Chapter-5

SUMMARY AND CONCLUSIONS

The present study was carried out to elucidate the mechanism(s) of chronic carbofuran neurotoxicity and neuroprotective potential of N-Acetylcysteine in male rats. Keeping in view the above objective male wistar rats weighing about 120-140 g were randomly segregated into four groups viz. control, carbofuran, NAC and carbofuran + NAC treated. Rats were administered carbofuran (1mg/kg body weight) and NAC (200mg/kg body weight) for a period of 28 days. Important findings of this investigation are summarized as follows:

1. Administration of carbofuran for a period of 28 days caused a detrimental effect on the growth of animals in terms of gain in body weight. Carbofuran administration also resulted in decrease in brain weight. NAC on the other hand had protective effect on carbofuran-induced changes in body and brain weight.

2. Acetylcholinesterase is a known biochemical marker of carbofuran poisoning. The activity of acetylcholinesterase was found to be inhibited in all the brain regions studied following chronic carbofuran treatment. The decrease was maximal in cerebellum, followed by cerebral cortex and brain stem. NAC treatment along with carbofuran resulted in partial recovery of the enzyme in different brain regions.

3. A significant increase in lipid peroxidation in terms of TBARS was observed in different brain regions after carbofuran exposure, maximum being in cerebral cortex followed by brain stem and cerebellum. NAC administration had decreased the carbofuran-induced lipid peroxidation.

4. A significant depletion in the levels of glutathione and total thiols in the brain regions was observed after carbofuran treatment. Beneficial role of NAC in protecting brain against free radical damage was observed as the content of GSH and total thiols increased in the brain.
of NAC plus carbofuran treated animals as compared to the carbofuran treated animals.

5. The activities of superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase were significantly decreased in the brain regions after carbofuran exposure, whereas NAC administration along with carbofuran resulted in a significant increase in the activities of these enzymes.

6. Calcium homeostasis in synaptosomes was studied in carbofuran treated animals. There was a significant increase in $^{45}$Ca$^{2+}$ uptake through voltage operated calcium channels with a concomitant decrease in Ca$^{2+}$-ATPase activity resulting in marked elevation in the intracellular free Ca$^{2+}$ levels. NAC administration had beneficial effect on carbofuran-induced alterations in calcium homeostasis.

7. A significant decrease in the levels of total lipids was observed after carbofuran exposure. This decrease was attributed to the reduction in the phospholipid levels although total cholesterol levels were found to be slightly increased. Furthermore, NAC administration ameliorated carbofuran-induced alterations in lipid composition. All the other lipid fractions studied viz. glycolipids, triglycerides and gangliosides were not affected in the carbofuran treated group.

8. Structural alterations in terms of modified lipid composition affects the activity of membrane bound enzyme, receptors and proteins. The ratio of cholesterol to phospholipid, the major determinant of membrane fluidity, was increased in response to carbofuran exposure leading to impairment of Na$^+$-K$^+$-ATPase activity. NAC had shown protection against carbofuran toxicity by restoring the cholesterol to phospholipid ratio and Na$^+$-K$^+$-ATPase activity.

9. Neurobehavioral deficits were observed in terms of motor and memory functions. Marked impairment in the motor function was seen following carbofuran exposure, which were evident by the significant decrease in the locomotor activity and reduction in the retention time of the rats on rotating rods. Cognitive deficits were also seen as
indicated by the significant decrease in active and passive avoidance response. NAC treatment significantly attenuated carbofuran-induced neurobehavioral deficits.

10. Histopathological studies of carbofuran exposed brain revealed high frequency of pyknotic neurons in the cerebral cortex and degeneration of the purkinje cells in the cerebellum. Effect of carbofuran on brain stem was manifested by slight microhemorrhages. NAC supplementation along with carbofuran resulted in normalization of the brain architecture as seen by reduction in the number of pyknotic nuclei in the cerebral cortex and no visible changes in the cerebellum and brain stem.

These findings demonstrate the biochemical alterations produced by chronic carbofuran exposure and the beneficial effects of NAC administration. It is evident that oxidative stress plays a pivotal role in carbofuran-induced neurotoxicity. In addition, carbofuran-induced alterations in calcium influx and efflux mechanisms result in marked accumulation of intracellular calcium, which is a sensitive index of neurotoxic damage. These changes may eventually be responsible for the histopathological changes and neurobehavioral deficits following carbofuran exposure. It can thus be concluded from the present study that carbofuran exerts its neurotoxic effects by augmenting the oxidative stress and modifying the calcium homeostasis, which could be ameliorated by the NAC treatment showing its potential therapeutic effects against carbofuran neurotoxicity.