Chapter-1

INTRODUCTION

Owing to increased food demand pesticides have become an integral part of modern agriculture as effective crop protectants. Currently, among all the classes of pesticides, carbamates are the most commonly used pesticides and have replaced organochlorines, which are persistent in nature. Carbamates have an advantage over organophosphates which although are less persistent but have serious neurotoxic, neurobehavioral and neuropsychological consequences (Abou-Donia, 2003). As a result of widespread use of carbamates, many incidences of their accidental poisoning in humans, animals, birds and fishes are reported worldwide. In addition to accidental poisoning, occupational exposure also takes place during manufacture and use of carbamates (Huang et al., 1998). The environmental exposure, involving general population is mainly due to the ingestion of the contaminated foods and water (Erickson and Norton, 1990; Kumari et al., 2002). Unfortunately, because of their easy availability and accessibility, they have also been commonly abused for suicidal purpose in the developing countries.

Among carbamates, carbofuran is the most commonly used in agriculture as a broad-spectrum insecticide and nematicide (Kuhr and Dorrough, 1976). Carbofuran has high mammalian toxicity through the oral and inhalation routes of exposure and therefore may pose a serious threat to those in immediate contact (Fahmg et al., 1970). Discovery of carbofuran in ground water and accidental poisoning from ingesting contaminated vegetables and fruits have spurred interest and concern about this pesticide (Chapalmadgu et al., 1992). Residues of carbofuran and its metabolites have been found in sugarcane plant and soil after application at 1 and 2 kg/ha (Battu et al., 2000). Carbofuran-containing insecticides are widely used by Indian farmers, thus endangering fishes by exposing them to the hazards after entering the aquatic environment through runoff during agricultural use (Guhathakurta and Bhattacharya, 1988). Carbofuran has been detected in California ground water at levels as high as 5
mg/L and in Sacramento-San Joaquin Delta at a maximum concentration of 1.33 mg/L (HSDB, 1998).

Carbofuran can produce life-threatening effects on the brain by virtue of acetylcholinesterase inhibition (Tobin, 1970). Acetylcholinesterase inhibition results in accumulation of acetylcholine, which exerts signs of cholinergic hyperactivity, including tremors, convulsions, muscle fasciculations and status epilepticus causing neuronal injury and death (Olney et al., 1986). Carbofuran exposure may also cause debilitating syndromes like headaches, memory loss, proximal muscle weakness, cramps and anorexia with marked weight loss (Gupta et al., 1994). Carbofuran administration has been shown to cause significant changes in neurotransmitter concentrations viz. gamma-aminobutyric acid, epinephrine, norepinephrine and dopamine (Gupta et al., 1984). It is also shown to cause changes in isoenzyme patterns of creatine kinase and lactate dehydrogenase and a significant decrease in the levels of total adenine nucleotides (Gupta et al., 1991; Gupta et al., 1994).

Despite the history of research on the cholinergic effects of carbofuran, there is relatively little information on mechanisms of its action other than the AchE inhibition. Various mechanisms involved in the pathogenesis of neuronal damage appear to be linked to free radical-mediated injury (Braughler and Hall, 1989). It is well documented that almost all the intracellular ATP is generated in the mitochondria and chemicals causing mitochondrial damage/dysfunction also cause ATP depletion and excessive generation of reactive oxygen species, which leads to oxidative stress (Pederson, 1999; Murphy et al., 1999). The detailed investigation into the possible brain damage following chronic carbofuran exposure is required to be studied as brain is highly susceptible to oxidative stress because of its high demand of oxygen, large amount of peroxidizable fatty acids and relatively low antioxidant capacity (Floyd, 1999). In one of the studies, correlation has been found between the accumulation of acetylcholine and extent of lipid peroxidation (Yang and Dettbarn, 1996). Lipid peroxidation, mitochondrial damage and reduction of neuronal energy level support the contention that AchE inhibitors such as carbofuran cause neuronal injury by excessive formation of reactive oxygen species (Yang and Dettbarn, 1998; Gupta et al., 2001a).
Calcium plays an important role in nervous system by regulating neurotransmitter release, neuronal membrane excitability and regulation of proteins (Somlyo and Himpens, 1989). Increasing evidence suggests that carbamates might in part exert its toxic effects by modifying calcium messenger system, which has serious consequences on neuronal functioning (Srinivas et al., 1989a; Babu et al., 1990). Disruption of the mechanism that regulates calcium homeostasis is often an early event in the development of reversible cell injury (El Fawal et al., 1990). Several studies have indicated the importance of increased intracellular calcium level in the mechanism of toxic cell death in a variety of animal tissues and cells (Farber, 1981). Increased calcium ion has been reported as a definite event in the etiopathogenic mechanism of the neurodegenerative diseases and is reported for activation of myriad arrays of events associated with deleterious effects (Choi, 1992; Liu et al, 1994). Therefore, it was thought imperative to study the effect of carbofuran on calcium homeostasis.

N-Acetylcysteine is a known thiol antioxidant that acts as a precursor for the natural antioxidant glutathione and is shown to be a scavenger of hydrogen peroxide and hydroxyl radical in vitro (Aruoma et al., 1989; De Flora et al., 1991). Flora et al. (2004) have demonstrated beneficial role of NAC against lead-induced diminished antioxidant defence system. It has been successfully used in various models of adult respiratory distress syndrome (Zhang et al., 1994). Aydin et al. (2002) have shown that NAC protects against the toxic effects of ethanol by increasing the brain glutathione level and activities of various antioxidant enzymes. Xiong et al. (1999) have also shown that NAC protects against traumatic brain injury by restoring the mitochondrial damage. Very preliminary evidence indicates that NAC may help in slowing the progression of Alzheimer's disease and restoring the memory deficit associated with aging (Grassi et al., 1980; Martinez et al., 2000).

In order to elucidate the biochemical mechanisms of chronic carbofuran neurotoxicity in rats and to evaluate the possible beneficial effects of NAC in protection against carbofuran neurotoxicity, the present study was designed with the following objectives.
Objectives

1. To understand the effect of chronic carbofuran exposure on lipid peroxidation and antioxidant defence system in rat brain.
2. To study the effect of chronic carbofuran exposure on neuronal calcium homeostasis.
3. To study the alterations in lipid composition and membrane bound enzymes following chronic carbofuran treatment.
4. To correlate the effect on biochemical changes with histopathological and behavioral alteration following chronic carbofuran exposure.
5. To study the protective role of NAC against neurotoxicity of chronic carbofuran exposure.
6. To isolate and identify carbofuran-degrading bacteria from carbofuran pretreated soil.