Chapter-1

General Introduction

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1.0 Introduction

With the increase of urbanization and industrialization in rapid pace, living bodies as well as human beings are exposed in excess to polluted environment. Among several pollutants, heavy metals including chromium show adverse effects on human health (Hopkins, 1991). In foremost, chromium, seventh most abundant metal available in the crust of the earth surface and a potent environmental contaminant, primarily in the form of a range of alloys are used widely in various industries for prolonged times. In the environment, chromium (Cr) is available in two different forms of oxidation states; one is trivalent form of chromium [Cr (III)] and other one is hexavalent form chromium [Cr (VI)]. Compounds of Cr (III) are extensively used in different industries, cause much mucosal irritations and cutaneous allergies, but has no carcinogenic effect, whereas Cr (VI) can take action as sensitive allergens and carcinogenic agents to humans (Dayan and Paine, 2001). Excessive exposure has been noticed in association with chromate production (Hopkins, 1991). In nature, chromate elements are toxic to cells, toxic to genes and also carcinogenic. In response to cytotoxic, genotoxic, and carcinogenic effect; chromates are proposed to affect through generation of strongly reactive oxygen species (ROS), damage of DNA and several changes like DNA-protein crosslink, DNA strand breaking and chromosomal instability (Dayan and Paine, 2001).

It has been well accepted that most of those toxic effects are mainly due to hexavalent Chromium. Many experimental studies have clearly indicated about the increase incidence of dermatitis, lung diseases and various cancers in the allied industries using chromium compounds. In various other experimental research and biochemical studies regarding incidence, explorable causes and prevention of skin

allergy, lung cancer and asthma clearly correlate the mechanisms of those toxic effects with wide exposure to chromium in such industries. The published literatures from 1985 to 2000 have been reviewed to acquire the knowledge about the probable role in pathogenesis of cutaneous allergies, various cancers and irritant toxic hazards of chromium (Dayan and Paine, 2001).

Physical and Biochemical properties of chromium

Chromium ions (relative atomic mass 51.996,atomic number 24) remain in different states of oxidation from +2 to +6, but only elemental metal form (0 valence), divalent chromium (Cr^{2+}), trivalent chromium (Cr^{3+}) and hexavalent chromium (Cr^{6+}) states are commonly found. Most unstable divalent chromium (Cr^{2+}) is easily oxidized to the trivalent (Cr^{3+}) form in atmosphere [Fig.1]. Trivalent Cr (III) and hexavalent Cr (VI) are useful for human health and prevention of illness. It is important to understand the different oxidation states of chromium which have varied physical and chemical properties and hence wide biological effects on living beings. The electrical potential difference between Cr (III) and Cr (VI) shows the hexavalent chromium has strong oxidizing potential energy than trivalent chromium. For oxidation of trivalent chromium to hexavalent chromium required energy is [1.33 eV] and it rarely occurs in biological systems. In the contrary, Cr (VI) reduces spontaneously in the living cells of the organism unless remain in insoluble complex form. Immediately after gaining entry in circulation, few of the entered Cr (VI) are reduced to Cr (III) in plasma of blood and other Cr (VI) ions after penetrating the erythrocyte membrane are reduced to Cr (III).Then reduced trivalent chromium ions are irreversibly bound to intracellular constituents of red blood corpuscles (Dayan and Paine, 2001).

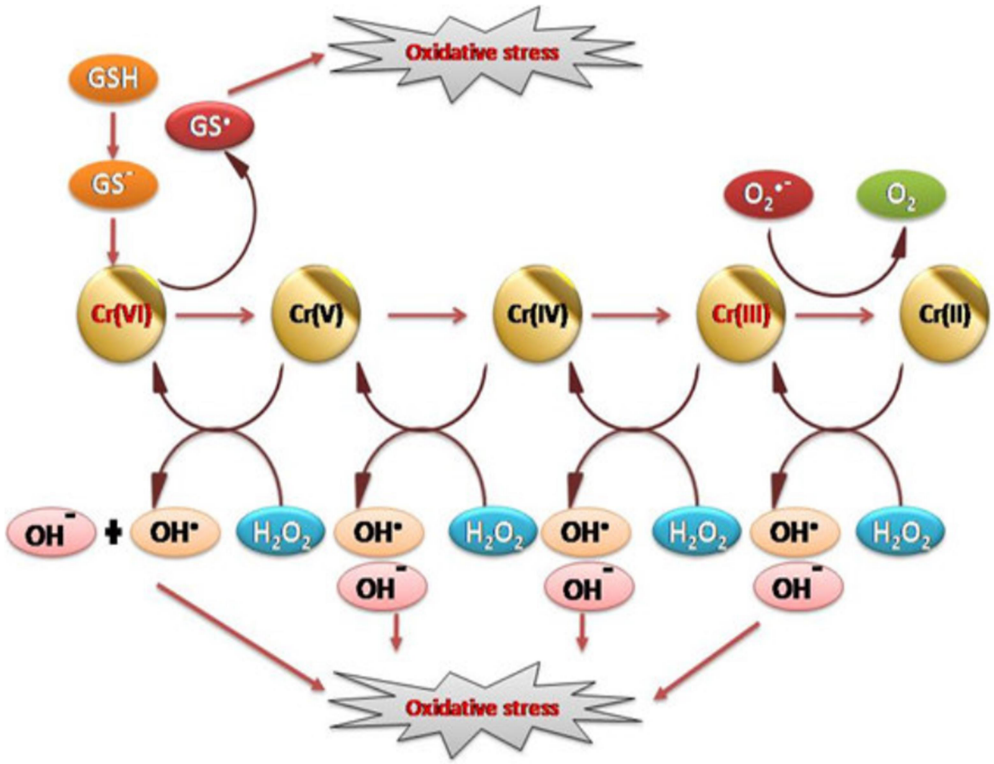

Figure 1: Diagrammatic presentation of chromium ion reduction and free radical generation.

Human exposure to chromium

In the adult human being, about 50–70 mg of soluble chromate compounds per kilogram of body weight is lethal oral dosage. Cr (III) in the dose of (50–200 microgram/day) is essential as one of the important micronutrients for insulin secretion and action for maintaining glucose metabolism. Exposure of Cr (VI) is possible through various routes in the human body and distributed throughout the body. They undergo intracellular metabolism and then eliminated through urine, faeces and exhalation (Fig-2). Toxicity is possible when there is either excess

accumulation of Cr (VI) compounds and intermediate metabolites beyond elimination or inadequate detoxification process.

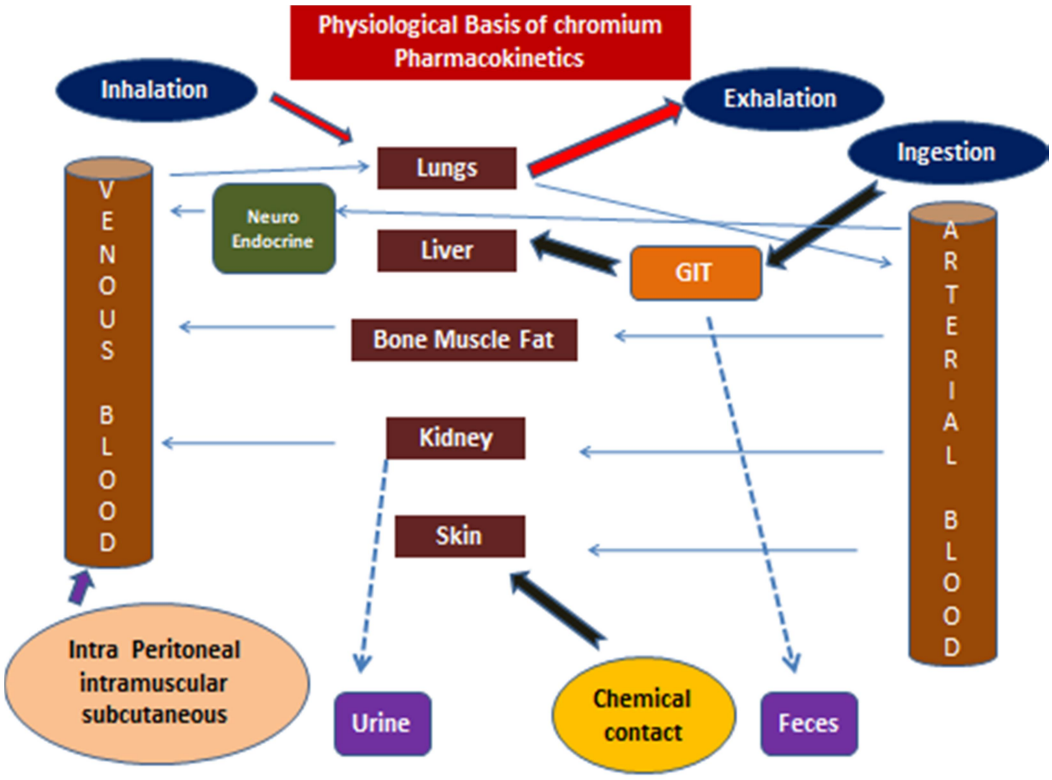

Figure 2: Exposure and elimination routes of chromium (VI).

Absorption, distribution, metabolism and excretion

After exposure to Chromium by inhalation in humans, increased urinary excretion of Cr (III) indicates higher respiratory absorption. However, the amount of the uptake of Cr (III) through lungs is mainly depended on the nature of the Chromium compound (Kiilunen et al, 1983). Various animal experiments show that Cr (III) salts are poorly absorbed after exposure through oral and dermal than inhalation routes (Myers, 1999). After oral application of tracer doses of sodium chromate salt, only 10% of the administered doses have shown to be absorbed through alimentary

tract. After administration of radioactive chromium via duodenum, almost half of the administered doses are excreted in faeces and only 10% is found in urine in the first 24 hours. This clearly correlates slower absorption through gastrointestinal tract. The process of reduction of Cr (VI) to the trivalent chromium form is clearly demonstrated (Donaldson and Barreras, 1966). There is significant correlation between pulmonary exposure to Cr (VI) and its excretion through kidneys in people working in chrome plating and wielding industries (Aitio et al, 1988). Uptake of the chromium compound by the respiratory tract mainly depends on its solubility (Aitio et al, 1988). In the lower respiratory tract, Cr (VI) is reduced by the epithelial cells and alveolar macrophages. In contrast to Cr (V), after entering in the circulation, Cr (VI) is immediately transformed into Cr (III) which is bound to transferrin, a plasma protein. Other few Cr (VI) ions, after reaching the blood stream, is selectively taken up by the erythrocytes, then reduced to Cr (III), and bound irreversibly to haemoglobin. Reduction of Cr (VI) and binding with haemoglobin during transport in the circulation corroborates with the findings that only Cr (III) is excreted in the urine. The important biological effects of hexavalent chromium by analysing the results of exposure have been reviewed by Aitio et al, (1988). Chromium is retained within the skin following topical dermal application of chromium compound in the form of sodium chromate (Dayan and Paine, 2001).

Exposure through Gastrointestinal tract

Oral uptake of chromium does not render much toxicity problem, because of faecal elimination of more than half of the absorbed chromium through gastrointestinal tract. Acute poisoning after ingestion of chromium nausea, vomiting, watery diarrhoea, haemorrhage into the gastrointestinal tract, blood loss with dehydration

causing cardiovascular shock are clinically seen in majority of the affected people (WHO, 1990). Thereafter, in patients who survive more than 8 days, necrosis of liver and kidneys are the major toxic effects of oral intake of lethal doses of chromium. When chromium is experimentally administered through parenteral routes in animals, teratogenicity is commonly observed but not so much birth defects are seen in human exposure to Chromium (Clarkson et al, 1985).

Dermal exposure

Chromium exposure to skin usually causes chronic cutaneous ulcers and acute itchy dermatitis in workers regularly exposed to chromium compounds (Clarkson et al, 1985). Chromates and Cr (VI) from chrome alloys, paints and chrome plated objects commonly induce allergic skin diseases, like contact dermatitis. It is assumed that hexavalent chromium is essential for early dermal sensitization, but both Cr (VI) and Cr (III) may perpetuate chronic dermatitis in those individuals who were sensitised early. The primary irritant actions on skin and epithelia are mainly due to strong oxidizing properties and acidic nature of soluble chromium ions (Dayan and Paine, 2001).

Respiratory exposure

Cr (VI) inhaled through respiratory tract can easily be deposited on the epithelial lining fluid in the airways and cause irritation. Not only mucosal irritation of bronchi but also necrosis and the septal perforation of nose are noticed frequently in employees in the industries using chromate and Cr (VI) compounds. As per report of International Agency for Research on Cancer, employees exposed to Cr (VI) are prone to excess risk for occurrence of some rare tumors like sino nasal neoplasia,

rhinitis, pneumonia and bronchospasm etc. The probable mechanism of pathogenesis of those pulmonary illnesses is loss and impairment of mucocilliary functions of respiratory airways during respiration. Though the exact role of chromium is not certain in such workers, because they are exposed to various other chemicals too (WHO, 1990). However, occupational exposure to chromium compounds clearly confirms the excess incidence of bronchial asthma in certain industries. Epidemiological studies have shown a constant relation between excess risks for development of lung cancer to chromium exposure in workers of chromate processing industry. Also some experimental studies in animals have proved a definitive role of Cr (VI) for lung cancer, mainly by pulmonary inhalation but not through oral uptake or dermal route. There is no conclusive proof either from experimental studies or observational analysis in animals and humans for development of cancer due to trivalent chromium compounds or elemental metallic chromium (Hopkins, 1991).

General mechanisms of chromium toxicity

It is a well-known fact to all that hexavalent Cr (VI) is usually more toxic than trivalent Cr (III). Cr (VI) can be readily transported across the cell membranes specifically aided by different membrane carrier systems, like carboxylate, phosphate and sulphate. The intracellular uptake of Cr (VI) administered in the form of sodium dichromate is dependent on the rate of reduction to Cr (III). Potentially more soluble form of Cr (VI) undergoes serial intracellular reduction through production of short-lived Cr (V), Cr (IV) and eventually reduced to trivalent chromium ions which have greater binding affinities to different intra cellular constituents. During this sequential reduction process of chromium compounds,

various highly reactive oxygen species (ROS) are generated and huge oxidative stress is created within the cell (Fig-1). Glutathione and cysteine mediated antioxidant mechanism stabilizes oxidative stress and prevents cellular damages (Dayan and Paine, 2001). However, this Cr (VI) reduction actually serves as a useful detoxification process. Once chromium is absorbed and ultimately reduced to Cr (III) which is accumulated in different tissues beyond the capability of detoxification and elimination, results in wide spectrum toxic effects of chromium compounds. Mitochondria and microsomes play significant role in intracellular reduction of Cr (VI). Ascorbate (vitamin C) in presence of important cofactors like NAD/NADH, cytochrome P450, haemoglobin and enzyme glutathione reductase actively hasten the chromium Cr (IV) reduction process (Hopkins, 1991).

Genotoxicity by chromium:

Water solubility of various chromium compounds is different. Cr (VI) compounds are potentially more water soluble than Cr (III) compounds. Numerous genetic studies show a wide range of effects on genes consistently by both of those chromium compounds (WHO, 1990). Cr (III) compounds generally have much toxicity than Cr (VI) compound in isolated cell nuclei and purified DNA. Besides, *in-Vivo* cell line test system experiments, Cr (III) gives less positive results. More often strict treatment conditions and very high dose of Cr (III) exposure are needed to get the equivalent genetic effects caused by Cr (VI) compounds. DNA damage is not seen in the intact animal cells after chromic chloride administration and micronuclei are not formed in cells after chromic nitrate administration. Compounds of trivalent chromium generally do not produce cellular transformation, gene mutation, DNA damages, exchange of genetic materials between sister chromatids

in cultured animal and human cells, but chromosomal aberrations are often seen with high concentration of Cr (III) compounds. Cr (VI), which is a strong oxidizing agent, breaks the DNA polynucleotide chain; whereas trivalent Cr (III) physio-chemically alters nucleic acids interacting with the phosphate groups and nitrogen bases. Breaking of DNA strands, DNA-DNA interaction, DNA -protein cross-linking and nucleotide modification, such as 8-hydroxyguanine indicate generation of free oxygen radicals by Cr (VI). However, these reactions are not seen in cell-free experimental systems without presence of reducing agents. Strongly reactive intermediate oxidants such as Cr (V) and Cr (IV) generated during intracellular Cr (VI) reduction are principally responsible for the noted genotoxicity. Intracellular reducing agents such as Vitamin-C and sulfhydryl compounds like cysteine and glutathione modify Cr (VI) reduction (Dayan and Paine, 2001). Although hydroxyl, thionyl and cysteinyl radicals are formed during Cr (VI) reduction, it is not clearly known whether these intermediate reactants have any role in development of cancers in chromium exposure (Standeven and Wetterhahn, 1991). The highly reactive free radicals generated during chromium reduction reactions inside cells activate the important transcription factor NFk β which plays as a crucial gene activator involved in pathogenesis of apoptosis, inflammation and immunity. Thus, chromium induced pathogenesis of various cancer is a poorly explained complex mechanism (Dayan and Paine, 2001). Accordingly, although it is now well established that Cr (VI) inhalation may cause cancer, the mechanisms involved, the exact role of other valence states of chromium and their solubility are still debatable.

Reactive oxygen species

Reactive oxygen species (ROS) are very important group of free radical substances (Halliwell, 2007). ROS and reactive nitrogen species (RNS) may play dual role of either harmful or beneficial in living organisms (Guo et al, 2013). One example of beneficial role of ROS is induction of cell mitogenesis at very low concentrations. In contrary, at high concentrations, ROS can act as intermediaries to break cell architecture, per oxidation of membrane lipids, proteins and breaking nucleic acid chains (Droge, 2002). Various non-enzymatic and intracellular enzymatic antioxidants try to salvage the ROS mediated adverse effects in tissues (Halliwell et al, 1992).

Oxidative stress in biological system can simply be defined as the imbalance between the production of reactive oxygen species and antioxidant defence mechanism of the body (Mittler, 2002). There is enough evidence confirming heavy metals, like iron, lead, cadmium, nickel, chromium, mercury, copper, aluminium and vanadium are capable to generate ROS / RNS and cause lipid peroxidation, alteration of calcium homeostasis, DNA damage, intracellular depletion of antioxidants of sulfhydryl groups (Ranjbar et al, 2008). The mitochondria are very sensitive to free radical induced stress, prone to altered mitochondrial transcription, lipid per oxidation and kinetics of energy production. Oxidative stress induced mitochondrial impairment and damage play a central role in development of heavy metals toxicities (Ott et al, 2007; Orrenius et al, 2007).

Metals-induced oxidative stress

Free radicals are defined as particular ionic state of atoms or molecules containing one or more unpaired electrons. The toxicity of xenobiotics, usually heavy metals including chromium are associated with generation of many reactive free radicals which cause tissue toxicities and development of many diseases (Mittler, 2002). The possible role and mechanism of oxidative cellular damage may explain pathogenesis of metal toxicity (Huang et al, 2004). Augmented ROS generation has been suggested to contribute to the heavy metal induced toxicities at high dose, including lead, cobalt, chromium, mercury, nickel, aluminium, vanadium and molybdenum, and, also other few elements such as arsenic and selenium (Valko et al, 2005), However, the evidence is lacking and poorly convincing for elucidating primary role of oxidative stress related toxicity heavy of metals. Although increased lipid per oxidation has been demonstrated in isolated cells and tissues exposed to metals, primarily depletion of intracellular antioxidants by the metals may cause tissue injury and contribute to metal toxicity (Jomova and Valko, 2011). Several research studies have mainly focused on heavy metal-induced tissue toxicities and carcinogenesis, explaining the enhanced production of oxidative ROS / free radicals in living biological systems (Jomova and Valko, 2011).

Mitochondria: The key source of cellular oxidative stress

Mitochondria are the principal cytoplasmic organelle for cellular uptake and utilization of Oxygen to produce energy currency of body, ATP. As estimated (Hansford et al, 1997), about 0.2–2% of the oxygen is converted to ROS by mitochondria, mainly through generation of superoxide anions. On average 85–90% of oxygen entering into a cell are consumed by mitochondria to undergo

oxidative phosphorylation for synthesis of adenosine triphosphate (Requejo et al, 2010). Hence, the mitochondrial electron transport chain serves as a store of ROS, ensuing as a major source of the disproportionate superoxide anions (Ranjbar et al, 2010). Many studies have clearly depicted the sites of ROS production along the respiratory chain (Starkov et al, 2004). Complex I and complex III of electron transport chain are two major sites of superoxide anion production (Jang *et al*, 2010). As described in previously published reports (Abdollahi et al, 2004), oxidative stress usually refers to both oxidative stress and oxidative damage and impact on cell signalling, nuclear transcription and other intracellular homeostatic processes; the term has also encompassed the oxidative effects of RNS. There are at least three distinct superoxide dismutase isoenzymes, one manganese form (Mn-SOD) present in the mitochondrial matrix. Out of the other two isoenzymes, copper (Cu –SOD) and zinc forms (Zn-SOD), first one is located only in cytoplasm of the cell and the second one is distributed in various extracellular fluids respectively (Young and Woodside, 2001). SOD catalyses the dismutation reaction of superoxide $O_2^{\bullet-}$ to O_2 and hydrogen peroxide (H_2O_2). Thereafter hydrogen peroxides are removed by Glutathione peroxidase (GPx) and catalase (CAT). In the presence of oxidising metals, H_2O_2 can be converted to highly reactive hydroxyl ($OH^{\bullet}$) radical (Yon et al, 2008; Fantel et al, 1998). Metabolizing water and corresponding alcohols (R-OH) need to reduce H_2O_2 and generation of a wide range of organic hydro peroxides (-ROOH) through some reactions catalysed by GPx. Another group of reactive Nitric oxide ($NO^{\bullet}$) radical is responsible for diverse physiological processes within the mammalian cell. ($NO^{\bullet}$) acts as an oxidative signalling molecule, having a significant ability to control a wide variety of physiological functions such as neurotransmission, regulation of blood pressure,

protective immune mechanisms, smooth muscle contraction (Szabo et al, 2007). Nitric oxide ($NO^{\bullet}$) is generated by the nitric oxide synthetase (NOS) and has a very short half-life of only a few seconds in an aqueous medium. Leaving aside those above free radicals, in certain circumstances, oxidizing peroxynitrite anion ($-ONOO^{-}$) free radicals are produced through binding ($NO^{\bullet}$) with the $O_2^{\bullet-}$, being capable of DNA fragmentation and lipid peroxidation (Webb et al, 2008).

Epigenetic and oncogene activation by Cr (VI) exposure

Previously non-microarray based studies have been used to report the function of mutations in oncogene. The reports have suggested that several oncogenes like ras, p53, Bcl-2; cyclin-D1 altered their expression in Cr (VI) carcinogenesis. This possibility has been supported after several research studies, conducted in experimental test systems or cancer tissues of Cr (VI) exposed workers. In lung cancer due to exposure of Cr (VI), activated ras oncogene has been observed. Though it is considered a rare event and not involved in Cr (VI) carcinogenesis. Changes in Bcl-2 and p53 expression level are noted although these have been found to be nonspecific to Cr (VI) carcinogenesis (Katabami et al, 2000). Further investigations have revealed correlation of mutant of p53 gene to cancer of lung in workers exposed to chromate compounds (Kondo et al, 1997). It has been illustrated that the elevated levels of pantropic p53 (pan-p53) proteins in serum of Cr (VI) workers (Hanaoka et al, 1997); and induction of p53 level up to 6-fold in Cr (VI) exposed human lung fibroblasts (Carlisle et al, 2000). The principal role of p53 gene in chromate toxicity or carcinogenesis has been demonstrated using p53 deficient transgenic mice (Bagchi et al, 2002); interventional studies have revealed that the loss of crucial p53 gene increased the genomic DNA fragmentation (Bagchi et al,

2001). The toxic effects of short term high dose (0.05 and 0.25 μM) Cr (VI) exposure on benzo alpha pyrene (B-a-P), shows DNA damage directed gene alteration in mouse hepatoma cells (Fan et al, 2012). RTPCR based analysis shows up regulation in genes related to apoptosis (Aifm, Bid, Bak, Bcl2, Fas, Apaf1, Tnf, Bax), cell cycle control (Rad17, Mdc1), tumor suppression (p15, p16, p18, p19, p21, p27), DNA damage (Brca1, Brca2, ATM, Gadd45, Mgmt) and down-regulation in genes related to drug metabolism (Cyp1b1, Cyp1z2, Gsta1, Nqo1, Aldh3, Cyp1a1). In an earlier study, the exposure of Cr (VI) has been found to increase the carcinogen-DNA adduct formation in mouse hepatoma cells (Schnekenburger et al, 2007). These observations indicate that Cr (VI) exposure facilitated the carcinogen-DNA adducts formation causing DNA damage. With respect to epigenetic changes, Cr (VI) induced methylation of p16 promoter and repression of DNA-mismatch-repair or tumour suppressor genes mut L homologue 1 (MLH1) and MLH2 have been reported besides the genetic instability in chromate lung cancer (Kondo et al, 2006). Sun et al (2009), have reported an increase in protein as well as mRNA level of G9a. G9a is a histone methyl-transferase enzyme that able to methylate H3K9 (histone H3 lysine 9) and accounted for global elevation of its dimethylated type and silencing of tumour suppressor gene MLH1 transcription. Others also show that Cr (VI) repressed the transcription co-activators (Kimura, 2010). Klein et al, (2002) have shown methylation of genes and modulation of gene cyclin-D1 by Cr (VI) in transgenic cells; study has revealed the responsiveness of cell cycle regulation to the toxic metal. A crucial role of cyclin D1 in Cr (VI) toxicity has been noticed in a study on ex-chromate workers affected with lung cancer wherein cyclin-D1 expression is found to be more as compared to non-exposed subjects harbouring other disease like pneumoconiosis. The altered expression of ATM (ataxia

telangiectasia mutated) gene (Ha et al, 2003), aneuploidy and dysregulation in spindle assembly checkpoint bypass are reported in Cr6+ exposed cells (Wise et al, 2006) ; these changes normally carry apoptosis, cell cycle ruling, because these are rudiments of cells responding to DNA damage and to genomic instability. In cell signalling MAPK (mitogen activated protein kinase) pathway, activation of ERK, JNK, p38 (regulators of cell differentiation and growth, proliferation, as well as apoptosis) have been observed. The activation of changes depends on toxicant's profiles, resulting ROS generation or oxidative stress (Tessier and Pascal, 2006). Their activation has also been reported in Cr (VI) exposed mouse embryonic stem cells (Chen et al, 2009) ; lower level of toxicant activated JNK (c-Jun-N-terminal kinase) via leukocyte C-terminal Src kinase (LCK), an associate of the Src family unit of protein tyrosine kinases or the Fyn-Cas-Crk (FAK/Src-Yes-Fyn/p130 CAS/CRK) signalling cascade. LCK can activate STAT3 (signal transducer and activator of transcription) and interleukin-6 (IL-6) which has contributed to inflammation and cancer (O'hara et al, 2007). Another study investigating ROS dependent changes has observed that Cr (VI) exposure activated nuclear factor kappa beta (Nfkβ) and p38 pathway. Nfkβ is essential for apoptosis. It has also measured as an indicator of Cr (VI) induced cytotoxicity (Liu et al, 2001). Using cultured cells, investigators have also showed activation of activator protein-1 (AP-1) but HOGG1 (8-oxoguanine DNA glycosylase) gene has been found to be unbiased. It is conditional that Nfkβ does not participate in tumour genesis; it is somewhat linked with a diminished cell proliferation and initiation of apoptosis (Kim et al, 2003). Over expression of inflammation specific COX-2 via Nfk β / c-Jun / AP-1 mediated pathway has been observed in normal epithelial cells of human bronchi and embryonic fibroblasts of mouse after Cr (VI) exposure (Zuo et al,

2012). The signalling molecule, vascular endothelial growth factor (VEGF) has been found to be over expressed by Cr (VI) exposure. The VEGF is involved in angiogenesis. It is usually over articulated in lung cancer, and used as prognostic marker (Papaioannou et al, 2006). One study on the divergence has showed the repression of VEGF expression by Cr (VI) exposure. In signalling pathway, other types of genes that are activated in response to Cr (VI) are Fyn and LCK and the initiation of an interferon signalling mechanism (Nemec and Barchowsky, 2009). Activation of AKT (α serine threonine protein kinase) has also been noticed by Cr (VI) in human lung fibroblast transformation. AKT is known to override G1/S checkpoint bypass, prevent Cr (VI) induced decrease in localization of retinoblastoma protein and p27 (cycline dependent kinase inhibitor 1B) the key factors of G1/S checkpoint, and contribute to toxicant induced genomic instability (Lal et al, 2009). Levels of Apo J / CLU (a senescence biomarker apolipoprotien J and an oxidative stress responsive gene protein (clusterin) in serum are noted to be high in shipyard wielders during the oxidative stress and are found to be lower after worksite intervention (Alexopoulos et al, 2008).

Genomic alteration by Chromium exposure

In the foremost work on Cr (VI) induced cellular gene expression modulation, Ye and Shi et al, (2001) have examined genomics in human lung type II epithelial A549 cell. By following microarray of 2400 genes after using potassium dichromate as a source of Cr (VI), it has been investigated that the molecular basis of Cr (VI) provoked ROS generation and the resultant oxidative tension, and have established a significant dysregulation of 220 genes which are part of the pathways of oxidative stress, apoptosis, and also carcinogenesis. In stress response pathway, an

unregulated transcription has been seen in Cu/Zn superoxide dismutase (SOD), GPx, metal-regulatory transcription factor-1, p53, heat shock proteins (HSP60, HSP70, HSP75), and activating transcription factor-3. The oxidative stress reactive proteins seclude the right conformation of lately produced proteins; their main function is to prevent tissue damage from ROS mediated oxidative stress and preserves the vitality of cells. In apoptosis pathway, Ye and Shi et al, (2001) have reported up regulation of only the hSIAH1 gene. Functionally, this apoptotic gene has been facilitated to label the protein for proteasomal degradation and the programmed cell death through induction of p53 signalling during Cr (VI) induced stress. In cell cycle regulatory pathway, the unregulated genes have transcribed protein products significantly for cellular endurance, cell division, cell proliferation and differentiation, G1 phase arrest in cell cycle, and tumour suppressor function. By underneath expression of the individual proteins, the down regulated genes have deregulated cell cycle control via hold back of cyclin dependent kinase activation causing cell cycle arrest, and demanding epithelial cell veracity for apoptosis. In DNA repair and metabolism pathway, only three genes are found to be dysregulated. The up regulated gene encoded polymerase has been involved in DNA repair. The down regulated casein kinase 2 (CK2) and cell division cycle (CDC) have decreased the function of serine / threonine kinase. It is also required for DNA replication. To standardize the signalling mechanisms in cell propagation and growth, cell cycle dependent kinase 4 (CDK4), CDK5 binds with CK2/CDC45 encoded a protein (Nigam et al, 2014).

The toxicology of inorganic metal compounds has become ubiquitous fields of research in environmental pollution, occupational health and clinical

pharmacotherapuetics. With numerous industrial applications of chromium, the adverse effect of chromium compounds in human health is dangerous (Baruthio, 1992).

***Andrgraphis paniculata* (Burm.f) Wall.ex Nees**: **Wonder Indian Herbs**

India has been always applauded for its praiseworthy rich ancient cultural history, knowledge and skills in all areas of human development. Since predawn era of the human civilisation, India has great natural resources and knowledge about traditional alternative systems of medicine and also acknowledged globally. *Andrographis paniculata* Nees (Family- Acanthaceae) is one of the extensively studied species of Genus *Andrgraphis* for its huge medicinal values and it has been used for centuries in several traditional systems of medicine, namely Ayurveda, Siddha, Unani, Homeopathy, Naturopathy etc. *Andrographis paniculata* Nees, also known as 'King of Bitters', is an annual herb and is extensively grown in tropical South Asian countries like India, Pakistan, China, Srilanka, Myanmar and Malaysia(Vijaykumar et al, 2007) and also in some parts of Europe. In traditional folk medicine and home remedies, *Andrographis paniculata* is commonly used to lower high body temperature, remove toxins from the body; cure common cold and flu (Gabrielian et al, 2002) and as an anti-snake venom agent and insect bites (Samy et al, 2008). The plant also exhibits various other biological activities *in vivo* as well as *in vitro* viz., antiviral (Wiart et al, 2005), antibacterial (Roy et al, 2010; Abubakar et al, 2011), immunomodulating / immunostimulatory (Calabrese et al, 2000), anti-inflammatory (Wen et al, 2010), anti-Human Immunodeficiency virus (Chao et al, 2010) and antineoplastic (Li et al, 2007). The plant shows potential therapeutic

action in curing liver disorders, cough and common cold in humans (Geethangili et al, 2008).

In Indian Ayurveda system of medicine, it is used as a bitter elixir for management of diabetes and debility, pain abdomen reliever, hepatic disorders and as an anti-helminthic (Bensky and Gamble, 1993). *Andrographis paniculata* extract has been used traditionally in various forms, such as tablet, syrup or injection. In Indian pharmacopoeia, about 26 Ayurvedic formulations are recorded (Maunwongyathi, 1994). Unani system of medicine also apply this plants useful as carminative, gastric and liver tonic, laxatives, anti-helminthic, anti-inflammatory, antipyretic, emollient, astringent, diuretic, and emmenagogue. Due to the assumption of its purifying actions on blood, it has been usually recommended for use in treatment of leprosy, gonorrhoea, boils, skin eruptions, scabies, chronic and seasonal fevers (Kumar et al, 2004).

Pharmacognosy

On the background of worldwide acceptance of broad medicinal uses of *Andrographis paniculata*, search for important bioactive phytochemicals responsible behind such beneficial roles in treatment and prevention of various diseases is constantly going on. Isolated and purified standard bioactive compounds of *Andrographis paniculata* have a great potential for providing useful drugs for human use. For setting a standard method of isolation, purification and pharmacothrapuetics of crude drugs, sensitive quantitative pharmacognostic parameters are to be determined (Sivananthan and Elamaran, 2013).

Therapeutic applications of *Andrographis paniculata* Nees

Antibacterial activity

Wide and unreasonable use of antibiotics today, bacterial resistance to available antibiotics is currently common. Even in the last decade, only few new antibiotic molecules are discovered. In this background scenario, it is urgently necessary to search for new antibacterial agents to combat both newer as well as previous common microorganisms. Thus special emphasis is given on exploration of the extensive therapeutic and antimicrobial potential of natural plant products. These medicinal plants have been widely used for treatment of many types of acute and chronic diseases and many other indigenous plants with particular antibacterial activity have been reported too (Dharmadasa et al, 2013).

Antioxidant activity

An antioxidant is a substance capable of maintaining redox balance within the cell by blocking chain of reduction reactions of oxidative agents/ heavy metals and removing free radical intermediates. Thus *Andrographis paniculata* Nees is assumed to have antioxidant property to avert oxidative stress and have ability to prevent occurrence of many acute and chronic diseases (Mishra et al, 2013).This antioxidant role and mechanism can further be investigated.

Anti-inflammatory activity

Extensive review of literatures revealed anti-inflammatory activity of *Andrographis paniculata,* by blocking the pathway of trans activation of NF-kappa beta (Chao et al, 2010) and by its ability to inhibit neutrophil adhesion and emigration through vascular endothelium. This prospective anti-inflammatory activity is due to the

inhibition of synthesis of capillary and leukocyte adhesion molecules mediated by down regulation of ROS production via PKC-dependent mechanism. Andrographolide may improve many acute and chronic inflammatory disorders by controlling the initial phase of inflammation i.e. leukocytes adhesion and transmigration (Prakash et al, 2011).

Anticancer activity

Cancer is a condition of unusual excessive growth of tissues in the body due to abnormal and uncontrolled cell division. Up till now, there is no true pathological cure of this condition in modern systems of medicine. So, there is great opportunity to develop new effective drugs from natural traditional plants, those are discussed to have bioactive compounds active against neoplastic and related diseases (Choudhury and Poddar, 1985). WHO has suggested that addiction to tobacco and alcohol, low intake of fruits and green leafy vegetables, chronic hepatitis B virus (HBV) and hepatitis C virus (HCV) infections and few types of human papilloma virus (HPV) are causative risk factors for development of various cancers in poor and developing countries. Worldwide deaths from cancer are projected to rise with an estimated 12 million deaths by 2030 A.D (Butler, 2008), mostly due increase in incidence and poor health infrastructure with escalating health expenditure.

Hepatoprotective and chloretic activity

In Ayurveda *Andrographis paniculata*t raditionally has been used in various formulations to treat jaundice and liver disorders. It has been shown as hepatoprotective in mice against carbon tetrachloride induced toxicity (Kapil et al, 1993). The heavy metals and other toxic chemical compounds damage the liver through membrane lipid peroxidation (Choudhury and Poddar, 1984), as a result of

production of free radicals. *Andrographis paniculata* extracted compounds show significant liver protection in experimental animals against chemical induced toxicity. This protective action is assumed to be due to the potential antioxidant capacity of some active compounds in *Andrographis paniculata* plant (Rana and Avadhoot, 1991). Andrographolide, the major active component in *Andrographis paniculata,* is reported to cause hepatic protection against carbon-tetrachloride administration (Handa and Sharma, 1990). *Andrographis paniculata* also protects the liver against paracetamol toxicity (Visen et al, 1993).

Activity on respiratory system

Andrographis paniculata preparations are used as a common home remedy, before modern antibiotic usage, in treatment of upper respiratory tract infections which includes sore throat, runny nose, allergy, cough, sinusitis, and earache. Upper respiratory infection due to viruses and bacteria during seasonal variation can be prevented by use of *Andrographis paniculata* (Ozkan and Dweik, 2004). Both crude extracts of *Andrographis paniculata* prepared in water or alcohol and isolated andrographolide have effective role in the treatment of upper respiratory tract infections (Cáceres et al, 1997; Melchior et al, 1997). These beneficial effects are especially due to anti-inflammatory, anti-microbial and immunomodulatory properties of the bioactive principles in this plant.

Cardiovascular activity

Andrographis paniculata plant extracts are effective in lowering of blood pressure. The active compound, andrographolide, inhibits platelet activating factor (PAF) and platelet aggregation (Amroyan et al, 1999). Due to this mechanism, intravascular

thrombosis in arteries of heart, brain, eyes and peripheral arteries in limbs can be avoided. As a result, major cardiovascular diseases like heart attack, cerebral stroke can be prevented to a large extent through use of *Andrographis paniculata*. Some studies have also noticed protective efficacy of *Andrographis paniculata* in restoring myocardial damage in chromate induced toxicity. This mechanism of protective efficacy of *Andrographis paniculata* against cardiovascular diseases is most likely due to its combined antithrombotic and direct cardiovascular activity.

1.1 Background of the Research Study

Impact of environmental pollution on human health is one of the most discussed topics of various researches nowadays. Increasing metal toxicities including increasing toxic effects of chromium compounds commonly used in various industries like steel, chrome industries, metal finishes, paints, wood polishes and tanneries are well established today. Thus, this study has potential contribution to knowledge in the field of social science, environmental research and national public health. It analyses increasing toxic effects of chromium compounds commonly used in various industries and also able to guide us to monitor the adverse impact of such heavy metals on environmental pollution, threat to human health and development of diseases.

However no ideal drugs have been developed so far to control the toxicity of Cr (VI). In developing countries alternative medicinal herbs are the best choice of common people for its low cost, easy availability and having no adverse effect upon the body. So, it has the social relevance on the basis of herbal (*Andrographis paniculata* Nees) management of chromium toxicity.

1.2 Aims and Objectives

On the background of wide acceptance of chromium toxicity, its potential protection by herbal compounds and with great interest to the role of the indigenous plant *Andrographis paniculata Nees*, broadly the goal is targeted to elucidate the role of this plant to chromium toxicity.

Towards fulfilling the above goal following specific aims and objective are-

- To observe the toxicity of Cr (VI) on liver, lungs tissues and mitochondria in male albino rats.
- To determine whether different solvent extracts of *Andrographis paniculata* Nees have any efficient protective role against Cr (VI) induced toxicity.
- Comparative study of protective efficacy of crude extract of *Andrographis paniculata* Nees in different proportions of mixed solvent on Cr (VI) induced toxicity.
- Exploration of specific ameliorative role of the most effective proportion of mixed solvent extract of *Andrographis paniculata* against chromium (VI) induced toxicity.
- Detection of effective phytochemical in most effective proportion of mixed solvent extracts of *Andrographis paniculata* Nees counteracting against toxic effects of Cr (VI) on tissues.

1.3 Experimental Design

For systematic experiment on the research topic following works are planned sequentially to attain the defined goal.

1. Induction of chromium toxicity by treatment with standard dose and duration study in male albino rat and its confirmation by bio-chemical analysis.
2. Assessment of chromium induced toxicity in liver and lungs.
3. Exploration of chromium toxicity on tissue mitochondria.
4. To find out the effective solvent extract / extracts of *Andrographis paniculata* Nees to protect liver and lungs against the toxicity of Cr (VI).
5. Comparative evaluation of remedial effect of mixed solvent extracts of *Andrographis paniculata* Nees in different proportion.
6. To study ameliorative role of most effective proportion of mixed solvent extract of *Andrographis paniculata* Nees on Cr (VI) induced toxicity.
7. Isolation, identification and quantification of active compound in the effective extract of *Andrographis paniculata* Nees by HPTLC, FTIR and HPLC.

In this experimental study, preservation and preparation of plant parts for extraction in various solvents as well as animal experiments were done in the laboratory of the Department of Human Physiology, Vidyasagar University, following the requisite guidelines of the University.

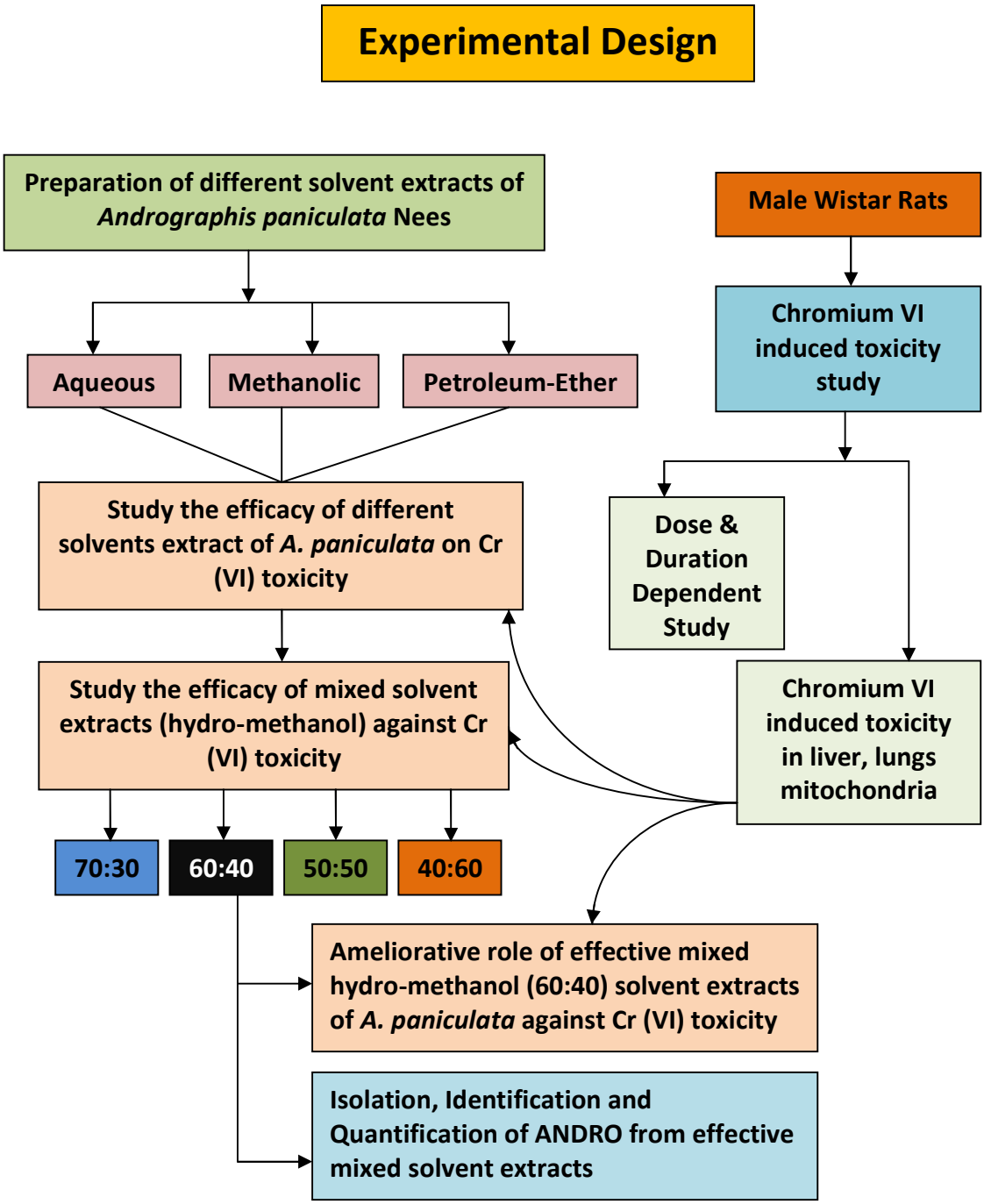

Figure 3: Experimental Design of the research study