Chapter 1

INTRODUCTION
The epilepsies are an important, common and diverse group of symptom complexes characterized by recurrent spontaneous seizures. Although many patients with epilepsy have their seizures controlled effectively by antiepileptic drugs (AEDs), about one-third of patients continue to have seizures, despite trying a range of AEDs (Sisodiya, 2007). Use of AEDs is frequently associated with a number of adverse drug reactions including hepatic, cognitive and neurological dysfunctions and electrolyte disturbances (Weinstein et al., 1984; Ahmed and Siddiqi, 2006; Koenig et al., 2006; Iorio et al., 2007; Shannon and Love, 2007). Therefore, there is a continued need to develop improved therapies for the effective and safe management of epilepsy, which is possible only through a better understanding of the basic mechanisms involved in the pathophysiology of the disease.

**Oxidative stress**

Oxidative stress and mitochondrial dysfunction are increasingly being recognized to have important roles in the pathophysiology of neurological diseases like epilepsy (Patel, 2004; Baron et al., 2007; Gao et al., 2007). A mitochondrial dysfunction causes an abatement in adenosine triphosphate (ATP) production, oxidative damage and the induction of apoptosis by opening the mitochondrial permeability transition pores, which are sensitive to stimuli like oxidative stress, calcium (Ca$^{2+}$) overload or ATP depletion (Sas et al., 2007). Reactive oxygen species (ROS) have been implicated in the initial phases of seizure induced pathology (Bruce and Baudry, 1995) and several studies have reported oxidative stress in different brain regions following experimental seizures (Frantseva et al., 2000; Rola et al., 2002; El-Abhar and El Gawad, 2003;
Marini et al., 2004; Gulati et al., 2005; Rajasekaran, 2005; Tejada et al., 2007). The ability of antioxidants to protect against the seizure manifestations and the accompanying biochemical changes further highlights a role of free radicals in seizures (Tan et al., 1998; Gupta et al., 2002a; Mohanan and Yamamoto, 2002; Barros et al., 2007; Xavier et al., 2007). In addition to this, resveratrol, a polyphenolic compound with potent antioxidant activity, has been reported to potentiate the effects of sodium valproate (SVP) and diazepam against pentylenetetrazole (PTZ) induced seizures in rats (Gupta et al., 2002b).

Phenytoin (PHT) and SVP are among the widely used first line AEDs effective in the treatment of both generalized tonic-clonic (GTC) and focal onset seizures (Lowenstein, 1998). However, these drugs have a narrow margin of safety and their use in epileptic patients has occasionally been associated with disturbances in the blood antioxidant defense systems and increased lipid peroxidation (Mahle and Dasgupta, 1997; Liu et al., 1998; Ono et al., 2000; Hamed et al., 2004).

N-acetylcysteine (NAC) is a thiol-containing compound, which has been used in clinical practice for several years (Moldeus and Cotgreave, 1994). It has antioxidant properties and a few studies have reported the beneficial effects of NAC administration against lipid peroxidation both in the peripheral tissues and in the central nervous system (CNS) (Demir and Inal-Erden, 1998; Nehru and Kanwar, 2004; Kamboj et al., 2006a). N-acetylcysteine in high doses has been reported to improve and stabilize the neurological symptoms in patients with Unverricht-Lundborg disease, a type of progressive myoclonic epilepsy in which oxidative stress has been thought to be an
important factor (Ben-Menachem et al., 2000). Additionally, NAC administration has been reported to reverse the memory impairment in aged SAMP8 mice (Farr et al., 2003). In preclinical studies, NAC has shown effectiveness against aminophylline (Gulati et al., 2005) and PTZ induced seizures (Devi et al., 2006a). We previously reported a facilitatory action of NAC on the anticonvulsant effects of SVP against the PTZ (Devi et al., 2006a) and the electroconvulsive threshold model (Devi et al., 2006b) of seizures. However, there is no report to date on the role of NAC in antiseizure therapy along with PHT and SVP against maximal electroshock (MES) induced seizures, an animal model of GTC seizures. Thus, the present study was planned to analyze the type of interactions of NAC with PHT and SVP in the mouse MES test and further to investigate the role of oxidative stress in the mediation of above effects. The latter is relevant in view of a role of oxidative stress in seizures and the evidence for the involvement of peroxidative injury in the adverse effects of AEDs. Further the effects of PHT, SVP, NAC and their combinations on neuromuscular function, memory, serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and calcium levels were also investigated.

**Histaminergic mechanisms**

A significant amount of research in epilepsy has focused on the gamma-aminobutyric acid (GABAergic) and the glutamatergic systems, while the role of brain histamine in regulating the seizure susceptibility has only recently been documented. Several lines of evidence, including clinical and experimental have suggested an anticonvulsant action of endogenous histamine (Tuomisto and Tacke, 1986; Yokoyama et al., 1992, 1994a;
Interestingly, a few reports in literature have suggested an involvement of free radicals in the process of endogenous histamine release, a phenomenon reported to be blocked by free radical scavengers like reduced glutathione (GSH) and D-mannitol (Masini et al., 1987; Mannaioni et al., 1988). However, contrary to this, there are also reports in literature, where NAC, known to have free radical scavenging properties (Aruoma et al., 1989) was found to enhance the release of histamine from mast cells (Barrett et al., 1985; Hong et al., 1991). Therefore, the role of brain histamine in the effects of PHT, SVP, NAC and their combinations against MES induced seizures was also investigated.

H₃ receptors have an important role in mediating the endogenous histamine release (Arrang et al., 1983). Clobenpropit, a H₃ receptor antagonist has been reported to increase the histamine release (Jansen et al., 1998). Additionally, it has also been reported to increase the activity of histidine decarboxylase (HDC) (Yokoyama et al., 1994b), an enzyme that requires pyridoxine in the form of pyridoxal-5-phosphate as a coenzyme (Lee et al., 1988). While clobenpropit has shown effectiveness against both electrically induced seizures (Yokoyama et al., 1994b) and kindling (amygdala and PTZ) models (Kamei, 2001; Zhang et al., 2003), there are also scientific reports that mention pyridoxine deficiency as a risk factor for seizures (Schlesinger and Lieff, 1975; Sharma and Dakshinamurti, 1992; Sharma et al., 1994). Experiments were thus designed to study the effects of clobenpropit, pyridoxine and their combinations on MES induced seizures and subsequent changes in brain histamine levels.
Intracellular calcium

Calcium ions play a pivotal role in normal neuronal function. The large difference between intracellular and extracellular Ca$^{2+}$ concentration highlights the importance of the mechanisms controlling influx and efflux of this ion (DeCoster, 1995). Loss of the regulatory ability of these mechanisms and the subsequent increased intracellular calcium [Ca$^{2+}$]$_i$, may be involved in the pathological events of epilepsy, including its induction and maintenance (DeCoster, 1995; DeLorenzo et al., 2005). Inhibition of Ca$^{2+}$ currents is thought to underlie the mechanism of action of several conventional and newer AEDs (Moshe, 2000; Rogawski and Loscher, 2004; Broicher et al., 2007; Meldrum and Rogawski, 2007).

Oxidative stress causes an increase in the Ca$^{2+}$ concentration in the cytoplasm either via release of Ca$^{2+}$ from internal cellular stores or influx from extracellular environment. Rising Ca$^{2+}$ concentration in the cytoplasm causes Ca$^{2+}$ influx into mitochondria and nuclei, thereby disrupting their normal function (Ermak and Davies, 2001). Jayalakshmi et al. (2005) have reported the ability of NAC to prevent the hypoxia induced increase in [Ca$^{2+}$]$_i$ levels in primary hippocampal culture.

Excitotoxicity is thought to be a major mechanism in many human disease states including epilepsy and contributes to neuronal degeneration (Arundine and Tymianski, 2003; Fujikawa, 2005; Gardoni and Di Luca, 2006). Recently, Garduno-Torres et al. (2007) have reported the inhibition of Ca$^{2+}$ dependent glutamate release from thalamic synaptosomes by histamine and immepip (a H$_3$ agonist), an effect that was reversed by clobenpropit. Clobenpropit and pyridoxine have shown protective effects against
N-methyl-D-aspartate (NMDA) induced excitotoxicity and domoic acid induced seizure activity respectively. Reduction in Ca\(^{2+}\) influx was considered to be one of the probable mechanisms for the above observed protective effects of these drugs (Dakshinamurti et al., 2003; Dai et al., 2007). In view of the above reports, we also investigated the effects of PHT, SVP, NAC, clobenpropit, imetit and pyridoxine on [Ca\(^{2+}\)]\(_{i}\) concentrations in the mouse brain synaptosomes.