MUSCARINIC M1 AND GLUTAMATE RECEPTOR GENE EXPRESSION AND THEIR FUNCTIONAL ROLE IN PILOCARPINE INDUCED TEMPORAL LOBE EPILEPSY IN RATS: REGULATION BY BACOSIDE A AND *Bacopa monnieri* EXTRACTS

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CERTIFICATE

This is to certify that the thesis entitled " **Muscarinic M1 and Glutamate Receptor Gene Expression and their Functional Role in Pilocarpine Induced Temporal Lobe Epilepsy in rats: Regulation by Bacoside A and *Bacopa monnieri* extracts**" is a bonafide record of the research work carried out by **Mr. Reas Khan S**, under my guidance and supervision in the Department of Biotechnology, Cochin University of Science and Technology and that no part thereof has been presented for the award of any other degree.

(C.S. PAULOSE)

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DECLARATION

I do hereby declare that the thesis entitled "**MUSCARINIC M1 AND GLUTAMATE RECEPTOR GENE EXPRESSION AND THEIR FUNCTIONAL ROLE IN PILOCARPINE INDUCED TEMPORAL LOBE EPILEPSY IN RATS: REGULATION BY BACOSIDE A AND *Bacopa monnieri* EXTRACTS**" is based on the original research carried out by me at the Department of Biotechnology, Cochin University of Science and Technology, under the guidance of Dr. C.S. Paulose, Director, Centre for Neuroscience, Reader & Head, Department of Biotechnology and no part thereof has been presented for the award of any other degree, diploma, associateship or other similar titles or recognition.

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DEDICATED TO MY MOTHER

ABRREVIATIONS USED IN THE TEXT

5-HT	5-Hydroxy tryptamine
ACh	Acetylcholine
AChE	Acetylcholine esterase
AD	Alzheimers disease
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
B_{max}	Maximal binding
CA	Cornu Ammonis
cAMP	Cylic Adenosine Monophosphate
CNS	Central Nervous System
CT	Computed tomography
DA	Dopamine
DBH	Dopamine β hydroxylase
DEPC	Di ethyl pyro carbonate
DNA	Deoxy ribonucleic acid
DTT	Dithiothreitol
EEG	Electroencephalogram
EPI	Epinephrine
EPSCs	Excitatory postsynaptic current
GABA	Gamma amino butyric acid
GEPRS	Genetically epilepsy prone rats
GFAP	Glial fibrillary acidic protein
GLUR2	Glutamate Receptor-2
Gq PRC	Gq Protein coupled receptors
i.p.	Intraperitoneally

IP3	Inositol 1,4,5-triphosphate
KA	Kainate
K_d	Dissociation constant
K_i	Inhibitory coefficient
K_m	Michaelis constant
LTLE	Lateral temporal lobe epilepsy
MRI	Magnetic resonance imaging
mRNA	Messenger Ribonucleic acid
MTLE	Mesial temporal lobe epilepsy
NADH	Nicotinamide adenine dinucleotide, reduced form
NADPH	Nicotinamide adenine dinucleotide phosphate, reduced form
NE	Norepinephrine
NGF	Nerve growth factor
NMDA	N-methyl-D-aspartate
NPY	Neuropeptide Y
p	Level of significance
PA	Passive avoidance
PFC	Prefrontal cortex
Pi	Inorganic phosphate
PIP2	Phospatidyl 4,5-bisphosphate
PKC	Protein kinase C
PLC	Phospolipase C
RT-PCR	Reverse-transcription-polymerase chain reaction
S.E.M	Standard error of mean
SMOCCs	Second messenger operated calcium channels
SRS	Spontaneous recurrent seizures

SSRLs	Selective serotonin reuptake inhibitors
SUDEP	Sudden unexpected death in epilepsy patient
TLE	Temporal lobe epilepsy
VICCs	Voltage insensitive calcium channels
V_{max}	Maximal velocity
VOCC	Voltage sensitive calcium channels
VTA	Ventral tegminal area
α_2-AR	α_2 Adrenergic receptor

CONTENTS

Introduction

Epilepsy is one of the oldest medical disorders known. The word *epilepsy* derived from the Greek word *epilambanein*, meaning to be seized or to be overwhelmed by surprise. Epilepsy is one of the most common serious disorders of the brain, affecting at least 50 million people worldwide. It knows no geographical, racial or social boundaries. Epilepsy accounts for 1% of the global burden of disease, determined by the number of productive life years lost as a result of disability or premature death. Among primary disorders of the brain, epilepsy ranks with depression and other affective disorders, Alzheimer's disease, other dementias and substance abuse. Among all medical conditions, it ranks with breast cancer in women and lung cancer in men. Eighty per cent of the burden of epilepsy is in the developing world, where 80–90% of people with epilepsy receive no treatment at all. It is also necessary to recognize that epilepsy consists of more than seizures for the affected individual and affects his or her family. Epilepsy leads to multiple interacting medical, psychological, economic and social repercussions, all of which need to be considered. (WHO epilepsy Atlas 2005)

Epilepsy is characterized by a tendency to recurrent seizures. The word 'seizure' is derived from the Latin word *sacire meaning* 'to take possession of' is the clinical manifestation of an abnormal, excessive, hypersynchronous discharge of a population of neurons. The hypersynchronous discharges that occur during a seizure may begin in a very discrete region of brain and then spread to neighbouring regions. Seizure initiation is characterized by two concurrent events: 1) high-frequency bursts of action potentials and 2) hypersynchronization of a neuronal population. The synchronized bursts from a sufficient number of neurons result in 'spike discharge' on the EEG. At the level of single neurons, epileptiform activity consists of sustained neuronal depolarization resulting in a burst of action

potentials, a plateau-like depolarization associated with completion of the action potential burst and then a rapid repolarization followed by hyperpolarization. This sequence is called the paroxysmal depolarizing shift. The bursting activity resulting from the relatively prolonged depolarization of the neuronal membrane is due to influx of extracellular Ca^{2+}, which leads to the opening of voltage-dependent Na^{+} channels, influx of Na^{+} and generation of repetitive action potentials (Schiller *et al.*, 2004). Seizure propagation is the process by which a partial seizure spreads within the brain, occurs when there is sufficient activation to recruit surrounding neurons. This leads to a loss of surrounding inhibition and spread of seizure activity into contiguous areas *via* local cortical connections and to more distant areas *via* long association pathways such as the corpus callosum.

Because of the well organized and relatively simple circuits within the entorhinal-dentate-hippocampal loop, the limbic system has been intensively studied in experimental models of epilepsy. These investigations have led to two theories regarding the cellular network changes which cause the hippocampus, among the most common sites of origin of partial seizures, to become hyper-excitable. The first proposes that a selective loss of interneurons decreases the normal feed-forward and feed-back inhibition of the dentate granule cells, an important group of principal neurons (Stief *et al.*, 2007). The other theory suggests that synaptic reorganization follows injury and creates recurrent excitatory connections, *via* axonal "sprouting," between neighboring dentate granule cells (Cohen *et al.*, 2003). More recently, it has been proposed that the loss, rather than being of GABAergic inhibitory neurons, is actually of excitatory neurons which normally stimulate the inhibitory interneurons to, in turn, inhibit the dentate granule cells. These mechanisms of hyperexcitability of the neuronal network are not mutually exclusive, could act synergistically and coexist in the human epileptic brain.

Temporal lobe epilepsy (TLE) is one of the most common forms of intractable epilepsy. Patients affected often have similar clinical history, including an initial precipitating injury such as childhood febrile convulsions, *status epilepticus* (SE) or trauma. Between this injury and the emergence of recurrent complex partial seizures, there is usually a latent period of several years (Turski *et al.*, 1989). Frequently associated with this epilepsy is the presence of hippocampal sclerosis (HS). HS is defined by specific neuronal loss throughout the hippocampus, with severe damage in the prosubiculum, CA1, CA4 and hilus in contrast with slighter damage in granule cells and relative sparing of CA3 and especially CA2 region. Human studies strongly support the view that HS probably initiates or contributes to the generation of most TLEs (Engel *et al.*, 1996). However, there is a growing body of evidence that amygdala, limbic thalamus and entorhinal cortex may be injured in TLE (Jutila *et al.*, 2001). The respective role of various hippocampal or extrahippocampal structures in the genesis of the disease remains unknown.

Induction of SE by systemic application of pilocarpine and subsequent occurrence of spontaneous seizures is probably the most attractive animal model, for the study of temporal lobe epilepsy. Pilocarpine treatment is characterized by generalized convulsive SE in rodents, which represents the initial precipitating injury. After a latent period, adult rats exhibit spontaneous recurrent seizures (SRS) during the remainder of their life. The EEG and behavioral features of these seizures resemble those of complex partial seizures (Leite & Cavalheiro, 1995). This model shares many histopathological and molecular changes that have been characterized in neurosurgical resections and post mortem specimen from TLE patients. Surprisingly little is known on the molecular and cellular signaling during induction of SE and the role of muscarinic and glutaminergic functional regulation in chronic epilepsy models.

Bacopa monnieri (Brahmi) has been used by Ayurvedic medical practitioners in India for almost 3000 years. The earliest chronicle mention is in the Ayurvedic treatise, the Charaka Samhita (100 A.D.), in which Brahmi is recommended in formulations for the management of a range of mental conditions including anxiety, poor cognition, lack of concentration and epilepsy. According to Charaka, Brahmi acts as an effective brain tonic that boosts one's capabilities to think and reason. The Sushruta Samhita (200 A.D.) attributes the plant with efficacy in maintaining acuity of intellect and memory. Pharmacologically, it is understood that Brahmi has an unusual combination of constituents that are beneficial in mental inefficiency and illnesses and useful in the management of convulsive disorders like epilepsy. Bacosides, Brahmi's active principle component responsible for improving memory related functions, are attributed with the capability to enhance the efficiency of transmission of nerve impulses, thereby strengthening memory and cognition (Kishore *et al.*, 2005).

The present work is to understand the alterations of total muscarinic, muscarinic M1 and glutamate receptors in the brain regions of pilocarpine induced epileptic rats. The work focuses on the evaluation of the antiepileptic activity of extracts of *Bacopa monnieri*, Bacoside A and Carbamazepine *in vivo*. The molecular changes in the muscarinic M1 receptors in the pre- and post-treated epileptic model with *Bacopa monnieri*, Bacoside A and Carbamazepine were also studied. These studies will help us to elucidate the functional role of muscarinic and glutamate receptors in epilepsy.

OBJECTIVES OF THE PRESENT STUDY

1. To study the antiepileptic activity of whole plant extract of *Bacopa monnieri* and Bacoside A in pilocarpine induced Temporal Lobe Epileptic rat model.

2. To study the cholinergic activity using acetylcholine esterase assay in the brain regions- hippocampus and brainstem in epilepsy, Carbamazepine, *Bacopa monnieri* and Bacoside A, post-treated epileptic rats.

3. To study the muscarinic general receptor binding parameters in the hippocampus, cerebellum and brainstem in epilepsy, Carbamazepine, *Bacopa monnieri* and Bacoside A, post-treated epileptic rats.

4. To study the muscarinic M1 receptor binding parameters in the hippocampus, cerebellum and brainstem in epilepsy, Carbamazepine, *Bacopa monnieri* and Bacoside A, pre- and post- treated epileptic rats.

5. To study the muscarinic M1 receptor gene expression in the hippocampus in epilepsy, Carbamazepine, *Bacopa monnieri* and Bacoside A, pre- and post-treated epileptic rats.

6. To study the muscarinic M1 receptor gene expression in the Cerebellum and Brainstem in epilepsy, Carbamazepine, *Bacopa monnieri* and Bacoside A, post-treated epileptic rats

7. To study the glutamate dehydrogenase activity in the brain regions-hippocampus, cerebellum and brainstem in epilepsy. *Bacopa monnieri* post-treated epileptic rats.

8. To study the glutamate receptor binding parameters in the hippocampus, cerebellum and brainstem in epilepsy, *Bacopa monnieri* post-treated epileptic rats.

9. To study the NMDA R1 and metabotrophic glutamate 8 receptor gene expression in the hippocampus, cerebellum and brainstem in epilepsy, *Bacopa monnieri* post-treated epileptic rats.

10. To perform the Neo-Timm Staining in the hippocampus in epilepsy, Carbamazepine, *Bacopa monnieri* and Bacoside A, post-treated epileptic rats to observe reversal of mossy fibre sprouting.

11. To perform neurophysiologic analysis of the electrical activity of the brain using electroencephalogram in epilepsy, Carbamazepine, *Bacopa monnieri* and Bacoside A, pre- and post-treated epileptic rats.

12. To study the spatial learning ability by Morris water maze experiment in epilepsy, *Bacopa monnieri* post-treated epileptic rats.

Literature Review

Definition

Epilepsy is characterized by recurrent, unprovoked, paroxysmal episodes of brain dysfunction manifesting as a large number of clinical phenomena, like altered levels of consciousness, involuntary movements, abnormal sensory phenomena, autonomic changes and transient disturbances of behaviour. It is a variety of symptoms arising from different kinds of pathologic processes of the brain rather than a specific disease or even a syndrome. During epileptic seizures, there are excessive discharges of electrical activity of the neurons in the brain and the clinical manifestations depend on the origin and the localization of those pathological discharges (Browne & Feldman, 1983; Waltimo *et al.*, 1983; Engel & Pedley, 1997).

Epidemiology

The incidence epilepsy is higher during the first years of life, falls dramatically thereafter and increases again in the elderly. Approximately 50% of cases of epilepsy begin in childhood or adolescence (Hauser *et al.*, 1993). Many studies suggest that males are at a greater risk for unprovoked seizures and epilepsy than female subjects (Hauser *et al.*, 1997). The prevalence of epilepsy is 0.7-0.8% worldwide and the lifetime cumulative incidence is 1-3%.

Etiology

The most common etiologic factors of epilepsy that can predispose a person to epilepsy are head traumas, neoplasms, degenerative diseases, infections, metabolic

diseases, ischemia and hemorrhages (Vinters *et al.*, 1993). At present, more and more genetic factors underlying different types of epileptic syndromes are revealed. It is also known that certain brain areas, i.e. temporal and frontal lobes are more susceptible to produce epileptic seizure activity than the other regions (Larsen & Iivanainen, 1994). However, there are also patients with unresolved etiology of epilepsy (Hauser *et al.*, 1997). Etiology of epilepsy is also a factor in determining cognitive function and intellectual changes over time. The main distinction is between symptomatic epilepsy which has an identified cause such as stroke or cortical dysplasia and idiopathic epilepsy which has no identified cause other than genetic factors. Lennox *et al.*, (1942) recognized that cognitive function was twice as likely to deteriorate in the presence of a known cause of epilepsy even if the idiopathic group had more frequent seizures.

Classification

The term "epilepsy" encompasses a number of different syndromes whose cardinal feature is a predisposition to recurrent unprovoked seizures. Although specific seizures can be classified according to their clinical features like complex partial seizures and generalized tonic-clonic seizures, (Commission on Classification and Terminology of the International League against Epilepsy, 1981). Epilepsy syndromes also classified according to the type of seizure, the presence or absence of neurological or developmental abnormalities and electroencephalographic (EEG) findings. Epilepsy syndromes fall into two broad categories: generalized and partial syndromes (Benbadis *et al.*, 2001). In generalized epilepsies, the predominant type of seizures begins simultaneously in both cerebral hemispheres. Many forms of generalized epilepsy have a strong genetic component. In partial epilepsies, by contrast, seizures originate in one or more localized foci, although they spread to

involve the entire brain. Most partial epilepsies are believed to be the result of one or more central nervous system insults, but in many cases the nature of the insult is never identified.

Partial seizures

Seizures that begin in a focal region of the cerebral cortex, often within one lobe of the brain are termed partial seizures. Partial seizures remain focal throughout the duration of the seizure or propagate *via* neuronal pathways and networks to various regions of the hemisphere (Chabolla *et al.*, 2002). When partial seizure spreads to involve the majority of both cerebral hemispheres, it is said to be secondary generalized (Chabolla *et al.*, 2002). Partial epilepsies comprise slightly over 50% of all epilepsies (Keranen *et al.*, 1988, Hauser *et al.*, 1997, Williamson *et al.*, 1997).

The symptoms associated with a partial seizure depend on the brain regions involved. In theory, any function produced by the cortex (e.g. somatosensory, motor, autonomic, psychic phenomena) can be a symptom of a seizure. The first sign or symptom of a seizure is often, but not always, the best indicator of the site of seizure origin. The most common sites of producing epileptic discharges are temporal and frontal lobes (Chabolla *et al.*, 2002).

Generalized seizures

When epileptic seizure involves both cerebral hemispheres at the onset, it is termed as generalized seizure. At the onset of a generalized seizure, patients experience a vague, indescribable warning, although the vast majority of patients lose consciousness without premonitory symptom. With the loss of consciousness, tonic-clonic convulsions occur. A generalized seizure also manifest itself as absence, atonic

or myoclonic seizures. Idiopathic generalized epilepsies are often childhood idiopathic syndromes, some of which have an excellent prognosis, whereas some of them are thought to require life long medication (Chabolla *et al.*, 2002).

Diagnosis

The procedures needed for the diagnosis of epilepsy include medical history with information on the possible predisposing events, a detailed description of the seizures and clinical evaluation with special respect paid to the cardiovascular and neurological examination. EEG-recording reveals focal or generalized spikes and slow waves or other epileptic phenomena. Magnetic resonance imaging (MRI) is recommended as the first line imaging method of the brain when seizures are thought to be of focal origin. MRI detects pathologic conditions that cannot be diagnosed with CT.

Prognosis

The prognosis of epilepsy depends greatly on the underlying cause. At the beginning of the last century, all epilepsies were considered chronic. Later, however, the prognosis has become markedly better with the better epidemiological studies of less selected populations (Liow *et al.*, 2007). The studies indicate that when treated, more than two thirds of the patients with newly diagnosed epilepsy soon enter long-term remission. Symptomatic epilepsies are more likely to relapse than idiopathic or cryptogenic epilepsies. An abnormal EEG pattern increase the risk for the recurrence of seizures (Sander & Sillanpaa, 1997).

It is well known that the mortality of the patients with epilepsy exceeds 2-3 times that among the general population. The increased mortality is due to excess

morbidity, accidents during the seizures, status epilepticus and suicides. The most important epilepsy-related death is 'Sudden Unexpected Death in Epilepsy Patients' (SUDEP) that accounts for as much as 10-15 % of the deaths among epilepsy patients. Many childhood epilepsies have a better prognosis than epilepsies in adults, but there are also severe childhood epilepsies that eventually lead to increasing neurological deficits and even to death (Sander & Sillanpää, 1997).

Status Epilepticus

Status epilepticus is defined as a condition characterized by an epileptic seizure frequently repeated and prolonged as to create a fixed and lasting condition (Gastaut *et al.*, 1970). Lowenstein and Alldredge (1998) advocated the use of an operational definition of status epilepticus: either continuous seizures lasting at least 5 minutes or two or more discrete seizures between which there is incomplete recovery of consciousness (Lowenstein & Alldredge, 1998). Any type of seizure develop into status epilepticus, although the form most often seen in adults is tonic-clonic status epilepticus. The morbidity and mortality from status epilepticus are related to three factors: damage to the central nervous system (CNS) caused by acute insult precipitating the status epilepticus, systemic stress from repeated generalized tonic-clonic convulsions and injury from repetitive electrical discharges within the CNS. Systemic effects of repeated generalized seizures influence cardiovascular, respiratory and renal failure (Leppik *et al.*, 1990). In addition, a number of biochemical changes not related to systemic effects of tonic-clonic activity occur in the CNS. Sixty minutes of repeated neuronal discharge results in severe neuronal death (Meldrum & Brierley, 1973). Neuropathological and imaging studies have shown damage in the hippocampus and in the amygdala, piriform cortex, thalamus, cerebellum and cerebral cortex after convulsive and nonconvulsive status epilepticus episodes in patients (Tien

& Felsberg, 1995; Wieshman *et al.*, 1997). *In vivo*, the measurement of neuron-specific enolase provides further evidence of acute cerebral damage following status epilepticus. The enzyme was elevated in the serum of both generalized convulsive and nonconvulsive status epilepticus patients within 24 to 48 hours after the onset of status (DeGiorgio *et al.*, 1995; Rabinowicz *et al.*, 1995). The therapy for status epilepticus currently consists of agents which stop seizures (benzodiazepines, phenytoin, barbiturates) (Bleck *et al.*, 1991). In a study trial comparing treatments for generalized convulsive status epilepticus, diazepam plus phenytoin, lorazepam or phenobarbital were equally effective therapies (Treiman *et al.*, 1998). Due to the significant morbidity and mortality associated with the insult despite current medical treatment, status epilepticus remains one of the most serious disorders affecting the CNS.

Structures and Connections of the Medial Temporal Lobe

The limbic system forms a border around the upper brain stem, diencephalon and corpus callosum. The main components of the limbic system are the hippocampal formation, limbic association cortices including parahippocampal gyrus and the amygdaloid complex (Braak *et al.*, 1996). Both the amygdala and hippocampus are located in the medial part of the temporal lobe, adjacent to the parahippocampal gyrus. This gyrus is a "continuation" of the cingulate gyrus onto the inferior surface of the brain. The cortex of the parahippocampal gyrus includes the subiculum and entorhinal cortex, both of which are functionally related to the hippocampus. The hippocampal formation is a prominent, bulging eminence in the floor of the temporal horn of the lateral ventricle. During development, the hippocampal formation undergoes an enfolding into the temporal lobe. This results in the interdigitation of two c-shaped structures, the hippocampus and the dentate gyrus. There is further subdivision of the

hippocampus into three regions, which are referred to as Cornu Ammonis (CA) fields. The CA3 field borders the hilus of the dentate gyrus; a short CA2 field follows; and a more extensive CA1 merges with the subiculum (Amaral & Insausti, 1990). The major source of cortical inputs to the hippocampal circuit is formed by the entorhinal cortex (Witter & Amaral, 1991). The human entorhinal cortex is made up of six layers, of which layer IV does not appear throughout all subfields of the entorhinal cortex (Insausti *et al.*, 1995). In the most classical hippocampal pathway, cells in layers II and III of the entorhinal cortex give rise to the perforant path that distributes to all subfields of the hippocampal formation, including the dentate gyrus (Witter & Amaral, 1991). From the dentate gyrus, granule cells project to the CA3 field of the hippocampus. The CA3 pyramidal cells in turn send a major projection to the CA1 field. Much of the input from the CA1 field is then sent on to the subiculum. From the subiculum, information conveyed to the deep layers of the entorhinal cortex (Amaral *et al.*, 1984; Witter *et al*, 1993). The entorhinal cortex receives prominent cortical innervation (Amaral & Insausti, 1990). Two-thirds of this cortical input originates in the perirhinal and parahippocampal cortices (Insausti *et al.*, 1987). The perirhinal cortex areas 35 and 36 (Brodmann *et al.*, 1909)) is bounded medially by the entorhinal cortex and laterally by temporal association areas. It also extends anteriorly to include the medial portion of the temporal pole (Suzuki *et al.*, 1996a). The parahippocampal cortex is caudally adjacent to both the entorhinal cortex and the perirhinal cortex, and is made up of a smaller, medially situated area TH and a larger, laterally situated area TF (Suzuki & Amaral, 1994). Direct input to the entorhinal cortex orginates in several cortical regions in the frontal and temporal lobes, and in the insular and cingulated cortices, as well as in the adjacent perirhinal and parahippocampal cortices (Insausti *et al.*, 1987). All these projections are reciprocal. There are extensive reciprocal connections with the hippocampus, the entorhinal cortex and the amygdala (Amaral *et*

al., 1987). The amygdaloid complex is composed of more than ten nuclei and their subdivisions which have different cytoarchitectonic, chemoarchitectonic, and connectional characteristics (Amaral *et al.*, 1992; Pitkänen *et al.*, 1997). The lateral nucleus of the amygdala gives rise to a prominent projection to layer III of the entorhinal cortex (Amaral & Insausti, 1990). There are also additional projections from the amygdaloid complex to the hippocampus and to the subiculum (Aggleton *et al.*, 1986) Conversely, the subiculum and the entorhinal cortex originate return projections to the amygdala (Aggleton *et al.*, 1986; Amaral *et al.*, 1986). In general, the amygdaloid complex projects to a greater number of cortical regions than those from which it receives projections (Amaral *et al.*, 1987). Essentially, all major divisions of the temporal cortex receive a projection from the amygdala. The perirhinal cortex, particularly, has prominent interconnections with the amygdala nuclei (Suzuki *et al.*, 1996b). Moreover, there is evidence of reciprocal connectivity with the amygdala and portions of the frontal and insular cortices. The general conclusion about the functional connectivity is that the amygdaloid complex is directly and reciprocally linked to a wide variety of cortical regions and can influence the sensory information processed to various degrees. In contrast, cortical information is funneled into and out of the hippocampal formation through polysensory border regions and highly processed before reaching the hippocampus (Amaral *et al.*, 1987; Insausti *et al.*, 1987).

Temporal lobe epilepsy (TLE)

Temporal lobe epilepsy is considered the most common epileptic syndrome and it is estimated that approximately 80% of patients with partial seizures have temporal lobe epilepsy (Williamson *et al.*, 1997). TLE can be subclassified into mesial temporal lobe epilepsy (MTLE) and lateral temporal neocortical epilepsy (LTLE).

MTLE comprises the majority of the cases of epilepsy refractory to pharmacotherapy (Babb & Brown, 1987). However, it may be remediable to surgery because hippocampal sclerosis can often be seen as an underlying pathology in MTLE (Thadani *et al.*, 1995, Benbadis *et al.*, 1996). In fact, surgical treament can abolish seizures in 80-90% of patients with MTLE (Wieser & Williamson, 1993).

Temporal lobe epilepsy is characterized clinically by the progressive development of spontaneous recurrent seizures (SRS) from temporal lobe foci (Engel *et al.*, 1989, 1996). Before exhibiting SRS, patients with TLE usually experience epileptic status early in life, followed by a seizure-free period ranging from months to years (Engel *et al.*, 1989; Lothman & Bertram, 1993). TLE is also characterized pathologically by unique morphological alterations in the hippocampus. The most frequently observed alteration is massive neuronal loss in the hilus of the dentate gyrus and in the CA1 and CA3 pyramidal cell layers (Engel *et al.*, 1989; Lothman & Bertram, 1993; Ben-Ari & Cossart, 2000). Another common morphological alteration is mossy fiber sprouting, the growth of aberrant collaterals of granule cell axons into the inner molecular layer of the dentate gyrus (Sutula *et al.*, 1989; Houser *et al.*, 1990; Babb *et al.*, 1991; Isokawa *et al.*, 1993). A prominent hypothesis states that hippocampal neuronal loss and mossy fiber sprouting play a critical role in the genesis and progression of TLE (Lothman & Bertram, 1993; Wasterlain *et al.*, 1993, 1996; Engel, 1996; Lowenstein *et al*, 1996; Coulter *et al.*, 1999; Houser *et al.*, 1999; Ben-Ari & Cossart, 2000). This hypothesis is supported by several lines of evidence. First, partial hippocampal removal including the site of neuronal damage results in a seizure-free state or a marked reduction of seizure frequency in many patients (Flaconer & Serafetinides, 1963; Rasmussen *et al.*, 1983; King *et al.*, 1986; Brown & Babb, 1987; Engel *et al.*, 1987, 1989; Bruton *et al.*, 1988; Babb *et al.*, 1990). Second, the sprouted mossy fibers seemingly form a powerful monosynaptic recurrent

excitatory pathway, through which seizures is generated (Sperk *et al.*, 1994; Lowenstein *et al.*, 1996; Lynch & Sutula, 2000). This suggestion is supported by the findings that the intensity and time course of mossy fiber sprouting positively correlate with the severity and time course of SRS (Sutula *et al.*, 1989) and that the sprouted mossy fibers seemingly form recurrent excitatory circuits (Tauck & Nadler, 1985; Cronin *et al.*, 1992; Lynch & Sutula, 2000).

Cholinomimetics

The relationship of cholinergic mechanisms to epilepsy was suspected by neurologists by the turn of the 19th century (Langley & Kato, 1915). Decades later it was suggested that acetylcholine (ACh) may be primarily involved in human convulsive disorders (Brenner & Merrit, 1942). In 1945, it was demonstrated experimentally in cats that intracisternal injection of acetylcholine resulted in motor seizures (Forster *et al.*, 1945). An epileptogenic potential of ACh was discovered that chronically isolated monkey cortex demonstrated an increased sensitivity to locally administered Ach (Echlin *et al.*, 1959). In later years, evidence from electrophysiological experiments reinforced the idea that ACh may be involved in the cellular mechanisms of epilepsy (Dichter & Ayala, 1987). In terms of specific mechanisms, it has been shown experimentally that muscarinic cholinergic excitation in the brain occurs as a result of a reduced voltage-dependent and Ca^{2+}-dependent K^{+} conductance and is mediated by voltage dependent Ca^{2+} and K^{+} conductance (Benardo & Prince, 1982). Acetylcholine functions by promoting the inward flow of Ca^{2+} and Na^{+} into neurons which is responsible for the membrane depolarization that leads to epileptic events (Pumain *et al.*, 1983). Muscarinic blockade slows and degrades the

location-specific firing of hippocampal pyramidal were reported early (Brazhnik *et al.*, 2003)

Pilocarpine

Pilocarpine is a potent cholinergic agonist originally isolated from the leaflets of *Pilocarpus microphyllus*. It is commonly used in the treatment of acute glaucoma in humans (Hardman *et al.*, 1996). Single systemic high dose (300-400 mg/kg) pilocarpine injection as a novel animal model of TLE was established (Turski *et al.*, 1983). The systemic administration of this muscarinic cholinergic agonist produced electroencephalographic and behavioral seizures, accompanied by widespread brain damage similar to that observed in autopsied brains of human epileptics. The electroencephalographic findings indicate that one of the most sensitive structures to the convulsant effect of pilocarpine is the hippocampus, while other structures remain unaffected or only slightly affected at early time points following injection. It is generally accepted that the hippocampus is indeed one of the earliest structures affected following pilocarpine treatment. Later studies confirmed that the hippocampus is the earliest structure to be activated according to electroencephalogaphic recordings (Turski *et al.*, 1983, 1989). One of the main features of the pilocarpine model that makes it very relevant for comparison to the human epileptic condition is the reproducible occurrence of spontaneous recurrent seizures (SRS) in rats injected with pilocarpine following a delay or silent period of about 2 weeks (Turski *et al.*, 1983, 1989; Cavalheiro *et al.*, 1991; Mello *et al.*, 1993). Spontaneity is one of the prominent signs of human epilepsy, therefore strengthening the clinical importance of this model (Turski *et al.*, 1983; Loscher & Schmidt, 1988). Pilocarpine seizures also provide an opportunity to study the involvement of the

cholinergic system in the onset, propagation and pathological consequences of limbic seizures (Clifford *et al.*, 1987). Behaviorally, pilocarpine seizures resemble other models of limbic seizures beginning with facial automatisms, head nodding and progressing to forelimb clonus with rearing and falling (Clifford *et al.*, 1987). In terms of neuropathology, the cell damage that results from seizures was identical whether they are initiated with a high-dose pilocarpine injection or a lower dose of pilocarpine administered with lithium (Clifford *et al.*, 1987). Lithium-pilocarpine is an analogous model to pilocarpine injection alone, except that lithium in combination with pilocarpine has been reported to produce a 20-fold shift in the pilocarpine dose-response curve for producing seizures (Clifford *et al.*, 1987) thereby permitting the use of a much lower dose of pilocarpine. In terms of cell damage reported at the light microscope level, pilocarpine-induced seizures consistently produce damage in the olfactory nucleus, pyriform cortex, entorhinal cortex, thalamus, amygdala, Hippocampus, lateral septum, bed nucleus of stria terminalis, claustrum, substantia nigra and neocortex (Clifford *et al.*, 1987; Turski *et al.*, 1989; Turski *et al.*, 1983). In the hippocampus, the CA3 and CA1 regions are involved and damage has been noted to be greater in ventral as opposed to dorsal hippocampal regions. Interestingly, the highest cholinergic receptor densities are in CA1 and the dentate gyrus, while the region most consistently and severely damaged is CA3 (Clifford *et al.*, 1957). This clearly indicates that the spread of seizure activity beyond the initial focus must entail activation of non-cholinergic pathways. Electron microscopic studies indicate the cellular changes include swelling of dendrites, swelling or vacuolar condensation of neuronal cell bodies and marked dilatation of astroglial elements with relative sparing of axonal components (Clifford *et al.*, 1987). The neuropathology reported with the pilocarpine model is consistent with prolonged seizures produced by other means (Ben-Ari *et al.*, 1985; Kapur *et al.*, 1989; Hajnal *et al.*, 1997) These findings support

that pilocarpine SE model is useful in studying the molecular mechanisms of neuropathology and screening neuroprotectants following cholinergic agonist exposure (Tetz *et al.*, 2006).

Focal structural lesions in temporal lobe

It has been established that hippocampal damage is the most common pathology underlying TLE (Babb & Brown, 1987). Neuron loss is usually located in the fields H1 and H3 of the hippocampus and when the neuron loss is restricted to those areas, it is regarded as the classic hippocampal cell loss (Babb & Brown, 1987; Sutula *et al.*, 1989). However, more wide spread neuron loss is often seen in resected temporal lobes of patients with TLE (Margerison & Corsellis, 1966; Bruton *et al.*, 1988). The structures suffering from neuron loss in addition to hippocampus include the amygdala, the uncus of the hippocampus and the parahippocampal gyrus. This form of neuron damage is called as mesial temporal lobe sclerosis. (Engel *et al.*, 1992 ; Wieser *et al.*, 1993 ; Williamson *et al.* 1993 ; Thadani *et al.*, 1995)

The neuronal reorganization continues with recurrent seizures and clinical observations on the development of medical intractability of MTLE also suggest an ongoing process (French *et al.*, 1993; Engel *et al.*, 1997). Recent studies have shown that, at least in some patients there is an association between an initial precipitating injury prior to habitual seizure onset and hippocampal sclerosis (Trenerry *et al.*, 1993; Mathern *et al.*, 2002). However, patients with episodes of generalized tonic-clonic status epilepticus and prolonged partial seizure activity may develop progressive hippocampal neuronal loss in a widespread distribution that is dissimilar to classic Ammon's horn sclerosis. It is concluded that hippocampal sclerosis is presumably both the cause and effect of seizures (Bruton *et al.*, 1988, Gloor *et al.*, 1991).

Pathophysiology of Temporal Lobe Epilepsy

EEG studies shows that the hippocampus is one of the earliest structures to be activated during seizures. In addition, the cure of epilepsy by surgical resection of the hippocampus in properly selected individuals led to the idea that hyperexcitability intrinsic to the hippocampus contribute to the development of epilepsy (Bausch & McNamara, 1999). Thus it is not surprising that from the perspective of mechanisms, the best studied form of seizure is the seizure activity in the hippocampus. Recent report states that different neuronal populations react differently to SE induction. For some brain areas most, if not all, of the vulnerable cells are lost after an initial insult leaving only relatively resistant cells and little space for further damage or cell loss (Covolan *et al.*, 2006)

Cell Loss

The most frequent lesion in patients with TLE is mesial temporal sclerosis or hippocampal sclerosis, consisting of gliosis and neuronal loss in the CA 1, CA3 and the hilus of the dentate gyrus (Houser *et al.*, 1990). This typical pattern of neuronal loss characteristic of hippocampal sclerosis (Kapur *et al*, 1999; Lewis *et al.*, 1999) can be produced experimentally by repeated or prolonged seizures and results presumably from excitotoxic damage subsequent to excessive activation of glutamate receptors (Olney *et al.*, 1986; Sloviter *et al.*, 1994). There are striking similarities between the pathology produced in experimental animals by prolonged seizures (Sloviter *et al.*, 1991) or head trauma (Coulter *et al.*, 1996) and the pathological changes seen in the hippocampi of many patients with TLE (Meldrum & Bruton, 1992). Seven days and two months post-status epilepticus rats showed significant neuron loss in the pre-endo piriform nucleus, layer III of the intermediate piriform cortex, and layers II and III of

the caudal piriform cortex (Chen *et al.*, 2007). There is an extensive loss of dentate hilar neurons (Bausch & Chavkin., 1997) and hippocampal pyramidal cells (DeGiorgio *et al.*, 1997) Recent data also demonstrated cases where some granule cells of experimental animals are also highly vulnerable (Sloviter *et al.*, 1996). Seizure-induced astrocytic damage has also been documented (Schmidt-Kastner & Ingvar, 1996). Interestingly, in contrast to the many studies showing cell loss, a recent study described an increased generation of hippocampal granule cells as a consequence of seizures (Parent *et al.*, 1997). Induction of limbic epilepsy resulted in an increased proliferation of granule cells using bromodeoxyuridine labelling. Therefore, although death of certain cell populations was suggested as a main event during or as a result of epileptogenesis, there is also evidence of neurogenesis.

Mechanistically, neuronal loss can occur with either active or passive participation of cellular constituents. This has been referred to as apoptosis or necrosis (Kerr *et al.*, 1972). Apoptosis is a form of gene-mediated death characterised by specific morphological features: early nuclear chromatin condensation, Cytoplasmic compaction with cell shrinkage, endonuclease-mediated DNA fragmentation into oligonucleosomes, apoptotic body formation and well-preserved organelles. In contrast, necrosis resulting from sudden injury with the cell unable to maintain homeostasis is characterized by early cytoplasmic vacuolization before any nuclear changes occurs and is associated with an inflammatory response (Tomei and Cope, 1991).

It appears that epileptic neuronal death is primarily but not exclusively apoptotic (Charriaut-Marlangue & Ben-Ari, 1995). Long-term repetitive stimulation of the perforant path induced apoptosis in the granule cells but necrosis in the hilar

and pyramidal cells (Sloviter *et al.*, 1996). The surviving granule cells showed dendritic deformations and shrinkage (Isokawa & Mello, 1991).

Axon sprouting

In addition to the neuronal loss, the second morphological change induced in the hippocampus by seizures is sprouting of dentate granule cell axons which are commonly referred to as mossy fibres. This occurs in both animal models of epilepsy (Bausch & Chavkin, 1997) as well as in human epilepsy (Babb *et al.*, 1991). Denervation of the inner molecular layer secondary to hilar cell loss is believed to constitute the initial stimulus for sprouting (Tauck & Nadler, 1985). The sprouted mossy fibre axons appear to make synaptic contacts with granule cells and GABAergic basket cells. It has been proposed that seizure induced expression of neurotropic genes which is suggested to underlie the sprouting of axons of the granule cell layer (Sutula *et al.*, 1996). It has been established that NGF protein levels in dentate granule cells are increased by seizure activity (Gall & Isackson, 1989).

Gliosis

Reactive gliosis occurs in response to injury, including pilocarpine- induced seizures, in the mature central nervous system (CNS). A salient manifestation of reactive gliosis is an increase in glial fibrillary acidic protein (GFAP), a protein subunit of glial intermediate filaments found exclusively in astrocytes in the CNS (Amaducci *et al.*, 1981). Glial proliferation characteristically accompanies neuronal loss seen in Ammon's horn sclerosis and after various insults including status epilepticus and contributes to epileptogenesis.

Dendritic Changes

Dendritic degeneration is another common pathological finding in TLE and its animal models (Isokawa *et al.*, 1998). Neurons from the hippocampus and neocortex from patients with chronic focal epilepsy showed dramatic dendritic abnormalities. Dendritic spine loss has been repeatedly reported and has been suggested to be more severe with an increased duration of a seizure disorder (Multani *et al.*, 1994). Dendrites of pyramidal cells have also been reported to have varicose swellings at irregular intervals along their length (Muller *et al.*, 1993). It was established that following initial acute seizures, surviving neurons undergo substantial changes in the morphology and density of dendrites and spines in the chronic phase, during which the gradual development of spontaneous seizure is established (Isokawa *et al.*, 1998). In the pilocarpine animal model of epilepsy, the membrane time constant of neurons, which can assess a cells total surface area and geographic extent of dendritic branches was reported to be significantly reduced in rats that experienced many spontaneous seizures in the chronic phase (Isokawa *et al.*, 1996). This suggests that the higher the frequency of spontaneous seizures, the more severe the local dendritic shrinkage.

Impaired Inhibition.

Repeated intense seizures caused an attenuation of gamma-aminobutyric acid (GABA) - mediated inhibition of the granule cells and in the pyramidal cells of the hippocampus (Coulter *et al.*, 1996). This change cannot be explained by a selective loss of GABAergic inhibitory interneuron, since the GABA immunoreactive neurons

were shown to be more resistant to seizure-induced injury than other hippocampal neurons (Sloviter *et al.*, 1987). Preservation of GABAergic cells in surgical specimens from patients with epilepsy was confirmed (Babb *et al.*, 1989). The neurons among the most sensitive to the seizure-induced neuronal death are the mossy cells in the dentate hilus (Lowenstrin *et al.*, 1992; Sloviter *et al.*, 1989). These cells receive synaptic input from granule cells *via* collaterals of mossy fibres and from the entorhinal cortex *via* the perforant path.

To account for the paradoxical loss of GABA-mediated inhibition with preservation of GABAergic neurons, the dormant basket cell hypothesis (Sloviter *et al.*, 1987) suggests that the seizure-induced loss of hilar excitatory neurons removes tonic excitatory projection to GABAergic basket cells, the inhibitory interneuron in the dentate hilus. Being deafferented these cells then lie dormant with the end result being disinhibition (Sloviter *et al.*, 1987). Loss of mossy cells which govern lateral inhibition in the dentate area cause functional delamination of the granule cell layer and result in synchronous multilamellar discharges in response to excitatory input (Sloviter *et al.*, 1994). Therefore, there are 3 premises to this theory: 1) the general preservation of the inhibitory network. 2) the loss of excitatory afferents to GABAergic interneuron, 3) decreased inhibition on principal cells (Bernard *et al.*, 1998).

ROLE OF NEUROTRANSMITTERS IN EPILEPSY

Epinephrine and Norepinephrine (NE)

The modification of the seizure activity by the noradrenergic system were reported early (Chen *et al.*, 1954). Four major observations have supported an anticonvulsant role for norepinephrine (NE): (1) selective lesioning of noradrenergic

neurons (with 6-hydroxydopamine or DSP-4) increases seizure susceptibility to a variety of convulsant stimuli (Arnold *et al.*, 1973; Jerlicz *et al.*, 1978; Mason & Corcoran, 1979; Snead *et al*, 1987; Trottier *et al.*, 1988; Sullivan & Osorio, 1991; Mishra *et al.*, 1994) (2) direct stimulation of the locus coeruleus (LC), the major concentration of noradrenergic cell bodies in the CNS and the subsequent release of NE reduce CNS sensitivity to convulsant stimuli (Libet *et al.*, 1977; Turski *et al.*, 1989) (3) genetically epilepsy-prone rats (GEPRs), a widely used animal model of epilepsy, have deficient presynaptic NE content, NE turnover, tyrosine hydroxylase levels, dopamine β-hydroxylase (DBH) levels and NE uptake (Jobe *et al.*, 1984; Dailey & Jobe, 1986; Browning *et al.*, 1989; Lauterborn & Ribak, 1989; Dailey *et al.*, 1991) (4) adrenergic agonists acting at the α_2 adrenoreceptor (α_2-AR) have anticonvulsant action (Baran *et al.*, 1985; Loscher & Czuczwar, 1987; Fletcher & Forster, 1988; Jackson *et al.*, 1991). α_2-AR is known to have a regulatory role in the sympathetic function (Das *et al.*, 2006) . The lesioning studies (i.e., chemical destruction of noradrenergic terminals) reduce the amount of NE release, this manipulation also reduces the release of other transmitters released with NE. The neuropeptides galanin and neuropeptide Y (NPY) and the neurotransmitter adenosine (i.e., ATP) are released at noradrenergic terminals and have been shown to exert anticonvulsant effects against several convulsant stimuli (Murray *et al.*, 1985; Mazarati *et al.*, 1992, 1998; Dichter *et al.*, 1994; Erickson *et al.*, 1996; Baraban *et al.*, 1997).

Dopamine

The mammalian prefrontal cortex (PFC) receives a substantial dopaminergic innervation from the midbrain ventral tegmental area (VTA) (Bjorklund & Lindvall

1984). Dopamine is an endogenous neuromodulator in the cerebral cortex and is believed to be important for normal brain processes (Bjorklund & Lindvall, 1986; Williams & Goldman-Rakic, 1995). There is strong evidence that alterations in dopamine function play a role in pathogenesis of a number of neuropsychiatric diseases including epilepsy (Starr *et al.*, 1996). *In vivo* studies have shown that dopamine increase and decrease spontaneous firing of neocortical neurons (Bunney & Aghajanian, 1976; Reader *et al.*, 1979;Ferron *et al.*, 1984; Bradshaw *et al.*, 1985; Sesack & Bunney, 1989; Bassant *et al.*, 1990; Yang & Mogenson, 1990; Thierry *et al.*, 1992; Pirot *et al.*, 1992). Dopamine favor long-lasting transitions of PFC neurons to a more excitable up state (Lewis & O'Donnell, 2000). *In vitro* electrophysiological experiments suggest that dopamine has multiple effects on PFC neurons. Both increases (Penit-Soria *et al.*, 1987; Yang & Seamans, 1996; Ceci *et al.*, 1999; Wang & O'Donnell, 2001; Gorelova & Yang, 2000; Henze *et al.*, 2000; Gonzalez-Burgos *et al.*, 2002; Tseng & O'Donnell, 2004) and decreases (Geijo-Barrientos & Pastore, 1995) in postsynaptic excitability of pyramidal neurons have been reported following D1 receptor activation. In addition, changes in excitability mediated by D2 receptors have been reported (Gulledge & Jaffe 1998; 2001; Tseng & O'Donnell, 2004). The effects of dopamine on synaptic responses are also complex and species-specific. AMPA receptor mediated excitatory postsynaptic currents (EPSCs) in layer V pyramidal cells are depressed by a D1 receptor–mediated effect of dopamine (Law-Tho *et al.*, 1994; Seamans *et al.*, 2001) Whereas *N*-methyl-D-aspartate (NMDA) responses have been reported to be both enhanced (Seamans *et al.*, 2001) and depressed (Law-Tho *et al.*, 1994). EPSCs in layers II/III are enhanced by dopamine in rats (Gonzalez-Islas & Hablitz, 2003) but decreased in primates (Urban *et al.*, 2002). The cerebral cortex contains interconnected local and distant networks of excitatory and inhibitory neurons. Stability of activity in such networks depends on the balance

between recurrent excitation and inhibition (Durstewitz *et al.*, 2000 ; Shu *et al.*, 2003). A shift of the balance toward excitation may lead to the generation of epileptiform activity. The presence of massive recurrent excitatory connections that depend on inhibition for regulation has been implicated in the susceptibility of the neocortex and the hippocampus to develop epileptiform activity and seizures (McCormick & Conteras, 2001). Modulatory influences strongly influence activity in thalamocortical (McCormick 1992; McCormick & Pape, 1990) and neocortical circuits (McCormick *et al.*, 1993). Dopamine is known to modulate epileptiform discharges both *in vivo* (Alam & Starr, 1992, 1993b, 1994a; George & Kulkarni, 1997) and *in vitro* (Alam & Starr 1993b, 1994b; Cepeda *et al.*, 1999; Siniscalchi *et al.*, 1997; Suppes *et al.*, 1985). In vivo studies in different models of epilepsy have suggested that dopamine may have a pro-convulsant effect mediated by D_1 receptors and an anti-convulsant effect via D_2 receptors (Starr *et al.*, 1996). Dopamine-mediated recruitment of neurons in local excitatory circuits and synchronization of activity in these neurons underlie these effects of dopamine in neocortex. Local excitatory neocortical networks are complexes of interconnected pyramidal neurons.

Several anti-epileptic drugs increase extracellular levels of dopamine (DA) and/or serotonin (5-HT) in brain areas involved in epileptogenesis (Smolders *et al.*, 1997). Behavioural and electrocorticographic studies in rats have shown that DA controls hippocampal excitability via opposing actions at D1 and D2 receptors (Bo *et al.*, 1995). Seizure enhancement is presumed to be a specific feature of D1 receptor stimulation, whereas D2 receptor stimulation is anticonvulsant (Alam & Starr, 1992, 1993). Decreased D2 receptor binding in the brainstem were reported in other neurological diseases like diabetes (Shankar *et al.*, 2006)

Serotonin

Central 5-HT_{1A} receptors function both as somatodendric presynaptic autoreceptors in the raphe nuclei as postsynaptic receptors in terminal field areas such as the hippocampus and many have different functional and regulatory characteristics, depending on the structures innervated (Barnes *et al*., 1999). In the raphe nuclei activation of 5-HT_{1A} autoreceptors produces inhibition of serotonergic neurons and decreases 5-HT release and neurotransmission. In contrast, postsynaptic 5-HT_{1A} receptor activation in the hippocampus increases 5-HT neurotransmission (Clarke *et al*., 1996). The 5-HT_{1A} somatodendric autoreceptors and postsynaptic receptors differ in their adaptive response to prolonged stimulation during long term treatment with selective serotonin reuptake inhibitors (SSRIs) such as fluoxetine, which has antiseizure effects in several models (Hernandez *et al*., 2002). The fluoxetine effect is not dependent on GABA receptors, may be mediated by multiple receptor subtypes and shows regional variation (Pasini *et al*., 1996). Rats treated over the long term with fluoxetine showed desensitation of 5-HT_{1A} somatodendric autoreceptors in the dorsal raphe nucleus but not of postsynaptic 5-HT_{1A} receptors in the hippocampus (Le Poul *et al*., 2000).

5-HT_{1A} receptor activation elicits a membrane hyper polarization response related to increase potassium conductance (Beck *et al*., 1991) and has an anticonvulsant effect in various experimental *in vivo* as well as *in vitro* seizure models. Including hippocampal kindled seizures in cats, intrahippocampal kainic acid induced seizures in freely moving rats and picrotoxin-bicuculline and Kainic acid induced seizures in rat hippocampal slice preparations (Wada *et al*., 1992). The anticonvulsant effects of 5-HT_{1A} receptor activation differ from region to region and from model to model. 5-HT is reported to inhibit low Mg^{2+} -induced epileptiform

activity, by reduction of NMDA receptor-mediated excitatory postsynaptic potentials in the subiculum and entorhinal cortex but not on areas CA3 and CA1 of hippocampus (Behr *et al.*, 1996).

The genetically epilepsy prone rat model (GEPR) illustrates 5-HT effects on seizure susceptibility. GEPRs have decreased 5-HT_{1A} receptor density in the hippocampus compared to non epileptic control rats (Statnick *et al.*, 1996). In addition the SSRI sertraline produces a dose dependent reduction in the intensity of audiogenic seizures in GEPRs, correlating with increased extracellular thalamic 5-HT concentrations (Yan *et al.*, 1995). However the model is complex and other neurotransmitters play a role, as 5-HT receptor activation increases release of catecholamines (Yan *et al.*, 1998). 5-HT_{1B} receptor was reported to inhibit rat ventral tegmental GABA release and 5-$HT_{1B/1D}$ activation increases nucleus accumbens dopamine release (Yan *et al.*, 2001).

Other receptor subtypes have received less attention. One study suggested an excitatory role of 5-HT_3 receptors in a rat kindling model (Wada *et al.*, 1997). Blockade of a number of receptors- 5-HT_3 and 5-$HT_{2A/C}$ was reported to not alter the reduction in seizure severity and increase in the threshold produced by fluoxetine (Watanabe *et al.*, 1998) Several knock out mouse models suggest a relation between 5-HT, hippocampal dysfunction and epilepsy. 5-HT_{1A} knockout mice display lower seizure thresholds and higher lethality in response to kainic acid administration. Furthermore, 5-HT_{1A} knockout mouse demonstrate impaired hippocampal dependent learning and enhanced anxiety related behaviors. Interactions between serotonergic and other neurotransmitters contribute to the behavioral phenotype (Sarnyai *et al.*, 2000). 5-HT_{2C} receptor knockout mice showed a combination of obesity and sound

induced seizures. Other receptor types are not altered in this model suggesting that the clinical effects are receptor subtype specific (Heiser *et al.*, 1998). In contrast activation of 5-HT_{2C} receptors potentates cocaine induced seizures (O'Dell *et al.*, 2000). The up-regulation of 5-HT_{2c} receptors were reported in the brain stem which induce sympathetic stimulation were reported (Pyroja *et al.*, 2007)

GABA

γ-Aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the CNS. It exerts an inhibitory action in all forebrain structures and plays a role in the physiopathogenesis of certain neurological conditions, including epilepsy. Impairment of GABA functions produces seizures, whereas enhancement results in an anticonvulsant effect. In tissue resected from patients with temporal lobe epilepsy, the number of GABA receptors are reduced in areas of hippocampus showing neuronal cell loss (McDonald *et al*, 1991; Johnson *et al*, 1992). Reduced BZD binding to GABA, receptors in mesial temporal lobe of such patients can be detected *in vivo* by noninvasive positron emission tomography imaging (Savic *et al.*, 1988). These changes are likely secondary to cell loss and not specific for GABA-receptive cells. Recent studies have shown some changes in $GABA_A$ receptors that occur in the neocortex of patients undergoing epilepsy surgery. These patients had TLE with severe damage and sprouting in limbic structures. Increased levels of steroid modulation of GABA, receptor ligand binding in neocortex were detected in patients with TLE. Increase in binding of diazepam- insensitive sites for the BZD ligand [3H]Ro15-45 13 associated with the *a4* GABA receptor subunit was also observed (Van Ness *et al*, 1995). Therefore, changes in the properties, rather than the number of GABA receptors possibly related to plastic changes in subunit combinations result in

an altered regulation of inhibitory function. Human focal epilepsy occurs commonly in the mesial temporal lobe often associated with Ammon's horn sclerosis. This is accompanied by severe gliosis and a sprouting in the molecular layer of the dentate gym (Babb *et al.*, 1989) as well as a dispersion of the granule cell layer (Houser *et al.*, 1990). This loss of neurons in the hippocampal formation is evident in CA3 and hilus, especially hilar mossy cells as evidenced by several neuronal markers including glutamic acid decarboxylase (GAD) and GABA receptors. One can mimic these changes in animals by producing lesions or using massive stimulation of hippocampal input (Sloviter *et al.*, 1991), kindling paradigms (Cavazos *et al*, 1991), or systemic kainite (Cronin *et al.*, 1992) or pilocarpine (Cavalheiro *et al.*, 1991). Like the human condition, these models involve end-folium sclerosis, including hilar interneuron loss and dentate granule cell hyperexcitability. The granule cells normally are inhibited laterally by hilar intemeurons, which are excited by mossy cells that innervate them longitudinally. Loss of these mossy cells has been proposed to make the surviving GABAergic basket cells "dormant," thus disinhibiting long stretches of granule cells (Sloviter *et al.*, 1991). In the pilocarpine model, there is loss of hilar cells, including GABAergic interneurons accompanied by decreased levels of mRNA and immunoreactivity of the GABAA receptor *a5* subunit in CA1/2 (Houser *et al.*, 1995). Loss of *a5* and *a2* mRNA was also observed by another group of investigators (Rice *et al.*, 1996) who demonstrated decreased GABA, synaptic activity in CA1. Therefore, in several of these animal models, there is evidence of reduced GABA-mediated inhibition.

Glutamate

Glutamate can cause convulsions when administered focally or systemically to experimental animals. Glutamate exerts its excitatory action *via* ligand-gated ion channels (NMDA and non-NMDA receptors) to increase sodium and calcium conductance. Reciprocal regulatory interactions exist between the activation of glutamatergic receptors and other transmitter systems, ion transport, gene activation and receptor modification. The flexibility and complexity of these interactions place glutamate-mediated transmission in a pivotal position for modulating the excitatory threshold of pathways involved in seizure generation. All classes of NMDA receptor antagonists (competitive NMDA antagonists, channel site antagonists, glycine site antagonists, polyamine site antagonists), as well as competitive and noncompetitive AMPA/kainate antagonists, display wide-spectrum anticonvulsant properties in acute and chronic animal epilepsy models, with varying degrees of behavioral side effects, ranging from minimal for some of the glycine site or competitive NMDA antagonists, to extensive for some of the high affinity open-channel NMDA antagonists.

Transgenic mice with an editing-deficient AMPA receptor subunit, GluR2, display early onset of epilepsy. The GluR2 subunit confers an almost complete block of calcium conductance in homomeric or heteromeric AMPA receptors. Both the GluR2 receptor level and the RNA editing process are reduced significantly, and the corresponding AMPA-evoked calcium current in pyramidal neurons increased significantly in accordance with the enhanced seizure susceptibility in these mice (Brusa *et al.*, 1995). Neuronal (EAAC-1) and glial (GLT-1 and GLAST) glutamate transporters facilitate glutamate and aspartate reuptake after synaptic release. A down-regulation of glutamate transporters would be compatible with enhanced excitatory activity. Transgenic mice with GLT-1 knockout display spontaneous epileptic activity (Tanaka *et al.*, 1997) and mice treated chronically with antisense probes to EAAC-1

shows reduced transporter levels and increased epileptic activity (Rothstein *et al.*, 1996). The reported changes in glutamate receptors and transporters subsequent to sustained or chronic epilepsy are less consistent and frequently transient in nature; some of these changes reflect patterns of cell loss. A functional enhancement of NMDA receptors is observed in amygdala-kindled rats and in resected tissue from humans with temporal lobe epilepsy (Mody *et al.*, 1998). The molecular alterations in the NMDA receptor responsible for this functional up regulation are not clearly defined but probably involve altered phosphorylation. Changes in the editing of the GluR2 AMPA subunit been reported in resected hippocampi from some patients with refractory epilepsy (Grigorenko *et al.*, 1997). The mRNA levels of multiple AMPA subunits are also altered in kindled rats and in rats after sustained seizure activity evoked by kainate or pilocarpine.

Metabotropic glutamate receptors are classified into three functional groups on the bases of their sequence homology, second messenger effectors and pharmacology (Dingledine & Conn 2000, Meldrum, 2000). Group I comprises mGluR1 and mGluR5, which are linked *via* G proteins to activation of phospholipase C. Group II (mGluR2 and mGluR3) and Group III (mGluR4, mGluR6, mGluR7, mGluR8) are both negatively linked to adenylyl cyclase activation. Activation of Group I mGluR enhances neuronal excitability by several mechanisms (blockade of accommodation to a steady current, potentiation of the effects of NMDA and AMPA and depolarization); accordingly, agonists acting on Group I receptors have convulsant activity (Ghauri *et al.*, 1996, Tizzano *et al.*, 1995). Conversely, Group I antagonists selective for mGluR1 have anticonvulsant activity in several experimental seizure models (Chapman *et al.* 1999 & 2000, Thomsen *et al.*, 1994). Activation of Group II and Group III receptors by reasonably selective agonists appears to have mixed convulsant/ anticonvulsant action, although a prolonged anticonvulsant action

seems to predominate (Tang *et al.*, 1997, Tizzano *et al.*, 1995). Down-regulation of mGluR8 in pilocarpine epileptic rats was reported early (Kral *et al.*, 2003). The anticonvulsant effect of metabotrophic glutamate 8 receptor agonist in the pilocarpine model of epilepsy was reported. (Jiang *et al.*, 2007)

L-Glutamate is the major excitatory neurotransmitter in the brain and serves a number of functions in the CNS (Nicholls & Attwell, 1990). This dicarboxylic amino acid is a precursor to the inhibitory amino acid neurotransmitter γ-aminobutyric acid (GABA) for the Krebs cycle intermediate a-ketoglutarate, and for the amino acid glutamine. Glutamate also functions as a detoxification agent for ammonia products in the brain. In addition to the many metabolic roles of glutamate, the most significant function of glutamate in the brain is its function as the primary excitatory neurotransmitter (Mayer & Westbrook, 1987). As a neurotransmitter, extracellular glutamate levels must be maintained at controlled levels. Although transporters exist to move glutamate into the brain across the blood-brain-barrier, the majority of glutamate is synthesized *de novo* from glucose, glutamine or aspartate (Lattera *et al.*, 1999). Glutamate is stored in synaptic vesicles. The signaling actions of glutamate are mediated at the neuronal membrane through specialized receptor macromolecules. The binding of glutamate to specific sites on its receptor molecule causes a conformational change that initiates signal transduction cascades in the neuron. Glutamate receptors are broadly categorized based on the signaling cascade they trigger. Ionotropic glutamate receptors are coupled to ion permeable channels which, under physiological conditions, depolarize neurons. In contrast, metabotropic receptors are coupled are coupled to guanosine triphosphate binding proteins (G proteins) and second messenger systems that modulate synaptic transmission (Dingledine *et al.*, 1999).

The ionotropic glutamate receptors are post-synaptic, ligand-gated ion channels. Three types of ionotropic glutamate receptors have been categorized and named according to selective ability of N-methyl-D-aspartate (NMDA), a-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) or kainate (KA) to activate them (Dingledine *et al.*, 1999). The AMPA receptor contributes to the early, fast component of the excitatory post-synaptic potential (EPSP). As a low affinity glutamate receptor, the AMPA receptor is typically permeable to the monovalent cations, sodium (Na^+) and potassium (K^+). However, AMPA receptors that lack a GluR2 subunit are also permeable to the divalent cation, Ca^{2+} (Wisden & Seeburg, 1993). This ligand-gated channel demonstrates little voltage dependence, and currents are very brief (a few milliseconds) due to the low glutamate affinity and a high rate of desensitization (Boulter *et al.*, 1990; Dingledine *et al.*, 1999). KA receptors are very similar in function to AMPA receptors. Like AMPA receptors, KA receptors are voltage-independent, monovalent cation permeable channels with low affinity and fast kinetics (Michaelis, 1998). KA receptor-mediated EPSPs have smaller peak amplitudes and slower decay kinetics than those derived from AMPA receptors (Frerking & Nicoll, 2000). The NMDA receptor is quite different from the AMPA and KA subtypes of glutamate receptor. First, in addition to their permeability to Na^+ and K^+, NMDA receptors have high permeability to Ca^{2+} (Dingledine *et al.*, 1999). NMDA receptors also have slower kinetics attributed to a much higher affinity for glutamate (Conti & Weinberg, 1999). The conductance through NMDA receptors can last several hundred milliseconds and constitutes a slower, later phase of the EPSP (Conti & Weinberg, 1999). Metabotropic receptors (mGluRs) are the other major category of glutamate receptors which is G-protein coupled. There are eight types of metabotropic glutamate receptors that are further classified according to the second messenger systems to which they are linked (Conn & Pin, 1997). These receptors are found both on the pre-

synaptic and post-synaptic membranes. Pre-synaptic mGluRs decrease neurotransmitter release, while mGluRs on the post-synaptic membrane regulate the function of ligand-gated ion channels including all three subtypes of ionotropic glutamate receptors (Anwyl, 1999). Thus, metabotropic glutamate receptors can act to modulate synaptic transmission in the CNS.

Calcium ion homeostasis

Calcium (Ca^{2+}) plays a fundamental role in the cell as a second messenger governing cellular functions such as differentiation and growth, membrane excitability, exocytosis, and synaptic activity. Neurons possess specialized homeostatic mechanisms to ensure tight command of cytosolic Ca^{2+} levels so that multiple independent Ca^{2+}-mediated signaling pathways can exist in the normal cell (Arundine and Tymianski, 2003). In excitotoxicity, excessive stimulation of glutamate receptors and an increase in extracellular glutamate concentration can lead to the disregulation of Ca^{2+} homeostasis (Arundine & Tymianski, 2003). An overwhelming increase in free intracellular calcium concentration can activate a self-destructive cellular cascade involving many calcium-dependent enzymes, such as phosphatases (eg, calcineurin), proteases and lipases. Lipid peroxidation can also cause production of free radicals which damage vital cellular proteins and lead to neuronal death (Choi, 1988; Michaels & Rothrnan, 1990; Tymianski & Tator, 1996; Delorenzo *et al.*, 2005).

The NMDA receptor mediates the vast majority of Ca^{2+} influx during excitatory neurotransmission (Ozawa, 1993). In addition, AMPA and **KA** receptors of certain subunit composition are permeable to Ca^{2+} (Jonas & Bumashev, 1995). Calcium extrusion across the plasma membrane. Two transport systems exist to pump

free intracellular Ca^{2+} out of the neuron into the extracellular space. Because Ca^{2+} extrusion acts against a large Ca^{2+} concentration gradient, these systems are energy-dependent and are, therefore highly susceptible to ischemic injury (Tymianski & Tator, 1996). The ATP-driven Ca^{2+} pump (Ca^{2+} - ATPase) expends one molecule of ATP for each Ca^{2+} ion extruded and is modulated by calmodulin, fatty acids, and protein kinases (Carafoli, 1992). The second transport system, the Na^{+}-Ca^{2+} exchanger, is indirectly coupled to ATP utilization in that it utilizes the Na+ concentration gradient maintained by the ATP driven Na^{+}- K^{+} exchanger. This electrogenic exchange system is triggered by increases in Ca^{2+} and extrudes one Ca^{2+} for every two or three Na^{+} that enters the neuron (Tymianski & Tator, 1996). Calcium buffering and sequestration can also reduce free intracellular Ca^{2+} levels. The endoplasmic reticulum (ER) functions as a Ca^{2+} store. Calbindin D-28k, one of the major CaBPs, is present at high cytosolic concentrations in neurons such as purkinje cells and hippocampal granule cells. Together with its high cytosolic concentration, the ability of calbindin to bind up to four Ca^{2+} ions at a time suggests that it plays an important role in Ca^{2+} buffering (Mattson *et al.*, 1995). The hippocampal fomiation is a locus of epileptic seizure activity (Lothman *et al.*, 1991). Recent research suggests that the absence of calcium buffer proteins results in marked abnormalities in cell firing (Bastianelli *et al.*, 2003). The calcium-binding proteins are present mainly in GABAergic intemeurons, thus their disturbance could result in an alteration of inhibitory mechanisms (Krsek *et al.*, 2004). Hippocampal neurons rich in the main Ca^{2+}-binding protein, calbindin D-28k, appear to be relatively resistant to degeneration in a variety of acute and chronic disorders (Sloviter, 1989; Hauser & Annegers, 1991; Magloczky *et al.*, 1997). Calbindin-like immunoreactivity is present in all dentate granule cells and some, but not all, CA1 and CA2 pyramidal cells in rat hippocampi. In area dentata, calbindin immunoreactivity is normally present in a

suggest that there is a loss of calbindin from granule cells of the dentate gyrus and select CA1 neuron populations in mouse models and in rat kindling models of epilepsy (Kohr *et al.*, 1991). Thus, the possible role of Ca^{2+} as a second messenger mediating some of these changes in hippocampal CA neurons, dentate granule neurons and interneurons is an important area of investigation.

Acetylcholine

The cholinergic system plays a crucial role in modulating cortical and in particular hippocampal functions including processes such as learning and memory (Ashe & Weimberger, 1991; Dunnett & Fibiger, 1993; Huerta & Lisman, 1993; Shen *et al.* 1994; Winkler *et al.*, 1995). Cholinergic actions are involved in the physiopathogenesis of epileptic discharges as suggested by the ability of some cholinergic agents to induce limbic seizures and histopathological changes resembling those seen in patients with temporal lobe epilepsy (Dickson & Alonso 1997; Liu *et al.* 1994; Nagao *et al.*, 1996; Turski *et al.*, 1989). Cholinergic stimulation of cortical neurons, including those located within the hippocampal formation, results in excitatory effects that are mediated mainly through the activation of muscarinic receptors (Krnjevic' *et al.* 1993; McCormick *et al.* 1993). Cholinergic innervation is present in the subiculum, which is a major synaptic relay station between the hippocampus proper and several limbic structures that are involved in cognitive processes (Amaral & Witter, 1989; Lopes da Silva, 1990). For instance, subicular cells recorded from freely moving animals generate "location specific" firing patterns; this indicates a possible contribution of this region to spatial learning (Barnes *et al.*,

1990; Sharp & Green, 1994). Subicular neurons are also involved in the spread of seizure activity within the limbic system (Lothman *et al.*, 1991). To date little is known about the effects of cholinergic agents in the subiculum. The EC is known to be a "gateway" for the bi-directional passage of information in the neocortical-hippocampalneocortical circuit (Van Hoesen *et al.*, 1982; Witter *et al.*, 1989; Lopes da Silva *et al.*, 1990) *via* a cascade of cortico-cortical projections, the superficial layers of the EC (II and III) receive an extensive input from polymodal sensory cortices (Jones & Powell, 1970; Van Hoesen & Pandya, 1975; Amaral *et al.*, 1983; Deacon *et al.*, 1983; Room & Groenewegen, 1986; Insausti *et al.*, 1987; Reep *et al.*, 1987) that is then conveyed to the hippocampal formation *via* the perforant path (Steward and Scoville,1976). In turn, the hippocampal formation projects back on the deep layers of the Entorhinal Cortex (EC) which provide output paths that reciprocate the input channels (Swanson & Cowan, 1977 ; Swanson & Kohler, 1986; Insausti *et al.*, 1997). In addition, the deep layers of the EC also project massively on the EC superficial layers (Kohler, 1986b; Dolorfo & Amaral, 1997) thereby closing an EC-hippocampal loop. Thus, by virtue of its extensive projection systems, the EC network may act powerfully in the generalization of temporal lobe seizures. The EC is also known to receive a profuse cholinergic input from the basal forebrain that terminates primarily in layers II and V (Lewis & Shute, 1967; Mellgren & Srebro, 1973; Milner *et al.*, 1983; Alonso & Ko"hler, 1984; Lysakowski *et al.*, 1989; Gaykema *et al.*, 1990), precisely those layers that gate the main hippocampal input and output. It is well known that the cholinergic system promotes cortical activation and the expression of normal population oscillatory dynamics. In the EC, *in vivo* electrophysiological studies have shown that the cholinergic theta rhythm is generated primarily by cells in layer II (Mitchell & Ranck, 1980; Alonso & Garcı´a-Austt, 1987a, b; Dickson *et al.*, 1995). In addition, *in vitro* studies have also shown that muscarinic receptor activation

promotes the development of intrinsic oscillations in EC layer II neurons (Klink & Alonso, 1997). On the other hand, some evidence indicates that altered activity of the cholinergic system is relevant to epileptogenesis.

Muscarinic receptors

Muscarinic receptors are a family of G protein-coupled receptors that have a primary role in central cholinergic neurotransmission. Specific agonists, which activate postsynaptic muscarinic receptors, stimulate cholinergic signaling (Valentin *et al.*, 2006). The muscarinic acetylcholine receptors are widely distributed throughout the body and subserve numerous vital functions in both the brain and autonomic nervous system (Hassal *et al.*, 1993). Activation of muscarinic receptors in the periphery causes decrease in heart rate, relaxation of blood vessels, constriction in the airways of the lung, increase in the secretions and motility of the various organs of the gastrointestinal tract, increase in the secretions of the lacrimal and sweat glands, and constriction in the iris sphincter and ciliary muscles of the eye (Wess, 1993). In the brain, muscarinic receptors participate in many important functions such as learning, memory and the control of posture.

Muscarinic receptors are members of a large family of plasma membrane receptors that transduce the intracellular signals *via* coupling to guanine nucleotide binding regulatory proteins. (Hulme *et al.*, 1990; Bonner, 1989; Nathanson, 1987). Molecular cloning studies have revealed the existence of five molecularly distinct mammalian muscarinic receptor proteins (Hulme *et al.*, 1990; Bonner, 1989).

All mammalian muscarinic receptor genes share one common feature with several other members of G-protein receptor gene family *i.e.*, their open reading frame

contained within a single exon (Bonner *et al.*, 1987). Like all other G protein coupled receptors, the muscarinic receptors are predicted to confirm to a generic protein fold consisting of seven hydrophobic transmembrane helices joined by alternating intracellular and extracellular amino-terminal domain and a cytoplasmic carboxy-terminal domain. The five mammalian muscarinic receptors display a high degree of sequence identity sharing about 145 amino acids. Characteristically all muscarinic receptors contain a very large third cytoplasmic loop, which, except for the proximal portions, displays virtually no sequence identity among the different subtypes (Bonner, 1989). Agonist binding to muscarinic receptors is thought to trigger conformational changes within the helical bundle, which are then transmitted to the cytoplasmic face where the interaction with specific G proteins occur. Site directed mutagenesis and receptor-modeling studies suggest that a conserved Asp residue present in TM II of almost all G protein coupled receptors plays a pivotal role in mediating the conformational changes associated with receptor activation (Wess, 1993).

The ligand binding to muscarinic receptors is predicted to occur in a pocket formed by the ring like arrangement of the seven transmembrane domains (Wess *et al.*, 1991; Hulme *et al.*, 1990). Ligand binding appears to be initiated by ion-ion interaction between positively charged amino head present in virtually all muscarinic receptor ligands and a conserved Asp residue located in TM III. In addition a previous mutagenesis study has shown that replacement of the conserved TM III Asp residue in the rat muscarinic M1 receptor with Asn results in a receptor unable to bind to [^{3}H] QNB.

Sequence analysis shows that the hydrophobic core of all muscarinic receptors contains a series of conserved Ser, Thr and Tyr residues, most of which do not occur in other G protein coupled receptors. Pharmacological analysis of mutant M3 muscarinic receptors showed that two Thr residues (Thr231 and Thr234) and four Tyr residues (Tyr148, Tyr506, Tyr529 and Tyr533) are important for high affinity acetylcholine binding (Wess *et al.*, 1991). It has been shown that a Pro 201 to Ala mutant M3 muscarinic receptor exhibits affinities for both muscarinic agonists and antagonists 80-450 times less than those of the wild type (Wess *et al.*, 1993).

In the periphery, among other effects, muscarinic receptors mediate smooth muscle contraction, glandular secretion and modulation of cardiac rate and force. In the central nervous system there is evidence that muscarinic receptors are involved in motor control, temperature regulation, cardiovascular regulation and memory. Interest in the classification of muscarinic receptors involved in functions at different locations has been heightened by the potential therapeutic application agents in areas such as Alzheimer's disease, Parkinson's disease, asthma, analgesia, and disorders of intestinal motility and cardiac and urinary bladder function (Caulfield & Birdsall, 1998).

Classification

Muscarinic receptors are widely distributed throughout the central and peripheral nervous system. They have critical functions in learning and memory, attention and motor activity (Levey, 1993; Weiner *et al.*, 1990; Bonner, 1989). The five muscarinic receptor subtypes are designated as M1 - M5. The odd-numbered receptors (M1, M3, and M5) couple to Gq/11, and thus activate phospholipase C,

which initiates the phosphatidyl inositol trisphosphate cascade. This leads to the dissociation of phosphatidyl 4, 5- bisphosphates (PIP2) into two components, i.e., IP_3 and DAG. IP_3 mediates Ca^{2+} release from the intracellular pool (endoplasmic reticulum), whereas DAG is responsible for activation of protein kinase C. On the other hand, PIP2 is required for the activation of several membrane protein, such as the "M current" channel and Na^{+}/Ca^{2+} exchangers and muscarinic receptor- dependent depletion of PIP2 inhibits the function of these proteins (Suh & Hille, 2005; Winks *et al.*, 2005; Fuster *et al.*, 2004; Meyer *et al.*, 2001; Caulfield & Birdsall, 1998; Bonner *et al.*, 1988; Bonner *et al.*, 1987;). The M1, M2 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

M1 receptors are predominantly expressed in the forebrain, including the cerebral cortex, hippocampus and corpus striatum, where this sub-type contributes by 50-60% to the total of the muscarinic receptors (Gerber *et al.*, 2001; Miyakawa *et al.*, 2001; Hamilton *et al.*, 1997). The M1 receptor subtype, which is also expressed in peripheral tissues, has been implicated in stress adaptive cardiovascular reflexes and central blood pressure control. Studies have shown that central administration of the M1 specific antagonist pirenzepine lowered the blood pressure (Buccafusco, 1996; Brezenoff & Xiao, 1986). A putative overexpression of the M1 subtype in selected brain areas of spontaneously hypertensive rats has been reported (Scheucher *et al.*, 1991). Muscarinic agonist depolarization of rat isolated superior cervical ganglion is mediated by M1 receptors (Brown *et al.*, 1980). M1 is one of the predominant

muscarinic receptor subtypes expressed in pancreatic islets (Gilon & Henquin., 2001). Studies in pancreatic islets revealed that activation of muscarinic receptors is pertusis toxin insensitive and Gq mediated. Muscarinic M1 receptor number decreased in the brainstem at time of pancreatic regeneration without any change in the affinity (Renuka *et al.*, 2006).

Muscarinic M2 receptor

Muscarinic receptor activation in guinea pig heart produces a reduction in force of contraction and a decrease in the rate of beating. These effects are probably the consequence of inhibition of voltage-gated Ca^{2+} channels and activation of inwardly rectifying K^{+} channels, respectively. Extensive studies with many antagonists have defined this response as being mediated by the M2 receptor (Caulfield, 1993). Muscarinic M2 receptors mediate both negative and positive ionotropic responses in the left atrium of the reserpinized rat, latter effect being insensitive to pertusis toxin (Kenakin & Boselli, 1990). Central cholinergic transmission can also be activated by inhibition of the presynaptic M2 acetylcholine autoreceptor using selective antagonists. The presynaptic M2 autoreceptor negatively influences the release of acetylcholine in several brain regions, including the striatum, hippocampus, and cerebral cortex (Kitaichi *et al.*, 1999; Zhank *et al.*, 2002; Billard *et al.*, 1995). A direct consequence of brain M2 autoreceptor inhibition is an elevation of acetylcholine release in the synaptic cleft. M2 receptor antagonists have been shown to enhance the release of acetylcholine in different brain structures (Stillman *et al.*, 1993, 1996).

Muscarinic M3 receptor

M3 muscarinic receptors are broadly expressed in the brain, although the expression level is not high, compared to those of the M1 and M2 receptors (Levey, 1993). Muscarinic M3 receptor is widely distributed in the peripheral autonomic organs with the highest expression found in the exocrine glands (Candell *et al.*, 1990; Pedder *et al.*, 1991; Kashihara *et al.*, 1992; Matsui *et al.*, 2000). Expression of the M3 receptor in the rat pancreatic islets and insulin secreting cell lines has been established (Lismaa, 2000). M3 receptor also triggers direct contractions of smooth muscle, however, it only represents a minor fraction of total muscarinic receptor population in smooth muscle. It is expressed in relatively low density throughout the brain. Studies using knock out mice for M3 receptors gave evidences for the primary importance of these receptors in the peripheral cholinergic system. In urinary bladder, pupillary muscles and intestinal smooth muscles the cholinergic contractions are mediated predominately by M3 receptors (Matsui *et al.*, 2000). Central Muscarinic M3 receptor subtypes functional balance is suggested to regulate sympathetic and parasympathetic activity (Renuka *et al.*, 2004).

Muscarinic M4 receptor

Muscarinic M4 receptor is known to be abundantly expressed in the striatum (Levey, 1993). Muscarinic M4 receptors act as inhibitory muscarinic autoreceptors in the mouse (Zhang *et al.*, 2002). The neuroblastoma-glioma hybrid cell line NG108–15 expresses M4 mRNA and M4 receptors can be detected readily in radioligand binding assays (Lazareno *et al.*, 1990). Inhibition of adenylyl cyclase activity by muscarinic agonists in rat corpus striatum is mediated by M4 receptors (Caulfield, 1993; Olianas *et al.*, 1996). Muscarinic M1 and M3 receptors function differently regulate glucose induced insulin secretion were reported (Renuka *et al.*, 2006).

Muscarinic M5 receptor

The M5 receptor was the last muscarinic acetylcholine receptor cloned. Localization studies have revealed that the M5R is abundantly expressed in dopamine-containing neurons of the substantia nigra par compacta, an area of the midbrain providing dopaminergic innervation to the striatum. Concordantly, oxotremorine-mediated dopamine release in the striatum was markedly decreased in M5R-deficient mice. More intriguingly, in M5R-deficient mice, acetylcholine induced dilation of cerebral arteries and arterioles was greatly attenuated (Yamada *et al.*, 2001), suggesting that the M5 receptor might be a suitable target for the treatment of cerebrovascular ischemia. Muscarinic M5 receptor subtype expressed at low levels in the brain (Hulme *et al.*, 1990; Hosey, 1992).

Studies of the M5 receptor have been hampered both by the lack of selective ligands and of tissues or cell lines that endogenously express the native receptor protein. Immunoprecipitation and RT-PCR studies have shown that the M5 receptor is expressed at very low densities in the mammalian brain. However, *in situ* hybridization studies have demonstrated that M5 transcripts are highly concentrated in the basal ganglia and are the only muscarinic receptor transcripts expressed on dopaminergic neurons in the substantia nigra pars compacta (SNc) and ventral tegmental area (VTA) (Reever *et al.*, 1997). Another potentially useful system is the eosinophilic leukemia cell line (EoL-1) where M5 receptors can be induced on differentiation with interferon-γ (Mita *et al.*, 1996).

Signal transduction by muscarinic activation

Gq-protein-coupled receptors (GqPCRs) are widely distributed in the CNS and play fundamental roles in a variety of neuronal processes. Their activation results in phosphatidyl inositol 4,5-bisphosphate (PIP2) hydrolysis and Ca^{2+} release from intracellular stores via the phospholipase C (PLC)-inositol 1,4,5-trisphosphate (IP_3) signaling pathway. Because early GqPCR signaling events occur at the plasma membrane of neurons, they might be influenced by changes in membrane potential (Billups *et al.*, 2006). Muscarinic receptors, which are G protein coupled, stimulate signaling by first binding to G protein complex ($\alpha\beta\gamma$) which provides specificity for coupling to an appropriate effector. The α subunit interacts with an effector protein or ion channel to stimulate or inhibit release of intracellular second messengers. Mutation analysis showed that the G protein is primarily but not exclusively acts through interaction with the third cytoplasmic loop. It is suggested that the short sequences, N terminal 16-21 and C terminal 19 amino acids of the loop play a key role in determining the specificity (Wess *et al.*, 1989).

Cyclic adenosine monophosphate

Adenylate cyclase can be either positively or negatively regulated by G protein coupled receptors resulting in an increase or decrease in the generation of the second messenger, Cyclic adenosine monophosphate (cAMP). The stimulation of muscarinic M2 and M4 receptors endogenously expressed in cell lines, results in the inhibition of adenylate cyclase. G protein reconstitution experiments have shown that M2 receptors inhibit adenylate cyclase through Gi and possibly through the pertusis toxin insensitive Gz. In neuroblastoma SK-N-SH cells which express endogenous

muscarinic M3 receptors stimulate adenylate cyclase activity (Baumgold & Fishman, 1988). The muscarinic M1 receptor which ectopically expressed at physiological levels in A9L cells, was shown to stimulate adenylate cyclase through an IP_3 and Ca^{2+} dependent mechanism (Felder *et al.*, 1989). In contrast, M1 receptors stimulate adenylate cyclase in CHO cells predominantly through an IP_3 and Ca^{2+} independent mechanism that also contained a small Ca^{2+} dependent component (Gurwitz *et al.*, 1994).

Phospholipase C

The family of phospholipase C (PLC) enzymes has been grouped into three classes, β, γ and δ (Rhee & Choi, 1992). PLC serves as the primary effector for the muscarinic M1 receptor that is coupled through Gq α subunits (Berstein *et al.*, 1992). Muscarinic M1, M3 and M5 receptors can stimulate the production of IP_3, independent of direct PLCβ and G protein interaction (Gusovsky, 1993). This alternate route for the generation of IP_3 involves the tyrosine kinase dependent phosphorylation of PLCγ, a mechanism normally stimulated by growth factors and their receptors (Meisenhelder *et al.*, 1989). Expression studies revealed that the cloned muscarinic M2 receptor stimulates PLC through a pertusis toxin-sensitive G protein although with lower efficiency than M1 or M3 receptors (Ashkenazi *et al.*, 1987). Inhibition of PLC by an endogenously expressed M2 receptor has been reported in FRTL5 cells suggesting that negative regulation may also occur in some cells (Bizzarri *et al.*, 1990).

Phospholipase A2

Phospholipase A2 catalyze the hydrolysis of membrane phospholipids to generate free arachidonic acid and the corresponding lysophospholipid. Muscarinic receptors have been shown to stimulate the release of arachidonic acid and its eicosanoid metabolites in a variety of tissues including heart, brain and muscle (Abdel-Latif, 1986). Ectopic transfection experiments indicates that the muscarinic M1, M3 or M5 receptors, but not M2 or M4 receptors are linked to phospholipase A2 activation (Conklin *et al.*, 1988; Felder *et al.*, 1990; Liao *et al.*, 1990). Muscarinic receptor stimulated release of arachidonic acid occurs predominantly through the activation of phospholipase A2 and phosphatidylcholine serves as the primary substrate. Studies suggested that calcium influx, through voltage independent calcium channel activation and diacylglycerol, through PLC activation were essential for phospholipase A2 activation (Felder *et al.*, 1990; Brooks *et al.*, 1989). In ileal smooth muscle cells, carbachol stimulated phospholipase A2 itself caused calcium influx, implicating an amplification mechanism in phospholipase A2 regulation (Wang *et al.*, 1993).

Phospholipase D

Muscarinic receptor stimulated phospholipase D has been reported in a number of cell types including canine synaptosomes (Qian & Drewes, 1989), rat astrocytoma cells (Martinson, 1990), human neuroblastoma cells (Sandmann & Wurtman, 1991) and rat parotid cells (Guillemain & Rossignol, 1992). Association of muscarinic subtypes with phospholipase D has been shown in human embryonic kidney cells transfected with the M1-M4 receptors. In most cells studied,

phospholipase C and D are usually stimulated simultaneously following receptor activation (Liscovitch, 1991).

Calcium influx and release from intracellular stores

Muscarinic receptors typically stimulate biphasic increases in intracellular calcium in most cells. The transient phase represents the release of calcium from IP_3 sensitive intracellular calcium stores. Calcium influx through calcium channels play a central role in the regulation of multiple signaling pathways activated by muscarinic receptors. In excitable cells such as neurons and muscle cells calcium passes predominantly through voltage sensitive calcium channels (VOCC). In non-excitable cells, such as fibroblasts and epithelial cells, calcium passes through a family of poorly characterised voltage - insensitive calcium channels. VICCs open in response to receptor activation and have been classified into (1) receptor operated calcium channels which are second messenger independent, (2) second messenger - operated calcium channels (SMOCCs) and (3) depletion operated calcium channels which open following IP_3 mediated depletion of intracellular stores and provide a source of calcium for refilling the stores.

***Bacopa monnieri* (Linn.) Pennell**
Family: Scrophulariaceae

Bacopa monnieri commonly called as 'Brahmi' in Malayalam and Hindi is a small creeping, glabrous, punctuate herb numerous ascending branches, commonly growing in most parts of India. It is being cultivated as a commercial crop. The main stem is green or slightly purplish, obtuse-angular and 10-30 cm long with rooting at

nodes. Leaves are opposite, short-petioled, obovate-oblong and somewhat succulent 1-2.5 cm long and 0.4-1 cm broad, glabrous on both sides and dotted with minute black specks. Flowers are solitary axially, white or purple-tinged. Fruits are ovoid capsules, about 5 mm long and glabrous. The plant flowers and fruits throughout the year, though mostly during February to April. Drug consists of fresh or dried whole plant.

The plant elaborates several triterpenoids of dammarane group which occur mostly as glycosides (saponins) and are present to the extent of 2-3% on dry herb basis and are considered medicinally valuable. Around 10 of these have been obtained pure (Bacosaponins A-F, Bacoposides III-IV). Betulinic acid a triterpene with known anticancer activity has also been obtained from the plant (Brown *et al.*, 1960). Four glycosides based on phenylethanol as basic unit have been isolated (Chakravarthy *et al.*, 2002). Of other secondary metabolites attention is drawn to flavonoids (luteolin and its glycosisdes), sugars (D-mannnitol) usual sterols (β-sitosterol, stigmasterol and its esters) and paraffins (heptacosane, hentriacontane).

The pharmacological properties of *Bacopa monnieri* were studied extensively and the activities were attributed mainly due to the presence of characteristic saponins called as Bacosides (Deepak & Amit, 2004). Bacosides are complex mixture of structurally closely related compounds, glycosides of either jujubogenin or pseudojujubogenin. Bacosides have been found to offer protective role in the synaptic functions of the nerves in hippocampus (Kishore *et al.*, 2005). There are few methods reported in the literature for quantification of Bacosides in plant extracts and formulations. Spectrophotometric methods (Pal & Sarin, 1992) developed based on the hydrolysis of Bacosides to an aglycone that has an absorption maximum at 278

nm. A high performance thin-layer chromatographic method was developed for the estimation of Bacoside A in *Bacopa monnieri* plant and its formulations (Shrikumar *et al.*, 2004). A few high performance liquid chromatographic (HPLC) methods were also developed for the quantification of Bacosides in *Bacopa monnieri* extracts and formulations (Pal *et al.*, 1998).

Due to the importance of *Bacopa monnieri* in the indigenous system of medicine, systematic chemical examinations of the plant have been carried out by several groups of researchers. The major chemical entity shown to be responsible for the memory-facilitating action of *Bacopa monnieri*, Bacoside A, was assigned as 3-(a-L-arabinopyranosyl)-O-b-D-glucopyranoside-10, 20-dihydroxy-16-keto-dammar-24-ene (Chatterji *et al.*, 1965). Bacoside A usually co-occurs with bacoside B; the latter differing only in optical rotation and probably an artifact produced during the process of isolating Bacoside A (Rastogi *et al.*, 1990). The chemical composition of bacosides, contained in the polar fraction, has been established on the basis of chemical and physical degradation studies. On acid hydrolysis, bacosides yield a mixture of aglycones, Bacogenin A1, A2, A3 (Kulshreshtha & Rastogi, 1973, 1974; Chandel *et al.*, 1977) and two genuine sapogenins, jujubogenin and pseudojujubogenin (Rastogi *et al.*, 1994).

Bacopa monnieri extracts and isolated bacosides have been extensively investigated in several studies for their neuropharmacological effects and a number of reports are available confirming their nootropic action. Preliminary studies established that the treatment with crude extract (Malhotra & Das, 1959) and with the alcoholic extract of *Bacopa monnieri* plant (Singh & Dhawan, 1982) enhanced learning ability in rats. Subsequent studies indicated that the cognition-facilitating effect was due to

two active saponins, Bacosides A and B, present in the ethanol extract (Singh & Dhawan, 1992). These active principles, apart from facilitating learning and memory in normal rats, inhibited the amnesic effects of scopolamine, electroshock and immobilization stress (Dhawan & Singh, 1996). It has been suggested that the Bacosides induce membrane dephosphorylation with a concomitant increase in protein and RNA turnover in specific brain areas (Singh *et al.*, 1990). Further, *Bacopa monnieri* has been shown to enhance protein kinase activity in the hippocampus which could also contribute to its nootropic action (Singh & Dhawan, 1997). A study of Bhattacharya *et al.*, (1999) reported that a standardized Bacoside-rich extract of *Bacopa monnieri*, administered for 2 weeks in rats, reversed cognitive deficits induced by intracerebroventricularly administered colchicines and by injection of ibotenic acid into the nucleus basalis magnocellularis. The central cholinergic system is considered the most important neurotransmitter involved in the regulation of cognitive functions. Cholinergic neuronal loss in hippocampal area is the major feature of Alzheimer's disease (AD) and enhancement of central cholinergic activity by anticholinesterase is presently the mainstay of the pharmacotherapy of AD-type senile dementia. Administration of *Bacopa monnieri* for two weeks reversed the depletion of acetylcholine, the reduction in choline acetylase activity and the decrease in muscarinic cholinergic receptor binding in the frontal cortex and hippocampus induced by neurotoxin, colchicines (Bhattacharya *et al.*, 1999). It has been suggested that the behavioral effects of cholinergic degeneration can be alleviated by a reduction in noradrenergic function (Sara *et al.*, 1989). *Bacopa monnieri* is known to lower norepinephrine and increase 5-hydroxytryptamine levels in the hippocampus, hypothalamus and cerebral cortex (Singh & Dhawan, 1997). *Bacopa monnieri* is suggested to indirectly modify Ach concentrations through its influence on other neurotransmitter systems. In a recent study, standardized extract of *Bacopa monnieri*

used to evaluate the antidementic and anticholinesterase activities in adult male Swiss mice (Das *et al.*, 2002). Antidementic activity was tested against scopolamine (3 mg/kg ip)-induced deficits in passive avoidance (PA) test.

The present work is to understand the alterations of total muscarinic, muscarinic M1 and glutamate receptors in the brain regions of pilocarpine induced epileptic rats. The work focuses on the evaluation of the antiepileptic activity of extracts of *Bacopa monnieri*, Bacoside A and Carbamazepine *in vivo*. The molecular changes in the muscarinic M1 receptors in the pre- and post-treated epileptic model with *Bacopa monnieri*, Bacoside A and Carbamazepine were also studied. These studies will help us to elucidate the functional role of muscarinic and glutamate receptors in epilepsy.

Materials and Methods

BIOCHEMICALS AND THEIR SOURCES

Biochemicals used in the present study were purchased from Sigma Chemical Co., St. Louis, USA. All other reagents were of analytical grade purchased locally.

CHEMICALS USED IN THE STUDY

Biochemicals: (Sigma Chemical Co., St. Louis, USA.)

Acetylthiocholine iodide, ethylene diamine tetra acetic acid (EDTA), Tris HCl, calcium chloride, pirenzepine, atropine, Glutamic acid, Carbamazepine, Pilocarpine

Radiochemicals

Quinuclidinylbenzilate, L-[Benzilic-4,4'-^{3}H] (Sp. Activity 42 Ci/mmol) was purchased from NEN life sciences products Inc., Boston, U.S.A. L-[G-^{3}H] Glutamic acid (Sp. Activity 49 Ci/mmol) was purchased from Amersham Biosciences, UK

Molecular Biology Chemicals

Random hexamers,, human placental RNAse inhibitor and dNTPS were purchased from Bangalore Genei, India. Reverse transcriptase enzyme MuMLV, was obtained from Amersham Life Science, UK. Tri-reagent kit was purchased from MRC, USA. Real Time-PCR Taqman probe assays on demand were purchased from Applied Biosystems, Foster City, CA, USA.

ANIMALS

Adult wistar rats of 250-300g body weight purchased from Amrita Institue of Medical Sciences, Cochin and Kerala Agriculture Unviersity, Mannuthy were used for all experiments. They were housed in separate cages under 12 hrs light and 12 hrs dark periods and were maintained on standard food pellets and water *ad libitum*.

PLANT MATERIAL

Specimens of *Bacopa monnieri* were collected from Cochin University area. The plants were taxonomically identified and authenticated by Mr. K.P. Joseph, Head of the Dept. of Botany (Retd), St. Peter's College, Kolenchery and voucher specimens are deposited at the herbarium of the Centre for Neuroscience, Dept. of Biotechnology, Cochin University of Science and Technology, Cochin, Kerala (No. MNCB3).

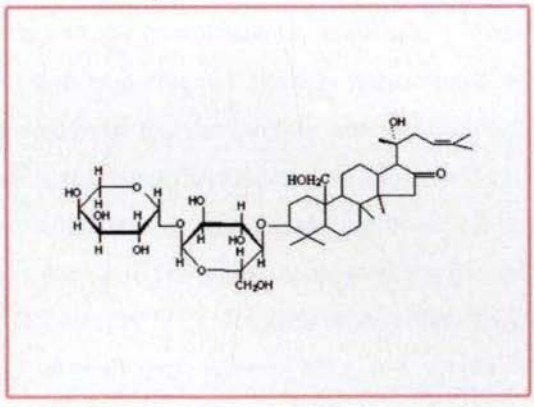
PREPARATION OF *Bacopa monnieri* PLANT EXTRACT

Whole plant *Bacopa monnieri* 10 g was mixed with 100ml of distilled water and homogeised well. The homogenate was filtered through cheese cloth This crude whole plant extract was used to study the anti epileptic effect in pilocarpine induced temporal lobe epilepsy in rats.

PREPARATION OF Bacoside A

Bacoside A was a generous gift from the Natural Remedies Pvt Ltd. Veerasandra Industrial Area, Bangalore, India and the extraction procedure was follows. The whole plant of *Bacopa monnieri* was dried in shade and then powdered plant material was extracted with distilled water. The aqueous extract was discarded and the residual plant material was extracted thrice with 90% ethanol. The residue obtained after removing the solvent was dried in vacuum and macerated with acetone to give a free-flowing power. The extract of *Bacopa*

Bacopa monnieri (L.) Pennel



Structure of Bacoside A (Levorotatory)

monnieri contained 40-50% bacoside estimated as Bacoside A. The estimation method involves acid hydrolysis of bacosides,which yields quantitatively a transformed aglycone-ebelin lactone which contained a conjugated triene system and was estimated by UV spectrophotometer at 278 nm (Pal & Sarin, 1992).

EPILEPSY INDUCTION

Adult male Wistar rats, weighing 250 to 300 g, were housed for 1 to 2 weeks before experiments were performed. Experimental animals were injected with pilocarpine (350 mg/kg i.p.), preceded by 30 min with atropine (1 mg/kg i.p.) to reduce peripheral pilocarpine effects. Within 20 to 40 min after the pilocarpine injection, essentially all of the animals developed *status epilepticus* (SE). Behavioral observation continued for 5 hrs after pilocarpine injection. Pilocarpine-induced seizures were graded according to the Racine scale using stage 1-5: Stage 0, in which the rats showed no convulsion; stage 1, in which rats showed Facial Automatism; stage 2, Head nodding 3, unilateral forelimb clonus 4, bilateral forelimb clonus 5, rearing, falling and generalized convulsions. The occurrence of stage 3-5 was considered as one complete seizure. SE was allowed to continue for 1 hr and then control and experimental animals were treated with diazepam (4 mg/kg i.p.). Animals recovered from this initial treatment within 2 to 3 days and were observed for the next 3 weeks. Animals were monitored by video recording and by clinical observation to evaluate the development of seizure discharge. Seizures were scored on a scale from 1 to 5, as used for the scoring of kindled seizures as described Racine, (1972). Over 80% of the animals were found to have recurrent partial and generalized seizures after 3-4 weeks after the initial pilocarpine injection. No seizures were observed in control animals.

24 days after pilocarpine treatment, the rats were continuously video monitored for 72 hrs. The behavior and seizures were captured with a CCD camera and a Pinnacle PCTV capturing software card and stored in the hard disk of the computer. One trained technician, blind to all experimental conditions,

viewed all videos. Seizure activity was rated according to Racine Scale. (Racine, 1972). Seizures were assessed by viewing behavioral postures (i.e. lordosis, straight tail, jumping/ running, forlimb clonus and/or rearing during fast forward observation of the videos. Once a behavioral posture was observed the video was rewound to the beginning of the behavior and examined at real-time speed.

DETERMINATION OF ANTI-EPILEPTIC POTENTIAL OF *Bacopa Monnieri* and Bacoside A

Animals used in this study were randomly divided into two groups

1) Pre-treated group
2) Post-treated group

Each of these group were subdivided into the following groups

a) Group 1: Control (given saline injection)

b) Group 2: Epileptic

c) Group 3: Epileptic rats treated with Carbamazepine

d) Group 4: Epileptic rats treated with *Bacopa monnieri*

e) Group 5: Epileptic rats treated with Bacoside A

Post-treatment

The rats were initially divided into two groups- Control and Epileptic. The epileptic group was injected with Pilocarpine according to the previously established protocols as described earlier. The control group received saline instead of pilocarpine. The epileptic group sowed spontaneous recurrent seizures approximately 20 minutes after pilocarpine injection. Those rats that did not show spontaneous seizures after pilocarpine treatment were excluded form the study group. The rats were singly housed and maintained for 24 days with standard food and water *ad libitum* after pilocarpine treatment. After 24 days the rats were

subjected continuous video monitoring for 72 hrs. The behavior and seizures were observed. Those experimental rats that did not show seizures were excluded from the study group. The experimental group was again divided into four. The first group that did not receive the treatment was epileptic. Carbamazepine was given orally to the third group of epileptic rats (150 mg/ kg body weight/day). Extract of *Bacopa monnieri* was given orally to the 4th group of epileptic rats in the dosage of 300 mg/Kg body weight/ day for 15 days. Bacoside A extract was given orally to the 5th group of epileptic rats in the dosage of 150mg/ Kg body/ day for 15 days. After 15 days of treatment the rats were again subjected to continuous video monitoring for 72 hrs. The rats were sacrificed after the video observation.

Pre-treatment

Extract of *Bacopa monnieri* was given orally to the 4th group of epileptic rats in the dosage of 300 mg/Kg body weight at 24 hour intervals. Bacoside A extract was given orally to the 5th group of epileptic rats in the dosage of 150mg/ kg body for 15 days and the rats were injected with pilocarpine at the 16th day. The rats were subjected to continuous video monitoring for 24 hrs and were sacrificed 24 hours after *status epilepticus*

SACRIFICE AND TISSUE PREPARATION

The animals were sacrificed by decapitation. The cerebral cortex, hippocampus, corpus striatum, brain stem, cerebellum and hypothalamus were dissected out quickly over ice according to the procedure of Glowinski & Iversen, (1966). Tissue samples were kept at -70° C until assay.

ACETYLCHOLINE ESTERASE ASSAY

Acetylcholine esterase asssay was done using the spectrophotometric method of Ellman *et al.*, (1961). The homogenate (10%) was prepared in sodium phosphate buffer (30mM, pH-7.0). One ml of 1% Triton x 100 was added to the

homogenate to release the membrane bound enzyme and centrifuged at 10,000 Xg for 30 minutes at 4°C. Different concentrations of acetylthiocholine iodide were used as substrate. The mercaptan formed as a result of the hydrolysis of the ester reacts with an oxidising agent 5,5' -dithiobis (2-Nitrobenzoate) absorbs at 412 nm.

MUSCARINIC RECEPTOR BINDING STUDIES USING [^{3}H] RADIOLIGANDS

Binding studies in the Brain regions

[^{3}H]QNB binding

[^{3}H]QNB binding assay in hippocampus, brain stem, cerebellum and cerebral cortex was done according to the modified procedure of Yamamura & Snyder (1981). Brain tissues were homogenised in a polytron homogeniser with 20 volumes of cold 50mM Tris-HCl buffer, containing 1mM EDTA (pH.7.4). The supernatant was then centrifuged at 30,000xg for 30 minutes and the pellets were resuspended in appropriate volume of Tris-HCl-EDTA buffer.

Total muscarinic receptor binding parameter assays were done using different concentrations i.e., 0.1-2.5nM of [^{3}H] QNB in the incubation buffer, pH 7.4 in a total incubation volume of 250µl containing appropriate protein concentrations (200-250µg). Non-specific binding was determined using 100µM atropine. Tubes were incubated at 22°C for 60 minutes and filtered rapidly through GF/C filters (Whatman). The filters were washed quickly by three successive washing with 5.0 ml of ice cold 50mM Tris-HCl buffer, (pH 7.4). Bound radioactivity was counted with cocktail-T in a Wallac 1409 liquid scintillation counter. The non-specific binding determined showed 10% in all our experiments.

Muscarinic M1 receptor binding assays were done using different concentrations i.e., 0.1-2.5nM of [^{3}H]QNB in the incubation buffer, pH 7.4 in a total incubation volume of 250µl containing appropriate protein concentrations (200-250µg). Non-specific binding was determined using 100µM pirenzepine.

Tubes were incubated at 220C for 60 minutes and filtered rapidly through GF/C filters (Whatman). The filters were washed quickly by three successive washing with 5.0 ml of ice cold 50mM Tris-HCl buffer, (pH 7.4). Bound radioactivity was counted with cocktail-T in a Wallac 1409 liquid scintillation counter.

Protein determination

Protein was measured by the method of Lowry *et al.*, (1951) using bovine serum albumin as standard. The intensity of the purple blue colour formed was proportional to the amount of protein, which was read in a spectrophotometer at 660nm.

GLUTAMATE DEHYDROGENASE ASSAY

Glutamate Dehydrogenase activity was estimated according to the procedure of Kaur and Kanungo, (1970). Sample extracts were prepared by making 5% homogenate of the tissue in ice cold Phosphate buffered saline, pH 7.4. The homogenate was centrifuge at 1000xg for 10 minutes to discard the nuclear pellet. The supernatant was centrifuged at 10,000xg for 20 minutes and the enzyme fraction was collected. The reaction mixture in the experimental and reference cuvettes contained triethanolamine buffer, pH 8.0, EDTA, ammonium acetate and enzyme sample of appropriate concentration. The reaction mixture of 1 ml volume was assayed at 366 nm using spectrophotometer by adding different concentration of α-ketoglutarate and 10mM NADH. Decrease in optical density due to oxidation of NADH was measured at 15 seconds interval for one minute at room temperature. The decrease in absorbance was linear during the course of all assays. One unit of enzyme activity was equal to the change in OD of 0.1 in 100 seconds at 366nm. Activity of enzyme was expressed as specific activity represented by units/mg of protein.

GLUTAMATE RECEPTOR BINDING STUDIES USING [^{3}H] RADIOLIGANDS

Membranes were prepared according to the modified method of Timothy *et al* (1984). Tissues were homogenized in 20 volumes of 0.32 M sucrose, 10 mM Tris/HCl and 1 mM $MgCl_2$ buffer, pH 7.4, with a polytron homogeniser. The homogenate was centrifuged twice at 1,000 *g* for 15 min at 4°C and the pellets were discarded. The supernatants were pooled and centrifuged at 27,000 x*g* for 15 min. The resulting pellet was lysed in a 10 mM Tris/HCl buffer, pH 7.4, for 30 min and centrifuged at 27,000 x*g* for 15 min. The resultant pellet was washed three times in 10 mM Tris/HCl buffer, pH 7.4, and centrifuged at 27,000 x*g* for 15 min. All steps were carried out at 4°C.

[^{3}H]-Glutamate binding. [^{3}H]-Glutamate binding assays were carried out according to Timothy *et al* (1984). Membranes were incubated in 0.25 ml reaction mixture containing 25 mM Tris/HCl, pH 7.4, 5 mM $MgCl_2$ and 10-350 nM [^{3}H]-glutamate. Incubation was carried out at 30°C for 15 min and the reaction was stopped by centrifugation at 27,000 x*g* for 15 min. The pellet and the wall of the tube were quickly and carefully washed with ice-cold distilled water. SDS (0.1%) and scintillation liquid were added to the dry pellet and radioactivity incorporated was determined with a Wallac scintillation counter. All the assays were carried out in triplicate. Nonspecific binding was determined by adding 350 μM nonradioactive glutamate to the reaction mixture in a parallel assay. Specific binding was considered to be the difference between total and nonspecific binding.

ANALYSIS OF THE RECEPTOR BINDING DATA

Linear regression analysis for Scatchard plots

The data were analysed according to Scatchard (1949). The specific binding was determined by subtracting non-specific binding from the total. The binding parameters, maximal binding (B_{max}) and equilibrium dissociation constant

(K_d), were derived by linear regression analysis by plotting the specific binding of the radioligand on X-axis and bound/free on Y-axis. The maximal binding is a measure of the total number of receptors present in the tissue and the equilibrium dissociation constant is the measure of the affinity of the receptors for the radioligand. K_d is inversely related to receptor affinity.

Nonlinear regression analysis for displacement curve

The displacement data were analysed by nonlinear regression using GraphPad Prism software, GraphPad Inc., USA. The concentration of the competing drug that competes for half the specific binding was defined as EC_{50} which is same as IC_{50} (Unnerstall, 1990). The affinity of the receptor for the competing drug is designated as K_i and is defined as the concentration of the competing ligand that will bind to half the binding sites at equilibrium in the absence of radioligand or other competitors (Cheng, 1973).

REVERSE TRANSCRIPTASE POLYMERASE CHAIN REACTION (RT-PCR)

Isolation of RNA

RNA was isolated from the brain regions of control and experimental rats using the Tri reagent (MRC., USA). 25-50 mg tissue homogenates were made in 0.5 ml Tri Reagent. The homogenate was kept in the room temperature for 5 minutes. 50µl of bromochloropropane (BCP) was added to the homogenate, kept in the RT for 10-15 minutes and was centrifuged at 12,000xg for 15 minutes at 4°C. The upper aqueous phase was transferred to a fresh tube and 250µl of isopropanol was added and the tubes were kept at room temperature for 10 minutes. The tubes were centrifuged at 12,000xg for 8 minutes at 4°C. RNA precipitate forms a pellet on the sides and bottom of the tube. The supernatant was removed and the RNA pellet was washed with 500µl of 75% ethanol, vortexed and centrifuged at 12,000xg for 5 minutes at 4°C. The pellets were semi

dried and dissolved in minimum volume of DEPC-treated water. 2 µl of RNA was made up to 1 ml and absorbance was measured at 260nm and 280nm. For pure RNA preparation the ratio of absorbance at 260/280 was ≥ 1.7. The concentration of RNA was calculated as one absorbance $_{260}$ = 42µg.

REAL-TIME POLYMERASE CHAIN REACTION

cDNA synthesis

Total cDNA synthesis was performed using ABI PRISM cDNA arhive kit in 0.2ml microfuge tubes. The reaction mixture of 20 µl contained 0.2µg total RNA, 10 X RT buffer, 25 X dNTP mixture, 10 X random primers, MultiScribe RT (50U/µl) and RNase free water. The cDNA synthesis reactions were carried out at 25 °C for 10 minutes and 37 °C for 2 hours using an Eppendorf Personal Cycler. The primers and probes were purchased from Applied Biosystems, Foster City, CA, USA.

Quantitative real-time PCR assays

Real time-PCR assays were performed in 96-well plates in a ABI 7300 real-time PCR instrument (Applied Biosystems). The TaqMan reaction mixture of 20 µl contained 25 ng of total RNA-derived cDNAs, 200 nM each of the forward primer, reverse primer, and TaqMan probe for muscarinic M1 receptor gene/ metabotrophic glutamate 8 receptor gene, endogenous control (β-actin) and 12.5 µl of Taqman 2X Universal PCR Master Mix (Applied Biosystems) and the volume was made up with RNAse free water. The thermal cycle conditions were as follows:

50 °C	---	2 min	
95 °C	---	10 min	
95 °C	---	15 sec	40 cycles
60 °C	---	01 min	

Fluorescence signals measured during amplification were considered positive if the fluorescence intensity was 20-fold greater than the standard deviation of the baseline fluorescence. The ΔΔCT method of relative quantification was used to determine the fold change in expression . This was done by first normalizing the resulting threshold cycle (CT) values of the target mRNAs to the CT values of the internal control β- actin in the same samples ($\Delta CT = CT_{Target} - CT_{\beta\text{- actin}}$). It was further normalized with the control ($\Delta\Delta CT = \Delta CT - CT_{Control}$). The fold change in expression was then obtained ($2^{-\Delta\Delta}C$ T).

EEG ANALYSIS IN CONTROL AND EXPERIMENTAL RATS

Spontaneous electrical activity of brain regions of the control and experimental rats were carried with Neurocare^{TM} Wingraph Digital EEG system. EEG analysis was done by placing electrodes in right and left frontal, parietal, occipital and temporal areas of the scalp of experimental rats and electrode placed on the ear was considered as reference. Each electrode was placed 10-20 percent away from the neighbouring electrode. The EEG recording data were analysed for the brain activity in different brain areas of control and experimental rats.

NEO-TIMM STAINING

The whole brains of the different experimental groups were stored in 4 % paraformaldehyde at room temperature for 3-5 days. They were sliced in coronal plane, processed for paraffin embedding in an automated tissue processor and 15 μm thick slices were collected on poly-L-lysine coated glass slides. Deparaffinised sections were rehydrated through graded alcohol and brought to glass distilled water. Batches of sections from the control and various test groups were placed in a glass jar containing a freshly prepared developer, maintained at 24-28°C in the dark. The developer contained a mixture of 240 ml gum Arabic solution (50% in distilled water), 40 ml citrate solution (9.4 g sodium citrate, 10.2 g citric acid in 40

ml dw), 120 ml of 5.6 g percent hydroquinone solution and 2.0 ml of 21.25 g percent silver nitrate solution. Batches of slides were developed for 30 to 45 min. washing the slides in running tap water terminated development of stain. The stained slides were dehydrated through graded series of alcohols, cleared in xylene and cover slipped in DPX mounting medium. Two investigators examined all the sections blind coded and the features were recorded. The staining procedure was repeated 3-4 times and slides showing consistent results were analyzed for evidence of neuronal sprouting

MORRIS WATER MAZE EXPERIMENT

Water maze experiment was conducted during post-treatment. The custom-constructed water maze pool measured 100 cm in diameter by 50 cm in depth and was filled with water to a depth of 35 cm. A 10-cm-diameter white platform was located 1.5 cm below the surface of the water. Nontoxic white paint was added to the water to visually obscure the location of the platform. The pool had been divided into four quadrants of homogeneous size and the platform was located in the center of one of the quadrants, halfway between the center and the wall of the pool. All swim latencies were recorded with a manual stopwatch, a technique routinely employed by others (Hort *et al.*, 1999). The water maze task consisted of 15 sessions conducted once daily over 15 successive days. Each session consisted of four trials separated by approximately 60 second. Rats were placed manually into the pool, facing the pool wall in the center of one of the quadrants that did not contain the platform. . For any given rat, the location of the platform remained fixed across all trials and all sessions. The latency to find the platform was recorded as the time from release into the pool until the rat had reached the platform. A maximum of 60 second was allowed for each trial. Rats not reaching the platform within 60 second were guided to the platform and a score of 60 second was recorded for each of these experimenter-terminated trials. The rat was allowed to remain on the platform for the duration of the inter-trial

interval. A 60-second probe test to determine the time spent in the platform quadrant after removing the platform from pool was conducted on the 13^{th} , 14^{th} and 15^{th} day of the study (24 h after the last hidden platform session). Rats were released into the pool in the quadrant opposite to that previously associated with the escape platform. A manual time-sampling procedure (one measurement per second) was utilized to record the swimming bias of the rat in each of the four quadrants of the pool.

STATISTICS

Statistical evaluations were done by ANOVA using InStat (Ver.2.04a) computer programme. Linear regression Scatchard plots were made using SIGMA PLOT (Ver 2.03).

Results

DRUG EFFECTS ON EPILEPTIC RATS

I. Seizure frequency after Carbamazepine, *Bacopa monnieri* and Bacoside A, in post-treated epileptic rats.

Seizure frequency per 4 hours over 72 hours video recording period showed a significant increase in epileptic group. Treatment with Carbamazepine, *Bacopa monnieri* and Bacoside A significantly ($p<0.01$) reduced the seizure frequency when compared to epileptic group (Table-1; Figure- 1-4).

II. Magnitude of Drug Effect

Mean difference of seizure frequency showed a significant decrease ($p<0.01$) in the Carbamazepine, *Bacopa monnieri* and Bacoside A, post-treatment when compared to epileptic group (Figure- 5).

III. Seizure onset latency after Carbamazepine, *Bacopa monnieri* and Bacoside A, pre-treatment.

Seizure onset latency showed a significant decrease ($p<0.01$) in the epileptic group when compared to Carbamazepine, *Bacopa monnieri* and Bacoside A, pre-treated groups (Table- 2, Figure- 6).

IV. Duration of *Status epilepticus* after Carbamazepine, *Bacopa monnieri* and Bacoside A, pre-treatment.

Duration of *Status epilepticus* significantly increased ($p<0.01$) in epileptic group when compared to Carbamazepine, *Bacopa monnieri* and Bacoside A, pre-treated groups (Table- 3, Figure- 7).

V. Effect of different dosage of Carbamazepine, *Bacopa monnieri* and Bacoside A, in post-treated epileptic rats

Carbamazepine and Bacoside A treatment in 150 and 300 mg/kg/day showed a significant decrease ($p<0.01$) in the seizure frequency when compared to epileptic group (Table-4a, Figure- 8). *Bacopa monnieri* treatment in 300 and 500 mg/kg/day showed a significant decrease ($p<0.01$) in the seizure frequency when compared to epileptic group (Table-4b, Figure- 8).

ACETYLCHOLINE ESTERASE ACTIVITY IN THE BRAIN REGIONS OF EXPERIMENTAL RATS

Hippocampus

Acetylcholine esterase kinetic studies showed that V_{max} significantly increased ($p<0.001$) in the hippocampus of epileptic group with no significant difference in the K_m when compared to control. Carbamazepine and extract of *Bacopa monnieri* treatment significantly reversed the V_{max} ($p<0.001$) to near control when compared to epileptic group (Table- 5, Figure- 9). Bacoside A treatment significantly reversed the V_{max} ($p<0.001$) to near control when compared to epileptic group (Table-6, Figure- 10). K_m showed no significant change in all the treated groups.

Brainstem

V_{max} of acetylcholine esterase significantly increased ($p<0.01$) in the brainstem of epileptic rats with no significant change in K_m when compared to control. Carbamazepine and *Bacopa monnieri* treatment significantly reversed the V_{max} ($p<0.01$) to near control when compared to epileptic group (Table-7, Figure- 11). Bacoside A treatment significantly reversed the V_{max} ($p<0.01$) to near control when compared to epileptic group (Table- 8, Figure- 12).

GLUTAMATE DEHYDROGENASE ACTIVITY IN THE BRAIN REGIONS OF EXPERIMENTAL RATS

Hippocampus

Glutamate dehydrogenase kinetics studies showed that V_{max} significantly increased ($p<0.01$) in the hippocampus of epileptic group with no significant change in K_m. Extract of *Bacopa monnieri* treatment significantly reversed the V_{max} ($p<0.01$) to near control when compared to epileptic group (Table- 9, Figure- 13).

Cerebellum

Glutamate dehydrogenase kinetics studies showed that V_{max} significantly increased ($p<0.01$) in the cerebellum of epileptic group with no significant change in K_m. Extract of *Bacopa monnieri* treatment significantly reversed the V_{max} ($p<0.01$) to near control when compared to epileptic group (Table- 10, Figure- 14).

Brainstem

Glutamate dehydrogenase kinetics studies showed that V_{max} significantly increased ($p<0.001$) in the brainstem of epileptic group with no significant change in

K_m. Extract of *Bacopa monnieri* treatment significantly reversed the V_{max} ($p<0.001$) to near control when compared to epileptic group (Table- 11, Figure- 15).

CENTRAL MUSCARINIC RECEPTOR ALTERATIONS IN THE BRAIN REGIONS OF EXPERIMENTAL RATS

Hippocampus

I) Total Muscarinic receptor analysis in post-treated epileptic rats

a) *Scatchard analysis of [^{3}H] QNB binding against atropine in the hippocampus of Control, Epileptic, Epileptic+Carbamazepine, Epileptic+Bacopa monnieri and Epileptic+Bacoside A, post-treated group rats*

The total muscarinic receptor status was assayed using the specific ligand, [^{3}H]QNB and muscarinic general antagonist, atropine. Scatchard analysis showed that the B_{max} increased significantly ($p<0.001$) in epileptic rats with a significant increase ($p<0.01$) in the K_d when compared to control. In Carbamazepine treated epileptic rats, B_{max} significantly ($p<0.001$) reversed to near control when compared to epileptic group. K_d also significantly ($p<0.05$) reversed to near control when compared to epileptic group. Extract of *Bacopa monnieri* treatment significantly reversed the B_{max} ($p<0.001$) and K_d ($p<0.01$) to near control when compared to epileptic group (Table-12 & Figure- 16). Bacoside A treatment significantly reversed the B_{max} ($p<0.001$) and K_d ($p<0.01$) to near control when compared to epileptic group (Table- 14 & Figure- 18).

b) Displacement analysis of [^{3}H] QNB against atropine

The competition curve for atropine against [^{3}H] QNB fitted for one site model in all groups. The log (EC_{50}) and K_i increased in epileptic condition and reversed to near control in Carbamazepine and Bacoside A treated epileptic rats (Table- 13,15 & Figure- 17,19).

II) Muscarinic M1 receptor analysis in post-treated epileptic rats

a) Scatchard analysis of [^{3}H] QNB binding against pirenzepine in the Hippocampus of Control, Epileptic, Epileptic+Carbamazepine, Epileptic+Bacopa monnieri and Epileptic+Bacoside A post- treated Epileptic rats

Binding analysis of muscarinic M1 receptors was done using [^{3}H]QNB and M1 subtype specific antagonist pirenzepine. The B_{max} increased significantly ($p<0.001$) in epileptic group when compared to control group. The K_d also increased significantly when compared to control group ($p<0.001$). In Carbamazepine treated epileptic rats B_{max} significantly ($p<0.001$) reversed back to near control when compared to epileptic group. K_d also significantly ($p<0.001$) reversed back to near control when compared to epileptic group. Extract of *Bacopa monnieri* treatment significantly reversed the B_{max} ($p<0.001$) and K_d ($p<0.01$) to near control when compared to epileptic group. (Table- 16 & Figure- 20). Bacoside A treatment significantly reversed the B_{max} ($p<0.001$) and K_d ($p<0.001$) to near control when compared to epileptic group (Table- 18 & Figure- 22).

b) Displacement analysis of [^{3}H] QNB using pirenzepine

The competition curve for pirenzepine against [^{3}H]QNB fitted for one site model in all groups. The log (EC_{50}) increased in epileptic group and reduced during Carbamazepine and *Bacopa monnieri* treatment. The K_i increased in epileptic condition and reversed to near control in Bacoside A treated epileptic rats (Table- 17, 19 & Figure- 21, 23).

III) Real Time-PCR analysis of Muscarinic M1 receptor mRNA in post-treated epileptic rats

Real Time -PCR analysis showed that the muscarinic M1 receptor mRNA increased significantly ($p<0.001$) in epileptic condition. It was reversed to near control in Carbamazepine ($p<0.001$), *Bacopa monnieri* ($p<0.01$) and Bacoside A ($p<0.01$) treated epileptic rats (Table-20, 21 & Figure- 24, 25).

IV) Muscarinic M1 receptor analysis in pre-treated epileptic rats

a) Scatchard analysis of [^{3}H] QNB binding against pirenzepine in the Hippocampus of Control, Epileptic, Epileptic+Carbamazepine, Epileptic+Bacopa monnieri and Epileptic+Bacoside A pre-treated Epileptic rats

Binding analysis of Muscarinic M1 receptors was done using [^{3}H]QNB and M1 subtype specific antagonist pirenzepine. B_{max} decreased significantly ($p<0.001$) in epileptic group when compared to control group. K_d also decreased significantly when compared to control group ($p<0.01$). In Carbamazepine treated epileptic rats B_{max} ($p<0.001$) and K_d ($p<0.01$) reversed significantly to near control when compared to epileptic group. Extract of *Bacopa monnieri* treatment significantly reversed the B_{max}

($p<0.001$) and K_d ($p<0.01$) to near control when compared to epileptic group (Table-22 & Figure- 26). Bacoside A treatment significantly reversed the B_{max} ($p<0.001$) and K_d ($p<0.01$) to near control when compared to epileptic group (Table- 24 & Figure-28).

b) Displacement analysis of [^{3}H] QNB using pirenzepine

The competition curve for pirenzepine against [^{3}H]QNB fitted for one site model in all groups. The log (EC_{50}) decreased in epileptic group and reversed to near control in Carbamazepine, *Bacopa monnieri* and Bacoside A treated epileptic rats. K_i increased in epileptic group and reversed to near control in Carbamazepine, *Bacopa monnieri* and Bacoside A treated epileptic rats (Table-23, 25 & Figure-27, 29).

V) Real Time-PCR analysis of Muscarinic M1 receptor mRNA in pre-treated epileptic rats

Real Time -PCR analysis showed that the muscarinic M1 receptor mRNA significantly ($p<0.001$) decreased in epileptic condition and it reversed to near control in Carbamazepine ($p<0.001$) *Bacopa monnieri* ($p<0.001$) and Bacoside A ($p<0.001$) treated epileptic rats (Table-26, 27& Figure- 30, 31).

Cerebellum

I) Total Muscarinic receptor analysis in post-treated epileptic rats.

a) Scatchard analysis of [3H] QNB binding against atropine in the Cerebellum of Control, Epileptic, Epileptic+Carbamazepine, Epileptic+Bacopa monnieri and Epileptic+Bacoside A post- treated Epileptic rats

Scatchard analysis of cerebellar total muscarinic receptors status showed that the B_{max} decreased significantly ($p<0.01$) in epileptic condition when compared to control group. The K_d showed no significant change in the epileptic group compared to control. In Carbamazepine treated epileptic condition B_{max} significantly ($p<0.01$) reversed to near control when compared to epileptic group. Extract of *Bacopa monnieri* treatment significantly reversed the B_{max} ($p<0.01$) to near control when compared to epileptic group without any change in K_d (Table- 28 & Figure- 32). Bacoside A treatment also significantly reversed the B_{max} ($p<0.01$) to near control when compared to epileptic group with out any change in K_d (Table-30 & Figure-34).

b) Displacement analysis of [^{3}H]QNB against Atropine

In the displacement analysis, the competitive curve fitted to a one-site model in all groups with Hill slope values near to unity. The K_i and EC_{50} decreased in epileptic condition and reversed to near control in Carbamazepine, *Bacopa monnieri* and Bacoside A treated epileptic rats (Table- 29, 31 & Figure- 33, 35).

II) Muscarinic M1 receptor analysis in post-treated epileptic rats

a) Scatchard analysis of [^{3}H] QNB binding against pirenzepine in the Cerebellum of Control, Epileptic, Epileptic+Carbamazepine, Epileptic+Bacopa monnieri and Epileptic+Bacoside A, post-treated Epileptic rats

Scatchard analysis of muscarinic M1 receptors showed that there was a significant decrease in B_{max} ($p<0.001$) and K_d ($p<0.001$) in epileptic rats compared to control group. In Carbamazepine treated epileptic group B_{max} significantly ($p<0.001$) reversed to near control when compared to epileptic group. The K_d also reversed to the near control level. Extract of *Bacopa monnieri* treatment significantly reversed the B_{max} ($p<0.001$) and K_d ($p<0.01$) to near control when compared to epileptic group (Table-32 & Figure- 36). Bacoside A treatment significantly reversed the B_{max} ($p<0.001$) and K_d ($p<0.01$) to near control when compared to epileptic group (Table-34 & Figure- 38).

b) Displacement analysis of [^{3}H] QNB against pirenzepine

In the displacement analysis, the competitive curve fitted to a one-site model in all groups with Hill slope values near to unity. The log (EC_{50}) and K_i showed a decrease in all the epileptic group. Treatment with Carbamazepine, *Bacopa monnieri* and Bacoside A reversed the log (EC_{50}) and K_i to near control when compared to epileptic group (Table-33, 35 & Figure-37, 39).

III) Real Time-PCR analysis of Muscarinic M1 receptor mRNA in post-treated epileptic rats

Real Time -PCR analysis showed that the muscarinic M1 receptor mRNA significantly decreased ($p<0.001$) in epileptic condition and it reversed to control level in Carbamazepine ($p<0.01$), *Bacopa monnieri* ($p<0.001$) and Bacoside A ($p<0.001$) treated epileptic rats (Table- 36, 37 & Figure- 40, 41).

IV) Muscarinic M1 receptor analysis in pre-treated epileptic rats

a) *Scatchard analysis of [^{3}H] QNB binding against pirenzepine in the Cerebellum of Control, Epileptic, Epileptic+Carbamazepine, Epileptic+Bacopa monnieri and Epileptic+Bacoside A, pre- treated Epileptic rats*

Scatchard analysis of Muscarinic M1 receptors showed that there was a significant increase in B_{max} ($p<0.001$) in epileptic rats when compared to control group. K_d showed no significant change. In Carbamazepine treated epileptic group B_{max} significantly ($p<0.01$) reversed to near control when compared to epileptic group. Extract of *Bacopa monnieri* treatment significantly reversed the B_{max} ($p<0.01$) to near control when compared to epileptic group (Table- 38 & Figure- 42). Bacoside A treatment also significantly reversed the B_{max} ($p<0.01$) to near control when compared to epileptic group with out any significant change in K_d (Table-40 & Figure- 44).

b) Displacement analysis of [^{3}H] QNB against pirenzepine

In the displacement analysis, the competitive curve fitted to a one-site model in all groups with Hill slope values near to unity. The log (EC_{50}) and K_i showed no significant change in the epileptic group compared to control. Carbamazepine, *Bacopa monnieri* and Bacoside A treatment decreased the log (EC_{50}) and K_i (Table-39, 41 & Figure-43, 45).

Brainstem

I) Total Muscarinic receptor analysis in post-treated epileptic rats.

a) *Scatchard analysis of [^{3}H] QNB binding against atropine in the Brainstem of Control, Epileptic, Epileptic+Carbamazepine , Epileptic+Bacopa monnieri and Epileptic+Bacoside A treated Epileptic rats*

Scatchard analysis showed that the B_{max} decreased significantly ($p<0.001$) in the brainstem of epileptic rats with a significant decrease ($p<0.01$) in the K_d when compared to control group. In Carbamazepine treated epileptic rats B_{max} significantly ($p<0.001$) reversed back to near control when compared to epileptic group. The K_d also reversed to near control when compared to epileptic group. *Bacopa monnieri* treatment significantly reverse the B_{max} ($p<0.001$) to near control when compared to epileptic group with a significant increase in K_d (Table- 42 & Figure- 46). Bacoside A treatment significantly reversed the B_{max} ($p<0.01$) and K_d ($p<0.01$) to near control when compared to epileptic group (Table-44 & Figure- 48).

b) Displacement analysis of [^{3}H]QNB using Atropine

In the displacement analysis, the competitive curve fitted to a one-site model in all groups with Hill slope values near to unity. The log (EC_{50}) and K_i showed no change in all the experimental groups. (Table- 43, 45 & Figure- 47, 49).

II) Muscarinic M1 receptor analysis in post-treated epileptic rats.

a) *Scatchard analysis of [^{3}H] QNB binding against pirenzepine in the Brainstem of Control, Epileptic, Epileptic+Carbamazepine, Epileptic+Bacopa monnieri and Epileptic+Bacoside A, post-treated rats*

Scatchard analysis showed that the B_{max} decreased significantly ($p<0.001$) in epileptic condition when compared to control group. K_d also decreased significantly when compared to control group ($p<0.01$). In Carbamazepine treated epileptic condition B_{max} ($p<0.001$) and K_d ($p<0.05$) were significantly reversed to near control when compared to epileptic group. *Bacopa monnieri* extract treatment significantly reversed the B_{max} ($p<0.001$) to near control when compared to epileptic group. K_d also reversed to near control when compared to epileptic group ($p<0.01$) (Table- 46 & Figure- 50). Bacoside A treatment also significantly reversed the B_{max} ($p<0.001$) and K_d ($p<0.01$) to near control when compared to epileptic group (Table- 48 & Figure- 52).

b) Displacement analysis of [^{3}H]QNB using pirenzepine

The competition curve for pirenzepine against [^{3}H]QNB fitted for one site model in all groups. The K_i showed an increase in epileptic group which reversed to

control in the *Bacopa monnieri* and Bacoside A treated rat groups (Table- 47, 49 & Figure- 51, 53). Carbamazepine did not reverse the increased K_t in epileptic rats.

III) Real Time-PCR analysis of Muscarinic M1 receptor mRNA in post-treated epileptic rats

Real Time-PCR analysis showed that the muscarinic M1 receptor mRNA significantly decreased ($p<0.001$) in epileptic condition and it reversed to near control in Carbamazepine treated ($p<0.001$), *Bacopa monnieri* ($p<0.001$) and Bacoside A ($p<0.001$) treated epileptic rats (Table-50, 51 & Figure- 54, 55).

GLUTAMATE RECEPTOR ALTERATIONS DURING EPILEPSY AND AFTER THE TREATMENT WITH *Bacopa monnieri* EXTRACT

Hippocampus

I) Total Glutamate receptor analysis in post-treated epileptic rats.

a) ***Scatchard analysis of [^{3}H] Glutamate binding against glutamate in the Hippocampus of Control, Epileptic and Epileptic+Bacopa monnieri treated Epileptic rats***

Scatchard analysis showed that the B_{max} decreased significantly ($p<0.01$) in the hippocampus of epileptic rats with out a significant change in K_d. Extract of *Bacopa monnieri* treatment significantly reversed the B_{max} ($p<0.01$) to near control when compared to epileptic group with out any change in K_d (Table-52 & Figure-56).

b) Displacement analysis of [^{3}H] Glutamate against glutamate

In the displacement analysis, the competitive curve fitted to a one-site model in all groups with Hill slope values near to unity. The log (EC_{50}) and K_i showed a significant decrease in the epileptic group which reversed to near control by *Bacopa monnieri* treatment (Table- 53 & Figure- 57).

II) Real Time-PCR analysis of NMDA R1 receptor in post-treated epileptic rats.

Real Time-PCR analysis showed that the NMDA R1 receptor mRNA significantly decreased ($p<0.01$) in epileptic group and it reversed to control in *Bacopa monnieri* treated ($p<0.05$) epileptic rats (Table- 54 & Figure- 58).

Cerebellum

I) Total Glutamate receptor analysis in post-treated epileptic rats.

a) *Scatchard analysis of [^{3}H] Glutamate binding against glutamate in the Cerebellum of Control, Epileptic and Epileptic+Bacopa monnieri post- treated Epileptic rats*

Scatchard analysis showed that the B_{max} decreased significantly ($p<0.001$) in the cerebellum of epileptic rats with out a significant change in K_d. *Bacopa monnieri* treatment significantly reversed the B_{max} ($p<0.001$) to near control when compared to epileptic group with out a change in K_d (Table- 55 & Figure- 59).

b) Displacement analysis of [^{3}H] Glutamate against glutamate

In the displacement analysis, the competitive curve fitted to a one-site model in all groups with Hill slope values near to unity. The log (EC_{50}) and K_i showed a

decrease in the epileptic group. Treatment with *Bacopa monnieri* reversed the K_i to near control. *Bacopa monnieri* treatment also increased the EC_{50} compared to the epileptic group (Table-56 & Figure-60).

II) Real Time-PCR analysis of NMDA R1 and metabotrophic glutamate 8 receptor in post-treated epileptic rats.

Real Time-PCR analysis showed that the NMDA R1 receptor mRNA significantly decreased ($p<0.001$) in epileptic condition and it reversed to near control in *Bacopa monnieri* ($p<0.01$) treated epileptic rats (Table- 57 & Figure- 61). Real Time-PCR analysis showed that the metabotrophic glutamate 8 receptor mRNA significantly decreased ($p<0.01$) in epileptic condition and it reversed to near control in *Bacopa monnieri* ($p<0.05$) treated epileptic rats (Table- 58 & Figure- 62)

Brainstem

I) Total Glutamate receptor analysis in post-treated epileptic rats.

a) *Scatchard analysis of [^{3}H] Glutamate binding against glutamate in the brainstem of Control, Epileptic and Epileptic+Bacopa monnieri treated Epileptic rats*

Scatchard analysis showed that the B_{max} decreased significantly ($p<0.01$) in the Brainstem of epileptic rats with out a significant change in K_d. *Bacopa monnieri* treatment significantly reversed the B_{max} ($p<0.01$) to near control when compared to epileptic group with out a change in K_d (Table- 59 & Figure- 63).

b) Displacement analysis of [^{3}H] Glutamate against glutamate

In the displacement analysis, the competitive curve fitted to a one-site model in all groups with Hill slope values near to unity. The log (EC_{50}) and K_i showed no significant change in experimental groups. (Table- 60 & Figure- 64).

II) Real Time-PCR analysis of NMDA R1 and metabotrophic glutamate 8 receptor in post-treated epileptic rats.

Real Time-PCR analysis showed that the NMDA R1 receptor mRNA significantly decreased ($p<0.001$) in epileptic condition and it reversed to near control in *Bacopa monnieri* ($p<0.01$) treated epileptic rats (Table- 61 & Figure- 65). Real Time-PCR analysis showed that the metabotrophic glutamate 8 receptor mRNA significantly decreased ($p<0.001$) in epileptic condition and it reversed to near control in *Bacopa monnieri* ($p<0.05$) treated epileptic rats (Table- 58 & Figure- 62)

Cerebral Cortex

I) Total Glutamate receptor analysis in post-treated epileptic rats.

a) *Scatchard analysis of [^{3}H] Glutamate binding against glutamate in the Cerebral Cortex of Control, Epileptic and Epileptic+Bacopa monnieri treated Epileptic rats*

Scatchard analysis showed that the B_{max} decreased significantly ($p<0.01$) in the cerebral cortex of epileptic rats with out any significant change in K_d. *Bacopa monnieri* treatment significantly reversed the B_{max} ($p<0.01$) to near control when compared to epileptic group with out a change in K_d (Table- 63 & Figure- 67).

b) Displacement analysis of [^{3}H] Glutamate against glutamate

In the displacement analysis, the competitive curve fitted to a one-site model in all groups with Hill slope values near to unity. The log (EC_{50}) and K_i showed no significant change in experimental groups. (Table- 64 & Figure- 68).

NEO-TIMM STAINING IN THE HIPPOCAMPUS IN POST-TREATED EPILEPTIC RATS

Neo-Timm silver staining in the Hippocampus showed that densely stained CA1 region of the epileptic rats compared to control which confirms mossy fibre sprouting. Treatment with Carbamazepine and *Bacopa monnieri* did not show reversal to the control status (Figure- 69 a-d).

ELECTROENCEPHALOGRAM ANALYSIS IN PRE- AND POST-TREATED EPILEPTIC RATS.

Electroencephalogram analysis showed that there is a change in the brain activity of temporal areas of epileptic rats when compared to control. Treatment with Carbamazepine, *Bacopa monnieri* and Bacoside A decreased the change in the brain activity to near control range in both pre-treated and post-treated groups (Figure- 70 a-e, 71 a-e).

MORRIS WATER MAZE EXPERIMENT IN THE POST-TREATED EPILEPTIC RATS

Morris water maze experiment showed a significant increase in the escape latency of epileptic group when compared to control. *Bacopa monnieri* treatment significantly ($p<0.001$) reversed the escape latency to near control (Table- 65, Figure-72).

Time spent in the platform quadrant of the epileptic rats showed a significant decrease ($p<0.01$) when compared to control. *Bacopa monnieri* post-treatment ($p<0.01$) reversed the time spent in the platform quadrant to near control (Table- 66, Figure- 73).

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_{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 an another suggestive evidence of the receptor enhanced susceptibility to the ligand.

Septo-hippocampal cholinergic fibers ramify extensively through out 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 potentates 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_{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 hyperexitability 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 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 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 & Dingledine, 1981). Moreover, muscarinic receptor stimulation significantly enhanced the spontaneous firing of the hippocampal GABA-ergic 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 B_{max} was observed when compared to the Epileptic group. 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 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 hyperexitability 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 hyperexitability 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 cyclohexamide 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_{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' diaschisis 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-mercaptopropionic acid was reported reversible increases in [^{3}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_{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 hyperexitability 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_{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 hyperexitability. Our previous report suggests that the acetylcholine esterase activity is increased in the brainstem of the epileptic rats compared to control. The hyperexitability 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_{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_{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_{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_{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 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_{max} reversed to the control level. NMDA R1 and metabotrophic 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_{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_{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. Metabotrophic 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.*, 199 l) 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*

al.,1999, 2000). 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.

Summary

1) Pilocarpine induced Temporal lobe epileptic rats were used as a model to study the alterations of muscarinic, muscarinic M1, glutamate receptors and their functional regulation by *Bacopa monnieri* and Bacoside A.

2) Antiepileptic activity of whole plant extract of *Bacopa monnieri* and Bacoside A were evaluated by seizure frequency over 72 hours video recording period, magnitude of drug effect in post-treatment and seizure onset latency and seizure duration in pre-treatment.

3) Acetylcholine esterase activity has been used as a marker for cholinergic activity. Acetylcholine esterase activity was measured in the brain regions. In epileptic rats the acetylcholine esterase activity was increased in the hippocampus and brainstem. In epileptic rats treated with Carbamazepine, *Bacopa monnieri* and Bacoside A, the activity of the enzyme reversed to near control.

4) Muscarinic receptor functional status was analyzed by Scatchard and displacement analysis using specific ligands [^{3}H] QNB- general muscarinic antagonist, atropine-non radioactive general muscarinic antagonist. In epileptic rats, total muscarinic receptors were up-regulated in the hippocampus whereas it was down regulated in cerebellum and brainstem during post-treatment with Carbamazepine, *Bacopa monnieri* and Bacoside A.

5) Muscarinic M1 receptor functional status was analyzed by scatchard and displacement analysis using specific ligands [^{3}H] QNB- general muscarinic antagonist and pirenzepine-non radioactive muscarinic M1 antagonist. During epilepsy muscarinic M1 receptors were up regulated in the hippocampus whereas it was down regulated in cerebellum and brainstem. Post-treatment with Carbamazepine, *Bacopa monnieri* and Bacoside A reversed the receptor changes to near control level. Muscarinic M1 receptors were down regulated in the hippocampus during the initial phase whereas it was up regulated in cerebellum. Pre-treatment with Carbamazepine, *Bacopa monnieri* and Bacoside A reversed the receptor changes to near control.

6) Receptor binding parametres were confirmed by studying the mRNA status of the correponding receptor using Real Time-PCR . In hippocampus, the muscarinic receptors were up regulated in epilepsy and it was down regulated during the initial phase. In Carbamazepine, *Bacopa monnieri* and Bacoside A pre- and post- treated epileptic rats, the receptor activity reversed to near control.

7) Real Time-PCR studies in the cerebellum and brainstem showed that the muscarinic receptors are down regulated in epileptic rats. In Carbamazepine, *Bacopa monnieri* and Bacoside A post-treated epileptic rats, the receptor activity reversed to near control.

8) Glutamate dehydrogenase activity in the hippocampus, cerebellum and brainstem showed a significant increase in epileptic rats. In *Bacopa monnieri*

post-treated epileptic rats, glutamate dehydrogenase activity reversed to near control.

9) Glutamate receptor binding parameters in the hippocampus, cerebellum and brainstem showed a significant decrease in the binding in epileptic rats. In *Bacopa monnieri* post-treated epileptic rats, glutamate receptor binding parametrs reversed to near control.

10) Real Time–PCR studies of NMDA R1 in the hippocampus, cerebellum and brainstem showed that the NMDA R1 receptors were down regulated in the epileptic rats. In *Bacopa monnieri* post-treated epileptic rats, the receptor activity reversed to near control.

11) Real Time–PCR studies of metabotrophic glutamate 8 receptor in the cerebellum and brainstem showed down regulation in the epileptic rats. In *Bacopa monnieri* post-treated epileptic rats, the receptor activity reversed to near control.

12) Neo-Timm staining in the hippocampus of epileptic rats showed a dense stainning in the CA1 region when compared to control which confirms mossy fibre sprouting. Post- treatment with Carbamazepine and *Bacopa monnieri* did not show reversal to the control.

13) A prominent brain activity difference was observed in epileptic rats compared to control by EEG analysis. In Carbamazepine, *Bacopa monnieri* and

Bacoside A pre- and post-treated epileptic rats, the brain activity reversed to near control.

14) Spatial learning ability of the rats were studied by Morris Water Maze experiment. In epileptic rats the learning ability of rats were impared compared to control. *Bacopa monnieri* post-treatment reversed the learning ability to near control.

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, Muscarinic M1 and glutamate receptors. This has immense clinical significance in the therapeutic management of epilepsy.

Conclusion

We conclude from our studies that cholinergic system through muscarinic, muscarinic M1 and glutamate receptors play an important role in the pathophysiology of pilocarpine induced temporal lobe epilepsy in rats. Cholinergic activity indicated by acetylcholine esterase as a marker for cholinergic system increased in brain regions- hippocampus and brainstem in epilepsy. Treatment of epileptic rats with Carbamazepine, *Bacopa monnieri* and Bacoside A reversed the enzyme status to near control. The functional changes in muscarnic receptors studied in the brain regions showed that total muscarinic and muscarinic M1 receptors were up regulated in the hippocampus and were down regulated in the cerebellum and brainstem in the epileptic state. Post-treatment with Carbamazepine, *Bacopa monnieri* and Bacoside A, reversed the receptor changes to near control. Glutamate receptors were down regulated in the hippocampus, cerebellum and brainstem in epileptic rats and post-treatment with *Bacopa monnieri* reversed the receptor changes to near control. Pre-treatment with Carbamazepine, *Bacopa monnieri* and Bacoside A significantly reduced the occurance of seizures mediated through muscarinic receptors. Electrophysiological studies confirmed the reversal observed with enzyme and receptor studies. Morris water maze experiment also showed the impairment in the spatial learning task during epilepsy and the treatment with *Bacopa monnieri* reversed the effect to near control. Thus our studies suggest that central muscarinic, muscarinic M1 and glutamate receptor subtype functional balance play an important role in pilocarpine induced temporal lobe epilepsy in rats. *Bacopa monnieri* and Bacoside A extracts have regulatory role in epilepsy through muscarinic and glutamate receptors. This has therapeutic application in the management of epilepsy.

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- **Reas Khan S,** Gireesh G and C.S.Paulose (2005) Decreased Glutamate decarboxylase activity in the cerebellum of pilocarpine induced epileptic rats. International Symposium of Advances in research on neurodegenerative diseases at University of Madras, Chennai

- Dr. Jerry Ignatius, P. S. John, Dr. C. S. Paulose, Santhosh K Thomas, **Reas Khan**, Gireesh G. Upregulation of neurotransmitter receptors- a possible mechanism for accelerated fracture healing. The journal of Kerala Orthopaedic Association (KOA) ISSN No. 0973-1709, 20: 31-39 (2006).

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Figure- 1

Seizure frequency per 4 hours interval over the 72 hours video recording period of Pilocarpine induced Epileptic rats

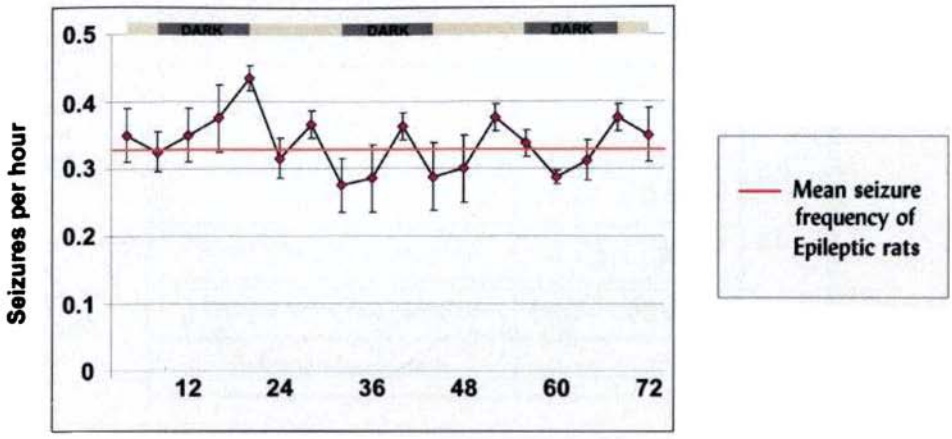


Figure- 2

Seizure frequency per 4 hours interval over the 72 hours video recording period of Pilocarpine induced Epileptic rats after 15 days Carbamazepine post-treatment

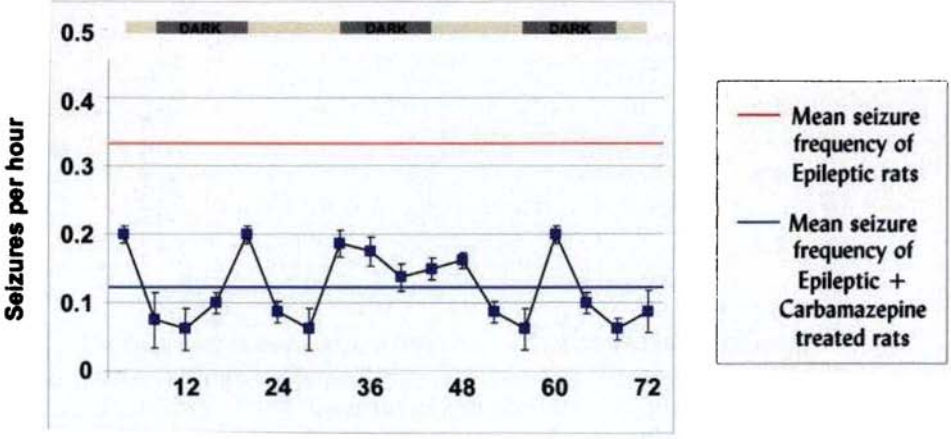


Figure- 3

Seizure frequency per 4 hours interval over the 72 hours video recording period of Pilocarpine induced Epileptic rats after 15 days *Bacopa monnieri* post-treatment

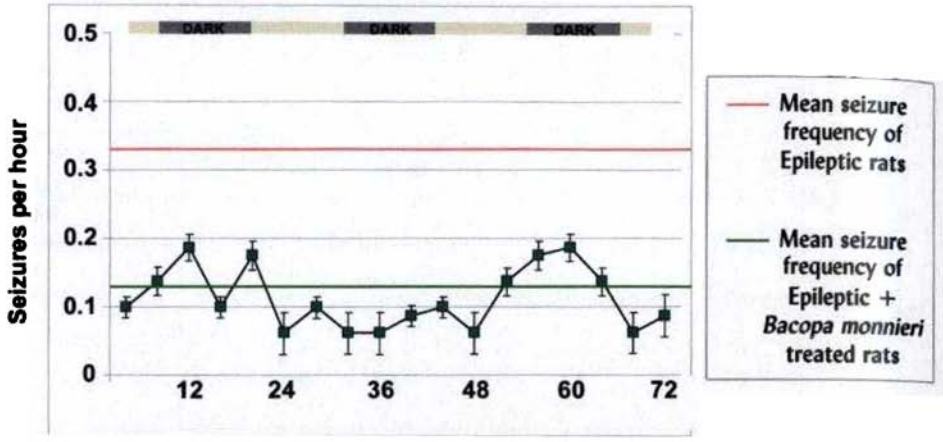


Figure- 4

Seizure frequency per 4 hours interval over the 72 hours video recording period of Pilocarpine induced Epileptic rats after 15 days Bacoside A post-treatment

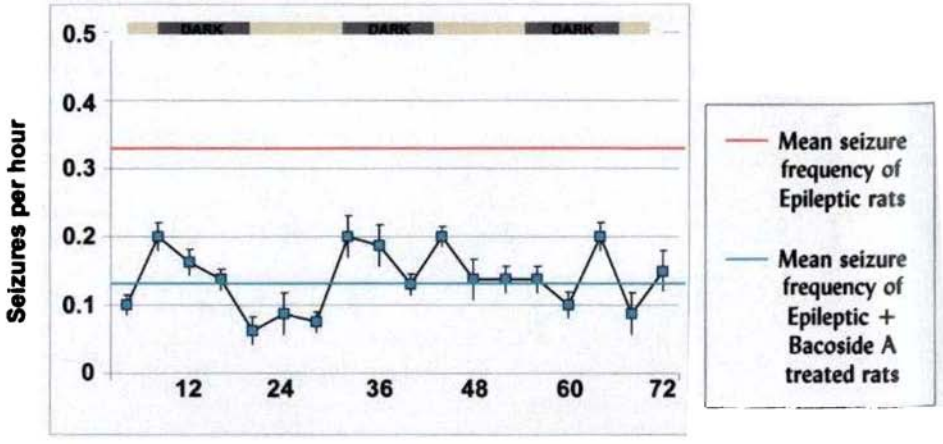


Table- 1

Mean seizure frequency per 4 hours interval over 72 hours video recording period of Pilocarpine induced Epileptic, Epileptic + Carbamazepine, Epileptic + *Bacopa monnieri* and Epileptic + Bacoside A post-treated group rats.

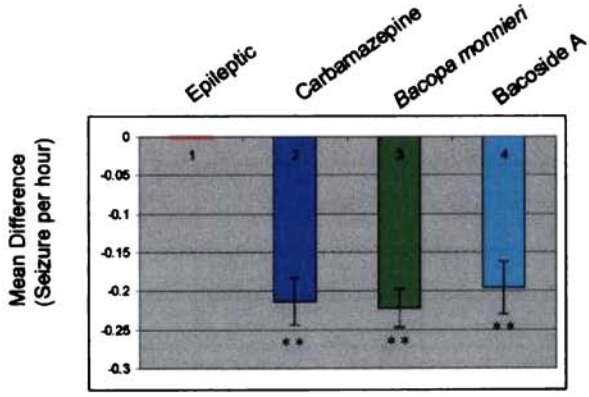
Experimental group	Mean seizure frequency (hrs)
Epileptic	0.33 ± 0.05
Epileptic+Carbamazepine	0.12 ± 0.02 **
Epileptic+*Bacopa monnieri*	0.11 ± 0.04 **
Epileptic+Bacoside A	0.13 ± 0.02 **

Values are mean ± S.E.M of 4-6 separate experiments.

** P<0.01 when compared to Epileptic group

Figure- 5

Magnitude of drug effect : Difference in mean seizure frequency



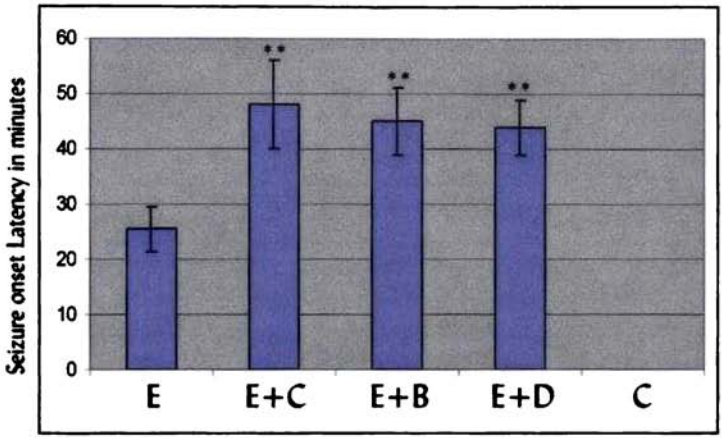
The difference in mean seizure frequency was calculated by substracting the average seizure frequency during post-treatment from the average seizure frequency of epileptic rats.

Values are mean ± S.E.M of 4-6 separate experiments.

** P<0.01 when compared to Epileptic group

Figure- 6

Seizure onset latency of Epileptic, Epileptic+Carbamazepine, Epileptic + *Bacopa monnieri* and Epileptic + Bacoside A pre-treated group rats



E- Epileptic
E+C- Epileptic+ Carbamazepine
E+B- Epileptic+*Bacopa monnieri*
E+D- Epileptic+ Bacoside A
C- Control

Table- 2

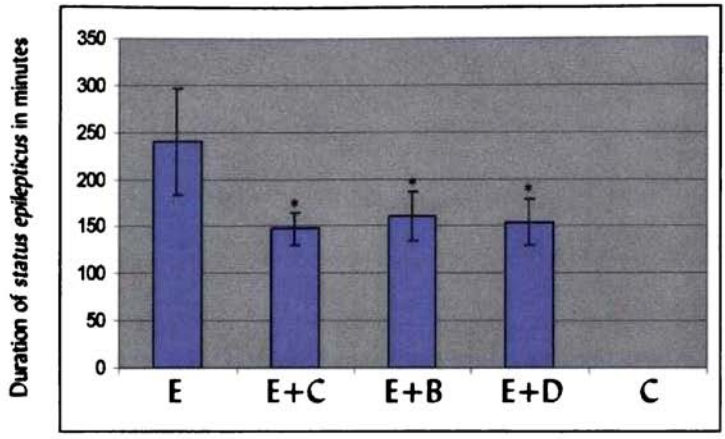
Seizure onset latency of Epileptic, Epileptic+Carbamazepine, Epileptic + *Bacopa monnieri* and Epileptic + Bacoside A pre-treated group rats

Experimental Group	Seizure onset Latency in minutes
Control	0
Epileptic	25.2 ± 2.1
Epileptic+Carbamazepine	52.0 ± 4.0 **
Epileptic+*Bacopa monnieri*	45.7 ± 3.2 **
Epileptic+Bacoside A	47.3 ± 2.8 **

Values are mean ± S.E.M of 4-6 separate experiments
** P<0.01 when compared to Epileptic group

Figure- 7

Duration of *status epilepticus* in Epileptic, Epileptic+Carbamazepine, Epileptic + *Bacopa monnieri* and Epileptic + Bacoside A pre-treated group rats after pilocarpine treatment



E- Epileptic
E+C- Epileptic+ Carbamazepine
E+B- Epileptic+ *Bacopa monnieri*
E+D- Epileptic+ Bacoside A
C- Control

Table- 3

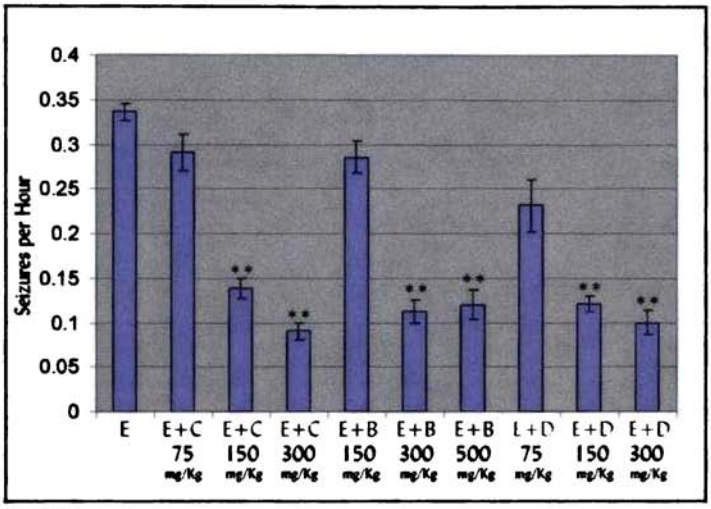
Duration of *status epilepticus* in Epileptic, Epileptic+Carbamazepine, Epileptic + *Bacopa monnieri* and Epileptic + Bacoside A pre-treated group rats after pilocarpine treatment

Experimental Group	Seizure onset Latency in minutes
Control	0
Epileptic	240 ± 28.0
Epileptic+Carbamazepine	147 ± 08.5 *
Epileptic+*Bacopa monnieri*	161 ± 13.4 *
Epileptic+Bacoside A	154 ± 12.4 *

Values are mean ± S.E.M of 4-6 separate experiments
* P<0.05 when compared to Epileptic group

Figure- 8

Effect of different dosage of Carbamazepine, *Bacopa monnieri* and Bacoside A on Mean seizure frequency over 72 hours observation period in post-treated group rats



E- Epileptic
E+C- Epileptic+Carbamazepine
E+B- Epileptic+*Bacopa monnieri*
E+D- Epileptic+Bacoside A

Table- 4

Effect of different dosage of Carbamazepine, *Bacopa monnieri* and Bacoside A on Mean seizure frequency over 72 hours observation period in post-treated group rats

a)

Experimental Group	75mg/Kg/day	150mg/Kg/day	300mg/Kg/day
Epileptic+Carbamazepine	0.29 ± 0.02	0.14 ± 0.01**	0.09 ± 0.01 **
Epileptic+Bacoside A	0.23 ± 0.03	0.12 ± 0.01**	0.10 ± 0.02 **

b)

Experimental Group	150mg/Kg/day	300mg/Kg/day	500mg/Kg/day
Epileptic+*Bacopa monnieri*	0.28 ± 0.02	0.11 ± 0.01**	0.12 ± 0.02 **

Values are mean ± S.E.M of 4-6 separate experiments
** P<0.01 when compared to Epileptic group

Figure- 9

Acetylcholine esterase activity in the Hippocampus of Control, Epileptic, Epileptic+ Carbamazepine and Epileptic+*Bacopa monnieri* post-treated group rats

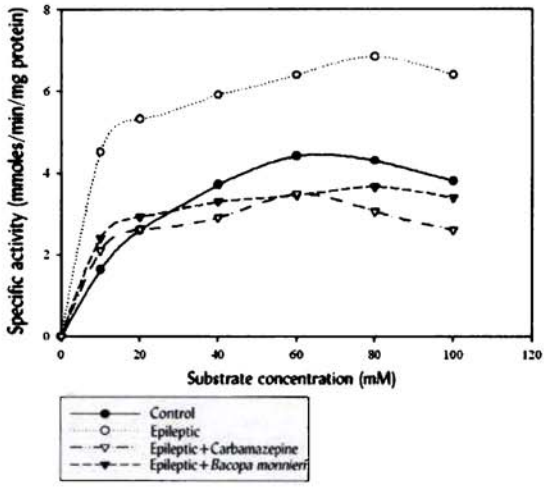


Table- 5

Acetylcholine esterase activity in the Hippocampus of Control, Epileptic, Epileptic+ Carbamazepine and Epileptic+*Bacopa monnieri* post-treated group rats

Experimental Group	V_{max} (mmoles/min/mg protein)	K_m (mM)
Control	3.66 ±0.09	9.8 ± 1.2
Epileptic	6.51 ±0.12***	11.9 ± 1.7
Epileptic+Carbamazepine	3.49 ±0.11@@@	8.7 ± 1.9
Epileptic+*Bacopa monnieri*	3.71 ±0.09@@@	10.5 ± 1.9

Values are mean ± S.E.M of 4-6 separate experiments.

*** P<0.001 when compared to Control group,
@@@ P<0.001 when compared to Epileptic group

Figure- 10

Acetylcholine esterase activity in the Hippocampus of Control, Epileptic, Epileptic+ Carbamazepine and Epileptic+Bacoside A post-treated group rats

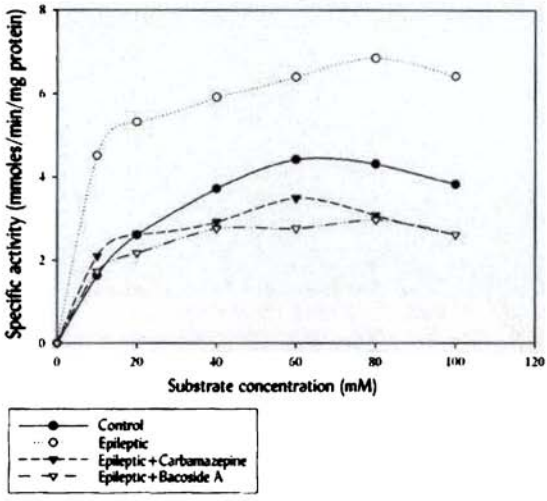


Table- 6

Acetylcholine esterase activity in the Hippocampus of Control, Epileptic, Epileptic+ Carbamazepine and Epileptic+Bacoside A post-treated group rats

Experimental Group	V_{max} (mmoles/min/mg protein)	K_m (mM)
Control	3.665 ± 0.09	9.8 ± 1.2
Epileptic	6.515 ± 0.12***	11.9 ± 1.7
Epileptic+Carbamazepine	3.490 ± 0.11@@@	8.7 ± 1.9
Epileptic+Bacoside A	2.875 ± 0.08@@@	9.4 ± 1.5

Values are mean ± S.E.M of 4-6 separate experiments.

*** P<0.001 when compared to Control group.
@@@ P<0.001 when compared to Epileptic group

Figure- 11

Acetylcholine esterase activity in the Brainstem of Control,Epileptic, Epileptic+Carbamazepine and Epileptic+*Bacopa monnieri* post-treated group rats

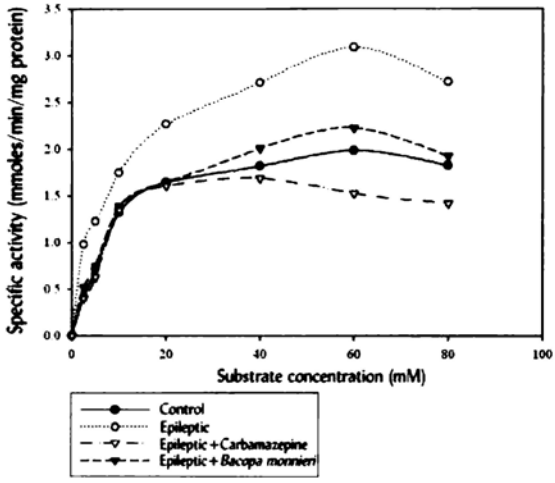


Table- 7

Acetylcholine esterase activity in the Brainstem of Control,Epileptic, Epileptic+Carbamazepine and Epileptic+*Bacopa monnieri* post-treated group rats

Experimental Group	V_{max} (mmoles/min/mg protein)	K_m (mM)
Control	1.97 ± 0.03	10.5 ± 1.41
Epileptic	3.07 ± 0.06 **	12.8 ± 2.51
Epileptic+Carbamazepine	1.71 ± 0.09 @@	9.7 ± 2.74
Epileptic+*Bacopa monnieri*	2.29 ± 0.08 @@	11.2 ± 1.82

Values are mean ± S.E.M of 4-6 separate experiments.

** P<0.01 when compared to Control group,
@@ P<0.01 when compared to Epileptic group

Figure- 12

Acetylcholine esterase activity in the Brainstem of Control, Epileptic, Epileptic+Carbamazepine and Epileptic+ Bacoside A post-treated group rats

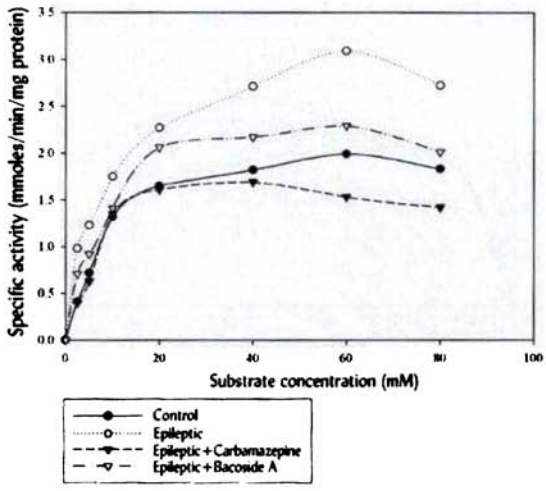


Table- 8

Acetylcholine esterase activity in the Brainstem of Control, Epileptic, Epileptic+Carbamazepine and Epileptic+ Bacoside A post-treated group rats

Experimental Group	V_{max} (mmoles/min/mg protein)	K_m (mM)
Control	1.970 ± 0.03	10.5 ± 1.41
Epileptic	3.075 ± 0.06 **	12.8 ± 2.51
Epileptic+Carbamazepine	1.715 ± 0.09 @@	9.7 ± 2.74
Epileptic+Bacoside A	2.340 ± 0.11 @@	10.2 ± 1.55

Values are mean ± S.E.M of 4-6 separate experiments.

** P<0.01 when compared to Control group,
@@ P<0.01 when compared to Epileptic group

Figure- 13

Glutamate dehydrogenase activity in the Hippocampus of Control, Epileptic and Epileptic+ *Bacopa monnieri* post-treated group rats

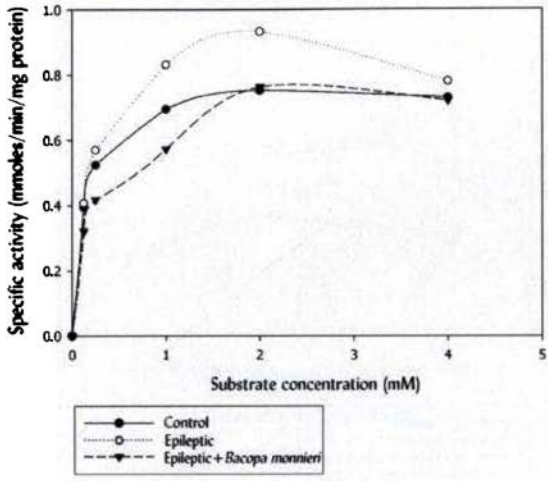


Table- 9

Glutamate dehydrogenase activity in the Hippocampus of Control, Epileptic and Epileptic+ *Bacopa monnieri* post-treated group rats

Experimental Group	V_{max} (mmoles/min/mg protein)	K_m (mM)
Control	0.780 ± 0.04	0.071 ± 0.19
Epileptic	0.915 ± 0.03 **	0.162 ± 0.09 *
Epileptic+*Bacopa monnieri*	0.765 ± 0.02 @@	0.106 ± 0.10 @

Values are mean ± S.E.M of 4-6 separate experiments.

** P<0.01, *P<0.05 when compared to Control group
@@P<0.01, @P<0.05 when compared to Epileptic group

Figure- 14

Glutamate dehydrogenase activity in the Cerebellum of Control, Epileptic and Epileptic+ *Bacopa monnieri* post-treated group rats

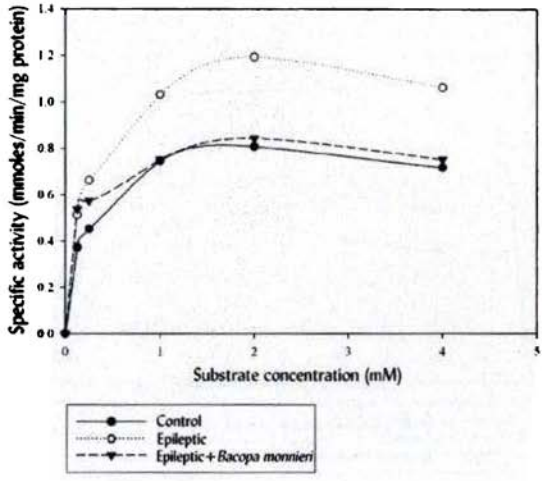


Table- 10

Glutamate dehydrogenase activity in the Cerebellum of Control, Epileptic and Epileptic+ *Bacopa monnieri* post-treated group rats

Experimental Group	V_{max} (mmoles/min./mg protein)	K_m (mM)
Control	0.79 ± 0.04	0.59 ± 0.09
Epileptic	1.23 ± 0.03 **	0.65 ± 0.07
Epileptic+*Bacopa monnieri*	0.84 ± 0.02 ⊕⊕	0.51 ± 0.09

Values are mean ± S.E.M of 4-6 separate experiments.

** P<0.01 when compared to Control group
⊕⊕ P<0.01 when compared to Epileptic group

Figure- 15

Glutamate dehydrogenase activity in the Brainstem of Control, Epileptic and Epileptic+ *Bacopa monnieri* post-treated group rats

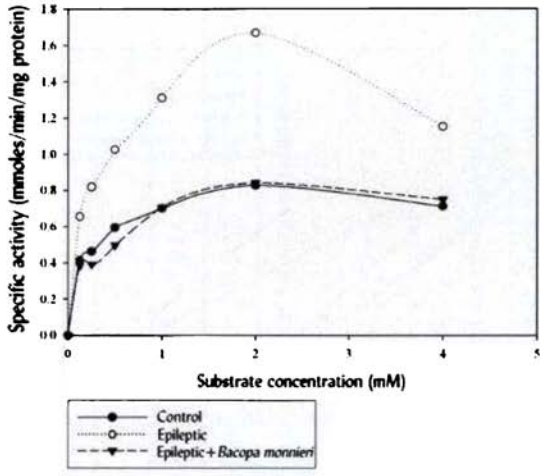


Table- 11

Glutamate dehydrogenase activity in the Brainstem of Control, Epileptic and Epileptic+ *Bacopa monnieri* post-treated group rats

Experimental Group	V_{max} (mmoles/min/mg protein)	K_m (mM)
Control	0.955 ± 0.13	0.071 ± 0.09
Epileptic	1.570 ± 0.14***	0.062 ± 0.07
Epileptic+*Bacopa monnieri*	0.810 ± 0.12@@@	0.086 ± 0.09

Values are mean ± S.E.M of 4-6 separate experiments.

*** P<0.001 when compared to control group
@@@ P<0.001 when compared to Epileptic group

Figure- 16

Scatchard analysis of [^{3}H] QNB binding against atropine in the Hippocampus of Control, Epileptic, Epileptic+Carbamazepine and Epileptic+*Bacopa monnieri* post-treated group rats

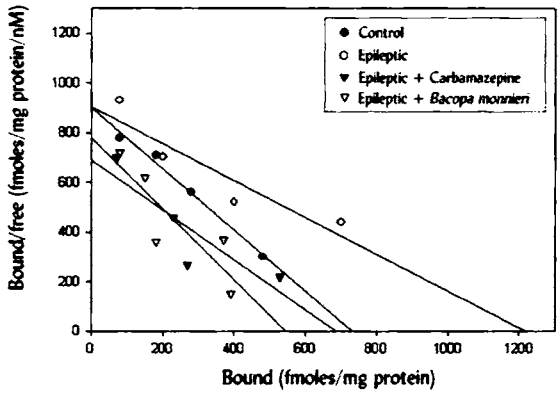


Table-12

Scatchard analysis of [^{3}H] QNB binding against atropine in the Hippocampus of Control, Epileptic, Epileptic+Carbamazepine and Epileptic+*Bacopa monnieri* post-treated group rats

Experimental Group	B_{max} (fmoles/mg protein)	K_d (nM)
Control	711 ± 32	0.71 ± 0.05
Epileptic	1257 ± 48 ***	1.25 ± 0.04 **
Epileptic+Carbamazepine	735 ± 44 @@@	1.03 ± 0.01 @
Epileptic+*Bacopa monnieri*	556 ± 51 @@@	0.74 ± 0.06 @@

Values are mean ± S.E.M of 4-6 separate experiments.

***P<0.001, **P<0.01 when compared to Control group
@@@P<0.001, @@P<0.01, @P<0.05 when compared to Epileptic group

Table- 13

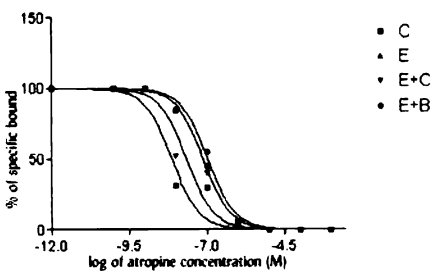
Binding parameters of [^{3}H]QNB against atropine in the Hippocampus of Control, Epileptic, Epileptic+Carbamazepine and Epileptic+*Bacopa monnieri* post-treated group rats

Experimental Group	Best-fit model	Log (EC_{50})	K_i	Hill slope
Control	One-site	-8.127	$3.07X\ 10^{-9}$	-0.953
Epileptic	One-site	-7.122	$3.10X\ 10^{-8}$	-0.998
Epileptic+Carbamazepine	One-site	-7.914	$9.13X\ 10^{-9}$	-0.953
Epileptic+*Bacopa monnieri*	One-site	-6.972	$4.39X\ 10^{-8}$	-0.996

Values are mean of 4-6 separate experiments

Figure- 17

Binding parameters of [^{3}H]QNB against atropine in the Hippocampus of experimental rats



Data were fitted with iterative nonlinear regression software (Prism, GraphPad, San Diego, CA).

Ki – The affinity of the receptor for the competing drug. EC_{50} is the concentration of the competitor that competes for half the specific binding.

Figure- 18

Scatchard analysis of [^{3}H] QNB binding against atropine in the Hippocampus of Control, Epileptic, Epileptic+Carbamazepine and Epileptic+Bacoside A post-treated group rats

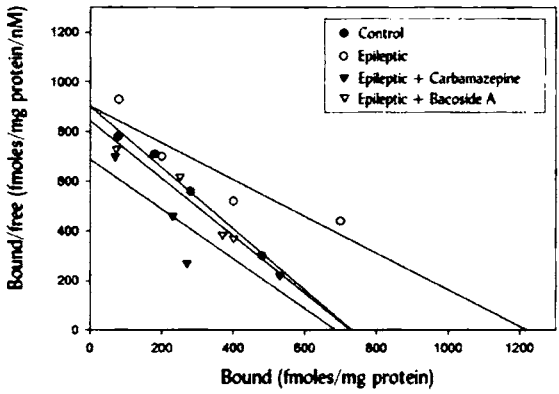


Table- 14

Scatchard analysis of [^{3}H] QNB binding against atropine in the Hippocampus of Control, Epileptic, Epileptic+Carbamazepine and Epileptic+Bacoside A post-treated group rats

Experimental Group	B_{max} (fmoles/mg protein)	K_d (nM)
Control	711 ± 32	0.71 ± 0.05
Epileptic	1257 ± 48 ***	1.25 ± 0.04 **
Epileptic+Carbamazepine	735 ± 44 @@@	1.03 ± 0.01 @
Epileptic+Bacoside A	705 ± 42 @@@	0.76 ± 0.04 @@

Values are mean ± S.E.M of 4-6 separate experiments.

***P<0.001, **P<0.01 when compared to Control group
@@@P<0.001, @@P<0.01, @P<0.05 when compared to Epileptic group

Table- 15

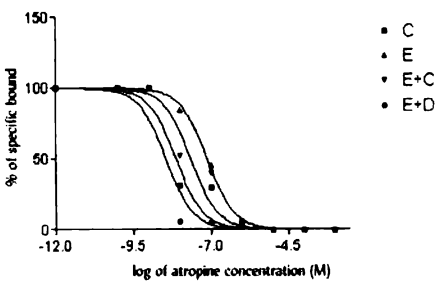
Binding parameters of [^{3}H]QNB against atropine in the Hippocampus of Control, Epileptic, Epileptic+Carbamazepine and Epileptic+Bacoside A post-treated group rats

Experimental Group	Best-fit model	Log (EC_{50})	K_i	Hill slope
Control	One-site	-8.127	$3.07X\ 10^{-9}$	- 0.953
Epileptic	One-site	-7.122	$3.10X\ 10^{-8}$	- 0.998
Epileptic+Carbamazepine	One-site	-7.914	$9.13X\ 10^{-9}$	- 0.953
Epileptic+Bacoside A	One-site	-8.451	$1.45X\ 10^{-9}$	- 0.952

Values are mean of 4-6 separate experiments

Figure- 19

Binding parameters of [^{3}H]QNB against atropine in the hippocampus of experimental rats



Data were fitted with iterative nonlinear regression software (Prism, GraphPad, San Diego, CA).

Ki – The affinity of the receptor for the competing drug. EC_{50} is the concentration of the competitor that competes for half the specific binding.

Figure- 20

Scatchard analysis of [^{3}H] QNB binding against pirenzepine in the Hippocampus of Control, Epileptic, Epileptic+Carbamazepine and Epileptic+*Bacopa monnieri* post-treated group rats

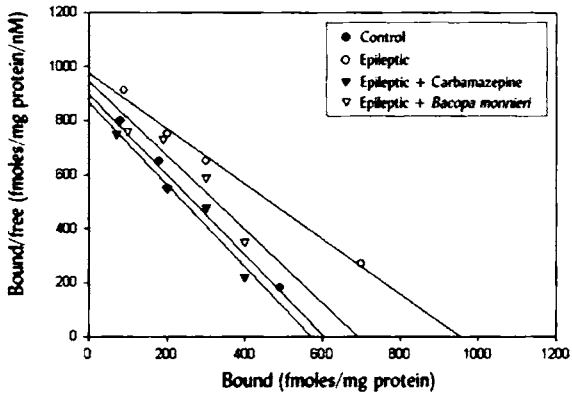


Table- 16

Scatchard analysis of [^{3}H] QNB binding against pirenzepine in the Hippocampus of Control, Epileptic, Epileptic+Carbamazepine and Epileptic+*Bacopa monnieri* post-treated group rats

Experimental Group	B_{max} (fmoles/mg protein)	K_d (nM)
Control	615 ± 15	0.64 ± 0.04
Epileptic	1005 ± 30 ***	1.07 ± 0.03 ***
Epileptic+Carbamazepine	545 ± 20 @@@	0.70 ± 0.01 @@@
Epileptic+*Bacopa monnieri*	695 ± 25 @@@	0.58 ± 0.02 @@

Values are mean ± S.E.M of 4-6 separate experiments.

*** P<0.001 when compared to Control group
@@@ P<0.001, @@ P<0.01 when compared to Epileptic group

Table- 17

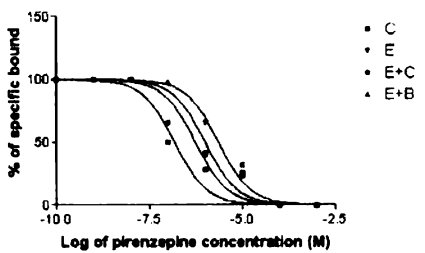
Binding parameters of [^{3}H]QNB against pirenzepine in the Hippocampus of Control, Epileptic, Epileptic+Carbamazepine and Epileptic+*Bacopa monnieri* post-treated group rats

Experimental Group	Best-fit model	Log (EC_{50})	K_i	Hill slope
Control	One-site	-6.822	$6.20X\ 10^{-8}$	-0.928
Epileptic	One-site	-5.642	$9.38X\ 10^{-7}$	-0.910
Epileptic+Carbamazepine	One-site	-6.262	$2.25X\ 10^{-7}$	-0.947
Epileptic+*Bacopa monnieri*	One-site	-6.001	$4.10X\ 10^{-7}$	-0.953

Values are mean of 4-6 separate experiments

Figure- 21

Binding parameters of [^{3}H]QNB against pirenzepine in the Hippocampus of experimental rats



Data were fitted with iterative nonlinear regression software (Prism, GraphPad, San Diego, CA).

Ki – The affinity of the receptor for the competing drug. EC_{50} is the concentration of the competitor that competes for half the specific binding.

Figure- 22

Scatchard analysis of [^{3}H] QNB binding against pirenzepine in the Hippocampus of Control, Epileptic, Epileptic+Carbamazepine and Epileptic+Bacoside A post-treated group rats

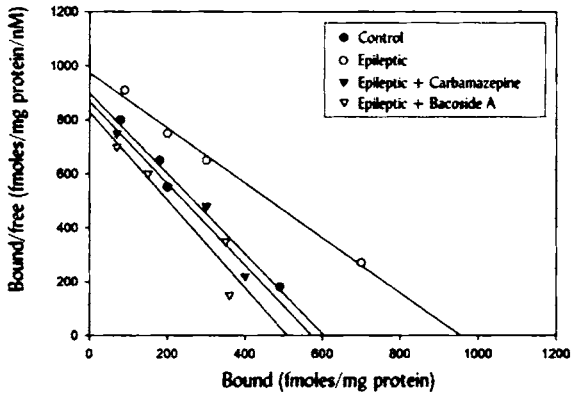


Table- 18

Scatchard analysis of [^{3}H] QNB binding against pirenzepine in the Hippocampus of Control, Epileptic, Epileptic+Carbamazepine and Epileptic+Bacoside A post-treated group rats

Experimental Group	B_{max} (fmoles/mg protein)	K_d (nM)
Control	615 ± 15	0.64 ± 0.04
Epileptic	1005 ± 30 ***	1.07 ± 0.03***
Epileptic+Carbamazepine	545 ± 20 @@@	0.70 ± 0.01@@@
Epileptic+Bacoside A	550 ± 50 @@@	0.65 ± 0.05@@@

Values are mean ± S.E.M of 4-6 separate experiments.

*** P<0.001 when compared to Control group
@@@ P<0.001 when compared to Epileptic group

Table- 19

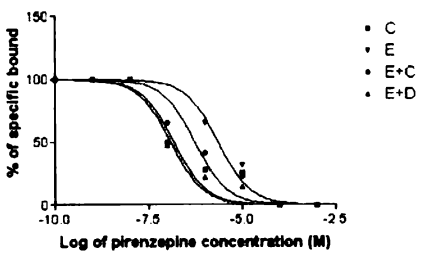
Binding parameters of [^{3}H]QNB against pirenzepine in the Hippocampus of Control, Epileptic, Epileptic+Carbamazepine and Epileptic+Bacoside A post-treated group rats

Experimental Group	Best-fit model	Log (EC_{50})	K_i	Hill slope
Control	One-site	-6.822	$6.20X\ 10^{-8}$	- 0.928
Epileptic	One-site	-5.642	$9.38X\ 10^{-7}$	- 0.910
Epileptic+Carbamazepine	One-site	-6.262	$2.25X\ 10^{-7}$	- 0.947
Epileptic+Bacoside A	One-site	-6.910	$5.06X\ 10^{-8}$	- 0.971

Values are mean of 4-6 separate experiments

Figure- 23

Binding parameters of [^{3}H]QNB against pirenzepine in the Hippocampus of experimental rats

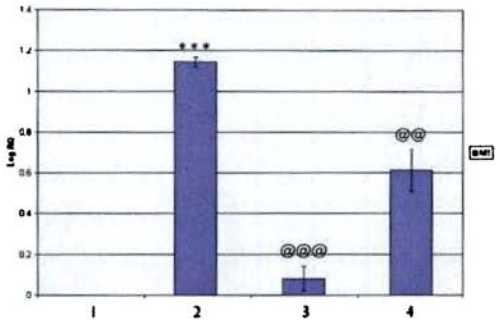


Data were fitted with iterative nonlinear regression software (Prism, GraphPad, San Diego, CA).

Ki – The affinity of the receptor for the competing drug. EC_{50} is the concentration of the competitor that competes for half the specific binding.

Figure- 24

Real Time PCR amplification of Muscarinic M1 receptor mRNA from the Hippocampus of Control, Epileptic, Epileptic+Carbamazepine and Epileptic+*Bacopa monnieri* post-treated group rats



1- Control
2- Epileptic
3- Epileptic+Carbamazepine
4- Epileptic+*Bacopa monnieri*

Table- 20

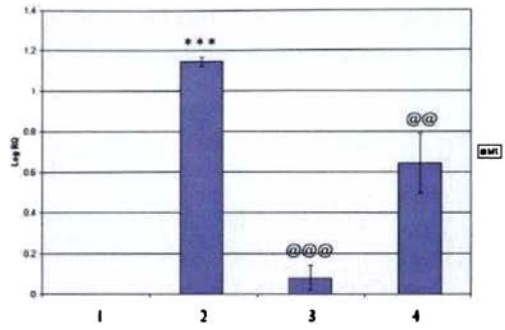
Experimental group	Log RQ Value
Control	0
Epileptic	1.152 ± 0.22***
Epileptic+Carbamazepine	0.092 ± 0.61@@@
Epileptic+*Bacopa monnieri*	0.607 ± 0.11 @@

Values are mean ± S.D of 4-6 separate experiments. Relative Quantification values and standard deviations are shown in the table. The relative ratios of mRNA levels were calculated using the ΔΔCT method normalized with β-actin CT value as the internal control and Control CT value as the calibrator.

*** $P<0.001$ when compared with Control group
@@@ $P<0.001$, @@ $P<0.01$ when compared to Epileptic group

Figure- 25

Real Time PCR amplification of Muscarinic M1 receptor mRNA from the Hippocampus of Control, Epileptic, Epileptic+Carbamazepine and Epileptic+Bacoside A post treated group rats



1- Control
2- Epileptic
3- Epileptic+Carbamazepine
4- Epileptic+Bacoside A

Table- 21

Experimental group	Log RQ Value
Control	0
Epileptic	1.152 ± 0.22***
Epileptic+Carbamazepine	0.092 ± 0.61@@@
Epileptic+Bacoside A	0.643 ± 0.12 @@

Values are mean ± S.D of 4-6 separate experiments. Relative Quantification values and standard deviations are shown in the table. The relative ratios of mRNA levels were calculated using the ΔΔCT method normalized with β-actin CT value as the internal control and Control CT value as the calibrator.

*** P<0.001 when compared with Control group

@@@ P<0.001, @@ P<0.01 when compared to Epileptic group

Figure- 26

Scatchard analysis of [^{3}H] QNB binding against pirenzepine in the Hippocampus of Control, Epileptic, Epileptic+Carbamazepine Epileptic+*Bacopa monnieri* pre-treated group rats

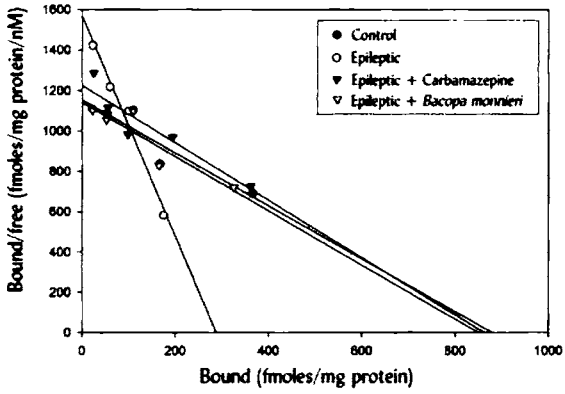


Table- 22

Scatchard analysis of [^{3}H] QNB binding against pirenzepine in the Hippocampus of Control, Epileptic, Epileptic+Carbamazepine Epileptic+*Bacopa monnieri* pre-treated group rats

Experimental Group	B_{max} (fmoles/mg protein)	K_d (nM)
Control	860 ± 22	0.77 ± 0.06
Epileptic	330 ± 43 ***	0.23 ± 0.06 **
Epileptic+Carbamazepine	854 ± 27 @@@	0.70 ± 0.05 @@
Epileptic+*Bacopa monnieri*	860 ± 23 @@@	0.74 ± 0.02 @@

Values are mean ± S.E.M of 4-6 separate experiments.

*** P<0.001, P<0.01 when compared to Control group
@@@ P<0.001, @@ P<0.01 when compared to Epileptic group

Table- 23

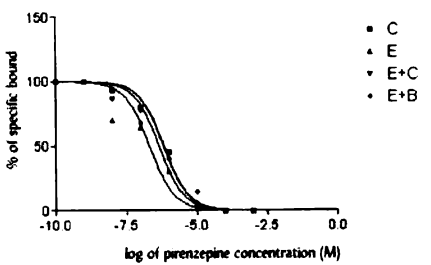
Binding parameters of [^{3}H]QNB against pirenzepine in the Hippocampus of Control, Epileptic, Epileptic+Carbamazepine and Epileptic+*Bacopa monnieri* pre-treated group rats

Experimental Group	Best-fit model	Log (EC_{50})	K_i	Hill slope
Control	One-site	-6.160	$2.84X\ 10^{-7}$	−0.992
Epileptic	One-site	-6.673	$8.73X\ 10^{-7}$	−0.936
Epileptic+Carbamazepine	One-site	-6.180	$2.72X\ 10^{-7}$	−0.981
Epileptic+*Bacopa monnieri*	One-site	-6.339	$1.88X\ 10^{-7}$	−0.961

Values are mean of 4-6 separate experiments

Figure- 27

Binding parameters of [^{3}H]QNB against pirenzepine in the Hippocampus of experimental rats



Data were fitted with iterative nonlinear regression software (Prism, GraphPad, San Diego, CA).

Ki – The affinity of the receptor for the competing drug. EC_{50} is the concentration of the competitor that competes for half the specific binding.

Figure- 28

Scatchard analysis of [^{3}H] QNB binding against pirenzepine in the Hippocampus of Control, Epileptic, Epileptic+Carbamazepine and Epileptic+Bacoside A pre-treated group rats

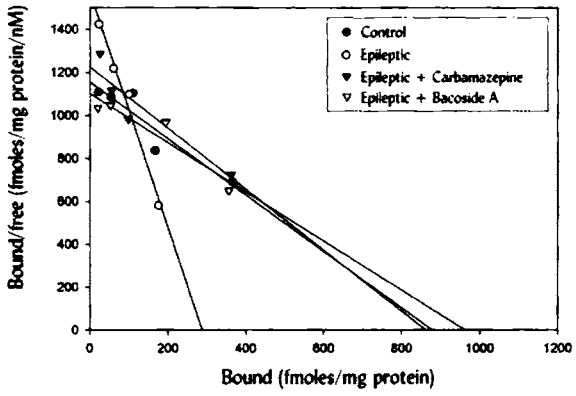


Table- 24

Scatchard analysis of [^{3}H] QNB binding against pirenzepine in the Hippocampus of Control, Epileptic, Epileptic+Carbamazepine and Epileptic+Bacoside A pre-treated group rats

Experimental Group	B_{max} (fmoles/mg protein)	K_d (nM)
Control	860 ± 22	0.77 ± 0.06
Epileptic	330 ± 43 ***	0.23 ± 0.06 **
Epileptic+Carbamazepine	854 ± 27 @@@	0.70 ± 0.05 @@
Epileptic+Bacoside A	930 ± 29 @@@	0.84 ± 0.03 @@

Values are mean ± S.E.M of 4-6 separate experiments.

*** P<0.001, ** P<0.01 when compared to Control group
@@@ P<0.001, @@ P<0.01 when compared to Epileptic group

Table- 25

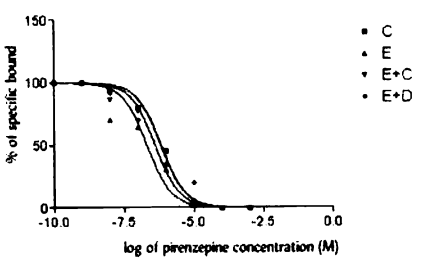
Binding parameters of [^{3}H]QNB against pirenzepine in the Hippocampus of Control, Epileptic, Epileptic+Carbamazepine and Epileptic+Bacoside A pre-treated group rats

Experimental Group	Best-fit model	Log (EC_{50})	K_i	Hill slope
Control	One-site	-6.160	$2.84X\ 10^{-7}$	-0.992
Epileptic	One-site	-6.673	$8.73X\ 10^{-7}$	-0.936
Epileptic+Carbamazepine	One-site	-6.180	$2.72X\ 10^{-7}$	-0.981
Epileptic+ Bacoside A	One-site	-6.382	$1.70X\ 10^{-7}$	-0.967

Values are mean of 4-6 separate experiments

Figure- 29

Binding parameters of [^{3}H]QNB against pirenzepine in the Hippocampus of experimental rats

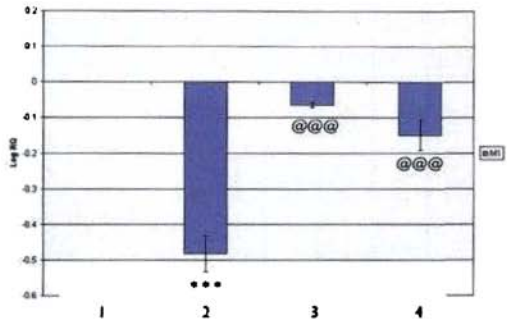


Data were fitted with iterative nonlinear regression software (Prism, GraphPad, San Diego, CA).

Ki – The affinity of the receptor for the competing drug. EC_{50} is the concentration of the competitor that competes for half the specific binding.

Figure- 30

Real Time PCR amplification of Muscarinic M1 receptor mRNA from the Hippocampus of Control, Epileptic, Epileptic+Carbamazepine and Epileptic+*Bacopa monnieri* pre-treated group rats



1- Control
2- Epileptic
3- Epileptic+Carbamazepine
4- Epileptic+*Bacopa monnieri*

Table- 26

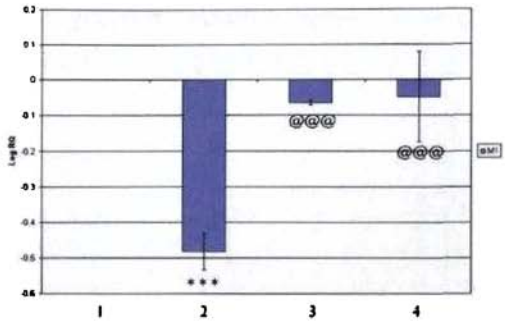
Experimental group	Log RQ Value
Control	0
Epileptic	$-0.482 \pm 0.05^{***}$
Epileptic+Carbamazepine	$-0.063 \pm 0.01^{@@@}$
Epileptic+*Bacopa monnieri*	$-0.149 \pm 0.04^{@@@}$

Values are mean ± S.D of 4-6 separate experiments. Relative Quantification values and standard deviations are shown in the table. The relative ratios of mRNA levels were calculated using the ΔΔCT method normalized with β-actin CT value as the internal control and Control CT value as the calibrator.

*** P<0.001 when compared with Control group
@@@ P<0.001 when compared to Epileptic group

Figure- 31

Real Time PCR amplification of Muscarinic M1 receptor mRNA from the Hippocampus of Control, Epileptic, Epileptic+Carbamazepine and Epileptic+Bacoside A pre-treated group rats



1- Control
2- Epileptic
3- Epileptic+Carbamazepine
4- Epileptic+Bacoside A

Table- 27

Experimental group	Log RQ Value
Control	0
Epileptic	$-0.482 \pm 0.05^{***}$
Epileptic+Carbamazepine	$-0.063 \pm 0.01^{@@@}$
Epileptic+Bacoside A	$-0.047 \pm 0.12^{@@@}$

Values are mean ± S.D of 4-6 separate experiments. Relative Quantification values and standard deviations are shown in the table. The relative ratios of mRNA levels were calculated using the ΔΔCT method normalized with β-actin CT value as the internal control and Control CT value as the calibrator.

*** P<0.001 when compared with Control group
@@@ P<0.001 when compared to Epileptic group

Figure- 32

Scatchard analysis of [^{3}H] QNB binding against atropine in the Cerebellum of Control, Epileptic, Epileptic+Carbamazepine and Epileptic+*Bacopa monnieri* post-treated group rats.

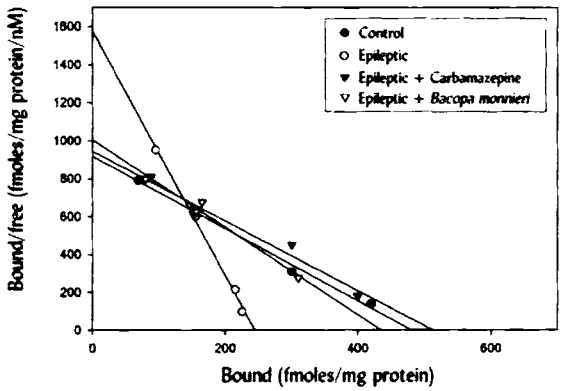


Table- 28

Scatchard analysis of [^{3}H] QNB binding against atropine in the Cerebellum of Control, Epileptic, Epileptic+Carbamazepine and Epileptic+*Bacopa monnieri* post-treated group rats.

Experimental Group	B_{max} (fmoles/mg protein)	K_d (nM)
Control	487 ± 32	0.39 ± 0.06
Epileptic	259 ± 37 **	0.28 ± 0.07
Epileptic+Carbamazepine	453 ± 38 ⊕⊕	0.36 ± 0.09
Epileptic+*Bacopa monnieri*	421 ± 39 ⊕⊕	0.37 ± 0.05

Values are mean ± S.E.M of 4-6 separate experiments.

** P<0.01 when compared to Control group,
⊕⊕ P<0.01 when compared to Epileptic group

Table- 29

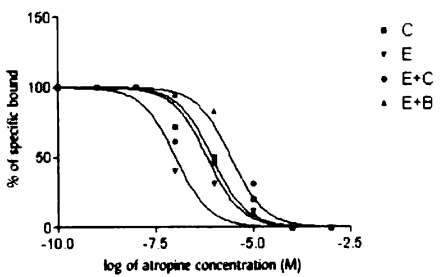
Binding parameters of [^{3}H]QNB against atropine in the Cerebellum of Control, Epileptic, Epileptic+Carbamazepine and Epileptic+*Bacopa monnieri* post-treated group rats

Experimental Group	Best-fit model	Log (EC_{50})	K_i	Hill slope
Control	One-site	-6.051	$3.65X\ 10^{-7}$	-0.965
Epileptic	One-site	-6.988	$4.25X\ 10^{-8}$	-0.945
Epileptic+Carbamazepine	One-site	-6.180	$2.71X\ 10^{-7}$	-0.896
Epileptic+*Bacopa monnieri*	One-site	-5.570	$8.92X\ 10^{-7}$	-0.991

Values are mean of 4-6 separate experiments

Figure- 33

Binding parameters of [^{3}H]QNB against atropine in the Cerebellum of experimental rats



Data were fitted with iterative nonlinear regression software (Prism, GraphPad, San Diego, CA).

Ki – The affinity of the receptor for the competing drug. EC_{50} is the concentration of the competitor that competes for half the specific binding.

Figure- 34

Scatchard analysis of [^{3}H] QNB binding against atropine in the Cerebellum of Control, Epileptic, Epileptic+Carbamazepine and Epileptic+Bacoside A post-treated group rats

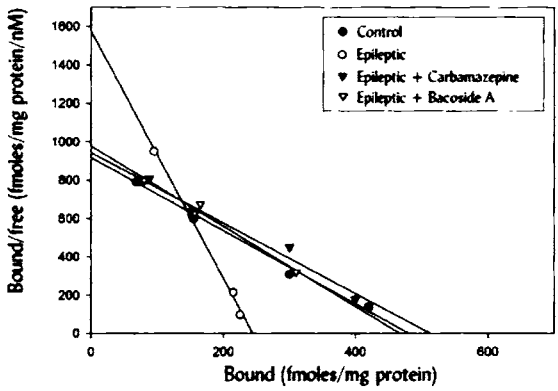


Table- 30

Scatchard analysis of [^{3}H] QNB binding against atropine in the Cerebellum of Control, Epileptic, Epileptic+Carbamazepine and Epileptic+Bacoside A post-treated group rats

Experimental Group	B_{max} (fmoles/mg protein)	K_d (nM)
Control	487 ± 32	0.39 ± 0.06
Epileptic	259 ± 37 **	0.28 ± 0.07
Epileptic+Carbamazepine	453 ± 38 @@	0.36 ± 0.09
Epileptic+Bacoside A	429 ± 41 @@	0.38 ± 0.07

Values are mean ± S.E.M of 4-6 separate experiments.

** P<0.01 when compared to Control group
@@ P<0.01 when compared to Epileptic group

Table- 31

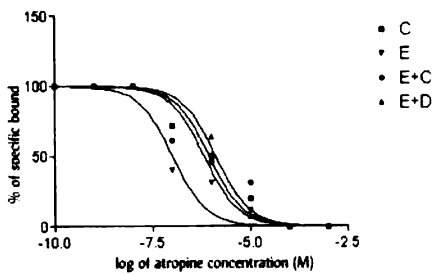
Binding parameters of [^{3}H]QNB against atropine in the Cerebellum of Control, Epileptic, Epileptic+Carbamazepine and Epileptic+Bacoside A post-treated group rats

Experimental Group	Best-fit model	Log (EC_{50})	K_i	Hill slope
Control	One-site	-6.051	$3.65X\ 10^{-7}$	-0.965
Epileptic	One-site	-6.988	$4.25X\ 10^{-8}$	-0.945
Epileptic+Carbamazepine	One-site	-6.180	$2.71X\ 10^{-7}$	-0.896
Epileptic+Bacoside A	One-site	-5.887	$5.34X\ 10^{-7}$	-0.965

Values are mean of 4-6 separate experiments

Figure- 35

Binding parameters of [^{3}H]QNB against atropine in the Cerebellum of experimental rats



Data were fitted with iterative nonlinear regression software (Prism, GraphPad, San Diego, CA).

Ki – The affinity of the receptor for the competing drug. EC_{50} is the concentration of the competitor that competes for half the specific binding.

Figure- 36

Scatchard analysis of [^{3}H] QNB binding against pirenzepine in the Cerebellum of Control, Epileptic, Epileptic+Carbamazepine and Epileptic+*Bacopa monnieri* post-treated group rats

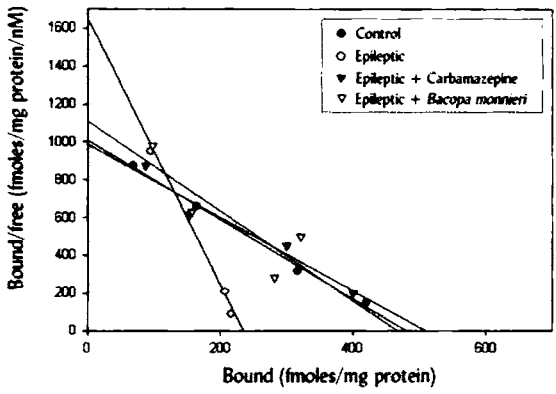


Table- 32

Scatchard analysis of [^{3}H] QNB binding against pirenzepine in the Cerebellum of Control, Epileptic, Epileptic+Carbamazepine and Epileptic+*Bacopa monnieri* post-treated group rats

Experimental Group	B_{max} (fmoles/mg protein)	K_d (nM)
Control	470 ± 16	0.47 ± 0.03
Epileptic	240 ± 29 ***	0.14 ± 0.04 ***
Epileptic+Carbamazepine	465 ± 21 @@@	0.42 ± 0.02 @@
Epileptic+*Bacopa monnieri*	498 ± 52 @@@	0.49 ± 0.05 @@

Values are mean ± S.E.M of 4-6 separate experiments.

*** P<0.001 when compared to Control group
@@@ P<0.001, @@ P<0.01 when compared to Epileptic group

Table- 33

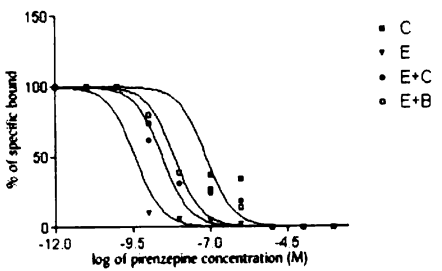
Binding parameters of [^{3}H]QNB against pirenzepine in the Cerebellum of Control, Epileptic, Epileptic+Carbamazepine and Epileptic+*Bacopa monnieri* post-treated group rats

Experimental Group	Best-fit model	Log (EC_{50})	K_i	Hill slope
Control	One-site	-7.124	$3.08X\ 10^{-8}$	-0.914
Epileptic	One-site	-9.413	$1.57X\ 10^{-10}$	-0.960
Epileptic+Carbamazepine	One-site	-8.541	$2.25X\ 10^{-9}$	-0.938
Epileptic+*Bacopa monnieri*	One-site	-8.154	$4.10X\ 10^{-9}$	-0.963

Values are mean of 4-6 separate experiments

Figure- 37

Binding parameters of [^{3}H]QNB against pirenzepine in the Cerebellum of experimental rats



Data were fitted with iterative nonlinear regression software (Prism, GraphPad, San Diego, CA).

Ki – The affinity of the receptor for the competing drug. EC_{50} is the concentration of the competitor that competes for half the specific binding.

Figure- 38

Scatchard analysis of [^{3}H] QNB binding against pirenzepine in the Cerebellum of Control, Epileptic, Epileptic+Carbamazepine and Epileptic+Bacoside A post-treated group rats

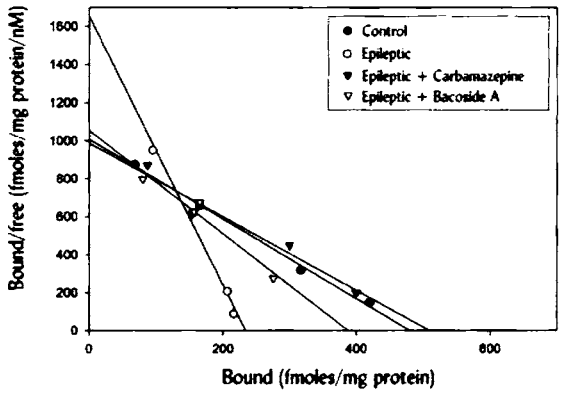


Table- 34

Scatchard analysis of [^{3}H] QNB binding against pirenzepine in the Cerebellum of Control, Epileptic, Epileptic+Carbamazepine and Epileptic+Bacoside A post-treated group rats

Experimental Group	B_{max} (fmoles/mg protein)	K_d (nM)
Control	470 ± 16	0.47 ± 0.03
Epileptic	240 ± 29 ***	0.14 ± 0.04***
Epileptic+Carbamazepine	465 ± 21 @@@	0.42 ± 0.02 @@
Epileptic+Bacoside A	402 ± 39 @@@	0.38 ± 0.06 @@

Values are mean ± S.E.M of 4-6 separate experiments.

*** P<0.001 when compared to Control group
@@@ P<0.001, @@P<0.01 when compared to Epileptic group

Table- 35

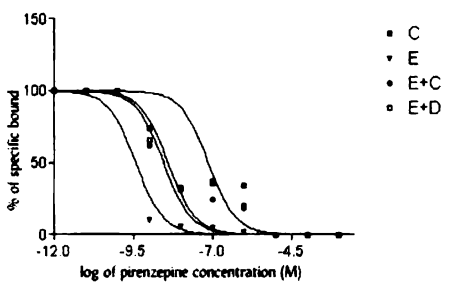
Binding parameters of [^{3}H]QNB against pirenzepine in the Cerebellum of control, Epileptic, Epileptic+Carbamazepine and Epileptic+Bacoside A post-treated group rats

Experimental Group	Best-fit model	Log (EC_{50})	K_i	Hill slope
Control	One-site	-7.124	$3.08X\ 10^{-8}$	-0.914
Epileptic	One-site	-9.413	$1.57X\ 10^{-10}$	-0.960
Epileptic+Carbamazepine	One-site	-8.541	$2.25X\ 10^{-9}$	-0.938
Epileptic+Bacoside A	One-site	-8.395	$1.64X\ 10^{-9}$	-0.900

Values are mean of 4-6 separate experiments

Figure- 39

Binding parameters of [^{3}H]QNB against pirenzepine in the Cerebellum of experimental rats

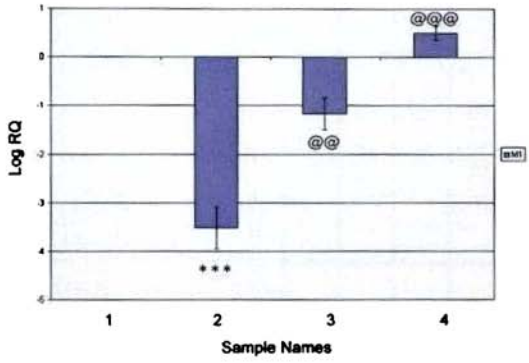


Data were fitted with iterative nonlinear regression software (Prism, GraphPad, San Diego, CA).

Ki – The affinity of the receptor for the competing drug. EC_{50} is the concentration of the competitor that competes for half the specific binding.

Figure- 40

Real Time PCR amplification of Muscarinic M1 receptor mRNA from the Cerebellum of Control, Epileptic, Epileptic+Carbamazepine and Epileptic+*Bacopa monnieri* post-treated group rats



1- Control
2- Epileptic
3- Epileptic+Carbamazepine
4- Epileptic+*Bacopa monnieri*

Table- 36

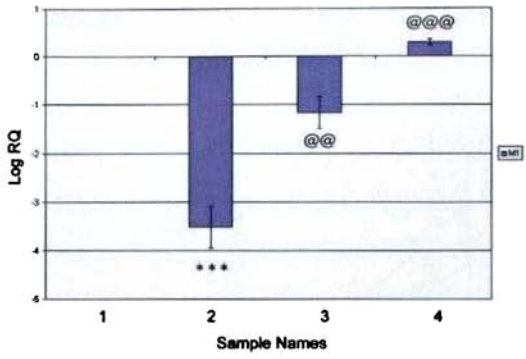
Experimental group	Log RQ Value
Control	0
Epileptic	$-3.520 \pm 0.42^{***}$
Epileptic+Carbamazepine	$-1.162 \pm 0.32^{@@}$
Epileptic+*Bacopa monnieri*	$0.505 \pm 0.14^{@@@}$

Values are mean ± S.D of 4-6 separate experiments. Relative Quantification values and standard deviations are shown in the table. The relative ratios of mRNA levels were calculated using the ΔΔCT method normalized with β-actin CT value as the internal control and Control CT value as the calibrator.

*** $P<0.001$ when compared with Control group
@@@ $P<0.001$, @@ $P<0.01$ when compared to Epileptic group

Figure- 41

Real Time PCR amplification of Muscarinic M1 receptor mRNA from the Cerebellum of Control, Epileptic, Epileptic+Carbamazepine and Epileptic+Bacoside A post-treated group rats



1- Control
2- Epileptic
3- Epileptic+Carbamazepine
4- Epileptic+Bacoside A

Table- 37

Experimental group	Log RQ Value
Control	0
Epileptic	$-3.520 \pm 0.42^{***}$
Epileptic+Carbamazepine	$-1.162 \pm 0.32^{@@}$
Epileptic+Bacoside A	$0.293 \pm 0.06^{@@@}$

Values are mean ± S.D of 4-6 separate experiments. Relative Quantification values and standard deviations are shown in the table. The relative ratios of mRNA levels were calculated using the ΔΔCT method normalized with β-actin CT value as the internal control and Control CT value as the calibrator.

*** $P<0.001$ when compared with Control group

@@@ $P<0.001$, @@ $P<0.01$ when compared to Epileptic group

Figure- 42

Scatchard analysis of [^{3}H] QNB binding against pirenzepine in the Cerebellum of Control, Epileptic, Epileptic+Carbamazepine and Epileptic+*Bacopa monnieri* pre-treated rats

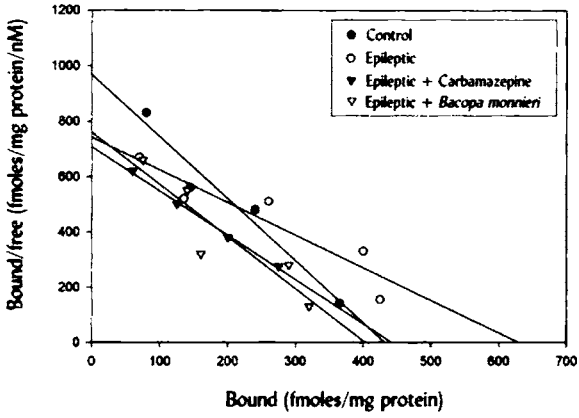


Table- 38

Scatchard analysis of [^{3}H] QNB binding against pirenzepine in the Cerebellum of Control, Epileptic, Epileptic+Carbamazepine and Epileptic+*Bacopa monnieri* pre-treated rats

Experimental Group	B_{max} (fmoles/mg protein)	K_d (nM)
Control	437 ± 12	0.54 ± 0.19
Epileptic	610 ± 11 ***	0.66 ± 0.09
Epileptic+Carbamazepine	422 ± 13 @@	0.51 ± 0.10
Epileptic+*Bacopa monnieri*	430 ± 30 @@	0.42 ± 0.08

Values are mean ± S.E.M of 4-6 separate experiments.

*** P<0.001 when compared to Control group,
@@P<0.01 when compared to Epileptic group

Table- 39

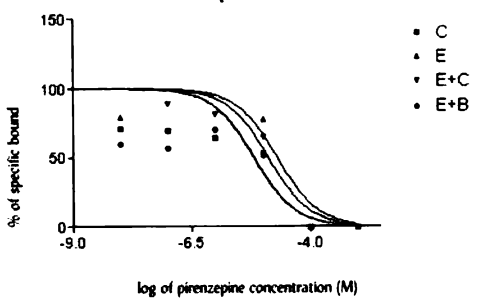
Binding parameters of [^{3}H] QNB against pirenzepine in the Cerebellum of Control, Epileptic, Epileptic+Carbamazepine and Epileptic+*Bacopa monnieri* pre-treated group rats

Experimental Group	Best-fit model	Log (EC_{50})	K_i	Hill slope
Control	One-site	-4.981	$4.30X\ 10^{-6}$	-0.934
Epileptic	One-site	-5.117	$3.14X\ 10^{-6}$	-0.608
Epileptic+Carbamazepine	One-site	-6.043	$3.73X\ 10^{-7}$	-0.751
Epileptic+*Bacopa monnieri*	One-site	-5.961	$4.48X\ 10^{-7}$	-0.874

Values are mean of 4-6 separate experiments

Figure- 43

Binding parameters of [^{3}H]QNB against pirenzepine in the Cerebellum of experimental rats



Data were fitted with iterative nonlinear regression software (Prism, GraphPad, San Diego, CA).

Ki – The affinity of the receptor for the competing drug. EC_{50} is the concentration of the competitor that competes for half the specific binding.

Figure- 44

Scatchard analysis of [^{3}H] QNB binding against pirenzepine in the Cerebellum of Control, Epileptic, Epileptic+Carbamazepine and Epileptic+Bacoside A pre-treated group rats

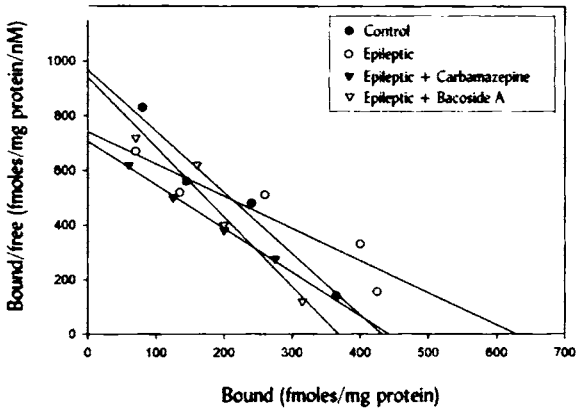


Table- 40

Scatchard analysis of [^{3}H] QNB binding against pirenzepine in the Cerebellum of Control, Epileptic, Epileptic+Carbamazepine and Epileptic+Bacoside A pre-treated group rats

Experimental Group	B_{max} (fmoles/mg protein)	K_d (nM)
Control	437 ± 12	0.54 ± 0.19
Epileptic	610 ± 11 ***	0.66 ± 0.09
Epileptic+Carbamazepine	422 ± 13 @@	0.51 ± 0.10
Epileptic+Bacoside A	405 ± 30 @@	0.46 ± 0.07

Values are mean ± S.E.M of 4-6 separate experiments.

*** P<0.001 when compared to Control group
@@P<0.01 when compared to Epileptic group

Table- 41

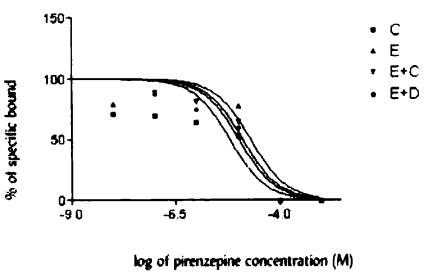
Binding parameters of [^{3}H]QNB against pirenzepine in the Cerebellum of Control, Epileptic, Epileptic+Carbamazepine and Epileptic+Bacoside A pre-treated group rats

Experimental Group	Best-fit model	Log (EC_{50})	K_i	Hill slope
Control	One-site	-4.981	$4.30X\ 10^{-6}$	-0.934
Epileptic	One-site	-5.117	$3.14X\ 10^{-6}$	-0.608
Epileptic+Carbamazepine	One-site	-6.043	$3.73X\ 10^{-7}$	-0.751
Epileptic+Bacoside A	One-site	-6.046	$3.70X\ 10^{-7}$	-0.891

Values are mean of 4-6 separate experiments

Figure- 45

Binding parameters of [^{3}H]QNB against pirenzepine in the Cerebellum of experimental rats



Data were fitted with iterative nonlinear regression software (Prism, GraphPad, San Diego, CA).

Ki – The affinity of the receptor for the competing drug. EC_{50} is the concentration of the competitor that competes for half the specific binding.

Figure- 46

Scatchard analysis of [^{3}H] QNB binding against atropine in the Brainstem of Control, Epileptic, Epileptic+carbamazepine and Epileptic+*Bacopa monnieri* post-treated group rats

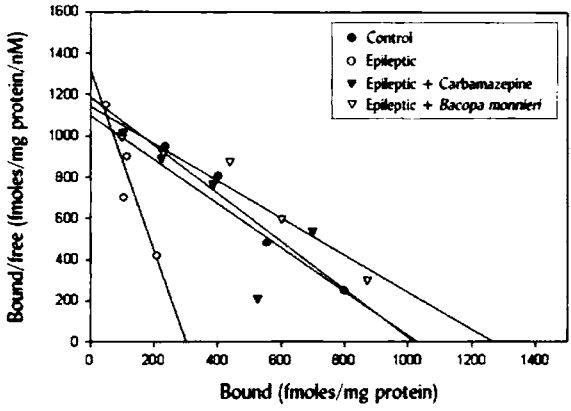


Table- 42

Scatchard analysis of [^{3}H] QNB binding against atropine in the Brainstem of Control, Epileptic, Epileptic+carbamazepine and Epileptic+*Bacopa monnieri* post-treated group rats

Experimental Group	B_{max} (fmoles/mg protein)	K_d (nM)
Control	1030± 22	0.93 ± 0.05
Epileptic	280 ± 19 ***	0.21 ± 0.01 **
Epileptic+Carbamazepine	1100± 49 @@@	0.95 ± 0.09 @@
Epileptic+*Bacopa monnieri*	1205 ± 50 @@@	0.96 ± 0.09 @@

Values are mean ± S.E.M of 4-6 separate experiments,

*** P<0.001, **P<0.01 when compared to Control group,
@@@ P<0.001, @@ P<0.01 when compared to Epileptic group

Table- 43

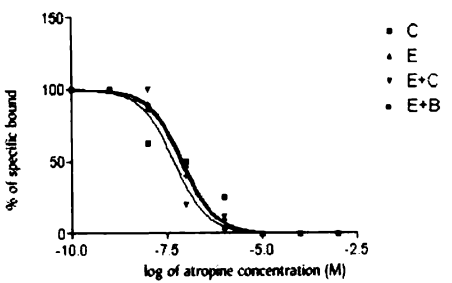
Binding parameters of [^{3}H]QNB against atropine in the Brainstem of Control, Epileptic, Epileptic+Carbamazepine and Epileptic+*Bacopa monnieri* post-treated group rats

Experimental Group	Best-fit model	Log (EC_{50})	K_i	Hill slope
Control	One-site	-7.093	$3.32X\ 10^{-8}$	-0.920
Epileptic	One-site	-7.069	$3.51X\ 10^{-8}$	-0.998
Epileptic+Carbamazepine	One-site	-7.314	$1.99X\ 10^{-8}$	-0.969
Epileptic+*Bacopa monnieri*	One-site	-7.143	$2.96X\ 10^{-8}$	-0.998

Values are mean of 4-6 separate experiments

Figure- 47

Binding parameters of [^{3}H]QNB against atropine in the Brainstem of experimental rats



Data were fitted with iterative nonlinear regression software (Prism, GraphPad, San Diego, CA).

Ki – The affinity of the receptor for the competing drug. EC_{50} is the concentration of the competitor that competes for half the specific binding.

Figure- 48

Scatchard analysis of [^{3}H] QNB binding against atropine in the Brainstem of Control, Epileptic, Epileptic+Carbamazepine and Epileptic+Bacoside A post-treated group rats

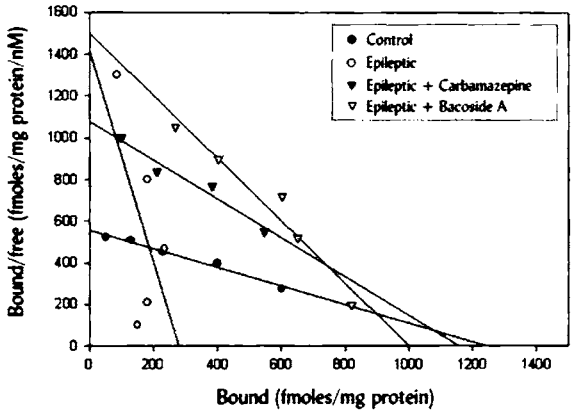


Table- 44

Scatchard analysis of [^{3}H] QNB binding against atropine in the Brainstem of Control, Epileptic, Epileptic+Carbamazepine and Epileptic+Bacoside A post-treated group rats

Experimental Group	B_{max} (fmoles/mg protein)	K_d (nM)
Control	1225±125	2.10 ± 0.05
Epileptic	270 ± 21 ***	0.21 ± 0.01 **
Epileptic+Carbamazepine	1150 ± 49 @@@	1.04 ± 0.08 @@
Epileptic+Bacoside A	975 ± 24 @@@	0.73 ± 0.13 @@

Values are mean ± S.E.M of 4-6 separate experiments,

*** P<0.001, **P<0.01 when compared to Control group,
@@@ P<0.001, @@P<0.01 when compared to Epileptic group

Table- 45

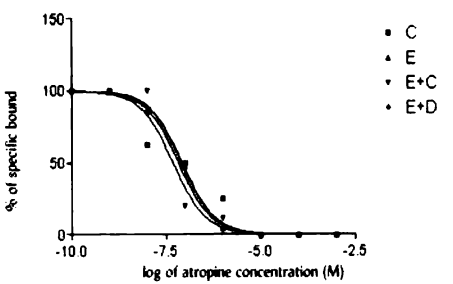
Binding parameters of [^{3}H]QNB against atropine in the Brainstem of Control, Epileptic, Epileptic+Carbamazepine and Epileptic+Bacoside A post-treated group rats

Experimental Group	Best-fit model	Log (EC_{50})	K_i	Hill slope
Control	One-site	-7.093	$3.32X\ 10^{-8}$	-0.920
Epileptic	One-site	-7.069	$3.51X\ 10^{-8}$	-0.998
Epileptic+Carbamazepine	One-site	-7.314	$1.99X\ 10^{-8}$	-0.969
Epileptic+Bacoside A	One-site	-7.167	$2.80X\ 10^{-8}$	-0.999

Values are mean of 4-6 separate experiments

Figure- 49

Binding parameters of [^{3}H]QNB against atropine in the Brainstem of experimental rats



Data were fitted with iterative nonlinear regression software (Prism, GraphPad, San Diego, CA).

Ki – The affinity of the receptor for the competing drug. EC_{50} is the concentration of the competitor that competes for half the specific binding.

Figure- 50

Scatchard analysis of [^{3}H] QNB binding against pirenzepine in the Brainstem of Control, Epileptic, Epileptic+Carbamazepine and Epileptic+*Bacopa monnieri* post-treated group rats

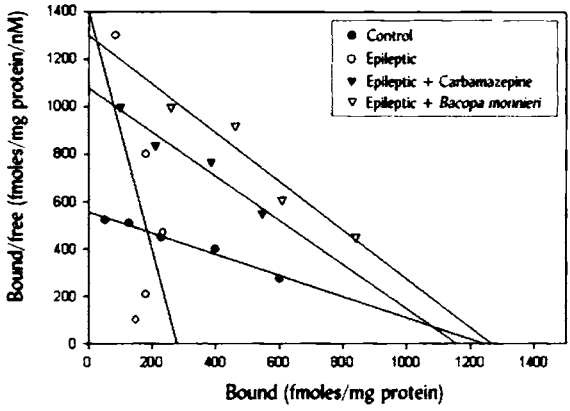


Table- 46

Scatchard analysis of [^{3}H] QNB binding against pirenzepine in the Brainstem of Control, Epileptic, Epileptic+Carbamazepine and Epileptic+*Bacopa monnieri* post-treated group rats

Experimental Group	B_{max} (fmoles/mg protein)	K_d (nM)
Control	1225±123	2.10 ± 0.05
Epileptic	270 ± 19 ***	0.21 ± 0.01 **
Epileptic+Carbamazepine	1145 ± 47 @@@	0.91 ± 0.13 @@
Epileptic+*Bacopa monnieri*	1193 ± 46 @@@	0.88 ± 0.11 @@

Values are mean ± S.E.M of 4-6 separate experiments.

*** P<0.001, **P<0.01 when compared to Control group,
@@@ P<0.001, @@ P<0.01 when compared to Epileptic group

Table- 47

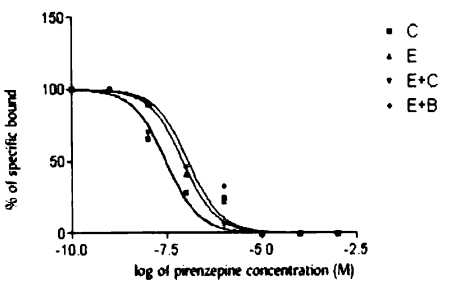
Binding parameters of [^{3}H]QNB against pirenzepine in the Brainstem of Control, Epileptic, Epileptic+Carbamazepine and Epileptic+*Bacopa monnieri* post-treated group rats

Experimental Group	Best-fit model	Log (EC_{50})	K_i	Hill slope
Control	One-site	-7.531	$1.21X\ 10^{-8}$	-0.955
Epileptic	One-site	-7.075	$3.42X\ 10^{-8}$	-0.984
Epileptic+Carbamazepine	One-site	-7.511	$4.71X\ 10^{-8}$	-0.947
Epileptic+*Bacopa monnieri*	One-site	-6.936	$1.23X\ 10^{-8}$	-0.960

Values are mean of 4-6 separate experiments

Figure- 51

Binding parameters of [^{3}H]QNB against pirenzepine in the Brainstem of experimental rats



Data were fitted with iterative nonlinear regression software (Prism, GraphPad, San Diego, CA).

Ki – The affinity of the receptor for the competing drug. EC_{50} is the concentration of the competitor that competes for half the specific binding.

Figure- 52

Scatchard analysis of [^{3}H] QNB binding against pirenzepine in the Brainstem of Control, Epileptic, Epileptic+Carbamazepine and Epileptic+Bacoside A post-treated group rats

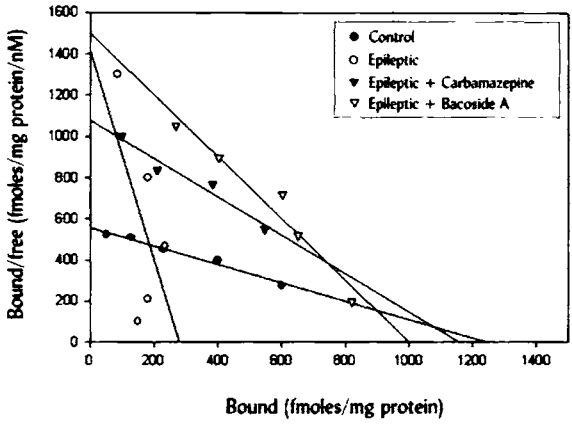


Table- 48

Scatchard analysis of [^{3}H] QNB binding against pirenzepine in the Brainstem of Control, Epileptic, Epileptic+Carbamazepine and Epileptic+Bacoside A post-treated group rats

Experimental Group	B_{max} (fmoles/mg protein)	K_d (nM)
Control	1225±123	2.10 ± 0.05
Epileptic	270 ± 19 ***	0.21 ± 0.01 **
Epileptic+Carbamazepine	1145 ± 47 @@@	0.91 ± 0.13 @@
Epileptic+Bacoside A	972 ± 27 @@@	0.73 ± 0.11 @@

Values are mean ± S.E.M of 4-6 separate experiments.

*** P<0.001, **P<0.01 when compared to Control group,
@@@P<0.001, @@P<0.01 when compared to Epileptic group

Table- 49

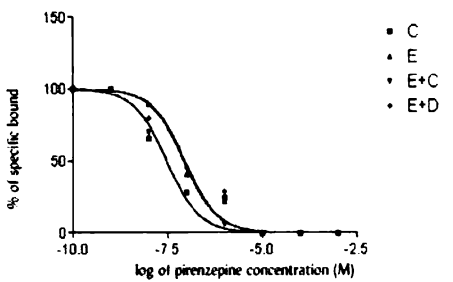
Binding parameters of [^{3}H]QNB against pirenzepine in the Brainstem of Control, Epileptic, Epileptic+Carbamazepine and Epileptic+Bacoside A post-treated group rats

Experimental Group	Best-fit model	Log (EC_{50})	K_i	Hill slope
Control	One-site	-7.531	$1.21X\ 10^{-8}$	-0.955
Epileptic	One-site	-7.075	$3.42X\ 10^{-8}$	-0.984
Epileptic+Carbamazepine	One-site	-7.511	$3.83X\ 10^{-8}$	-0.947
Epileptic+Bacoside A	One-site	-7.031	$1.23X\ 10^{-8}$	-0.963

Values are mean of 4-6 separate experiments

Figure- 53

Binding parameters of [^{3}H]QNB against pirenzepine in the Brainstem of experimental rats

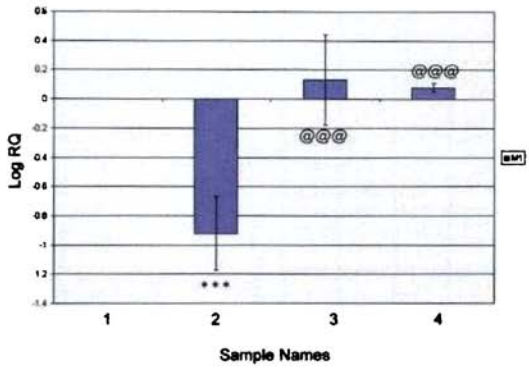


Data were fitted with iterative nonlinear regression software (Prism, GraphPad, San Diego, CA).

Ki – The affinity of the receptor for the competing drug. EC_{50} is the concentration of the competitor that competes for half the specific binding.

Figure- 54

Real Time PCR amplification of Muscarinic M1 receptor mRNA from the Brainstem of Control, Epileptic, Epileptic+Carbamazepine and Epileptic+*Bacopa monnieri* post-treated group rats



1- Control
2- Epileptic
3- Epileptic+Carbamazepine
4- Epileptic+*Bacopa monnieri*

Table- 50

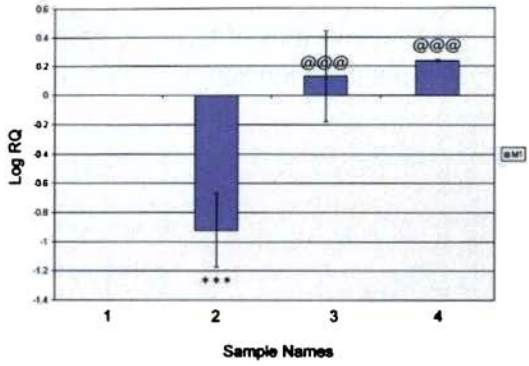
Experimental group	Log RQ Value
Control	0
Epileptic	$-0.921 \pm 0.25^{***}$
Epileptic+Carbamazepine	$0.132 \pm 0.31^{@@@}$
Epileptic+*Bacopa monnieri*	$0.081 \pm 0.02^{@@@}$

Values are mean ± S.D of 4-6 separate experiments. Relative Quantification values and standard deviations are shown in the table. The relative ratios of mRNA levels were calculated using the ΔΔCT method normalized with β-actin CT value as the internal control and Control CT value as the calibrator.

*** P<0.001 when compared with Control group
@@@ P<0.001 when compared to Epileptic group

Figure- 55

Real Time PCR amplification of Muscarinic M1 receptor mRNA from the Brainstem of Control, Epileptic, Epileptic+Carbamazepine and Epileptic+Bacoside A post-treated group rats



1- Control
2- Epileptic
3- Epileptic+Carbamazepine
4- Epileptic+Bacoside A

Table- 51

Experimental group	Log RQ Value
Control	0
Epileptic	$-0.921 \pm 0.25^{***}$
Epileptic+Carbamazepine	$0.132 \pm 0.31^{@@@}$
Epileptic+Bacoside A	$0.237 \pm 0.06^{@@@}$

Values are mean ± S.D of 4-6 separate experiments. Relative Quantification values and standard deviations are shown in the table. The relative ratios of mRNA levels were calculated using the ΔΔCT method normalized with β-actin CT value as the internal control and Control CT value as the calibrator.

*** P<0.001 when compared with Control group
@@@ P<0.001 when compared to Epileptic group

Figure- 56

Scatchard analysis of [^{3}H] Glutamate binding against glutamate in the Hippocampus of Control, Epileptic and Epileptic+*Bacopa monnieri* post-treated group rats

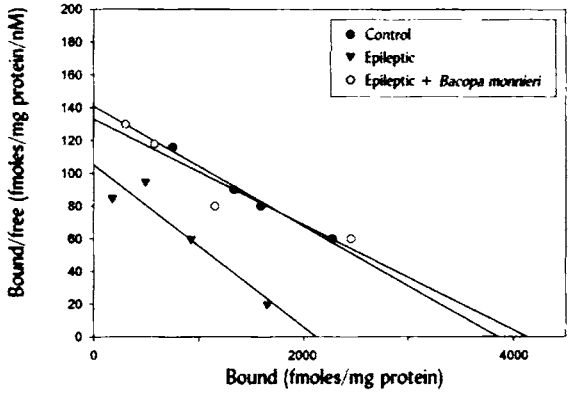


Table- 52

Scatchard analysis of [^{3}H] Glutamate binding against glutamate in the Hippocampus of Control, Epileptic and Epileptic+*Bacopa monnieri* post-treated group rats

Experimental Group	B_{max} (fmoles/mg protein)	K_d (nM)
Control	3864± 96	59 ± 7.8
Epileptic	2170± 60 **	45 ± 7.2
Epileptic+*Bacopa monnieri*	4132±128 @@	55 ± 6.3

Values are mean ± S.E.M of 4-6 separate experiments.

** P<0.01 when compared to control group

@@ P<0.01 when compared to epileptic group

Table- 53

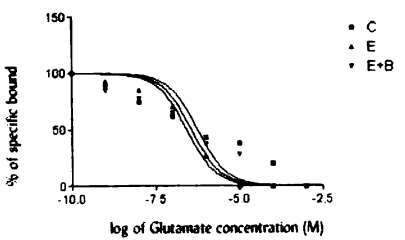
Binding parameters of [^{3}H] Glutamate against glutamate in the Hippocampus of Control, Epileptic and Epileptic+*Bacopa monnieri* post-treated group rats

Experimental Group	Best-fit model	Log (EC_{50})	K_i	Hill slope
Control	One-site	-6.247	$1.41X\ 10^{-7}$	-0.899
Epileptic	One-site	-6.577	$1.32X\ 10^{-8}$	-0.984
Epileptic+*Bacopa monnieri*	One-site	-6.435	$1.83X\ 10^{-7}$	-0.857

Values are mean of 4-6 separate experiments

Figure- 57

Binding parameters of [^{3}H] Glutamate against Glutamate in the Hippocampus of experimental rats

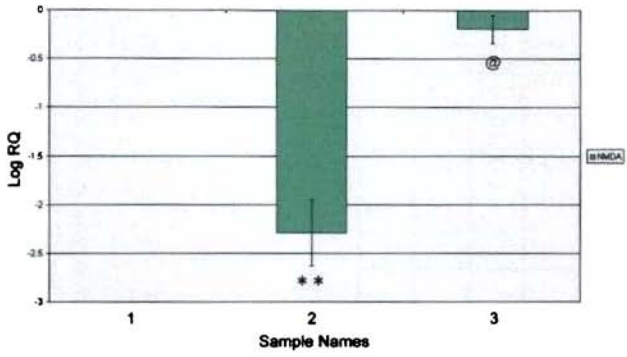


Data were fitted with iterative nonlinear regression software (Prism, GraphPad, San Diego, CA).

Ki – The affinity of the receptor for the competing drug. EC_{50} is the concentration of the competitor that competes for half the specific binding.

Figure- 58

Real Time PCR amplification of NMDA R1 receptor mRNA from the Hippocampus of Control, Epileptic and Epileptic+*Bacopa monnieri* post-treated group rats



1- Control
2- Epileptic
3- Epileptic+*Bacopa monnieri*

Table- 54

Real Time ampilfication of NMDA R1 receptor mRNA from the Hippocampus of Control, Epileptic, Epileptic+*Bacopa monnieri* post-treated group rats

Experimental group	Log RQ Value
Control	0
Epileptic	−2.290 ± 0.34 **
Epileptic+*Bacopa monnieri*	−0.192 ± 0.14 @

Values are mean ± S.D of 4-6 separate experiments. Relative Quantification values and standard deviations are shown in the table. The relative ratios of mRNA levels were calculated using the ΔΔCT method normalized with β-actin CT value as the internal control and Control CT value as the calibrator.

** $P<0.01$ when compared with Control group
@ $P<0.05$ when compared with Epileptic group

Figure- 59

Scatchard analysis of [^{3}H] Glutamate against glutamate in the Cerebellum of Control, Epileptic and Epileptic+*Bacopa monnieri* post-treated group rats

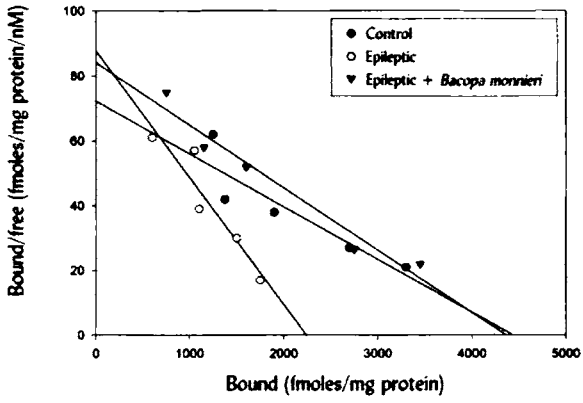


Table- 55

Scatchard analysis of [^{3}H] Glutamate against glutamate in the Cerebellum of Control, Epileptic and Epileptic+*Bacopa monnieri* post-treated group rats

Experimental Group	B_{max} (fmoles/mg protein)	K_d (nM)
Control	4586±110	54 ± 4.5
Epileptic	2233± 95 ***	37 ± 5.3
Epileptic+*Bacopa monnieri*	4396±87 @@@	51 ± 5.7

Values are mean ± S.E.M of 4-6 separate experiments.

*** P<0.001 when compared to Control group.
@@@ P<0.001 when compared to Epileptic group

Table- 56

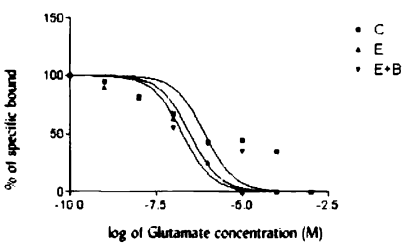
Binding parameters of [^{3}H]Glutamate against glutamate in the Cerebellum of Control, Epileptic and Epileptic+*Bacopa monnieri* post-treated group rats

Experimental Group	Best-fit model	Log (EC_{50})	K_i	Hill slope
Control	One-site	-6.081	$4.12X\ 10^{-7}$	- 0.587
Epileptic	One-site	-6.729	$9.32X\ 10^{-8}$	- 0.971
Epileptic+*Bacopa monnieri*	One-site	-6.515	$6.92X\ 10^{-7}$	- 0.859

Values are mean of 4-6 separate experiments

Figure- 60

Binding parameters of [^{3}H]Glutamate against Glutamate in the Cerebellum of experimental rats

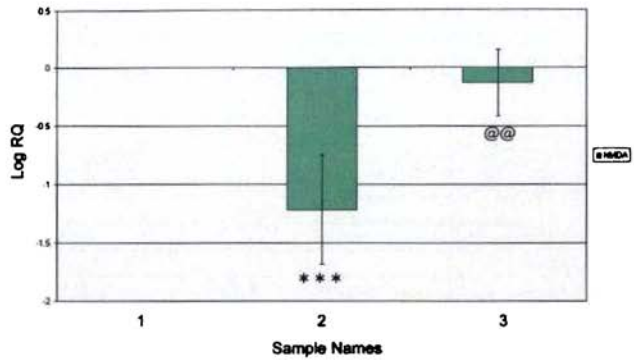


Data were fitted with iterative nonlinear regression software (Prism, GraphPad, San Diego, CA).

Ki – The affinity of the receptor for the competing drug. EC_{50} is the concentration of the competitor that competes for half the specific binding.

Figure- 61

Real Time PCR amplification of NMDA R1 receptor mRNA from the Cerebellum of Control, Epileptic, Epileptic+*Bacopa monnieri* post-treated group rats



1- Control
2- Epileptic
3- Epileptic+*Bacopa monnieri*

Table- 57

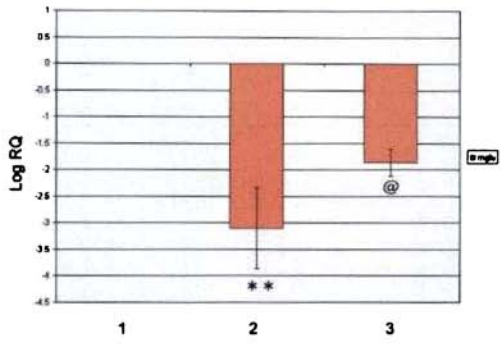
Experimental group	Log RQ Value
Control	0
Epileptic	$-1.215 \pm 0.46^{***}$
Epileptic+*Bacopa monnieri*	$0.130 \pm 0.28^{@@}$

Values are mean ± S.D of 4-6 separate experiments. Relative Quantification values and standard deviations are shown in the table. The relative ratios of mRNA levels were calculated using the ΔΔCT method normalized with β-actin CT value as the internal control and Control CT value as the calibrator.

*** P<0.001 when compared with Control group
@@ P<0.01 when compared to Epileptic group

Figure- 62

Real Time PCR amplification of Metabotropic Glutamate 8 receptor mRNA from the Cerebellum of Control, Epileptic and Epileptic+*Bacopa monnieri* post-treated group rats



1- Control
2- Epileptic
3- Epileptic+*Bacopa monnieri*

Table- 58

Experimental group	Log RQ Value
Control	0
Epileptic	−3.099 ± 0.07 **
Epileptic+*Bacopa monnieri*	−1.862 ± 0.25 @

Values are mean ± S.D of 4-6 separate experiments. Relative Quantification values and standard deviations are shown in the table. The relative ratios of mRNA levels were calculated using the ΔΔCT method normalized with β-actin CT value as the internal control and Control CT value as the calibrator.

** P<0.01 when compared with Control group
@ P<0.05 when compared to Epileptic group

Figure- 63

Scatchard analysis of [^{3}H] Glutamate against glutamate in the Brainstem of Control, Epileptic and Epileptic+*Bacopa monnieri* post-treated group rats

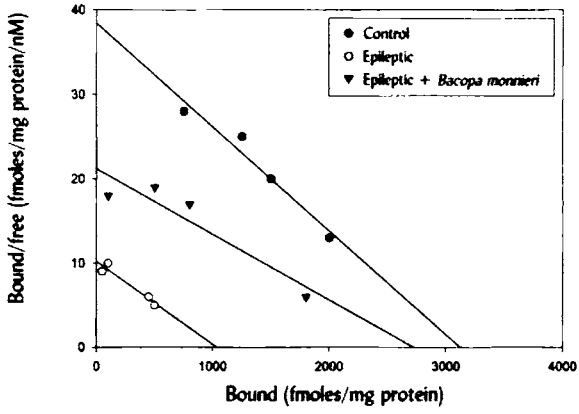


Table- 59

Experimental Group	B_{max} (fmoles/mg protein)	K_d (nM)
Control	3152± 57	81 ± 23
Epileptic	821 ±198 **	140 ± 52
Epileptic+*Bacopa monnieri*	2951 ±254 @@	100 ± 35

Values are mean ± S.E.M of 4-6 separate experiments.

** P<0.01 when compared to Control group,
@@ P<0.01 when compared to Epileptic group

Table- 60

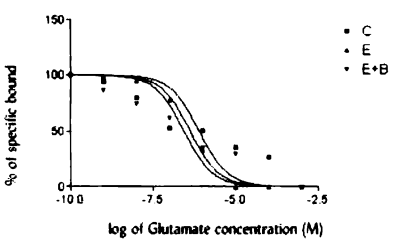
Binding parameters of [^{3}H]Glutamate against glutamate in the Brainstem of Control, Epileptic and Epileptic+*Bacopa monnieri* post-treated group rats

Experimental Group	Best-fit model	Log (EC_{50})	K_i	Hill slope
Control	One-site	-6.100	$1.79X\ 10^{-7}$	-0.912
Epileptic	One-site	-6.373	$2.12X\ 10^{-7}$	-0.997
Epileptic+*Bacopa monnieri*	One-site	-6.560	$1.37X\ 10^{-7}$	-0.836

Values are mean of 4-6 separate experiments

Figure- 64

Binding parameters of [^{3}H]Glutamate against Glutamate in the Brainstem of experimental rats

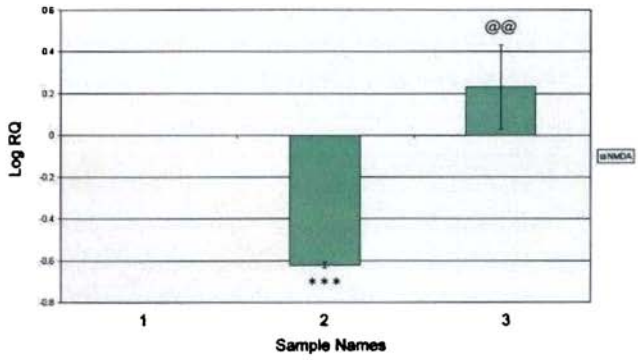


Data were fitted with iterative nonlinear regression software (Prism, GraphPad, San Diego, CA).

Ki – The affinity of the receptor for the competing drug. EC_{50} is the concentration of the competitor that competes for half the specific binding.

Figure- 65

Real Time PCR amplification of NMDA R1 receptor mRNA from the Brainstem of Control, Epileptic and Epileptic+*Bacopa monnieri* post-treated group rats



1- Control
2- Epileptic
3- Epileptic+*Bacopa monnieri*

Table- 61

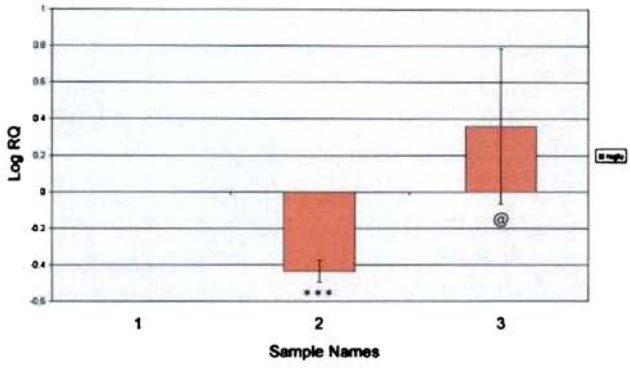
Experimental group	Log RQ Value
Control	0
Epileptic	$-0.621 \pm 0.01^{***}$
Epileptic+*Bacopa monnieri*	$0.231 \pm 0.20^{@@}$

Values are mean ± S.D of 4-6 separate experiments. Relative Quantification values and standard deviations are shown in the table. The relative ratios of mRNA levels were calculated using the ΔΔCT method normalized with β-actin CT value as the internal control and Control CT value as the calibrator.

*** $P<0.001$ when compared with Control group
@@ $P<0.01$ when compared to Epileptic group

Figure- 66

Real Time PCR amplification of Metabotropic Glutamate 8 receptor mRNA from the Brainstem of Control, Epileptic and Epileptic+*Bacopa monnieri* post-treated group rats



1- Control
2- Epileptic
3- Epileptic+*Bacopa monnieri*

Table- 62

Experimental group	Log RQ Value
Control	0
Epileptic	−0.436 ± 0.05 ***
Epileptic+*Bacopa monnieri*	0.358 ± 0.42 @

Values are mean ± S.D of 4-6 separate experiments. Relative Quantification values and standard deviations are shown in the table. The relative ratios of mRNA levels were calculated using the ΔΔCT method normalized with β-actin CT value as the internal control and Control CT value as the calibrator.

*** P<0.001 when compared with Control group
@ P<0.01 when compared to Epileptic group

Figure- 67

Scatchard analysis of [^{3}H] Glutamate against glutamate in the Cerebral Cortex of Control, Epileptic and Epileptic+*Bacopa monnieri* post-treated rats

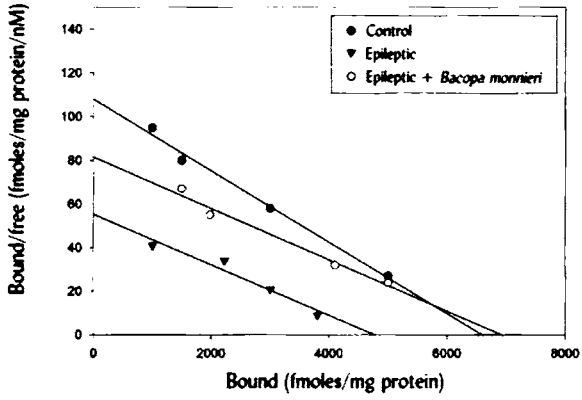


Table- 63

Scatchard analysis of [^{3}H] Glutamate against glutamate in the Cerebral Cortex of Control, Epileptic and Epileptic+*Bacopa monnieri* post-treated rats

Experimental Group	B_{max} (fmoles/mg protein)	K_d (nM)
Control	6525±168	59 ± 8.7
Epileptic	4777±211 **	62 ± 7.2
Epileptic+*Bacopa monnieri*	6834±178 @@	71 ± 14

Values are mean ± S.E.M of 4-6 separate experiments.

** P<0.001 when compared to Control group,
@@ P<0.001 when compared to Epileptic group

Table- 64

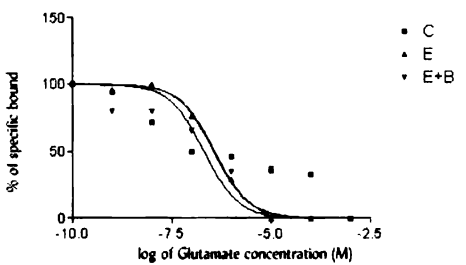
Binding parameters of [^{3}H]Glutamate against glutamate in the Cerebral Cortex of Control, Epileptic and Epileptic+*Bacopa monnieri* post-treated group rats

Experimental Group	Best-fit model	Log (EC_{50})	K_i	Hill slope
Control	One-site	-6.698	$1.89X\ 10^{-7}$	-0.892
Epileptic	One-site	-6.442	$1.81X\ 10^{-7}$	-0.997
Epileptic+*Bacopa monnieri*	One-site	-6.417	$1.91X\ 10^{-7}$	-0.792

Values are mean of 4-6 separate experiments

Figure- 68

Binding parameters of [^{3}H]Glutamate against Glutamate in the Cerebral Cortex of experimental rats



Data were fitted with iterative nonlinear regression software (Prism, GraphPad, San Diego, CA).

Ki – The affinity of the receptor for the competing drug. EC_{50} is the concentration of the competitor that competes for half the specific binding.

Figure- 69

Neo-Timm staining in the Hippocampus of Control, Epileptic, Epileptic+ Carbamazepine and Epileptic+ *Bacopa monnieri* post-treated group rats

a)

Control

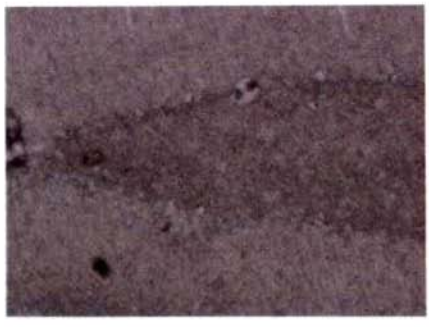
b)

Epileptic

c)

Epileptic+Carbamazepine

d)

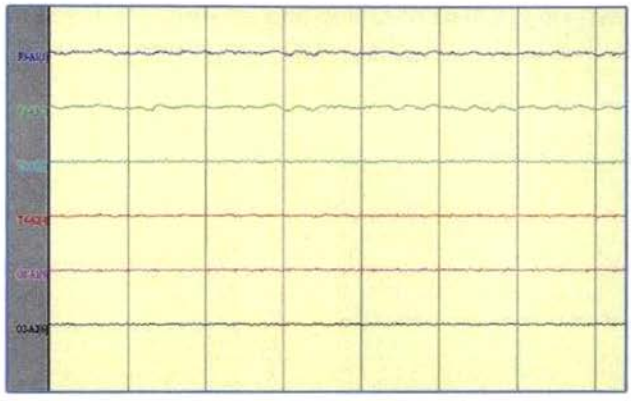


Epileptic+ *Bacopa monnieri*

Figure- 70

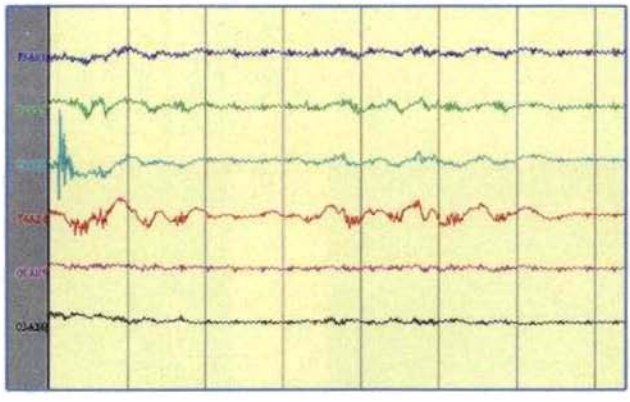
EEG of Control, Epileptic, Epileptic+Carbamazepine, Epileptic+*Bacopa monnieri* and Epileptic+Bacoside A post-treated group rats

a)



Control

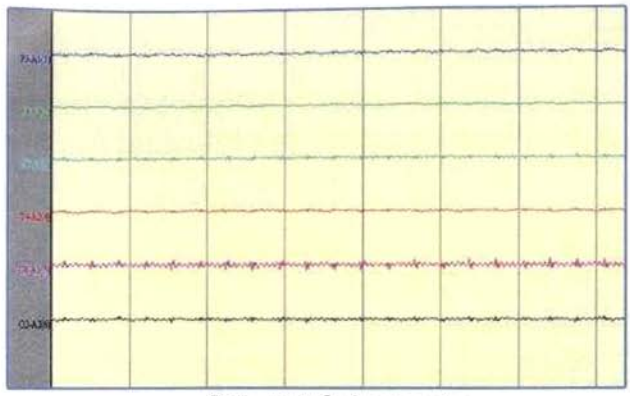
b)



Epileptic

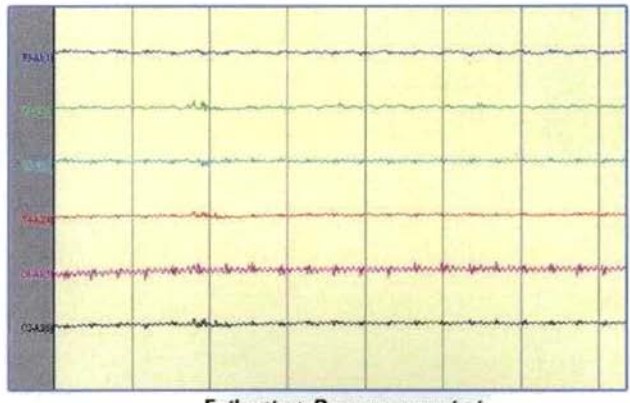
F3	Frontal lobe	left	F4	Frontal lobe	right
T3	Temporal lobe	left	T4	Temporal lobe	right
O1	Occipital lobe	left	O2	Occipital lobe	right
A1	Reference	left	A2	Reference	right

c)



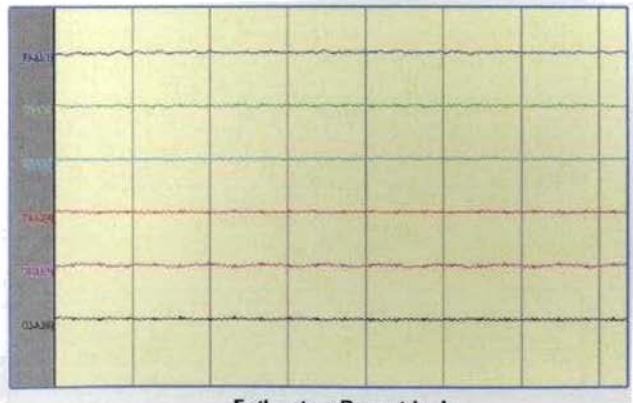
Epileptic + Carbamazepine

d)



Epileptic + *Bacopa monnieri*

e)



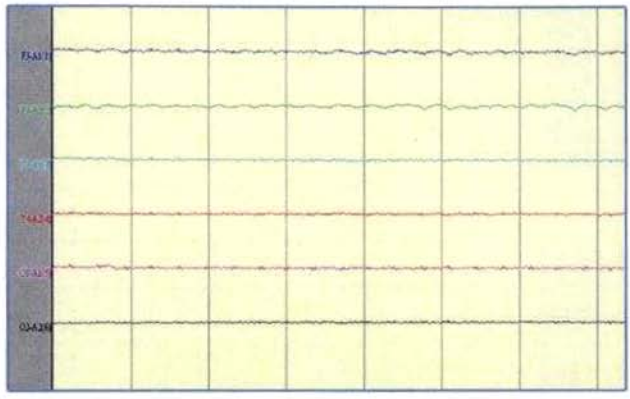
Epileptic + Bacoside A

F3	Frontal lobe	left	F4	Frontal lobe	right
T3	Temporal lobe	left	T4	Temporal lobe	right
O1	Occipital lobe	left	O2	Occipital lobe	right
A1	Reference	left	A2	Reference	right

Figure- 71

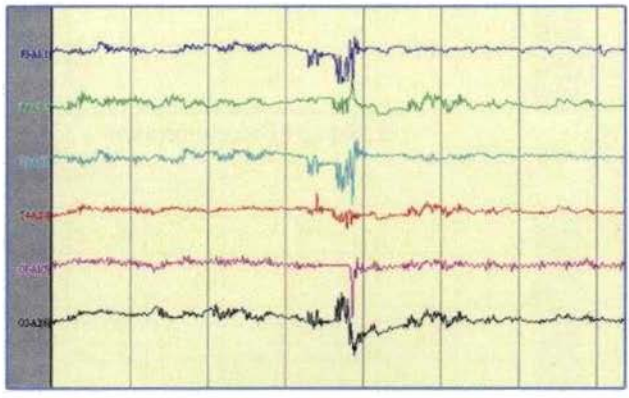
EEG of Control, Epileptic, Epileptic+Carbamazepine, Epileptic+*Bacopa monnieri* and Epileptic+Bacoside A pre-treated group rats

a)



Control

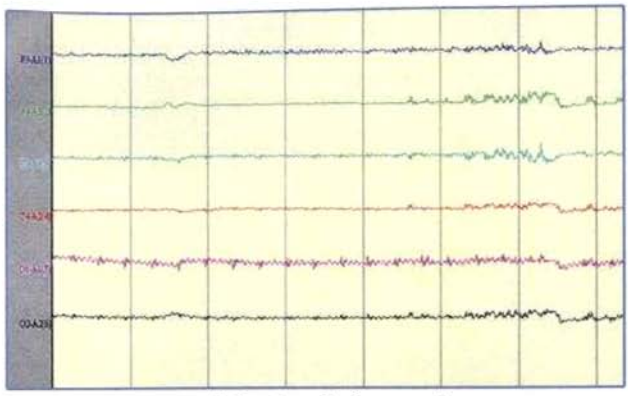
b)



Epileptic

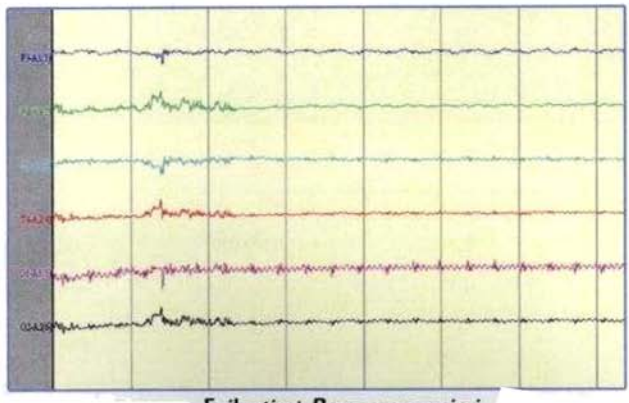
F3	Frontal lobe	left	F4	Frontal lobe	right
T3	Temporal lobe	left	T4	Temporal lobe	right
O1	Occipital lobe	left	O2	Occipital lobe	right
A1	Reference	left	A2	Reference	right

c)



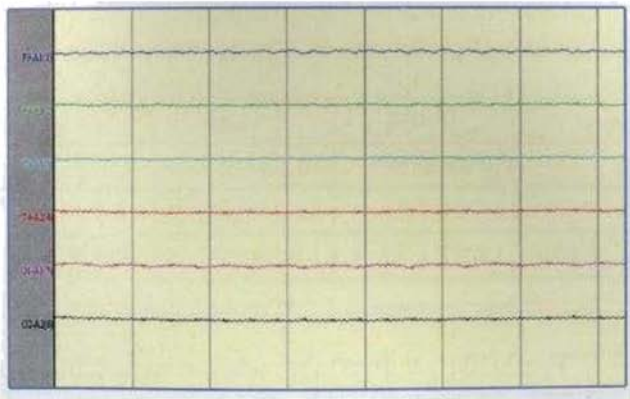
Epileptic + Carbamazepine

d)



Epileptic + *Bacopa monnieri*

e)



Epileptic + Bacoside A

F3	Frontal lobe	left	F4	Frontal lobe	right
T3	Temporal lobe	left	T4	Temporal lobe	right
O1	Occipital lobe	left	O2	Occipital lobe	right
A1	Reference	left	A2	Reference	right

Figure- 72

Escape Latency of Control, Epileptic and Epileptic+*Bacopa monnieri* post-treated group rats in Morris water maze Experiment

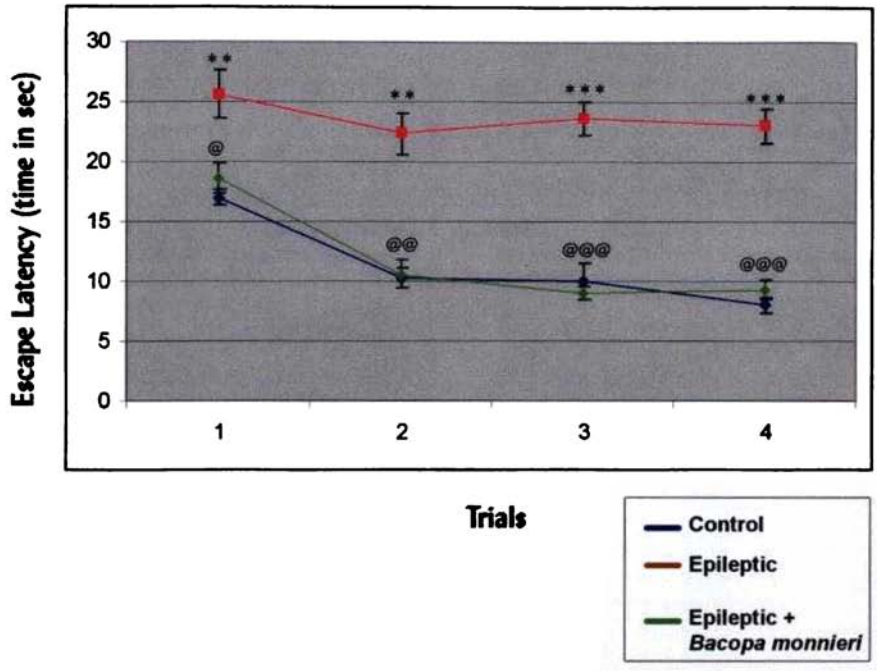


Table- 65

Escape Latency of Control, Epileptic and Epileptic+*Bacopa monnieri* post-treated group rats in Morris water maze Experiment

Experimental group	1st Trial	2nd Trial	3rd Trial	4th Trial
Control	16.0±0.57	10.3±0.88	10.0±1.52	8.0±0.57
Epileptic	25.6±2.02 **	22.3±1.76 **	23.6±1.45 ***	22.3±0.57 ***
Epileptic+Bacopa *monnieri*	18.6±1.20 @	10.6±1.20 @@	10.0±1.52 @@@	8.0±0.57 @@@

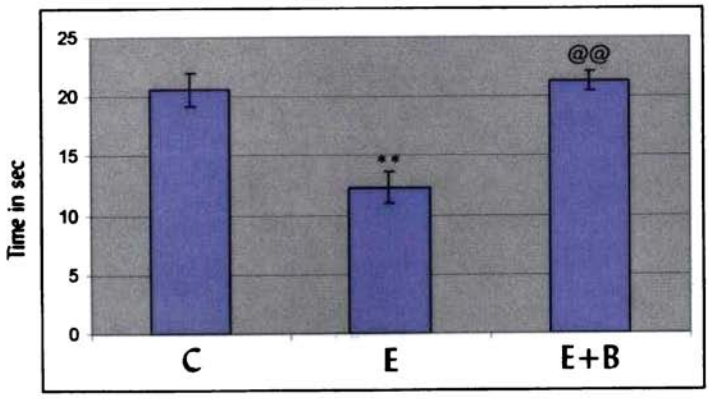
Values are mean ± S.E.M of 4-6 separate experiments.

*** P<0.001, ** P<0.01, when compared to Control

@@@ P<0.001, @@ P<0.01, @ P<0.05, when compared to Epileptic

Figure- 73

Time spent in platform quadrant by the Control, Epileptic and Epileptic+*Bacopa monnieri* post-treated group rats in Morris water maze experiment



C- Control
E- Epileptic
E+B- Epileptic+*Bacopa monnieri*

Table- 66

Time spent in platform quadrant by the Control, Epileptic and Epileptic+*Bacopa monnieri* post-treated group rats in Morris water maze experiment

Experimental Group	Time spent in platform quadrant
Control	20.6 ± 1.45
Epileptic	12.3 ± 1.30 **
Epileptic+*Bacopa monnieri*	21.3 ± 0.88@@

Values are mean ± S.E.M of 4-6 separate experiments

** P<0.01 when compared to Control group
@@ P<0.01 when compared to Epileptic group