Fluoride has been described as an essential element needed for normal development and growth of animals and extremely useful for human beings. Fluoride is abundant in the environment and the main source of fluoride to humans is drinking water. It has been proved to be beneficial in recommended doses, and at the same time its toxicity at higher levels has also been well established. Fluoride gets accumulated in hard tissues of the body and has been known to play an important role in mineralization of bone and teeth. At high levels it has been known to cause dental and skeletal fluorosis.

Fluorides are naturally occurring harmful contaminant in the environment. It is a cumulative poison and thus leads to fluorosis; a serious public health problem. Endemic fluorosis is prevalent in many parts of the world where drinking water contains more than 1 to 1.5 ppm of fluoride and causes damage not only to hard tissues of teeth and skeleton, but also to soft tissues, such as brain, liver, kidney, spleen and endocrine glands (Santhakumari and Subramanian, 2007; Ozsvath, 2009). Fluoride enters into the human body and animals mainly through the oral route along with food and water. It can be rapidly absorbed by passive diffusion through stomach, small intestine, mouth, lungs and skin. If fluoride is ingested beyond the permissible limits it may cause 3 types of fluorosis namely dental fluorosis, skeletal fluorosis and non-skeletal fluorosis.

The occurrence of fluoride in drinking water is reported from many parts of the world. Large scale incidences and severity of fluorosis in different parts of India suggest that out of the various components of the environment, water is the major contributor to the problem of fluorosis. Most of the freshwater sources are found to be contaminated with soluble inorganic and organic materials that make the groundwater unsuitable for drinking purpose. Fluoride is probably the first inorganic ion which drew attention of the scientific forum for its toxic effect and now the toxic effects due to fluoride in drinking water is a well recognized global problem, because of the number of health hazards it ultimately leads to. Fluoride concentration in drinking water up to 1.0 mg/L is beneficial for the human body. But a concentration of fluoride beyond 1.0 mg/L is considered to be deleterious to health and is the cause for dental and skeletal fluorosis.
The beneficial interference of fluoride in the caries development is directly dependent on a constant and permanent level of fluoride in the oral environment (Fejerskov, 2004). There is no need for a high concentration of fluoride because low concentrations can be sufficient for the control of mineral tooth loss. This is a key point when considering the use of fluoride for caries preventive purposes which is different from caries arrestment (Sampaio and Levy, 2011). The ideal or ‘optimal’ concentration of fluoride will be the one that will have maximum preventive effect with a minimal risk for dental fluorosis. This is rather difficult to consider since individual variations in fluoride bioavailability and cultural habits may influence the intake of fluoride from systemic methods such as water, salt or milk (Newbrun, 2010).

The severity of the disease will depend on several factors such as dosage, duration of exposition, the activity stage of the protein, age and individual susceptibility. Fluorotic enamel is characterized by the retention of amelogenins in the early maturation stage of development and the formation of enamel which have a hypomineralized subsurface and more porous tissue. This is a conflicting point. Since dental fluorosis is related to high consumption of fluoride during tooth development, many dental professionals make the assumption that this tooth condition is related to a high concentration of fluoride in the dental tissues (enamel and dentin). Actually, it is the opposite situation. The white chalk appearance of the enamel is related to the light refraction of this tissue that presents a high porous appearance due the hypomineralized subsurface (Levy, 2003).

The present study is aimed to elucidate the changes in some biochemical parameters which are involved in energy metabolism, hematology, histological and transmission of electron microscopic studies with the dosing of sodium fluoride and vitamin C.

In the present investigation an attempt is made to observe the effect of sodium fluoride on mammalian model, albino mice to extrapolate to human beings, beside survey made in an endemic fluorosis area.

Healthy adult male albino mice of same age (75±5 days) and weight (30±5 g) were divided into six groups having ten animals each. They were fed with rat feed. All treatments were given orally with a hypodermic syringe attached to an angular
needle. The first group animals treated as control and the second group of animals treated with De Ionized water + Vitamin C (15mg /animal/day) orally by gavage method. Third and fifth group animals were treated for 7 days and 30 days with 5 ppm (5 mg/L) sodium fluoride water respectively. This dose was selected as per Harvey (1962). Fourth and sixth group animals were treated with combined dose of sodium fluoride 5 ppm (5 mg /L) and vitamin C for 7 days and 30 days. According to him serious problem of fluorosis occurs when the element is present in drinking water at levels of 5 ppm or greater. In India fluoride content is exceeding 1.0 ppm and varies between 0.1 to 25 ppm (Teotia and Teotia, 1991). To study the effects of fluoride concentration of 5 ppm in albino mice considering it as a mammalian model and it is related to the problems in humans.

The control and experimental animals after the stipulated period (i.e. on 8th day and 31st day) were sacrificed and the tissues were isolated, cleaned in physiological saline and processed immediately for microscopic analysis. The tissues were also quickly isolated under ice cold conditions and stored in deep freezer at -80°C for biochemical analysis.

The investigation was carried out in two affected villages of Kurnool district (A.P.), of Tuggali mandal. The first village is Tuggali, located about 62 km from Kurnool city. The second village is Sabhashpuram, which is about 58 km from Kurnool city and 15 km from Tuggali town. Tuggali has a population of 750 approximately in various age groups and Sabhashpuram has about 400 approximately.

The main source of drinking water in Tuggali and Sabhashpuram villages are bore wells and dug wells. The fluoride content in Tuggali village is 4.3 (Mean) and Sabhashpuram village 3.9 (Mean) (Table 1.1) is observed in the present study. In these two villages Tuggali has more amount of fluoride content in water when compared to Sabhashpuram. In these two villages, dug wells have more amount of fluoride content when compared to bore wells.

The epidemiological survey conducted, reveals that in Tuggali village 68% of the people affected with dental fluorosis and 59.5% (Table 1.2) of the people are affected with skeletal fluorosis. In Sabhashpuram village 62.5% of the people are affected with dental fluorosis and 54% (Table 1.3) are affected with skeletal fluorosis.
The skeletal fluorosis is evidenced with crippled limbs and bending of stature in adults and people have experienced difficulty in walking because of stiffness and limitations of various joints. Some skeletal fluorosis persons are suffering from compression of spinal cord, vertebral crippling, osteophytosis. Some skeletal fluorosis patients are suffering from restricted movement of neck, trunk and rigidity of bones. In some cases weight loss, brittleness of bones, muscular wasting, anaemia and weakness are observed. In children crippling of limbs and stunted growth are observed in both the villages (Plates 1.1 to 1.5; Figs. A to J).

Dental fluorosis patients showed brownish colour stains, white flecks, abnormal patchy mottled teeth, dental carries, dental erosion, loss of enamel, damage of molar teeth, spongy gums etc (Plates 1.6 to 1.8; Figs. A to K).

In the present investigation a comparative study is aimed to understand the toxicity of 5ppm sodium fluoride for 7 days and 30 days. Stock solution of sodium fluoride was prepared in deionized water, 5ppm sodium fluoride was made by dissolving 5mg of sodium fluoride in one litre of deionized water.

Carbohydrates are the primary energy source of the organism. In the present study total carbohydrate contents were reduced in experimental animals when compared to control animals (Table 2.1; Fig. 2.1). Under any stress conditions, the organism needs high energy to tolerate the stress conditions, thus the decrease in tissue carbohydrates signifies its utilization possibly to meet higher energy demands.

Total Proteins content decreased in the present investigation (Table 2.2; Fig. 2.2). During stress condition, organisms needed more energy to detoxify the toxicants and to overcome stress, chronic period of stress, proteins also a source of energy. Generally the breakdown of proteins dominates over synthesis under enhanced proteolytic activity.

The increase in FAA content in the present study (Table 2.3; Fig. 2.3) is a clear indication of (a) stepped up proteolysis and or (b) fixation of ammonia into keto acids resulting in amino acid synthesis.

Total lipid content, somatic index of liver, testes, body weight were decreased (Tables 2.4 & 2.5; Figs. 2.4 – 2.6) due to reduction of fatty acids. Weight loss is due
to degeneration of structure of organs, and decreased protein levels. In fourth & sixth group of animals recovery was more observed in 30 days than 7 days of tissues when compared to experimental animals.

Energy metabolism occupies a key position in the metabolic machinery and its modulation leads to variations in the energy budget of the cell. Glucose molecule undergoes a series of enzyme-catalyzed reactions to release energy-rich electrons which are captured by coenzyme and pass to the cytochrome system to convert the chemical energy through phosphorylation from ADP to ATP. The total ATPases activity has shown a significant decreased trend in all the tissues of albino mice in experimental animals when compared to control animals (Table 3.1; Fig. 3.1), maybe due to the action of sodium fluoride which affect the mitochondrial integrity as well as the enzyme systems localized in mitochondria, leading to less synthesis of ATP molecules. ACP is a hydrolytic enzyme and ALP is a brush border enzyme. Alkaline phosphatase is the most active enzyme of the phosphatases. A significant increase in the activity levels of acid and alkaline phosphatases in experimental animals when compared to control animals (Tables 3.2 & 3.3; Figs. 3.2 & 3.3). May be due to increased activity of phosphorylase enzyme and subsequent breakdown of glycogen to release energy during sodium fluoride intoxication.

Transaminases are important enzymes in animal metabolism which are intimately associated with amino acid synthesis and lysis. Among these, aspartate and alanine transaminases are widely distributed to the cells of animals. AST, ALAT play an important role in the utilization of amino acids for the oxidation and or for gluconeogenesis. The increased levels of AAT & ALAT (Tables 3.4 & 3.5; Figs. 3.4 & 3.5) indicate that metabolism of amino acids towards the gluconeogenesis to meet the energy levels against the sodium fluoride stress besides tissue damage and also disruption of mitochondrial integrity & increased synthesis of enzymes.

Haematological parameters have exhibited gradual decrease in RBC, Hb, WBC, PCV values in experimental animals when compared to control group (Table 4.1; Fig. 4.1) and MCV is significantly increased. There was a slight fluctuation in MCH & MCHC values of mice exposed to fluoride due to fluoride poisoning. The fall in these parameters leads to anemia, and leucopenia. At the same time with the combination of NaF + Vit. C, RBC, Hb, WBC and PCV values are increased.
Histopathology has been used as an important diagnostic tool in biochemical pathology for many years and has been a corner stone in the large field of bio-clinical pathology. Histopathological studies are necessary for the elevation and description of possible damages caused to the mice exposure to various toxicants. Hence, in the present investigation histopathological studies were also carried out by light microscope and electron microscope to elucidate the toxic potentials of the sodium fluoride and Vit. C in mice exposed to individual and combination (Plates 5.1 – 5.22). Clear architectural changes in liver, testes of exposed mice were observed. In the present investigation liver showed cytoplasmic degeneration, cellular disarray, and severe necrosis in hepatocytes, pushing of nucleus to periphery, nuclear fragmentation and binucleated condition. Testes showed degeneration in seminiferous tubules, clumped spermatozoa, formation of large lumen and scattered spermatids. Histopathological changes of liver, testes were more damaged in 30 days when compared to 7days of animals with the combination of sodium fluoride and vit. C, all tissue recovery was more observed in 30 days than 7days.

Today, there is clear evidence that fluorosis is increasing worldwide. This concern raises doubts about the beneficial aspects of systemic fluoride methods. But there are clear evidences that these methods have more beneficial effects than risks. For instance, in spite of the potential risk for dental fluorosis, dietary fluoride supplements are regarded as effective in preventing caries and are still available in several countries. This method was not discussed in this paper but this issue is also relevant (Buzalaf, 2011). The recent reduction in fluoride levels in the water communities cannot be interpreted as a limitation of the method. Conversely, this adjustment proves that water fluoridation is still necessary. High fluoride contents in drinking water has created not only dealt problem but it has become a serious livelihood problem for the consumers which in turn has adversely affected the socio-economic status of the state. Therefore, for combating with this problem, following precautions must be taken:

✓ Inculcation of awareness amongst rural and urban population regarding harmful effects of quality parameters.
✓ Popularizing simple and handy defluoridation techniques at domestic and community level in endemic areas.
✔ Avoid the habit of open air defecation.
✔ Avoid the sewage disposal directly to ground water.
✔ Installation of reverse osmosis, ion-exchange and electrodialysis plants in affected areas.
✔ Avoid indiscriminate use of nitrogenous fertilizers and promote the use of bio – fertilizers and bio – pesticides.
✔ Construction of wells/tube wells in low nitrate and fluoride areas.
✔ Prohibition on use of fluoride rich edibles, cosmetics and luxury items.
✔ Supplementation of ascorbic acid.
✔ Promulgation of Aquifer Protection Policy (APP) to avoid over-exploitation of ground water to alleviate ingress of fluoride.
✔ Mandatory provision of construction of rain water harvesting structures at domestic, official and community levels.