1.1 An Overview

Chromium (Cr) is a naturally occurring element; it is present in soil, rock, volcanic dust and gases, animals, and plants. It exists in a variety of oxidation states ranging from -2 to +6. Most common is the hexavalent (6+) and trivalent (3+) forms (Cohen et al., 1993). It is released into environment both from natural and anthropogenic sources, but majorly is contributed by industrial emissions. The industries that contribute maximum are metal processing, tanneries, chromate production, stainless steel welding, and ferrochrome and chrome pigment production (ATSDR 2008).

Cr$^{3+}$ is also an essential nutrient required for glucose uptake and for energy metabolism. The Institute of Medicine (IOM) of the National Research Council, Washington DC, USA has identified an adequate intake of 20-45 μg Cr/day for adults (IOM 2001). Currently, the biological target for essential effects of Cr$^{3+}$ is unknown. The biological active form of chromium, called chromodulin, is referred as glucose tolerance factor (GTF) (Jacquamet et.al., 2003). Chromodulin is an oligopeptide complex and binds four Cr$^{3+}$ ions, however, the biological function of this peptide has not been established yet (Anderson 1998 and IOM 2001). It is proposed that chromodulin facilitates interaction of insulin with its cellular receptor sites. Reports of Cr$^{3+}$ deficiency are rare; there is no recognized disease (except diabetes) that is linked to its deficiency. Therefore, if Cr$^{3+}$ is a true essential element is still under debate (ATSDR 2008).

Inspite of being an essential element, hexavalent chromium (Cr$^{6+}$) is known as a human carcinogen (IARC 1990). Sufficient evidence exists for its carcinogenic
effect in humans. Cr$^{6+}$ enters the cells by facilitated uptake through anion transport channel located in cell membrane. The transport is specifically due to its structural similarity with phosphate anions. It is reduced to Cr$^{3+}$ via the intermediate forms of Cr$^{5+}$, Cr$^{4+}$. Reduction of Cr$^{6+}$ to Cr$^{3+}$ generates reactive intermediates, free radicals, and DNA-protein crosslinks. Prolonged exposure to Cr$^{6+}$ compounds can occur by ingestion, inhalation or topical contact; and is known to result in adverse health effects of respiratory, gastrointestinal, and immune system. Dermal and ocular irritation occurs via direct contact (ATSDR 2008). In USA, the Environmental Protection Agency (EPA), the Occupational Safety and Health Administration (OSHA), the food and Drug Administration (FDA), and the National Institute for Occupational Safety and Health (NIOSH) have developed recommendations for exposure to toxic substances. The federal maximum concentration level (MCL) for total Cr$^{6+}$ in drinking water is 100 μg/L, and the National Institute for Occupational Health and Safety (NIOSH, 2005) recommends an exposure limit for Cr (VI) of 1 μg/m$^3$ and an exposure limit for Cr (0), Cr (II) and Cr (III) of 500 μg/m$^3$ for a 8-hour workday, 40-hour week. The Food and Drug Administration (FDA, 2007) has determined that the chromium concentration in drinking water should not exceed 0.1 mg/L. Occupational Safety and Health Administration (OSHA, 2007) has set a legal limit for Cr$^{6+}$ of 0.0005mg/m$^3$ in air averaged over an 8-hour work day, for Cr$^{3+}$ it is 0.5mg/m$^3$ (ATSDR, 2008).

1.2 Environmental exposure to chromium in India

The mining sector is economically very important sector for many countries (Grove and Komljenovic 2007) but it is also considered as one of the most hazardous work environments in many countries around the world due to their frequent mining
injuries, illnesses and fatalities (Leigh et al., 2004). Daily employment in the Indian mining sector is 5,60,000, which includes 87% of the public sector and 13% in the private sector. The mine workers are regularly exposed to toxicants present in the mining environment such as chromium, lead, mercury, cadmium, manganese, aluminum, fluoride, arsenic, etc (Dhatrak and Nandi 2009). In India, the Sukinda valley of Odisha is one of the richest chromite producing area. It contains 98% of the countries chromite ore deposits. In Sukinda area, the lateralization process creates alkaline pore water which generates hazardous Cr$^{6+}$ contaminated water. Seventy percent of surface water sample and 60% discharge water sample contained hexavalent chromium over 0.1 mg/L. The Sukinda valley ranks fourth worst polluted places in the ‘Blacksmith Institute Pollution Report for 2007 which says, chromite mine workers are constantly exposed to contaminated dust and water which causes health problems like gastrointestinal bleeding, tuberculosis, asthma, infertility, and birth defects (Das and Singh 2011).

### 1.3 Biological effects

Lung cancer risk is prevalent in pigment chromate handlers, ferrochromium production workers, stainless steel welders, and chromeplaters (Sorahan et al., 1998). Apart from occupational cancer, risk of other kinds of adverse health effects are also reported in humans after short term or prolonged exposure through inhalation, ingestion, or topical contact; nasal itching, ulceration and perforation of nasal septum, nasal mucosa atrophy, chronic bronchitis, asthma, pneumoconiosis, skin problems like allergic dermatitis are the examples (Shelnutt 2007; Langard 1990; Olaguibel and Basomba 1989; Machle and Gregorius 1948).
Chromate compounds are cytotoxic, genotoxic, and carcinogenic in nature (Rodrigues et al., 2009; Xie et al., 2008; Xie et al., 2007; Wise et al., 2006a; Danadevi et al., 2004; Wise et al., 2002; Kowalski et al., 1996; Tsuda and Kato 1977). Mechanism of action is proposed to involve ROS generation, oxidative stress, and DNA damage; a variety of other changes like increased formation of DNA adducts and DNA-protein crosslinks, DNA strand breaks, chromosomal aberrations and instability (Hu et al., 2011; Macfie et al., 2010; Reynolds et al., 2007; Guerci et al., 2000), disruption of mitotic cell division, chromosomal aberration, premature cell division (Lai et al., 1998; Gomez et al., 1981; Koshi et al., 1979), S or G2/M cell cycle phase arrest (Wakeman et al., 2004; Katabami et al., 2000), and carcinogenesis (Rodrigues et al., 2009) are also reported in humans or experimental test systems.

1.4 Metabolism of Cr\(^{6+}\) and mechanism of its toxicity

In hexavalent form, it displays a strong oxidizing potential. In this way, Cr\(^{6+}\) interacts with cellular nucleophiles and undergoes a rapid metabolic reduction; ascorbic acid (AsA), low molecular weight thiols (e.g. glutathione, cysteine), and protein thiols are the natural nucleophiles in cells that bear the impact of Cr exposure. Cr\(^{6+}\) is rapidly reduced to Cr\(^{3+}\), forming Cr\(^{5+}\), and Cr\(^{4+}\) which can form ternary Cr-DNA adducts, DNA strand breaks (single or double). DNA double strand breaks lead to a cell cycle (G\(_2\) phase) arrest which causes both centrosome amplification and spindle assembly checkpoint bypass and could lead to aneuploidy and neoplastic transformation or cancer.
1.5 Amelioration efforts

During reductive metabolism, when reactive oxygen species (ROS) are generated, antioxidants, including enzymatic system (superoxide dismutase, catalase and glutathione peroxidase) and non enzymatic antioxidants system (ascorbate, vitamin E, thioredoxin and glutathione) will protect the body from the oxidative stress (Mates et al., 1999; McCall and Frei 1999). Supplementation of non enzymatic antioxidants are ideal to increase the protection of cells from oxidative stress due to easy dietary administration. For abrogation of Cr toxicity, targeting ROS-scavenging and oxidative stress mitigation have been endeavoured. Antioxidants, e.g. vitamin C, vitamin E, n-acetyl cysteine (NAC), alpha-lipoic Acid (LA), pyrrolidine-dithiocarbamate (PDTC), and poly-phenolic compounds e.g. epilgallo-catechin-3-gallate (EGCG) have been examined for their chemo preventive and therapeutic potential both in vitro and in vivo (Qi et al., 2000; Shi et al., 2000a; Shi et al., 2000b; Budwar and Kumar 2005a; 2005b). Several reports revealed the effectiveness of antioxidants to reduce toxicity in humans and animals (Nakamura et al., 1998; Sugiyama 1991). In last decade, few microarray based studies were conducted by other researchers to identify the Cr\textsuperscript{6+} related gene expression alterations, but mitigation of toxic effects (i.e. cell transformation, gene and pathway dysregulations) has rarely been evaluated. In light of these facts, following aims and objectives were proposed.

1.6 Aims and Objectives

- Study global gene expression profile in Cr\textsuperscript{6+} transformed cells and its modulation by antioxidants.
• Characterize, quantify and validate altered genes expression using qPCR

• Study comparative experimental-therapeutics potential of select antioxidants for mitigation of Cr$^{6+}$ genotoxicity and carcinogenicity.

1.7 Study Plan

1.7.1 Study in Cr$^{6+}$ transformed mouse peritoneal macrophage cells in vivo.

We determined the cell transforming dose of Cr and studied changes in global gene expression profile of Cr$^{6+}$ transformed mouse peritoneal macrophages in Host Mediated Cell Transformation Assay (Massa et al., 1990). Cell transformation was characterized by soft agar assay which characterizes acquisition of the anchorage independent cell growth potential in the transformed cells. In addition to the Giemsa staining method, the Cytoselect™ based method was also used to quantify soft agar assay. Fc receptor analysis was done for further characterization of transformed cells. Altered genomic profile of cells was investigated using microarray approach and validation of altered genes expression was done by quantitative Real-Time PCR (qPCR). Mitigation of cell transformation was investigated by the co-administration of antioxidants (Vitamin C or Vitamin E or Alpha Lipoic Acid) and the toxicant (Cr$^{6+}$). Quantitative Real-Time PCR (qPCR) was used for comparative study of the ameliorative effect of antioxidants (Chapter 3).
1.7.2 Study in Cr\(^{6+}\) transformed C3H10T1/2 mouse embryo fibroblast cells in vitro.

We determined the cell transforming dose of Cr and studied changes in global gene expression profile of Cr\(^{6+}\) transformed C3H10T1/2 mouse embryo fibroblast cells. Cell transformation was characterized by soft agar assay which characterizes acquisition of the anchorage independent cell growth potential in the transformed cells. Altered genomic profile of cells was investigated using microarray approach and validation of altered genes expression was done by quantitative Real-Time PCR (qPCR). Mitigation of cell transformation was investigated by the co-administration of antioxidants (Vitamin C or Vitamin E or Alpha Lipoic Acid) and the toxicant (Cr\(^{6+}\)). Quantitative Real-Time PCR (qPCR) was used for comparative study of the ameliorative effect of antioxidants. Results of this study (C3H10T1/2) were also validated in another mouse cell line BALB/c 3T3 (Chapter 4).