CHAPTER I
INTRODUCTION

The lifestyle of the modern society has improved greatly with the help of tremendous industrialization, extensive use of automobile and advanced drugs and fertilizers. For such facilities, mankind is paying the price in the form of genetic mutation and toxicity. Hence it is now imperative to direct the attention to toxicity research.

Today in the era of global warming, when the environment is not very favorable for the total health of the human being it is more relevant to study toxic effect of metal ions in detail.

Metal Toxicity:

Metals are probably the oldest toxins known to humans. Metals differ from other toxic substances that they are neither created nor destroyed by humans. About 80 of the 105 elements in the periodic table are regarded as metals, but less than 30 have been reported to produce toxicity in humans. Copper, Lead, Arsenic, Mercury and Nickel are some of common metal toxicants, whose toxicity has been extensively reported.

Earlier, aluminium was thought to be a non-toxic and relatively safe metal (Hewitt et al., 1990), but later it was proved to be neurotoxic by Domingo (1995). It also affects other body structures like skeletal tissues and blood cells.

It has been suggested that Aluminium is a cause of Alzheimer's disease as some brain plaques have been found to contain this metal. Research in this
area has been controversial and inconclusive. Aluminium accumulation may be a consequence of the Alzheimer's damage, not the cause. This ambiguity regarding the toxicity of aluminium particularly at low doses triggered the present study which was conducted to determine the toxic effect of aluminium on some vital and reproductive organs.

Chromium is also used extensively in our day to day life with aluminium in form of drugs and chromium plated utensils. Hence, the combined study was carried out to determine the antagonistic, synergistic or additive effect of Aluminium and Chromium.

**Aluminium:**

Aluminium, as a metal has been extensively used throughout the globe with little regard to its possible toxicity. Aluminium is the most abundant metal on the earth, comprising about 8 % of the earth's crust. It is found in a variety of minerals and as a normal constituent of soil, plant and animal tissue. The metal has been used for the production of a wide variety of articles including building and construction materials, can and packaging materials. Salts of aluminium are used by the pharmaceutical industries as a major ingredient of antacid and antidiarrhoeal drugs. The main food sources of Aluminium are hard cheese, grain product (flour), herbs and tea leaves. Concentration of Aluminium in food ranges widely from < 0.001 to 69.5 mg / 100 g, depending on the nature of the food stuffs (Pennington,1988). Golub et al.(1995) identified 500 µg Al/ g diet, equivalent to about 100 mg Al/kg bodyweight per day, as the lowest observed – adverse effect level)LOAEL.
Exposure:

Since aluminium is ubiquitous in the environment and is used in a variety of products and processes, daily exposure of the general population to aluminium is inevitable. Nutritionally, it is nonessential, however it is used in the treatment of drinking water, pharmacological preparations and in the manufacture of cooking utensils (WHO, 1997). According to Miller et al. (1984), Aluminium is more likely to exist in surface water than in ground water. Only 9% of ground water had a detectable amount of aluminium where as 78% of surface water has detectable aluminium. However, humans would absorb only 3% of their total daily uptake of aluminium from drinking water, a relatively minor source compared to food (Yokel et al., 2008)

Aluminium compounds and materials have a wide variety of uses, including production of glass, ceramics, rubber, wood preservatives and waterproofing, textiles. Natural aluminium minerals are used in water purification, sugar refining, brewing and paper industries.

Aluminium containing adjuvants are widely used in a variety of vaccine products, such as recombinant protein, conjugated polysaccharide and recently DNA vaccines (Zho and Sitrin, 2001). The commonly used vaccines diphtheria tetanus, hepatitis, rabies and anthrax all are based on aluminium adjuvant.

Some plants naturally accumulate relatively high amounts of aluminium compounds in their leaves from the soil in which they grow. One such example is tea, which accumulate high concentration of aluminium in leaves.
High levels of aluminium hydroxide are present in some antacid preparation used to treat indigestion and in buffered aspirin tablets which are designed to reduce irritation of the stomach.

Aluminium sulphate is widely used in the water purification treatment. It is used as a flocculating agent to remove suspended particles, including the spores of some infectious organisms which are difficult to remove by other means. Most of the aluminium is removed in the later stage and the final concentration is usually much less.

From these natural and man made sources of aluminium, it is estimated that the average person has an oral intake of around 10 mg of aluminium each day. It widely depends on dietary habits and medication.

**Absorption of Aluminium by intestine:**

Van der Voet (1992) suggested that the degree of aluminium absorption in an animal depends on a number of parameters including pH, aluminium speciation and dietary factors (Martin, 1992). According to Rodger et al. (1991), more aluminium is absorbed at low pH than neutral or high pH. However in the intestine at near neutral pH, most of aluminium changes into insoluble form and is not available for uptake.

The gastrointestinal absorption of aluminium compounds has been studied by using radioactive isotope Al^{26}. From this study it was concluded that humans absorb about 3% of their daily intake of aluminium from the source depending upon the level found in food and the frequency of antacid use. The composition of the food eaten in conjugation with drinking water containing aluminium has a strong effect on the absorption of aluminium. Walton et al. (1995) found that aluminium containing water with lemon juice, orange juice,
coffee or wine has tendency to increase absorption about 1800%, 1700%, 250% and 188% respectively. According to Slanina et al. (1986) increased level of aluminium in combination with lemon juice and orange juice is due to the formation of non-ionized aluminium citrate, which is expected to cross the gastrointestinal barrier. Domingo et al. (1993) have reported that ascorbic acid and lactic acid promotes absorption of aluminium.

Gastrointestinal absorption of aluminium is also enhanced by certain disease. Wills and Savory (1989) had reported increased level of aluminium in patients suffering from chronic renal failure. It is also observed that aluminium is absorbed more readily in individuals suffering from uremia (Lindholm et al., 1983).

According to Day et al. (1991), absorbed aluminium in the blood stream binds with plasma protein like albumin and transferrin. Fatemi et al. (1991) suggested that from absorbed aluminium approximately 60 % binds to Transferrin, 34 % binds to albumin and remainder to citrate in normal human blood serum. There are indications that aluminium binds to protein in a dose dependent manner (Hohr et al., 1989). Aluminium distribution depends on the animal species used, route of administration and the aluminium compound administered.

Crapper et al. (1980) have found out that the highest level of aluminium in human body are in skeleton, lungs, kidneys, spleen, thyroid and parathyroid gland. Experience with dialysis patients has shown that aluminium has the potential to accumulate in the skeleton and brain (Zumkley et al., 1987). The normal blood aluminium levels in humans are reported to be between 1 to 16 μg/L.
Excretion of Aluminium:

Usually, the absorbed aluminium in human body is excreted by the kidneys. Monteagudo et al. (1988) investigated that aluminium excretion occurs in the distal tubule of the kidney and is situated close to the sites of maximal sodium and calcium ion reabsorption. Renal excretion is inefficient owing to the significant reabsorption of aluminium in the proximal tubules. It was suggested that the route of excretion also varies with the route of administration.

Toxic effect of aluminium:

There is no known biological role for aluminium. It does not appear to be an essential trace element and the body has highly effective barriers to exclude aluminium. When these barriers do not work properly or when the kidneys' ability to excrete aluminium is impaired, the accumulation of aluminium cause adverse health effects.

Aluminium is an important element with respect to the human use and exposure, with known toxicity in the human body, mainly in the central nervous system (Domingo, 1995). Some toxic effects reported to caused by aluminium are microcytic anemia, osteomalacia, glucose intolerance of uremia and cardiac arrest (Starkey, 1987). In addition aluminium has been shown to inhibit a number of enzyme activities including key enzymes involved catecholamine synthesis, such as dihydropteridine reductase (Altmann et al., 1987).
Extensive use of aluminium containing antiperspirants is more likely cause of toxicity. Aluminium increases estrogen related gene-expression in human breast cancer cell grown in laboratory (Darbre, 2006). These salts have estrogen like effects which have led to their classification as metalloestrogen. Use of aluminium in some antiperspirants and food additives is controversial. According to Exley et al. (2007) aluminium in antiperspirants may increase the risk of breast cancer.

**Dialysis Encephalopathy:**

Dialysis encephalopathy is the most studied aluminium related syndrome. Some symptoms of this syndrome include speech disorder, neuropsychiatric abnormalities and multifocal myoclonus(Dewberry et al.,1980).

Alfrey et al. (1976) suggested that patients with dialysis dementia have elevated serum aluminium level with increased concentration in many tissues such as cerebral cortex.

**Amyotropic Lateral Sclerosis (ALS) and Parkinson’s Dementia (PD):**

Garruto et al. (1990) investigated that in cases of ALS and PD, severe neurodegenerative disease have a relation to aluminium accumulation in brain. These diseases are observed at very high incidence among the Chamorro people on Guam, and both disease shows loss of motor neuron function and the presence of neurofibrillary tangles in the brain.

**Alzheimer’s disease (AD):**

Aluminium accumulation may be cause for the development and of onset of Alzheimer’s disease (AD). The main symptoms of AD are memory lapses, disorientation, confusion and frequent depression. Genetic and
environmental factors have also been suggested as a cause of AD but none of them has been proven. This condition affects about 5% of the population aged over 65 years.

It has been suggested that aluminium content of the brain is elevated in AD. This aluminium is associated with the plaques and tangles which occur in the Alzheimer's brain (Good et al., 1992). Wisniewski and Kozlowski (1982), investigated that AD patients may have an altered blood-brain barrier that allows excess aluminium to accumulate in the brain. Flow chart 1 depicts the possible mechanism of action of Aluminium induced toxicity, in neurodegenerative disorders.

In addition to evaluating the possible toxic effects of aluminium, the present study was also aimed at determining the toxicity related to chromium exposure, as well as, the synergistic or potentiated effect if any of the combined administration of both metals. Hence, the toxicity related to chromium intake is also an important aspect of this study.

While extensive clinical evidence is available for aluminium toxicity there is a paucity of information through basic research. Few experimental studies have been carried out, particularly with low doses of aluminium (Patel, 2000).
Flow chart 1: Mechanism of Aluminium Toxicity (Source: Molecular toxicity of aluminium in relation to neurodegeneration, Bharathi et al., 2008)

Chromium:

Chromium is one of the most common elements in the earth's crust and sea water and exists in the environment in several oxidation states. Chromium was first discovered in the Siberian red lead ore in 1798 by the French chemist Vauquelin. The stable forms of chromium are the trivalent Cr (III) and the hexavalent Cr (VI). Cr (VI) is considered the most toxic form of Cr which usually occurs associated with oxyanions.

According to Becquer et al. (2003), Cr(III) is less mobile, less toxic and mainly found to exits in organic matter in soil and aquatic environment. Hamilton and Wetterhahn (1988) investigated that Cr(III) is poorly absorbed regardless of the route of exposure where as Cr(VI) is more readily absorbed. Chromium acetate, Chromium chloride, Chromium oxide and chromium sulfate
are of Cr(III) state while the Cr(VI) state includes ammonium chromate, calcium chromate, potassium chromate and potassium dichromate.

**Exposure:**

Trivalent chromium has been detected in a wide range of food including egg yolk, whole grain product, coffee, nuts, green beans, broccoli, meat and some brands of wine and beer. Chromium is also present in many multivitamin and mineral supplements.

Chromium is used as catalyst in ammonia synthesis, in the production of chromium steel and chromium alloys and for electroplating. Organic compounds are used as development dyes in colour photography. Inorganic chromium compounds are used as paint pigment. Chromium (VI) salts are widely used in wood preservation and leather industry.

A number of people who live around such industries suffer indirectly through contamination of drinking water due to percolation of untreated or incompletely treated effluents into the ground. There are several such large scale industries in India and other developing countries, where pollution control laws may not be implemented effectively.

Chromium was thought to be a toxic metal until it was discovered in 1957 to be the essential part of glucose tolerance factor (GTF). GTF and chromium are vital molecule in regulating carbohydrate metabolism by enhancing insulin function for proper use of glucose in the body. GTF is composed of one chromium molecule in trivalent state, two niacin molecules and three amino acid i.e. glycine, cysteine and glutamic acid.
The U.S. National Academy of Sciences has established the recommended Daily Allowance for chromium as 50-200 μg/day for adult men and women.

Absorption of Chromium:

Trivalent chromium is very poorly absorbed (0.5 – 2 %) and many trials with animals and humans have not demonstrated toxicity where as hexavalent chromium is readily absorbed (Hamilton and Wetterhahn, 1988). According to Langard (1982), animals absorbed approximately 10% of an orally administered dose of Cr (VI). Stoecker(1999) investigated that absorption of chromium is affected by various factors in the gastrointestinal tract such as ascorbic acid and fiber components.

The absorbed chromium is distributed to various tissues of body but appears to be more concentrated in the kidneys, muscle and Liver(Hepburn and Vincent, 2003). Bragt and van Dura(1983) found that animals exposed by intratracheal injection distributed both Cr(III) and Cr(VI) throughout the body, but mainly to lungs, spleen, bone marrow, liver and kidney.

Excretion of Chromium:

Hamilton and Wetterhahn(1988) suggested that Cr(VI) undergoes enzymatic reduction, resulting in the formation of intermediates and Cr(III).Cr(VI) is reduced by ascorbic acid, thiols, glutathions, cystein, cystramine, lipoic acid , coenzyme A and coenzyme M at a significant rate.
Love (1983) investigated that chromium administered orally or intravenously is excreted mainly in the urine, whereas chromium administered by inhalation or intratracheal injection is excreted in both urine and feces. A little amount of aluminium is also excreted in milk, sweat, hair and nails (Guthrie, 1982).

Some toxic effects of Chromium:

Due to its insolubility, metallic chromium is not toxic in water. The toxicity of chromium is due to the oxidizing properties of hexavalent compounds and the pH (chromic acid has pH of 5 and ammonia dichromate has pH 13).

The hexavalent chromium in dichromate which binds non specifically to protein and nucleoproteins, is specifically taken up into red cells and platelets intracellularly. Then it is reduced to trivalent chromium but this conversion and formation of intermediate pentavalent chromium combines to produce severe free radical damage to mitochondria particularly in the kidney tubules and hepatocytes (Michie et al., 1991).

The hexavalent form of chromium crosses the cell membrane and the placenta and may be found in breast milk. Ingestion of hexavalent compounds usually leads to abdominal pains, vomiting, diarrhea and intestinal bleeding. It can cause initial circulatory collapse which leads to death (Ellenhorn, 1997) and if the patient survives the initial phase, some renal tubular damage may occur.

Chromate dust causes conjunctivitis, chronic penetrating lesions of skin, ulcerations of the nasal septum and respiratory cancer (Cooper, 1974). Acute
toxicity of chromium includes vertigo, thirst, abdominal pain, vomiting, oligurin, shock, seizures and intravascular hemolysis.

While investigating the toxicity of Aluminium and Chromium, alone and in combination, it was also thought necessary to identify a suitable ameliorating agent, which could work to overcome the induced toxicity. Antioxidants were preferred for trials.

**Antioxidants:**

Antioxidants work to protect lipids from peroxidation by radicals. Antioxidants are effective because they are willing to give up their own electrons to free radicals. When a free radical gains the electron from an antioxidant it no longer needs to attack the cell and the chain reaction of oxidation is broken (Dekkers, 1996). After donating an electron an antioxidant becomes a free radical by definition but antioxidant in this state are not harmful because they have the ability to accommodate the change in electron without becoming reactive.

There are several known antioxidants, many of which have already been investigated as possible ameliorative agents on the toxic effect of Aluminium. However the role of β-carotene in this regard has not been reported. Hence, the possible ameliorative action of β-carotene has been experimentally verified in the present study. The antioxidant properties of β-carotene are manifold as mention here.
β-carotene:

β-carotene is one of the most important naturally occurring antidotes. The name carotene was given by scientist Wachroder after he crystallized this compound from carrot root. It is a fat-soluble pigment found in fruits, grains, oil and vegetables like carrot and spinach. Alpha, Beta and Gamma carotene are considered provitamins because they can be converted to active vitamin A. After consumption, β-carotene converts to retinol, a readily usable form of vitamin A.

Deficiency of vitamin A leads to abnormal bone development, disorders of the reproductive system and Xerophthalmia (a drying condition of the cornea of eye). β-carotene is converted to retinol, which is essential for vision and is subsequently converted to retinoic acid, which is used for processes involving growth and cell differentiation. β-carotene apart from being the main source of retinol, have been reported to be potent free radical quenchers, singlet oxygen scavengers and lipid antioxidants. It has therefore been recommended that β-carotene be used for protection against certain types of human cancers and photosensitized oxidative damage.

Weitberg et al. (1985) suggested that β-carotene gave protection against oxygen free radical induced chromosome damage that was measured by frequencies of sister chromatid exchange. Gey (1994) and Kohlmeir and Hastings (1995) investigated that food rich in antioxidant vitamins and β-carotene have an important role in the prevention of cardiovascular disease as well as in cancer.
Cozzi et al. (1997) concluded that ascorbic acid and β-carotene is effective in reducing H₂O₂ induced sister chromatid exchanges. Both vitamins act as scavengers of endogenous and H₂O₂ induced reactive oxygen species.

In the present study the toxic effects of these two metals viz., Aluminium and Chromium have been investigated focusing on the biochemical and histoarchitectural changes in the target tissues such as testis, cauda epididymis, caput epididymis, seminal vesical, vas deferens, liver and kidney. Specifically the biochemical changes in protein, carbohydrate, nucleic acid metabolism/biosynthesis was investigated with special reference to evaluating oxidative stress as indicated by the level of Lipid peroxidaes, SH groups and free radical protective enzyme viz., superoxide dismutase activity. In addition the possibility of Al or Cr toxicity induced DNA damage was determined in spermatozoa using the comet assay. Specifically the toxicity of aluminium and chromium alone and in combination was evaluated by assaying sperm quantitative and qualitative changes. This included determination of cauda epididymal sperm count, motility, viability and certain functional parameters which are known to be sensitive indices of toxic exposures.

There is a paucity of information in literature on the combined toxicity of aluminium and chromium. Hence the purpose of this study was to assess the toxicity of aluminium and chromium individually as well as in combination. Certain toxicants manifest their toxicity alone while others are known to have synergistic or potentiating effects on the toxic action of another agent. The aim of the present investigation therefore was to provide evidence of possible mediated toxicity of chromium and aluminium alone and in combination. This finding has specific significance in throwing light on the probable synergistic or
potentiating action of two metal ions, whose toxicity previously has been a much neglected area of research.