Chapter-5

Comparison of the protective efficacy of mixed solvent extracts of *Andrographis paniculata* Nees in different proportions against chromium (VI) induced toxicity

Contents

5.0 Introduction

Oxidative stress refers to cellular alteration of function as a consequence of mismatch between generation of reactive free radicals and cellular defence mechanism against it. In oxidative stress, reactive free radical generation increases, but scavenging of those free radicals diminishes or reduction of modified macromolecules to repair oxidative stress or both. Cr (VI) alters mitochondrial functions, responsible for oxidative stress and alteration of Immune function resulting in disorders of different organs and diseases.

In previous study, efficacy of different (Aqueous, Methanol, Petroleum-ether) solvent extract of *Andrographis paniculata* has been observed prominently against Cr (VI) toxicity developed in liver and lung mitochondria of male albino rats. Out of the three different solvent extract supplementation groups, namely aqueous (AE-AP), methanol (ME-AP) and petroleum-ether (PEE-AP); supplemented group with methanol extract of *Andrographis paniculata* has resulted in significant prevention of superoxide generation, lipid peroxidation, NO-release, elevated GSH level and improvement of SOD activity in liver and lung to protect Cr (VI) induced toxicity. On the other hand, aqueous extract of *Andrographis paniculata* (AE-AP) has showed a little more potential effectiveness than Petroleum-ether extract (PEE-AP) of *Andrographis paniculata* against chromium-induced toxicity in liver and lungs mitochondria. It has been well explored that *Andrographis paniculata* has many established pharmacotherapuetic activities such as analgesic, anti-inflammatory, antipyretic, antidiarrheal, antispasmodic and antiulcer agent (Madav et al, 1995). Dey et al, (2011) have observed that the methanol extract of *Andrographis paniculata* effectively restores the alterations of liver impairment induced by

hexavalent chromium. It has been reported that aqueous extract of *Andrographis paniculata* supplementation can aptly overcome oxidative stress in liver and kidney after nicotine exposure (Dey et al, 2016). Andrographolide, principal effective compound of *Andrographis paniculata,* can protect (CCl_4) induced lipid peroxidation of rat liver (Kapil et al, 1993).

So, the present chapter of study is intended to explore the mixed water and methanol extract of *Andrographis paniculata* in different ratio that can be used as potential ameliorative agent against *in vivo* chromium-induced tissue toxicity in male albino rats.

5.1 Methodology

5.1.1 Chemicals

Required amount of various chemicals, reagents and consumables were collected as described in chapter II.

5.1.2 Preparation of Mixed solvent extract

Mixed solvent extract of *Andrographis paniculata* were prepared by mixing crude dry powdered leaves with solvent water and methanol in the ratio of (70:30), (60:40), (50:50), (40:60).

5.1.3 Animals and diet

Animals and their diet were described in Chapter-II.

5.1.4 Mode of treatment

Rats of almost equal average body weight were divided into six groups. The animals of five groups were injected with $K_2Cr_2O_7$ (800 microgram / 100 gram of body weight/day) and 6th group as vehicle control were injected with 0.9% NACL solution as described in Chapter-II. Amongst the five chromium treated animals groups, 1st group served as chromium control group and the rest four as supplemented groups were given the mixed hydro-methanol *Andrographis paniculata* extract orally in the following schedule, rats in 2nd group-water and methanol (ratio of 70:30), 3rd group-water and methanol (ratio of 60:40), 4th group-water and methanol (ratio of 50:50) and 5th group-water and methanol (ratio of 40:60) at a dose of 500 mg/kg body weight daily at an interval of six hours after injection of $K_2Cr_2O_7$ for 28 days.

5.1.5 Animals sacrifice and sample preparation

Animal sacrifice and sample preparation were described in Chapter-II.

5.1.6 Homogenization of tissues

Homogenization of tissues was described in Chapter-II.

5.1.7 Isolation of Mitochondria

Mitochondria isolation procedure was described in Chapter-II.

5.1.8 Analytical methods

Following modalities of biochemical parameters, like Lipid peroxidation, Conjugated dienes, NO release, SOD, GSH, GSSG, GPx, GR, GST and protein were estimated, described as per previous chapter-II.

5.1.9 Statistical Analysis

Statistically data analysis was described in Chapter-II.

5.2 Results

Following chromium exposure, the MDA and conjugated dienes concentration are significantly elevated in all the organs (Figure 1 & 2). It was observed that the MDA and conjugated dienes content were restored markedly with supplementation of different ratio of mixed hydro-methanol solvent *Andrographis paniculata* extract in tested tissue mitochondria after chromium (VI) treatment and it was maximum in the ratio (40:60) of hydro-methanol mixed solvent extract of *Andrographis paniculata* rather than other ratio.

The nitric oxide (NO) release and activity of SOD in tested tissue mitochondria are also observed to be significantly increased and decreased respectively in response to chromium (Figure 3 & 4). It was observed that significantly decreased production of NO and increased the SOD activity in chromium-treated rats after supplementation with the different ratio of mixed solvent extract. Maximum counteraction was found in NO production at the ratio (40:60) and SOD activity at the ratio (60:40) of mixed hydro-methanol solvent extract in liver and lungs mitochondria in chromium exposed rats.

The GSH and GSSG contents are noticed to be significantly diminished in liver and lungs mitochondria of rats when exposed to chromium (Figure 5 & 6). In hydro-methanol supplemented groups of rats, it was observed that the GSH and GSSG content remarkably increased in all mixed solvent ratio but was maximum at (60:40) in both liver and lungs mitochondria.

Figures (7, 8 & 9) show that the GPx, GR and GST activities are prominently diminished in tested organs' mitochondria in chromium exposed rats. On the other hand, GST, GP_X and GR activities have regained markedly in both the tested organs after supplementation with the mixed hydro-methanol extract of *Andrographis paniculata* in different ratio. But such supplementation has shown to be maximum effective at the ratio (60:40) of mixed hydro-methanol solvent extract in tested tissue mitochondria in chromium exposed rats.

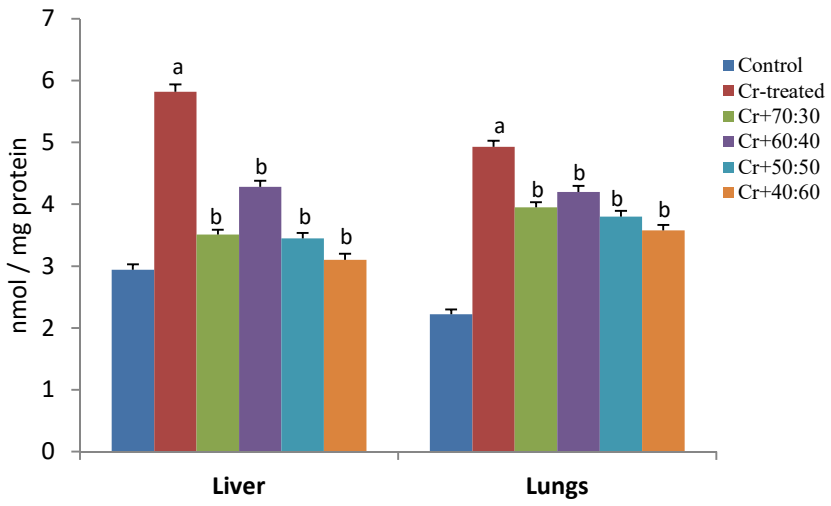

Figure 1: Changes of MDA concentration in liver and lungs mitochondria after co-administration of mixed solvent water and methanol in the ratio of (70:30), (60:40), (50:50) & (40:60) in chromium treated rats. [a] noted significant difference ($P<0.05$) compared to control; [b] noted significant difference ($P<0.05$) compared to chromium treated.

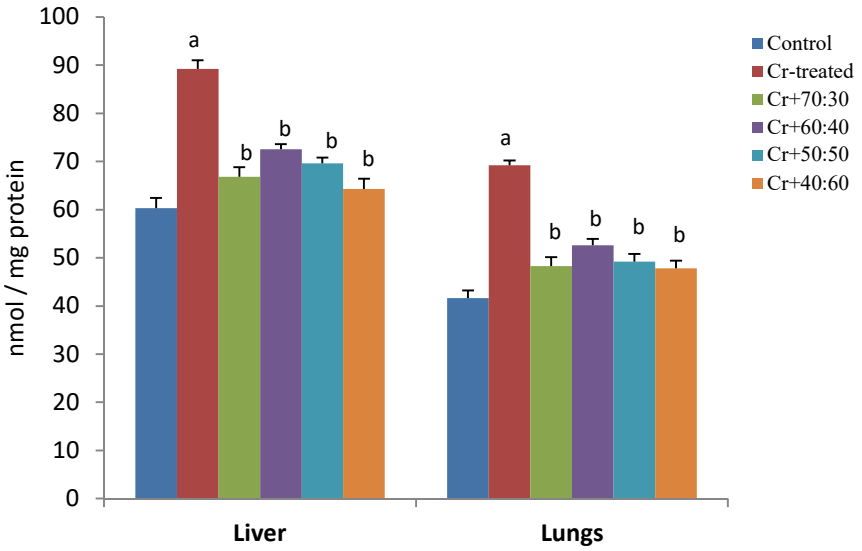

Figure 2: Shows the conjugated dienes content in liver and lungs mitochondria after co-administration of mixed solvent water and methanol in the ratio of (70:30), (60:40), (50:50) & (40:60) in chromium treated rats. [a] noted significant difference (P<0.05) compared to control; [b] noted significant difference (P<0.05) compared to chromium treated.

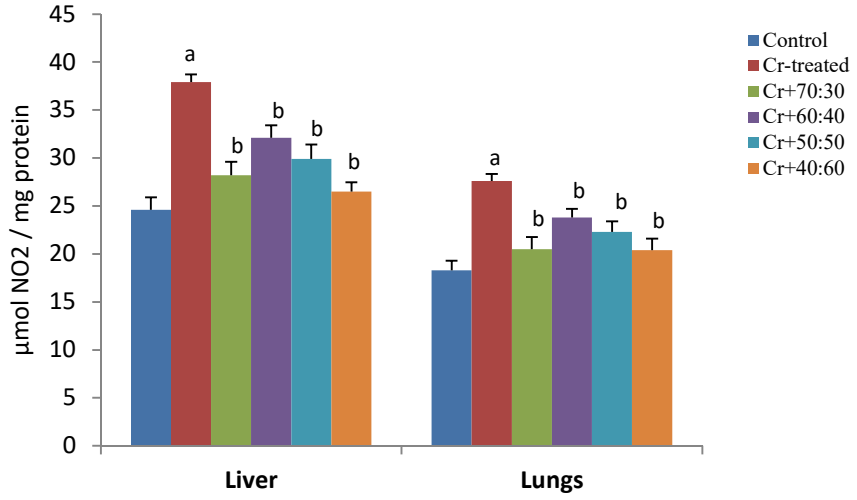

Figure 3: Shows the production of nitric oxide (NO) in tested organ mitochondria after co-administration of mixed solvent water and methanol in the ratio of (70:30), (60:40), (50:50) & (40:60) in chromium treated rats. [a] noted significant difference (P<0.05) compared to control; [b] noted significant difference (P<0.05) compared to chromium treated.

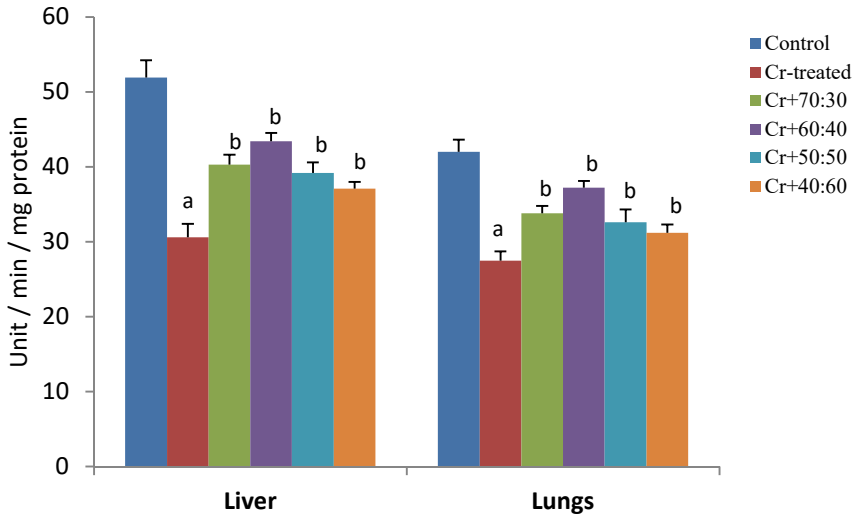

Figure 4: Changes of the SOD activity in liver and lungs mitochondria after co-administration of mixed solvent water and methanol in the ratio of (70:30), (60:40), (50:50) & (40:60) in chromium treated rats. ^{a} noted significant difference ($P<0.05$) compared to control; ^{b} noted significant difference ($P<0.05$) compared to chromium treated.

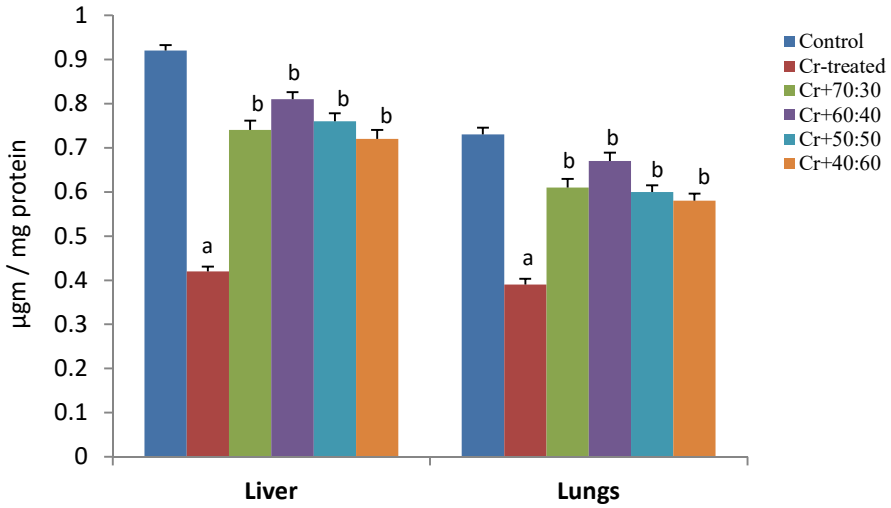

Figure 5: Shows the level of GSH in tested organ mitochondria after co-administration of mixed solvent water and methanol in the ratio of (70:30), (60:40), (50:50) & (40:60) in chromium treated rats. ^{a} noted significant difference ($P<0.05$) compared to control; ^{b} noted significant difference ($P<0.05$) compared to chromium treated.

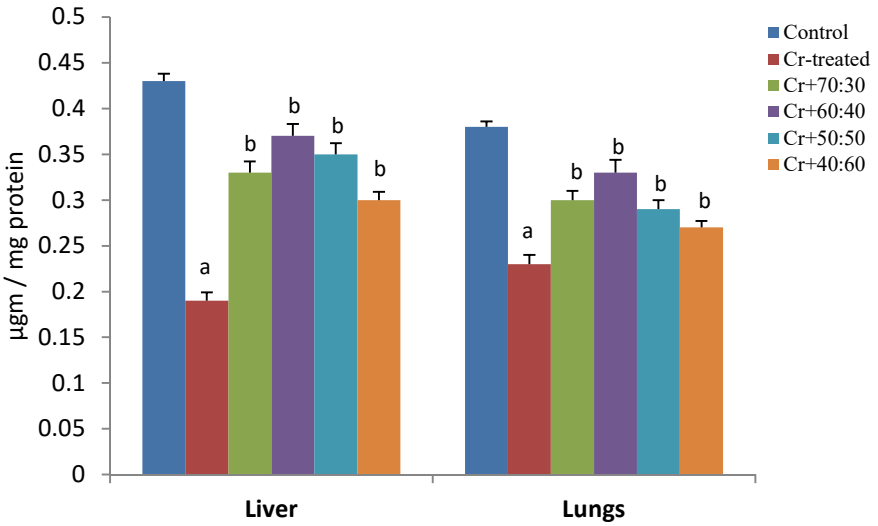

Figure 6: Variation in the GSSG level in liver and lungs mitochondria after co-administration of mixed solvent water and methanol in the ratio of (70:30), (60:40), (50:50) & (40:60) in chromium treated rats. ^{a} noted significant difference ($P<0.05$) compared to control; ^{b} noted significant difference ($P<0.05$) compared to chromium treated.

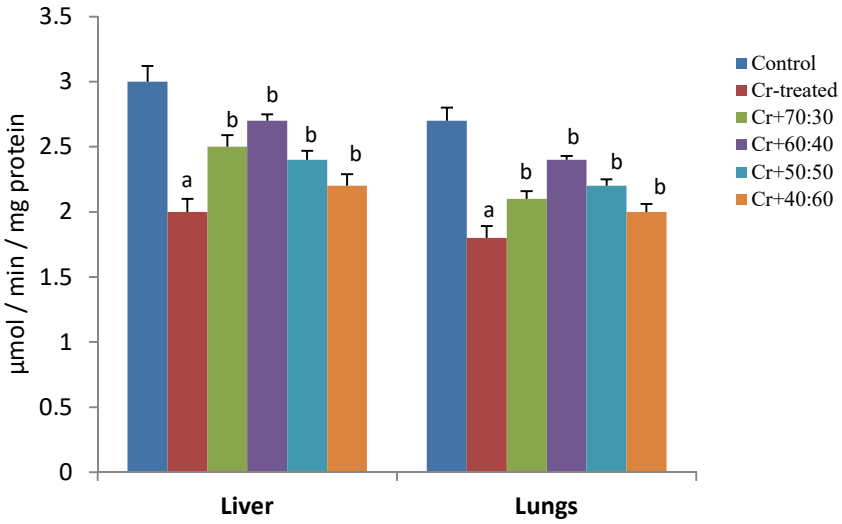

Figure 7: Variations of GPx activity in liver and lungs mitochondria after co-administration of mixed solvent water and methanol in the ratio of (70:30), (60:40), (50:50) & (40:60) in chromium treated rats. ^{a} noted significant difference ($P<0.05$) compared to control; ^{b} noted significant difference ($P<0.05$) compared to chromium treated.

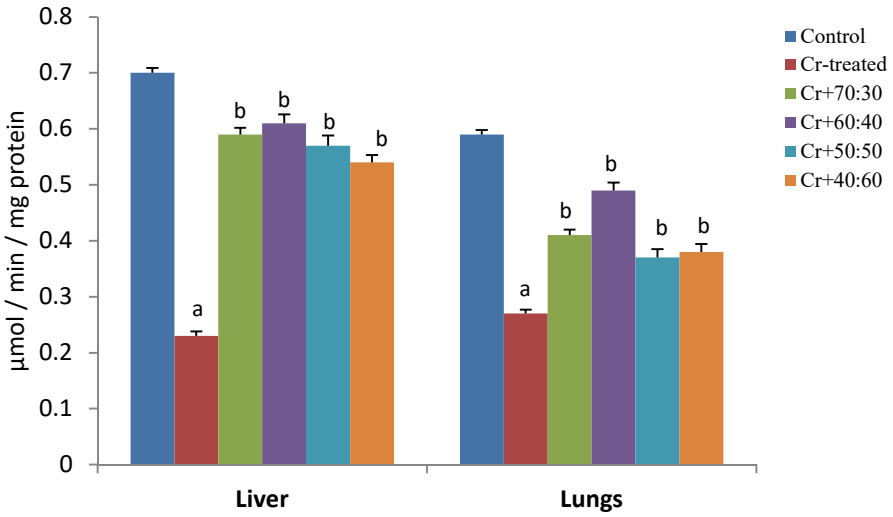

Figure 8: Changes in GR activity in liver and lungs mitochondria after co-administration of mixed solvent water and methanol in the ratio of (70:30), (60:40), (50:50) & (40:60) in chromium treated rats. [a] noted significant difference (P<0.05) compared to control; [b] noted significant difference (P<0.05) compared to chromium treated.

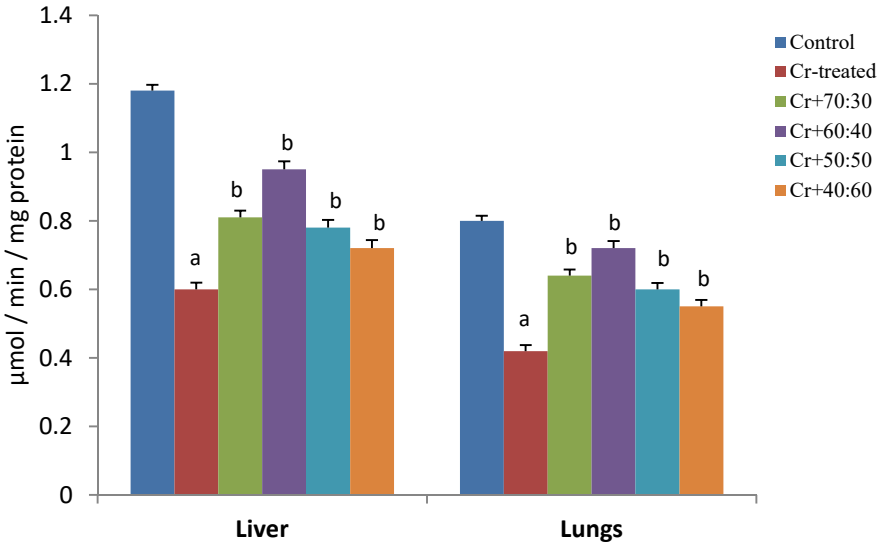

Figure 9: Shows the changes of GST activity in tested organ mitochondria after co-administration of mixed solvent water and methanol in the ratio of (70:30), (60:40), (50:50) & (40:60) in chromium treated rats. [a] noted significant difference (P<0.05) compared to control; [b] noted significant difference (P<0.05) compared to chromium treated.

5.3 Discussion

Oxidative stress is a consequence of either alteration of inherent enzymatic and nonenzymatic antioxidant system or enhanced lipid peroxidation or both. However, formation of large amounts of reactive oxygen and nitrogen species during initial stress may directly enhance lipid peroxidation (Braughler and Hall, 1989); as demonstrated in the liver and heart (Hu et al, 2000). From the present research work, salient features have been observed that MDA and conjugated dienes concentration are markedly increased both in liver and lungs mitochondria in response to chromium exposure (Figure 1 & 2). On the other hand, it has been found that the NO production increases significantly in both the tested organs (Figure 3). Excess formation and release of NO alters rat brain function by inhibiting mitochondrial respiration, because this compound may irreversibly bound to the haem group of cytochrome enzymes, especially cytochrome oxidase (Poderoso et al, 1996). Excess NO, forming oxidant peroxinitrite radicals, may inhibit important enzymes and impair mitochondrial integrity (Radi et al, 2002). Significant diminution in the concentration of MDA, including CD and NO production in liver and lungs mitochondria of rats supplemented with mixed hydro-methanol solvent extract at different ratio indicate that the *Andrographis paniculata* has important properties for scavenging free radicals in response to chromium. Much reduction of indicators of lipid peroxidation is noticed after supplementation of hydro-methanol (40:60) extract of *Andrographis paniculata* in Cr (VI) treated rats.

So much deleterious effects, as a result of excess ROS formation in the mitochondria, largely can be prevented by various endogenous and exogenous antioxidants. Initial defence against chromium-induced intracellular ROS generation

is provided by SOD. Superoxide dismutase (SOD) enzymes can efficiently convert superoxide to H_2O_2 (Fridovich, 1995). Reduced transition metal ions may react with H_2O_2 to produce hydroxyl radicals which spontaneously produce peroxynitrite combining with NO. It is most essential to maintain a steady concentration of $O_2^{\cdot -}$ at lowest possible level, through balanced antioxidant enzyme activities, like SOD. The results of current study have also supported the above findings. The mitochondrial SOD activity has been detected low in liver and lungs mitochondria during chromium administration to rats (Figure 4), which may be as a result of overproduction of $O_2^{\cdot -}$, H_2O_2 and excess utilisation of SOD enzymes to counter the oxidative stress. Administration of mixed solvent extract of *Andrographis paniculata* significantly increases the activities of SOD in liver and lungs mitochondria. These results may suggest that mixed hydro-methanol (60:40) solvent herbal extract of *Andrographis paniculata* attenuates oxidative stress in experimental rat tissues.

GSH is also one of the main intracellular oxidants and it directly scavenges free radicals to protect important biomolecules from free radical attack. Reduced GSH level in this study has been noted in liver and lungs mitochondria of tested rats after chromium injection (Figure 5) and may signify the impairment of GSH synthesis and more utilization of GSH for detoxification of chromium-induced free radicals. Decreased activity of the GSSG (Figure 6) indicates the severe disruption of GSSG to GSH conversion (Singh et al, 2001). Moreover, noticeable decrease of GPx, GR and GST activities has also been noted in isolated mitochondria of all the tested organs of rat in response to chromium (Figures 7, 8 & 9). These responses may be triggered by low levels of NADPH which is a co-factor of the enzyme GR that

convert GSSG to GSH. H_2O_2, the product of $O_2^{\cdot -}$ dismutation and the main precursor of $-OH^{\cdot}$ in the presence of reduced transition metals, is mostly detoxified by the enzyme GPx. The mitochondria in liver have showed about one third of the total GPx activity (Chance et al, 1979). Other GPx enzymes are attached with the mitochondrial membrane, known as phospholipid–hydroperoxide GPx, used specifically to be involved in reducing membrane lipid peroxides (Nomura et al, 2000). On the other hand, supplementation with different hydro-methanol solvent extract of *Andrographis paniculata* at different ratio has recovered the GSH and GSSG level and the activities of GPx, GR and GST in response to chromium in liver and lungs mitochondria. This depletion may have resulted deleterious oxidative changes in the tissue mitochondria due to involvement of the accumulated toxic product of Cr (VI) compound. So, the increased level of antioxidants indicates the much protective efficacy of *Andrographis paniculata* against chromium (VI) driven oxidative stress and significant variation of different parameters are seen more in mixed hydro-methanol solvent extract at the ratio (60:40) of *Andrographis paniculata*.

5.4 Conclusion

In reference to previous chapter, methanol and aqueous extracts of *Andrographis paniculata* have showed more potential remedial actions in alleviating oxidative stress induced by Cr (VI) in liver and lung mitochondria. In the present experiment the protective property of *Andrographis paniculata* extract prepared in mixed methanol and aqueous solvent in different proportions was studied against Cr (VI) induced toxicity of liver and lung mitochondria of male albino rat. For this experiment crude extract 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) mediated liver and lung toxicity to search for most effective ratio of hydro-methanol extract of *Andrographis paniculata*. The important findings in this study has corroborated the fact that particular (60:40) mixed hydro-methanol solvent extract of *Andrographis paniculata* has greater potential benefit than other ratio of mixed hydro-methanol solvent extract like (70:30), (50:50) and (40:60) in maintenance of oxidative equilibrium, scavenging of ROS and augmented anti-oxidant defence against ROS mediated chromium (VI) toxicity in liver and lungs mitochondria.

So, ameliorative role against chromium-induced tissue toxicity and isolation of effective compound from mixed hydro-methanol solvent extract at the ratio (60:40) of *Andrographis paniculata* can be studied further.