**Discussion**

Epilepsy, a common neurological disorder characterized by recurrent spontaneous seizures, is considered to be a major health problem that affects approximately one to two percent of the population worldwide. The epileptogenesis is a dynamic process with major modifications taking place at multiple levels, which include synaptic plasticity, aberrant reorganization of the neuronal circuitry, alterations in interneuron number and function and changes in dentate neurogenesis. The latency period found between the initial precipitating insult and the development of chronic epilepsy offers a useful window for application of promising neuroprotective strategies. On basis of behavioural, electrophysiological and histopathological findings obtained with different protocols and by comparing mortality rates, onset of spontaneous recurrent seizures (SRSs), neuronal damage, and network reorganization, pilocarpine model is considered as a valuable tool to investigate the mechanisms involved in TLE.

We observed a decrease in the body weight of epileptic rats compared to control and *Bacopa monnieri* treated control rats. Also, the feed and water intake of the epileptic rats were comparatively reduced. *Bacopa monnieri* treatment and carbamazepine treatment reversed these changes to near control level. There was no significant change in the blood glucose in the control and experimental groups of rats. PET imaging studies of glucose metabolism and cerebral blood flow in patients with TLE and depression have so far mainly focused on frontal and temporal lobes, with hippocampus and amygdala being the regions of main interest (Kondziella *et al.*, 2007). The same stress-responsive CNS mediators that produce emotional, cognitive and behavioral manifestations also lead to inhibition of endocrine programs for
growth and reproduction to preserve energy, a catabolic state to provide glucose to the brain and increased heart rate and blood pressure (Gold, 2005)

The serotonergic innervation of the cerebral cortex in the rat has been studied by immunohistochemistry employing an antibody directed against the neurotransmitter, serotonin. All regions of the cerebral cortex appear to be innervated by serotonergic axons which have a distinctive morphology: they are fine (0.1–0.5 µm), varicose and extremely convoluted. Serotonergic axons of passage are thicker and comparatively straight.

The serotonergic afferents to the cortex appear to have at least two different modes of distribution, a relatively uniform pattern in the anterior cingulate and the lateral neocortex and a restricted, laminar pattern in the posterior cingulate and the hippocampus. The density and extent of the serotonin innervation is such that the raphe neurons may contact every cell in the cortex. The widespread arborization of serotonin axons contrasts with the spatially restricted termination of thalamic afferents. The distribution of serotonin-containing fibers also differs substantially from the terminal patterns of noradrenergic and dopaminergic fibers. The differences in axonal morphology and distribution amongst the monoamine afferents reflect differences in their contributions to cortical circuitry. The present findings indicate that the serotonin-containing neurons may exert a profound and global, but not necessarily uniform, influence upon cortical function (Lidov, 1980).

**Serotonin content**

We observed a decrease in the 5-HT content in the cerebral cortex hippocampus, cerebellum and brainstem of epileptic rats when compared to control and Bacopa monnieri treated control rats. The monoamine content in brain structures has been related to neuronal excitability and several approaches have been used to
study this phenomenon during seizure vulnerability. An increase in the serotonin content was found in cerebral cortex hippocampus, cerebellum and brainstem of *Bacopa monnieri* treated epileptic rats and carbamazepine treated epileptic rats. 5-HT plays an important regulatory role in epileptic mechanism; as demonstrated from studies in both animal models of epilepsy and humans. Reciprocal interactions between the motor system and the serotonergic modulatory system are well documented (Jacobs & Fornal, 1997). In the genetically epilepsy-prone rat model of generalized epilepsy, a decrease is found in brain concentration of serotonin (Dailey *et al*., 1989). There has been increasing evidence that serotonergic neurotransmission modulates a wide variety of experimentally induced seizures. Generally, agents that elevate extracellular serotonin levels, such as 5-hydroxytryptophan and 5-HT reuptake blockers, inhibit both focal and generalized seizures. Conversely, depletion of brain 5-HT lowers the threshold to audiogenically, chemically and electrically evoked convulsions (Bagdy *et al*., 2006). Moreover 5-HT is a key modulatory neurotransmitter and has been implicated in the pathophysiology and treatment of anxiety and mood disorders (Neumeister *et al*., 2002). Both *Bacopa monnieri* and carbamazepine treatment to epileptic rats increased the 5-HT content in brain regions of epileptic rats. *Bacopa monnieri* plant extract is a nerve tonic used extensively in the traditional Indian medicinal system Ayurveda. *Bacopa monnieri* has been used for centuries as a memory-enhancing, antioxidative, adaptogenic (Jyoti & Sharma, 2004), antiinflammatory, analgesic, antipyretic, sedative, and antiepileptic agent. *Bacopa monnieri* is currently recognized as being possibly effective in the treatment of mental illness and epilepsy (Russo *et al*., 2003).
Cerebral Cortex

5-HT\textsubscript{2C} receptors are involved in a diversity of physiological functions such as the control of nociception, motor behaviour, endocrine secretion, thermoregulation, modulation of appetite and the control of exchanges between the central nervous system and the cerebrospinal fluid (Tecott \textit{et al.}, 1995; Fone \textit{et al.}, 1998). The cerebellar cortex, like all other motor structures, receives serotonergic innervations in the form of a plexus of fine varicose fibers. (Dieudonné & Dumoulin, 2000).

Our findings report an increase in 5-HT\textsubscript{2C} receptor function in the cerebral cortex with no significant change in \(K_d\) which is supported by the gene expression studies. Increased 5-HT\textsubscript{2C} receptor function in cortical regions is implicated in mood disorders and anxiodepressive states (Millan, 2005). It has been reported that increased 5-HT\textsubscript{2C} receptor contributes to the enhanced response to anxiety (Fone \textit{et al.}, 1996) in the epileptic rats. 5-HT\textsubscript{2C} receptor agonist 1-(m-chlorophenyl) piperazine has been reported to produce hypolocomtion, hypophagia, and anxiogenesis (Samad \textit{et al.}, 2008) in rats. The changes in the receptors have been confirmed using immunofluorescent antibodies specific to 5-HT\textsubscript{2C} receptors. Behavioural and neurochemical evidence for anxiogenic actions of 5-HT\textsubscript{2C} agonists have been well documented in rodents (Hackler \textit{et al.}, 2007).

The serotonin 5-HT\textsubscript{2C} receptor subtype signals activate phospholipase C (PLC), leading to the intracellular accumulation of inositol trisphosphate and subsequent \(Ca^{2+}\) release. Increased \(Ca^{2+}\) release triggers the oxidative damage and excitotoxicity (Bishnoi \textit{et al.}, 2008). Thus up-regulated 5-HT\textsubscript{2C} receptor function in epileptic cortex leads to excessive \(Ca^{2+}\) overload in cells leading to apoptosis. Earlier findings from our lab also implicate overactivation of 5-HT\textsubscript{2C} receptors in cell proliferation by acting as a co-mitogen (Sudha & Paulose, 1998). Hence, the up-regulated 5-HT\textsubscript{2C} receptors could play a significant role in neuronal hypertrophy.
(Julius et al., 1989) which has been previously reported in cortical regions of patients with temporal lobe epilepsy (Bothwell et al., 2001). At present it is unknown why cortical tissues become epileptogenic. It has been previously demonstrated that in the lateral temporal lobe of TLE patients which are supposed to be normal (Babb et al., 1984) there is fine disorganization in the synaptic circuits that consists of increase and decrease in excitatory and inhibitory synapses respectively (Marco et al., 1997). Neuronal hypertrophy leads to formation of tumours which are reported to be potential causes for onset of secondary seizures (Loscher, 2002). Focal cortical dysplasia is associated with vulnerability to epilepsy, and suggested to augment hippocampal epileptogenicity (Takase et al., 2008) However, its epileptogenicity remains unclear. Therefore, we created a novel rat Serotonin can inhibit events triggered by glutamate release by acting at postsynaptic receptors of the 5-HT$_{2C}$ subtype. Experiments in human cerebral cortex support the view that serotonergic activation through 5-HT$_{2C}$ receptors interacts with glutamate transmission through NMDA receptor (Maura et al., 2000).

Our investigation revealed a down regulation in the NMDA receptor binding in the cerebral cortex of the epileptic rats compared to control and *Bacopa monnieri* treated without a change in its affinity. Also, there was increase in the gene expression of 5-HT$_{2C}$ and mGlu5 gene expression whereas a decrease in the expression NMDA2b of in the cerebral cortex of epileptic. *Bacopa monnieri* and carbamazepine treatment to epileptic rats showed a reversal of the receptor expressions to near control. These have been confirmed using immunofluorescent antibodies specific to 5-HT$_{2C}$, NMDA2b and mGlu5 receptors in our study. Conflicting reports exist regarding the status of NMDA receptors in the epileptic cortex. Guzman et al., (2008) reported neuronal network hyperexcitability due to NMDA receptors in the deep layers of entorhinal cortex of pilocarpine treated rats. But we report a decrease in the NMDA
receptor activity in the epileptic cortex. A possible explanation to the loss of NMDA binding sites is suggested to be due to a compensatory down-regulation of the receptors because of an excessive release of glutamate (Otoya et al., 1997) during epilepsy. Hypofunction of NMDA receptor has been indicated to produce schizophrenic symptoms, including deficits in working memory (Olney and Farber, 1995). NMDA-receptor antagonism could adversely affect storage and processing of information (Javitt et al., 1996) in the cerebral cortex of epileptic rats. Our recent studies involving Water Maze Test (Reas et al., 2008) confirms the deficits in memory and cognitive impairments observed in the epileptic rats.

Immunohistochemical studies have previously identified positive staining for presynaptic mGlu5 receptors in the rat cerebral cortex (Romano et al., 1995). The mGluR5 is reported to mediate a G-protein-dependent release of intracellular calcium stores (Valenti et al., 2002). Moreover, NMDA receptor function is inhibited by a rise in intracellular calcium (Rosenmund et al., 1995). Yu et al., (1997) pointed out that mGlu5 mediated direct inhibition via G-proteins also leads to NMDA receptor inhibition. We observed an increased expression of the mGluR5 receptor gene expression in the cerebral cortex of epileptic rats compared to control and Bacopa monneiri treated control rats. Hence, it is likely that the G-protein-dependent release of intracellular Ca^{2+} through mGlu5 activation depresses NMDA responses as seen in our present study.

Our experimental findings also demonstrate an increase in intracellular IP3 content in the epileptic cerebral cortex. Inositol phosphates are known to regulate membrane trafficking, glucose metabolism, cytoskeletal organisation and intracellular Ca^{2+} homeostasis—particularly the release of stored Ca^{2+} via IP3 receptors. Intracellular Ca^{2+} regulation by both inositol 1,4,5-triphosphate receptors (IP3R) and ryanodine receptors (RyR) has been implicated in epileptogenesis and the
maintenance of epilepsy (Nagarkatti et al., 2008). This leads to excess Ca\(^{2+}\) release from IP3-sensitive stores in response both 5-HT\(_{2C}\) receptor hyperfunction and mGluR5 stimulation. Excessive Ca\(^{2+}\) overload in cells have been reported to cause apoptosis. Boehning et al., (2003) demonstrated a small amount of cytochrome C released from mitochondria binds to and promote Ca\(^{2+}\) conductance through IP3 in the endoplasmic reticulum membrane. The released Ca\(^{2+}\) further triggers mass exodus of cytochrome C from all mitochondria in the cell and thus activating the caspase and nuclease enzyme that finalize the apoptotic process. Glutamate uptake into neurons and glial cells is important for the termination of glutamatergic transmission. They are essential for the maintenance of low extracellular levels of glutamate (Lo´pez-Bayghen et al., 2003). We observed an increase in the expression of GLAST receptor gene expression of epileptic rats. The increase in the glutamate transporter is suggested to be a compensatory mechanism to facilitate the fast removal of the glutamate and thus prevents its prolonged action at the synapse during the epileptic condition.

Treatment with *Bacopa monnieri* to epileptic rats caused a reversal in the B\(_{\text{max}}\) of 5-HT\(_{2C}\) receptors and NMDA receptors to near control level. It is also evident that *Bacopa monnieri* has neuroprotective action mediated through the 5-HT\(_{2C}\), NMDA2b, mGlu5 and GLAST receptor at the transcription level. Singh et al., 2006 have demonstrated the anti-oxidant properties of *Bacopa monnieri* in rodents. *Bacopa monnieri* treatment has been demonstrated to modulate serotonin level (Singh et al., 1997) which renders protection against seizures (Reas et al., 2008) during epilepsy. However, its traditional memory-enhancing claim could be established experimentally by our findings on 5-HT\(_{2C}\) and NMDA receptors. *Bacopa monniera* treatment to epileptic rats significantly increases NMDA receptors which play an important role in cognition and memory. Also, *Bacopa monnieri* treatment has been reported to possess
anxiolytic and antidepressant property which is evident from modulation of 5-HT$_{2C}$ receptors. Up regulation of the 5-HT$_{2C}$ receptor renders the epileptic rat anxiogenic which is supported by elevated plus maze test. Bacopa monnieri treatment to epileptic rats caused the upregulated 5-HT$_{2C}$ receptors and IP3 content to near control level and thus provides support at the molecular level to the antidepressive and anxiolytic property as provided by reports of Bhattacharya & Ghosal, (1998) and Sairam et al., (2002).

To determine anxiety in experimental groups, rats were subjected to the elevated plus maze. Behaviour in rodents is determined by the conflict between the drive to explore the unknown area/object and the motivation to avoid potential danger. Elevated open alleys arouse greater avoidance responses than elevated closed alleys. Voluntary passage onto the open arms of an elevated, plus shaped maze is associated with neurobiological changes indicative of a decreased anxiety (Olivier et al., 2008). In elevated plus maze test, the epileptic rats exhibit significant alterations in its behavioural response due to epileptic insult to the cortical neurons. The epileptic rats remained for longer period in closed arms of elevated plus-maze which is characteristic to anxio-depressive traits. It has been demonstrated that the preference shown for the closed arms reflects an aversion toward the open arms, caused by fear or anxiety induced by the open space in the elevated plus maze test (de Souza et al., 2007). The head dipping attempt, stretched attend posture and grooming attempts were also greatly reduced supporting the anxiogenic condition as a result of epileptic stress. But an increased attempt made towards open arm entry by epileptic rats indicates an abnormal behavioural profile. 5-HT$_{2C}$ selective antagonists show robust anxiolytic-like effects in conflict-based paradigms, so the activity of S32006 in the Vogel’s Confidence Test can confidently be ascribed to 5-HT$_{2C}$ receptor blockade (Martin et al. 2002; Millan 2003). The Bacopa monnieri treatment during epilepsy
reversed the behavioural abnormalities to control. *Bacopa monnieri* treatment to epileptic rats had anti-anxiety effects, as indicated by increase of the time spent in the open arm and increase in head dipping attempt, stretched attend posture and grooming attempts compared to epileptic rats.

Thus we show evidence for dysfunction of the epileptic cerebral cortex that is a reflection for manifestation of abnormal behavioural patterns and possibility for cellular proliferation. The receptor analysis and gene expression studies along with the behavioural data implicate a role for 5-HT$_{2C}$ receptors in the manifestation of anxiety and NMDA receptors for the cognitive and memory deficits associated with epileptic rats. It is evident that neuroprotective role of *Bacopa monnieri* in epilepsy involves the interaction of 5-HT$_{2C}$ and NMDA receptors, with modulation of mGlu5 and GLAST receptor gene expression at the mRNA level and IP3 and cGMP activation at the second messenger level. Thus our results suggest that anti-epileptic effect of *Bacopa monnieri* extract is mediated through 5-HT$_{2C}$, NMDA receptors, mGlu5, GLAST, IP3 and cGMP functional regulation which has clinical significance in the treatment of epilepsy.

**Hippocampus**

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. A wide range of neuropsychological deficits follow the SE, which typically include learning and memory dysfunction and other cognitive deficits (Holmes *et al.*, 2004; Holmes, 2006). The hippocampal formation contains a rich glutamatergic and GABA-ergic input, GABA-ergic interneurones containing peptide co-transmitters and the glutamatergic perforant pathway interconnects with entorhinal cortex, subiculum,
CA1, CA3 fields and dentate gyrus (Ottersen & Storm-Mathisen, 1984). 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). Recent findings suggest that the SE induced by pilocarpine is triggered by changes in the blood-brain barrier permeability.

We observed a decrease in the serotonin content in the hippocampus of epileptic rats when compared to control and *Bacopa monnieri* treated control rats. Studies of the serotonergic modulation of hippocampal function have been complicated by the marked heterogeneity of 5-HT receptor subtypes, with at least 14 distinct subtypes expressed in the central nervous system. Psychological stress activates the serotonergic neurons in the hippocampus and the amygdala through the cortical association areas and through ascending catecholaminergic neurons from the brain stem (Feldman & Weidenfeld 1998; Koob & Heinrichs 1999). Serotonergic neurotransmission exerts a considerable influence on hippocampal function. This structure is influenced powerfully by serotonergic projections from midbrain raphe nuclei (Tecott *et al.*, 1998) which modulate hippocampal electrical activity, hippocampal-dependent behaviours, and long-term potentiation (LTP), a form of hippocampal plasticity that has been implicated in memory formation (Vanderwolf & Baker, 1986). There is good evidence that noradrenaline and 5-HT interact to influence neuroplasticity in the brain (Delgado 2004). Many modern antidepressants including mirtazapine, milnacipran, venlafaxine, and duloxetine have been developed based on their interaction with both 5-HT and noradrenaline (Tran *et al.* 2003).

In this study, we focused on the 5-HT$_{2C}$ receptor, which is abundantly expressed throughout the hippocampal formation and the subiculum. An involvement of this receptor subtype is suggested in the regulation of neuronal plasticity (Tecott,
We observed a significant increase in the $B_{\text{max}}$ and $K_d$ of 5-HT$_{2C}$ receptors in the hippocampus of epileptic rats compared to control and *Bacopa monnieri*. 5-HT$_{2C}$ receptors gene expression patterns were similar to the receptor binding studies. Treatment with *Bacopa monnieri* and carbamazepine reversed the receptor alterations in $B_{\text{max}}$ and $K_d$ to near control levels. Anxiolytic-like actions of 5-HT$_{2C}$ receptor antagonists, transduced in the amygdala and hippocampus (Menard & Treit 1999; Campbell & Merchant 2003; Millan 2003; Alves *et al.* 2004), may be expressed against certain forms of clinical anxiety. 5-HT$_{2C}$ receptor antagonism have been reported to improve insomnia and sexual dysfunction comorbid to depression (Dekeyne *et al.*, 2008). *Bacopa monnieri*’s multiple active constituents have multifunctional properties, making its pharmacology complex. *Bacopa monnieri* treatment has been demonstrated to increase serotonin levels in hippocampus (Singh & Dhawan, 1997) preventing the compensatory increase in the number of 5-HT$_{2C}$ receptor in the epileptic hippocampus and thus provide protection to hippocampus during seizures.

Based on extensive supportive experimental data, the release of high levels of glutamate by neurons at a seizure focus is thought to be the underlying mechanism for the initiation and maintenance of seizures. Imbalances between excitatory and inhibitory synaptic transmission in key brain areas such as hippocampus are implicated in the pathophysiology of TLE, in which fast synaptic excitatory neurotransmission is mediated via activation of ionotropic glutamate receptors like NMDA receptors.

We report a down regulation in the NMDA receptor binding in the hippocampus of the epileptic rats compared to control with an increase in its affinity. The gene expression of NMDA2b sub unit was also found to be decreased in the epileptic hippocampus. *Bacopa monnieri* treatment and carbamazepine treatment...
caused an increase in the NMDA receptors and NMDA2b gene expression to near control level. There was no significant change in $K_d$ of epileptic rats treated with carbamazepine compared to epileptic rats. The mGlu5 and GLAST gene expression was also found to be increased in the epileptic hippocampus. *Bacopa monnieri* treatment and carbamazepine treatment reversed these changes in the gene expression to near control level. Our experimental results also show an increase in the IP3, cGMP and cAMP content in the hippocampus of epileptic rats compared to control and *Bacopa monnieri* treated control rats. *Bacopa monnieri* and carbamazepine treatment to epileptic rats showed a reversal in the alterations to control level.

A possible explanation to the loss of NMDA binding sites in the epileptic hippocampus is suggested to be due to a compensatory down-regulation of the receptors because of an excessive release of glutamate in the epileptic hippocampus (Otoya *et al*., 1997; Reas *et al*., 2008) or loss of cells in the sclerotic hippocampus (Steffens *et al*., 2005) during epilepsy. Hippocampal area CA3 is critically involved in the formation of nonoverlapping neuronal subpopulations to store memory representations as distinct events. Efficient pattern separation relies on the strong and sparse excitatory input from the mossy fibers to pyramidal cells and feed forward inhibitory interneurons (Galván *et al*., 2008). Several studies have shown an increase in the density of hippocampal and cortical NMDA receptors in some animal models of epilepsy (Yeh *et al*., 1989; Ekonomou & Angelatou, 1999). Other experiments in epileptic human and rat neocortex, showed that components of the NMDA-receptor complex were downregulated (Wyneken *et al*., 2003). Our experimental results support the latter. Earlier studies described that intrahippocampal administration of pilocarpine resulted in a decrease of the extracellular glutamate and shows simultaneous slowing of the rhythmic activity recorded on the electroencephalogram (EEG), showing theta and delta waves. Intrahippocampal pilocarpine perfusion
followed by a significant and sustained enhancement of the extracellular glutamate concentrations was reported. (Smolders et al., 2004). Reports also suggest that 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). Enhanced glutamate in the EC is suggested to be associated with the development of epileptic condition (Thompson et al., 2007). Recent research suggests that the consolidation of fear and extinction memories depends on NMDA receptors. Using a fear conditioning and extinction paradigm in healthy normal volunteers, it was shown that post-learning administration of the NMDA partial agonist D-cycloserine facilitates fear memory consolidation (Kalisch et al., 2009).

Our experimental results showed that Bacopa monnieri treatment caused an increase in total NMDA receptors and K_d as well as gene expression of NMDA2b to near control level. We had previously reported a down regulated NMDAR1 gene expression during epilepsy which was reversed to control level after Bacopa monnieri treatment (Reas et al., 2008). NMDA receptor in the hippocampus is reported to modulate some forms of memory formation, with the NR2b subunit being particularly relevant to this process (White & Yougentoba, 2004). It is evident that Bacopa monnieri has a definite role in decreasing glutamate mediated excitotoxicity in the hippocampus of epileptic rats. Treatment with Bacopa monnieri extract reduced the increase in glutamate dehydrogenase activity to near-control levels and was effective in memory enhancement in epileptic rats which was observed through the Water Maze Test (Reas et al., 2008; Paulose et al., 2008) This is consistent with earlier reports (Singh et al., 1988) that Bacopa monnieri treatment can induce membrane dephosphorylation and a concomitant increase in mRNA turnover and protein synthesis. It can also enhance protein kinase activity in the hippocampus, which is critically involved in learning and memory.
The mGluR5 is reported to mediate a G-protein-dependent release of intracellular calcium stores (Valenti et al., 2002). Moreover, NMDA receptor function is inhibited by a rise in intracellular calcium (Rosenmund et al., 1995). Yu et al., (1997) pointed out that mGlu5 mediated direct inhibition via G-proteins also leads to NMDA receptor inhibition. mGluR5 receptors may modulate NMDA receptor function because both receptors have been linked as signaling partners (Kotecha et al. 2003; Movsesyan et al. 2001). We observed an increased expression of the mGluR5 receptor in the cerebral cortex of epileptic rats compared to control and Bacopa monnieri treated control rats. Hence, it is likely that the G-protein-dependent release of intracellular Ca\(^{2+}\) through mGlu5 activation depresses NMDA responses as seen in our present study. MGluR5 antagonists are promising candidates for anti-depressants (Stachowicz et al. 2006).

Our experiment with the second messengers revealed an increased level of IP3, cGMP and cAMP in the hippocampus of epileptic rats compared to control and Bacopa monnieri treated control rats. Both Bacopa monnieri and carbamazepine treatment to epileptic rats reversed these changes to control values. Mossy fiber sprouting, gliosis, and synaptic reorganization in the hippocampus, amygdala, parahippocampal cortices and thalamus during temporal lobe seizures participates in the constitution of hyperexcitable circuits underlying spontaneous seizures and emotional disturbances (Roch et al. 2002). Antiepileptic agents reduce aggression in rats (Keele 2001) and humans (Stanford et al. 2005).

**Cerebellum**

In our earlier studies, we reported the therapeutic action of Bacopa monnieri on glutamate receptors especially in the cerebellum and hippocampus of pilocarpine induced epilepsy in rats (Reas et al., 2008, Paulose et al., 2008). In the present study,
we have demonstrated the therapeutic role of *Bacopa monnieri* in epileptic motor dysfunction through its effect on 5-HT$_{2C}$ receptor gene expression and binding in cerebellum. Electrophysiologists have reported that serotonergic agonists affect directly the firing of cerebellar neurons (Cumming-Hood *et al*., 1993) and are able to modulate the effect of excitatory amino acids. Experimental evidence indicate the involvement of the cerebellum in variety of human mental activities including language, attention, cognitive affective syndromes (Gowen and Miall, 2005) fear and anxiety caused by threats of pain (Ploghaus *et al*., 1999) and motor relearning (Imazumi *et al*., 2004). There is also accumulating evidence to suggest that the cerebellum plays a role in more cognitive, social and emotional functions (Allen *et al*., 1997; Schmahmann and Sherman, 1998). Some of the most frequent signs of cerebellar hypoplasia include poor fine motor skills, hypotonia and autistic features (Wassmer *et al*., 2003). The cerebellar vermis integrates and processes the inputs from the vestibular, visual and proprioceptive systems to coordinate muscle timing as a result of which the centre of gravity stays within the limits of stable upright standing (Diener *et al*., 1989). Damage to the cerebellum, in particular the vermis (Balogh *et al*., 1998) results in more postural sway than in control subjects (Ho *et al*., 2004, Marvel *et al*., 2004). Decreased postural stability would correspond with abnormalities of the vermis observed in autistic subjects (Gowen & Miall, 2005).

5-HT$_{2C}$ receptors exist in the rat cerebellum and they participate in the processing and integration of sensory information, regulation of the monoaminergic system modulation of neuroendocrine regulation, anxiety and feeding behaviour (Tecott *et al*., 1995). Our investigation revealed a decrease in the 5-HT content and 5-HT$_{2C}$ receptor binding in the cerebellum of the epileptic rats compared to control with an increased affinity. Decreased serotonin in the brain has previously been implicated in development and spread of seizures (Dailey *et al*., 1989). This decreased
5-HT_2C receptor binding in the cerebellum is suggested to contribute towards a lowered threshold and a rapid progression of seizure activity in the epileptic rats. Previously, mesulergine (2 or 4 mg/kg), administered prior to electroshock testing, have shown to recapitulate epileptic syndrome associated with sporadic spontaneous seizures in the 5-HT_2C mutant phenotype in wild-type mice (Applegate & Tecott, 1998).

Treatment with *Bacopa monnieri* to epileptic rats caused a reversal in the B_max of 5-HT_2C receptors to near control level. Rohini et al., (2004) have demonstrated the anti-oxidant properties of *Bacopa monnieri* in rodents. This is in consistence with the earlier reports (Reas et al., 2008) which states that *Bacopa monnieri* treatment induces membrane dephosphorylation and concomitant increase in mRNA turnover and protein synthesis. Moreover, *Bacopa monnieri* treatment has been demonstrated to increase serotonin level (Singh & Dhawan, 1997) which renders protection against seizures (Bagdy, 2006) during epilepsy.

We also observed an increase in the NMDA receptors binding, NMDA2b and mGlu5 gene expression in the cerebellum of epileptic rats compared to control rats and *Bacopa monnieri* treated control rats. In the cerebellum of pilocarpine induced epileptic rats, extracellular glutamate was reported to be elevated significantly during the pilocarpine-induced convulsions. 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 and thus glutamate concentrations in ipsilateral cerebellum.

Pharmacological tools now allow for the examination of the role of metabotropic glutamate receptors (mGluRs) in the development of sensitization (Spooren et al. 2000). mGluRs regulate synaptic transmission by modulating calcium
and potassium channels and the activity of ionotropic glutamate receptors. mGluR5 receptors modulate NMDA receptor function because both receptors have been linked as signaling partners (O’Leary et al. 2000; Movsesyan et al. 2001; Kotecha et al. 2003). The activation of mGluR5 receptors leads to the potentiation of NMDA currents (Bleakman et al. 1992; Cerne and Randic 1992), possibly through the activation of protein kinase C and the subsequent increase in intracellular Ca$^{2+}$, thereby acting as an indirect agonist of NMDA receptors (Benquet et al. 2002; Fujii et al. 2004). NMDA receptor activation in the cerebellum leads to an increase in the Ca$^{2+}$ also via IP3 receptors. Glutamate uptake into neurons and glia cells is important for the termination of glutamatergic transmission. Several glutamate transporters have been characterized, the Na$^+$-dependent glutamate/aspartate transporter (GLAST) being the major uptake system within the cerebellum. We observed a down regulation in the GLAST expression in the cerebellum of epileptic rats. Bacopa monnieri treatment and carbamazepine treatment reversed the changes to near control. The decreased expression of the GLAST gene expression causes a low uptake of glutamate in the cerebellum. Purkinje cells make synaptic contacts with the parallel fibers, which are the axons of the granule cells and that these synapses are glutamatergic, it is possible that glutamate synaptic levels regulate the Bergmann glia glutamate transporter, GLAST. In fact, it has been suggested that glutamate regulates GLAST translocation process in these cells in a receptor-independent manner (Gonzalez & Ortega, 2000). Thus, Bacopa monnieri treatment renders protection by increasing the glutamate re-uptake into cerebellar cells and thus reducing the extracellular glutamate which cause excitotoxicity.

Our experimental results also show an increase in the IP3, cGMP and cAMP content in the cerebellum of epileptic rats compared to control and Bacopa monnieri
treated control rats. *Bacopa monnieri* and carbamazepine treatment to epileptic rats reversed the increase in the IP3, cGMP and cAMP levels to near control.

The increased cGMP content in the epileptic cerebellum is due to the increased NMDA receptors. Suvarna and O’Donnel, (2002) reported the NMDA mediated increase in the cGMP in the neuronal culture studies. Baltrons *et al*., (1997) and Oh *et al*., (1997) reported an NMDA induce cGMP formation in the cultured cerebellar granule cells. Increased IP3 activation leads to Ca$^{2+}$ influx which in turn activates neuronal nitric oxide synthase (nNOS) to produce NO (Garthwaite, 2005). NO activates soluble guanylyl cyclase (sGC) to generate increased levels of cGMP which in turn activates protein kinase G (PKG) (Garthwaite, 2005) in the cerebellum during epilepsy. The role of NO during epileptogenesis is controversial as it has been shown to have both anticonvulsant (Sardo & Ferraro, 2007; Royes *et al*., 2007)) and proconvulsant (De Sarro *et al*., 1993; Tutka *et al*., 1996). This rise in cAMP could be mainly due to the influence of increased mGlu5 (Winder & Conn, 1992) receptors as is seen our studies. Thus the neuroprotective effect of *Bacopa monnieri* involves the modulation at the second messenger level also.

We observed a down regulation of the 5-HT$_{2C}$ receptor expression using immunofluorescent antibodies specific to 5-HT$_{2C}$. *Bacopa monnieri* and carbamazepine treatment to epileptic rats reversed the alteration to near control, thus confirming the gene expression studies in the cerebellum of epileptic rats. Also, there was increase in the gene expression of NMDA2b and mGlu5 gene expression in the cerebellum of epileptic and these have been confirmed using immunofluorescent antibodies specific to NMDA2b and mGlu5 receptors in our study. *Bacopa monnieri* and carbamazepine treatment to epileptic rats reversed the alteration to near control, thus confirming the gene expression studies in the cerebellum of epileptic rats.
Rotarod test has been previously used to examine motor incoordination (Cendelin et al., 2008). The rotarod experiment demonstrated the impairment in the motor function and coordination in the epileptic rats. Epileptic rats showed lower fall off time from the rotating rod when compared to control suggesting impairment in their ability to integrate sensory input with appropriate motor commands to balance their posture and at the same time adjust their limb movements on the metallic rod and is indicative of cerebellar dysfunction. Many other brain regions have been associated with timing tasks including the dorsal lateral premotor cortex, inferior parietal lobe, supplementary motor area, superior temporal gyrus, caudal putamen, ventrolateral thalamus and inferior frontal gyrus (Rao et al., 1997; Jancke et al., 2000; Lewis and Miall, 2003). Abnormalities of some of these areas, such as the inferior frontal gyrus and superior temporal gyrus (Abell et al., 1999; Castelli et al., 2002) have been reported in autistic subjects rendering it difficult to isolate the cerebellum in this task. However, increased timing variance has been observed in patients with cerebellar disorders (Ivry et al., 1988). Loss of coordination of motor movement, inability to judge distance and timing, incapacity to perform rapid alternating movements and hypotonia has been reported during cerebellar damage (Gowen and Miall, 2005). Poor limb-eye coordination in patients with cerebellar dysfunction has been earlier report (Van Donkelaar and Lee, 1994).

Thus perturbations of the 5-HT2C receptor system directly modulate seizure susceptibility. This study demonstrates the involvement of 5-HT2C receptor which has modulating effect on the seizure susceptibility and associated motor defects. The administration of crude extract of Bacopa monnieri to epileptic rats increased the fall off time from the rod when compared to control rats. Bacopa monnieri not only possesses memory enhancing properties but also alleviate their stress levels which assist in lowering their time for spatial recognition (Shankar & Singh, 2000) and helps
to maintain their posture during movement on the rod. It is also reported to facilitate the acquisition, consolidation, retention and recall of learned asks (Diener et al., 1989) and improves the speed at which visual information is processed.

To summarize, our findings suggest dysfunction of the epileptic cerebellum that is a reflection of cerebellar serotonergic and glutamatergic abnormality. The receptor analysis and gene expression studies along with the behavioural data implicate a role for serotonin, 5-HT$_{2C}$, NMDA and mGlu5 receptors in the modulation of neuronal network excitability and seizure propagation via changes in IP3, cGMP and cAMP. These neurofunctional deficits are one of the key contributors to motor deficits and stress associated with epilepsy. Our results suggest that *Bacopa monnieri* extract treatment reverses the 5-HT$_{2C}$ receptor mediated motor dysfunction in epilepsy and cerebellar excitotoxicity due to glutamatergic modulation mediated through changes in IP3, cGMP and cAMP. This will have clinical significance in the management of epilepsy.

**Brain stem**

We found a decrease in the serotonin content in the brainstem of epileptic rats when compared to control and *Bacopa monnieri* treated control rats. The cell bodies of the 5-HT containing neurons are confined primarily to the raphe nucleus of the brainstem (Duan *et al*., 1989; Jocobs and Azimitia, 1992) where the level of extracellular 5-HT are determined (Sharp *et al*., 1990). 5-HT plays an important regulatory role in epileptic mechanisms; as demonstrated from studies in both animal models of epilepsy and humans. Selective serotonin reuptake inhibitors (SSRIs) such as fluoxetine, which has antiseizure effects in several models (Hernandez *et al*., 2002). In the genetically epilepsy-prone rat model of generalized epilepsy, a decrease is found in brain concentration of serotonin (Dailey *et al*., 1989). Serotonin is reported to
play a role in the mechanism of action of some antiepileptic drugs (AEDs). Studies in genetically epilepsy prone rats GEPRs suggest that carbamazepine (CBZ) and valproate (VPA) release 5-HT. (Yan, 2002, Yan et al., 1992, Dailey et al., 1997). Decrease in the serotonin content is thus involved the seizure generation in epileptic rats. After Bacopa monnieri and carbamazepine treatment, an increase in the serotonin content towards control level was found. Thus, Bacopa monnieri treatment to epileptic rats causes an increase in the serotonin content in the brainstem.

Our experimental results showed a decrease in the $B_{\text{max}}$ and $K_d$ and gene expression pattern of 5-HT$_{2c}$ receptors in the brainstem of epileptic rats compared to control and Bacopa monnieri treated control rats. Bacopa monnieri and carbamazepine treatment reserved these receptor changes to control level.

There was an increase in the $B_{\text{max}}$ of NMDA receptors with a decreased $K_d$ when compared to control and Bacopa monnieri treated control rats. The NMDA2b mGlu5 and GLAST gene expression was found to be up-regulated in the brainstem of epileptic rats when compared to control and Bacopa monnieri treated control rats. Our results showed that there is an increased glutamatergic activity in the brainstem during chronic epileptic state. Increased NMDA receptors in the brain regions are involved in repetitive tonic seizures during epilepsy. In the epileptic rats group treated with Bacopa monnieri and carbamazepine the $B_{\text{max}}$ reversed to the control. NMDA2b and mGlu 5 and GLAST gene expression gene expression also reversed to the control values. These results suggest the therapeutic effect of Bacopa monnieri in epilepsy through NMDA and mGlu5 receptors. It is widely accepted that excitatory amino-acid neurotransmitters 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) in brainstem.

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). 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.

We observed an increase in the IP3 and cGMP levels while a decrease in the cAMP level in the brainstem of epileptic rats compared to control and *Bacopa monnieri* treated control rats. NMDA receptor activation has been implicated in IP3 mediated and cGMP signalling pathway by Suvarna and O'Donnel, (2002). Winder and Conn (1993) reported the involvement of mGlu5 receptors on cAMP activation. But in our study, the cAMP levels are low inspite of increased mGlu5 gene expression indicating the involvement of other receptors on the functional modulation of cAMP in the epileptic brainstem.

Feelings of despair, depressive mood, aggressive behaviour, anxiety, memory impairment and overt psychosis are among the common psychiatric features in patients with TLE (Kanner 2006; Blumer et al. 2004). Monoaminergic neurotransmitters such as 5-HT and noradrenaline interact with glutamatergic metabolite, which leads to disturbances of neuronal circuits. Atrophy of hippocampus and memory impairment develops, as do transient hypertrophy of amygdala and impaired fear processing. As the hippocampus has critical implications for both seizure activity and mood disorders, this area provides a link between epilepsy and depression (Hajszan & MacLusky 2006). The structural and functional alterations from one disease evoke the other and *vice versa*. In TLE, for instance, hyperexcitability and neuronal cell loss in the limbic system evokes mood disturbances, whereas hippocampal atrophy and neurotransmitter disturbances in depression decreases the seizure threshold and ultimately lead to TLE. Hence, the social interaction test and forced swim test which are commonly used paradigms for models of anxiodepressive states, was conducted.
In the social interaction test, epileptic rats spent less time in active interactions in the novel environment. Attempts at allogrooming, sniffing the partner, following were reduced when compared to control rats and *Bacopa monnieri* treated control rats. Administration of *Bacopa monnieri* treatment to epileptic rats resulted in an increase in the time spent in social interaction to near control values. Patients with intractable TLE exhibit an increased risk of psychiatric comorbidity, including depression, anxiety, psychosis, and learning disorders (Gastens *et al.*, 2008). 5-HT$_{2C}$ receptor agonists likewise decrease social interaction. Though an inhibitory influence upon motor function suggests caution in the interpretation of these data, their influence on cellular markers and clinical studies also suggest anxiogenic-like effects (Hackler *et al.* 2007). 5-HT$_{2C}$ antagonists consistently enhance active social interaction in rats.

The forced swim test, the space for rat’s movement was restricted from which they cannot escape. The period of immobility was greater in epileptic rats compared to control and *Bacopa monnieri* treated control rats. *Bacopa monnieri* treatment once daily over a period of 15 days decreased the period of immobility in the epileptic rats. Carbamzepine treatment to epileptic rats also showed a similar effect. Immobility in rats is considered to be a state of lowered mood or hopelessness which the rodent experience when they are forced to swim in a constrained space from they cannot escape. This is believed to indicate a failure or reduced attempts towards escape directed behaviour from persistent stress. It also causes the development of passive behaviour that disengages the animal from coping up with stressful stimuli. This form of immobility which is a state of despair is reported to be reduced by a broad spectrum of anti-depressant drugs (Porsolt *et al.*, 1977).

Our experimental results thus support the anticonvulsant property of *Bacopa monnieri* at the molecular level. We conclude from our studies that *Bacopa monnieri*
extract treatment potenates a therapeutic effect by reversing the alterations in 5-HT\textsubscript{2C} and NMDA receptor binding, gene expression for NMDA2b, mGlu5 receptor and GLAST along with IP3, cGMP and cAMP that occur during epilepsy, resulting in reduced glutamate-mediated excitotoxicity in the overstimulated brain regions. Thus, it is evident that *Bacopa monnieri* treatment to epileptic rats renders protection against seizure related excitotoxicity, associated with motor and cognitive deficits which will have therapeutic significance in the management of epilepsy.