CHAPTER V

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The present study explores the following areas:

1. \textit{In vivo} effects of arsenic trioxide (\(\text{As}_2\text{O}_3\)) in cerebral hemisphere (CH) and cerebellum (C) of mice brain and \textit{in vitro} human peripheral blood lymphocyte culture for assessment of genotoxic end points.

2. To evaluate protective effect of antioxidants viz., vitamin A and kalmegh \textit{in vivo} and \textit{in vitro}.

Part I: \textit{In vivo} studies

This study was carried out to:

a. Investigate the effects of \(\text{As}_2\text{O}_3\) at two dosage levels (0.5 and 1 mg/kg body weight) for 45 days on Swiss albino mice (\textit{Mus musculus}) in CH and C regions of brain, serum and whole blood; to explore a battery of parameters.

b. Oral supplementation of vitamin A (0.2 mg/kg body weight) on arsenic neurotoxicity to study its possible beneficial effects.

The results of this study are summarised as follows:

A. Studies on arsenic toxicity

Gravimetric and biochemical parameters together with haematological indices were studied in cerebral hemisphere and cerebellum of brain, serum and blood. Arsenic intoxicated mice demonstrated a significant reduction in their body and organ weights. Arsenic also caused oxidative stress by
generating reactive oxygen species and increased free radical toxicity as evidenced by depletion of antioxidant enzymes - superoxide dismutase and catalase activities. This was accompanied by increased lipid peroxidation and reduced levels of glutathione, total -SH levels and ascorbic acid levles.

Decrease in the activities of enzymes - adenosine triphosphatase (ATPase), succinic dehydrogenase (SDH), phosphorylase and cholinesterase (ChE) was noted in the brain of arsenic intoxicated mice. Metabolic parameters like protein, cholesterol and total lipids observed a significant depletion together with an elevation in glycogen levels after As$_2$O$_3$ treatment. Inhibition of SDH by arsenic as noted in our study may have led to slower rate of TCA cycle, thus producing insufficient acetyl CoA, for the synthesis of cholesterol and other lipids, thus explaining their depletion. Arsenic is known to react and bind with sulphydryl containing molecules such as glutathione, amino acids and proteins thus leading to the destruction of these molecules and enzymes. This way, it destroys the antioxidant enzymes, carbohydrate metabolism system together with proteins and total -SH levels.

Serum ChE along with protein, cholesterol, total lipids and total -SH groups also indicated a sharp decline after As$_2$O$_3$ exposure. Arsenic retention was determined after 45 days of treatment in cerebral hemisphere and cerebellum regions of brain; and in the whole blood. Its retention was correlated negatively with the various indices after arsenic exposure in a dose dependent manner. Haematological parameters - RBC, WBC and haemoglobin also depleted sharply, following arsenic treatment due to its toxicity probably on heamopoietic tissue.
B. Amelioration by vitamin A upon arsenic induced neurotoxicity

Vitamin A (0.2 mg/kg body weight) was supplemented orally along with both low and high dose arsenic for 45 days. Results established considerable comparison with the control group in tissue, serum as well as in whole blood parameters revealing its mitigation over arsenic exerted toxicity. Thus, vitamin A effectively mitigated arsenic induced neurotoxicity by quenching the free radicals, thereby reducing oxidative stress in brain and blood of mice.
Part II: *In vitro* studies

*In vitro* studies were carried out to study:

1. The mutagenic role of arsenic trioxide at three different doses and
2. Evaluation of probable beneficial role of antioxidants - vitamin A and kalmegh (30% andrographolide).

Parameters included were sister chromatid exchange (SCE), cell cycle proliferative index (CCPI), average generation time (AGT), population doubling time (PDT), micronuclei (MN), chromosomal aberrations (CA) together with aneuploidy.

Genotoxic studies with arsenic showed a remarkable increase in the frequency of SCE (SCE/cell and SCE/chromosome), MN, CA and aneuploidy, while average CCPI decreased. Both - PDT and AGT increased with arsenic trioxide exposure in the lymphocyte cultures, indicating a lag in the cell cycle kinetics of lymphocytes. These effects were found to be dose dependent. Though exact mechanism of arsenic induced genotoxicity remains elusive, it might be attributed to generation of reactive oxygen species (ROS), reactive nitrogen species (RNS) which in turn induced oxidative stress leading to genotoxicity. It has been found to be an antioxidant and a free radical scavenger in many mammalian studies. Vitamin A has also been indicated as a potent inhibitor of lipid peroxidation thereby diminishing the level of DNA damage and frequency of chromosomal aberrations. Thus addition of vitamin A to arsenic cultures has shown protective effects to certain level.

*Andrographis paniculata* (kalmegh), a herbal product is also a compelling antioxidant. This plant extract has been reported to prevent
oxygen radical and $\text{H}_2\text{O}_2$ production in human neutrophils, thus suggesting its protective role. Findings have also suggested the antioxidant potential of this plant, where it curbed the DNA damage induced by various test chemicals. Andrographolide, a key principle of the extract is known to curtail lipid peroxidation and scavenge free radicals and their formation. These *in vitro* studies thus indicated that vitamin A and kalmegh could be used to combat arsenic induced genotoxicity in a varied manner.

**Conclusion**

This data on *in vivo* studies revealed that arsenic certainly leads to free radical toxicity in brain together with imbalances in other biochemical events as well, followed by its effect on blood. Arsenic also caused a range of genotoxic effects in lymphocyte cultures. Both vitamin A and kalmegh proved to be favourable against arsenic toxicity as seen from the results of *in vitro* and *in vivo* studies. This role of vitamin and kalmegh is attributed towards their antioxidant properties. Both these compounds can be further studied to be developed as a possible antidote/antioxidant over arsenic poisoning in arsenic contaminated areas of India and other places. Hence, the results of this work have a noteworthy bearing on the amelioration of arsenic by these antioxidants. Thus, the work embodied in the thesis is an important contribution to our knowledge in the field of arsenic toxicology.