shown previously in studies where fluoride intake was accompanied by calcium deficiency.Therefore calcium intake in the older rats was no different from that in the younger rats. So they concluded thatthere was possibilityof aging

effects and fluoride incorporation in the bone which act synergistically to decrease bone strength.

Teotia *et al*. (1998) revealed that fluoride toxicity afflicts children more severely over a shorter period of exposure (about 6 months) as compared to adults. This wasdue to rapidly growing of bones of children ware metabolically active and more vascular and thus absorbed and accumulate fluoride faster and in greater amounts than older bones, particularly at the sites of bone growth and physiological calcifications.

2.2.7 Pregnancy/lactation

Roholm (1937) opined that a large amount of calcium requirement increases the sensitivity to fluorine. Bone symptoms ware noticed most readily in young and growing individuals. The toxic effect on cattle became visible especially during pregnancy and lactation.

Christie (1980) reported thatexcessive fluoride ingestion in pregnant women may possibly poisonous and there must be an alteration in activity of enzyme and hormonal systems in the fetus causing disturbances in osteoid formation and mineralization of bones.

Ream *et al*. (1983) indicated that fluoride ingestion during lactation created a high state of calcium homeostatic stress. As a result, bone mineral was mobilized by resumption of the endosteal surface and by cavitation of the interior of the cortex.

2.3 Clinical signs

2.3.1 Dental fluorosis

Shearer *et al*. (1978) conducted a histological study on incisor teeth on 5 to 6 year old Holstein-Friesian cows. The cows had been given NaF to supply F equivalent to a yearly average of 40 mg/kg in forage, with range 20 to 120 mg/kg, from 4 months old. Features of fluorotic enamel noted were: hypomineralized outer enamel, coronal cementum hyperplasia, disrupted

subsurface pigment band, hypoplastic pits, puckered incremental lines, periodic radiolucent regions, positive protein staining and decreased micro-hardness of the outer enamel.

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

Maylin *et al*. (1987) studied calves born to the fluoride intoxicated cows and reported the manifestation of congenital fluorosiswith clinical signs of by brown discoloration of enamel, enamel hypoplasia, brown mottling of bone, severe retardation of cartilage cell differentiation, atrophy of osteoblasts, osteopenia, atrophy of bone marrow cells, serous atrophy of bone marrow fat and severely stunted growth.

Shupe *et al*. (1987) examined theteeth from cattle, sheep, and horses suffering from fluorosis and teeth from field studies of these species and deer, elk, and bison for abnormalities. The findings like severity of fluoride-induced mottling, hypoplasia, and abnormal abrasion of paired permanent incisor teeth were correlated with abrasion of premolar and molar (cheek) teeth that form and mineralize at approximately the same age. These irregular wear of cheek teeth impaired mastication and resulted in poor utilization of feed and unthriftiness. These abnormalities might be due to excessive amounts of fluoride ingestion during tooth formation and mineralization.

Susheela and Bhatnagar (1993) investigated the effect of fluoride toxicity on the morphology as well as inorganic chemical constituents of rabbit teeth. The scanning electron micrographs studies revealed hypoplastic, rough, uneven, pitted and cracked enamel surfaces covered with granular deposits due to excessive intake of fluoride.

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

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 situated near by Bhubaneswar, Odisha.

Choubisa (1999) observed chronic fluoride toxicity in the form of osteo-dental fluorosis in cattle, buffaloes, sheep and goats from 21 villages of Banswara, Dungarpur and Udaipur districts of southern Rajasthan. The prevalence of dental fluorosis in calves (< 1 year age) was greater than that in adult cattle and buffaloes. At a fluoride concentration in the water of 4.0 ppm, 100% of calves, 65.6% of buffaloes and 61.0% of cattle were found to be affected with dental fluorosis to varying degrees. In the older group of buffaloes, their teeth were brownish black instead of creamy yellow as found in calves and cattle.

Muralidhar *et al*. (2000) identified dental fluorosis cases in bovine in field based on the lesions of dental mottling, irregular wear, tear of teeth and premature ageing of teeth.

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.

Maiti *et al*. (2003) studied chronic fluoride toxicity in the form of dental fluorosis was observed in cattle from nine villages under two blocks of Nayagarh district of Orissa. The commonly observed signs of dental fluorosis were brown discoloration, mottling, attrition or uneven wearing of teeth 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, NALCO, Odisha.

Choubisa *et al*. (2012) investigated chronic toxic effects of fluoride in the form of osteo-dental and non skeletal fluorosis in 99 domesticated *Bos taurus* cattle rearing in Chani village, Bikaner district of Rajasthan, India located nearby Thar desert. Their anterior teeth were bilaterally striated and horizontally have light to deep yellowish in colour. In some calves, dental staining was found to be light brown todeep brownish or dark. In severe forms of dental fluorosis, irregular wearing of teethand recession and swelling of gingiva were also observed.

Narwaria and Saksena (2012) studied Karera block of Shivpuri district, India that was facing the problem of ground water fluoride contamination. They investigated fluoride content present in drinking water and found (higher content) positive in 10 villages and incidence of dentalfluorosis in domestic animals were studied.They revealed that 41.34% adult buffaloes, 40.0% adultcows and 36.12% immature buffaloes and 36.72% cows were found to have dental fluorosis withlight to deep yellowish brown teeth, striated and horizontal lines starting from the base of teeth.

Choubisa (2014) conducted an observational survey of 2,747 mature and 887 immature domestic animals of diverse species living in areas with naturally fluoridated (>1.5 ppm F) drinking water of Rajasthan. Out of these mature and immature animals, 899 (32.7 %) and 322 (36.3 %) showed evidence of dental fluorosis with varying degrees, respectively and also foundlight to deep brownish color staining in incisor teeth.

Modasiya (2014) investigated toxic effects of chronic fluoride exposure in the form of osteo-dental and non-skeletal fluorosis in 85 domesticated animals living in Udasar village, Bikaner district of Rajasthan, India. They observed that the anterior teeth showed light to deep yellowish staining and

striated lines. In severe forms of dental fluorosis, recession of gingival swelling and irregular wearing of teeth were also present.

2.3.2 Skeletal fluorosis

Sutie (1977) indicated that cattle were most commonly affected species, and the symptoms of excessive fluoride ingestion on that species include lesions in the developing dentition, skeletal lesions and lameness.

Shlosberg *et al*. (1980) observed the symptoms of lameness, exostoses of hind legs and ribs in fluorotic dairy herds fed with non-deflourinated rock phosphate as mineral supplement in Israel.

Shupe (1980) reported that, lameness and stiffness were inconclusive measures of fluoride toxicosis. The intermittent lameness and stiffness that were noticed in more advanced cases of fluoride toxicosis appear to be associated with the osteofluorotic lesions and the calcification of periarticular structures and tendon insertions. The bones usually were chalky white and have a roughened irregular periosteal surfaceand heavier than normalshowing the changes of osteosclerosis, osteoporosis, hyperostosis, osteo- phytosis and osteomalacia. These changes showed increased density, excessive porosity, abnormal hyperostosis, osteophytosis and osteomalacia, which were demonstrated by radio- graphically as well as grossly in chronic fluoride toxicity in animals.

Maylin *et al*. (1987) noticed the signs of 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.

Boivin *et al*. (1989) reported bone formation rate and adjusted apposition rate were significantly decreased in skeletal fluorosis. On stained sections and microradiographs, bone tissue showed typical modifications for skeletal fluorosis viz., linear formation defects and mottled bone. The volume

of cancellous interstitial mineralization defects and the proportion of mottled periosteocytic lacunae were markedly increased in skeletal fluorosis.

Nyssen-Behets (1989) found exostoses in the 5th rib, the cannon bone and the mandible in a 6-year-old fluorotic cow. Microradiographic alterations showed lamellar bone in these skeletal region included both matrix modifications and mineralization troubles.

Zhi (1989) studied in forty four domestic pigs which revealed that the bone dynamics, both bone formation rate and bone mineralization rate were inhibited, indicating the toxic effects of fluoride in tremendous amount on bone remodeling. Authors also suggested that the existence of osteosclerosis of axial and osteoporosis of peripheral bone in fluorosis might be related to the redistribution of calcium within the body. Furthermore, fluoride may be an osteomalacic factor for the development of osteomalacia in endemic fluorosis.

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

Schultz *et al*. (1998) investigated the animals reared from a highly fluoride polluted area situated in Central Europe (Ore mountains and their southern foreland, Czech-German border region). The osteofluorotic changes recorded by them include comprised extended apposition of periosteal bone onto the mandibular cortex as well as deformation of the mandibular body, which was attributed to a fluoride-induced osteomalacia.

Muralidhara *et al*. (2000) recognized skeletal fluorosis in bovine basing on lameness, pain on palpation of bone and joint, dislocated limbs and history of frequent fractures.

Patra *et al*. (2000) recorded the signs of dental discolouration, difficulty in mastication, bony lesions, lameness, debility and mortality in domesticated animals, reared around superphosphate fertiliser plants located approximately

15 km north of Udaipur, Rajasthan prompted us to investigate for the occurrence of fluorosis.

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

Beveridge (2006) observed clinical signs of lameness in eastern grey kangaroos (*Macropus giganteus*) inhabiting heathland and farmland surrounding an aluminium smelter at Portland, Victoria, Australia were exhibiting osteophytosis of the distal tibia and fibula, tarsal bones, metatarsus IV, and proximal coccygeal vertebrae; osteopenia of the femur, tibia, and metatarsus IV; incisor enamel hypoplasia; stained, uneven, and abnormal teeth wear; abnormal bone matrix mineralization and mottling; increased bone density; and elevated bone fluoride levels. Microradiography of affected kangaroos exhibited “black osteons,” which weresignificant manifestation of fluorosis.

Kant *et al*. (2009) reported that fluoride toxicity caused dissociation of the normal sequence in osteogenesis resulting in production of abnormal bone due to stimulation of alkaline phosphatase, acceleration of remodeling, bonefracture, enlargement of sternum and lower jaw, callus formation on the ribs, increase in diameter ofmetacarpals, metatarsals and phalanges, and sometimes osteoporosis. The affected animals and human beings showed lameness with increased immobilization of joints, pain during palpation of affected bones, stiffness which was associated with oesteofluorotic lesions and calcification of tendons and peri-articular structures. Osteophytosis and sclerosis of the vertebral column might because of compression of spinal cord and nerve roots and could produce neurological signs and they suspected the compression of nerve root could lead to atrophy of various muscles.

Choubisa*et al*. (2012) studied chronic toxic effects of fluoride in the form of osteo-dental and nonskeletal fluorosis in 99 domesticated cattle (*Bos*

taurus) living in Chani village, Bikaner district of Rajasthan state (India), nearby Thar desert. They recordedthe signs of skeletalfluorosis as intermittent lameness and snapping sound of legs, wasting of bodymuscles and excessive periosteal exostoses of the mandibles, ribs, metacarpus, and metatarsus regions.

2.3.3 Non skeletal fluorosis

Krook and Maylin (1979) reported chronic fluoride poisoning in Cornwall Island cattle which were clinically manifested by stunted growth and dental fluorosis to a degree of severe interference with mastication.

Maylina and Krookb (1982) investigated sales records of milk in a dairy herd which showed that milk production did not decrease during the first 4 yr of exposure of cows to fluoride pollution nearby an aluminium plant. During the next 3 yr, milk production was not decreased significantly. From 8 yrs of exposure there was a significant decrease, which persisted upto 19 yrs, when the dairy operation was terminated. From 15 yrs to 19yrs, the milk yield averaged less than 60% of the expected values were recorded.

Kessabi *et al*. (1984) observed the effects of chronic fluorosis on serum biochemical parameters of 50 cattle in the Darmous area of Morocco. Their study revealed an increased level of potassium, urea, gamma-globulins, lactate dehydrogenase, alkaline phosphatase and aspartate aminotransferase were observed, whereas calcium, total proteins and albumin were decreased. Such disturbances drew attention towards kidney, liver and mineral metabolism and have to be taken into account to improve the management of cattle herds in areas of chronic fluorosis.

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

Dasarathy *et al*. (1996) performed a prospective case-controlled study to evaluate the gastrointestinal symptoms and mucosal abnormalities

occurring in patients with osteofluorosis. Ten patients with documented osteofluorosis and ten, age- and sex-matched healthy volunteers were included in their study. All patients with osteofluorosis had gastrointestinal symptoms with abdominal pain. Endoscopic abnormalities were found in seven patients with osteofluorosis. In all 7 of these patients, chronic atrophic gastritis was seen on histology. Electron microscopic abnormalities were observed in all 10 patients with osteofluorosis showing microvilli, cracked-clay appearance, and the presence of surface abrasions on the mucosal cells.

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.

Zhavoronkov (1977) presented descriptions of pathology of the central nervous system, skeletal musculature, stomach, liver, kidney, cardiovascular system, retina, and the skin. The evidences obtained indicate that disturbances in soft tissues in chronic intoxication with fluorine develop early. He estimated the fluoride content in eight-week old male Wistar rats in liver, kidney, brain, testis, and serum from beginning of the experiment and after 2, 4, and 12 weeks of exposure and observed a dose and time-dependent increasing manner of fluoride deposition in these tissues.

Jagadish *et al*. (1998) recorded the clinical signs of 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 content.

Attia *et al*. (2004) noticed that high fluoride intake significantly depressed body weight, egg number, egg weight, egg mass, feed consumption and feed conversion ratio in laying hens.

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 ageing.

Xu *et al*. (2007) studied the effect of fluoride on intracellular free calcium ([Ca2+]i) and Ca2+-ATPase of renal cells. They concluded that the effect of fluoride on Ca2+-ATPase was similar to a dose-effect relationship phenomenon characterized by low-dose stimulation and high-dose inhibition, and the increase of [Ca2+]i probably plays a key role on the mechanism of renal injury in fluorosis.

Madan *et al*. (2008) examined twelve male buffalo calves between 10 to 12 months of age divided into 3 groups of four each. They were fed wheat straw, concentrate mixture and 3 Kg greens daily. The chemical composition of the diet was same in all the three groups except fluoride which was added (as NaF) in concentrate mixture of group B and C to make the final fluoride concentration 30 ppm and 60 ppm, respectively. Analysis of data revealed that the dry matter intake decreased non significantly in group B and C, as compared to control group.

Reddy (2009) reported some neurological complications of endemic skeletal fluorosis, namely radiculopathy, myelopathy or both ware mechanical in nature.

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

Choubisa *et al*. (2012) examined chronic toxic effects of fluoride in the form of osteo-dental and nonskeletal fluorosis in 99 domesticated cattle (*Bos taurus*) living inChani village, Bikaner district of Rajasthan state, India nearby Thar desert. In these animals, colic, intermittent diarrhoea, excessive

urination, irregular reproductive cycles, repeated abortions, and stillbirths were found as clinical signs of nonskeletal fluorosis.

Roy and Dass (2013) indicated that fluoride toxicity could also cause non-skeletal diseases like aches and pain in joints, non-ulcer dyspepsia, polyurea and polydipsia, muscle weakness, fatigue, anemia with very low hemoglobin levels, etc. besides other reasons.

2.4 Haematological changes

Hillman *et al*. (1979) collected blood and urine from 72 cows in six dairy herds with varying severity of dental and bone fluorotic lesions. They found an increasing urinary fluoride level, increased eosinophils count, and decreased cholesterol level. Cattle afflicted with fluorosis developed hypothyroidism, anemia, and eosinophilia of leukocytes.

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) and decreased in total erythrocytes (RBC), haemoglobin (Hb), packed cell volume (PCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) levels. 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 an elevated erythrocyte counts, packed cell volume, haemoglobin level and decreased total leucocyte count in blood of fluorotic hen reared near a superphosphate fertilizer producing factory.

Metwalli *et al*. (1995) performed a haematological examination of affected cows that showed significant decrease in haemoglobin and haematocrit.

Jagadish *et al*. (1998) observed mild microcytic, hypochromic anaemia, and mild eosinophilia in severely fluoride toxicity affected animals.

Mishra and Mohapatra (1998) estimated the fluoride concentration present in bones and differential haemotological characteristics in amphibians, *Bufo melanostictus*, collected from fluoride-contaminated and uncontaminated area. The average haemoglobin content, total RBC count and haematocrit (%) level of blood samples were found to be significantly reduced, while mean corpuscular concentration and volume were significantly elevated in individuals from the contaminated area in comparison to those from the uncontaminated area.

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 affected with fluorosis kept near an aluminium smelting plant in Orissa, India.

Upadhyay *et al*. (2005) studied the effect of high fluoride intake on cattle and buffaloes and revealed that affected animals exhibited mild anemia with few exceptions of mild leukopenia and eosinophilia.

Kant *et al*. (2009) studied the effect of sodium fluoride alone and with aluminium sulfate as ameliorative agent administered orally daily for 30 days in healthy goats of group 1 and 2, respectively to access the effect of the hematological profile on different days of exposure. Exposure of sodium fluoride alone produced significant reduction in hemoglobin (Hb), packed cell volume (PCV), total leucocytes count (TLC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) and increased blood clotting time. However, values of total erythrocyte count (TEC) and differential leucocytes count (DLC) were not significantly changed.

Nurgül *et al*. (2014) studied the protective effects of resveratrol on hematological and biochemical changes induced by fluoride in rats. A total of 28 rats were divided into 4 groups, viz. control, resveratrol, fluoride, and fluoride/resveratrol (n = 7 each), for a total of 21 days of treatment.Compared to the control group, the fluoride-treated group showed significant differences in several hematological parameters, including decreases in WBC, RBC, and

PLT counts and neutrophil ratio. The group that received resveratrol alone showed a decrease in WBC count compared to the control group.

2.5 Biochemical changes

Kessabi *et al*. (1984) observed the effects of chronic fluorosis on serum biochemical parameters of 50 cattle in the Darmous area of Morocco. They noticed an increase in potassium, urea, gamma-globulins, lactate dehydrogenase, alkaline phosphatase and aspartate aminotransferase whereas calcium, total proteins and albumin were lowered.

Surendra *et al*. (1986) revealed biochemical findings as raised plasma levels of fluoride, alkaline phosphatase and iPTH, and increased urinary fluoride excretion.

Hamid *et al*. (1994) recorded a reduced albumin/globulin ratio, total proteins and Ca in fluorotic hens reared near a superphosphate fertilizer producing factory.

Singh and Swarup (1994) reported the occurrence of fluorosis in buffaloes in several villages of Ghaziabad district of U.P. Affected animals had significantly lower serum calcium and higher inorganic phosphorus, 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 calcium and total protein were significantly decreased, whereas serum alkaline phosphatase activity, fluoride content, urea, and creatinine were significantly increased, compared with control animals.

Jagadish *et al*. (1998) revealed lower serum calcium level and 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).

Krook and Minor (1998) reported that serum alkaline phosphatase increases in fluoride therapy against osteoporosis.

Sing and Swarup (1999) investigated biochemical changes of serum and urine influorotic cattle and buffaloes 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, creatnine, inorganic phosphorus, alkaline phosphatase and manganese. The concentration of calcium, magnesium, iron and copper were low in fluorotic animals. Changes in urine included increased level of calcium, phosphorus and alkaline phosphatase in fluorotic animals.

Mehedintu *et al.* (2000) recorded paraclinical changes in industrial fluorosic ruminants as anemia, hypocalcaemia, hypophosphotaemia and increased serum alkaline phosphatase activity.

Singh *et al*. (2002) indicated that fluoride affected goats showed a significant decrease in total protein, albumin, glucose and cholesterol 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.

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, inorganic phosphorus, plasma urea nitrogen, ALP and AST activities whereas the concentrations of calcium, total cholesterol, total protein, albumin and globulin level were decreased in the affected animals. The levels of plasma magnesium, creatinine and A/G ratio remained

unchanged. The affected animals had higher concentrations of calcium, phosphorus and ALP activity in urine.

Madan *et al*. (2009) investigated some biochemical changes in twelve male buffalo calves of 10 to 12 months of age divided into 3 groups of four each. They were fed wheat straw, concentrate mixture and 3 Kg greens. The chemical composition of the diet was same in all the three groups except fluoride which was added (as NaF) in concentrate mixture of group B and C to make the final fluoride concentration 30 ppm and 60 ppm respectively.They revealed that there was a significant increase of alkaline phosphatase activity in group C animals, decrease in serum calcium and a significant increase in phosphorus concentration in group C animals.

Nurgül *et al*. (2014) study showed a significant increased level of ALT enzyme activity and decreased inorganic phosphorus level in fluorotic animals in comparison to healthy ones.

2.6 Microminerals

Ranjan *et al*. (2008) analysed soil, fodder and water samples collected from some localities of Udaipur district, Rajasthan, India for fluoride content. Concentration of micro and macro minerals were estimated in blood samples collected from cattle reared in these localities, and with clinical lesions suggestive of chronic fluoride toxicity. In comparison to healthy controls, zinc, copper and manganese levels were significantly lower, while cobalt and magnesium concentrations were significantly higher in fluoride-intoxicated cattle.

Meral *et al*. (2004) investigated the serum copper, zinc, manganese, and magnesium status in a group of men with chronic fluorosis.Men with chronic fluorosis had lower serum Cu, Zn, Mn, and Mg levels than did the controls, and it was therefore concluded that chronic fluorosis is associated with decreased serum levels of these minerals.

Ersoy *et al*. (2011) aimed to determine the serum levels of trace elements including serum copper, zinc and serum levels of minerals including calcium, phosphorus, magnesium, sodium, potassium in patients with endemic fluorosis.Serum Cu levels (89.14±16.77 µg/dL vs. 102.69±25.04 µg/dL, respectively, P=0.017), serum Zn levels (77.98±20.58 µg/dL vs. 94.57±35.87µg/dL, respectively, P=0.032), and serum Mg levels (1.92±0.18 mg/dL vs. 2.07±0.31 mg/dL, respectively, p=0.022) was significantly lower in chronic fluorosis patients than in controls. There were no statistically significant differences between the fluorosis group and control group with respect to serum levels of Na, K, Ca, and P.

2.7 Concentrations of fluoride

2.7.1 Plasma/serum

Patra *et al*. (2000) observed the clinical signs of fluorosis in domesticated animals, reared around superphosphate fertiliser plants located approximately 15 km north of Udaipur, Rajasthan andreported that the mean fluoride concentrations in serum and urine were 1.53 +/- 1.27 and 26.4 +/- 6.17 mg l(-1) in calves below 1 year of age, 0.56 +/- 0.17 and 26.2 +/- 3.86 mg l(-1) in cattle of 1-3 years, 0.49 +/- 1.13 and 27.5 +/- 4.63 mg l(-1) in cattle above 3 years and 0.60 +/- 0.07 and 28.6 +/- 4.73 mg l(-1) in buffalo over 1 year, respectively.

Milhaud *et al*. (1980) studied 12 goats aged about 8 months, nine were given 2.5 mg/kg daily of sodium fluoride added to the feed, and killed after 1, 2 or 3 years. Three goats not fed fluoride were controls. Plasma fluoride content in experimental animals was generally 0.8-0.9 mg/l, against 0.1-0.2 mg/l in controls.

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 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+or-0.00 and 0.08+or-0.00 ppm, respectively.

Kanungo (2005) recorded fluoride content in serum of affected cattle near aluminium smelter of NALCO, Angul, 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. Abruptly high level of serum fluoride has been recorded in the affected cattle indicating constant chronic exposure of the animals to the fluoride pollutants. The serum fluoride level of fluorotic cattle was found significantly higher than the apparently healthy and non-fluorotic cattle.

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 ppmagainst 0.055 + 0.002 ppm in healthy control.

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).

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 1.37 + 0.07 ppm, 0.47 + 0.02 ppm, 0.48 + 0.02 ppm and 0.5 + 0.05 ppm on Chitradura, Ramnagar. Kolar and Magadi in 1999, respectively.

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 were 0.59 + 0.02 ppm and 0.73 + 0.02 ppm, respectively against was 0.18 + 0.01 ppm, in healthy animals.

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.

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.

2.7.2 Urine

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.

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.

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 of age.

Fidanc and Sel (2001) found the concentrations of fluoride ion in the urine samples to be 7.86±0.77 ppm which indicated the presence of chronic industrial fluorosis in sheep near the coal burning power station in Mugla-Yatagan, Turkey.

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+or-0.27 and 2.37+or-0.83 ppm, respectively.

2.7.3 Water

Singh (1994) found an average fluoride concentration of water samples collected from well, ponds 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.

Patra *et al*. (2000) studied around superphosphate fertiliser plants located approximately 15 km north of Udaipur, Rajasthan for the occurrence of fluorosis.Fluoride concentrations in the environmental sample collected from the affected locality were 534.4±74.9 mg kg in fodder, 1.19±0.29 mg l in pond water and 0.479±0.351 mg lin tube well water.

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

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.

Sabal *et al*. (2008) investigated the fluoride content in drinking water in (200 samples of) 40 villages of Phulera tehsil having fluoride content more than permissible limits (> 1.5 mg l).The water samples were alkaline with pH ranging from 7.05 to 10.16. Electrical conductivity (EC) ranged from 157 micromhoScm to 1018 micromhoS cm. Calcium hardness (Ca-H) ranged from 10 to 127 mg l. Total hardness (TH) varied from 69 to 572 mg l. Chloride varied from 92.00 mg lto 1422.00 mg land fluoride from 1.20 to 18 mg l.

Choubisa *et al*. (2012) studied thechronic toxic effects of fluoride in the form of osteo-dental and nonskeletal fluorosis were observed in 99 domesticated cattle (*Bos taurus*) living in Chani village, Bikaner district of Rajasthan state, India, located in the Thar desert. F in drinking water sources (bore wells) of this village varies between 1.5 and2.5 ppm (mean 2.0 ppm).

Sadat (2012) assessed the fluoride concentration in the river (Godavari) and groundwater of the Nanded City, in Maharashtra, which is a prominent city of Marathwada region. The fluoride is responsible for many diseases ranging from simple dental carries reduction to kidney failures and deaths. In the city, the fluoride was found to be in the range of 0.43 mg/L to 2.0 mg/L. while in water it was found to be in the range of 0.23-1.76 mg/L.

Arif *et al*. (2013) performed assessment of water quality with special reference to fluoride in Ladnu block of Nagaur district in Rajasthan. The maximum fluoride concentration (7.1 mg/l) was recorded in groundwater of the Roja, while minimum (0.5 mg/l) was recorded in Hudas.

Pandey *et al*. (2013) reports the ground water quality deterioration due to fluoride in the parts of Chhattisgarh state. Out of selected 60 sites, high contamination of fluoride content up to 7.00 ppm was found in Kolam, Muragaon and Saraitola villages and in the range of 1.0 to 1.2 ppm in Basanpalli, Bhalumuda, Dolesara, Penkapara, Kunjhemura villages.

Modasiya *et al*. (2014) Toxic effects of chronic fluoride (F) exposure in the form of osteo-dental and non-skeletal fluorosis were observed in 85 domesticated animals living in Udasar village, Bikaner district of Rajasthan, India located in the That desert of India where fluoride in drinking water sources (bore wells) varies between 1.6 ppm and 2.2 ppm (mean 1.9 ppm).

2.7.3 Soil

Kessabi and Ouhsine (1986) recorded the fluoride content of the upper layers of the soil to be on average 2000 mg/kg around the industrial town of Safi, Central Morocco. Animals are contaminated by grazing dusty herbage, drinking water containing suspended particles of soil, inhalation of dust, and ingestion of soil while searching for roots.

Cronin *et al*. (2000) indicated that grazing sheep and cattle obtain over 50% of their dietary fluoride (and this may be >80% during winter) from soil ingestion and also suggested that at the extremes of the ranges of the

measured winter soil ingestion (143-300 g for sheep and 900-1600 g for cattle) and dietary F absorptivity (bioavailability) of soil F (20 -38%), total topsoil F concentrations in the range of 372-1461 micro g F could cause chronic fluorosis in sheep and 326-1085 micro g F in cattle.

Muralidhara *et al*. (2000) collected 374 soil samples from Mundargi, Chitradurga, Bagepalli, Pavagada, Ramnagar, Kolar, Magadi and Bidar in Karnataka, India in order to analyse their fluorine (F) contents. Soil F was highest in Chitradurga (4.55ppm) while the lowest in Ramnagar (0.25 pp) and Bidar (0.08 ppm), respectively.

2.7.4 Fodder

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.

Swarup *et al*. (1998) reported significantly higher fluoride level in the vegetation samples collected from different directions around aluminium smelter plant. Contamination of vegetation with fumes of aluminium smelter was the main source of fluoride intoxication.

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.

Kanungo (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).

2.8 Different ameliorative agents

Allcroft *et al*. (1965) investigated between 1954 and 1961 on a farm surrounded by industrial works which emit fluoride. The 3 groups each of 4 heifers and 4 cows and all got a similar intake of fluoride from pasture. The groups were given no mineral supplement, or hydrated aluminium sulfate about 1% of total intake of dry matter, alone or with 56 g calcium carbonate 56

and sodium acid phosphate 28 g per head daily. Calves were treated as their dams.In the groups given supplements fluoride in bone and urine was significantly less by about 22% than in the control group. In all groups cancellous bone had significantly more F than did compact. There was no greater reduction in F with Ca and P than with aluminium sulfate alone. The amount of aluminium sulfate given delayed but did not prevent accumulation of excess F in bone.

Elsair *et al*. (1981) conducted a study onrabbits intoxicated by administration of 30 mg/kg/day of fluoride for 3 months followed by 15 mg/kg/day for a subsequent 3 months (F). Boron was given alone (B) as preventive and simultaneously with fluoride prophylactically (F + Bp), as well as therapeutically namely midway during the experimental period (F + Bpc) while fluoride was being administered and after it was discontinued (Bc compared with fluoride interuption F*). Boron administered during fluoride intoxication or after its interruption, reduces fluoremia and increases urinary fluoride excretion. The high fluoride content in bone F decreased with addition of boron. It is still high in lot F* but returns to normal in locBc. Calcium content of bones remains normal in all lots. Posterior pad radiography shows a cortical thickness in lot F which is less pronounced in intoxicated prophylactic level group, and returns to normal in therapeutic group.

Zhai *et al*. (1985) conducted drug prevention and therapy trial in artificially produced cases of chronic fluorosis in sheep. Treated groups were given orally aluminium hydroxide, sodium selenite and borax for 390 days. The clinical signs were not so severe in treated groups as in untreated fluorotic group, but there was no significant difference in body wt., serum fluorine and bone fluorine.

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

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.

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.

Ouyang *et al*. (2000) raised Weanling rats on water containing 0.1 mol/L fluoride and food containing low, normal and high levels of calcium for 2 months. They revealed that with increasing food calcium ingestion during the development of teeth, the level of dental fluorosis is decreasing. The calcium supply was effective in protection from fluoride toxicity to a certain extent.

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

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 Na2SeO3 and ZnSO4 were more powerful than either Na2SeO3 or ZnSO4 alone.

Ekambaram *et al*. (2002) assessed the effect of calcium on the toxic effects of fluoride, adult female Wistar rats were treated with sodium fluoride (NaF, 500 ppm in drinking water) alone or in combination with calcium carbonate (CaCO3, 50 mg/ kg by oral intubation) daily for 60 days. NaF treatment decreased food and water intake, reduced body-weight gain and impaired exploratory motor activity and rota-rod performance. Dental lesions, inhibition of the activities of AchE and N+ K+ ATPase and a decrease in the concentration of protein, and serum calcium were also observed in these animals. These effects were accompanied by a marked elevation of fluoride concentration in the serum. CaCO3 decreased the concentration of fluoride in the serum of NaF-treated animals. A prevention of locomotor behavioural, biochemical and dental toxicities of fluoride was observed both in these

groups. It is concluded that the dose of CaCO3 used in the present study has a potential to prevent the 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 2 months.

Stanley *et al*. (2002) reported that administration of seed extract of *Moringa oleifera* (radish tree or drum stick) reduced the fluoride intoxicated changes and ameliorate fluoride toxicity in Wistar rats by providing NaF (25mg/kg. b.wt.) and seed extract (1 gm / kg. b.wt.).

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.

Bronckers *et al*. (2006) tested the hypothesis that high-calcium medium given prior to or immediately after exposure to fluoride (F) reduces the negative effects of F on secretory amelogenesis. Hamster molar tooth germs were grown in organ culture in media with different calcium levels. The result indicated that high calcium may protect against fluoride exposure by enhancing amelogenin secretion into the enamel space, thereby increasing the local buffering capacity at the mineralization front.

Bharti *et al*. (2007) assessed the ameliorative effects of boron on high fluoride intake in buffalo calves (*Bubalus bubalis*). Three groups of four 6-8 month-old male Murrah buffalo calves were fed a conventional concentrate mixture and roughage supplemented with sodium fluoride (NaF, 60 ppm elemental F), alone or in combination with borax (Na2B4O7•10H2O, 140 ppm elemental B) for three months.The findings suggested that supplementation of the feed with boron can counteract F-induced toxic effects on the haemogram and urinary biochemical profile of buffalo calves.

Ranjan (2007) made experimental study on evaluation of ameliorative potential of *Tamarindus indica* (Imali) in fluoride intoxicated rabbits, and found

useful in amelioration of fluorosis. Experiment was done in New Zealand white rabbits by treating with aqous 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 sulfate @ 20mg / kg. b.wt. orally for the same period and it was found that *T. indica* extract had more efficacy in ameliorating fluorosis.

Bharti *et al*. (2008) determinedthe beneficial effect of boron on nutrient utilization in buffalo calves exposed to high fluoride ration. They selected three groups of four male Murrah buffalo calves (body weight 98-100 kg, aged 6-8 month) each. Treatment I animals were fed basal diet, concentrate mixture, and F [as NaF, 60 ppm of dry matter (DM)]. The treatment II animals were fed basal diet, concentrate mixture, F (as NaF, 60 ppm of DM), and B (as sodium tetraborate, 140 ppm of DM). The findings of experiment suggested that fluoride-containing diet for short duration has effect on nutrient utilization, and boron at 140-ppm dose level, in general, antagonized the absorption and retention of F and also improved the feed intake in buffalo calves.

Gowda *et al*. (2012) reported boron, antioxidants and certain plant extracts like Tamarind pulp and *Moringa* seeds aspromising ameliorating agents.

Ranjan *et al*. (2009) indicated that the aqueous extracts of *T. indica* fruit pulp (100 mg/kg body weight) and *M. oleifera* seeds (50 mg/kg body weight) orally once daily for 90 days lowered plasma fluoride concentrations in rabbits receiving fluorinated drinking water (200 mg NaF/ Liter water). Changes in plasma biochemistry suggested less hepatic and renal damages in animals receiving plant extracts along with fluorinated water in comparison to that receiving fluorinated water alone. Preliminary results revealed these plant extracts have some potential to mitigate fluoride toxicity.

Amaravadi and Vasant (2011) studied the mitigative effects of *Mangifera indica* L. fruit powder as a dietary supplement in fluoride toxicity were investigated with reference to tissue lipid peroxidation and antioxidant

metabolism. *Mangifera indica* fruit possesses considerable antiperoxidative and antioxidant potential to mitigate the fluoride toxicity.

Gupta *et al*. (2013) studied the ameliorative effect of *Tamarindus indica* L. on biochemical parameters of serum and urine in cattle from fluoride endemic area Calcium, phosphorus, alkaline phosphatase and fluoride concentration in serum and collagen degradation marker i.e. hydroxyproline in urine were considered for evaluating the efficacy of tamarind fruit pulp. The present study found that dried powder of *Tamarindus indica* fruit pulp has ameliorative potential on management of fluorosis in cattle.

Jafari *et al*. (2014) investigated fluoride removal, using *Moringa oleifera seed* extract and the influence of *Moringa oleifera* dosage, initial F concentration, and pH were determined. With a constant initial F concentration, it was found that increasing the MO dose resulted in increased F removal. pH had no meaningful effect on F removal. Based on an optimized condition, for 85.4% removal of 8 mg/L of fluoride, a coagulant dosage of 900 mg/L is needed.

MATERIALS AND METHODS

The present study entitled "***Ameliorative efficacy of medicinal herbs in calves exposed to industrial fluorosis***" was carried out in the Department of Veterinary Clinical Medicine, Ethics and Jurisprudence, College of Veterinary Science and Animal Husbandry, Orissa University of Agriculture and Technology, Bhubaneswar during the period of December 2013 to July 2014. The investigation was carried out in the periphery villages of the Aluminium Smelter plant, National Aluminium Company (NALCO), Angul, Odisha, India.

3.1 Study sites

The present study sites were selected on the basis of previous report of endemic fluorosis in the villages around close proximity of NALCO smelter plant and NALCO captive power plant in Angul district of Odisha, India. The present study sites are located 133 km away from Bhubaneswar city at latitude 20.83°N and longitude 85.15°. Six villages namely Bonda, Tulasipal, Gardarkhai, Kulad, Jhajharibahal and Languliabeda present within 2 km radius of the NALCO (National Aluminium Company) smelter plant and 3km radius of the NALCO captive power plant were selected for the present study. Healthy animals reared in Bhubaneswar area were served as health control group. Laboratory estimation was done in the College of Veterinary Science and Animal Husbandry, O.U.A.T. Bhubaneswar.

3.2 Sampling

The biotic and abiotic samples were collected from the selected areas. The biotic samples include blood, urine and faecal materials were collected from the above selected area. The abiotic samples include feed, fodder, soil and water.

3.3 Screening of animals

The calves reared in the industrial fluorotic areas i.e. vicinity of National Aluminium Company (NALCO), Angul, were screened on the basis of their

clinical signs and higher plasma fluoride level. A total number of thirty (n=30) fluorotic animals having clinical signs of discoloration, mottling, pitting of teeth, difficulty in mastication and plasma fluoride level (more than 2 ppm) were selected and divided equally into 3 groups of 10 animals each for the present research work named as Group-I, Group-II, Group-III. Ten calves without having any clinical signs of osteo-dental fluorosis residing in a fluorotic free zones at Bhubaneswar were selected and taken as healthy group, which were named as Group-IV.

For epidemiological study, 5 villages viz. Kulad, Jharjharibahal, Langudiabeda, Tulasipal and Bonda were selected to examine the clinical signs of dental fluorosis and skeletal fluorosis in calves. House to house surveys were conducted in the early morning and late evening when the animals generally remain available. A total of 107 calves were examined for characteristics dental fluorosis lesions and skeletal abnormal signs.

3.4 Plant materials

Fruits of *Moringa oeilifera* were procured from the local market and were dried at 40^0C in hot air oven and powder using an electronic grinder. 25 grams of dried Drumstick powder was poured into one packet and a total of 600 packets were made. Group- II animals were fed with 1 packet daily for 60 days to each animal.

3.5 Chemical antidote

Calcium in combination with boron were used as standard chemical antidote against fluorosis affected animals. Laboratory reagent grade Calcium Carbonate ($CaCo_3$) was used as the source of calcium and boric acid (H_3BO_3) as the source of boron which were manufactured by Merck Private Ltd. Six hundred number of packets of calcium were made containing 20gm in each and the same number of packet was made containing 5gm of Boric acid in each. Daily oral feeding of calcium was given @ 100 mg/Kg b.wt. for 60 days using calcium carbonate which contains 40% of elemental calcium to group IV

animals. Boron was administered orally once daily @ 10 mg/Kg b.wt. taking boric acid as the source to Group-II animals for 60 days (Maiti *et al.* 2003).

3.6 Collection of biotic samples

Biotic samples i.e. blood, urine and faeces were collected from 30 fluorosis affected calves those were having visible fluorotic lesions before starting treatment i.e. on day 0, during the treatment i.e. on day 30 and at the end of the treatment i.e. on day 60. These samples were also collected from ten adult healthy cattle on day 0, day 30 and day 60 during experimental period.

3.6.1 Collection of blood

Blood samples were collected from jugular vein of both affected and healthy cattle by sterile disposable syringe and 18 gauge needles during early hours of morning on 0, 30th and 60^{th} days of post experiment. About 8 ml of blood were collected from each selected animal from which 5 ml were kept in heparinized vial for biochemical estimation and 3 ml in EDTA vial for hematological estimation. Then the tubes were marked properly and transported to laboratory in an ice box.

Plasma samples were separated from the heparinized blood samples after centrifugation at 3000 rpm for 3 minutes in a thermostable refrigerated centrifuge machine by micro centrifuge (Eppendropf) tube. Plasma samples were used for estimation of fluoride content as well as biochemical parameters.

3.6.2 Collection of urine

Ten ml of urine samples were collected from screened animals directly in polyethylene bottle early in the morning hour before the commencement of the experiment i.e. on day 0, during the treatment i.e. on day 30 and at the end of the treatment i.e. on day 60 and were transported to the laboratory in an ice box.

3.6.3 Collection of faeces

Faecal samples were collected from the screened animals directly from the rectum in each pre-labelled plastic specimen bottle during morning hour, before the commencement of the experiment i.e. on day 0, during the treatment i.e. on day 30 and at the end of the treatment i.e. on day 60.

3.7 Collection of abiotic samples

Abiotic samples like feed, fodder, soil and water samples from ponds, tube wells and deep bore wells which were collected in polythene bags and porcelain vials for fluoride estimation.

3.8 Methodology

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

3.8.1 Solutions required

For fluoride estimation, the required solution

1. 10% HCl
2. 15% Sodium acetate
3. TISAB II
4. TSAB III

3.8.2 Preparation of electrode

Filling solution was filled just below to the ring mark of electrode. The solution was filled 1 inch above the level of the samples or standards in the beaker before measuring their concentrations. The sensor present at the lower end of the electrode was washed and soaked with a filter paper very carefully after each immersion into the standards or samples.

3.8.3 Preparation of standard solutions

1. 10 ppm + TISAB (1:1)
2. 1 ppm + TISAB (1:1)
3. 0.1 ppm + TISAB (1:1)

3.8.3.1 Preparations of working standards

For plasma and urine samples: 10 ppm + TISAB (1:1) solution is prepared by adding 9 ml of distilled water to 1 ml of fluoride standard (100ppm). Then, mix 1 ml of TISAB II with same amount of 10 ppm solution for standard solution.

- 1 ppm + TSAB (1:1) solution is prepared by adding 9 ml of distilled water to 1 ml of previously prepared 10 ppm solution, then mix 1 ml of TISAB II with same amount of 1 ppm solution for standard solution.
- 0.1 ppm + TSAB (1:1) solution is prepared by adding 9 ml of distilled water to 1 ml of previously prepared 1 ppm solution, then mix 1 ml of TISAB II with same amount of .1 ppm solution for standard solution.

For fodder/soil/faecal sample: 10 ppm and 1 ppm standard solutions were prepared by adding 10% HCl in place of distilled water for dilution.

3.8.3.2 Preparation of samples

1. Serum/Plasma - 0.2 ml serum + 0.8 ml distilled water + 1 ml TISAB II
2. Soil/Fodder - 1 ml sample+ 9 ml 15% Sodium acetate.

3.8.4 Procedure for plasma fluoride estimation

- Electrode operation (slope) must be checked and the instrument should be calibrated using 0.1 and 1ppm fluoride standard solutions every time before estimation.
- For standards, Place 1 ml of the standard and 1 ml TISAB II into a polypropylene beaker and calibrate the instrument.
- For estimation of fluoride in plasma, take 0.2 ml of plasma and 0.8ml of distilled water and add 1 ml of TISAB II in polypropylene vial, mix thoroughly, and put the electrode in it and record the reading once it became ready.
- The final concentration of fluoride in the samples is expressed as g/ml (ppm) after multiplying with dilution factor.

3.8.5 Procedure for soil/fodder fluoride estimation

- Dry the soil or fodder samples in an oven and make to powder.
- To 1 g of powdered soil/fodder add 10 ml of 10% HCI.
- Keep it for 48 hr after proper mixing.
- Centrifuge at 3000 rpm for 15 min and collect the supernatant in volumetric flasks.
- Again add 10 ml 10% HCI in tubes and after thorough mixing centrifuge for 15 min.
- Store the supernatant in corresponding flasks. The procedure should be repeated5 times and make the final volume to 50 ml 10% HCl.
- For calibration, take 1 ml suitable standard (1 and 10 ppm prepared in 10% HCI) with 9 ml of 15% sodium acetate and place under electrode for reading.
- For each sample, take 1 ml sample with 9 ml 15% sodium acetate and note down the reading.
- Final fluoride concentration is expressed as µg/g of soil/fodder after multiplying with respective dilution factors.

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.

3.9 Procedure for haematological parameters

After collecting the heparinised blood samples from all fluoroticcalves, haemoglobin (grams/dl) was estimated by Sahli's method as stated by Benjamin (1978). Packed cell volume (PCV) was determined using Wintrobe's method as described by Coles (1986). Total erythrocyte count (TEC) and 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).

3.10 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), total protein, were estimated by semi-autoanalyser (MERCK, MICROLAB 300) using commercial reagent kits.

3.10.1 Determination of alkaline phosphatase activity (ALP)

For the determination of ALP activity, Alkaline Phosphatase Kit was used which was determined by pNPP Kinetic method as described by the manufactured in the Kit which was supplied by Crest Biosystem, a division of Coral Clinical Systems, Goa, India.

Principle: Alkaline phosphatase hydrolyzes colourless para-nitrophenyl phosphate (pNPP) producing phosphate and p-nitrophenol. The speed at which the p-nitrophenolate anion (yellow) appears, read at 405 nm, which is directly proportional to the enzymatic activity of the sample.

$$\text{p-Nitrophenylphosphate} \xrightarrow{\text{ALP}} \text{Inorganic phosphate} + \text{p-Nitrophenol}$$

Calculations

ALP Activity in U/l= Δ A/min x 2754

3.10.2Alanine aminotransferase (ALT)

The activity of serum Alanine aminotransferase (ALT) was estimated by LDH-NADH and Kinetic UV method, using the reagent kit supplied by LAB KIT, Chemelex, S.A., Canovelles, Barcelona.

Principle: ALT catalyzes 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

3.10.3Aspartate amino transferase (AST)

The activity of serum Aspartate aminotransferase **(**AST) was estimated by MDH-NADH, Kinetic UV method as supplied by LAB KIT, Chemelex, S.A., Canovelles, Barcelona.

Principle: AST catalyzes the transfer of amino group between L-aspartate andα-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 propotional to the SGOT (AST) activity in the sample at wave length of 340 nm.

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

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

Calculations

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

3.10.4Urea

The serum urea was estimated by Modified Berthelot method, using the reagent kit supplied by Crest Biosystem, a division of Coral Clinical Systems, Goa.

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.

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

Ammonia +Phenolic chromogen + Hypochlorite →Green coloured complex

Calculations

Urea in mg/dl = Abs. T / Abs. S × 40

3.10.5Creatinine

The serum creatinine was estimated by Alkaline Picrate method, using the reagent kit supplied by Crest Biosystem, a division of Coral Clinical Systems, Goa.

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 →Orange Coloured Complex

Calculations

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

3.10.6Total protein

The serum total protein was estimated by Biuret method, using the reagent kit supplied by Crest Biosystem, a division of Coral Clinical Systems, Goa.

Principle: Proteins, in an alkaline medium bind with the cupric ions present in the biuret reagent to form a blue violet coloured complex. The intensity of the colour formed is directly proportional to the amount of proteins present in the sample at wave length 550 nm.

Proteins + Cu^{++}→Blue violet coloured complex

Calculations

Total protein (g/dl) = Abs.T / Abs. S× 8

3.10.7 Albumin

The plasma albumin was estimated by Bromocresol Green (BCG) method, using the reagent kit supplied by Crest Biosystem, a division of Coral Clinical Systems, Goa.

3.10.8 Estimation of calcium

Serum calcium was estimated by ARSENAZO III calorimetric method using calcium reagent kit supplied by LAB KIT, Chemelex, S.A., Canovelles, Barcelona.

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

3.10.9Estimation of phosphorus

Serum Phosphorus was estimated by Phosphomolybdate, U.V. method using calcium Kit supplied by LAB KIT, Chemelex, S.A., Canovelles, Barcelona.

Principle: Inorganic phosphate reacts in acid medium with ammonium molybdate to form a phosphomolybdate complex with yellow colour. The intensity of the color 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

3.10.10 Estimation of Magnesium

The plasma sample was digested by using triple acid (nitric acid, sulphuric acid and perchloric acid, 4:2:1) mixture and heated below 80 degree C till digestion. The digested samples were diluted with de-ionized triple glass distilled water and the concentration of magnesium in plasma samples was estimated by Double Beam Atomic Absorption Spectrophotometer [ELICO, India, Model No. SL243].

3.11 Experimental design

The research work was carried out on 30 selected fluorosis affected calves out of 232 calves with dental fluorotic lesions. The 30 calves were divided into 03 groups named Group-I and Group-II and Group-III.

Group-I

Ten calves showing without any clinical manifestations of fluorosis were taken as health control group those were reared at non fluorotic zone

Group-II

Ten calves showing clinical manifestations of dental fluorosis were not given any treatment and considered as disease control group.

Group-III

Ten calves showing clinical manifestations of dental fluorosis were treated with herbal therapy as dried fruit powder of *Moringa oleifera* (Drumstick), orally daily for 60 days.

Group-IV

Ten calves showing clinical manifestations of dental fluorosis were treated with standard therapy as calcium @ 20g/animal and boron @ 5g/animal, orally daily for 60 days.

Biotic samples like blood, urine and faeces were collected from the above animals on day 0, day 30 and day 60 of post-experiment for laboratory examinations.

Group	Days of samples collected		
	0	30	60
Group I(Healthy group)	✓	✓	✓
Group II(Disease control group)	✓	✓	✓
Group III(*Moringa oleifera* died fruit powder treated group)	✓	✓	✓
Group IV(Calcium+ Boron treated group)	✓	✓	✓

3.12 Statistical analysis

The data obtained from each parameter were compiled and statistically analyzed to find out the mean, standard error, range and significant difference of mean values between within the group and between the groups at P≤ 0.05 as per standard method described by Snedecor and Cochran (1975).

RESULTS

The aim of the study was to determine the ameliorative efficacy of medicinal herbs in calves exposed to industrial fluorosis through estimation of fluoride level in plasma, excretions (urine and faeces) and different haematobiochemical studies at College of Veterinary Science and Animal Husbandry, O.U.A.T., Bhubaneswar. The calves included in this study were selected at periphery villages of NALCO aluminium smelter plant (within 2 km distance) in Angul district of Odisha.

4.1 Prevalence

Out of 107 calves examined for clinical signs, 90 (84.112%) calves have either dental or skeletal lesions of fluorosis. The prevalence of dental (84.1%) and skeletal fluorosis (9.34%) is given in Table no. 2. Prevalence of dental fluorosis was higher in 12-18 month (94.8%) and 6-12 (84.7%) age group of calves than 0-6 (63.6%) age group. Skeletal fluorosis was not recorded within 0-6 month age group. Only 8.69% of calves in 6 to 12 month group and 15.38% in 12 to 18 month age group showed skeletal fluorosis.

The mean fluoride concentration in soil were 68.186, 55.025, 47.310, 41.230, 40.180 ppm and in green fodder (99.000, 130.370,31.067, 48.400, 34.530 ppm) recorded in Gadarkhai, Languliabeda, Tulsipal, Jharjharibahal and Bonda village respectively. Similarly water resources of Gadarkhai was recorded with higher fluoride content of 1.892 ppm than Languliabeda (1.068ppm), Tulsipal (1.219ppm), Jharjharibahal (1.165ppm) and Bonda (0.303ppm).

4.2 Therapeutic study

Twenty calves out of one hundred and seven no. of calves were randomly selected for therapeutic regimen with the consent of owner for study. These animals were divided into two groups of ten animals. Dry fruit powder of *Moringa oleifera* (drum stick) was given to group (n=10) @ 25g/calf and other group was given with calcium and boron @ 20g and @ 5g per

calve, orally daily for 60 days. Another ten fluorotic calves of same fluorotic zone were selected as disease control group. The blood, urine and faeces were collected on day 0, day 30 and day 60 of study.

4.3 Clinical observations

The clinical manifestations like mild to severe dental mottling, bilateral striation horizontally light to deep yellowish colour on anterior teeth, dental staining of light brown to deep brownish or dark colour, irregular wearing of teeth and recession and swelling of gingiva were examined for screening of dental fluorosis in calves. Secondary manifestations like inappetance, difficult in mastication, curd dropping were also noticed during observation.

The clinical manifestations of skeletal fluorosis were expressed as intermittent lameness and snapping sound of legs, wasting of body muscles, and excessive periosteal exostoses of the mandibles, ribs, metacarpus, metatarsus regions, short, thicker and broader phalanges. Periosteal hyperostosis or exostosis at joint or places of attachment of ligaments and tendons cause shifting pain and lameness.

4.4 Fluoride concentration

The plasma fluoride concentrations in calves of different experimental group at different observation periods are given in Table no. 4 and Figure no.4. The mean plasma fluoride concentration of group I and group II revealed no significance ($p<0.05$) from day 0 to end of the experiment. But a non-significant increasing trend was noticed in group II from day 0 (0.523±0.009 ppm) today60 (0.542±0.012 ppm). Fluoride affected calves revealed significantly ($p<0.05$) higher level of plasma fluoride as compared to normal calves at different observation periods. Significant ($p<0.05$) reduction in plasma fluoride concentration was recorded in group III and group IV from day 30 onwards.

The plasma fluoride concentration was decreased significantly ($p<0.05$) after supplementation of *Moringa oleifera* (Gr III) on day 30 (0.277 ± 0.02 ppm

vs. 0.537 ± 0.009 ppm) and day 60 (0.221 ± 0.027 ppm vs.0.542 ± 0.012 ppm) with respect to calves reared in fluorotic zone (Gr II). Significantly ($p<0.05$) decrease in fluoride level was recorded in standard treatment group i.e. calcium along with boron (Gr IV) on day 30 (0.295 ± 0.02 ppm) and 60 (0.215 ± 0.019 ppm) in relation to fluorotic calves (Gr II).

Table no. 5 and Figure no.5 depicted the urine fluoride concentrations in fluorosis affected calves before treatment on day-0, during treatment on day-30 and at end of treatment on day 60 along with normal calves from non fluorotic zone. The fluoride affected calves (Gr II) had significantly ($p<0.05$) higher urinary fluoride level as compared to healthy calves (Gr I) on day 0 (16.400 ±0.683 ppm vs. 2.383± 0.230 ppm), 30 (17.050±0.598 ppm vs.2.358± 0.232 ppm) and 60 (17.833±1.749 ppm vs. 2.383 ± 0.236 ppm) of the experiment. Significant ($p<0.05$) reduction in urine fluoride concentration in group III and group IV was recorded on day 30 (12.211 ± 0.760 ppm and 12.633± 1.310 ppm) and day 60 (8.473± 0.487 ppm and 9.633± 1.162 ppm) as compared to day 0 values (17.263±0.985 ppm and 16.816 ± 1.231 ppm).

Significant ($p<0.05$) decrease in urinary fluoride level was noted after supplementation of *Moringa* and Ca+B on day 30 (12.211 ± 0.760 ppm and 12.633 ± 1.310 ppm) and day 60 (8.473 ± 0.487 ppm and 9.633 ± 1.162 ppm) as compared to gr II on day 30 (17.050 ± 0.598 ppm) and day 60 (17.833±1.749 ppm), respectively.

The fluoride concentrations in faeces of fluorosis affected calves are presented in Table no. 6 and Fig. no 6.Calves from fluorotic area (Gr II) excreted higher level of fluoride through faeces on day 0, (23.533 ± 0.554 ppm vs. 9.933 ± 0.689 ppm), 30 (24.416± 0.522 ppm vs. 9.783 ± 0.601 ppm) and 60 (25.011 ± .641 ppm vs. 9.811± 0.560 ppm) compared to calves from non-fluorotic area (Gr I).

Significant ($p<0.05$) increase in fluoride concentration was recorded at the end of the experiment in both *Moringa* and Ca+B treatment group (34.990 ± 0.575ppm and 31.84 ± 0.743 ppm) as compared to disease control group (25.011 ± 0.641 ppm).

4.5 Haematological changes

Blood was collected from jugular vein in sterilized blood collection vial with heparin and EDTA as anticoagulant from the fluorosis affected calves along with normal calves from non-fluorotic zoneon day 0 just before treatment, during treatment on day 30 and day 60 at end of treatment. Haematological parameters i.e. haemoglobin, TEC, TLC, DLC, PCV were evaluated by standard methods (Jain, 1986).

Table no. 7 and Figure no.7 showed the significant reduced haemoglobin content in calves of fluoroticarea (Gr II) as compared to healthy calves (Gr I) on day 0 (7.866 ±0.223 g% vs. 11±.318 g%), day 30 (7.833 ± 0.189 g% vs. 11.133 ± 0.251g%) and day 60 (7.583 ± 0.161g% vs. 11.100±0.276g%). There was non-significant ($p<0.5$) increase in Hb (g%) in both the treatment groups (Gr III & IV) from day 0 to day 30 but significant ($p<0.5$) increase on day 60 was recorded. Mean Hb content was 7.733 ± 0.111, 7.866 ± 0.084and 9.433 ± 0.080 (in g %) in group III and 7.7 ± 0.217, 7.966 ± 0.181 and 8.533 ± 0.66 (g%) in group IV on day 0, day 30 and day 60, respectively. There wassignificant ($p<0.5$) increase in Hb content in group III (9.433±0.08g%) and group IV (8.533 ±0.66g%) as compared to Group II (7.583 ±0.161g%) on day 60 of the observation.

Packed cell volume (%) increased (Table no. 8 and Figure no. 8) significantly ($p<0.05$) at end of treatment i.e. day 60 of observation in *Moringa* treated (Gr III) calves as compared to day 0 (29.5 ± 0.921% vs. 23.333 ± 0.557 %). Normal calves showed (Gr I) higher PCV % than other groups at day 0 (33.166 ± 0.833 %), day 30 (33.500 ± 0.763 %) and day 60 (33.166 ± 0.792 %) of observation.

Table no. 9 and Figure no. 9 recorded lower Total leucocyte count (Cumm) in calves grown in fluorotic zone in comparison to non fluorotic zone on day 0 (5575 ± 224.629 vs. 8933.333 ± 294.863), 30 (5375 ± 106.262 vs. 8475 ± 243.84) and 60 (5933±392.994 vs. 8483.333±209.629). There was significant ($p<0.05$) increase in TLC count in *Morinaga* treated (Gr III) calves

(7550 ± 232.02) and Calcium + Boron treated (Gr IV) calves (6850 ± 172.723) on day 60 in comparison to calves of fluorotic zone (5933±392.994).

Lymphocyte count (%) in fluorosis affected calves (Table no. 10 and Fig. no. 10) showedhigher % in calves of fluorotic zone in comparison to normal calves on day 0 (66.5 ± 1.118% vs. 57.833 ± 0.401%), 30 (66.33 ± 1.145% vs. 58.166 ± 0.749%) and day 60 (66.166 ± 1.376% vs. 58.166 ± 0.401%). Supplementation of *Moringa* (Gr III) reduces lymphocyte % significantly ($p<0.05$) on day 30 compared to day 0 (63.670± 0.76% vs. 67. 670± 1.837%). Similar results were also observed in Ca + B treated group (Gr IV) on day 30 (64.17 ± 0.909% vs. 68.17 ± 1.351%). Significant ($p<0.05$) decrease in lymphocyte count was observed in both treatment groups compared to calves of affected areas at the end of the experiment.

Neutrophil count (%) in fluorosis affected calveswassignificantly ($p<0.5$) lower from normal calves on day 0 (22.83 ± 1.013% vs. 38.833 ± 0.166%), 30 (23.833 ± 0.833% vs. 37.5 ± 0 .718%) and 60 (22 ± 1.549% vs. 37.166 ± 0.477%). Supplementation of *Moringa* in calves (Gr III) increased neutrophil count at end of treatment compared to calves of affected areas (31.34 ± 0.802 vs. 22 ± 1.549%). Significant lower value in *Moringa* treated group was recorded than calcium and boron treated group (31.34 ± 0.802 vs. 31.17 ± 0.477%) but not comparable to normal animal (37.166 ± 0.477%) at end of treatment (Table no. 11 and Fig. no. 11).

Eosinophil percentage in fluorotic calvesdepicted a higher % as compared to normal calves on day 0 (9.833 ± 0.703%vs. 2.161 ± 0.307%), 30 (8.833 ± 0.477%vs. 3 ± 0.258%) and 60 (9.666% ± 0.421 vs. 2.167 ± 0.360%). *Moringa* supplementation reduced eosinophil count at end of treatment i.e. on day 60 as compared to day 30 values in both *Moringa* and Ca + B treated calves (4.833 ± 0.542% vs. 7.833 ± 0.401%) and (4.500 ± 0.342% vs. 7.833 ± 0.307%). Both treatment group recorded significant ($p < 0.05$) reduction in eosinophil count (4.833 ± 0.542 % and 4.500 ± 0.342 %) from calves of fluorotic areas (9.666 ± 0.421%) at end of treatment (Table no. 12 and Fig. no. 12).

4.6 Biochemical findings

Plasma was collected from heparinised blood samples from the fluorosis affected calves along with normal calves from non fluorotic zone on day 0 just before treatment, during treatment on day 30 and day 60 at end of treatment for estimation of biochemical parameters. Enzyme activities were recorded within 24 hours of blood collections.

Calcium concentration in fluorosis affected calves (Table no. 13 and Figure no. 13) was significantly ($p< 0.05$) lower in calves reared in fluorotic zone as compared to normal animals on day 0 (6.855 ± 0.147 mg/dLvs.10.856 ± 0.310 mg/dL), day 30 (6.766 ± 0.103mg/dL vs. 10.722 ± 0.182 mg/dL) and day 60 (6.745 ± 0.134mg/dL vs. 10.761 ± 0.189mg/dL) of experiment. *Moringa* supplementation (Gr III) seems to be increasing the plasma calcium concentration in calves on day 30 of experiment with respect to day 0 (7.84 ± 0.058mg/dL vs. 7.037 ± 0.064mg/dL). Ca+B treatment group (Gr IV) showed significant increase in calcium level on day 30 and 60 (8.039 ± 0.092 mg/dL and 8.497 ± 0.059mg/dL, respectively) with respect to day 0 value (7.031 ± 0.058mg/dL). *Moringa* and calcium + boron supplementation increased the calcium concentration on day 60 compared to claves of fluorotic zone (8.197 ± 0.112mg/dL and 8.497 ± 0.059mg/dL respectively vs.6.745 ± 0.134mg/dL).

Table no. 14 and Figure no. 14 indicated the significant ($p< 0.05$) increased phosphorus concentration in calves of fluoric zone on day 0 (7.053 ± 0.084 mg/dLvs.4.847 ± 0.198mg/dL), day 30 (7.015 ± 0.033mg/dLvs.4.834 ± 0.182mg/dL) and day 60 (7.042 ± 0.09mg/dLvs.4.763 ± 0.16mg/dL) of experiment. There was significant ($p<0.05$) decrease in phosphorus concentration in *Moringa* treated group (Gr III) on 30 day of experiment as compared to day 0 value (6.265 ± 0.18mg/dL vs 7.133 ± 0.092mg/dL). Similarly phosphorus concentration reduced in calcium treated groups from day 30 to day 60 (6.177 ± 0.141mg/dL and 5.783 ± 0.066mg/dL).

Total protein concentration was significantly ($p< 0.05$) lower in fluorosis affected calves as compared to normal calves on day 0 (5.456 ± 0.082g/dL

vs. 8.328 ± 0.046 g/dL), 30 (5.465 ± 0.083g/dL vs.8.343 ± 0.068 g/dL) and day 60 (5.486 ± 0.078g/dL vs. 8.389 ± 0.123 g/dL) of the experiment. There was significant ($p<0.05$) increase in total protein concentrations at end of experiment on supplementation of *Moringa* (6.723 ± 0.086g/dL) as well as Ca + B (6.81 ± 0.047g/dL) in calves compared to day 0 values (5.421 ± 0.139g/dL and 5.498 ± 0.105g/dL). The total protein concentration was lower at end of experiment in *Moringa* (6.723 ± 0.086g/dL) and Ca + B (6.81 ± 0.047g/dL) compared to healthy calves group (8.389 ± 0.123g/dL) but higher than affected calves of fluorotic zone (5.486 ± 0.078g/dL) (Table no.16 and Figure no. 16).

Albumin concentration was (Table no.17 and Figure no.17) significantly ($p< 0.05$) lower in fluorosis affected calves than normal calves on day 0 (2.367 ± 0.141 g/dL vs. 3.51 ± 0.12g/dL), 30 (2.239 ± 0.046g/dL vs. 3.452 ± 0.078g/dL) and day 60 (2.263 ± 0.04g/dL vs. 3.417 ± 0.06 g/dL) of experiment. There was significant ($p< 0.05$) increase in albumin concentration on day 60 compared to day 30 in *Moringa* (2.833 ± 0.04g/dL vs. 2.374 ± 0.048g/dL) as well as Ca + B (2.523 ± 0.039g/dL vs. 2.304 ± 0.045g/dL) group of calves. There was higher mean albumin concentration on day 60 of *Moringa* treatment group compared to Ca + B group (2.833 ± 0.04g/dL vs. 2.523 ± 0.039g/dL).

Urea concentration in plasma was (Table no. 18) significantly ($p<0.05$) higher in calves of affected areas than normal calves on day 0 (36.441 ± 0.951 vs. 27.050 ± 0.750), 30 (36.195 ± 0.894 vs. 27.153 ± 0.698) and 60 (36.354 ± 0.941 vs. 27.261 ± 0.748) of experiments. Treatment groups showed no significant differences in urea concentration in relation to fluorotic calves.

Creatinine concentration in plasma of fluorosis affected calves (Gr II) showed (Table no. 19 and Figure no. 18) significant ($p<0.05$) increased concentration than normal healthy calves on day 0 (3.784 ± 0.137 vs 1.181 ± 0.093mg/dL), on day 30 (3.76± 0.113 vs 1.166 ± 0.056 mg/dL) and day 60 (3.748 ± 0.089 vs. 1.177 ± 0.049 mg/dL) of study. Plasma creatinine concentration decreased significantly ($p< 0.05$) after 60 day supplementation of *Moringa* in group III (2.89 ± 0.03) and Ca + B treated group (3.308 ± 0.084mg/dL) when compared to group II (1.177 ± 0.049) animals. There was significantly ($p<0.05$) higher concentration of plasma creatinine concentration

recorded in Ca + B treated group with respect to *Moringa* in group III (3.308 ± 0.084 vs.2.890± 0.030 mg/dL).

Significant (p< 0.05) higher alkaline phosphatase activity (Table no. 20 and Figure no. 19) in plasma was recorded in calves of fluoride affected areas than normal calves on day 0 (298.153 ± 9.465 vs.113.882 ± 5.574), 30 (288.703 ± 6.689 vs.116.117 ± 4.9) and day 60 (289.418 ± 7.187 vs.114.862 ± 3.886) of experiment. The reduced activity of alkaline phosphatase was recorded after 30 day of supplementation of *Moringa* (208.53 ± 9.103) and Ca + B treated group (209.146 ± 5.499) compared to calves of fluoride affected calves (288.703 ± 6.689). Similar result was also recorded on day 60 in Gr III (176.141 ± 3.153), Gr IV (154.13 ± 3.98) compared to Gr II (289.418 ± 7.187). The activity of ALP was found to be significantly (p < 0.05) higher in Gr II on day 60 than Gr III (176.141 ± 3.153) and Gr IV (154.13 ± 3.98).

The Aspartate transaminase (U/L) in fluorosis affected calves (Table no. 22 and Figure no. 20) have significantly (p<0.5) higher activity than normal calves on day 0 (87.271 ± 0.621 U/Lvs. 87.271 ± 0.621 U/L), 30 (87.498 ±0.619U/L vs. 52.074 ± 1.401 U/L) and day 60 (87.584 ± 0.590 U/Lvs. 52.349 ± 1.432 U/L) of experiment. There was significant (p<0.05) reduction in AST activity in both *Moringa* as well as Calcium with Boron supplementation on day 30 (75.387 ± 0.378 U/L and 79.216 ± 0.405U/L) and day 60 (66.251 ± 0.238U/L and 70.300 ± 0.458U/L) as compared to day 0 (86.956 ± 0.690U/L and 86.375 ± 0.552U/L). There was significant lower AST activity on day 30 of *Moringa* supplemented group compared to Ca + B supplementedgroup (75.387 ± 0.378U/Lvs. 79.216 ± 0.405U/L) as well as on day 60 (66.251 ± 0.238U/Lvs. 70.300 ± 0.458U/L). AST activity was significantly lower in calves of *Moringa* treated group and Ca + B treated group at end of treatment as compared to calves of fluorotic areas (66.251 ± 0.238 U/L, 70.300 ± 0.458U/L,87.584 ± 0.590U/L, respectively).

Alanine transaminase activity in plasma showed (Table no. 21) significantly higher activity in calves of fluoride affected areas than normal calves on day 0 (20.721 ± 1.116 vs.16.983 ± 0.772), day 30(20.971 ± 0.912 vs.16.873 ± 0.877) and day 60 (21.265 ± 0.991 vs.16.966 ± 0.779). Non-significant increase in the activity of ALT was observed after supplementation of *Moringa* as well as standard treatment at different observation periods.

Table 1: Prevalence of fluorosis among calves reared in industrial fluorotic zone (NALCO, Angul)

No of calves screened	Calves with dental or skeletal lesions	Calves with no dental or skeletal lesions
107	84.112% (90/107)	15.88% (17/107)

Table 2: Prevalence of dental vs. skeletal fluorosis around NALCO smelter plant in different age group of calves

Age group (months)	Prevalence of dental fluorosis	Prevalence of skeletal fluorosis
0-6	63.6% (14/22)	0
6-12	84.7% (39/46)	8.69 % (4/46)
12-18	94.8% (37/39)	15.38 % (6/39)

Table 3: Mean fluoride concentration of soil, water, fodder in different villages

Villages	Soil (mean F conc.) in ppm	Water (mean F conc.) in ppm	Green fodder (mean F conc.) in ppm
Gadarkhai	68.186	1.892	99.000
Languliabeda	55.025	1.068	130.370
Tulsipal	47.310	1.219	31.067
Jharjharibahal	41.230	1.165	48.400
Bonda	40.180	0.303	34.530

Table 4: The Plasma fluoride concentration in fluorosis affected calves along with normal calves

Group	Day 0	Day 30	Day 60
I	0.084 ± 0.004^{A}	0.086 ± 0.007^{A}	0.087 ± 0.011^{A}
II	0.523 ± 0.009^{B}	0.537 ± 0.009^{C}	0.542 ± 0.012^{C}
III	0.531 ± 0.025^{cB}	0.277 ± 0.027^{bB}	0.221 ± 0.027^{aB}
IV	0.549 ± 0.029^{cB}	0.295 ± 0.02^{bB}	0.215 ± 0.019^{aB}

Table 5: The concentration of fluoride in urine (ppm) in fluorosis affected animals along with normal animal

Group (n=10)	Day 0	Day 30	Day 60
I	2.383 ± 0.230^{A}	2.358 ± 0.232^{A}	2.383 ± 0.236^{A}
II	16.400 ± 0.683^{B}	17.050 ± 0.598^{C}	17.833 ± 1.749^{C}
III	17.263 ± 0.985^{cB}	12.211 ± 0.760^{bB}	8.473 ± 0.487^{aB}
IV	16.816 ± 1.231^{cB}	12.633 ± 1.310^{bB}	9.633 ± 1.162^{aB}

Table 6: The concentration of fluoride in faeces (ppm) in fluorosis affected animals along with normal animal

Group (n=10)	Day 0	Day 30	Day 60
I	9.933 ± 0.689^{A}	9.783 ± 0.601^{A}	9.811 ± 0.560^{A}
II	23.533 ± 0.554^{B}	24.416 ± 0.522^{B}	25.011 ± 0.641^{B}
III	23.505 ± 0.605^{aB}	32.118 ± 0.564^{bD}	34.990 ± 0.575^{bC}
IV	23.630 ± 0.606^{aB}	29.740 ± 0.681^{bC}	31.840 ± 0.743^{bC}

Group I: Healthy control; Group II: Disease control; Group III: *Moringa*t reatment; Group IV: Ca + B treatment. Values (mean ± S.E.) having no common superscript (small letter in a row and capital letter in a column) differ significantly at $p<0.05$.

Table 7: Haemoglobin concentration in (g%) in fluorosis affected animals along with normal animal

Group (n=10)	Day 0	Day 30	Day 60
I	11.000 ± 0.318^{B}	11.133 ± 0.251^{B}	11.100 ± 0.276^{C}
II	7.866 ± 0.223^{A}	7.833 ± 0.189^{A}	7.583 ± 0.161^{A}
III	7.733 ± 0.111^{aA}	7.866 ± 0.084^{aA}	9.433 ± 0.080^{bB}
IV	7.700 ± 0.217^{aA}	7.966 ± 0.181^{aA}	8.533 ± 0.660^{bB}

Table 8: Packed cell volume in (%) in fluorosis affected animals along with normal animal

Group (n=10)	Day 0	Day 30	Day 60
I	33.166 ± 0.833^{B}	33.500 ± 0.763^{B}	33.166 ± 0.792^{C}
II	23.500 ± 0.763^{A}	24.500 ± 0.763^{A}	$24.\ 333 \pm 0.802^{A}$
III	23.333 ± 0.557^{A}	24.500 ± 0.428^{A}	29.500 ± 0.921^{bB}
IV	23.166 ± 1.42^{A}	25.666 ± 1.977^{A}	25.666 ± 0.421^{A}

Table 9: Total leucocyte count (per Cu mm of blood) in fluorosis affected calves along with normal calves

Group (n=10)	Day 0	Day 30	Day 60
I	8933.333 ± 294.863^{B}	8475.00 ± 243.84^{B}	8483.333 ± 209.629^{C}
II	5575.000 ± 224.629^{A}	5375.000 ± 106.262^{A}	5933.000 ± 392.994^{A}
III	$5533.333 \pm 463.621^{aA}$	$5700.000 \pm 157.056^{aA}$	7550.000 ± 232.02^{bB}
IV	$5483.333 \pm 149.257^{aA}$	$5783.333 \pm 177.795^{aA}$	$6850.000 \pm 172.723^{bB}$

Group I: Healthy control; Group II: Disease control; Group III: *Moringa* treatment; Group IV: Ca + B treatment. Values (mean ± S.E.) having no common superscript (small letter in a row and capital letter in a column) differ significantly at $p<0.05$.

Table10: Lymphocyte count (%) in fluorosis affected calves along with normal calves

Group(n=10)	Day 0	Day 30	Day 60
I	57.833 ± 0.401A	58.166 ± 0.749 A	58.166 ± 0.401A
II	66.500± 1.118 B	66.330± 1.145 B	66.166 ± 1.376 C
III	67. 670± 1.837 bB	63.670± 0.76 aB	62.340± 0.557 aB
IV	68.170± 1.351 bB	64.170± 0.909 aB	61.840± 0.401 aB

Table 11: Neutrophil count (%) in fluorosis affected calves along with normal calves

Group(n=10)	Day 0	Day 30	Day 60
I	38.833 ± 0.166 ^{B}	37.500 ± 0 .718 ^{C}	37.166 ± 0.477^{C}
II	22.830 ± 1.013 ^{A}	23.833 ± 0.833^{A}	22.000 ± 1.549^{A}
III	23.666 ± 0.421^{aA}	26.833 ± 0.792^{bB}	31.340 ± 0.802^{cB}
IV	23.000 ± 0.447^{aA}	26.833 ± 1.137^{bB}	31.170 ± 0.477^{cB}

Table 12: Eosinophils count (%) in fluorosis affected calves along with normal calves

Group(n=10)	Day 0	Day 30	Day 60
I	2.161 ± 0.307^{A}	3.000 ± 0.258^{A}	2.167 ± 0.360^{A}
II	9.833 ± 0.703^{B}	8.833 ± 0.477^{B}	9.666 ± 0.421^{C}
III	9.333 ± 0.614^{bB}	7.833 ± 0.401^{bB}	4.833 ± 0.542^{aB}
IV	8.5 ± 1.087^{bB}	7.833 ± 0.307^{bB}	4.500 ± 0.342^{aB}

Group I: Healthy control; Group II: Disease control; Group III: *Moringa* treatment; Group IV: Ca + B treatment. Values (mean ± S.E.) having no common superscript (small letter in a row and capital letter in a column) differ significantly at $p<0.05$.

Table 13: The Calcium concentration (mg/dL) in fluorosis affected calves along with normal calves

Group(n=10)	Day 0	Day 30	Day 60
I	10.856 ± 0.310^{B}	10.722 ± 0.182^{C}	10.761 ± 0.189^{C}
II	6.855 ± 0.147^{A}	6.766 ± 0.103^{A}	6.745 ± 0.134^{A}
III	7.037 ± 0.064^{aA}	7.84 ± 0.058^{bB}	8.197 ± 0.112^{bB}
IV	7.031 ± 0.058^{aA}	8.039 ± 0.092^{bB}	8.497 ± 0.059^{cB}

Table 14: The Phosphorus concentration (mg/dL) in fluorosis affected calves along with normal calves

Group(n=10)	Day 0	Day 30	Day 60
I	4.847 ± 0.198^{A}	4.834 ± 0.182^{A}	4.763 ± 0.160^{A}
II	7.053 ± 0.084^{B}	7.015 ± 0.033^{C}	7.042 ± 0.090^{C}
III	7.133 ± 0.092^{bB}	6.265 ± 0.180^{aB}	5.985 ± 0.091^{aB}
IV	7.126 ± 0.074^{cB}	6.177 ± 0.141^{bB}	5.783 ± 0.066^{aB}

Table 15: The Magnesium concentration (ppm) in fluorosis affected calves along with normal calves

Group(n=10)	Day 0	Day 30	Day 60
I	23.70 ± 0.59^{B}	22.84 ± 0.23^{C}	22.92 ± 0.20^{C}
II	19.33 ± 0.44^{A}	19.33 ± 0.59^{A}	18.68 ± 0.59^{A}
III	19.73 ± 0.33^{aA}	20.79 ± 0.4^{aB}	22.40 ± 0.28^{bC}
IV	19.77 ± 0.35^{A}	20.23 ± 0.43^{AB}	20.89 ± 0.34^{B}

Group I: Healthy control; Group II: Disease control; Group III: *Moringa* treatment; Group IV: Ca + B treatment. Values (mean ± S.E.) having no common superscript (small letter in a row and capital letter in a column) differ significantly at $p<0.05$.

Table 16: The Total Protein (g/dL) in fluorosis affected calves along with normal calves

Group(n=10)	Day 0	Day 30	Day 60
I	8.328 ± 0.046^{B}	8.343 ± 0.068^{B}	8.389 ± 0.123^{C}
II	5.456 ± 0.082^{A}	5.465 ± 0.083^{A}	5.486 ± 0.078^{A}
III	5.421 ± 0.139^{aA}	5.695 ± 0.158^{aA}	6.723 ± 0.086^{bB}
IV	5.498 ± 0.105^{aA}	5.741 ± 0.095^{aA}	6.810 ± 0.047^{bB}

Table 17: The Albumin (g/dL) in fluorosis affected calves along with normal calves

Group(n=10)	Day 0	Day 30	Day 60
I	3.51 ± 0.12^{B}	3.452 ± 0.078^{B}	3.417 ± 0.060^{D}
II	2.367 ± 0.141^{A}	2.239 ± 0.046^{A}	2.263 ± 0.040^{A}
III	2.214 ± 0.046^{aA}	2.374 ± 0.048^{aA}	2.833 ± 0.040^{bC}
IV	2.158 ± 0.032^{aA}	2.304 ± 0.045^{aA}	2.523 ± 0.039^{bB}

Table 18: Urea (mg/dL) in fluorosis affected calves along with normal calves

Group(n=10)	Day 0	Day 30	Day 60
I	27.050 ± 0.750^{A}	27.153 ± 0.698^{A}	27.261 ± 0.748^{A}
II	36.441 ± 0.951^{B}	36.195 ± 0.894^{B}	36.354 ± 0.941^{B}
III	36.290 ± 0.686^{B}	35.824 ± 0.556^{B}	35.119 ± 0.523^{B}
IV	36.465 ± 0.642^{B}	36.049 ± 0.563^{B}	34.941 ± 0.721^{B}

Group I: Healthy control; Group II: Disease control; Group III: *Moringa* treatment; Group IV: Ca + B treatment. Values (mean ± S.E.) having no common superscript (small letter in a row and capital letter in a column) differ significantly at $p<0.05$.

Table 19: The Creatinine (mg/dL) in fluorosis affected calves along with normal calves

Group(n=10)	Day 0	Day 30	Day 60
I	1.181 ± 0.093^{A}	1.166 ± 0.056^{A}	1.177 ± 0.049^{A}
II	3.784 ± 0.137^{B}	3.76 ± 0.113^{C}	3.748 ± 0.089^{D}
III	3.766 ± 0.077^{bB}	3.524 ± 0.037^{bBC}	2.890 ± 0.030^{aB}
IV	3.726 ± 0.077^{bB}	3.534 ± 0.076^{abB}	3.308 ± 0.084^{aC}

Table 20: Theactivity of Alkaline phosphatase (U/L) in fluorosis affected calves along with normal calves

Group(n=10)	Day 0	Day 30	Day 60
I	113.882 ± 5.574^{A}	116.117 ± 4.9^{A}	114.862 ± 3.886^{A}
II	298.153 ± 9.465^{B}	288.703 ± 6.689^{C}	289.418 ± 7.187^{D}
III	303.257 ± 12.921^{cB}	208.53 ± 9.103^{bB}	176.141 ± 3.153^{aC}
IV	314.197 ± 9.646^{cB}	209.146 ± 5.499^{bB}	154.13 ± 3.98^{aB}

Table 21: The Alanine transaminase in (U/L) in fluorosis affected calves along with normal calves

Group(n=10)	Day 0	Day 30	Day 60
I	16.983 ± 0.772^{A}	16.873 ± 0.877^{A}	16.966 ± 0.779^{A}
II	20.721 ± 1.116^{B}	20.971 ± 0.912^{B}	21.265 ± 0.991^{B}
III	21.461 ± 1.010^{B}	19.593 ± 0.880^{AB}	19.560 ± 0.691^{AB}
IV	21.126 ± 0.934^{B}	19.736 ± 0.859^{AB}	18.685 ± 0.893^{AB}

Group I: Healthy control; Group II: Disease control; Group III: *Moringa* treatment; Group IV: Ca + B treatment. Values (mean ± S.E.) having no common superscript (small letter in a row and capital letter in a column) differ significantly at $p<0.05$.

Table 22: The Aspartate transaminase (U/L) in fluorosis affected calves along with normal calves

Groups(n=10)	Day 0	Day 30	Day 60
I	51.790 ± 1.415^{A}	52.074 ± 1.401^{A}	52.349 ± 1.432^{A}
II	87.271 ± 0.621^{B}	87.498 ± 0.619^{D}	87.584 ± 0.590^{D}
III	86.956 ± 0.690^{cB}	75.387 ± 0.378^{bB}	66.251 ± 0.238^{aB}
IV	86.375 ± 0.552^{cB}	79.216 ± 0.405^{bC}	70.300 ± 0.458^{aC}

Group I: Healthy control; Group II: Disease control; Group III: *Moringa* treatment; Group IV: Ca + B treatment. Values (mean ± S.E.) having no common superscript (small letter in a row and capital letter in a column) differ significantly at $p<0.05$.

Table 23: Proximate analysis of dried *Moringa* fruit powder

Sl. No.	Constituents	Percentage composition (%)
1	Moisture	15.49
2	Crude Protein	13.27
3	Ether extract	1.45
4	Crude Fiber	36.64
5	Total ash	7.12
6	Sand and Silica	0.04

DISCUSSION

Fluorosis has become a worldwide health problem in both animals and human beings causing severe economic losses and silent sufferings in endemic zones. In India, fluorosis is now slowly enveloping millions of Indians and livestock in both rural and urban parts of country. Due to rapid industrialization, health problems are increasing among the livestock and humans due to fluoride toxicity. As water is the major source of fluoride poisoning, animals are in greater risk of toxicity than humans because of non availability of defluorinated water for animal consumption. Water is not the only source rather air, fodder, soil are also contribute heavily towards occurrence of fluorosis (Patra *et al.,* 1999 and Gupta *et al.,* 2013). Emission of fluoride dust and fumes from the smelters of aluminium producing factories are the major source for fluoride pollution. Use of cryolite (trisodium hexafluoroaluminate), as a flux in conversion of alumina to aluminium, is responsible for industrial fluorosis (Susheela *et al.,* 2013).

This present study recorded the symptoms of fluorosis like rough body coat, stunted growth, emaciation, lameness and wasting of hip and shoulder. The dental lesions included yellowish discolouration of incisors, brown pigmentation, chalk-like dull white discoloration of incisors, wearing, mottling and pitting of enamel, abrasion of enamel and defective dental development. The skeletal lesions in the fluorotic calves (low prevalence) were found as osteophytosis of ribs, mandible, metacarpal, metatarsal and pelvic vertebrae and deformed over growth of hooves. These clinical findings were in corroboration with the findings of Sahu (1995), Singh (1995), Choubisa *et al.* (1996), Dwivedi *et al.* (1997), Mohapatra (1997), Sharma *et al.* (1997), Muralidhara *et al.* (2000), Patra *et al.* (2000), Maiti *et al.* (2004) Kanungo (2005) and Ranjan *et al.* (2009). Enamel mineralization is highly sensitive to free fluoride ions, which uniquely promote the hydrolysis of acidic precursors such as octacalcium phosphate and precipitation of fluoridated apatite crystals. Once fluoride is incorporated into enamel crystals, the ion likely affects the subsequent mineralization process by reducing the solubility of the mineral and thereby modulating the ionic composition in the fluid surrounding

the mineral. In the light of evidence obtained in human and animal studies, it is now most likely that enamel hypomineralization in fluorotic teeth is due predominantly to the aberrant effects of excess fluoride on the rates at which matrix proteins break down and/or the rates at which the by-products from this degradation are withdrawn from the maturing enamel. Any interference with enamel matrix removal could yield retarding effects on the accompanying crystal growth through the maturation stages, resulting in different magnitudes of enamel porosity at the time of tooth eruption. Secondly, the fluoride uptake interferes, indirectly, with the protease activities by decreasing free Ca (2+) concentration in the mineralizing milieu. The Ca (2+)-mediated regulation of protease activities is consistent with the in situ observations that (a) enzymatic cleavages of the amelogenins take place only at slow rates through the secretory phase with the limited calcium transport and that, (b) under normal amelogenesis, the amelogenin degradation appears to be accelerated during the transitional and early maturation stages with the increased calcium transport (Aoba and Fejerskov, 2002). Skeletal form of fluorosis can be attributed to reduction in cortical bone density, de-bonding of mineral-collagen interface, damage to collagen, hypo-mineralization (increase in unmineralized osteoid), hyper-mineralization (brittle bone), non-uniformity of mineralization, osteocyte damage (Gordon and Corbin, 1992; Fratzl, 1996; Chachra,1999; Kindt, 2009 and Turner, 2001).

In prevalence study, there was overall higher prevalence of dental fluorosis than skeletal fluorosis in all age group of calves as deposition of fluoride in teeth occurs during or before eruption (Radostits *et al.*, 2000). But the bone lesions are revealed after gradual deposition of fluoro-appatite crystals in bones for a longer period causing deformity. Calves exhibited the greatest prevalence of fluorosis and this may be because calves are more sensitive and susceptible and less tolerant to fluoride and also ingest more fluoridated milk and water, so having a greater chance of exposure to fluoride (Chaubasia, 1999; Patra *et al.,* 2000; Radostits *et al.,* 2000; Maiti *et al.,* 2004 and Chaubasia, 2008).

Fluoride level in soil decreased as the distance from smelter plant increases. This may be due to centrifugal spread of fluoride in effluents around the plant. Similar trend in concentration of fluoride in soil according to distance has been reported by Muralidhara *et al.* (2000). Reduced fluoride level in water and fodder in relation to distance from smelter was observed in this study. Lowering of fluoride in green fodder with increasing distance from aluminium factory may be due to decrease in fluoride content of the soil.

In the present investigation, it was observed that there were elevated level of plasma fluoride, urine fluoride and faecal fluoride in fluorotic calves than calves from non-fluorotic area. Calves are in greater risk for affection of dental fluorosis and this may be because calves are more sensitive, susceptible and less tolerant to fluoride. The higher plasma fluoride level in affected calves might be due to intake of more fluoridated milk, water and fodder (Chaubasia, 1999 and Patra *et al.*, 2000). Fluoride appears readily in urine after absorption and generally the urine fluoride reflects the absorbed fluoride on same day (Gopal and Ghosh, 1985). However, the findings indicate that determination of fluoride levels in urine is a valuable tool for diagnosing early stages of fluoride intoxication, thus enabling the introduction of suitable preventive and curative measures to minimize suffering and losses in animals (Bharti *et al.,* 2007). Fluoride concentrations in faeces reflect current fluoride intake and correlate with length of exposure and level of fluoride in the diet.

Supplementation of *Moringa oleifera* fruit powder @ 25 g/calve was able to reduce the plasma fluoride level in affected calves. Interference with fluoride absorption from the gut might have played a role in reducing plasma fluoride concentrations. The lower molecular weight water soluble proteins in *Moringa* seeds have strong positive charge that attracts highly electronegative fluoride ions resulting in formation of flocculants (Mangale *et al.,* 2012). Also the presence of tannins, fibers and high concentration of minerals in *Moringa* like calcium, aluminium, phosphorus, manganese, potassium, cupper and iron (Anjorin *et al.,* 2010 and Kawo *et al.,* 2009) which are reported to form insoluble complexes with fluoride in gut. This justified the enhanced fluoride

elimination in faeces and reduction of absorption, thereby reduction in urinary fluoride concentration.

There was significant reduction in Hb, TLC and PCV in fluorotic calves than healthy ones. The presence of anemia in fluoride affected animal was also reported in calves (Bharti *et al.,* 2007 and Gill and Dumka, 2013), buffaloes (Swarup and Singh, 1989), cows (Singh and Swarup, 1994) and goats (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 fluoride intoxication depresses bone marrow activity in cattle (Radostis *et al.,* 2000) resulting in normocytic and normochromic anemia due to reduced erythropoesis (Dwivedi *et al.,* 2000). Fluoride-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 fluoride 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 fluoride on the RBC cell membrane and subsequently shrinkage of cell. Studies on cattle, goats and sheep in relation to haematological alterations in fluorotic animals by various authors like Maiti *et al.,* 2003; Singh *et al.,* 2002 and Vinay kant *et al.,* 2009 also revealed similar changes.

In the present investigation, there was increase in Hb, TLC and PCV value in both the treatment groups after 60 days of treatment. This might be due to prevention of oxidative damage to cell membrane of RBC by Boron (Bharti *et al.,* 2007) in Ca+B supplementation group. But the more significant increase in *Moringa* supplementation group than Ca+B might be due to high Fe, Cu and antioxidant like ascorbic acids, polyphenols, flavanoids and organosulfer compounds present in *Moringa* (Aja *et al.,* 2014, Kumar *et al.,* 2010 and Anwar *et al.,* 2007).

A significant increase in lymphocyte %, eosinophil % and decrease in neutrophil % was revealed in the study which was also reported by other workers viz. Hoogstratten *et al*., 1965; Hillman *et al*., 1979; Jagadish *et al.,*

1998, Upadhyay *et al.,* 2005 and Agha *et al.,* 2012. But significant alterations in above parameters towards their normal values were observed in both the treatment groups.

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 excretion causing hypocalcaemia (Singh and Swarup, 1999). These findings are in agreement with those of earlier workers (Maiti and Das, 2004; Bharti *et al*., 2007) who also reported a decline in serum calcium in fluorotic animals. However, supplementations of dried fruit powder of *Moringa* to the fluorotic animals produced significant increase in calcium concentration which was also found by Ranjan *et al.* (2009) in experimental animals. The beneficial effect of *Moringa* might be due to its richness in calcium (Anjorin *et al.,* 2010 and Kawo *et al.,* 2009) and reduced absorption or an increase in elimination of fluoride from the body. But Ca+B supplementation is more efficient in increasing calcium concentration in blood which was also found by Maiti and Das (2004). This might be due to directly increased absorption and decreased urinary excretion of Ca due to high calcium intake and protective effect of B against the inhibitory effect of F on Ca ATPase in renal tissues, respectively.

In fluorotic calves there is decreased concentration of Mg than healthy calves which also matches with the findings of Meral *et al.* (2004), Tao *et al.* (2005), Bharti *et al.* (2006) and Ranjan *et al.* (2008). This might be due to the reaction of the highly electronegative F with this mineral, thereby reduction of their absorption. Increase in urinary and faecal excretion of various minerals may be another factor responsible for their decreased status in the body (Ranjan *et al.,* 2008). But treatment with *Moringa* fruit powder significantly increased the Mg content in plasma. As *Moringa* not only enhances fluoride

elimination, but also contains high amount Mg (Anjorin *et al.,* 2010 and Kawo *et al.,* 2009), it might be reducing the elimination of these minerals from body.

Alkaline phosphatase activity was significantly higher in fluorotic calves as compared to those from the non fluorotic area. This finding was in agreement with many other observations reported in fluorotic animals by Maiti and Das, 2004; Upadhyaya *et al.,* 2005. Since fluoride stimulates osteoblastic activity (Araya *et al.,* 1990), the increase in alkaline phosphatase can probably be related to abnormal bone formation and stimulated osteoblastic activity with increased fluoride concentration in the serum (Farley *et al.,* 1987, Radostis *et al*., 2000 and Singh and Swarup, 1999).

Supplementation of calcium and boron has decreased the serum alkaline phosphatase activity in fluorotic calves and the same was reported by Maiti and Das (2004). Supplementation of dried fruit powder of *Moringa* to the fluorotic calves significantly reduced the activity of alkaline phosphatase. The beneficial effect of *Moringa* on reduction of alkaline phosphatase activity might be due to the presence of high calcium content in the dried *Moringa* fruit powder (Gopalan *et al.,* 1982 and Ishola *et al.,* 1990).

The significant enhancement of plasma AST and reduction of total protein and albumin, 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). Decrease in total protein level in fluoride affected calves might be due to impairment of polypeptide chain inhibition, weak incorporation of amino acids into proteins and abnormal accumulation or inhibition of DNA synthesis (Chinoy *et al.,* 1994). Disruption of protein metabolism in liver through increased activity of glutamate dehydrogenase (GLDH) enzyme that is responsible for deamination of amino acids may also contribute towards lower plasma protein level in fluoride intoxication as previously reported in rats by Brikner *et al.* (2000). But the ALT level remained within normal range in fluorotic calves and this finding is at one with the findings of Radostits *et al*. (2000) and Swarup *et al*. (2001).

There was significant decrease in AST level and significant increase in plasma total protein and albumin level upon treatment with Ca and B. This might be due to decreased plasma F burden, thereby reversing the hepatic metabolic status. The anti-oxidative property of Boron might also contribute this change.

Supplementation of *Moringa* significantly decreased the AST level and increased the total protein and albumin in plasma. It has been reported that *Moringa* contains monoterpenes, glycosides, organic acids, lipids, alkaloids, xanthenes, flavanoids (quercetin), β-carotenes and ascorbic acid (Aja *et al.,* 2014, Buraimoh *et al.,* 2011 and Rookmani *et al.,* 1998) which have hepato-protective effect due to their anti-oxidant property (Ranjan *et al*., 2009 and Gupta and Mishra, 2006, Eidi *et al.,* 2012). The presence of phytochemicals like oleic acid, ascorbic acid, dihexadecanoic acid octadecanoic acid, methyl ester hexadecanoic acid and octadecenamide in *Moringa* seeds (Aja *et al.,* 2014) also reported to have hepatoprotective effect by preventing oxidative damage to liver tissues (Kumar *et al.,* 2010).

In this present study, a significant higher level of urea and creatinine in the fluorotic calves was recorded than the calves from non fluorotic zone 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 disturbance of protein metabolism due to increased activity of glutamate dehydrogenase (GLDH) enzyme which catalyzes transamination and oxidative deamination of aminoacids in liver. GLDH enzyme activity is allosterically inhibited by GTP and NADH and activated by ADP and GDP. But fluoride intoxication results in reduction of intracellular high energy compounds (Chitra and Ramana, 1984) resulting increased GLDH activity in liver and urea concentration in blood (Brikner *et al.,* 2000). The increased

catabolism of protein because of partial starvation in affected animals (Swarup *et al*., 2001) may also contribute towards higher urea level in plasma.

Consequent upon the treatment with *Moringa* dried fruit powder, the creatinine level decreased significantly at 60 days of treatment and the urea level decreased non-significantly. This might be due to antioxidative property, reduced fluoride burden and higher Ca content of dried *Moringa* fruit powder which protects renal (Ranjan *et al.,* 2009) and muscular damage. The Ca supplementation causes reduction in absorption of ingested F and inhibition of phosphodiasterase, thereby augmenting c-AMP levels which are involved in the activation of several phsophokinase in liver and muscle (chinoy *et al.,* 1993). However, the protective effect of Ca+B might not be strong enough to alter the urea and creatinine level as compared to *Moringa* treatment.

SUMMARY AND CONCLUSION

Environmental degradation often tends to become irreversible and imposes damaging effect in form of ill- health to human as well as livestock and loss of crop output. Deleterious effects of the pollution are reflected upon the health status of both human being and livestock inviting the intervention in the management of the physical components of environments like soil, water and air. Fluorosis, characterized by chronic fluoride intoxication is a worldwide health problem and is endemic in areas where fluoride content of drinking water and fodder are high. Chronic ingestion of fluoride rich fodder and water in endemic area leads to development of fluorosis in animals. During last several years, numerous reports of fluorosis in animals and humans from India, China and other countries were observed. 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. Fluoride rich drinking water feed supplements and mineral mixtures can also be taken into account for fluoride toxicity.

Angul, Talcher attracted large industries because of large deposits of coal and high flow in river Brahmani. Heavy industrialization in Angul-Talcher industrial complex has become the principal cause of environmental pollution. Fluorosis comes out as the principal adverse health effect on animals and human beings of this aluminium industrialization.

The present study entitled "***Ameliorative efficacy of medicinal herbs in calves exposed to industrial fluorosis***" was carried out in the Department of Veterinary Clinical Medicine, Ethics and Jurisprudence, College of Veterinary Science & Animal Husbandry, Orissa University of Agriculture and Technology, Bhubaneswar during the period of December 2013 to July 2014. The investigation was carried out in the periphery villages (within 2 km radius) of the Aluminium Smelter plant, National Aluminium Company, Angul, Odisha, India.

One hundred and seven calves reared in the industrial fluorotic areas i.e. Vicinity of National aluminium company (NALCO), Angul, was screened

for dental and skeletal lesions and higher plasma fluoride level. A total of thirty (n=30) calves having clinical signs of discoloration, mottling, pitting of teeth, difficulty in mastication and higher plasma fluoride level (more than 2 ppm)were selected and divided equally into 4 groups of 10 animals each, given below; Group I : Healthy group

Group II: Disease Control group

Group III: *Moringa oleifera* dried fruit powder supplemented group

Group IV: Calcium+ Boron supplemented group

Blood samples were collected from jugular vein in sterilized blood collection vial with heparin and EDTA as anticoagulant from the fluorosis affected calves along with normal calves from non fluorotic zone on day 0 just before treatment, during treatment on day 30 and day 60 at end of treatment. Plasma fluoride level, haematological parameters i.e. haemoglobin, TLC, DLC, PCV were evaluated by standard methods. Biochemical parameters like Ca, P, TP, albumin, ALP, ALT, AST, urea, creatinine were evaluated using commercial kits as per manufacturer's instruction. Faecal samples were collected directly from the rectum of calves, in pre-labelled plastic specimen bottle with the above mentioned schedule for estimation of fluoride content. Urine samples were also collected in sterilized containers for measurement of urinary fluoride excretion level. Soil, water and fodder samples were collected in separate containers for fluoride level estimation.

The overall prevalence of fluorosis (dental and skeletal fluorosis) in calves in the vicinity of NALCO aluminium plant, Angul, Odisha was 93.457 %. The prevalence of dental fluorosis was almost predominant with 84.11% in calves out of which 36.67%, 84.7% and 94.8% in 0-6 months, 6-12 months and 12- 18 months calves respectively were affected with it.

Plasma fluoride estimation showed no significance from day 0 to end of the experiment in normal calves. Fluoride affected calves revealed significantly ($p<0.05$) higher level of plasma fluoride as compared to normal

calves at different observation periods with non-significant increasing trend from day 0 to end of experiment. Significant ($p<0.05$) reduction in plasma fluoride concentration was recorded in (*Moringa* treated) group III and (Ca+B) group IV from day 30 onwards.

The urine fluoride concentrations in fluorosis affected calves had significantly higher urinary fluoride level as compared to healthy calves (Gr I) on day 0, 30 and 60 of the experiment. Significant reduction in urine fluoride concentration in *Moringa* supplemented and Ca+B supplemented was recorded on day 30 and day 60 as compared to day 0 values. Significant ($p<0.05$) decrease in urinary fluoride level was noted after supplementation of *Moringa* and Ca+B on day 30 and day 60 as compared to gr II on day 30 and day 60, respectively.

Calves from fluorotic area (Gr II) excreted higher level of fluoride through faeces on day on different observational days of experiments compared to calves from non-fluorotic area (Gr I). Significant increase in fluoride concentration was recorded at the end of the experiment in both *Moringa* and Ca+B treatment group as compared to disease control group.

Calves of fluorotic area (Gr II) showed reduced haemoglobin content in as compared to healthy calves (Gr I). There was non-significant increase in Hb (g%) in both the treatment groups (Gr III & IV) from day 0 to day 30 but significant increase on day 60 was recorded. There was significant increase in Hb content in group III and group IV as compared to Group II on day 60 of the observation.

Packed cell volume (%) increased significantly at end of treatment i.e. day 60 of observation in *Moringa* treated (Gr III) calves as compared to day 0. Normal calves showed (Gr I) higher PCV % than other groups. Lower, Total leucocyte count (Cumm) in calves grown in fluorotic zone in comparison to non fluorotic zone on day 0, 30 and 60. There was significant increase in TLC count in Morinaga treated (Gr III) calves and Calcium + boron treated (Gr IV) calves on day 60 in comparison to calves of fluorotic zone.

Lymphocyte count (%) in fluorosis affected calves showed higher % in calves of fluorotic zone in comparison to normal calves. Supplementation of *Moringa* (Gr III) reduces lymphocyte % significantly on day 30 compared to day 0. Similar results were also observed in Ca + B treated group (Gr IV) on day 30. Significant decrease in lymphocyte count was observed in both treatment groups compared to calves of affected areas at the end of the experiment.

Neutrophil count (%) in fluorosis affected calves was significantly lower from normal calves. Supplementation of *Moringa* in calves (Gr III) increased neutrophil count at end of treatment compared to calves of affected areas. Significant lower value in *Moringa* treated group was recorded than calcium and boron treated group but not comparable to normal animal at end of treatment.

Eosinophil percentage in fluorotic calves depicted a higher % as compared to normal calves. *Moringa* supplementation reduced eosinophil count at end of treatment i.e. on day 60 as compared to day 30 values in both *Moringa* and Ca + B treated calves. Both the treatment group recorded significant reduction in eosinophil count from calves of fluorotic areas at end of treatment period.

Calcium concentration in fluorosis affected calves was significantly lower in calves reared in fluorotic zone as compared to normal animals. *Moringa* supplementation (Gr III) seems to be increasing the plasma calcium concentration in calves on day 30 of experiment. Ca+B treatment group (Gr IV) showed significant increase in calcium level on day 30 and 60 of experimentation. *Moringa* and calcium + boron supplementation increased the calcium concentration on day 60 compared to claves of fluorotic zone.

The significant high phosphorus concentration was recorded in calves of fluoric zone than normal calves in this experiment. There was significant decrease in phosphorus concentration in *Moringa* treated group (Gr III) on 30 day of experiment. Similarly phosphorus concentration reduced in Ca+B treated groups from day 30 to day 60 of experimentation.

Total protein concentration was significantly lower in fluorosis affected calves as compared to normal calves. There was significant increase in total protein concentrations at end of experiment on supplementation of *Moringa* as well as Ca + B in calves compared to day 0. The total protein concentration was lower at end of experiment in *Moringa* and compared to healthy calves group but higher than affected calves of fluorotic zone.

Albumin concentration was significantly lower in fluorosis affected calves than normal calves. There was significant increase in albumin concentration on day 60 compared to day 30 in *Moringa* as well as Ca + B group of calves. There was higher mean albumin concentration on day 60 of *Moringa* treatment group compared to Ca + B group.

Urea concentration in plasma was significantly higher in calves of affected areas than normal calves. Treatment groups showed no significant differences in urea concentration in relation to fluorotic calves.

Creatinine concentration in plasma of fluorosis affected calves (Gr II) showed significant increased concentration than normal healthy calves. Plasma creatinine concentration decreased significantly after 60 day supplementation of *Moringa* and Ca + B treated group when compared to group II animals. There was significantly higher concentration of plasma creatinine concentration recorded in Ca + B treated group with respect to *Moringa* supplemented calves.

Significant higher alkaline phosphatase (ALP) activity in plasma was recorded in calves of fluoride affected areas than normal calves. The reduced activity of alkaline phosphatase was recorded after 30 day of supplementation of *Moringa* and Ca + B compared to calves of fluoride affected zone. Similar result was also recorded on day 60 in Gr III, Gr IV compared to Gr II. The activity of ALP was found to be significantly higher in Gr II on day 60 than Gr III and Gr IV.

Alanine transaminase (ALT) in plasma showed significantly higher activity (U/L) in calves of fluoride affected areas than normal calves. Non-

significant increase in the activity of ALT was observed after supplementation of *Moringa* as well as standard treatment at different observation periods.

The Aspartate transaminase (AST)in fluorosis affected calveshave significantly higher activity (U/L) than normal calves. There was significant reduction in AST activity in both *Moringa* as well as Calcium with Boron supplementation on day 30 and day 60 as compared to day 0. There was significant lower AST activity on day 30 of *Moringa* group compared to Ca + B group as well as on day 60. AST activity was significantly lower in calves of *Moringa* treated group and Ca + B treated group at end of treatment as compared to calves of fluorotic areas.

The finding of the present study revealed that dry fruit powder of *Moringa oleifera* has ameliorative potential in chronic fluoride toxicity. Further studies on screening, isolation and purification of bioactive phytochemicals compounds followed by in-depth laboratory and field bioassays are needed as the present investigation shows that there is scope to use *Moringa* fruits for prevention as well as amelioration of fluorosis.

The following conclusions are drawn from the present investigation

- The soil, fodder and water contains high amount of fluoride near the aluminium smelter plant area, which is above permissible level.
- The prevalence of dental and skeletal fluorosis increases with age of animal.
- Considering the favourable ameliorative efficacy of calcium + boron and *Moringa* fruit powder to reduce fluoride load in calves. It may be wise to suggest that, farmers around NALCO smelter plant, Angul, may be advised to administer one of the above formulations to calves to ward off the threat of juvenile fluorosis and its progression.
- However the present investigation showed that *Moringa* fruit powder supplementation is superior to calcium and boron in ameliorating fluorosis effectively.
- As supplementation of *Moringa* is a herbal therapy and the fruit is available in the rural area a plenty, in Odisha, therefore farmers may use this *Moringa* fruit powder in young calves as preventive measure against fluorosis and it will be more economical.

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Plate 1: Calf showing emaciation

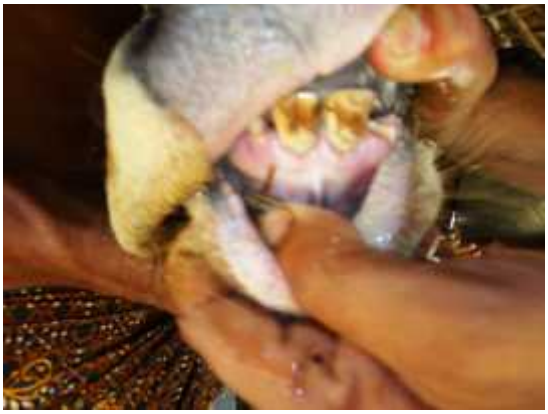

Plate 2: Calf showing sign of dental fluorosis

Plate 3: Fluoride estimation

Plate 4: Biochemical estimation

Plate 5: Magnesium estimation