Review of Literature
2.1. Epilepsy Historical perspective

Epilepsy oldest account dates back to 2000 BC from a Babylonian textbook, which emphasizes its supernatural nature. Babylonians associated seizure type with the name of spirit or god-usually evil. Treatment was therefore, largely a spiritual matter. In 5th Century BC Greeks termed epilepsy as "The Sacred disease" and the word epilepsy is derived from the Greek "epilepsia" which means "to take hold of" or "to seize." In ancient times, patients were treated by saints (fig-1), prayed to St. Cornelius, St. Gilles, and St. Valentine for relief (Streeter, 1922). Hippocrates first described that epilepsy was not sacred, but a disorder of the brain: a revolutionary view. He recommended physical treatments and stated that if the disease became chronic, it turns incurable. Hippocrates' view of epilepsy as a brain disorder did not begin to take root until the 18th-19th centuries. In 19th century with the advent of neurology as a new discipline, distinct from psychiatry the concept of epilepsy as a brain disorder became more widely accepted especially in Europe and North America. In 1857 Sir Charles Locock introduced bromide, as the world's first effective antiepileptic drug which later became popular in Europe and North America during the second half of the last century. Hughlings Jackson (1873) a London neurologist, laid foundation of our modern understanding of pathophysiology associated with epilepsy. After the discovery of EEG "Brain waves" by Hans Berger (1920) different patterns of brainwave discharges associated with different types of seizure types were identified. This helped to locate the site of seizure discharges and expanded the possibilities of neurosurgical treatments. During the first half of this century the main drugs for the treatment of epilepsy were phenobarbitone (1912) and phenytoin (1938). In the last few decades, horizon of the understanding and treatment of epilepsy has been increased due to the developments in structural and functional neuroimaging, especially computer tomography (CT) scanning,
magnetic resonance imaging (MRI) and MRI spectroscopy and positron emission tomography. Of the estimated 40 million people in the world with epilepsy, 32 million have no access to treatment at all - either because of lack of services or because epilepsy was not viewed as a medical problem or a treatable brain disorder. Most of the advances in developed economies are of little or no relevance to the 80% of people with epilepsy who live in developing countries. For most of these people the older supernatural views, social stigma and discrimination still prevail. Even in the developed world, the disorder is still shrouded in secrecy, and people prefer not to reveal or discuss their illness. Temkins (1971) has rightly said that the history of epilepsy epitomizes the long struggle between magical and scientific concepts of disease, and only over the last two centuries has epilepsy found himself wearing the winner, colour with increasing frequency. Recently in 1997, two organizations, International League Against Epilepsy (ILAE), International Bureau for Epilepsy (IBE) joined World Health Organization in the Global Anti-Epilepsy Campaign aimed at improving prevention, treatment, care and services for those with epilepsy and raising public awareness of the disorder and its acceptability.

2.2. Definition and types of epilepsy

Epilepsy is a disorder of the brain characterized by an enduring predisposition to generate seizures, and by the neurobiologic, cognitive, psychological, and social consequences of the condition. The ILAE and IBE proposed the definitions of epileptic seizures and epilepsy, stating that “an epileptic seizure is a transient occurrence of signs and/or symptoms due to abnormal excessive or synchronous neuronal activity in the brain. The definition of epilepsy requires the occurrence of at least one epileptic seizure” (Fisher et al., 2005). Patients with epilepsy are prone to cognitive and neurobehavioral deficits (Devinsky, 2004). For example, temporal lobe epilepsy (TLE), the most common type of epilepsy in adults, is associated with memory impairment and behavioral problems, including depression, anxiety and psychoses (Gaitatzis et al., 2004). Epilepsy could not be cured but controlled with medication. In severe cases surgery options are usually considered.

According to the causal etiology, epilepsy is classified into idiopathic and symptomatic epilepsies. Most types of idiopathic epilepsy, including absence seizures, are age-dependent
and benign. They are thought to have a genetic predisposition, for which several candidate genes have recently been identified, mainly at ion channel loci (see review of Hirose et al., 2000; Berkovic and Scheffer, 2001; Moulard et al., 2001). Idiopathic epilepsy is a common and heterogeneous neurobiological disorder arising due to biochemical and molecular alterations that are yet to be completely understood. The development of the molecular markers and genomic resources has facilitated the isolation of genes responsible for rare monogenic epilepsies in human and mouse. Many of the identified genes encode for Na⁺, K⁺ and Ca²⁺ channel proteins or other components of neuronal signaling (Meisler et al., 2001; Park et al., 2003). Idiopathic epilepsy should not be understood as a single disorder, but rather as a syndrome with vastly divergent symptoms involving episodic abnormal electrical activity in the brain. In contrast, a variety of etiologies are known in symptomatic epilepsies (Engel, 2001), which involve birth accidents, abnormal brain development (e.g., migration disorder), infection, vascular diseases, head trauma, brain tumors, and neurodegeneration. However, there are also many examples of epilepsy without such clear epileptogenic lesions (cryptogenic epilepsy). Though epilepsy is idiopathic but it is estimated that up to 50% of all epilepsy cases are initiated by neurological insults and are called acquired epilepsy (DeLorenzo et al., 2005). Acquired (symptomatic) epilepsy develops in three phases: (1) the initial brain insult, (2) a latency period, during which epileptogenesis takes place, and (3) spontaneous recurrent seizures (SRS), i.e., the chronic epileptic phase. Status epilepticus (SE), stroke, and traumatic brain injury are three major examples of common brain injuries that can lead to the development of acquired epilepsy (DeLorenzo et al., 2005).

Epileptic seizures are generally classified into two groups, focal (partial) and generalized. The clinical manifestation of focal seizures varies depending upon the origin of epileptic discharges (the epileptic focus), and motor, sensory, autonomic, and psychic symptoms. Seizures are defined as simple partial if there is no loss of consciousness, and complex partial if there is a loss of consciousness. Generalized seizure (widespread seizure discharge), includes a strong participation of motor system circuitry, which results in a convulsive response, that includes both tonic (sustained contractions) and clonic (oscillating contractions and relaxations) components. Absence seizures are primary generalized seizures that do not include strong motor system recruitment, and appears to be
pre-generalized seizures. Even focal seizures, however can become generalized (secondary generalized seizures) (Morimoto et al., 2004). In contrast to absence seizures, myoclonic seizures are brief and sudden which involve involuntary muscle contractions of variable distribution and manifestations (Fahn et al., 1986). They may involve whole body (generalized) and differ from tonic clonic seizures, as they are of much shorter duration and involve much less movement. Myoclonic seizures may be focal or regional, single or repetitive jerks, unilateral or bilateral and symmetrical or asymmetrical (Leppik et al., 2003).

2.3. Post-Traumatic Epilepsy

Post traumatic epilepsy is a symptomatic development of seizures due to secondary damage to the brain after head injury. Therefore, prevention of post traumatic epilepsy is of primary importance to reduce the degree of functional morbidity associated with traumatic brain injury (TBI). After TBI, it’s important to differentiate between provoked and unprovoked seizures, in order to assess and treat post-traumatic epileptogenesis. By definition, seizures occurring within 24h of TBI (immediate seizures) and seizure occurring between 4 h and the first 7 days after head injury (early post-traumatic seizures) are provoked seizures, whereas seizures seen after this (late post-traumatic seizures) are unprovoked seizures (Beghi, 2003). An unprovoked seizure occur in the absence of one or more precipitating factors, whereas a provoked (acute symptomatic) seizure occur in close temporal relation with an acute systemic, toxic, or metabolic insult (including TBI). Computerized tomography (CT) scan studies have demonstrated that the most powerful factor of early and late epilepsy is focal hemorrhagic brain damage (D’Alessandro et al., 1982).

Adams et al., 1997 have defined different types of head injury which can result into post-traumatic epileptogenesis. For example, closed head injury, in which nothing penetrates the skull; an open head injury, where the skull is penetrated by an object, as seen in a gunshot or shrapnel wound; concussion, resulting from injury by violent shaking or jarring of the brain with an associated transient functional impairment; and contusion by bruising of cerebral tissue with preservation of its original architecture. A universally accepted classification for head injury severity is yet to be developed, although a number of alternate strategies have been used. Hahn and coworkers (1988) proposed a severity
overall risk of seizures after TBI ranges from 2 to 5% in civilian populations; it ranges from ~39% in patients with cortical injury and neurologic sequel and < 50% with dural penetration (Caveness et al., 1979). All the above statistics shows that prevention of PTE is necessary to reduce the seizure risk associated with head injury. A population based study reported that the cumulative probability of developing late seizures after TBI, significantly correlated to the severity of the head injury. One of the study have shown that, incidence ratio of late seizures was 1.5 after mild injuries and 2.9 after moderate injuries and 17.0 after severe head injuries (Annegers, 1998). Men are more commonly affected than women, and the incidence peaks between the ages of 15 and 24. (Langendorf and Pedley, 1997).

2.3.2. Mechanisms of Post-traumatic epileptogenesis

Since the days of Hippocrates, the mechanism of PTE is unclear. There is usually a latent or clinically silent period of variable duration (ranging from weeks to years) between the brain insult and the occurrence of the first late unprovoked seizure. Variety of changes like cell death, axonal sprouting, changes in excitatory and inhibitory neurotransmitters, neurogenesis, and network reorganization, which result in hyperexcitability and spontaneous seizures generation have been shown to occur during latent period (Chang and Lowenstein, 2003). There are multiple theories that have been developed to explain the mechanisms behind the development of chronic seizures after a head injury. Of which formations of deleterious free radicals by blood in the parenchyma of the brain, resulting in excitatory activity following injury, and changes in the inhibitory functions of the brain is widely accepted. Mechanical effects of trauma causes bulk displacement of tissue with secondary responses that include alterations in cerebral blood flow, changes in intracranial pressure, and altered vascular permeability (Willmore, 1990). Histopathological studies of material obtained from traumatized brain show formation of axonal retraction balls, reactive gliosis, wallerian degeneration and microglial scar formation within cystic white matter lesions (Tornheim et al., 1983). Head injury or
Figure 3. Showing mechanism of development of epileptogenesis (Modified by Mori et al., 1990).

Hemorrhagic cortical infraction results in extravasations of blood and breakdown of red blood cells (RBC) and hemoglobin (Hb). Biologic iron is normally protein bound in hemoglobin and transferring but free iron liberated are sequestered in the form of hemosiderin, a prominent histopathologic feature of human post-traumatic epilepsy (Payan et al., 1970). Iron liberated from hemoglobin is thought to be associated with the generation of reactive oxygen species. Moreover, Hb itself may promote oxygen free radicals, especially OH, are mainly responsible for the peroxidation of neuronal lipids (an injury to neuronal membrane). On the other hand hydroxyl radicals can also accelerate the production of guanidine compounds (endogenous convulsants). These alterations followed by excitatory and inhibitory neurotransmitter release disorder are believed to be a probable cause for the development of epileptic discharges in the epileptogenic focus (Mori et al., 1990).

2.4. Animal models of post-traumatic epileptogenesis.

There are several animals model of post-traumatic epilepsy reproducing the human post-traumatic condition exists to elucidate the pathophysiological substrates of epileptogenesis and to perform preclinical screening of anti epileptic drugs (AEDs) (Schmidt and
Rogawaski, 2002; Stables et al., 2002; White, 2003). Of which three are well established as listed in table-1.

Table-1. Showing methods to induce epileptogenesis in rat model.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Animal Model</th>
<th>Procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cortical undercut model (Prince and Tseng, 1993; Hoffman et al., 1994; Graber and prince, 1999).</td>
<td>A small region of neocortex is partially isolated by using a needle bent at 90° and transecting the underlying white matter.</td>
</tr>
<tr>
<td>2</td>
<td>Fluid percussion injury model (D’Ambrosio et al., 2004; Nilsson et al., 1994)</td>
<td>A brief (10ms) pressure pulse of 3.75-4 atm on the intact dura (Burr hole = 3mm)</td>
</tr>
<tr>
<td>3</td>
<td>Iron injection model (Willmore et al., 1978)</td>
<td>100mM FeCl₃ injected intracortically to induce seizures</td>
</tr>
</tbody>
</table>

Iron-induced epilepsy model (3) was first proposed by Willmore et al., (1978). They have shown that unilateral injection of iron into the somatosensori cortex can induce epileptiform activity in the electrocorticogram (ECoG). Injection of FeCl₃ mimics the release of iron by breakdown of hemoglobin, released from blood in the neuronal tissue after head injury (Willmore et al., 1978a,b). Generally epileptiform discharges are induced 15 minutes after ferric chloride injection into the rat sensorimotor cortex, with discharges detected for more that six months (Moriwaki et al., 1992). Therefore, in the present study FeCl₃ induced epileptogenesis model has been used to study the antiepileptogenic efficacy of curcumin and L-deprenyl.

2.4.1. Pathophysiology associated with Iron induced epileptogenesis

Deposition of ferrous compounds into neuronal tissue after head injury or hemorrhagic cortical infarction is known to initiate a Haber-Weiss iron-catalyzed reaction that results in the hyperproduction of reactive oxygen species (ROS) like superoxide anion(O₂•⁻), hydroxyl radicals (OH* ) (Kucukkaya et al., 1998; Willmore and Rubin 1981), and reactive nitrogen species (RNS) at the focal site of injection / infarction (Mori et al., 2004), which lead to the initiation of lipid peroxidation (Willmore et al., 1978a,b) of the neuronal membrane which was observed as increased TBARS content (Kabuto et al., 1998; Hiramatsu et al., 1990), fluorescent product (Triggs and Willmore, 1984) and protein
oxidation (Wu et al., 2006). DNA damage was also observed in the form of elevated levels of 8-hydroxy-2'-deoxyguanosine (8-OH dG) (Komatsu et al., 2000). Iron is also shown to disrupt cellular thiol functions and affect the activities of superoxide dismutase (SOD), Glutathione reductase (GR) and Glutathione peroxidase (GPx) (Singh and Pathak 1990). The induction of an epileptic focus by iron deposition is also related to decreased nitric oxide synthase activity (Kabuto et al., 1998) as well as activation of dopaminergic neurons. The increased production of reactive species and initiation of lipid peroxidation accelerated production of guanidine compounds in the brain, which in turn leads to epileptogenicity (Mori et al., 1990). Hence, biochemical alteration could be one of the reasons for electrophysiological impairments during epileptogenesis.

Intracortical injection of FeCl₃ elicits a continuum of epileptic seizure consisting of bursts, spikes and multiple spike wave complexes in a high percentage of animals (Willmore et al., 1978; Engstrom et al., 2001). Rats exhibiting isolated spikes do not show any behavioral abnormality, whereas, rats showing spike and wave complexes exhibited vibrissa tremors and head nodding but no other abnormality (Moriwaki et al., 1992; Willmore et al., 1978). The iron induced epileptiform activity starts from the ipsilateral side (Injection site) and after some time become generalized and involves almost entire cerebral cortex of both hemispheres (Moriwaki et al., 1990, 1992). With time epileptiform activity spreads to intracortical areas like thalamus, locus cerulus and substantia nigra (Sharma et al., 1999a, b). Several workers have reported that subcortical injection of FeCl₃ into hippocampus/amygdala potentially initiates epileptogenesis process. For example, Castigligoni et al., (1990) reported that unilateral injection of FeCl₃ into the hippocampus of rats induced spontaneous spiking activity ipsilaterally which later propagates to contralateral hippocampi. Recently, it has been shown that Generalized convulsive seizures starts within the first week post-operation, and spontaneous epileptiform activity and generalized seizures lasted as long as 2 weeks post-operation (Yao et al., 2006). In addition amygdalal nuclear injection of 100 mM FeCl₃ has been reported to induce chronic, spontaneous, recurrent focal seizures with generalized limbic behaviors similar to those induced by electrical kindling (Ueda et al., 1998). They proposed that typical rodent limbic seizures began within 5th day after injection and progressed as stage-I, II, III and IV epileptic discharges throughout the duration of observation. Electrophysiological recording
exhibited propagation of epileptic focus from the injection site to the contralateral amygdala. Intense behavioral seizures, matching stage 4 patterns behavioral score of kindling (Racine et al., 1972), were associated with presence of epileptiform discharges in both hippocampi and both amygdala (Ueda et al., 1998).

Neurotransmitter imbalance also plays a crucial role in the development of epileptogenesis. Studies have shown elevated levels of excitatory amino acids (EAA) like glutamate and aspartate after brain injury in epileptogenic rats (Nilsson et al., 1994; Hillered and Persson, 1999; Janjua et al., 1990) as well as humans (Ronne-Engstrom et al., 1992). Increased EAA causes a large calcium dependent increase in extracellular potassium (Katayama et al., 1990) which increases neuronal excitability and, leads to seizures and cell death (Langendorf and Pendley, 1997). Thus, a physical insult (trauma) to the brain initiates cascade of excitatory events that results in neurotoxicity and seizure activity. Increased extracellular levels of glutamate further enhance the synaptic release of EAA in patients and animal models (During and Spancer 1993). Glutamate present in brain as well as extracellular fluid is involved in many metabolic functions, however, excess glutamate exerts an excitotoxic effect (Evans et al., 1962). The extracellular level of glutamate is normally regulated by a rapid uptake into astroglial cells by specific glutamate transport proteins (Rothstein et al., 1996). EAA excessively activates their neurotransmitter receptors, to initiate generation of NO and ROS, which is followed by accelerated production of guanidine compounds (Lafoz-cazal et al., 1993; Lancelot et al., 1998). The accelerated release of EAA can trigger excitotoxicity of the NMDA receptors in acute seizures, which is followed by the formation of a chronic epileptogenic focus (Janjua et al., 1990). Contrarily, lipid oxidation causes severe damage to GABA release than GABA uptake, Hence imbalance between release and uptake causes reduction of GABA inhibition (Zhang et al., 1989) inside the epileptic area. Decreased inhibition by GABA and increased excitation by glutamate explains the mechanism for generation of seizures (Janjua et al., 1990; Zhang et al., 1989) in iron-induced epileptogenesis or epilepsy in general. It has been reported that EAA transporter proteins GLT-1 and GLAST protein were transiently decreased during epileptogenesis in TBI animal models (Ueda et al., 2001). This also contributes elevation of EAA level at the focal site of injury. The above report was
substantiated by the report that genetically GLT-1 deficient mice die from status epilepticus, and are more prone to excitotoxic brain injury after TBI.

In the iron induced epileptic brain, cortical area near the injection site was shown to be positively stained for iron, whereas no iron was detected in any other part of cerebrum including corpus callosum and contralateral cortex (Moriwaki et al., 1992). Iron injection is associated with a variety of histopathological alterations like astrogliosis, neuronal loss, and iron filled macrophages and with modest fibroblast growth at the pial surface. Numerous shrunken slightly angulated, hyper-chromatic neurons (pyknotic) have been demonstrated within the zone of tissue adjacent to the glial lined cavity (Willmore et al., 1981). Furthermore, golgi staining of epileptogenic tissue have been shown to exhibit dendritic abnormalities, including loss of spines, nodularity, and string of beads deformity (Reid et al., 1979). The most common reaction of astrocytes to injury in CNS is the striking hypertrophy and/or proliferation of these cells, generally referred to as reactive astrogliosis or astrocytosis. Generalized astrocytic enlargement in the cerebral cortex in human and animals with temporal lobe epileptic foci (Garzillo and Mello, 2002), and their containment of great amount of glial fibrillary acidic protein (GFAP) are considered to be an indicative of alteration in the functional state of astrocytes (Otani et al., 2003; Pekny and Pekna, 2004; Pekny and Nilsson, 2005).

2.4.2. Prophylaxis of epileptogenesis

Prophylaxis is the process of protecting against the development of a specific disease by an action or treatment that affects pathogenesis (Willmore, 1990). The current treatment of epilepsy focuses exclusively on the prevention or suppression of seizures. Recent findings regarding the sequence of neurological alterations leading to epilepsy and its molecular basis have raised the question, whether or not process of epileptogenesis and its progression could be prevented, or modified in a way, that its development is milder, easier to treat, non-progressive and without cognitive decline and drug-resistance (Loscher and Schmidt, 2004; Pitkanen, 2004). There are reports demonstrating that intense seizure activity associated with status epilepticus causes hippocampal damage in part by excessive activation of glutamate receptors and resultant excitotoxicity (Slovitor et al., 1985; Olney, 1986; Meldrum and Chapman 1999). The view that neuronal death can cause epilepsy gain support from earlier studies demonstrating that surgical removal of a damaged
hippocampus improves the condition of epilepsy patients (Bruton, 1988). In addition, it is believed that seizure-induced neuronal death involves overlapping processes of necrosis and apoptosis (Bengzon et al., 1997; Slovitor et al., 1996), which are strongly influenced by mitochondrial function and oxidative stress. Seizure activity initiates the mitochondrial pathway of apoptosis via involvement of proapoptotic factors, cytochrome c translocation, and caspase-3/9 activation (Henshall et al., 2001; 2002). Hence, therapies for the various types of epilepsies are largely aimed at decreasing neuronal excitability and thereby controlling the occurrence of epileptic seizures. A recent therapeutic approach is to search for antiepileptogenic rather than antiepileptic drugs, which could target underlying processes that lead to the development of epilepsy (Stables et al., 2003).

2.5. Aging process and Brain

Aging is an inevitable process which leads to a general decline in the structural, molecular (including genomic), biochemical and physiological functions of different organs and brain is at the top. Brain is more prone to aging due to oxidative stress contributed from relative high concentration of peroxidizable fatty acids, high oxygen consumption, high level of oxidizable dopamine molecules and poor cellular defense system i.e. antioxidant concentration. Aging is associated with several alterations in the neuronal networks including synaptic connectivity (Rapp and Gallagher 1996; Smith et al., 2000), electrotonic coupling (Barnes et al., 1987), receptor and channel properties (Potier et al., 1992; Gutierrez et al., 1996) and the number of functionality of specific types of neurons (Shetty and Turner, 1998; Cadacio et al., 2003). Aging-related changes in potassium and calcium buffering (Roberts and Feng, 1996; Thibault et al., 1998) and their extracellular (Sykova et al., 1998) are reported to affect neuronal synchronization and excitability (Dudek et al., 1999).

Aging is associated with alterations in neural electrophysiologic parameters such as action potentials, synaptic potentials, spontaneous field potentials (EEG). These changes signify age related impairment and disorganization of electrical signals leading to decrement in neurological functions (Singh and Sharma, 2005). EEG represents integrated post synaptic potentials and reflects the state of neurophysiological activity of the cerebral cortex. A higher frequency in EEG activity (increased alpha and beta activity) indicates
increased vigilance promoting influences (Siwak et al., 2000) and lower frequencies may be associated with the cognitive decline (Radeck et al., 1994). The human EEG is reported to show altered frequencies and synchronization with aging (Duffy et al., 1996; Mc Evoy et al., 2001). For example, the resting membrane potential of nerve cells remain unaffected (Potier et al., 1993), whereas, the synaptic resting membrane potential decreases with age (Tanaka and Ando 1990). Similarly, excitability of neuron decrease, while the rheobase was shown to increase in the CA1 hippocampal neurons in aged Wistar and Fischer 344 rats (Potier et al., 1993). Velocity of nerve impulse conduction (action potential) in peripheral nerve fibers decreases during aging (Johnson and Murray 1992). Factors such as axon shrinkage, demylination and intra-nodal distance influence conduction velocity (Aston-Jones et al., 1985; Nodera et al., 2004; Johnson and Murray 1992). Several neurons have ability to change their firing pattern during different states of arousal. Neurons which fire non-rhythmically during slow wave sleep, adopts burst-firing patterns during waking and REM sleep. This functional plasticity however shown to decrease with aging (Apartis et al., 2000).

Aging associated increase in lipid peroxidation significantly influences membrane excitability (Pellmer et al., 1995), and synaptic transmission (Avshalumov 2000, Zoccarto et al., 1995). As lipid peroxidation causes membrane’s lipid environment change it adversely affects the membranes Na-K ATPase activity (Mattson, 1998). Age related impairment of NA-K ATPase activity may also results from age related mitochondrial bioenergetics impairment (Sapolsk 2003). This enzyme is involved in maintaining the resting membrane potential. Age related increase of lipid peroxidation was correlated with age related decrease in GPx and GST activity (Singh and Sharma 2005). These antioxidant enzymes have been shown to play important role in scavenging free radicals.

Aging is associated with significant synaptic loss in both the middle and inner molecular layers of the dentate gyrus of the aged rat has been demonstrated, with reductions in the mean number of synapses per neuron for the entire synaptic population and within specific synaptic categories, such as perforated and non-perforated axospinous synapses (Morrison and Hof, 1997; Geinisman et al., 1995).

Aging is associated with increased load of oxidative damage, which accelerates expression of glial fibrillary acidic protein (GFAP) mRNA and proteins in humans and
inbred lab rodents (Finch et al., 2002; Finch and Longo, 2002; Morgan et al., 1997). The increased GFAP expression during aging is due to increased transcription of GFAP, as shown by in situ hybridization at a cellular level with intronic cRNA probes (Morgan et al., 1997). Rabbits also showed increased GFAP during aging (Woodruff-Pak and Trojanowsk, 1996). Age-related alterations in molecular functions are believed to modulate genetic expression of channel proteins. For example number of channels and their subunit composition alter electrical signaling (Murchinson and Griffith 1995).

Behavioral Studies have also shown poor synaptic plasticity for long-term potentiation in older rats' comparison to young ones (Davis et al., 1993). Aging process is also reported to alter inhibitory and excitatory post synaptic potential which mainly depends upon the number of binding sites (receptor density) available and the presynaptic release mechanism (Shen and Barnes 1996; Taylor and Griffith 1993). Candy et al., (2001) have shown that aged rats exhibited a 30%-40% decrease in NMDA receptor binding density. With age decline in the levels of NMDA receptors in CA1 / CA2 and subiculum (Wenk and Barnes 2000, Riedel et al., 1999) have been shown to correlate with the learning abilities.

2.5.1. Affect of aging on epilepsy

Epilepsy is a disease with common onset in the extremes of life. With aging, the incidence of epileptic seizures and epilepsy is highest, even exceeding that seen in childhood (Tallis et al., 1991). The mechanisms involved in seizure susceptibility and expression in the elderly are largely unclear. A pubmed search report published by Leppik et al (2006) shows that only 30 reports were published from 1965-2003, which directly modeled seizures and/or epilepsy in aged animals. This finding suggests that there is a relatively modest interest and limited progress in basic science research regarding epilepsy and aging. Until recently, seizure disorders and epilepsy in the elderly were one of several age-related medical problems that generated little interest. However, several epidemiologic studies conducted within the past 10–15 years have revealed that the elderly experience the highest incidence and prevalence of seizure disorders in developed countries (Luhdorf et al., 1986; Hauser et al., 1997) and this population exhibits an increased likelihood for
developing status epilepticus and status-related morbidity and mortality (DeLorenzo et al., 1992; Treiman et al., 1997).

Whether rodent’s exhibits a similar increase in seizure susceptibility/severity during aging is debatable, because of the contradictory data (Holtkamp et al., 2004; Darbin et al., 2004; Chiba et al., 1992). Various in vivo studies suggest both, an increased (Dawson et al., 1992; Klioueva et al., 2001) and a decreased (Kitani et al., 1985) susceptibility with aging. Also, several in vitro studies have been carried out evaluating neuronal excitability of old tissue in stimulation experiments. Again, increases (Landfield et al., 1986; Barnes et al., 1987) and decreases (Deupree et al., 1993; Potier et al., 1992; 1993) of neuronal excitability with aging have been reported. Holtkamp et al., (2004) employed a non-lesional in vitro epilepsy model to study seizure susceptibility, spread pattern, and propagation velocities in combined hippocampal-entorhinal cortex slices of aged rats and controls using electrophysiological methods and imaging of intrinsic optical signals. In aged animals, less extensive spread of seizure-like events into areas adjacent to the region of onset of activity and a decreased spread velocity in various anatomical regions were reported. In addition, both the activity-dependent shrinkage of the extracellular space (ECS)-volume and the extracellular K⁺ concentration were significantly reduced compared to controls. Neuromodulatory systems may also be altered during aging (Cunha et al., 1995; Klapstein and Colmers, 1997) and these systems have been shown to regulate epileptiform activity in vitro, as well as seizure severity/susceptibility and spread in vivo (Williamson and Patrylo, 1999; Patrylo et al., 1999; Rubinstein et al., 2001). Changes in the peripheral organs (e.g. renal clearance), blood brain barrier, and/or pharmacokinetics also are involved with producing altered EEG and behavioral characteristics in aged rodents during Kainate-induced status epilepticus (Darbin et al., 2004). Apoptotic neurodegeneration plays important role in neuropathological outcome of head trauma during aging. Differential vulnerability of neurons / glia to apoptosis and degree of myelinization and brain water content determines the impact of traumatic forces to transmit easier and deeper brain structures in the aging brain (Bittigau et al., 2003; 2004). Since, aging process leads to variety of derangements in the brain and makes it more vulnerable for dysfunctions, it is important to investigate the affect of aging on post-traumatic epileptogenesis in terms of its development and progression.
2.5.2. Pharmacological interventions

Clinical PTE is characterized by a latent period between injury and seizure development which provide an opportunity for the treatment of epilepsy with probable antiepileptic drugs (AEDs). Anticonvulsants are frequently administered soon after brain injury in an effort to prevent seizure activity. This procedure is known as anticonvulsant prophylaxis. It is truly prophylactic as drug administration occurs prior to observable seizures. None of the AEDs available in the market show effective protection against development of late seizures (spontaneous seizures), hallmark of PTE. One of the major problems associated with prophylaxis of PTE is that these AEDs directly or indirectly exerts adverse effects, which is enhanced in the presence of brain injury (Massagli, 1991). Negative effects of AEDs have been observed as cognitive and behavioral abnormalities in patients with head injury (Brunbech and Sabers, 2002). Prevention of epilepsy has been an obscure target. Majority of antiepileptogenic trials on experimental animals (Pitkanen, 2002) and in humans (Temkin, 2001) are still unsatisfactory.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Model</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbamazepine</td>
<td>Amygdala-kindled rats</td>
<td>Weakly attenuated</td>
</tr>
<tr>
<td></td>
<td>Amygdala-kindled cats</td>
<td></td>
</tr>
<tr>
<td>Diazepam</td>
<td>Amygdala-kindled rats</td>
<td>Attenuated</td>
</tr>
<tr>
<td></td>
<td>Pentyleneetetrazol-induced kindling in rats</td>
<td>Ineffective</td>
</tr>
<tr>
<td>Ethosuximide</td>
<td>Pentyleneetetrazol-induced kindling in rats</td>
<td>Attenuated</td>
</tr>
<tr>
<td>Felbamate</td>
<td>Amygdala-kindled rats</td>
<td>Weakly attenuated</td>
</tr>
<tr>
<td>Lamotrigine</td>
<td>Homocysteine thiolactone administration</td>
<td>Ineffective</td>
</tr>
<tr>
<td>Levetiracetam</td>
<td>Amygdala-kindled rats</td>
<td>Attenuated</td>
</tr>
<tr>
<td>Phenobarbital</td>
<td>Pentyleneetetrazol-induced kindling in rats</td>
<td>Attenuated</td>
</tr>
<tr>
<td></td>
<td>Amygdala-kindled cats</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Amygdala-kindled cats</td>
<td></td>
</tr>
</tbody>
</table>
Hippocampal injection of penicillin in rats

Attenuated

Alumina-gel injection in monkeys

Attenuated

Phenytoin

Amygdala-kindled rats

Matrix attenuated

Amygdala-kindled rats

Ineffective

Homocysteine thiolactone administration

Attenuated

Flurothyl seizures in mice

Attenuated

Kindling induced by cortical penicillin in rats

Attenuated

Alumina-gel injection in monkeys

Mixed

Tiagabine

Amygdala-kindled rats

Attenuated

Topiramate

Amygdala-kindled rats

Ineffective

Valproate

Amygdala-kindled rats

Markedly attenuated

Pentylenetetrazol-induced kindling in rats

Attenuated

Flurothyl seizures in mice

Attenuated, reorganized

Rat-brain slice

Mixed

Vigabatrin

Amygdala-kindled mice

Attenuated

Amygdala-kindled rats

Ineffective

Corneally kindled rats

Attenuated

Table summarizes the effect of old and new AEDs on epileptogenesis in different animal models of PTE. Based on results from animal experiments, the antiepileptogenic effect have been considered definite for some antiepileptic drugs like diazepam, levetiracetam, Phenobarbital, tiagabine, valproate. The antiepileptogenic effect is also probable for other compounds like Phenytoin, topiramate, vigabatrin and absent for carbamazepine (CBZ), felbamate, gabapentin, oxcarbazepine (Beghi, 2003).

Results of experimental studies and clinical trials for the prophylaxis of PTE showed that AEDs are thought to reduce the rate of occurrence of early seizures, but fail to exert a significant effect on the prevention of PTE. Clinical trials for the prevention of epilepsy demonstrated that Phenytoin and valproate can suppress early seizures after severe
traumatic brain injury, but does not affect the development of late epilepsy (Temkin et al., 1990; Temkin et al., 1999).

There are several drawbacks associated with the clinical trials for screening of AEDS, described as follows:

1) Patients enrolled for clinical trials are heterogeneous and have different propensity to develop seizures (Temkin, 1998).
2) Prolonged period is required for development of PTE, which affect patient compliance.
3) Diagnosis of epilepsy is not clear (Temkin, 2001).
4) Significant heterogeneity of the study designs is well documented in systemic reviews (Schierhout et al., 1998; Beghi 2003)
5) Limited numbers of drugs have been tested for their clinical efficacy against PTE (Temkin, 2001).

Therefore, keeping in view the need for the development of effective treatment for epileptogenesis variety of other compounds (listed in table-2), having antioxidative potential were tried, but non- could reveal direct evidence of antiepileptic activity especially in the light of electrophysiology, biochemical and behavioral parameters.

Post-traumatic epileptogenesis is closely associated with the generation of ROS and RNS. ROS initiates lipid peroxidation and cascade of alterations which results in development of epileptogenic focus. Scientists have invariably reported that antioxidants treatment

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Compound</th>
<th>Reported antiepileptic activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>α- tocopherol</td>
<td>Pretreatment with tocopherol prevents the development of iron-induced epileptiform activity in rats, decreases the formation of peroxides at the iron injection site (Rubin and Willmore, 1980), hastens the resolution of brain edema, and also prevents the development of cavitation and gliosis (Willmore et al., 1981).</td>
</tr>
<tr>
<td>2</td>
<td>Melatonin</td>
<td>Melatonin inhibits iron-induced epileptic discharges in rats by suppressing peroxidation (Kabuto et al., 1998).</td>
</tr>
<tr>
<td>3</td>
<td>Condensed tannins</td>
<td>Epigallocatechins (50 mg/kg v) and (-) epigallocatechin 3-O-gallate (200mg/kg iv.) prevented or slowed the occurrence of epileptiform discharges induced b iron ion injection into the rat brain (Yokoi et al., 1989). Condensed tannins are widely distributed n the plant kingdom and are present in high amounts in tea, red wine and fruits.</td>
</tr>
</tbody>
</table>
4 Adenosines Adenosine is known to act as a neurotransmitter and neuromodulator in the peripheral and CNS. Adenosine depresses neuronal activity by acting at specific extracellular receptors. Adenosine (5mg/kg) of 2 Chloroadenosine (1mg/kg), injected intraperitoneally 30 min prior to the iron injection into rats, suppress or delays the occurrence of epileptiform discharges induced by iron ions (Yokoi et al., 1995).

6 Fermented papaya (PS-501) PS-501 scavenged OH⁻ and inhibited lipid peroxidation, oxidative DNA damage and rat brain tissue injury induced by iron ions, suggesting an inhibitory effect on iron-induced seizures (Imao et al., 1999).

7 Gastrodia elata BL GE is a traditional herbal medicine widely used to treat convulsive disorders and dizziness in China. GE significantly inhibits the increase in lipid peroxide levels and increase SOD activity in the rat brain with ferric chloride induced epilepsy (Mori et al., 2004).

8 Gulingji Pretreatment of gulingji has been reported to decrease levels of TBA-RS and increases SOD activity in the brain. This shows that gulingji could be used as an antiepileptogenic agent (Liu et al., 1990).

9 Zonisamide Zonisamide is known to be very effective as an anticonvulsant in a wide variety of animal model of epilepsy. Zonisamide has potential to protect neurons from free radical damage (OH, NO) and stabilization of neuronal membrane (Mesuda et al., 1998; Mori et al., 1999).

10 EPC-K α-tocopherol-L-ascorbate-2-O-phosphate diester EPC-K prevented increase in TBA-RS content and significantly lowered percent induction of epileptic discharges in electrocotico-grams until 6 months after iron injection (Yamamoto et al., 2002). The occurrence of epileptic discharges could be delayed and/ or suppressed by prior and simultaneous administration of EPC-K.

Table 3. List of natural antioxidants possessing antiepileptic activity against iron induced epilepsy.

inhibits the ROS mediated development of epileptogenesis. Table 2 represents a list of antioxidants investigated and known to prevent the formation of epileptogenic foci, or to attenuate activation of seizures in iron-injected rat brain. These natural antioxidants can be very useful alternative medications or supplements for preventing or attenuating the occurrence of epileptic seizures without any side effects (Mori et al., 1998).
In addition, there are varieties of plant products, being used as spice for flavoring food and used for variety of medicinal purposes in India. For example Garlic, turmeric, Brahmi, Tulsi etc. are being consumed since time immorial and proved to have no side effects. Since, they are known for their antioxidative potential it would be of great interest to study whether or not these natural antioxidants of plant origin if used as food supplement could prevent brain dysfunctions like epilepsy. In the present study attempt were made to investigate the affect of curcumin and L-deprenyl on epileptic young and old rats.

2.6. Curcumin: Source and Structure

Curcumin is a flavoring compound extracted from the rhizome (root) of *Curcuma longa* (turmeric) plant. It is commonly used as a food additive and herbal medicine in Indian system of medicine “Ayurveda” and “Sidha” for the treatment and healing of chronic ulcers and scabies. The characteristic yellow color of turmeric is due to the curcuminoids, first isolated by Vogel in 1842 (Vogel and Pelletier, 1918). Turmeric contains curcumin along with other chemical constituents known as the “curcuminoids” (Srinivasan, 1952). Curcumin is an orange-yellow crystalline powder practically insoluble in water. The structure of curcumin (C_{21}H_{20}O_{6}) was first described in 1910 by Lampe and Milobedeska and shown to bediferuloylmethane (Aggarwal et al., 2003).
The major curcuminoids present in turmeric are demethoxycurcumin (curcumin II), bisdemethoxycurcumin (curcumin III), and the recently identified cyclocurcumin (Kiuchi et al., 1993). Commercial available curcumin contains curcumin I (~77%), curcumin II (~17%), and curcumin III (~3%) as its major components. The curcuminoid complex is also referred to as Indian saffron, yellow ginger, yellow root, \textit{kacha haldi}, ukon, or natural yellow 3.

2.6.1. Biotransformation of curcumin

Earlier literature, assert that curcumin has low bioavailability and rapid biotransformation in human and rat studies. For example: Investigation of the pharmacokinetic properties of curcumin in mice reported that after intraperitoneal (i.p.) administration of curcumin (0.1 g/kg) to mice, about 2.25 mg/ml of curcumin appeared in the plasma in the first 15 min. One hour after administration, the levels of curcumin in the intestines, spleen, liver, and kidneys were 177.04, 26.06, 26.90, and 7.51 µg/g, respectively. Only traces (0.41 µg/g) were observed in the mice brain at 1 hour (Lin et al., 2000). Lin et al. (2000) showed that curcumin was first biotransformed to dihydrocurcumin and tetrahydrocurcumin and than these compounds were subsequently converted to monoglucuronide conjugates. Thus, curcumin–glucuronide, di-hydrocurcuminglucuronide, tetrahydrocurcumin glucuronide,
and tetrahydrocurcumin are major metabolites of curcumin in mice. It has been proposed that the biotransformation of curcumin and the stability of tetrahydrocurcumin (THC) play important roles in the biological effects of curcumin and the microsomal enzyme reactions such as reduction and glucuronidation. Further experimental testings are underway to describe the efficacy of the metabolites of curcumin (Pan et al., 1998).

In humans, the serum concentration of curcumin usually peaked at 1 to 2 h after oral intake of curcumin and gradually declined within 12 h. The average peak concentrations in serum after taking 4000 mg, 6000 mg, and 8000 mg of curcumin were 0.51 ± 0.11 μM, 0.63 ± 0.06 μM, and 1.77 ± 1.87 μM, respectively. The levels of curcumin in the brain is 0.1-1 μM, similar to those required to inhibit central nervous system AP-1 mediated transcription in vivo (Luo et al., 1999) and related suppression of inducible nitric-oxide synthase (Chan et al., 1998) and antioxidative activities. Curcumin is highly lipophilic and can cross blood brain barrier (Kelloff et al., 1996).

2.6.2. Antioxidative potential of curcumin

Several studies investigated antioxidative abilities of curcumin by showing the in vitro protection against H₂O₂ induced oxidative stress in renal cell line (Cohly et al., 1998), induction of hemeoxygenase in endothelial cells (Motterlini et al., 2000) suppressive effects of trichloroethylene- induced oxidative stress (Watanabe and Fukui 2000), and inhibition of oxidative damage to cellular DNA (Kelly et al., 2003). The antioxidant activity of curcumin is due to the presence of two electrophilic, β-unsaturated carbonyl groups in its structure, which can react with nucleophiles such as glutathione. This provides curcumin the potential to inhibit lipid peroxidation, neutralize reactive oxygen and nitric-oxide-based free radicals (Butterfield and Lauderback, 2002; Sreejayan et al., 1997). Presence of phenolic group with a methoxy group at the ortho-position is also shown to be responsible for antioxidant potential of curcumin (Motterlini et al., 2000).

In Indian traditional medicine Ayurveda, curcumin is used as a natural alternative to vitamin E (Kelloff et al., 1996). In fact, curcumin has been reported to be at least ten times more active antioxidant or free radical scavenger than vitamin E (Khopde et al., 1998; Zhao et al., 1989). Earlier researchers on curcumin have proved its potential for: induction of
GST in liver (Susan and Rao, 1992; Sharma et al., 2001), inhibition of ROS in ferrous ion induced oxidation (Reddy and Lokesh, 1994), induction of GSH, MDA, SOD, CAT in the ischemic zone of cat heart (Dikshit et al., 1995), modulation of lipid peroxidation and glutathione contents in cyclophosphamide-induced lung injury (Venkatesan and Chandrakaran 1995). Curcumin has been shown to serve as a Michael acceptor, reacting with glutathione and thioredoxin. Reaction of curcumin with these agents reduces intracellular glutathione in the cells (Adams et al., 2005).

In addition to curcumin, primary metabolite THC, also act as an antioxidant with β-diketone moiety, by cleavage of the C-C bond at the active methylene carbon between the two carbonyl groups (Pan et al., 1998). Curcumin is a potential scavenger of free radicals and increases the level of glutathione during apoptosis (Jaruga et al., 1998). Its ability to inhibit nuclear factor kappa B (NF_B)-mediated transcription of inflammatory cytokines (Xu et al., 1998), inducible nitric oxide synthase (iNOS) [Chan et al., 1998], and cyclooxygenase 2 (Cox-2) (Plummer et al., 1999) are indicative of its anti-oxidative and anti-inflammatory capabilities.

Since lipids are more prone to free radical mediated oxidative damages, scientists have reported inhibitory effect of curcumin on lipid peroxidation induced by paracetamol-induced cytotoxicity in rat hepatocytes (Donatus et al., 1990), atherosclerotic rabbits (Quiles et al., 1998), carbon tetrachloride induced rat liver injury (Park et al., 2000) and hyperlipidemia rats (Wang et al., 2000). Curcumin potential to scavenge free radicals like superoxide and hydroxyl radicals was also reported (Ruby et al., 1995). Effective antioxidant property of curcumin decreases the utilization of vitamins C and E in the liver and thus maintains their levels (Rukkumani et al., 2003).

2.6.3. Therapeutic use of Curcumin

Prevalence of AD between 70 to 79 years of age in human is reported to be 4.4 fold less in India than that of the United States (Ganguly et al., 2000), which has been linked to an extensive use of curcumin as spice and herbal medicine in India. Curcumin has a long history of safe and well tolerated use in human with very limited or no side effects (Kelloff et al., 1996; 2000). In rats, there are studies showing that even higher doses of curcumin in rat and mice do not have vulnerable side effects. For example Srimal and Dhawan (1972)
reported no mortality in any of experimental mice fed with curcumin at a dose of 2000mg/kg body wt. Similarly, Lim et al., (2001) had noticed any adverse effect of curcumin fed at a dose of 5000ppm, on the presynaptic markers as well as GFAP in any brain region. Therefore, effects of curcumin can be investigated for amelioration of variety of neurological disorders.

Curcumin (diferulomethane) being known for its antioxidant and anti-inflammatory activities with favorable toxicity profile, has been currently extensively studied for its anticancerous properties (Kelloff et al., 1996). The use of curcumin as chemopreventive agents to cancer prevention and control is attractive because conventional therapy alone has not been fully effective in combating either the high incidence or the low survival rate of several forms of cancer (Boone et al., 1990; Rao et al., 1995). Furthermore, variety of mechanisms have been suggested for the anticarcinogenic effect of curcumin, including antioxidative activities, modulation of the cell cycle, inhibition of the enzymes related to tumor promotion such as ornithine decarboxylase, protein kinase C, modulation of transcription factors and inhibition of angiogenesis (Huang et al., 1991; Plummer et al., 1999; Arbiser, et al., 1998; Mohan et al., 2000). In addition, several studies in India have proved curcumin as anticancerous (Kuttan et al., 1985, Anto et al., 1996), and antitumor (Krishnaswamy et al., 1998), including preventive in colorectal cancer (Chauhan et al., 2002).

Curcumin’s ability to inhibit CNS AP-1 mediated transcription in vivo ( Luo et al., 1999), suppress inducible nitric oxide synthase (iNOS) (Chan et al., 1998), inhibit COX-2 expression in gastrointestinal cancer cells and mouse skin (Goel et al., 2001, Zhang et al., 1999; Chun et al., 2003 ), formation of COX- and LOX-dependent metabolites along with decreases activities of PLA2 and PLCg1 (Huang et al., 1991; Rao et al., 1995), inhibiting the activity of EGFR and HER2/neu (Korutla and Kumar, 1994; Korutla et al., 1995) and to deplete the cells with HER2/neu protein (Hong et al., 1999) have been suggested to be a key mechanism for its anticarcinogenic action (Cuendet et al., 2000; Huang et al., 1997; Conney et al., 1991). In addition, modulation of arachidonic acid metabolism by phosphorylation of cPLA2, expression of COX-2 and 5-LOX also contribute to their anticarcinogenic actions (Hong et al., 2004).
Cyclin D1 ↓ S-LOX ↓ COX2 ↓ iNOS ↓ MMP9 ↓ IL-8 ↓ IL-6 ↓ TNF ↓ IL-12 ↓

Gene expression

IKK ↓ EGFR ↓ HER2 ↓ AKT ↓ Src ↓ JNK ↓ JAK2 ↓ TYK2 ↓ PKC ↓ JAK2 kinases

Curcumin

Protein kinases

NF-κB ↓ AP-1 ↓ STAT ↓ ERK ↓ STAT-3 ↓ STAT-5 ↓ EpRE ↓ CBF ↓ β-catenin ↓ Nrf2 ↑

Others

Enzymes

TF ↓ AR/ARp ↓ P53 ↓ MDR ↓ ELAM ↓ FTPase ↓ GST ↓ GSH-px ↓ Hemeoxygenase ↓ Xanthine oxidase ↓ uPA ↓

Fig-6. Showing different molecular targets of curcumin make it a probable anticancerous agent (Adapted from manuscript by Aggarwal et al., 2003).

In addition to anticancerous properties, several studies from national publications have demonstrated effectiveness of curcumin on variety of diseases. For example: on hypercholesteremic rats (Patil and Srinivasan, 1971), blood sugar (Srinivasan, 1972), rheumatitic activity (Deodhar et al., 1980). Curcumin exerts its protective, effect against nicotine-induced lung toxicity by modulating the extent of lipid peroxidation and augmenting antioxidant defense system (Kalpana and Menon, 2004). International publications also reported several pharmacological effects like anti-inflammatory (Srimal and Dhawan, 1973), antinephrotoxic, antimutagenic (Ruby, 1995), anti-viral (Vlietinck et al., 1998), anti-atherosclerotic (Kuttan et al., 1987).

Curcumin act like non-steroidal anti-inflammatory drug (NSAIDs) based on the inhibition of nuclear factor–kappa B mediated transcription of inflammatory cytokine (Xu et al., 1998), iNOS (Chan et al., 1998) and cyclooxygenase (Zhang et al., 1999). Curcumin treatment up regulates MMP-9 and downregulates MMP-2 to block gastric damage (Swarnakar et al., 2005).
Several clinical trials have also shown that curcumin treatment is quite effective in preventing several diseases without having any side effects. A short term, double blind, cross over study carried out by Deodar et al., (1980), reported that curcumin dose of 1200 mg curcumin/day was well tolerated, had no side effects, and showed anti-rheumatic activity in humans. Lal et al. (1999) administered curcumin orally to patients suffering from chronic anterior uveitis (CAU) at a dose of 375 mg three times a day for 12 weeks. None of the patients reported any side effects. The efficacy of curcumin and recurrences of CAU following treatment are comparable to corticosteroid therapy, which is currently considered the only available standard treatment for this disease.

Satoskar et al., (1986) evaluated the anti-inflammatory properties of curcumin in patients with post-operative inflammation. Curcumin dose (500mg/day) was found to be quite safe, produced a better anti-inflammatory response than placebo (Satoskar et al., 1986). Soni et al., (1992) reported effect of curcumin treatment with a dose of 500 mg/day for 7 days decreased the level of serum lipid peroxides (33%), total serum cholesterol (11.63%), increased the HDL cholesterol (29%). The results suggest curcumin as a chemopreventive substance against arterial diseases. Lal et al. (2000) reported the clinical efficacy of curcumin in the treatment of patients suffering from idiopathic inflammatory orbital pseudotumors (IIOP). Oral administration of curcumin at a dose of 375 mg/three times/day for a period of 6 to 22 months is safe and effective in the treatment of IIOP. Cheng et al., (2001) examined the toxicology, pharmacokinetics, and biologically effective dose of curcumin in humans. There was no treatment related toxicity for doses up to 8000 mg/day. Beyond 8000 mg/day, the bulky volume of the drug was unacceptable to the patients. In conclusion, this study demonstrated that curcumin is not toxic to humans at doses up to 8000 mg/day when taken orally for three months.

2.6.4. Neuroprotective potential of curcumin.

Oral administration of curcumin has been shown to have neuroprotective capabilities (Kaul et al., 1997; Rajakrishnan et al., 1999) as it has protective effects against ischemia/reperfusion insult in the rat forebrain. Ability of curcumin to cross the blood–brain barrier and bind to redox active metal ions has been demonstrated as a direct evidence for its
neuroprotective capabilities (Yang et al., 2005). This effect was suggested to be result of its antioxidative properties and/or its inhibitory effects on xanthine dehydrogenase/xanthine oxidase conversion and resultant superoxide radical production (Ghoneim et al., 2002). It is believed that TBI is associated with low levels of BDNF and of its downstream effectors synapsin I and CREB which determines synaptic plasticity and required for normal learning in the Morris water maze. Curcumin administration at a dose of 500 ppm can normalize the levels of BDNF and its downstream genes and prevents the cognitive decline associated with the traumatic brain injury is well documented (Wu et al., 2003). These observations suggest that curcumin supplementation might be an effective therapy to counteract the deleterious effects of TBI on neuronal plasticity and function (Wu et al., 2006). Experimental allergic encephalomyelitis (EAE), a CD4+ Th1 cell-mediated inflammatory demyelinating autoimmune disease of the CNS, serves as an animal model for of multiple sclerosis (MS). The destruction of oligodendrocytes and myelin sheath in the CNS is the pathological hallmark of MS. Latter is an inflammatory autoimmune disease of the CNS resulting from myelin antigen-sensitized T-cells in the CNS. Curcumin inhibits EAE by blocking IL-12 signaling in T-cells and suggest its use in the treatment of MS and other Th1 cell-mediated inflammatory diseases (Natarajan and Bright, 2002).

Etiology of AD and PDs are linked to increased oxidative damage, including NO-based damage to a specific protein synuclein. Yang et al., (2006) have shown that curcumin directly binds small β-amyloid species to block aggregation and fibril formation in vitro and in vivo. The effect of curcumin does not depend on amyloid β (Aβ) sequence but on fibril related conformation. Low dose of curcumin (0.1-1 μm range) effectively disaggregates Aβ as well as prevent fibril and oligomer formation (Yang et al., 2005). These findings support the rationale for curcumin use in clinical trials for preventing or treating Alzheimer’s disease (Yang et al., 2005). Administration of curcumin at a dose of 500 ppm prevented Aβ-infusion induced spatial memory deficits in the Morris water maze, post-synaptic density (PSD)-95 loss and reduced Aβ-deposits. Therefore, curcumin’s least side-effect profile and long history of safe use, made it reliable for clinical application of AD prevention (Frautschy et al., 2001).

Lim et al. (2001) found that curcumin reduces oxidative damage and amyloid pathology in an Alzheimer transgenic mouse (APPs) model. Both low (160 ppm), and high
doses (5000 ppm), were shown to lower oxidized proteins and IL-1β, a proinflammatory cytokine. They had reported that low-dose (160 ppm) curcumin treatment also reduced the astrocytic marker glial fibrillary acidic protein, insoluble beta-amyloid (Aβ), soluble Aβ, and plaque burden by 43 to 50%. However, levels of amyloid precursor in the membrane fraction were not reduced. Microgliosis was also suppressed in neuronal layers but not adjacent to plaques. In view of curcumin’s efficacy and without apparent toxicity, this Indian spice component is believed to have promising neuroprotective capability against Alzheimer’s disease.

Despite of above discussed antioxidative, therapeutic and neuroprotective capabilities there are hardly any study which demonstrates the affect of curcumin on epilepsy. Therefore, investigating whether dietary intake of curcumin (500 ppm & 1500ppm) can influence seizures and associated alterations or not would be a meaningful study. These doses have already been reported to possess antioxidative potential and shown to provide protection against amyloid-β formation.

2.7. L-Deprenyl

L-Deprenyl (phenyl-isopropyl-N-methyl-propargylpynylamine) was first synthesized in 1962 by Ecseri in the Chinoin Pharmaceutical Works laboratory, Hungary. (-)-deprenyl has become the golden standard of MAO-B inhibitors. It possesses dopamine potentiating (Paterson et al., 1991), antidepressant neuroprotective and neurorescue effects as reviewed by Magyar et al., (2006). L-deprenyl is readily absorbed from the gastrointestinal tract (GIT) and rapidly enters the brain and spinal cord following oral administration. It is extensively metabolized in the liver (human), to form desmethyl-L-deprenyl (DMS) and methamphetamine, which are further metabolized to amphetamine (Magyar and Szende, 2004). Yasar et al., (1996) reported that the metabolites of L-deprenyl, L-amphetamine and L-methamphetamine have endogenous beneficial clinical effects and also complement beneficial clinical actions of L-deprenyl itself. This suggests that L-deprenyl is completely metabolized in human system. L-deprenyl is free of the ‘cheese effect’ and devoid of amphetamine like dependence and catecholamine releasing activity, which makes it a safe drug (Ebadi, 1998; Magyar et al., 1998; Ebadi & Hiramatsu, 2000; Ebadi et al., 2002).
Deprenyl is indicated as a safe therapeutic drug for the treatment of Parkinson's disease (Kotake et al. 1998) and reported to reduce functional decline in Alzheimer's disease (Sano et al. 1997). Therefore, investigations on the generalized effects of chronic L-deprenyl treatment on post-traumatic epilepsy would be of great interest.

2.7.1. Antioxidant and anti-aging potential

(−)-Deprenyl acts as an antioxidative agent as it can both directly inhibit reactive oxygen species formation by blocking the normal metabolism of biogenic amines (Magyar, 1996) and indirectly by activating antioxidant enzyme activity (Kaur et al., 2003). It is reported, that lower concentration of (−)-deprenyl is required to prevent oxidative damage than needed to inhibit MAO-B (Chiueh et al., 1994; Wu et al., 1993). Long-term treatment with L-deprenyl enhances the synthesis of SOD1, SOD2 and catalase activity in experimental animals (Carrillo et al., 1991, 1992, 1993). L-deprenyl does not have a generalized upregulating effect on antioxidant enzymes (Bickford et al. 1997; Carrillo et al. 1992b). For example, it elevates SOD in the striatum but not in the hippocampus (Bickford et al. 1997; Carrillo et al. 1991; Carrillo et al. 1993), increases catalase in the substantia nigra but not in the striatum and decreases GPx in substantia nigra but has no effect in the striatum (Thiffault et al. 1995; Carrillo et al. 1991). In fact, L-deprenyl act as an effective antioxidant against hydroxyl radical formation and protects dopaminergic neurons against cell damage induced by neurotoxins (Nomoto et al., 2003). Deprenyl protects neuronal mitochondria against respiratory chain dependent oxygen stress by enhancing the activity of mitochondria-protecting SOD and CAT, increases the expression of glutathione peroxidase and preserves the mitochondrial membrane potential. As mitochondrial dysfunction is centrally involved in triggering apoptosis, an effective prevention of mitochondrial oxidative damage by L-deprenyl may provide a reasonable explanation for its antiapoptotic effect in models of neuronal apoptosis (Suh et al., 2000). L-Deprenyl counters oxidative stress by reducing lipid peroxidation (Wu et al., 1996), protein oxidation (Jyoti et al., 2006).

Experimental studies revealed that L-deprenyl treatment extends life expectancy (Bickford et al. 1997). Deprenyl treatment in low doses increased the life span of the laboratory animals like aged mice, old male rats, dogs and monkeys (Freisleben et al.,
1994; Kitani et al., 1993; Knoll et al., 1994; Milgram et al., 1990; Yen and Knoll, 1992, Archer and Harrison 1996) but contradictory data were also published (Gallagher et al., 1998). Chronic treatment with L-deprenyl also counters the decline in sexual activity as well as memory decline in aged male rats (Brandeis et al., 1991). The anti-aging actions of L-deprenyl may be mediated through a combination of activities: prevention of lipid peroxidation and accumulation of lipofuscin, stimulation of the activities of Na-K ATPase, GST and multiple unit action potentials (Kaur et al., 2003). Lipofuscin accumulation within the cytoplasm of pyramidal neurons of hippocampus was also reported to be reduced (Amenta et al., 1994, Biagini et al., 1994). Neuroactive agents such as L-deprenyl decrease lipofuscin and ceroid pigment dissolution by cytoplasm rehydration, optimization of the brain cellular recycling system activities. Therefore, L-deprenyl exhibit therapeutical solutions in brain aging deceleration and in age associated diseases (Riga and Riga., 1995).

L-deprenyl induces neuronal differentiation in undifferentiated pluripotent embryonic stem cells (ESCs) in dose-dependent manner and induces neurotrophin expression. This study suggests that both L-deprenyl and stem cell therapy can together be used to improve deficits in neurodegenerative diseases related to aging in future. Therefore, there is a growing interest in applying stem cell therapy in aging (Esmaeili et al., 2006). Prophylactic (-)deprenyl medication may improve the quality of life in the later decades, delaying the time of natural death and decreasing the susceptibility to age-related neurological diseases. Hence, potentials of (-) deprenyl, for human use as anti-aging drugs remain worthy of exploration in the future (Kitani et al., 2002).

2.7.2. Therapeutic use of L-deprenyl

Based on clinical observations, Birkmayer and his group first reported that (-)-deprenyl potentiates the neuroprotective effect of levodopa and improves the quality of life of Parkinsonian patients (Birkmayer et al., 1987). L-deprenyl possesses dopamine sparing effects (DSE), so it is used as a safe therapeutic drug for the treatment of Parkinson’s disease in many countries (Kotake et al. 1998). (-)-deprenyl has been used in more than 50 countries alone or in combination with levodopa for the treatment of Parkinson’s disease. Parkinsonian patients on levodopa supplemented with (-)deprenyl (10 mg daily) live significantly longer than those on levodopa alone. Maintenance on (-)-deprenyl
significantly improves the performance of patients with Alzheimer's disease as well (Knoll 1994). With the possible exception of coenzyme Q10 and L-deprenyl, no other effective neuroprotective agent is available for the treatment of PD (Slawek, 2000; Ebadi et al., 2001). L-deprenyl treatment in chronic heart failure, exhibits reduced plasma norepinephrine, cardiac oxidative stress and myocyte apoptosis, prevented the changes of Bcl-2 and Bcl-2 to Bax ratio, and improved left ventricular fractional shortening and dP/dt (Qin F et al., 2003).

2.7.3. Neuroprotective role

The neuroprotection by (-)-deprenyl has been considered to be associated with several intracellular mechanism, such as a direct anti-oxidant effect (Mytilineou et al., 1998), enhancement of anti-oxidant enzymes like SOD and catalase (Carrillo et al., 1992), inhibition of the uptake of dopamine, preservation of mitochondrial membrane potential (Wadia et al., 1998), as well as increase in mRNA of trophic factors like neurotrophic growth factor (NGF) (Sernkova et al., 1996) and ciliary neurotrophic factor (CNTF) (Seniuk et al., 1994). Maia et al., 2004 have shown a significant protective effect of L-deprenyl on memory deficits (T-maze test & passive avoidance test) and lipid hyperperoxidation observed after cerebral ischemia. L-deprenyl is also reported to prevent the formation of amyloid plaques in Alzheimer's patients (Sienkiewicz-Jarosz & Kostowski, 2000). L-deprenyl induced a significant (p<0.001) increase in the number of branching points and intersections in both apical and basal dendrites in treated monkeys compared to controls. Such an enriched dendritic arborization in prefrontal cortical neurons is responsible for the enhancement of cognitive functions in Alzheimer disease patients following (-) deprenyl treatment (Shankaranarayana et al., 1999). Melatonin and deprenyl collaborate to prevent nigrostriatal pathway damage against MPTP by acting on different aspects of MPTP-induced damage. Melatonin maintains mitochondrial respiration (by reversal of complex I inhibition) and reduces oxidative stress, deprenyl partially prevents DA turnover and TH activity changes, avoiding locomotor activity reduction (Khaldy et al., 2003). Similarly, co-administration of deprenyl and estradiol caused a synergistic effect on spatial memory (Kiray et al., 2004). L-deprenyl significantly improved learning and memory deficits associated with old age and studies suggest that it possesses potential
cognitive enhancement abilities probably due to an increase in dopaminergic activity (Brandeis et al., 1991).

L-deprenyl maintains intra-mitochondrial redox balance by regulating mitochondrial membrane potential (ψ) and Ca²⁺ by increasing the synthesis of proteins such as BCl₂ (Wadia et al., 1998). The protection does not depend on inhibition of MAO-B activity, since the cells contain only type-A MAO (Maruyama et al., 1997). In addition to antioxidant properties, it also acts like a trophic factor (Tatton et al., 1996). It increases the survival of degenerating neurons and enhances neuritic outgrowth in cultured rat spinal ventral horn neurons and induces the (CNTF) gene expression in vitro (Seniuk et al., 1994). In addition, deprenyl compensates for target-deprived trophic support, delays apoptosis in serum-deprived cells, and blocks apoptosis-related reduction in cell size (Ebadi, 1998; Ebadi et al., 2002), suggesting that L-deprenyl has a complex pharmacokinetic profile. Chronic treatment of deprenyl administration has been shown to induce acetylcholine esterase (AChE) activity which in turn modulates dendritic branching pattern in specific (CA1 of hippocampus) brain regions (Lakshmana et al., 1998). Hence, (-)-deprenyl can also be used against AD which exhibit decreased activity of acetylcholine-esterase (AChE). L-deprenyl has been shown to provide neuroprotection in transient ischemia by preventing motor neuron degeneration and increased its performance in Wistar rats (Ravikumar et al., 1998).

Neuroprotective effect of L-deprenyl can also be due to diminished production of H₂O₂ through MAO-B inhibition (Cohen and Spina, 1981). Munirathinam et al., 1996 have shown that L-deprenyl provides protection to cortical neurons (in vitro) exposed to aluminium chloride (AlCl₃) by significantly attenuating both the morphological alterations and the lactate dehydrogenase (LDH) efflux induced by AlCl₃. This suggests its potential role to prevent aluminium induced alterations in neurons. The results of the study of Piccinin et al., 1990 suggest that the higher and statistically significant effects of L-deprenyl on memory and attention in AD patients seem to be due to an improved function of monoaminergic systems involved in the process of neuronal degeneration. L-deprenyl provided neuroprotection against N-methyl (R) salsolinol, 6-hydroxydopamine, and peroxynitrite induced apoptosis in SH-SY5Y cells (Maruyama et al., 1997; Naoil et al., 2000a, b; Naoil & Maruyama, 2001). Tatton and Chalmers-Redman (1996) reported that
Deprenyl mediated activation of transcriptional program to prevent apoptogenesis is crucial for neuroprotective action.

2.7.4. Antiepileptic effect

L-deprenyl administration has been shown to reversibly suppress the epileptiform activity evoked by picrotoxin (Hsu et al., 1996). L-deprenyl at a micromolar range could produce a significantly inhibitory effect on the epileptiform discharges evoked by picrotoxin in the hippocampal CA1 neurons in vivo (Hsu et al., 1996). Loscher and Honack (1995) investigated the anticonvulsant and antiepileptogenic effect of L-deprenyl in the kindling model of partial seizures (epilepsy) (Loscher et al., 1999). L-deprenyl was also effective in phenobarbital and pentylenetetrazole induced myoclonic and clonic seizures (Loscher and Lehmann, 1996, 1998). Loscher and coworkers (1999) have shown that the anticonvulsant activity of L-deprenyl is not related to MAO-B inhibition, but to other effects of this drug, such as inhibition of MAO-A by comparing its action with other selective MAO inhibitors. L-Deprenyl per se was anxiogenic, and in combination with diazepam (1 mg/kg) potentiated the anti-anxiety effect of the latter. In epilepsy, L-deprenyl might be acting partially by influencing the GABA<sub>A</sub>/benzodiazepine mechanism in the brain (similar to diazepam and phenytoin), and the cholinergic system which might be playing a role in its cognition enhancing effect. Thus, L-deprenyl could prove to be an adjuvant in the antiepileptic therapy and beneficial in dementia associated with epilepsy (Gupta and Kulkarni 2000). Gliosis in neurodegenerative diseases and epilepsy is characterized by an increased expression of the enzyme MAO-B. Therefore, ligand $^{11}$C-L-deuterium-deprenyl can be used for in vivo and in vitro binding and then further analyzed by positron emission tomography (PET). The above method can correctly identify the epileptic site in human patients suffering from temporal lobe epilepsy (BergstrÄm et al., 1998). Long term administration of deprenyl also inhibit the pentylenetetrazol (PTZ)-induced maximal seizures in Lewis rats, indicates that the anticonvulsant effect of deprenyl is related to changes in levels of certain endogenous compounds or down or up-regulation of relevant receptor/effector units (Hoffman et al., 1997).

In summary, L-deprenyl has been reported to possess antioxidative, antiageing and neuroprotective abilities but its potential to inhibit post-traumatic epileptogenesis is hardly
known. It has been reported that L-deprenyl prevent pilocarpine and PTZ induced seizures in animal models (Loscher and Lehmann 1996;1998), therefore, in order to ascertain the efficacy of curcumin, we also gave L-deprenyl to another set of same age group animal. Since affect of L-deprenyl on post-traumatic seizures is hardly known, it would be interesting to investigate the effect of L-deprenyl on iron-induced epilepsy.