6. Discussion

The present study was performed to investigate the antiepileptic potential of curcumin and L-deprenyl. Epilepsy was induced with FeCl₃ according to the method of Willmore et al., (1978a,b) and was studied in the cortex and hippocampus regions of the brain as both are involved in learning and memory functions (Barnes, 1987; Fukui et al., 2003). Though both brain regions are predominantly affected in head trauma (Ozdemir et al., 2005; Tong et al., 2002) but hippocampus in particular, has been reported to play a key role in encoding, retrieval and long-term spatial memory consolidation in the Morris water maze tests (Reidel et al., 2003). Earlier literature reveals that most of the studies have been done either on young rats or rats randomly selected on the basis of their body weight. For example Willmore and Rubin, (1981), Kabuto et al., (1998) and Doi et al., (2001) used rats weighing between 250-300g for their studies on epilepsy whereas others have used rats of 350-450g (Nilsson et al., 1994; Yokoi et al., 1995). In the present work we induced epilepsy in young (4 months) as well as old (18 months) rats and also studied the response of dietary curcumin and L-deprenyl in the light of electrophysiological, biochemical, microscopic and behavioral observations. Earlier studies have monitored epileptogenesis for short-term for example one week (Willmore et al., 1982) or to one month (Willmore et al., 1978; Pathak and Singh, 1990). Since, post-traumatic epileptic patients exhibits spontaneous seizures months to years after traumatic head injury (D’Ambrosio et al., 2004), it was of interest to observe epileptogenesis on long term basis. Therefore, in the present study, we have characterized long-term progression of seizures with the help of synchronized video EEG monitoring.

In the present study, biochemical parameters related to oxidative damage like lipid peroxidation and protein oxidation were measured, as iron-induced epileptogenesis was reported to be mediated by free radicals (Willmore et al., 1990), generated due to ferric ions at the epileptogenic foci. Alterations in Na-K ATPase and membrane fluidity were also assessed as these correlate with lipid peroxidation (Sharma et al., 1993) and influence the electrophysiological properties of the membrane (Pellmer, 1995, 1995; Singh and Sharma, 2005). In fact, decrease in Na-K ATPase activity has been linked with epilepsy in human (Grisar et al., 1992), and animal models of experimental epilepsy (Vaillend et al., 2002). Cytosolic PKC activity was estimated in both brain regions of epileptic as well as
curcumin and L-deprenyl treated epileptic animals groups, as PKC shown to be involved in electrical kindling (Chen et al., 1992; Daigen et al., 1991; Vernet et al., 1992), cerebral ischemia (Oster et al., 2004), pilocarpine-induced seizures (Tang et al., 2004).

In behavioral studies, Morris water maze and open field tests were performed to assess the cognitive and anxiety status in epileptic and curcumin treated epileptic rats which is hardly reported in the literature. Cognitive dysfunctions are usually the most disabling squeal of head trauma, which is not limited to severe injury but can also accompany mild to moderate injury (Guthrie et al., 1999; Capruso et al., 1999). Therefore, behavioral studies would be of great significance. Microscopic studies were also undertaken to look for the ultra-structural alterations in epileptic rats. None of the previous studies have described ultrastructural alterations in iron-induced epileptic model. We have attempted to find whether curcumin and L-deprenyl does have any affect on epilepsy induced alterations at cellular level.

In this study, effect of two doses of curcumin [low dose (500 ppm) and high dose (1500 ppm)] was investigated for its antiepileptogenic potential. Dose of 500 ppm curcumin was shown to be protective by aggregating amyloid-β precursor proteins in animal models of Alzheimer's disease (Frautschy et al., 2001). Similar dose was reported to possess redox metal binding (Baum et al., 2004), anticancerous (Ammon and Wahl, 1991), anti-inflammatory (Lin, 2007) activities. Higher dose (1500 ppm) of curcumin was administered just to optimize the outcome of these two doses of curcumin. Both doses of curcumin treatment was given for two durations i.e. long-term & Short-term. Long-term treatment commenced 16 weeks prior to FeCl₃ injection and continued for 22 weeks in young & 18 weeks in old until control epileptic rat started exhibiting grade IV epileptic seizures. This helped in determining the effect of long-term intake of curcumin on epileptogenesis. In short-term treatment group, curcumin was administered after FeCl₃ injection for only 5 weeks to ascertain the antiepileptic potential of curcumin. Effect of L-deprenyl was investigated in order to elucidate comparison between the two i.e. curcumin and L-deprenyl. The dose (1 mg/kg/day) used in this study was previously reported to have neuroprotective effect in aging (Kaur et al., 2003) and aluminium induced neurotoxicity (Jyoti and Sharma, 2006; Jyoti et al., 2007).
6.1. Characterization of seizures

We employed synchronized video-EEG recording to monitor the arrival of seizures and its associated behavioral manifestation in ferric chloride induced young and old epileptic rats. Previous reports suggested that in iron-induced epilepsy seizures are initiated as isolated spikes in the ipsilateral (FeCl$_3$/FeCl$_2$ injection site) side and later spread to the contralateral side and than to the different brain regions for example: hippocampus (Castiglino et al., 1990), thalamus, locus cerulus and substantia nigra (Sharma and Singh, 1999a,b). Very few studies have investigated long-term progression of seizures in iron-induced epileptic rats. Only Moriwaki et al., (1990, 1992) have reported long-term assessment of arrival of seizures in the iron induced epileptic rats. They proposed that isolated spikes first appears in the ipsilateral side, and after 30 days to several months, spike and wave complexes discharges becomes generalized seizures. In our study, we observed that saline injected or control rat did not exhibit seizures. This confirms previous findings which suggested that the seizures originate due to injection of FeCl$_3$ intracortically (Willmore et al., 1978; 1981, Pathak and Singh, 1990) in iron induced epileptic models.

Earlier literature reveals that none of studies so far have completely characterized different grades of seizures on long-term video-EEG monitoring in iron induced epileptic rats. Similarly, no report is available which describes the spectral composition of transient seizure discharges appeared in iron induced epileptic rats. Therefore, in this study we have characterized electrobehavioral seizure progression and simultaneously analyzed the spectral composition of epileptic discharges. Our electrophysiological studies are novel with respect to previous studies (Sharma and Singh, 1999a,b; Willmore et al., 1978a,b; 1986; Willmore and Rubin 1981) because we have clearly demonstrated the time taken by young and old rats to exhibit higher grades of seizures. We have observed that isolated spikes starts within one week and later develop into spontaneous seizures several weeks (18 weeks in young & 12 weeks in old) after FeCl$_3$ injection. FFT and CWT analysis of the typical traces shows that isolated spikes are mainly 3-4 Hz signals whereas frequencies in the range of 6.5-7.5 Hz are dominating during Grade III seizures and 7-10 Hz is dominating in Grade IV seizures of both young and old rats. Our FFT and CWT analysis of transient seizures confirmed that it is the progression, manifestation and spread of seizures which differs in old epileptic rats but the basic frequency content of transient discharges remain
same in both young and old epileptic rats. Moriwaki et al., (1992) have proposed that no behavioral alteration is associated with isolated spikes but epileptic rats exhibiting spike and wave complexes shows vibrissa tremors and head nodding. In accordance to this study, we found similar behavioral alterations except shivering movement which was synchronized with arrival of grade IV transient discharge. We have never observed any overt myoclonic behavior which is in agreement with the previous work published on iron-induced epileptic models (Moriwaki et al., 1992; Sharma and Singh, 1999a,b)


Epilepsy is third most common neurologic disorder in the elderly (Tallis et al., 1991), therefore it is believed that aging of the nervous system may affect seizure susceptibility (Hauser et al., 1993). Studies on different models of experimental epilepsy have shown alterations of seizure susceptibility/severity with age (Holtkamp et al., 2004; Darbin et al., 2004). Various in vivo studies showed an increased (Dawson et al., 1992; Klioueva et al., 2001) and a decreased susceptibility for seizures with aging (Kitani et al., 1985) in different experimental epilepsy model of rats. In contrast, vulnerability of brain to develop post traumatic seizures in young and old rats is not known. Therefore, one of the main objectives of this study was to investigate the epileptogenic vulnerability in young and old rats. Kelly et al., (2003) have suggested that for aging study, selection of 18 month age group minimizes the individual variations in old age survivors and provides true generalized trend. Therefore, in the present study we considered 18 month rats as old only. Our data obtained from older epileptic rats exhibits faster seizure maturation to higher grades and spread to contralateral cortex / hippocampus than younger rats. On the basis of identified epileptic grades of seizures described in results section, we found that older epileptic rats exhibited grade IV seizures in 12 weeks while young epileptic rats take 18 weeks to exhibit grade IV seizures. This suggested that propagation of much faster in older rats. There are reports which accentuate differential vulnerability for epilepsy in young and old rats. For example, Chiba et al., (1992) in his studies have shown that the rate of pentylenetetrazole-induced seizure is slower in aged rodents compared to young ones and aged animals exhibit longer duration after discharge as well as faster propagation of seizures to the contralateral side. Darbin et al., (2004) induced status epilepticus in rats with
kainate and demonstrated that that aged epileptic rats exhibited shorter latency to onset of all seizure classes compared to young ones. Therefore, inconsistency in the development of seizures observed in young and old rats in earlier as well as our study might be attributed to the different methods of inductions of seizures employed in different model of epilepsy.

Earlier studies demonstrating age-related alteration in synaptic connectivity (Rapp et al., 1999; Smith et al., 2000), electrotonic coupling (Barnes et al., 1987), receptor and channel properties (Potier et al., 1992; Gutierrez et al., 1996), potassium and calcium buffering (Roberts and Feng, 1996; Thibault et al., 1998), as well as our’s showing increase in the lipid oxidation, protein oxidation, and decrease in the membrane fluidity, Na-K ATPase activity and PKC activity in old rats could be responsible for the observed differential susceptibility for seizures. Lipid peroxidation (Pellmer, 1995; Seutin et al., 1995), Na-K ATPase (Beal, 1995; Riddle et al., 1993), membrane fluidity (Faroqui and Horrocks, 1998) are shown to play important role in determining the electrophysiological properties of the neuronal membrane (Sharma et al., 1993; Smith et al., 1991; Singh and Sharma, 2005).

Our electron microscopic observation reveals cytoplasmic vacuolization, lipofuscin pigment accumulation and mitochondrial swelling in old control rats which is in accordance with several previous reports suggesting necrotic changes like extensive vacuolation, chromatin condensation, mitochondrial swelling in the hippocampus (Deloncle et al., 2001) and cortex regions of the brain in old rats (Struys-Ponsar et al., 1994). Therefore, we infer that observed cellular alterations could be one of the causes for faster seizure progression in old epileptic rats. No such cytormorphological changes were observed in young rats.

6.1.2. Biochemical alterations in epileptic rats

It has been widely demonstrated that iron-induced epilepsy involve free radical generation and leads to increased lipid peroxidation in the cortex (Willmore and Rubin, 1981; 1982, 1984, Triggs and Willmore 1984; Mori et al., 1990; Pathak and Singh, 1990) and hippocampus (Willmore et al., 1986) of young rats. Lipid peroxidation is also believed to increase with age in experimental rats (Cini and Moretti, 1995; Babusikova et al., 2007; Tian et al., 1998; Liu et al., 2003). Increased lipid peroxidation influences membrane
electrophysiology by altering action potential production (Pellmer 1986), membrane excitability (Pellmer 1995; Seutin et al., 1995), and synaptic transmission (Avshalumov 2000; Zoccarto et al., 1995). Our data indicating significant enhancement of lipid peroxidation in old controls compared to young controls signifies the age related increase whereas significant increase in both young and old epileptic rats compared to age-matched control may be suggested as a result of iron-induced epileptogenesis.

Proteins are also believed to be the favorite site for free radical damage and reported to be elevated with aging in rats (Stadtman et al., 1992; Levine, 2002), monkeys (Levine and Stadtman, 2001), human tissues (Chevion et al., 2000; Head et al., 2007). Increase in protein oxidation has been reported in traumatic brain injury (Wu et al., 2003), fluid percussion injury (Wu et al., 2006) and Alzheimer’s disease (Lim et al., 2001). In vivo studies indicated that oxidative modification of proteins affect variety of cellular functional proteins like receptors, signal transduction mechanisms, transport systems, and enzymes (Evans and Halliwell 1999; Butterfield and Lauderback, 2002). Unlike lipid peroxidation, not much is known about the level of protein oxidation in iron induced epilepsy, except regarding the elevation of protein carbonyl within 60 min. which later attains normal level after 3 days of iron injection (Yamamoto et al., 2002). Our results showing increased protein oxidation in young and old controls compared to young controls indicate age-related increase in protein oxidation. In epileptic rats this increase was registered in both young and old rats which suggest that iron-induced epilepsy induces protein oxidation.

Fluidity is an important physical property of cell membranes closely related to permeability and may affect membrane embedded enzyme activities (Moriyama et al. 1989). Fong and McNamee (1986) reported that altered membrane fluidity induce conformational changes in the receptor proteins associated Na$^+$ channels. Therefore, any alteration in the membrane fluidity may lead to epileptic discharge as shown in the freeze-lesion model in cat (Nelson and Delgado-Escueta, 1986). There are also reports suggesting that membrane fluidity decreases with age (Choi and Yu, 1995; Mantha et al., 2006, Fukaya et al., 2007). Significant decrease of membrane fluidity in both young and old epileptic brain irrespective of age in the present study is an interesting finding. Increased lipid peroxidation and protein oxidation could be the reason for the decrement in fluidity.
This is in accordance with previous study, suggesting that increase in the lipid peroxidation correlate with decrease in the membrane fluidity (Farooqui and Horrocks, 1998).

Na-K ATPase is known plays a pivotal role in the maintenance of cellular ionic gradients. Age related decrement in the Na-K ATPase activity (Bala et al., 2006; Mantha et al., 2006) have been suggested to be responsible for alterations in neuronal excitability in the old rats (Beal 1995; Deboer and Tobler, 1995; Riddle et al., 1993). Therefore, our data showing decrement in Na-K ATPase activity in old rats compared to young rats is in agreement to the report. There are reports indicating inhibition of Na-K ATPase activity in experimental epilepsy induced with PTZ (Schneider Oliveira et al., 2004), intrastratal methylmalonic acid injection (Malfatti et al., 2003), cortical freezing lesion (Grisar et al., 1992) and ferric ion injection (Mori et al., 1986). Na-K ATPase Inhibition enhances neuronal excitability and facilitates the appearance of excitatory activity and convulsions (Vasilets and Schwarz, 1993). In the present study, we observed that both young and old epileptic rats exhibited significant inhibition of Na-K ATPase activity compared to their age-matched controls. Possibly, age or epilepsy related inhibition of Na-K ATPase could be due to several factors for example, increased lipid peroxidation (Bala et al., 2006; Mori et al., 1986), direct effect of free radical (Lees, 1991) and decrease in the ATP availability, mitochondrial bioenergetic impairment (Tretter and Adam-Vizi, 1996; Martinez et al., 2000; Sapolsky, 2003). Therefore, oxidative inactivation of membrane Na-K ATPase in young and old epileptic rats may be one of the reasons for enhanced neuronal excitability and seizure manifestation. Inhibition of Na-K ATPase has been shown, linked with intracellular accumulation of Na+, which reverses the direction of the Na+/Ca2+ exchange and exacerbate intracellular Ca2+ accumulation (Goddard and Robinson, 1976; Dipolo and Beauge, 1983; Akerman and Nicolls, 1981), which could further increases lipid peroxidation, membrane derangement, excitotoxicity/ apoptosis (Choi, 1993; Farber 1981).

The PKC family of serine/threonine kinases is shown to be involved in a variety of physiological and pathophysiological processes in the brain including development (Oster et al., 2004), synaptic plasticity (Sossin, 2007), cerebral ischemia (Bright and Mochly-Rosen, 2005), and neuronal cell death (Zhang et al., 2007). A long-lasting increase of the PKC epsilon in the axons of granule cells has been linked with the kainate-induced seizures (Guglielmetti et al., 1997). PKC increase was also reported in electrical kindling model of
amygdala (Chen et al., 1992), hippocampus (Daigen et al., 1991) and cortex (Vernet et al., 1992). We observed increased PKC activity in both young and old epileptic rats in comparison to their age matched controls. Earlier studies demonstrated that redox modification of Zinc-binding and cystine-rich motifs at N-terminal regulatory domain by peroxide could disrupt auto-inhibitory function and stimulate PKC activity (Gopalakrishna and Jaken, 2000). Recently increased PKC activity in epileptic rats has been linked with Neuropeptide Y mediated inhibition of glutamate release (Silva et al., 2007) and mGluR1alpha-related excitoneurotoxicity (Tang et al., 2004). Therefore, our observations delineating significant increase in the PKC activity in young and old epileptic rats may be due to oxidative modification of N-terminus regulatory subunits. Since age-related decrement in PKC activity is widely reported (Fordyce and Wehner, 1993; Mizutani et al., 1998) decline in PKC activity in the old controls compared to the young controls may be suggested as affect of age.

6.1.3. Electron microscopic studies

Cytomorphological alterations at ultrastructural levels in FeCl3 induced epileptic rats have been very little described in the literature. Our electron microscopic observations revealing extensive cytoplasmic vacuolation, increase in lipofuscin, lysosomes, nucleolar budding hyper electron dense cytoplasm, chromatin condensation, nuclear membrane folding, swelling of RER and mitochondria in the epileptic rats is an indicative of apoptotic and necrotic changes. There are studies demonstrating similar changes in apoptotic cells of pilocarpine-induced seizures (Covolan et al. 2000; Clarke, 1990) as well as appearance of hyper electron dense shrunken cells 4-aminopyridine induced seizures (Fabene et al., 2006). Cytoplasmic vacuolization in the epileptic neurons may be described as compensatory adaptation to meet the higher demands for protein and amino acids by consuming their cytoplasm (Kanungo et al., 1970; Zs-Nagy et al., 1979). Epilepsy related increased gliosis observed as increased glial fibrillary acid protein staining (Willmore and Rubin, 1982; Willmore et al., 1978) in epileptogenic focus was confirmed by our electron microscopic studies. The perineuronal aggregation glial cells observed in the epileptic rats
is an indication to compensate stressful conditions (Olkowski and Manocha, 1972; Manocha and Sharma, 1978) induced due to hyperactivity of the epileptic neurons.

6.1.4. Micrometric studies (Cell Count)

Selective loss of cellular count was previously reported in human post-traumatic epileptic patients (Swartz et al., 2006), PTZ induced seizures (Park et al., 2006), fluid percussion model of closed head injury (Kharatishvili et al., 2006) and kainate acid induced temporal lobe epilepsies (Fedele et al., 2005). The selective loss of hippocampal neurons are linked with axonal sprouting (Fedele et al., 2006) and possibly reorganization of intrahippocampal circuits to increase excitability, typical to the epileptogenesis process (Bolkvadze et al., 2007). Hippocampal neuron losses is often observed in head trauma and have been recognized linked with memory deficits (Kotapka et al., 1991; Lowenstein et al., 1992). In accordance, our data showed significant decrease in cell count in CA1 region of epileptic rats in comparison to saline injected or controls in both young and old rats. No decrease in cell count was observed in the Layer III cortical cells was observed in epileptic rats compared to control. This implicates that hippocampus cells are more vulnerable to epilepsy induced neuronal damage compared to cortex.

6.1.5. Cognitive functions in epileptic rats

Epilepsy-related memory impairment have been widely demonstrated in human patients (Thompson and Corcoran, 1992; Giovagnoli et al., 1997) and animal models of epileptogenesis such as electrical kindling (Becker et al., 1992; Genkova-Papazova and Lazarova-Bakarova, 1995; Letty et al., 1995; Gilbert et al., 1996), PTZ induced kindling (Hamm et al. 1995; Lamberty and Klitgaard 2000; Omrani et al., 2007) and in animal models of PTE, fluid percussion injury (Hamm et al., 1992; Browne et al., 2006). None of the studies reported cognitive status in iron induced epileptic rats. Therefore, in this study we performed Morris water maze test to ascertain the learning and memory abilities. Both young and old epileptic rats exhibited higher latency to reach platform, implicating epilepsy coupled cognitive deficit. The observed learning and memory loss could be result of seizures manifestation (Gilbert et al., 2000) and free radical mediated biochemical alterations (Marklund et al., 2001; Paolin et al., 2002) in the hippocampus of epileptic rats.
In addition, selective neuronal loss in the CA1 field and necrotic changes at ultrastuctural level observed in the pyramidal neurons would be another reason for loss of hippocampus-dependent spatial learning and memory, as CA1 field neurons are believed to play crucial role in memory consolidation (Reidel et al., 1999).

Results obtained from open field tests indicate a characteristic profile of behavioral changes in young and old epileptic rats. Epileptic rats exhibits lower scores of ambulatory, rearing activity whereas higher defecation index compared to saline injected rats. This indicates high anxiety behavior in iron induced epileptic rats. In accordance to our result PTZ induced kindling exhibited significant decrease in open field exploratory activity (Franke and Kittner, 2001), whereas increase of exploratory activity was observed in kainic acid-induced status epilepticus (Thurber et al., 1992) and no change was observed in electrical kindling in mature rats (Holmes et al., 1993) and PTZ induced seizures in immature rats (Huang et al., 2002). Therefore, different studies have shown that behavioral alterations in open field parameters are related to the age of rats and method of epileptogenesis. We have found that in iron induced epilepsy resulted in significant decrease in exploratory activity and increase of anxiety in both young and old epileptic rats.

6.2. Effect of curcumin treatment on epilepsy

6.2.1. Effect of curcumin on electrobehavioral progression of seizure

In the previous section we demonstrated that intracortical injection of FeCl₃ induces spontaneous seizures which are accompanied by increase in lipid, protein oxidation and decrease of Na-K ATPase activity. In this section we observed that administration of curcumin completely abolished arrival of generalized seizures in both long and short-term (except LDST) curcumin treatment studies. We are first to report that curcumin has potential to inhibit spontaneous seizures in iron-induced model of post-traumatic epilepsy in rat model system. Earlier studies have reported curcumin efficacy in delaying (Sumanont et al., 2007) or completely inhibiting arrival of convulsive seizures (Shin et al., 2007) in kainic acid induced epilepsy in epileptic rats. Curcumin pretreatment ameliorate damage at the chromatin level, attenuating histone modifications, expression of immediate early genes to decrease the severity of kainic acid induced status epilepticus (Sng et al.,
Curcumin also blocks the signaling pathway for apoptotic cell death and indirectly affects BBB for protection of neuronal cell death in kainic acid induced epilepsy (Shin et al., 2007). In this study we observed that both high and low doses of long-term curcumin treatment epileptic rats exhibited normal EEG pattern in cortex and hippocampus. In short-term curcumin treated groups high dose of curcumin (1500 ppm) treatment was found to be more effective in inhibiting generalized seizures than low dose (500 ppm). In LDST curcumin treated rats, grade III seizures were observed in the ECoG recordings. Therefore, from our study it is clear that a lower concentration of curcumin would be effective only if administered for a longer time. Our correlation studies clearly indicates that the observed biochemical and histochemical effect of LDST curcumin treated epileptic rats hardly correlated with each other whereas in long-term (HDLT, LDLT) and HDST curcumin treated groups different parameters were strongly correlated with each other. Therefore, it could be concluded that curcumin’s antioxidative and neuroprotective effect could be the mechanism behind curcumin’s seizure suppressive capabilities.

6.2.2. Effect of curcumin on biochemical parameters

Curcumin has been reported to possess antilipidperoxidative potential in aging (Bala et al., 2006), cerebral ischemia (Shukla et al., 2008; Al-omar et al., 2006; Thiagarajan and Sharma, 2004), colchicines induced cognitive deficit (Kumar et al., 2007), diabetes encephalopathy (Kuhad and Chopra, 2007), neurotoxicity induced by nitropropinoic acid (2007), lead (Dairam et al., 2007; Shukla et al., 2008) in rats brain. In epileptic rats, antiperoxidative role of curcumin has been hardly described. Our data showed that both dose of curcumin (1500 ppm and 500 ppm) as well as long-term and short term treatment inhibited lipid peroxidation in young and old epileptic rats. The observed antilipidperoxidative effect of curcumin might have been attributed to curcumin’s redox metal binding activity (Yang et al., 2005), free radical scavenging properties (Masuda et al., 2001; Motterlini et al., 2000) and antioxidative potential (Kelloff et al., 1996). Our data showing lowering of LPO in epileptic rats after curcumin treatment suggested antilipidperoxidative abilities of curcumin in iron-induced post-traumatic epilepsy.

Protein oxidation has been previously reported to interfere with the normal functioning of cellular proteins (Stadtman, 1992). Curcumin has been shown to reduce
oxidative damage and amyloid pathology in Alzheimer's disease (Frautschy et al., 2001; Lim et al., 2001; Ono et al., 2004; Thiyagarajan and Sharma, 2004) and fluid percussion injury (wu et al., 2006). In the present study, we found that curcumin supplemented diet effectively inhibited the increase of protein oxidation due to iron-induced epilepsy. Presence of two electrophilic alpha, β-unsaturated carbonyl groups in curcumin structure must be involved in neutralizing the hydroxyl radicals. Hence, the observed anti protein oxidative effect of curcumin could be due to direct free radical scavenging potential (Reddy and Lokesh, 1994; Ruby et al., 1995). Curcumin's capacity of crossing blood brain barrier (Yang et al., 2005) and its ability to bind Fe2+/Fe3+ ions (Baum et al., 2004) could be another mechanism of its ability to prevent protein oxidation.

As discussed earlier, oxidative modification at N-terminus is responsible for the increased PKC activity in the young and old epileptic rats. Antioxidative potential of curcumin must have prevented oxidative modification of PKC regulatory subunit. Therefore, in the present study we found curcumin treatment potentially inhibited the increase of cytosolic PKC activity in both young and old epileptic rats to inhibit PKC mediated excitotoxicity. Earlier literature suggests that curcumin inhibit PKC activity in cancerous cells by competing with Ca2+ binding regulatory sites (Mahmmoud, 2007) or by competing with phosphatidyl residue (Liu et al., 1993). Our data showing lowering of PKC activity in epileptic rats could have resulted indirectly from antioxidative potential of curcumin and directly by interaction with regulatory subunit.

Membrane fluidity was observed to be significantly declined in both young and old iron-induced epileptic rats. Effect of curcumin treatment on membrane fluidity is hardly known. Kempaiah and Srinivasan (2006, 2005) have shown that curcumin can inhibit decline of membrane fluidity of erythrocytes in high fat fed as well as hypercholesterolaemic rats. In the present study, we observed that curcumin treatment significantly inhibited decrease of membrane fluidity associated with post-traumatic epilepsy in young and old rats. The observed protective effect of curcumin could be attributed to the antilipidperoxidative and anti protein oxidative effects. Both lipid and protein oxidation have been shown to adversely affect membrane fluidity (Farooqui and Horrocks, 1998).
Decrease in Na-K ATPase activity was reported to result in hyperexcitability (Renkawek et al. 1992), cerebral ischemia, epilepsy (Grisar, 1984), various neurodegenerative disorders (Lees, 1991), Ca\(^{2+}\) mediated excitotoxicity (Goddard and Robinson, 1976) and enhanced glutamate release (Lees, 1991; Henneberry et al., 1989) which ultimately lead to cell death. Curcumin treatment significantly activated Na-K ATPase activity in both young and old epileptic rats compared to age-matched untreated controls. Since, it has been widely documented that lipid peroxidation negatively correlate with Na-K ATPase activity (Sharma et al., 1993; Beal et al., 1995; Riddle et al., 1993). Therefore, our data showing lowering of lipid peroxidation in curcumin treated epileptic rats may be one of the reasons for significant inhibition of decline of Na-K ATPase activity in epileptic rats.

### 6.2.3. Effect of curcumin treatment on cytomorphological changes

Neurodegenerative changes have been described in several models of experimental epilepsy (Covolan et al. 2000; Clarke, 1990; Fabene et al., 2005), aging (Deloncle et al., 2001), aluminium toxicity (neurodegenerative diseases (Scheff and Price, 2006). In coherence we also observed necrotic and apoptotic changes in the cortex and hippocampus of iron induced epileptic rats. Both long and short-term treatment effectively inhibited neurodegenerative changes at the ultra-structural level. No report is available regarding curcumin potential to protect neuronal cells at ultrastructural level. Dietary curcumin treated epileptic rats exhibited homogeneous cytoplasm, containing organelles like ER, golgi apparatus, mitochondria and lysosomes free from lipofuscin pigments. Nucleus does not exhibit abnormal chromatin condensation or nucleolar budding. This ultrastructural study, confirmed curcumin neuroprotective potential in both cortex and CA1 field of hippocampus. The observed effect could be a result of its antioxidative potential.

In addition to ultrastructural studies, results of cellular count also showed that curcumin treatment effectively inhibited decrease of cellular count in CA1 field of epileptic rats. This indicates that dietary supplementation of curcumin ameliorated neuronal damage resulted from iron induced epilepsy. Similarly, curcumin has been previously reported to protect CA1 neurons in cerebral Ischemia (Wang et al., 2005). Curcumin neuroprotection in the CA1 field not only reflected amelioration of neuronal apoptosis but can also be
related improved neurobehavioral outcome. Non-significant increase in the cortical cell count was observed in the curcumin-treated epileptic rats.

6.2.4. Cognitive functions

We observed that both dose of curcumin treatments were effective in preventing cognitive deficit associated with epileptogenesis. Anti-dementic effects of curcumin treatment has been widely reported in experimentally induced cognitive impairment due to intracerebroventricular colchicines treatment (Kumar et al., 2007), experimental diabetic encephalopathy (Kuhad and Chopra, 2007), amyloid β induced cognitive damage (Frautschy et al., 2001) and fluid percussion induced cognitive impairment (Wu et al., 2006). Wu et al., (1996) have reported that curcumin effectively inhibits cognitive decline in fluid percussion model by modulating the BDNF and it’s down stream effectors synapsin I and CREB. BDNF system is reported to modulate synaptic plasticity (Bolton et al., 2000; Hariri et al., 2003) and is required for normal functioning in the MWM. Decreased lipid peroxidation and increased acetylcholinesterase activity (a marker of cholinergic functions) has been linked with the cognitive decline observed in MWM tests (Kuhad and Chopra 2007, Kumar et al., 2007). Therefore, our data showing lower latency in curcumin treated epileptic rats could be suggested as a result of antiperoxidative affects of curcumin.

In epileptic rats, we investigated the effect of curcumin intake on hippocampus region of brain as well, as this region is intimately associated with the processing of cognitive functions (Drapeau et al., 2003; Wilson et al., 2004; Sugaya et al., 1996). Our results obtained from microscopic studies showed prevention of neuronal loss in CA1 field and maintaining normal cellular ultrastructure of hippocampal neurons in curcumin treated epileptic rats, which would also be one of the reasons for inhibiting memory loss. Since hippocampus CA1 region receives signals from CA3 subfield via CA3-CA1 projections for short-term memory formation (Li et al., 1994), beneficial affect of curcumin on CA1 hippocampal neurons, may be suggested as direct/indirect implication of curcumin on learning and memory functions.

An overall data obtained from the electrophysiological, biochemical, microscopic and behavioral studies including correlation analysis demonstrated significant antiepileptic potential of curcumin. Curcumin has an ability to suppress both arrival and propagation of
seizures by decreasing lipid and protein oxidation. The increase of Na-K ATPase activity, membrane fluidity and decrease of PKC activity must have been contributed immensely in suppressing seizures. Curcumin treatment was also effective in protecting cellular structures. The observed neuroprotection in biochemical and microscopic levels by curcumin treatment was very well reflected in the behavioral studies. Open field tests confirmed anxiolytic properties while MWM tests showed memory consolidation potential of curcumin treatment in epileptic rats. Therefore, overall results indicate multiple target of curcumin's action and confirm its antioxidative potential.

6.3. L-deprenyl treatment

Free radical induced neuronal damage is implicated in the mechanism behind iron-induced epileptogenesis. Several studies have shown that L-deprenyl possess antioxidative potential (Mytilineou et al., 1998; Magyar et al., 1996; Nomoto et al., 2003; Carillo et al., 1993). Hence, in the present study the antiepileptic potential of L-deprenyl was investigated in iron-induced post-traumatic epilepsy in young and old rats.

6.3.1. Effect of L-deprenyl treatment on epileptic seizures

We observed that long-term administration of L-deprenyl for 32 weeks significantly inhibited generalization of seizures and completely inhibited arrival of grade III/IV seizures in both young and old treated groups. Our results from long-term treatment confirmed the seizure suppressing potential of L-deprenyl. It is reported that L-deprenyl treatment increases dopamine and nonadrenaline in the catecholaminergic neurons to reduce or suppress seizures (Kilian and Frey, 1973 and Przegalinski, 1985; Knoll et al. 1994). Therefore potential of L-deprenyl to modulate neurotransmitter could one of the reasons for seizure suppressing abilities observed in this study. L-deprenyl also decreases excitatory synaptic transmission and limits repetitive firing of action potentials in the hippocampus via a dopaminergic mechanism (Hsu et al., 1996 and Huang et al., 1997). This could be another reason behind observed seizure suppressing abilities of L-deprenyl in iron-induced epileptic rats.
Short-term treatment of L-deprenyl for 5 weeks also inhibited the arrival of grade IV seizures but failed to inhibit grade III seizures in young and old epileptic rats. The observed effect raised doubts regarding the antiepileptogenic potential of L-deprenyl as short-term treatment failed to inhibit grade IV seizures. Since this study was performed on lower dose (1mg/kg/day) of L-deprenyl, efficacy of higher doses for inhibiting arrival of grade IV seizures cannot be ruled out. Therefore, further studies with higher doses may confirm the antiepileptic action of L-deprenyl. The observed seizure suppressing abilities of short-term deprenyl treated rats could be related with its antiepileptic capabilities. Our observed anticonvulsive effects of L-deprenyl at doses of 1 mg/kg/day corroborates with the previous reports in kindling mice (Lösch and Hönack, 1995) and rats (Lösch and Lehmann, 1996).

6.3.2. Biochemical study

Administration of L-deprenyl for both long-term and short-term treatment significantly lowers lipid peroxidation in epileptic rats. This confirms antilipidperoxidative effect of L-deprenyl in iron-induced epileptic rats. Earlier literature reveals antilipidperoxidative potential of L-deprenyl in rodent model of aging (Kiray et al., 2004; Kaur et al., 2003; Alper et al., 1999), aluminium neurotoxicity (Jyoti and Sharma, 2006; Jyoti et al., 2007), Parkinson's disease (Ebadi et al., 2001; Chiueh et al., 1994) and Alzheimer's disease (Wu et al., 1993). L-deprenyl's ability to directly inhibit reactive oxygen species (Nomoto et al., 2003) or indirectly activating antioxidant enzyme activity (Jyoti et al., 2007; Kaur et al., 2003; Kiray et al., 2004) could be the possible reason behind its antilipidperoxidative potential observed in this study.

The present study revealed that protein oxidation was significantly elevated in both young as well as old epileptic rats. Administration of L-deprenyl led to a significant inhibition of protein oxidation linked with epilepsy. No other studies have described effect of L-deprenyl on protein oxidation in epilepsy. L-deprenyl potential to inhibit protein oxidation was previously reported in MPTP (Vizuete et al., 1993) and aluminium (Jyoti et al., 2007) induced neurotoxicity in different rat brain regions. The observed anti-protein oxidative effect could be attributed to the extensively reported antioxidative potential of l-deprenyl (Mytilineou et al., 1998; Magyar et al., 1996; Nomoto et al., 2003; Carrillo et al., 1993).
Earlier reports demonstrated that antioxidative effect of L-deprenyl could be either mediated through MAO-B inhibition (Cohen and Spina, 1989; Wadia et al., 1998) or through enhanced cellular antioxidant enzymes like SOD and Catalase (Carillo et al., 1991, 1992, 1993; Jyoti et al., 2007).

Both long and short-term L-deprenyl administration significantly inhibits decrease of membrane fluidity linked with iron-induced epilepsy. Only hippocampus of short-term L-deprenyl treated rats exhibited non-significant inhibition. This shows that hippocampus is less responsive to L-deprenyl treatment. As previously described lipid peroxidation plays important role in modulating membrane fluidity (Swapna et al, 2006; Garcia et al., 2000), the observed antilipidperoxidative effect of L-deprenyl could be vital for the observed increase of membrane fluidity in L-deprenyl treated epileptic rats.

L-deprenyl treatment significantly inhibited the iron-induced decrement of Na-K ATPase activity in young and old epileptic rats. Decreased Na-K ATPase has been linked with different type of experimental epilepsy (Vaillend et al. 2002; Renkawek et al. 1992). Therefore our observation showing, activation of Na-K ATPase activity in L-deprenyl epileptic rats seems to prevent seizure arrival. Earlier L-deprenyl administration was reported to stimulate catecholaminergic system (Knoll et al., 1998) which in turn activates brain Na-K ATPase (Swann et al., 1984). Na-K ATPase activation can modulate catecholaminergic and serotoninergic systems (Hernández, 1987) as well as the neural excitability and brain metabolic energy production. Therefore, Na-K ATPase stimulating effect of L-deprenyl could be one of the reasons for its seizure suppressing effects.

L-deprenyl was also found to inhibit the altered activation of cytosolic PKC in young and old epileptic rats. Altered activation of PKC has been reported to be involved in cerebral ischemia (Oster et al., 2004) as well as experimental animal model of epileptogenesis (Vernet et al., 1992; Daigen et al., 1991). Therefore, observed suppression of PKC activity by L-deprenyl could be crucial in inhibiting seizure arrival. Magnitude of L-deprenyl protection at biochemical level was significantly less in short-term treatment group compared to long-term treatment group. This explains the lower efficacy of short-term treatment group to suppress seizures.
6.3.3. Microscopic study

L-deprenyl known to prevent formation of alpha S fibrils (falphaS) in Parkinson’s patients (Ono et al., 2007), aluminium neurotoxicity (Jyoti et al., 2007; Jyoti and Sharma, 2006), MPTP induced alterations in gold fish brain (Goping et al., 1995). In the present study, we observed that both long-term and short-term L-deprenyl treatments were effective in preventing cellular vacuolization, apoptotic and necrotic changes in epileptic rats. L-deprenyl abilities to lower lipid peroxidation, protein oxidation, cytosolic PKC activity and increase in Na-K ATPase activity, membrane fluidity could be the reason for inhibition of neurodegenerative changes in L-deprenyl treated epileptic rats. Micrometric studies revealed significant decrease in cell count in hippocampal CA1 region of epileptic rats, while non-significant decrease was found in the cortex of both young and old epileptic rats. L-deprenyl treatment was observed to be effective in inhibiting cell loss in CA1 field. The observed L-deprenyl induced neuroprotection might be due to the inhibition in biochemical alterations resulted due to epilepsy.

6.3.4. Behavioral study

Both short term and long-term doses were found to significantly inhibit the cognitive decline in epileptic rats. The anti-dementic effects of L-deprenyl could be due to seizure suppressing ability observed in this study, as seizures arrival adversely affect cognitive status (Knoll., 1994; Helmstaedter, 2007). Earlier reports suggest that L-deprenyl treatment increases dopaminergic activity (Brandeis et al., 1991) and cholinergic activity (Knoll 1998; 1992; Yavich et al., 1993). Therefore, it could be suggested that observed effects in MWM latency to acquire hidden platform trials may be consequences of improved dopaminergic and cholinergic activity in L-deprenyl treated epileptic rats. In addition, neuroprotection of hippocampus CA1 neurons may also be another reason for improved performance in cognitive functions after L-deprenyl treatment in epileptic rats.

Overall data / results indicates that in both young and old epileptic rats L-deprenyl treatment delays onset and progression of seizures by way of counteracting the iron-induced alterations in biochemical parameters like lipid peroxidation, protein oxidation, membrane fluidity, cytosolic PKC and Na-K ATPase as well as cytomorphological impairments and
improves cognitive functions. This study substantiates the antiepileptic potential of L-deprenyl observed earlier in pilocarpine and PTZ induced epilepsy.

6.4. Correlation studies

Pearson correlation was performed between different parameters in order to investigate interrelationship between electrophysiological, biochemical, behavioral and microscopic parameters. Data obtained from young and old epileptic rats reveals that arrival of seizure discharges correlate positive with oxidized lipid and proteins, PKC activity, cell count degeneration and anxiety behavior while negatively correlated with Na-K ATPase activity. The high Pearson's correlative values indicate that iron-induced epilepsy is a cumulative resultant of multitude of changes at different levels. The observed decrease of membrane fluidity and Na-K ATPase activity positively correlated with increased lipid peroxidation in epileptic rats. Earlier studies suggest that increased lipid peroxidation is responsible for inhibition of Na-K ATPase (Sharma et al., 1993; Beal et al., 1995; Riddle et al., 1993) activity and decrease of membrane fluidity (Swapna et al., 2006; Garcia et al., 2000). Therefore, our data confirms the interrelationship between lipid peroxidation, Na-K ATPase and membrane fluidity. In addition, we observed that seizures in the epileptic rats positively correlated with hippocampal neuronal loss. Similar, neuronal loss was also reported in experimental kindling model (Bertram et al., 1990; Bolkvadze et al., 2007) as well as kainate induced model of epilepsy (Liang et al., 2007; Hoffman et al., 2003). The observed Pearson correlation studies clearly indicates that iron-induced epilepsy is a resultant of cumulative alterations of different parameters. All these changes are interrelated with each other.

As discussed in previous sections, both curcumin and L-deprenyl treatment was found to be effective in inhibiting epilepsy induced alterations at electrophysiological, biochemical, behavioral and microscopic levels. In HDLT curcumin treatment onset of grade III/IV seizures was completely inhibited while in LDST curcumin treated group grade III seizures were observed. The Pearson correlation results from HDLT curcumin treated epileptic group's different parameters were strongly and significantly correlated with each other whereas in LDST curcumin treated epileptic rats, parameters were either not correlated or exhibited poor correlation. The observed high correlative values ($r = +0.9$
or -0.9) in HDLT curcumin treated epileptic rats indicate that curcumin is more effective and protective if frd in high dose for long term in epileptic rats. Like curcumin, we observed that in L-deprenyl treated groups, different parameters studied correlated significantly in long term L deprenyl treated epileptic group compared to short-term treatment. Possibly low correlative values among different parameters in short-term L-deprenyl treated group would be the reason behind its failure to suppress grade III seizures. This concludes that an effective antiepileptic drug should have multiple target of action.