Summary and Conclusion

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Let us sum up the previous observations and arguments in a cogent manner. Chromium, the heavy metal, commonly in hexavalent and trivalent states are found in environmental systems. Cr (VI) compounds develop more toxicity to a variety of aquatic as well as terrestrial organisms. A large intake of hexavalent chromium has been found to have mutagenic and carcinogenic effects. The intracellular reduction reactions of Cr (VI) to Cr (III) lead to generation of strongly reactive intermediates culminating into oxidative tissue damage and cellular injury.

In this present research broadly the potential ameliorative role of popular Indian medicinal herb, *Andrographis paniculata* Nees against Cr (VI) induced toxicity were investigated in liver and lungs of male albino rats.

In the beginning, investigation has been conducted to select the dose and duration dependent response of chromium (VI) on perturbation of ALP, ALT and AST enzyme activities in serum, tissues of liver and lungs of adult male albino rats in various doses of chromium (200, 400, 600 and 800 µg / 100 gm body weight / day) to select most effective dose and duration of chromium treatment over and along 1st day, 3rd days, 7th days, 14th days, 21st days and 28th days. It has been noted that the activities of ALP, ALT and AST enzymes in serum were maximum at the dose of 800 µg / 100 gm body weight / day on 28 days treatment of hexavalent chromium. On the other hand, it has also been found that activities of ALP, ALT and AST enzymes were decreased in rat liver and lungs, in dosage 800 µg /100 gm body weight / day on 28 days treatment of hexavalent chromium. From the above observations it may be inferred that the toxicity of hexavalent chromium was

maximum at the dose of 800 µg / 100 gm body weight /day on 28th day of treatment. Also, it has been noted that the body weight significantly decreases in response to chromium and tested organs displays noticeable increase in chromium contents. The impact on body weight may be direct effects of chromium and not as a result of deficient food intake, as the control rats have been pair-fed to chromium-treated rats. Decreased body weight was not reflected in the loss of individual organ weights. On the other hand, during research study on the toxicity of Cr (VI) in liver and lungs tissues, increased lipid peroxidation and level of conjugated dienes were observed, probably associated with depletion of intracellular antioxidants in liver and lungs in chromium treated rats. The current study has pointed the excessive generation of ROS in response to chromium. Hence, it has been observed reasonably, the significant decrease of GSH and GSSG levels and also remarkably diminished activities of GST, GPx and GR. This depletion of the activities of such antioxidant enzymes like GSH, GSSG, GST, GPx, GR, CAT and SOD in liver and lungs tissues of chromium administered rats may be most probably due to increased utilization of those substances during metabolic detoxification of chromium within the cell.

The role of mitochondria is also considered in this connection. Mitochondrial dysfunction in cells challenged to Cr (VI) diminishes cellular energy (ATP) production. It has been earlier proved that a great amount of decrease of ATP in hamster fibroblasts and decreased mitochondrial ATP levels in rat thymocytes exposed to Cr (VI) are closely correlated to inhibition of cellular respiration. The inhibition of mitochondrial oxidative respiration has been detected in isolated rat liver mitochondria after Cr (VI) treatment.

Hexavalent chromium in the form of $K_2Cr_2O_7$ induced toxicity study, in liver and lungs mitochondria, clearly displays remarkable elevation of MDA and CD. These results may be due to generation of ROS and oxidative damage in inner mitochondrial membrane. Results are noticed that NO production increases in liver and lungs mitochondria. SOD is believed to play a major role in the first line of natural antioxidant cellular defence mechanism. In this study, decreased SOD activity in chromium-induced rat in liver and lungs mitochondria can reasonably be clarified by the massive generation of ROS. GSH and GSSG levels have significantly diminished in liver and lungs mitochondria. Also, the activities of GST, GPx and GR are diminished in selected tissue mitochondria after chromium treatment might be owing to increased oxidative stress.

Andrographis paniculata Nees popular Indian medicinal plant, belonging to the Acanthaceae family commonly known as "Kalmegh", has been most widely used in many Ayurvedic formulations for centuries in India for the prevention and treatment of varied number of diseases and is one of the frontier areas of research for alleviation of metal toxicities. So, the present study specifically has been aimed to select most potential effective solvent extract of *Andrographis paniculata* notably in three different solvents like aqueous, methanol and petroleum-ether to yield effective compound having powerful anti-peroxidative properties. During this experiment two doses (250 mg/kg body weight/day and 500 mg/kg body weight/day) of different solvent extracts (aqueous, methanol and petroleum ether) were chosen to observe supplementary effects in chromium- induced oxidative damage in selected tissue mitochondria of rats. This research clearly demonstrates marked rise of MDA, conjugated dienes levels and NO production in mitochondria of liver and lungs in response to chromium. But GSH and GSSG levels and also the

SOD, GPx, GR and GST activities in both liver and lungs mitochondria were profoundly reduced after toxic insult of hexavalent chromium. Interestingly, supplementations of preparation of *Andrographis paniculata* with different solvents, the extracts play vital roles to counteract the stress of oxidative challenge to chromium toxicity in liver and lungs mitochondria. Methanol and aqueous extract shows better effects than petroleum-ether in protecting Cr (VI) induced tissue toxicity. These findings may explain and guide further that methanol as well as aqueous extract may play a crucial role at the dose of AP_{500} which may contain some important active components having antioxidant property to diminish or prevent chromium induced oxidative stress in selectively isolated tissue mitochondria.

So, the next step in the present investigation is intended to explore the protective efficacy of aqueous-methanol solvent extract of *Andrographis paniculata* with selectively different ratios (70:30, 60:40; 50:50; 40:60) at a dose 500 mg/kg body weight. It can be used as potential ameliorative agent against chromium (VI) induced tissue toxicity. From the present study, noticeable variation of MDA, CD and NO production were seen in both liver and lungs mitochondria in chromium exposed rats. The major decrease of GSH and GSSG levels and altered antioxidant enzyme (SOD GPx, GR and GST) functions were well recorded in this study of chromium (VI) toxicity on isolated tissue mitochondria. During the experiment, extracts of *Andrographis paniculata* in the mixed hydro-methanol solvent ratios of (70:30, 60:40, 50:50 and 40:60) have been successively used for the supplementation against Cr (VI) induced toxicity of liver and lung to find out most effective ratio of hydro-methanol extract of *Andrographis paniculata*. The salient findings in this study has corroborated the facts that particular (60:40) mixed hydro-

methanol solvent extract of *Andrographis paniculata* has greater potential benefit than other ratios of mixed hydro-methanol solvent extract in maintenance of oxidative equilibrium, scavenging of ROS and augmentation of anti-oxidant defence against chromium induced tissue toxicity.

Thus, keeping in mind the main objective to reduce the Cr (VI) mediated tissue toxicity, by administering the most potent mixed hydro-methanol solvent extract in the ratio of (60:40) of *Andrographis paniculata* in rats, the changes in respect of certain alteration of plasma membrane structure and function, mitochondrial electron transport chain (Mito–ETC) complexes, expression of certain cytokines, apoptotic signalling pathway and histopathological status of liver and lungs are studied in detail. In the present investigation, few pertinent points were noted that chromium by altering the relative proportion of phospholipid and cholesterol may damage cell membrane structure in both liver and lungs tissues. Cr (VI) compound also alters the total ATPase and Na^{+}-K^{+} ATPase activities and indicates remarkable functional changes of plasma membrane in chromium-treated group. On the other hand, diminished activities of mitochondrial ETC-Complex I, II and III during Cr (VI) challenge, in consequence of leakage of electrons through mitochondrial respiratory chain, may have produced superoxide ions in liver and lung mitochondria. It is clearly evident that mixed hydro-methanol (60:40) solvent extracts of *Andrographis paniculata* have attenuated those damaged structure and function of the liver and lungs plasma membrane. Also the activities of mitochondrial ETC-Complex I, II and III were reconditioned by supplementation with hydro-methanol (60:40) *Andrographis paniculata* extract.

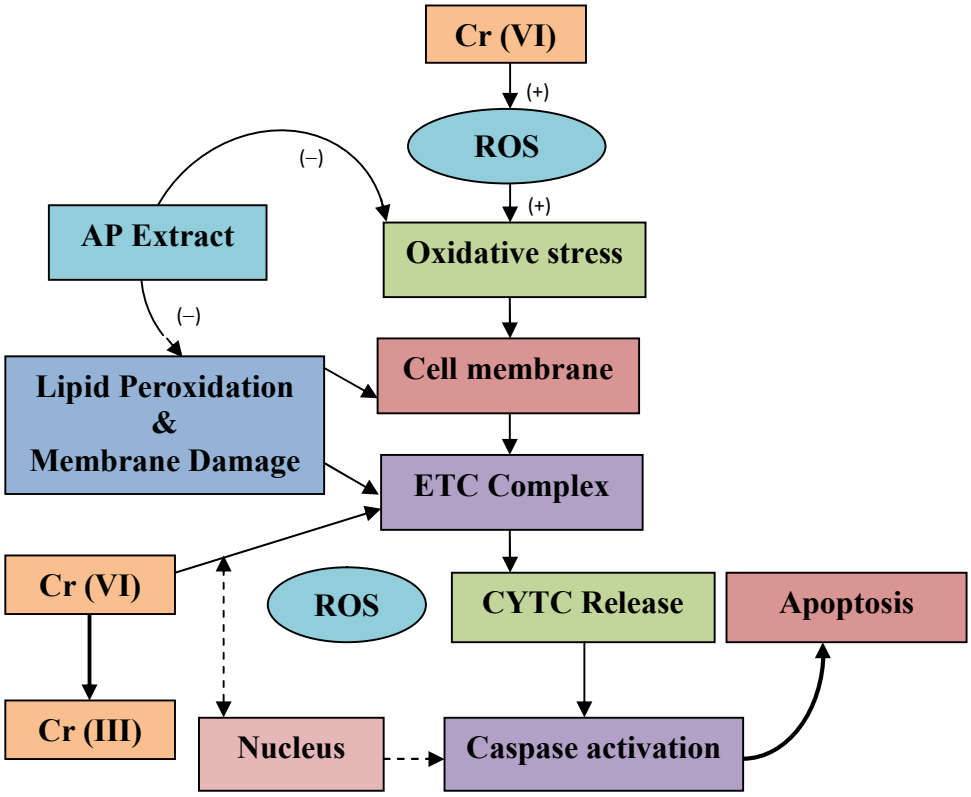
To investigate further the few cytokines pro-inflammatory (TNF-α and IL-12) and anti-inflammatory (TGF-β and IL-10) levels in liver and lungs tissues in chromium treated rats, it has been noticed that TNF-α and IL-12 levels were elevated significantly but the levels of anti-inflammatory cytokines like TGF-β and IL-10 were diminished markedly after chromium exposure. It has also been found that the ROS level of lymphocytes was elevated significantly after chromium treatment. ROS plays a major role for lymphocytic cell death. To notice the probable pathway of cell death occurrence due to apoptosis or necrosis, researcher analyses the lymphocytes by staining with EtBr-AO. In the study, it is evident that chromium treatment drastically decreases the number of viable cells. To examine changes of nuclear morphology of lymphocytes due to treatment of chromium, staining of the chromium-exposed lymphocytes were done by DAPI staining. It has been noticed about the significant morphological changes in nuclear chromatin in cells for the experimental period. After supplementation, it has been observed that hydro-methanol mixed solvent (60:40) extract of *Andrographis paniculata* plays a potent role as ROS inhibitor and efficiently protect the lymphocytes from chromium-induced cytotoxicity.

It becomes also necessary to discuss the role of cytokines. Inflammatory cytokines, mainly TNF-α, might have stimulated apoptosis pathway through caspase-8-mediated pathway. Potent anti-inflammatory cytokine IL-10 plays a central role in limiting host immune response to various pathogens. The current studies show increased concentration of TNF-α obviously, but the concentration of IL-10 decreases noticeably in chromium exposed rat lymphocytes. The augmented pro-inflammatory responses along with increased pro-apoptotic factors such as Caspase 3 and Caspase 8 are responsible for enhanced lymphocyte death. But

supplementation of hydro-methanol (60:40) mixed solvent extract of *Andrographis paniculata* one of the most important medicinal plants, containing potent compounds which have played a vital role to minimize the chromium-induced changes mediated by pro- and anti- apoptotic markers in rat lymphocytes. Besides this, it has been also found that hydro-methanol (60:40) mixed solvent extract of *Andrographis paniculata* supplementation plays a crucial role to protect the hepatic and lung alveolar damage observed in presence of chromium.

In fulfilling the purpose of pertaining above experimental research work entitled "Effect of *Andrographis paniculata* Nees extract on hexavalent chromium induced toxicity", isolation and standardised purification of the active compound from a complex multi-component mixture are needed to obtain from natural medicinal plants. Hence, the final objective of the present work was isolation, identification and quantification of potentially most bioactive ingredient from hydro-methanol (60:40) mixed solvent extract of *Andrographis paniculata* Ness by using HPTLC, FTIR and HPLC. In the present investigation, most potent compound andrographolide (ANDRO) was identified and quantified from the mixed hydro-methanol (60:40) solvent extract of *Andrographis paniculata* by comparing with the standard "Andrographolide".

Thus, it can be inferred after the research study that crude extract of *Andrographis paniculata* Nees in proper mixed solvent may yield more active remedial natural agent for cost effective management of chromium induced toxicity and related disorders in human beings. Possible mechanism of remedial actions of *Andrographis paniculata* against chromium induced tissue toxicity is depicted in the picture below next.



Probable mechanism of remedial actions of *Andrographis paniculata* Nees against Cr (VI) toxicity.

(+) Indicates increase toxicity

(–) Indicates decrease toxicity