Discussion

SEIZURE LATENCY AND MAGNITUDE OF DRUG EFFECT

Epileptic rats showed a lower seizure onset latency compared to the epileptic rats treated with Carbamazepine, *Bacopa monnieri* and Bacoside A. This indicates that the onset of the seizures is extended in the pre-treated groups. The seizure duration in the epileptic rats increased when compared to the pre-treated groups. The control rat group showed no seizures. The increase in the seizure onset latency and decrease in the duration of seizures of various antiepileptic drugs were reported earlier (Eric et al., 2002). Post-treated with Carbamazepine, *Bacopa monnieri* and Bacoside A reduced the number of seizures per hour compared to the epileptic rat groups. The severity of the seizures in the treated rat groups was also decreased. These results are the suggestive evidence of the ability of the *Bacopa monnieri* and Bacoside A in reducing the spontaneous seizures in both the pre-treated and post-treated groups which shows their antiepileptic property.

CENTRAL ACETYLCHOLINE ESTERASE ACTIVITY

Acetylcholine is the primary neurotransmitter of the cholinergic system and its activity is regulated by acetylcholine esterase. The termination of nerve impulse transmission is accomplished through the degradation of acetylcholine into choline and acetyl CoA by acetylcholine esterase (Weihua Xie et al., 2000). Acetylcholine esterase activity has been used as a marker for cholinergic activity (Goodman & Soliman, 1991; Ellman et al., 1961).

Central cholinergic activity was studied in experimental rats using acetylcholine esterase as marker. Our results showed an increase in $V_{\text{max}}$ in the
hippocampus and brainstem of epileptic rats when compared to control. The $K_m$ showed no significant change in both regions. The up regulated acetylcholine esterase gene and the subsequent increase in the acetylcholine depletion were earlier reported in Alzheimer's disease. (Von der Kammer et al., 2001). Treatment with Carbamazepine, *Bacopa monnieri* and Bacoside A reversed the $V_{max}$ near control. The anticholine esterase activity of the *Bacopa monnieri* was early reported (Hanumanthachar et al., 2006). Stimulation of ACh function by effective dose of Carbamazepine is involved in the antiepileptic and mood stabilizing mechanisms of action of Carbamazepine (Mizunok et al., 2000). Our results suggest that the antiepileptic activity of the *Bacopa monnieri* is attributed to its pro-cholinergic and anti-acetylcholine esterase properties.

**MUSCARINIC RECEPTOR ALTERATIONS DURING POST-TREATMENT IN THE HIPPOCAMPUS.**

Over the past decade, the role of muscarinic receptors in health was given much scientific study. The potential therapeutic value of various cholinergic agonists and antagonists have received increasing attention (Zwieten & Doods., 1995; Zwieten et al., 1995). Muscarinic receptors are a family of G protein-coupled receptors that have a primary role in central cholinergic neurotransmission. The muscarinic M1 receptor is one of five known muscarinic subtypes in the cholinergic nervous system (Bonner et al., 1987; Hulme et al., 1990; van Zwieten & Doods, 1995). The M1, M2 M3 and M4 subtypes of mACHRs are the predominant receptors in the CNS. These receptors activate a multitude of signaling pathways important for modulating neuronal excitability, synaptic plasticity and feedback regulation of ACh release (Volpivelli et al., 2004)
Muscarinic M1 receptor binding studies in the hippocampus of the epileptic rats showed a significant increase in the $B_{max}$ when compared to control. The $K_d$ also showed a significant increase. Increased $B_{max}$ indicates the increased number of receptors. The increased $K_d$ indicates the decreased affinity for the ligand. These results were further confirmed by the increase in the log $EC_{50}$ and the $K_i$ by displacement analysis. The Hill slope values of both epileptic and control groups showed a value near to unity which confirms a single binding site. The Real Time-PCR analysis showed that the muscarinic M1 receptors were up regulated in the hippocampus. The increased receptor binding through the up regulation of muscarinic M1 receptor in the hippocampus could be taken as suggestive evidence for the excitatory effect of muscarinic receptors in the propagation of seizures. The increase in the $K_d$ indicates that affinity of the receptors towards the ligand was low in epileptic rats which is another suggestive evidence of the receptor enhanced susceptibility to the ligand.

Septo-hippocampal cholinergic fibers ramify extensively throughout the hippocampal formation that are differentially expressed by distinct populations of neurons. The resultant modulation of cellular exitability and synaptic transmission with in hippocampal pyramidal cells. (Dodd et al., 1981; Cole & Nicoll, 1983). The ionic basis of this excitation has now been elucidated. Muscarinic receptors modulate a large number of ionic conductance in pyramidal neurons through both direct and indirect biochemical interactions. In addition muscarinic acetylcholine receptors activation also potentiates two mixed cation current ($I_{cat}$) the calcium dependent non specific cation current (Halliwell 1990, Colino & halliwell, 1993) and modulates activity of both voltage dependent $Ca^{2+}$ currents. (Toselli et al. 1989) and several ligand gated receptors including NMDA (Makram & Segal, 1990). Physiological
activation of muscarinic acetylcholine receptors also produces profound alteration in the second messenger cascade and intracellular calcium mobilization (Power & Sah, 2002) suggesting long term consequences for the neuronal excitability. Pharmacological activation of muscarinic acetylcholine receptor directly increases the frequency and amplitude of spontaneous IPSCs whilst at the same time depressing monosynaptically evoked IPSCs (Behrends & Bruggencate, 1993). Activation of muscarinic acetylcholine receptors directly excites GABAergic interneurons. It also has a depressant effect on the synaptic release of GABA. More recent studies have shown that in the majority of identified GABAergic interneurons, pharmacological activation of Muscarinic acetylcholine receptors resulted in a similar membrane depolarization to that seen in pyramidal cells.

In the experimental rat groups post-treated with Carbamazepine, *Bacopa monnieri* and Bacoside A, a significant decrease in the $B_{max}$ was observed when compared to the epileptic group. The $K_d$ also decreased and returned near to the control group. The Real Time - PCR analysis showed that the muscarinic M1 receptors are down regulated to a lower level when compared to the epileptic group. This could be taken as suggestive evidence that these drugs have effect against the pilocarpine induced seizures through muscarinic receptors. The reversal of the $K_i$ and log EC$_{50}$ to the near control values in the treated group further confirms this observation. There are evidences for the action of *Bacopa monnieri* on the cholinergic system. The effect of *Bacopa monnieri* incudes modulation of acetylcholine release, choline acetylase and muscarinic receptor binding were reported (Bhattacharya et al., 1999). The anti- acetylcholine esterase activity of the *Bacopa monnieri* was previously established (Mizunok et al., 2000). Our results suggest that *Bacopa monnieri* through its active component Bacoside A prevent the depletion of
acetylcholine in the brain. The reversal of the enhanced receptor binding in the hippocampus is suggested to be a compensatory mechanism to regulate the cholinergic activity.

MUSCARINIC RECEPTOR ALTERATIONS DURING PRE-TREATMENT IN THE HIPPOCAMPUS.

Muscarinic M1 receptor binding studies in the hippocampus of the epileptic rats showed a significant decrease in the $B_{\text{max}}$ when compared to control. The $K_d$ also showed a significant decrease. These results were further confirmed by the decrease in the log EC$_{50}$ and $K_i$ by displacement analysis. The Hill slope values of the both epileptic and control groups showed a value near to unity which confirms a single binding site. The Real Time - PCR analysis showed that the muscarinic M1 receptors were down regulated in the hippocampus. The decreased receptor binding through the down regulation of muscarinic M1 receptor in the hippocampus could be taken as suggestive evidence to compensate the hyper excitability by the muscarinic receptors in the initiation of seizures. The decrease in the $K_d$ and log EC$_{50}$ values and the increase in the $K_i$ values indicate that affinity of the receptors towards the ligand is high even 24 hours after pilocarpine induced recurrent seizures which confirms the hyperexcitability of the muscarinic receptors. Previous reports showed that in animal kindling model of epilepsy, a 10-30% decline in the muscarinic receptor occurs in the hippocampal formation and amygdala transiently after secondarily generalized seizures with in a duration of less than 4 days indicating that seizures alone can affect receptor gene expression (Dasheiff et al., 1982)
Hippocampus is an area of interest to investigate the pilocarpine-induced seizures, because it is one of the most vulnerable brain areas for epilepsy-related brain damage and plays a main role in the development and maintenance of limbic seizures. The projection from the medial septal area to the hippocampus is cholinergic (Moor et al., 1994). Muscarinic receptors (Rotter et al., 1984) and NMDA receptors (Cotman et al., 1987) are widely distributed in the hippocampal region. The hippocampal formation contains a rich glutamatergic and GABA-ergic input, GABA-ergic interneurons containing peptide co-transmitters and the glutamatergic perforant pathway interconnects with entorhinal cortex, subiculum, CA1, CA3 fields and dentate gyrus (Ottersen & Storm-Mathisen, 1984; Kupferman, 1991). Pilocarpine produced marked changes in morphology, membrane properties and synaptic responses of hippocampal rat neurons which are comparable to those observed in human epileptic hippocampal neurones (Isokawa & Mello, 1991). The presumed mechanism of action is muscarinic receptor stimulation being responsible for seizure initiation and for driving amino acids to sustain epileptic activity and to induce neuronal damage (Turski et al., 1989). Earlier studies described that intrahippocampal administration of pilocarpine resulted in a decrease of the extracellular glutamate and GABA levels and shows simultaneous slowing of the rhythmic activity recorded on the EEG, showing theta and delta waves. This effect was blocked by co-perfusion with the non-selective muscarinic receptor antagonist. The cholinergic nature and involvement of cholinergic receptors in hippocampal theta rhythm has been previously described in vitro (Konopacki et al., 1988). Presynaptic muscarinic M2 receptor on hippocampal glutamatergic nerve terminals that decreases the release of glutamate has been described by in vitro studies (Marchi et al., 1989; Marchi & Raiteri, 1989). Intra- and extracellular single cell recordings demonstrated that acetylcholine exerted a rapid and powerful muscarinic inhibitory effect upon both
excitatory and inhibitory afferents to hippocampal neurons and it suggested that this effect was mediated by a decrease in the amount of released neurotransmitter (Valentino & Dulingedine, 1981). Moreover, muscarinic receptor stimulation significantly enhanced the spontaneous firing of the hippocampal GABAergic interneurones, resulting in an increased frequency of spontaneous-activity-dependent inhibitory post-synaptic potentials (Pitler & Alger, 1992). Intrahippocampal pilocarpine perfusion followed by a significant and sustained enhancement of the extracellular glutamate concentrations was reported early. (Smolders et al., 2004) These elevations were associated with the onset of the limbic seizures, as evidenced from the patterns recorded on the EEG. Seizure related elevations of the extracellular glutamate concentration have also been observed in patients with complex partial seizures subjected to epilepsy surgery (Carlson et al., 1992; During & Spencer, 1993). Enhanced glutamate and GABA release in the EC is suggested to be associated with the development of epileptic condition (Thompson et al., 2007) Intrahippocampal perfusion with atropine for three hours did not further change the extracellular hippocampal GABA and Glutamate level during and after co-administration of pilocarpine and no specific limbic convulsions were noticed indicating muscarinic receptors as the primary site of action. Malanski et al., (1994) showed that atropine can block pilocarpine-induced seizures but is unable to interrupt already established convulsions.

In the experimental rat groups pre-treated with Carbamazepine, Bacopa monnieri and Bacoside A, a significant increase in the Bmax was observed when compared to the Epileptic group. The Kd also increased and reached to a value near to the control group. The Real Time - PCR analysis showed that the Muscarinic M1 receptor gene expression reversed back to the control value. This could be taken as a
suggestive evidence that these drugs have a neuroprotective ability against the pilocarpine induced seizures through the inactivation due to the hyperexcitability of the muscarinic receptors. The reversal of the $K_i$ and log EC$_{50}$ to the near control values in the treated group further confirms this observation. There were evidences for the action of *Bacopa monnieri* on the cholinergic system. The effect of *Bacopa monnieri* includes modulation of acetylcholine release, choline acetylase and muscarinic receptor binding were reported (Bhattacharya *et al*, 1999).

**MUSCARINIC RECEPTOR ALTERATIONS DURING POST-TREATMENT IN THE CEREBELLUM**

Total muscarinic receptor binding studies in the cerebellum of the epileptic rats showed a significant decrease in the $B_{max}$ when compared to control. The $K_d$ showed no significant change. Muscarinic M1 receptor binding studies in the cerebellum of the epileptic rats showed a significant decrease in the $B_{max}$ when compared to control. The $K_d$ also showed a significant decrease. The Hill slope values of the both epileptic and control groups showed a value near to unity which confirms a single binding site. The Real Time - PCR analysis showed that the muscarinic M1 receptors are down regulated in the hippocampus. The decreased receptor binding in the cerebellum could be taken as suggestive evidence of the differential regional specific alteration of the muscarinic receptors in the propagation of seizures. The hyperexcitability of the hippocampus and the modulation of the background firing frequency by the cerebellar units were already reported (Baratta *et al.*, 2004). The decreased muscarinic M1 and muscarinic general binding in the cerebellum during the chronic epilepsy is suggested to be a compensatory mechanism by the cerebellum in reducing the firing frequency.
Earlier studies established that rhythmic output from the cerebellum may contribute to the maintenance of generalized seizures (Kandel et al., 1993). Down regulation of the muscarinic M2 and M3 mRNA in cultured cerebellar granule cells by carbachol were studied earlier (Fukamauchi et al., 1991). These previous studies showed the down regulation of the muscarinic receptors by the agonist induction. Muscarinic receptor antagonist atropine and M1 specific antagonist pirenzepine prevented the carbachol induced muscarinic receptor down regulation (Fukamauchi et al., 1991). Decreased muscarinic M4 receptor binding was reported in the saturating concentration of agonist carbachol (Lenz et al., 1994). The receptor degradation kinetics in the presence of protein synthesis inhibitor cyclohexamidc showed that receptor down regulation sufficiently accounted by the increase receptor degradation. Wang et al., (1990) showed that muscarinic agonist carbamylcholine for 24 hrs decreased receptor density and mRNA levels in Chinese hamster ovary cells transfected with M1 receptor gene. Evidences showed that two kinds of response 10 and 16 msec and those with latencies around 30 m sec by gross electrodes inserted deep into the granule layer of cerebellum. When evoked by a hippocampal stimulus, the characteristic slow wave response consists of early and late component which can be influenced by conditioning suggest that separate conduction systems are involved. The relative fast time course of the early component can be attributed to mossy fibre relays through the reticular formation. The alternative pathway would be through the pontine nuclei and hence by mossy fibers to the posterior lobe. The relatively slow time of the late component suggest that a proportion of the hippocampal pathways reach the cerebellum by climbing fibre relays through the inferior olive.

In the experimental rat groups post-treated with Carbamazepine, Bacopa monnieri and Bacoside A, B_{\text{max}} reversed back to near control level in the cerebellum.
The $K_d$ also increased and reached to a value near to the control group. The Real Time - PCR analysis showed that muscarinic M1 receptor gene expression reversed back to the near control level. This could be taken as suggestive evidence that these drugs have effect against the pilocarpine induced seizures through muscarinic receptors.

**MUSCARINIC RECEPTOR ALTERATIONS DURING PRE-TREATMENT IN THE CEREBELLUM.**

Muscarinic M1 receptor binding studies in the Cerebellum of the epileptic rats showed a significant increase in the $B_{max}$ when compared to control. The $K_d$ showed no significant change. Displacement analysis showed no significant change in log $EC_{50}$ and $K_i$. The Hill slope values of the both epileptic and control groups showed a value near to unity which confirms a single binding site. The increase receptor binding in the cerebellum could be taken as suggestive evidence of the hyper excitability by the muscarinic receptors in the initiation of seizures.

In the cerebellum of pilocarpine induced epileptic rats, extracellular GABA and glutamate was reported to be elevated significantly during the pilocarpine-induced convulsions. Seizure-related stimulation of the hypothalamocerebellar GABA-ergic projection (Dietrichs *et al.*, 1992) is suggested to be responsible for the increased cerebellar GABA release, but does not directly explain simultaneous and longer lasting glutamate increase. During limbic seizures and during the interictal period, changes in metabolic activity and cerebral blood flow occur (Park *et al.*, 1992). Hyperperfusion at the ipsilateral side of epileptic focus, as demonstrated in a case report (Overbeck *et al.*, 1990) explain the increased amino acid concentrations in ipsilateral cerebellum. These increases were abolished when pilocarpine-induced
seizures were prevented by antiepileptic drug treatment. Pilocarpine, was reported to increase hydrolysis of phosphoinositol (PI) in the cerebellum (Johnson et al., 2000). The muscarinic cholinergic cascade in brain and other tissues appears to involve the hydrolysis of phosphoinositides to form diacylglycerol and inositol phosphates which can serve as second messengers (Berridge et al., 1983; Michell, 1975). This effect could be completely inhibited by the pretreatment with muscarinic antagonist scopolamine. It should be noted that the cerebellar but not the hippocampal pilocarpine induced rise in the PI hydrolysis. This indicate that the pilocarpine acting through Muscarinic M2 receptor to indirectly increase glutamate release from parallel fibers by inhibition of GABA releasing golgi cells. Previous study on the extracellular hippocampal amino-acid levels suggest that glutamate, aspartate and GABA are not involved in seizure onset, but play a role in seizure maintenance and/or spread in the pilocarpine animal model of epilepsy (Smolders et al., 2004). The increase in extracellular amino acids in ipsi- and contralateral cerebellum following limbic seizures provoked in the hippocampus, probably play a role in the 'reversed' diascchisis phenomenon. Muscarinic receptor stimulation is presumed to be responsible for the onset of pilocarpine-induced seizures, whereas amino acid mechanisms are presumed to maintain sustained seizure activity and to lead to neuronal damage (Turski et al., 1989; Smolders et al., 1997). The acetylcholine was reported to increase during the onset of SE in different regions of the brain. In the cerebellum of pilocarpine induced epileptic rats, extracellular GABA and glutamate was reported to be elevated significantly during the pilocarpine-induced convulsions. The administration of convulsant drug 3-mercaptopropionionic acid was reported reversible increases in [\(^1\)H]QNB binding to cerebellum (Schneider et al., 2000).
In the experimental rat groups Pre-treated with Carbamazepine, *Bacopa monnieri* and Bacoside A, a significant decrease in the $B_{\text{max}}$ was observed when compared to the Epileptic group in the cerebellum. The $K_d$ also decreased and reached to near to the control group. The Real Time PCR analysis showed that the muscarinic M1 receptors are down regulated to a lower level when compared to the epileptic group. This could be taken as suggestive evidence that these drugs have effect against the pilocarpine induced seizures through muscarinic receptors. There were evidences for the action of *Bacopa monnieri* on the cholinergic system. The decreased binding and gene expression in the hippocampus and the increased binding in the cerebellum could be explained by the highest vulnerability of hippocampus for epilepsy-related hyperexcitability and brain damage during the initial stage of the epilepsy.

**MUSCARINIC RECEPTOR ALTERATIONS DURING POST-TREATMENT IN THE BRAINSTEM.**

Muscarinic M1 and total muscarinic receptor binding studies in the brainstem of the epileptic rats showed a significant decrease in the $B_{\text{max}}$ when compared to control. The $K_d$ also showed a significant decrease. The decreased receptor binding in the brainstem can be taken as suggestive evidence of the hyper excitability by the muscarinic receptors. The decrease in the $K_d$ indicate the affinity of the receptors towards the ligand was increased as a compensatory mechanism to reduce the hyperexcitability. Our previous report suggests that the acetylcholine esterase activity is increased in the brainstem of the epileptic rats compared to control. The hyperexcitability and seizure onset by the infusion of muscarinic agonist on specific regions of the brainstem were already reported. The decreased receptor binding through the down regulation of muscarinic M1 receptor in the brainstem could be
taken as evidence to compensate the hyper excitability by the Muscarinic receptors in the chronic epilepsy. Earlier studies reported that iontophoretically applied acetylcholine inhibits neurons in the feline dorsolateral nucleus reticularis and that this inhibition, but not that evoked by GABA or glycine, can be accompanied by an increase in burst activity.

Muscarinic M1, M2 and M3 mAChR subtypes were distributed heterogeneously throughout the brainstem. For all 3 mAChR subtypes, the greatest levels of binding were found in the dorsal raphe and locus coeruleus and the least amount of binding was in the reticular formation (Baghdoyan et al., 1994). Previous studies reported that the repetition of running-bouncing and tonic-clonic seizures mediated by brainstem structures eventually elicits seizure activity in the forebrain. Periaqueductal gray (PAG) region is a component of the neural network through which brainstem seizures elicit forebrain seizures. Bilateral microinjection of carbachol into the PAG region of rats induced arrested, staring behavior accompanied by epileptiform electrocorticogram (ECoG) after discharge recorded from the parietal cortex. The carbachol effect was mediated by muscarinic receptors as bilateral atropine microinjection 1 min prior to carbachol microinjection inhibited all seizure activity. The occurrence of seizures consisting of facial and forelimb clonus with rearing and falling occur with minutes after single application of GABA antagonist, glutamate agonist or muscarinic agonist in the area tempestas (AT) in the discrete epileptogenic site in the deep prepiriform cortex (Gale et al., 1990; Piredda & Gale, 1985) However carbachol alone in AT was not effective for evoking seizures after extended mid- or precollicular transactions. Presumably, the combination of enhanced excitation with carbachol and blockade of inhibition with bicuculline was necessary for triggering seizures from AT. The inferior colliculus (IC) is the initiation site in the
neuronal network for the epileptic audiogenic seizure (AGS). Unilateral microinjections of carbachol into the IC elicited intense locomotor activity, contraversive rotations and myoclonic seizures. This indicates that the IC is the initiation site for the induction of myoclonic seizures and suggests that these myoclonic seizures result from activation of muscarinic M1 receptors.

In the experimental rat groups post-treated with Carbamazepine, *Bacopa monnieri* and Bacoside A, the $B_{\text{max}}$ reversed back to near control level in the brainstem. The $K_d$ also increased and reached to a value near to the control group. The Real Time - PCR analysis showed that the muscarinic M1 receptors gene expression reversed to near control level. These results suggest that these drugs have effect against the pilocarpine induced seizures through Muscarinic receptors in the brainstem.

**GLUTAMATE RECEPTOR ALTERATIONS DURING POST-TREATMENT IN THE HIPPOCAMPUS.**

Glutamate receptor binding studies in the hippocampus of the epileptic rats showed a significant decrease in the $B_{\text{max}}$ when compared to control. The $K_d$ showed no significant change. The decreased receptor binding in the hippocampus is suggested to due to the hyper excitability by the glutamate receptors in the initiation of seizures. Our result shows that the glutamate dehydrogenase activity is high in the hippocampus during chronic epileptic state. Previous studies showed that the glutamate decarboxylase activity was decreased in the hippocampus. Increased glutamate dehydrogenase and decreased glutamate decarboxylase result in the accumulation of glutamate in the rat hippocampus (Houser *et al.*, 1996). Our results
suggest that the decrease in the receptor binding and the gene expression of the NMDA was due to the vulnerability to the increased activity of the glutamate receptors in the hippocampus. In the epileptic rats treated with *Bacopa monnieri* the $B_{\text{max}}$ reversed to the control level. NMDA receptor gene expression also reversed to the control level. These results suggest the therapeutic effect of *Bacopa monnieri* in the treatment of epilepsy and its action on the glutamate receptors.

**GLUTAMATE RECEPTOR ALTERATIONS DURING POST-TREATMENT IN THE CEREBELLUM.**

Glutamate receptor binding studies in the cerebellum of the epileptic rats showed a significant decrease in the $B_{\text{max}}$ when compared to control. The $K_d$ showed no significant change. The NMDA R1 and metabotropic glutamate receptor 8 gene expression was down regulated in the cerebellum of epileptic rats. Previous studies showed that the glutamate decarboxylase activity was decreased in the cerebellum. There were reports showing the co-localization of ZnT3 and GAD in the cerebellar cortex which decreases the GAD activity. Increased glutamate dehydrogenase and decreased glutamate decarboxylase result in the accumulation of glutamate in the rat cerebellum (Ruiz *et al.*, 2004). Our results suggest that the decreased receptor binding and the gene expression of the NMDA was due to the vulnerability to the increased activity of the glutamate receptors in the cerebellum. In the epileptic rats treated with *Bacopa monnieri* the $B_{\text{max}}$ reversed to the control level. NMDA R1 and metabotropic glutamate receptor 8 gene expression also reversed to the control level. These results suggest the therapeutic effect of *Bacopa monnieri* in epilepsy through glutamate receptors.
GLUTAMATE RECEPTOR ALTERATIONS DURING POST-TREATMENT IN THE BRAINSTEM.

Glutamate receptor binding studies in the brainstem of the epileptic rats showed a significant decrease in the $B_{\text{max}}$ when compared to control. The $K_d$ showed no significant change. The NMDA R1 and metabotrophic glutamate receptor 8 gene expression was down regulated in the brainstem of epileptic rats. Our results showed that the glutamate dehydrogenase activity is high in the brainstem during chronic epileptic state. Our results suggest that the decrease in the receptor binding and the gene expression of the NMDA and metabotrophic glutamate receptor reflect the down regulation of glutamate receptors due to repetitive tonic seizures. In the epileptic rats group treated with *Bacopa monnieri* the $B_{\text{max}}$ reversed to the control. NMDA R1 and metabotrophic glutamate receptor 8 gene expression also reversed to the control. These results suggest the therapeutic effect of *Bacopa monnieri* in epilepsy through glutamate receptors.

It is widely accepted that excitatory amino acid transmitters such as glutamate are involved in the initiation of seizures and their propagation. Most attention is directed to synapses using NMDA receptors but more recent evidence indicates potential roles for ionotropic non-NMDA (AMPA/kainate) and metabotropic glutamate receptors (Ure *et al.*, 2006). Based on the role of glutamate in the development and expression of seizures, antagonism of glutamate receptors has long been thought to provide a rational strategy in the search for new, effective anticonvulsant drugs. Furthermore, glutamate receptor antagonists, particularly those acting on NMDA receptors, protect effectively in the induction of kindling. It was suggested that they have utility in epilepsy prophylaxis. However, many clinical trials with competitive and uncompetitive NMDA receptor antagonists in patients with
partial seizures showed that these drugs lack convincing anticonvulsant activity but induce severe neurotoxic adverse effects in doses which were well tolerated in healthy volunteers. The proconvulsant effects of NMDA were reported when administered 30 minutes before pilocarpine injection. Smaller and higher doses of NMDA drugs not protected but increased pilocarpine-induced seizures and mortality. (Frietas et al., 2006). NMDA antagonists, irrespective whether they are competitive, high- or low-affinity uncompetitive, glycine site or polyamine site antagonists, do not counteract focal seizure activity. They attenuate propagation to secondarily generalized seizures indicating that once kindling is established, NMDA receptors are not critical for the expression of fully kindled seizures (Locher & Honak, 1991). Recurrent seizures in animal models of early-onset epilepsy have been shown to produce deficits in spatial learning and memory (Bo et al., 2004). In early reports, seizures induced either by tetanus toxin or flurothyl were found to reduce the expression of NMDA receptor subunits in both the hippocampus and neocortex (Hashimoto et al., 2004). Taken together, the reports suggest that recurrent seizures produce persistent decreases in molecular markers for glutamatergic synapses - particularly components of the NMDA receptor complex implicated in learning and memory. Mitsuyoshi et al. (1993) reported that NMDA receptors were down regulated due to repetitive tonic seizures in double mutant spontaneously epileptic rats. The possible role of altered genetic expression in mediating symptomatic epilepsy represents a molecular mechanism that could account for long-lasting changes in neuronal function in response to environmental influences (DeLorenzo, 1991; DeLorenzo and Morris, 1999). If changes in genetic expression underlie epilepsy, long lasting alterations in transcriptional regulation should accompany epileptogenesis. Previous reports indicate that epilepsy induced by SE in the pilocarpine model is associated with a long lasting increase in the binding of the transcription factor SRF to its DNA consensus sequence.
SRE. The increase in DNA binding was present in both hippocampal and cortical nuclear enriched fractions but not in cerebellar nuclear-enriched fractions. The hippocampus and cortex both play roles in seizure generation and propagation (Mello et al., 1993). Both in vivo and in vitro studies established that NMDA receptor activation during SE is required for epileptogenesis (Sombati & DeLorenzo, 1995; DeLorenzo et al., 1998; Rice & DeLorenzo, 1998). Blockage of NMDA receptor activation during pilocarpine induced SE completely blocked the long-term increase in SRF binding. Thus, a long-lasting increase in SRF expression and DNA binding occurs in association with the persistent plasticity changes that underlie epilepsy. Long-term changes in epilepsy may be mediated by persistent changes in gene expression. The pathological over expression of SRF may also act to repress transcription of a number of genes.

Acetylcholine was reported to potentiate NMDA responses (Markram & Segal, 1990a) in CA1 pyramidal neurons indicate that the ACh-induced potentiation was mediated via muscarinic acetylcholine receptors (Markram & Segal, 1990b). Activation of muscarinic acetylcholine receptors in CA1 potentiates NMDA receptors both in acute slices (Marino et al., 1998) and in dissociated cells (Lu et al., 1999) as well as in the other cell types such as striatal spiny neurons (Calabresi et al., 1998), and in auditory neocortical cells (Aramakis et al., 1999). The muscarinic receptor subtype mediating the potentiation is likely M1 as specific M1 toxins blocked the carbachol-induced potentiation (Marino et al., 1998). Consistent with this finding, M1 receptors were also shown to co-localize with NR1A at specific postsynaptic sites (Marino et al., 1998) and muscarinic M1 receptors are highly expressed in the hippocampus (Levey et al., 1995). Markram & Segal (1990) reported that the M1-induced enhancement of NMDA responses required activation of PI turnover via
G_{q} subunits. Metabotropic glutamate receptors could stimulate PI turnover or lead to the mobilization of intracellular Ca^{2+} (Sladeczek et al., 1985; Nicoletti et al., 1986; Pearce et al., 1986; Sugiyama et al., 1987; Mayer and Miller, 1990). Previous studies strongly suggest the activity-dependent modifications of CA1 synapses mediated by NMDA receptors, play an essential role in the acquisition of spatial memories (Tsien et al., 1996). The behavioral and cognitive changes occur soon after SE, were permanent and are dependent on NMDA-receptor activation during SE (Rice et al., 1996).

Metabotropic glutamate (mGlu) receptors have multiple actions on neuronal excitability through G-protein-linked receptors, modifications of enzymes and ion channels. They act presynaptically to modify glutamatergic and GABAergic transmission and can contribute to long term changes in synaptic function. (Alexander et al., 2006) The classical agonists acting on group III mGlu receptors such as L-(+)-2-amino-4-phosphonobutyric acid and L-serine-O-phosphate shows anticonvulsant activity. The more recently identified agonists (R,S)-4-phosphonophenylglycine [(R,S)-PPG] and (S)-3,4-dicarboxyphenylglycine [(S)-3,4-DCPG] and (1S,3R,4S)-1-aminocyclopentane-1,2,4-tricarboxylic acid [ACPT-1] are all anticonvulsant without proconvulsant effects. These results suggest the anticonvulsive activity of III metabotrophic glutamate receptors. (Moldrich et al., 2003) The anticonvulsant effect of metabotrophic glutamate 8 receptor agonist in the pilocarpine model of epilepsy was reported (Jiang et al., 2007).
ELECTROPHYSIOLOGICAL CHANGES DURING EPILEPSY

Neuroelectrophysiological recordings represent a non-invasive and reproducible method of detecting central and peripheral nervous system alterations (Morano et al., 1996). Interictal spike discharges were seen intermittently in animals manifesting clinical seizure activity. (Rice et al., 1996). Cellular changes in the hippocampus underlie epileptogenesis established through EEG studies show that hippocampus is one of the early structures activated during seizures.

The control, epileptic, Carbamazepine, Bacopa monnieri and Bacoside A post treated and pre-treated rats underwent EEG analysis. The epileptic rats showed a change in the EEG pattern compared to control rats. Treatment with Carbamazepine, Bacopa monnieri and Bacoside A brought the wave patterns to near control levels. Interictal spikes are widely accepted diagnostically as a sign of epilepsy. It is easily generated in normal brain by pharmacologically reducing inhibition. Experimental studies of acquired epilepsy indicate that spikes precede seizures. Interictal spikes are correlated with epilepsy because they play a fundamental role in epileptogenesis following brain injury. Spikes may guide sprouting axons back to their network of origin increase and sustain the strength of the synapses formed by sprouted axons. They alter the balance of ion channels in the epileptic focus resulting in seizures (Staley & Dudek, 2006).

MOSSY FIBRE SPROUTING IN THE HIPPOCAMPUS OF EPILEPTIC RATS

The increased Timm staining density in the CA1 region in the hippocampus of the epileptic rats were observed when compared to control. Mossy fibre sprouting is the condition in which dentate granule cells as a consequence of a pathologic
rearrangement of neuronal circuitry in which the excitatory granule cells innervate
themselves, resulting in a recurrent excitatory circuit (Nadler et al., 1980; Tauck &
Nadler, 1985). This rearrangement would be attributable to the synapse elimination
resulting from death of neurons like the mossy cells that normally project to the
proximal third of the dendrites of the granule cells. The eliminated synapses would be
replaced by the mossy fiber axons of the granule cells themselves. The mossy fiber
axons contain high concentrations of zinc and can be readily identified by a Timm
stain, thereby facilitating detection of axonal rearrangements of these neurons. A
consistent increase in this projection in the supragranular layer of the dentate gyrus
has been identified with Timm staining following seizures induced by the glutamate
receptor agonist kainate (Tauck & Nadler, 1985) in kindling (Sutula et al., 1988;
Cavazos et al., 1991) and in specimens from humans with epilepsy (Sutula et al.,
1989). Some anatomical evidence suggests that sprouted mossy fibers innervate
GABAergic basket cells, which would be expected to enhance paired pulse inhibition
of the granule cells (Sloviter, 1992). Using field potential recordings in vivo in
kainate-treated rats, Sloviter (1992) found a reduction of granule cell inhibition and
increased excitability prior to the development of the sprouting. In the epileptic rats
post-treated with Carbamazepine and *Bacopa monnieri* even though the functional
reversal was observed in muscarinic and glutamate receptors, structural difference was
not reversed in these groups. The results suggest that long time treatment is required
for the structural reversal.

**MORRIS WATER MAZE EXPERIMENT**

Impairment of cognitive learning during the silent period between pilocarpine
induced SE and appearance of spontaneous recurrent seizures was reported (Hort et
Place navigation in the Morris water maze consists of two distinct components: declarative place representations as well as procedural learning (Morris et al., 1990). The procedural aspects include learning to inhibit inborn nonadaptive behavior, such as swimming along the wall (Paylor & Rudy 1990, Whishaw & Mittleman 1986), while selecting appropriate behavioral strategies, such as swimming across the pool or uniformly searching its surface. Other procedural components involved skills such as improved distance and angle judgment that are a necessary prerequisite for the cognitive demands of the task. The hippocampal formation is critical for computing place representations but is believed to be dispensable for procedural memories (O'Keefe & Nadel 1978). Previous reports suggest that declarative memory is seriously impaired by pilocarpine-induced SE (Hort et al., 1999). Some impairment of procedural components in addition to cognitive mechanisms is very probable. Persinger et al., (1994) described the deterioration of the declarative (radial-maze acquisition) and non-declarative (conditioned taste aversion) form of memory after seizures induced by a systemic injection of lithium pilocarpine. Levetiracetam treatment was reported to result in less histological damage in the hippocampus but had no effect on visual spatial function or place cell physiology in either control or SE rats (Zhou et al., 2007).

Thus from our results we conclude that central muscarinic, muscarinic M1 and glutamate receptor subtypes functional balance play an important role in the pathophysiology of pilocarpine induced Temporal lobe epilepsy in rats. 

Bacopa monnieri and Bacoside A extracts have a regulatory effect on epilepsy through muscarinic and glutamate receptors. This has immense clinical significance in the therapeutic management of Epilepsy.