Summary and Conclusions

Hexavalent chromium (Cr$^{6+}$) is a toxic metal; sufficient evidence exists for its carcinogenic effect in humans. Apart from cancer, risks of other kinds of adverse health effects are also reported. Toxicity occurs after short or prolonged exposure through inhalation, ingestion, or topical routes. Cr$^{6+}$ is understood to induce toxic effects via its metabolic reduction to Cr$^{3+}$, the resultant reactive oxygen species (ROS) generation, mutagenesis, DNA damage, epigenetic changes, and cell cycle alteration. The molecular basis of these changes culminating into biological / clinical effects, tissue lesions, or cancers has sporadically been examined. In relevant literature, the altered cellular gene expression profile has been proposed as the possible molecular basis navigating the onset or progress of Cr$^{6+}$ induced morbidities. Several studies using gene microarray have shown selective and strategic dysregulations of cellular genes and pathways induced by Cr$^{6+}$. These studies have provided a comprehensive view of critically altered genomic profile of Cr$^{6+}$ exposed cell that may lead to cell transformation, tumourogenesis or carcinogenesis. In this study toxicogenomics of Cr$^{6+}$ carcinogenesis has been investigated; the ameliorative potential of antioxidants on Cr$^{6+}$ induced cell transformation and gene dysregulation has also been studied.

Our aim was to elucidate the genomic profile of cell transformed by Cr$^{6+}$ both in vivo (using mouse peritoneal macrophages) and in vitro (using C3H10T1/2 and BALB/c 3T3 cells). The attenuation of transformation and gene dysregulation has been endeavoured using antioxidants Vitamin C, Vitamin E and Alpha lipoic acid. The proposed aims were as follows:
Summary and Conclusions

- Study global gene expression profile in Cr$^{6+}$ 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.

The conclusions are as follows:-

(a) Cr$^{6+}$ at test doses of (35 & 70 µM) induces cell transformation under in vivo conditions (using mouse peritoneal macrophages) and at test doses of Cr$^{6+}$ (0.01 &1µM) under in vitro conditions (using C3H10T1/2 & BALB/c cells).

(b) Cr$^{6+}$ dysregulated the gene expression profile of cells transformed at all the test doses. A large numbers of genes dysregulated in a dose dependent manner. RT-PCR based validation of select genes from microarray data shows similar results.

(c) Genes dysregulated in vivo at 35 or 70uM dose are involved in pathways of growth, oxidative stress, tumour suppression, apoptosis, DNA repair, cell cycle regulation, metabolism & biosynthesis, immune system, signal transduction, cell adhesion, cytoskeleton and morphogenesis. Genes dysregulated in vitro at 0.01 &1µM dose participate in different pathways i.e. DNA repair, cell cycle, tumour suppression, cell adhesion & cytoskeleton, immune-regulation, biosynthesis, and energy metabolism. The difference in number of participated genes and pathways could be due to the different test systems, test dose/concentrations, and exposure duration.
(d) Co-administration of antioxidants (Vitamin C or vitamin E or Alpha lipoic acid) and Cr$^{6+}$ in equimolar concentrations prevented cell transformation as well as the transcriptional dysregulation. Alpha lipoic acid provided greatest protection against Cr$^{6+}$ induced cell transformation & gene alteration in comparison to vitamin C & E. At test doses below PEL, Cr$^{6+}$ activated the phosphorylation of extracellular signal related kinase (ERK) and α- LA prevented the change.

(e) The influenced pathways seemed to be crucial for progression as well as mitigation of Cr$^{6+}$ toxicity and indicate their crucial role in mechanism of anti-carcinogenic action of these antioxidants. Influenced genes can be considered as the unique molecular signatures of exposure to carcinogenic dose of Cr$^{6+}$.

(f) This study emphasizes the pharmacotherapeutics efficiency of lipoic acid against Cr$^{6+}$ carcinogenesis in vivo and in vitro.