$GABA_A$ AND $GABA_B$ RECEPTOR SUBUNITS GENE EXPRESSION AND THEIR FUNCTIONAL ROLE IN PILOCARPINE INDUCED TEMPORAL LOBE EPILEPSY: EFFECTS OF *Bacopa monnieri* AND BACOSIDE A

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CERTIFICATE

This is to certify that the thesis entitled **"$GABA_A$ and $GABA_B$ Receptor Subunits Gene Expression and their Functional Role in Pilocarpine Induced Temporal Lobe Epilepsy: Effects of *Bacopa monnieri* and Bacoside A"** is a bonafide record of the research work carried out by **Mr. Jobin Mathew,** 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.

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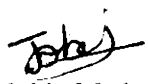
DECLARATION

I hereby declare that the thesis entitled **'$GABA_A$ and $GABA_B$ Receptor Subunits Gene Expression and their Functional Role in Pilocarpine Induced Temporal Lobe Epilepsy: Effects of *Bacopa monnieri* and Bacoside A'** is the authentic record of research work carried out by me for my doctoral degree, under the supervision and guidance of Dr. C. S. Paulose, Professor & Head, Department of Biotechnology, Director, Centre for Neuroscience, Cochin University of Science and Technology and that no part thereof has previously formed the basis for the award of any degree or diploma, associateship or other similar titles or recognition.



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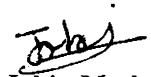


Jobin Mathew

Dedicated to my Beloved
Parents and Sisters. . .

ABBREVIATIONS

5-HT	5-Hydroxy tryptamine
ACh	Acetylcholine
AChE	Acetylcholine esterase
AEDs	Anti epileptic drugs
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ATP	Adenosine triphosphate
B_{max}	Maximal binding
BMR	Basal metabolic rate
bp	Base pair
BS	Brain stem
BZD	Benzodiazepine
CA	Cornu Ammonis
Ca^{2+}	Calcium
CB	Cerebellum
CC	Cerebral cortex
cAMP	Cylic adenosine monophosphate
CaBPs	Calcium-binding proteins
CBZ	Carbamazepine
CF	Cerebrospinal fluid
CNS	Central Nervous System

COPI	Coat protein-I
DA	Dopamine
DBH	Dopamine β hydroxylase
DNA	Deoxyribonucleic acid
DG	Dentate gyrus
E/I	Excitation/inhibition
EAAC-1	Excitatory amino acid carrier 1
EC	Entorhinal Cortex
ECD	Electrochemical detector
EDTA	Ethylene diamine tetra acetic acid
EEG	Electroencephalogram
EPI	Epinephrine
ER	Endoplasmic reticulum
GABA	Gamma amino butyric acid
$GABA_AR$	$GABA_A$ receptor
$GABA_BR$	$GABA_B$ receptor
GABA-T	GABA Transporter
GAD	Glutamic acid decarboxylase
GAD-IR	GAD- immunoreactive
GDPs	Giant Depolarizing Potentials
GEFS+	Generalized Epilepsy with Febrile Seizures Plus

GEPRs	Genetically epilepsy prone rats
GFAP	Glial fibrillary acidic protein
GIRKs	G protein-coupled inwardly rectifying K+ channels
GLAST	Glutamate/aspartate transporter
GLT-1	Glutamate transporter-1
GluR2	Glutamate Receptor-2
GLUT4	Glucose transporter type 4
GPCR	G protein-coupled receptor
HD	Heptahelical domain
HPLC	High performance liquid chromatography
Hsp70	Heat-shock protein 70
i.p.	Intraperitoneally
IPI	Initial Precipitating Injury
IPSCs	Inhibitory post-synaptic currents
KA	Kainate
KCCs	K^+Cl^- co-transporters
K_d	Dissociation constant
K_m	Michaelis constant
LDH	Lactate dehydrogenase
LFPs	Local field potential
LGICs	Ligand-gated ion channels

LTD	Long term depression
LTP	Long term potentiation
LTLE	Lateral temporal lobe epilepsy
MDH	Malate dehydrogenase
MF	Mossy fiber
mGlu	Metabotropic glutamate receptors
MPA	3-mercaptoproprionic acid
MR	Magnetic resonance
MRC	Mitochondrial respiratory chain
MRI	Magnetic resonance imaging
mRNA	Messenger ribonucleic acid
MRS	Magnetic resonance spectroscopy
MSN	Medium Spiny Neurons
MTLE	Mesial temporal lobe epilepsy
NE	Norepinephrine
NKCC1	Na-K-Cl cotransporter-1
NMDA	N-methyl-D-aspartate
P	Level of significance
P450	Cytochrome P450
PBS	Phosphate buffered saline
PBST	Phosphate buffered saline Triton X- 100

PC	Purkinje cell
PF	Parallel fiber
PFC	Prefrontal cortex
PWE	People Live With Epilepsy
RNA	Ribonucleic acid
ROS	Reactive oxygen species
SE	Status Epilepticus
S.E.M	Standard error of mean
SOD	Superoxide dismutase
SRMS	Spontaneous recurrent motor seizures
SSRIs	Serotonin reuptake inhibitors
T3	Triiodothyronine
T4	Thyroxine
TLE	Temporal lobe epilepsy
TM3	Transmembrane-3
VFT	Venus Flytrap domain
vGAT	Vesicular Transporter for GABA
VTA	Ventral tegmental area
WHO	World Health Organization
α_2-AR	α_2-Adrenergic receptor

CONTENTS

Introduction

Seizures and epilepsy have been documented since the earliest civilizations, before much was understood about the nervous system at all. Most individuals with epilepsy were thought to be possessed and the word "seizure" is derived from that notion, implying that gods take hold or "seizes" a person at the time a convulsion occurs. A general definition for the word "seizure" is a period of abnormal, synchronous excitation of a neuronal population. Seizures typically last seconds or minutes but can be prolonged and continuous in the case of status epilepticus. Importantly, the clinical manifestations vary and some seizures are not involving muscular contractions (convulsions) at all. In this modern era, epilepsy is the most frequent neurodegenerative disease after stroke. It afflicts more than 50 million people worldwide (Strine *et al.*, 2005). Epilepsy knows no geographical, racial or social boundaries and 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 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. Epilepsy leads to multiple interacting medical, psychological, economic and social repercussions, all of which need to be considered (WHO epilepsy Atlas 2005).

The difference between seizures and epilepsy is commonly confused. The two are not the same. Epilepsy is defined by a state of recurrent, spontaneous seizures. If one seizure occurs in an individual, it may not necessarily mean that they have epilepsy because the seizure may have been provoked and that individual may never have a seizure again. The concept of epileptogenesis refers to the development of the state of epilepsy. It refers to the sequence of events that converts the normal brain into one that can support a seizure. It is assumed that groups of neurons become hyperexcitable, poised to abnormally discharge impulse. Seizures can be caused by

multiple mechanisms and often they appear so diverse that one would suspect that no common theme applies. However, one principle that is often discussed is that seizures arise when there is a disruption of mechanisms that normally create a balance between excitation and inhibition. Thus, normally there are controls that keep neurons from excessive action potential discharge, but there are also mechanisms that facilitate neuronal firing so the nervous system can function appropriately. Disrupting the mechanisms that inhibit firing or promoting the mechanisms that facilitate excitation can lead to seizures. Conversely, disrupting the mechanisms that bring neurons close to their firing threshold, or enhancing the ways neurons are inhibited, usually prevents seizure activity.

The hypersynchronous discharges that occur during a seizure are beging 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, 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.

Temporal lobe epilepsy (TLE) is among the most frequent types of drug resistant epilepsy (Litt *et al.*, 2001). In a population of new patients presented with epilepsy, almost 30% of them had seizures originating from the temporal lobe of the brain (Manford *et al.*, 1992). Individuals affected with TLE typically have comparable clinical description; including an initial precipitating injury (IPI) such as the *status epilepticus* (SE), head trauma, encephalitis or childhood febrile seizures (Harvey *et al.*, 1997; Fisher *et al.*, 1998). In many individuals with TLE, an initial insult or injury leads to a period of time without evidence of overt seizures and then recurrent seizures begin. Thus, the fact that many individuals with TLE have experienced an event (typically early in life) that could be an initial insult is compelling. It suggests that an initial insult begins a process that ultimately leads to recurrent seizures. If it does not actually "begin" the process, at the very least it seems likely that it facilitates it. Examples include birth trauma, a febrile seizure, or infection such as encephalitis. However, the insult also may occur later and a common example is a war injury as an adult that leads to TLE decades later. The fact that these events are injurious, leading to neuronal damage and the fact that many patients with TLE have dramatic neuronal loss upon autopsy, have led many to conclude that damage to neurons is critical to the pathophysiology of TLE (Meldrum, 1997).

Furthermore, it appears that certain types of neurons are more vulnerable than others, such as hippocampal Cornu Ammonis-1 (CA1) and the endfolium relative to the dentate gyrus and CA2 (Scharfman & Pedley, 2006). This has led to the assumption that the pattern of neuronal loss may also be important in TLE and the idea that protection of vulnerable neuronal populations could be advantageous. However, there is no clear proof to date that the selective loss of CA1 pyramidal cells, for example, is a key component of TLE. The fact that a delay appears to occur between an initial insult and the first seizure, has led many to suggest that a progression of changes in response to the initial insult is critical in epileptogenesis. However, the latent period can be very long, even decades in some patients. That

observation has led some to suggest that additional environmental factors or "second hits" could be important in ultimately causing seizures to emerge (Walker *et al.*, 2002).

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. 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.

Gamma-aminobutyric acid (GABA) is the principal inhibitory neurotransmitter in the mammalian brain. It acts through 2 classes of receptors, $GABA_A$ receptors that are ligand-operated ion channels and the G-protein-coupled metabotropic $GABA_B$ receptors. Impairment of GABAergic transmission by genetic mutations or application of GABA receptor antagonists induces epileptic seizures, whereas drugs augmenting GABAergic transmission are used for antiepileptic therapy. In animal epilepsy models and in tissue from patients with temporal lobe epilepsy, loss in subsets of hippocampal GABA neurons is observed. Also at the level of the $GABA_A$ receptor, neurodegeneration-induced loss in receptors is accompanied by markedly altered expression of receptor subunits in the dentate gyrus and other

parts of the hippocampal formation, indicating altered physiology and pharmacology of $GABA_A$ receptors. Such mechanisms have highly relevant role in seizure induction, augmentation of endogenous protective mechanisms and resistance to antiepileptic drug therapy. Studies suggest a role of $GABA_B$ receptors in absence seizure Presynaptic $GABA_B$ receptors suppress neurotransmitter release. Depending on whether this action is exerted in GABAergic or glutamatergic neurons, there may be anticonvulsant or proconvulsant actions. It has therefore been suggested that dysfunction of the GABAergic system have a fundamental role in the propagation of acute seizures and in the manifestation of epilepsy syndromes. Indeed mutant mice lacking the enzyme glutamate decarboxylase (GAD) or certain subunits of $GABA_A$ receptors are prone to spontaneous epileptic seizures.

The ability to reproduce human diseases in animal models presents a great advantage for modern experimental medicine (Russell, 1964). A great deal of the knowledge that has improved our understanding of epileptic disorders has derived from appropriate animal models (Purpura *et al.*, 1972). This is certainly the case in TLE, the most common type of partial complex seizure in adulthood (Hauser *et al.*, 1996). The main features of TLE are: (i) the localization of seizure foci in the limbic system, particularly in the hippocampus, entorhinal cortex and amygdala (Bartolomei *et al.*, 2005); (ii) the frequent finding of an "initial precipitating injury" that precedes the appearance of TLE (Mathern *et al.*, 2002); (iii) a seizure-free time interval following the precipitating injury known as "latent period" and (iv) a high incidence of mesial or Cornu Ammonis sclerosis, i.e., a unilateral hippocampal lesion leading to atrophy, typically caused by neuronal loss and gliosis in Sommer's sector (the subiculum-CA1 transition zone) and the endfolium (dentate hilus) (Mathern *et al.*, 1997). Most of these characteristics can be reproduced in chronic animal models of TLE, particularly kindling or SE animal models. 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. 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 GABAergic functional regulation in chronic epilepsy models.

A significant number of people (25%) afflicted with epilepsy have seizures that cannot be controlled by antiepileptic drugs (Litt et al., 2001). Moreover, anti-epileptic drugs (AEDs) merely provide symptomatic treatment without having any influence on the course of the disease. Thus, there is a pressing need to develop alternative therapeutic approaches that prevent the epileptogenesis after the SE or an IPI. From this perspective, identification of compounds or approaches that are efficacious for providing neuroprotection to the hippocampus after the onset of SE has great significance (Acharya *et al.*, 2008). While pharmaceutical companies continue to invest enormous resources in identifying agents that could be used to alleviate debilitating disorders and retard mental deterioration afflicting numerous people around the world, a source of potentially beneficial agents, namely phytochemicals, would appear to have significant benefits that have yet to be fully exploited. Therefore, several plants have been selected based on their use in traditional systems of medicine, and research has identified a number of natural compounds that could act as nootropic agents. One plant that has been used as brain tonic and restorative in epileptic conditions is 'Brahmi' (*Bacopa monnieri*). *Bacopa monnieri* belonging to the family Scrophulariaceae, is a creeping annual plant found throughout the Indian subcontinent in wet, damp and marshy areas (Russo & Borrelli, 2005).

In the Indian medicinal system - Ayurveda, *Bacopa monnieri* has been used since 3000 years as a memory enhancing, antioxidative, adaptogenic, anti-inflammatory, analgesic, antipyretic, sedative and antiepileptic agent (Jyoti & Sharma, 2006). The earliest chronicle mention is in the Ayurvedic treatise, the Charaka Samhita (100 A.D.), in which *Bacopa monnieri* 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, *Bacopa monnieri* 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 *Bacopa monnieri* has an unusual combination of constituents that are beneficial in mental inefficiency, illnesses and useful in the management of convulsive disorders like epilepsy. Treatments with the plant (Malhotra & Das, 1959) and ethanol extract (Singh & Dhawan, 1982) have enhanced learning ability. Bacosides, *Bacopa monnieri'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 & Singh, 2005). But so far there are very few studies reporting the role of *Bacopa monnieri* treatment on the functional regulation of neurotransmitters and their receptors. In our previous studies we reported the down regulated expression of the N-methyl-D-aspartate receptor-1 (NMDA R1) in the hippocampus (Reas *et al.*, 2007), down regulated expression of the metabotropic glutamate receptor-8 (mGluR8) (Paulose *et al.*, 2008) and 5-HT receptor binding in the cerebellum of epileptic rats was reversed upon treatment with *Bacopa monnieri* (Amee *et al.*, 2009). Here the alterations of GABA receptor subtype and their gene expression were evaluated in the brain regions of pilocarpine model of TLE. Also, the antiepileptic effect of *Bacopa monnieri* and bacoside A was assessed at the molecular level.

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 measure the T3 and insulin content in the serum of control, epileptic, epileptic rats treated with *Bacopa monnieri*, Bacoside A and Carbamazepine.

3. To study the cholinergic activity using acetylcholine esterase assay in the muscle and heart and Malate dehydrogenase activity in the muscle of control and experimental rats.

4. To study the GABA content in the cerebral cortex, hippocampus, cerebellum, corpus striatum and brain stem of control and experimental groups of rats.

5. To study the general GABA, $GABA_A$ and $GABA_B$ receptor binding parameters in the cerebral cortex, hippocampus, cerebellum, corpus striatum and brain stem of control and experimental rats.

6. To study the gene expression of $GABA_{A\alpha 1}$, $GABA_{A\alpha 5}$, $GABA_{A\gamma 3}$, $GABA_{A\delta}$, $GABA_B$, and GAD in the cerebral cortex, hippocampus, cerebellum, corpus striatum and brain stem of control and experimental rats using Real-Time PCR.

7. To study the confocal imaging of $GABA_{A\alpha 1}$ receptor subunit in the cerebral cortex, hippocampus, cerebellum, corpus striatum and brain stem of control and experimental rats using immunofluorescent receptor specific antibodies in brain slices.

8. To study the behavioral changes in the control and experimental rats using Radial Maze and Y-Maze test, grid walk test, narrow beam test and open field test.

Temporal Lobe Epilepsy

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 (Lowenstein, 1996). Temporal lobe epilepsies are a group of medical disorders in which humans and animals experience recurrent epileptic seizures arising from one or both temporal lobes of the brain (Engel, 1992; Engel & Pedley, 1997). TLE is considered the most common epileptic syndrome and it is estimated that approximately 80% of patients with partial seizures have TLE (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) arises in the hippocampus, parahippocampal gyrus and amygdala which are located in the inner aspect of the temporal lobe. 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). LTLE arises in the neocortex on the outer surface of the temporal lobe of the brain.

TLE is also characterized pathologically by unique morphological alterations in the hippocampus (Engel *et al.*, 1989, 1996). It is a neurodegenerative disease that interrupts the normal electrical activity of the brain and promotes abnormal wiring in the brain. From time to time, multiple groups of neurons fire simultaneously in people with epilepsy, which triggers an electrical tempest within the brain culminating in seizures (Engel *et al.*, 1989; Lothman & Bertram, 1993). TLE with hippocampal sclerosis, one of the most frequent kinds of human epilepsy, is typified by a progressive development of spontaneous recurrent motor seizures (SRMS) (Engel *et*

al., 1989; Lothman & Bertram, 1993; Ben-Ari & Cossart, 2000). Usually the disease starts in early childhood and becomes pharmacologically intractable during the course of time (Engel, 2001). A classic sequence of seizures in TLE encompasses an aura followed by arrest of motor behavior, blank stare and automatisms. Furthermore, patients with TLE often show impairments in attention, memory, mental processing speed, executive functions, mood, personality and interictal depression (Devinsky, 2004).

Definition of Epilepsy

Epilepsy is a chronic disorder characterized by recurrent seizures, which may vary from a brief lapse of attention or muscle jerks, to severe and prolonged convulsions. The seizures are caused by sudden, usually brief, excessive electrical discharges in a group of brain cells (neurons) (Gastaut *et al.*, 1970). A seizure is a convulsive episode, which starts of as atypical, excessive hyper-synchronous discharges from an aggregate of neurons in the brain and then recruits surrounding neurons to comprise one or both hemispheres of the brain (Acharya *et al.*, 2008). During the seizure the person may experience the change or loss of consciousness, involuntary movements such as jerking, shaking or twitching. The International Classification of Epileptic Seizures (1981) recognizes two general categories of seizures based on the origin of the abnormal electrical discharge. Two broad categories of seizures are recognized, partial and generalized, with each category having different subtypes (Benbadis *et al.*, 2001).

1) Partial seizures, referred to as focal or local seizures, originate in one location of the brain and then may or may not spread to other brain areas. Partial seizures are further subdivided into simple partial and complex partial. In simple partial seizures consciousness is preserved. In complex partial seizures there is an alteration in consciousness, the person does not recall having the seizure and may be

very confused and fatigued in the aftermath. A partial seizure may also progress into a generalized motor seizure (Chabolla, 2002).

2) Generalized seizures, referred to as "grand mal" seizures, begin simultaneously in all areas of the brain. Consciousness is altered and the person may or may not show convulsions (Chabolla, 2002).

Other commonly used terms include ictal (of seizure itself) and interictal (between seizures). Convulsion implies ictal behaviour with vigorous motor activities. SE denotes a very prolonged seizure or series of seizures occurring so frequently that full recovery of brain function does not occur intricately.

Epidemiology of Epilepsy

Epilepsy is the commonest serious neurological condition affecting 0.5-1% of the population. Today, an estimated 50 million people live with epilepsy, 80% of whom in developing countries. Those most affected often do not come forward. Stigma, misconceptions and beliefs attached to this condition influence the open presentation of affected individuals in public meetings. The public health significance is particularly high in these settings because of its high prevalence, its seizure acuteness and frequency and the sociological, psychosocial and financial consequences for the households it affects (Hauser, 1997). Resource poor countries share demographic, sociological and economic features. They are particularly marked by ethnic, linguistic and religious richness, and their populations are frequently threatened by political instability and economic uncertainties. As a consequence health systems are typically weak and lack efficiency in addressing health needs (Quet *et al.*, 2008).

Temporal Lobe Epilepsy and Brain Injury

The TLE is characterized by hippocampal neurodegeneration, aberrant mossy fiber sprouting, spontaneous recurrent motor seizures, cognitive deficits and

abnormally enhanced neurogenesis during the early phase and dramatically declined neurogenesis during the chronic phase of the disease. Although the precise cause of TLE is unknown, it is typically seen after an initial precipitating injury (IPI) such as brain injury, tumors, meningitis, encephalitis, SE and febrile seizures (Engel *et al.*, 1989; French *et al.*, 1993; Mathern *et al.*, 1995; Mathern *et al.*, 1996; Wieser, 2004; Lewis, 2005). Nevertheless, it is difficult to predict the consequences of initial seizures in humans because of multiple contributing factors. These include differences in the etiology and age at the onset of seizures, the types, frequency and duration of seizures, interventions with antiepileptic drugs (AEDs) and genetic components (Avoli *et al.*, 2005; Guerrini *et al.*, 2005). On the other hand, it is well known that epilepsy or seizures are linked with neurodegeneration in several areas of the brain (Wasterlain & Shirasaka, 1994; Jacobs *et al.*, 2000; Armstrong, 2005). Furthermore, it is clear that both necrotic and apoptotic cell death contribute to neuronal damage in epileptogenic insults such as the SE, head injury or stroke (Fujikawa *et al.*, 2000a, 2000b; Henshall *et al.*, 2000; Sahuquillo *et al.*, 2004). It is supposed that multiple mechanisms and neurochemical modulators play roles in the initiation of epileptogenesis after an IPI. The term epileptogenesis refers to the transformation of the normal neuronal network into a long lasting chronically hyperexcitable state.

The brain injury resulting from seizures is a dynamic process that comprises multiple factors contributing to neuronal cell death. These include genetic factors, the extent of glutamate-mediated excitotoxicity leading to disturbances in the intracellular electrolyte metabolism, mitochondrial dysfunction, oxidative stress, growth factor withdrawal or depletion and increased concentration of cytokines (Ferriero, 2005). At cellular level, intense seizure activity typically initiates massive influx of calcium *via* voltage gated and N-methyl-D-aspartate (NMDA)-dependent ion channels (Van Den Pol *et al.*, 1996). Elevated intracellular ions lead to biochemical cascades which trigger acute neuronal cell death after the SE (Fujikawa *et al.*, 2000a). Additionally, high levels of intracellular calcium can induce generation of reactive oxygen species

(ROS), uncoupling of mitochondria and activation of a wide range of catabolic enzymes that are capable of deteriorating cell function (Gupta & Dettbarn, 2003; Niquet & Wasterlain, 2004; Acharya & Katyare, 2005; Niquet *et al.*, 2005).

Hippocampus and Temporal Lobe Epilepsy

The hippocampus has been implicated in TLE for three main reasons. First, seizure activity can be recorded from the hippocampus (Spencer & Spencer, 1994; Engel, 1995; Bertram, 1997). Second, removal of the affected hippocampus eliminates seizures in 80–90% of TLE patients exhibiting unilateral mesial temporal lobe sclerosis (Falconer *et al.*, 1964; Ojemann, 1987). Third, stereotypic neuropathology is found in the hippocampus of TLE patients that is recapitulated in animal models of TLE, termed hippocampal sclerosis (Schwartzkroin & Knowles, 1984; Sutula *et al.*, 1988; Houser *et al.*, 1990; French *et al.*, 1993; Mello *et al.*, 1993; Buckmaster & Dudek, 1997). Indeed, multiple hippocampal changes are apparent in both TLE and animal models of TLE (Sloviter, 2006). The structural changes in the hippocampus comprise partial degeneration of CA1 and CA3 pyramidal neurons and dentate hilar neurons (Rao *et al.*, 2006), reductions in gamma-amino butyric acid positive interneurons (Shetty & Turner, 1997, 2000), loss of the calcium binding protein calbindin in dentate granule cells (Nagerl *et al.*, 2000; Shetty & Hattiangady, 2007) and abnormal sprouting of dentate granule cell, entorhinal, and CA3 axons (Shetty & Turner, 1997; Shetty, 2002; Shetty *et al.*, 2005; Siddiqui & Joseph, 2005; Wozny *et al.*, 2005). The axons of dentate granule cells (hippocampal mossy fibers) branch out of the dentate hilus and abnormally innervate the dentate inner molecular layer in TLE, a phenomenon known as aberrant mossy fiber sprouting (Tauck & Nadler, 1985; Sutula *et al.*, 1989; Shetty & Turner, 1997; Shetty *et al.*, 2003; Koyama & Ikegaya, 2004, 2005). It appears that the aberrant mossy fiber sprouting contributes to the increased seizure susceptibility of the dentate gyrus, as the extent of aberrant mossy fiber sprouting positively correlates with both antidromically evoked burst firing and

spontaneous seizures in kainic acid (KA) models of TLE (Cronin & Dudek, 1988; Masukawa *et al.*, 1989; Mathern *et al.*, 1993; Dudek *et al.*, 1994; Buhl *et al.*, 1996). In some species, including certain bats (Buhl & Dann, 1990) and monkeys (Seress & Mrzljak, 1987; Frotscher *et al.*, 1988), a subpopulation of granule cells regularly has basal dendrites. In humans, basal dendrites normally extend into the hilus in 22-47% of granule cells (Seress & Mrzljak, 1987; Al-Hussain & Al-Ali, 1995; Lim *et al.*, 1997; Lauer *et al.*, 2003). Granule cell basal dendrites are more frequent in schizophrenics (Lauer *et al.*, 2003), but that might be a side-effect of treatment with neuroleptics, which stimulate granule cell neurogenesis (Dawirs *et al.*, 1998). Increasing the subpopulation of granule cells with basal dendrites might be pathogenic in patients with schizophrenia or epilepsy by augmenting positive-feedback circuits. Basal dendrites are ideally positioned to receive excitatory synaptic input from granule cell axons, which are concentrated in the hilus. In normal monkeys, granule cells with basal dendrites exhibit functional recurrent excitation (Austin & Buckmaster, 2004). Increased production of new granule cells after the SE typically results in abnormal migration of a substantial fraction of newly differentiated granule cells into the dentate hilus. The hilar granule cells get activated when animals display spontaneous seizures during the chronic epilepsy (Scharfman *et al.*, 2002b; Jessberger *et al.*, 2007). With perforant path stimulation, the ectopic granule cells display an unusually long latency to onset of evoked responses, suggesting polysynaptic activation of these cells (Scharfman *et al.*, 2003). Analysis of dendrites of hilar dentate granule cells confirms mossy fiber terminals as their source of afferent input (Pierce *et al.*, 2005). Further characterization implies that the synaptic connectivity of hilar granule cells predisposes them to discharge in epileptiform bursts. For instance, physiological recordings in hippocampal slices demonstrate spontaneous bursts of action potentials in ectopic granule cells. Because these spontaneous discharges are synchronized with area CA3 pyramidal cell population discharges, it suggests an abnormal activity (Scharfman *et al.*, 2000). Because of the above connectivity and

behavior, it is believed that the hilar granule cells contribute to spontaneous recurrent seizures (SRS) occurring in chronically epileptic animals. Indeed, a study by McCloskey et al, (2006) reports that the frequency of behavioral seizures in animals treated with pilocarpine is directly proportional to the size of the ectopic granule cell population in the dentate hilus. Additionally, Jung et al, (2004) demonstrate that inhibition of dentate neurogenesis after the SE *via* intracerebroventricular infusions of the mitotic inhibitor cytosine-β-D-arabinofuranoside reduces the frequency of chronic seizures, suggesting the involvement of ectopically integrated newly born neurons in the generation of spontaneous seizures. Therefore, strategies that prevent or reverse abnormal mossy fiber sprouting in the dentate gyrus have received considerable attention as a treatment for TLE (Shetty & Turner, 1997; Shetty *et al.*, 2005; Hattiangady *et al.*, 2006). Thus, involvement of the hippocampus in the pathophysiology of TLE is substantial.

PATHOPHYSIOLOGY OF TEMPORAL LOBE EPILEPSY

EEG studies show 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, 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 CA1, 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.*, 2000) 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 hippocampus of many patients with TLE (Meldrum & Bruton, 1992). Seven days and two months post-status epilepticus rats showed significant neuron loss in the pre-endopiriform 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. Cases have been demonstrated, where some granule cells of experimental animals are 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 study described an increased generation of hippocampal granule cells as a consequence of seizures (Parent *et al.*, 1997). It is hypothesized that the astrocytes in sclerotic tissue have activated molecular pathways that could lead to enhanced release of glutamate by these cells. Such glutamate release excites surrounding neurons and elicits seizure activity (Janigro, 2008). 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 & 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 nerve growth factor (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 SE 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.

Mossy Fibre Sprouting and Impaired Inhibition

Mossy fiber sprouting is a form of synaptic reorganization in the dentate gyrus that occurs in human TLE and animal models of epilepsy. The axons of dentate gyrus granule cells, called mossy fibers, develop collaterals that grow into an abnormal location, the inner third of the dentate gyrus molecular layer. Electron microscopy has shown that sprouted fibers form synapses on both spines and dendritic shafts in the inner molecular layer, which are likely to represent the dendrites of granule cells and inhibitory neurons. One of the controversies about this phenomenon is whether mossy fiber sprouting contributes to seizures by forming novel recurrent excitatory circuits among granule cells. Sprouting mossy fibers synapse almost exclusively with excitatory neurons in the granule cell layer and molecular layer of the dentate gyrus. Lesioning the synaptic input from the entorhinal cortex to granule cells also triggers mossy fiber sprouting and synaptogenesis in adult rats (Laurberg & Zimmer, 1981; Frotscher & Zimmer, 1983). A variety of experimental treatments that produce epilepsy also induce axon sprouting in other brain regions (Perez *et al.*, 1996; McKinney *et al.*, 1997; Esclapez *et al.*, 1999). Repeated intense seizures caused an attenuation of 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 (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.

Impaired Inhibition

Research into seizures has gravitated to mechanisms associated with synaptic transmission because of its critical role in maintaining the balance between excitation and inhibition. As more research has identified the molecular mechanisms of synaptic transmission, it has become appreciated that defects in almost every step can lead to seizures (Babb & Brown, 1987; Sutula *et al.*, 1989). Glutamatergic and GABAergic transmission, as the major excitatory and inhibitory transmitters of the nervous system, respectively, have been examined in great detail. It is important to point out, however, that both glutamate and GABA not have a simple, direct relationship to seizures. One reason is that desensitization of glutamate and GABA receptors can reduce effects, depending on the time course of exposure. In addition, there are other reasons (Coulter *et al.*, 1996). GABAergic transmission can lead to depolarization rather than hyperpolarization if the gradients responsible for ion flow through GABA receptors are altered. For example, chloride is the major ion that carries current through $GABA_A$ receptors and it usually hyperpolarizes neurons because chloride flows into the cell from the extracellular space (Sloviter *et al.*, 1987). However, the K^+Cl^- co-transporters (KCCs) that are pivotal to the chloride gradient are not constant. In development, transporter expression changes and this has led to evidence that one

of the transporters Na-K-Cl cotransporter-1 (NKCC1) which explains seizure susceptibility early in life. The relationship of glutamate to excitation is not always be simple either. One reason is that glutamatergic synapses innervate both glutamatergic neurons and GABAergic neurons in many neuronal systems (Sloviter *et al.*, 1987). Exposure to glutamate could have little net effect as a result, or glutamate paradoxically increases inhibition of principal cells because the GABAergic neurons typically require less depolarization by glutamate to reach threshold. It is surprisingly difficult to predict how glutamatergic or GABAergic modulation will influence seizure generation *in vivo*, given these basic characteristics of glutamatergic and GABAergic transmission. Repeated intense seizures caused an attenuation of 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 (Sloviter *et al.*, 1989).

Pilocarpine Model of Temporal Lobe Epilepsy

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 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 (Turski *et al.*, 1983; Clifford *et al.*, 1987; Turski *et al.*, 1989). 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.*, 1987). 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.

ROLE OF NEUROTRANSMITTERS IN EPILEPSY

GABA

GABA is the major inhibitory neurotransmitter in the CNS (Sivilotti & Nistri, 1991). Inhibitory inter-neurons that make use of GABA as their neurotransmitter are found throughout the brain, but in any region they comprise a wide range of morphological and functional types that participate in different circuits with principal neurons. Thus, in the CA1 area of the rat hippocampus it is possible to distinguish 16 different types of GABAergic interneurons on the basis of their morphology, specific protein content (e.g., calbindin, calretinin, parvalbumin), and pattern of firing in relation to ongoing rhythms and oscillatory firing of pyramidal neurons (Kaila, 1997). Through the mechanism of recurrent inhibitory feedback, GABAergic interneurons in the cortex terminate local sustained burst firing and, through inhibitory surround, limit

the lateral spread of seizure activity. Chemical agents that impair GABAergic inhibition are powerful convulsants.

Precise GABAergic synaptic signaling is critical to the accurate transmission of information within neural circuits and even slight disruptions can produce hypersynchronous activity (Chagnac-Amitai & Connors, 1989). Moreover, changes in ambient GABA can alter tonic inhibition and thus the overall synaptic tone of a brain region (Farrant & Nusser, 2005). The mechanisms of GABA synthesis and degradation are well understood. Glutamate is decarboxylated to GABA *via* glutamic acid decarboxylase (GAD). GABA that is released into the synaptic cleft, is transported in to both astrocytes and interneurons through specific transporters. Transported GABA can be repackaged for subsequent release in interneuronal terminals while astrocytic GABA is usually metabolized *via* GABA-transaminase (GABA-T). These metabolic cycles are reviewed (Martin & Tobin, 2000; Bak *et al.*, 2006). However, there is an increasing recognition that regulating neurotransmitter metabolism provides another avenue for neuromodulation. In terms of the GABAergic system, the anticonvulsant vigabatrin enhances the GABA content of neurons and glia by blocking its degradation, thereby increasing vesicular concentrations (French, 1999) while the expression of the synthetic enzyme, GAD, is enhanced following a seizure (Feldblum *et al.*, 1990; Esclapez & Houser, 1999). Moreover, both experimental and modeling studies have shown that modulating the intracellular content determines the degree of vesicular GABA release (Engel *et al.*, 2001; Wu *et al.*, 200; Axmacher *et al.*, 2004). Liang *et al* (2006) showed that blocking neuronal glutamine uptake reduces evoked inhibitory potentials (IPSCs) in a highly use-dependent fashion, presumably by limiting glutamate availability within interneurons. Similar data were presented by Fricke *et al* (2007). Studies using isotopically labeled compounds suggest that the bulk of GABA that is released during neurotransmission is freshly synthesized from glutamine rather than transported from the extracellular space (Waagepetersen *et al.*, 2001). In addition, there is evidence that GAD and the

vesicular transporter for GABA (vGAT) form a protein complex (Jin *et al.*, 2003). These data suggest that newly synthesized GABA is preferred substrate for vesicle loading. Consistent with the hypothesis that synaptic inhibition relies on newly synthesized GABA, transported glutamate is used for GABA synthesis (Mathews & Diamond, 2003) and blocking neuronal glutamate transport is associated with seizure activity *in vivo* (Sepkuty *et al.*, 2002). The consequences of this dependence on freshly synthesized GABA, as compared to transported and repackaged transmitter, have not been studied in detail using *in vitro* preparations where better controlled studies of physiological responses can be obtained. There is an extensive literature showing that seizures can be provoked by blocking GABA synthesis with 3-mercaptoproprionic acid (MPA) *in vivo* (Mares *et al.*, 1993). These studies were demonstrating the involvement of GABA in the prevention of the overstimulation of neuronal networks.

GABA Receptors

GABA mediates its actions *via* three distinct receptors: the ionotropic $GABA_A$ and $GABA_C$ receptors and the metabotropic $GABA_B$ receptors (Bormann, 2000). In addition to its CNS functions, the GABAergic system is also present in peripheral tissues, including the gastrointestinal tract (Gilon *et al.*, 1990; 1991; Harty *et al.*, 1991; Krantis *et al.*, 1994). Ionotropic GABA receptors are the most important Cl^- channels in the central nervous system CNS, and their expression has also been found in peripheral organs. These receptors mediate a fast inhibitory neurotransmission in the CNS (Akinci & Schofield, 1999). Ionotropic GABA receptors can be categorized into $GABA_A$ and $GABA_C$ receptors based on their subunit compositions and pharmacological properties (Bormann, 2000).

$GABA_B$ receptors mediate slow prolonged inhibition in the brain by activating postsynaptic G protein-coupled inwardly rectifying K^+ channels (GIRKs) and inactivating presynaptic voltage-gated Ca^{2+} channels. $GABA_B$ receptors also inhibit

adenylate cyclase, leading to diminished activity of PKA signaling pathways (Bowery, 2006). Structurally $GABA_B$ receptors are members of the class C family of G-protein coupled receptors (GPCR) and are encoded in vertebrates by two genes - $GABA_B$ receptor-1 ($GABA_BR1$) and $GABA_BR2$ respectively (Bettler *et al.*, 2004; Couve *et al.*, 2002).

In tissue resected from patients with temporal lobe epilepsy, the number of GABA receptors is 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 [^{3}H]Ro15-4513 associated with the α4 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 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 $GABA_A$ receptor α5 subunit in CA1/2 (Houser *et al.*, 1995). Loss of α5 and α2 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.

$GABA_A$ Receptors

$GABA_A$ receptors are pentameric in structure, with the five subunits arranged like spokes of a wheel around a central Cl^- selective pore (Barnard, 2001). Nineteen GABA receptor subunits have been cloned from rats, which include α1–6, β1–3, γ1–3, ρ1–3, δ, θ, ε, and π (Whiting *et al.*, 1999). The 19 subunits (α1–6, β1–3, γ1–3, δ, ε, θ, π, ρ1–2) are encoded by 19 distinct genes. Each subunit has four transmembrane segments, with both the amino and carboxy termini located extracellularly. These extracellular segments form the recognition sites (two per channel) for GABA and also, in some channel types, the recognition site (one per channel) for benzodiazepine-like allosteric modulators. The subunit composition determines both the biophysical properties of the receptor–channel complex and its pharmacology, most notably the sensitivity to benzodiazepines (BDZ) (Rudolph & Mohler 2004; Johnston, 2005). A typical benzodiazepine-sensitive $GABA_A$ receptor consists of two α1, α2, α3, or α5 subunits, two β2 or β3 subunits (or one each) and a γ2 subunit.

Classically, $GABA_A$ receptors have been recognized as mediating phasic inhibition through the generation of fast, transient, rapidly desensitizing currents (IPSCs) in postsynaptic neurons in response to synaptically released GABA. More recently, it has been recognized that $GABA_A$ receptors also contribute to tonic (extrasynaptic) inhibition, representing the Cl^- conductance activated at nonsynaptic sites in response to background concentrations of GABA (Farrant & Nusser, 2005). Phasic and tonic inhibitions are mediated by $GABA_A$ receptors with different subunit composition, GABA affinities and rates of desensitization. The most notable difference in subunit composition is that the receptors mediating tonic inhibition contain the δ subunit, rather than the γ subunit characteristic of synaptic $GABA_A$ receptors (Nusser *et al.,* 1998). Receptors containing α4, α5, or α6 are commonly found nonsynaptically. Pharmacologically, the most notable difference is that receptors with α4, α6, or δ subunits are not potentiated by benzodiazepines or by nonbenzodiazepine benzodiazepine receptor agonists (such as zolpidem), whereas those with α1, α2, α3, α5, or γ2 subunits are benzodiazepine sensitive. The benzodiazepine-sensitive α subunits (α1, α2, α3, α5) differ from the insensitive ones (α4, α6) in possessing a histidine residue at position 101.

Genetic studies in humans reveal a range of idiopathic generalized epilepsy syndromes linked to mutations in the $GABA_A$ receptor (Macdonald *et al.*, 2004). It is noted that these syndromes are phenotypically indistinguishable from those associated with mutations in voltage-gated ion channels. For example, a mutation in the $GABA_A$ receptor α1 subunit is associated with autosomal dominant juvenile myoclonic epilepsy (Cossette *et al.*, 2004); the mutation reduces peak current by decreasing trafficking of the subunit, so that there is deficient surface expression (Macdonald *et al.*, 2004). Mutations involving the γ2 subunit in two cases associated with generalized epilepsy with febrile seizures plus (GEFS+) and in two cases associated with childhood absence epilepsy with febrile convulsions have been found to alter the kinetic properties of the receptor and also their surface expression (Macdonald *et al.*,

2004). Three mutations in families with GEFS+ involving the δ subunit (Dibbens *et al.*, 2004) are associated with reduced current, suggesting that impairment of tonic inhibition by extrasynaptic GABA receptors can also produce epilepsy. Studies in genetically modified mice have revealed spontaneous seizures in β3 knockout mice (DeLorey *et al.*, 1998) supporting the interpretation that the seizures that are a prominent feature of the Angelman syndrome (which, in addition to other genetic abnormalities, lacks the β3 gene) are due specifically to defects in $GABA_A$ receptors.

$GABA_B$ Receptors

The $GABA_B$ receptor is part of the class C of GPCRs that also includes the mGlu, the Ca^{2+}-sensing and the sweet and umami taste receptors among others (Pin *et al.*, 2003). These receptors are dimers, either homodimers linked by a disulphide bond (mGlu and Ca^{2+}-sensing receptors) or heterodimers made of two similar, but distinct subunits (the $GABA_B$ and taste receptors). Indeed, the $GABA_B$ receptor was the first GPCR to be identified that requires two distinct subunits to function: the $GABA_{B1}$ and $GABA_{B2}$ subunits (Jones *et al.*, 1998; White *et al.*, 1998; Kaupmann *et al.*, 2003). Although the $GABA_{B1}$ subunit was soon shown to bind all known $GABA_B$ ligands (both agonists and antagonists) this protein did not form a functional $GABA_B$ receptor when expressed alone (Kaupmann *et al.*, 1997). Only when $GABA_{B1}$ was co-expressed with the homologous $GABA_{B2}$ subunit was a functional $GABA_B$ receptor observed, either in cell lines or in cultured neurons. The $GABA_B$ dimeric entity was confirmed in native tissue (Kaupmann *et al.*, 1998). Indeed, both $GABA_{B1}$ and $GABA_{B2}$ mRNAs are co-localized in most brain regions. Second, both proteins are found in the same neurons, even in the same subcellular compartments as observed at the electron microscopic level. Moreover, co-immunoprecipitation of $GABA_{B1}$ with a $GABA_{B2}$ antibody could be demonstrated from brain membranes. Eventually, mice lacking either $GABA_{B1}$ or $GABA_{B2}$ share very similar phenotypes and none of the known $GABA_B$-mediated responses could be measured in either mice (Prosser *et al.*,

2001; Schuler *et al.*, 2001). Although unusual baclofen mediated inhibition of GIRK channels could be observed in mice lacking $GABA_{B2}$, it is still not known whether this represents a natural response, or is the consequence of the absence of the $GABA_{B2}$ subunit. Taken together, these data demonstrate that the assembly between these two proteins is required to get a functional $GABA_B$ receptor in native tissues.

$GABA_B$ receptors play an important role in maintaining excitatory–inhibitory balance in brain and alterations can lead to seizures (Hossein *et al* 2008). $GABA_B$ receptors, the metabotropic receptors for GABA, are G protein-coupled receptors (GPCR) which regulate neuronal excitability both pre and postsynaptically. The action of GABA at presynaptic $GABA_B$ receptors is to reduce Ca^{2+} influx and thus inhibit neurotransmitter release (Takahashi *et al.*, 1998). These receptors exist on GABAergic terminals (autoreceptors), or on terminals arising from cells containing other neurotransmitters, such as glutamate (heteroreceptors). Postsynaptically, $GABA_B$ receptors are responsible for the generation of the late inhibitory postsynaptic potential (IPSP), *via* the opening of K^+ channels and inhibit adenylate cyclase (Bettler *et al.*, 1998). Abnormality in either of these functions could have consequences for the generation and/or prevention of epileptic seizures. Multiple laboratories have demonstrated altered expression of $GABA_BR1$ and $GABA_BR2$ in animal models for seizure disorders (Straessle *et al.*, 2003; Princivalle *et al.*, 2003; Han *et al.*, 2006). Han *et al.* (2006) found that as a result of multiple seizures, there was a long-term decrease in $GABA_BR1$ (15 days) and $GABA_BR2$ (30 days) expression in rat hippocampus. Following an injection of kainic acid into the dorsal hippocampus in mouse model of temporal lobe epilepsy, Straessle *et al.* (2003) observed a rapid decline in $GABA_BR1$ and $GABA_BR2$ in CA1, CA3c, and hilus. Princivalle *et al.* (2003) observed that the corticothalamic circuit, using genetic absence epilepsy rats from strasbourg (GARES), found increased $GABA_BR1$ and $GABA_BR2$ protein expression in somatosensory cortex, ventrobasal, and reticular thalamic nuclei (Princivalle *et al.*, 2003). Moreover, knockout mice lacking $GABA_BR1$ exhibit epilepsy, enhanced prepulse inhibition,

impaired memory and die prematurely largely as a result of development of generalized seizures (Prosser *et al.*, 2001; Schuler *et al.*, 2001). Taken together, these animal studies suggest that the changes in the number $GABA_B$ receptors lead to epilepsy, due to changes in transmitter release (presynaptic) and inhibition (postsynaptic). Accordingly, GABA neurons have been alternately proposed to be highly vulnerable or relatively invulnerable after insults known to cause epilepsy (Ribak *et al.*, 1979; Sloviter *et al.*, 1987) and GABA-mediated inhibition is reportedly decreased, in animal models of epilepsy (Dalby *et al* 2001).

$GABA_C$ Receptors

$GABA_C$ receptors, which are a subfamily of $GABA_A$ receptors, are members of the Cys-loop superfamily of ligand-gated ion channels (LGICs), an important group of receptors involved in rapid synaptic transmission and whose malfunction can result in a variety of neurological disorders; hence, understanding their mechanism of action is of considerable pharmacological interest. $GABA_C$ receptors are mostly located in retinal neurons where they play a role in retinal signaling involved in diseases such as macromolecular degeneration (Bormann, 2000). The receptors are activated by the binding of GABA, the main inhibitory neurotransmitter in the central nervous system. $GABA_C$ receptors have distinct pharmacological properties from $GABA_A$ receptors, e.g., they are not inhibited by bicuculline, the classic $GABA_A$ receptor antagonist (Barnard *et al.*, 1998; Chebib *et al.*, 2000). Like all the LGICs belonging to the Cys-loop superfamily, $GABA_C$ receptors are composed of five subunits arranged in a pentagonal array around a central ion-permeant pore. Each subunit has an extracellular N-terminal domain (ECD), a transmembrane domain composed of four α-helices, and an intracellular domain. Three subunits (ρ_{1-3}) have been identified; these can all form functional homomeric or heteromeric receptors (Enz, 2001).

Similar to $GABA_A$ receptors, they possess a high permeability to Cl^-, but in contrast to $GABA_A$ channels, they are insensitive to bicuculline, barbiturates and

benzodiazepines (Polenzani *et al.*, 1991). The activity of $GABA_C$ receptors is regulated by extracellular agents, such as Zn^{2+}, H^+, Ca^{2+} (Wang *et al.*, 1995; Kaneda *et al.*, 1997) and also by intracellular factors, such as Ca^{2+}, phosphatases and kinases (Feigenspan & Bormann, 1994*b*; Kusama *et al.*, 1995). Protein phosphorylation is postulated to be an important physiological mechanism for regulating GABA mediated synaptic inhibition (Moss *et al.*, 1992; Moss & Smart, 1996).

Glutamic Acid Decarboxylase

GABA the main inhibitory neurotransmitter in the brain, is synthesized by GAD. GAD exists in two isoforms termed GAD65 and GAD67 due to their molecular weights of 65 and 67 kDa, respectively. These enzymes are the products of two independently regulated genes sharing 65% sequence homology in rats (Erlander *et al.*, 1991a; 1991b, *Bu et al.*, 1992). Most GABAergic interneurons express both subtypes of GAD (Houser & Esclapez 1994; Esclapez *et al.*, 1994) which are simultaneously detectable in the rat brain as early as embryonic day 17 (Dupuy & Houser, 1996). GAD67 is found in axonal regions as well as in neuronal cell bodies, whereas GAD65 is mainly associated with synaptic terminals (Kaufman *et al.*, 1991). Therefore it has been suggested that GAD67 mostly provides a pool of GABA for general metabolic activity while GABA synthesized by GAD65 is likely to be more involved in synaptic transmission (Martin & Rimvall, 1993). Mice lacking GAD65 are vital and do not exhibit changes in their brain GABA content though they have an increased susceptibility to seizures (Asada *et al.*, 1996; Kash *et al.*, 1997). During embryogenesis the mRNA coding for GAD67 is regulated by alternative splicing (Bond *et al.*, 1990; Szabo *et al.*, 1994). At least two additional transcripts exist, I-80 and I-86 (summarized as EGAD), distinguished by insertions of 80 or 86 base pair (bp) in GAD67 mRNA, respectively. The two inserts are identical with exception of the 6 bp at the 3'-end of the larger fragment containing an overlapping stop-start codon. The complete coding region of embryonic GAD messages comprises

1,860 (80 bp insert) and 1,866 bp (86 bp insert). Both embryonic transcripts code for a short enzymatically inactive GAD protein of 25 kDa (GAD25) which corresponds to the amino-terminal regulatory region of GAD67 and therefore has putative regulatory functions. Termination-reinitiation at the stop-start codon of I-80 additionally produces an enzymatically active protein of 44 kDa (GAD44) corresponding to the carboxy-terminal catalytic domain of GAD67 that contains the pyridoxal phosphate cofactor binding site (Szabo *et al.*, 1994).

The early finding that baby food deficient in vitamin B6 (a cofactor of the GABA-synthesizing enzyme GAD) caused vitamin-reversible seizures provided one of the first clues that seizures might be caused by reduced synthesis of GABA (Hunt *et al.*, 1954, Bankier *et al.*, 1983). Accordingly, GABA neurons have been alternately proposed to be highly vulnerable or relatively invulnerable after insults known to cause epilepsy (Ribak *et al.*, 1979; Sloviter, 1987) and GABA-mediated inhibition is reportedly altered, in a variety of constantly compared but greatly dissimilar animal models (Dalby & Mody, 2001). The use of GAD immunocytochemistry in kindled and control tissue was used to allow direct anatomic confirmation for measuring changes in GAD-immunoreactivity (GAD-IR) which would represent GABA synthesis for release by the recurrent inhibitory system of the fascia dentata. Immediately after the last kindled seizure, optically detected GAD-IR puncta densities were significantly reduced in stratum granulosum. At 3 or 7 days after the last kindled seizure (Babb *et al.*, 1989). GAD65-/- mice develop spontaneous seizures that result in increased mortality. Seizures can be precipitated by fear or mild stress. Seizure susceptibility is dramatically increased in GAD65 mice backcrossed into a second genetic background, the nonobese diabetic strain of mice enabling electroencephalogram analysis of the seizures. The generally higher basal brain GABA levels in this backcross are significantly decreased by the GAD65 mutation, suggesting that the relative contribution of GABA synthesized by GAD65 to total brain GABA levels is genetically determined (Kash *et al.*, 1997).

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 TLE (Turski *et al.*, 1989; Liu *et al.*, 1994; Nagao *et al.*, 1996; Dickson & Alonso 1997). 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 (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 *et al.*, 1990). 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, 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) that is then conveyed to the hippocampal formation *via* the perforant path (Steward & 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, 1986) thereby closing an EC–hippocampal loop. Thus, by virtue of its extensive projection systems, the EC network acts 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 & Kohler, 1984; Lysakowski *et al.*, 1989; Gaykema *et al.*, 1990), recisely 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, 1987; 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.

Epinephrine and Norepinephrine

The modification of the seizure activity by the noradrenergic system was 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; Dailey *et al.*, 1991) (4) adrenergic agonists acting at the α_2 adrenoreceptor (α_2-AR) have anticonvulsant action (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 (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 (DA) is an endogenous neuromodulator in the cerebral cortex and is believed to be important for normal brain processes (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). Dopamine favour long-lasting transitions of PFC neurons to a more excitable up state. *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) and decreases (Geijo-Barrientos & Pastore, 1995) in postsynaptic excitability of pyramidal neurons have been reported following DA D1 receptor activation. In addition, changes in

excitability mediated by DA D2 receptors have been reported (Gulledge & Jaffe, 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 DA D1 receptor–mediated effect of dopamine (Law-Tho *et al.*, 1994; Seamans *et al.*, 2001). 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 leads 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). Dopamine is known to modulate epileptiform discharges both *in vivo* (Alam & Starr 1993) and *in vitro* (Suppes *et al.*, 1985; Alam & Starr 1993; 1994; Siniscalchi *et al.*, 1997; Cepeda *et al.*, 1999). *In vivo* studies in different models of epilepsy have suggested that dopamine have a pro-convulsant effect mediated by DA D_1 receptors and an anti-convulsant effect via DA 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 and/or serotonin in brain areas involved in epileptogenesis. Behavioural and electrocorticographic studies in rats have shown that DA controls hippocampal excitability via opposing actions at DA D1 and DA D2 receptors. Seizure

enhancement is presumed to be a specific feature of D1 receptor stimulation, whereas DA D2 receptor stimulation is anticonvulsant (Alam & Starr, 1993). Decreased DA D2 receptor binding in the brainstem were reported in other neurological diseases like diabetes.

Serotonin

Central 5-HT_{1A} receptors function both as somatodendric presynaptic autorecptors 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 autorecptors 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, 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. 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 behaviours. Interactions between serotonergic and other neurotransmitters contribute to the behavioural 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. The up-regulation

of 5-HT_{2c} receptors were reported in the brain stem which induce sympathetic stimulation were reported.

Glutamate

Glutamate 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 TLE (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 mGluR2 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 (mGluR) are classified into three functional groups on the bases of their sequence homology, second messenger effectors and pharmacology (Dingledine & Conn 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 (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 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 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. 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. 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 & 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 activates a self-destructive cellular cascade involving many calcium-dependent enzymes, such as phosphatases (eg, calcineurin), proteases and lipases. Lipid peroxidation also causes production of free radicals which damage vital cellular proteins and lead to neuronal death (Choi, 1988; Michaels & Rothrnan, 1990).

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). 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. 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. 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). 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 interneurons, 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, CAI and CA2 pyramidal cells in rat hippocampi. In area dentata, calbindin immunoreactivity is normally present in a small number of interneurons of the molecular and granule cell layers and in a small population of presumed basket cells in or below the granule cell layer. Recent studies suggest that there is a loss of calbindin from granule cells of the dentate gyrus and select CAI 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.

Epilepsy and Treatment

Antiepileptic drug therapy is ineffective to control seizures in a significant fraction of people (~25%) afflicted with epilepsy (Litt *et al.*, 2001; McKeown & McNamara, 2001). Besides, antiepileptic drugs have side effects and do not influence the course of the disease. Although surgical resection of the epileptic hippocampus is efficacious for controlling seizures in many cases of drug-resistant TLE (Clusmann *et al.*, 2002; Engel, 2003), this approach is associated with significant cognitive deficits

(Nunn *et al.*, 1999; Martin *et al.*, 2002; Richardson *et al.*, 2004). Therefore, developing alternative therapeutic approaches that prevent or restrain epileptogenic changes and SRMS after an IPI have considerable significance.

Neuroprotection following SE should encompass not only the prevention of neuronal death, but also preservation of neuronal and network function. This is critical because these aims are not necessarily equivalent; prevention of neuronal loss, for example, does not inevitably prevent epileptogenisis. Anticonvulsant drugs prevent or terminate seizures. In so doing, these agents act on the emergent properties of the epileptogenic network to alter or diminish their function (Walker, 2007). This involves modification of specific neuronal components that result in sufficient elevation of seizure threshold that prevents the usual initiating mechanisms from activating the network. Such an action can totally prevent the seizure or result in blockade of specific behavioural components of the seizure thereby reducing seizure severity (Graumlich *et al.*, 1999; Faingold, 1999). Long-lasting changes in neuronal networks are observed following repetitive experiences, including behavioural conditioning and repeated seizures. Seizure repetition can induce neurogenesis in susceptible brain sites, resulting in structural and functional network changes. Structural changes are partially mediated by neurotrophic factors and excitation increases and burst firing in network neurons can result.

By classifying epilepsy syndromes such as seizure type, age of onset, EEG evidence and associated impairments clinicians can begin to rationalise their approach and define the therapeutic options for each patient. When to start and/or stop drug treatment in epilepsy is a major issue, which requires detailed knowledge of the prognosis of the disorder. For 20-30% of sufferers, epilepsy is a chronic and disabling condition, refractory to drug treatment, which has immense social impact. Although currently available drugs are able to prevent seizures, there remains a clear medical need for new antiepileptic drugs. Experiments in intact animals provide a complementary approach for the application of mechanistic information obtained in *in*

vitro investigations. *In vivo* techniques allow the exploration of the regional selectivity of these mechanisms, since it has become quite clear that the anticonvulsant effects of certain drugs involve actions on a combination of specific currents and cellular properties selectively expressed in specific brain networks (Kao & Coulter, 1997). Whether such actions observed with acute anticonvulsant drug treatment continue during the duration of treatment is unknown, since it is becoming clear that chronic treatment with a number of drugs that modify neuronal properties often results in compensatory mechanisms that lead to tolerance or to additional chronic effects that contribute to the action of the drug (Chen *et al.*, 1999).

Epilepsy and cognitive impairment

Neuropsychological impairment is an important co-morbidity of chronic epilepsy (Elger *et al.*, 2004). Patients with epilepsy often experience cognitive dysfunction. Multiple factors can adversely affect cognition in epilepsy, including the etiology of the seizures, cerebral lesions acquired before the onset of seizures, seizure type, age at onset of epilepsy, seizure frequency, duration, severity, interictal physiologic dysfunction, structural cerebral damage caused by repetitive or prolonged seizures, hereditary factors, psychosocial factors and sequelae of treatment for epilepsy, including antiepileptic drugs AEDs and epilepsy surgery (Saling *et al.*, 1993; Helmstaedter & Kurthen 2001; Jones-Gotman, 2000; Elger *et al* 2004 Aldenkamp & Arends, 2004; Dodrill, 2004). All these interrelated factors make complex contributions to cognitive deficits (Helmstaedter & Kurthen 2001). AEDs affect cognition by suppressing neuronal excitability or enhancing inhibitory neurotransmission. Patients and even some clinicians, tend to blame cognitive problems on AEDs because they are more identifiable than other factors. However, AED effects should not be overrated. Psychosocial problems, which are common, can be overlooked as a source of cognitive impairment. The stigma of epilepsy and the fear of having seizures in public can lead to low self-esteem, social isolation, and

depression, all of which can negative affect cognitive function. Similarly, subclinical epileptiform activity is another important contributor to cognitive dysfunction that can go unrecognized, especially in patients with infrequent seizures. A rich literature has characterized relationships between adequacy of mental status and a variety of clinical epilepsy factors including etiology, age of onset, seizure type and severity, duration, antiepilepsy medications and other factors (Saling *et al.*, 1993; Jones-Gotman, 2000; Helmstaedter & Kurthen 2001; Jokeit & Ebner, 2002; Aldenkamp & Arends, 2004; Dodrill, 2004; Koop *et al.*, 2004, Marsh *et al.*, 2006). In addition, cognitive profiles have been derived for several syndromes of epilepsy and efforts have been undertaken to identify the shared versus unique cognitive abnormalities evident across these syndromes (Lassonde *et al.*, 2000; Nolan *et al.*, 2003; Elger *et al.*, 2004; Henkin *et al.*, 2005; Riva *et al.*, 2005; Hommet *et al.*, 2006; Sart *et al.*, 2006;).

The nature, timing and course of cognitive impairments in epilepsy remains an issue of substantial interest and concern, particularly the degree to which chronic medication-resistant epilepsy lead to progressive cognitive impairment (Pitkanen & Sutula 2002). While evidence to this effect has been reviewed (Dodrill, 2004), the early cognitive substrate upon which subsequent chronic epilepsy exert its effects is an important consideration. The possibility that early onset or childhood epilepsy adversely alter a child's cognitive substrate in a greater than expected fashion despite their increased plasticity is an issue of clinical interest.

Indirect evidence implicating an adverse neuro-developmental effect of childhood onset epilepsy has come from studies of adults with chronic epilepsy grouped by age of onset categories where fairly robust relationships have been reported between earlier age of onset of recurrent seizures and cognitive abnormality. This relationship, reported early in the last century (Fox, 1924), confirmed in studies of adult patients with diverse seizure types (Dikmen *et al.*, 1975, 1977; Dodrill & Matthews, 1992; Glosser *et al.*, 1997) and observed in neuropsychological studies of younger patients with complex partial and other types of seizures (Leary *et al.*, 1981;

Schoenfeld *et al.*, 1999; Cormack *et al.*, 2007). In addition, greater than expected neuropsychological abnormalities have been reported in adults with the syndrome of mesial TLE (Hermann *et al.*, 1997), a syndrome defined by a focal neuropathological substrate and early onset of recurrent seizures or IPI (Engel, 1996). These findings suggest that early onset epilepsy, including localization-related syndromes of epilepsy such as mesial temporal lobe epilepsy, is associated with widespread influence on brain development and structure.

In the case of mesial temporal lobe epilepsy, quantitative volumetric magnetic resonance (MR) imaging studies have reported abnormalities in neural regions involved in the genesis and propagation of seizures, including the hippocampus (Jack *et al.*, 1992; Quigg *et al.*, 1997; Woermann *et al.*, 1998; Tasch, *et al.*, 1999), amygdale (Kalviainen *et al.*, 1996), entorhinal cortex (Bernasconi *et al.*, 1999), fornix (Kuzniecky *et al* 1999), thalamus and basal ganglia (DeCarli *et al.*, 1998), and temporal lobe (Breier *et al.*, 1996; Marsh *et al.*, 1997; Lee *et al.*, 1997; Moran *et al.*, 2001). Additional investigations have reported abnormalities in more wide ranging volumes of gray and white matter in extratemporal lobar (Marsh *et al.*, 1997), regional (Sandok *et al* 2000) or total brain morphometrics (Lee *et al.*, 1997; Sisodiya *et al.*, 1997) These distributed volumetric abnormalities, interesting in their own right, are also consistent with the widespread cognitive abnormalities that can be observed in chronic temporal lobe epilepsy. The potential impact of early versus late age of seizure onset on quantitative MRI volumetrics in TLE has however, rarely been systematically investigated, surprisingly given the neuropsychological literature reviewed above as well as animal studies demonstrating that seizures in the immature brain adversely affect brain growth and development (Wasterlain & Plum, 1973; Dwyer & Wasterlain 1998; Wasterlain *et al.*, 1999).

However, more direct evidence of the neurodevelopmental impact of recurrent seizures on cognition has been provided by controlled studies of children and adolescents with chronic but substantially shorter duration epilepsy. Studies such

as these have also reported considerable neuropsychological impairment (Farwell *et al.*, 1985; Roeschl *et al.*, 2002; Smith *et al.*, 2002) consistent with an early adverse neurodevelopmental impact on cognition. However, even these effects could be a combination of pre-epilepsy onset (etiological) insults, factors which have contributed to the development of epilepsy and simultaneously contributed to abnormal mental status. In order to derive perhaps the clearest perspective of the natural course of cognitive status in epilepsy, it is important to characterize the earliest status of the cognitive substrate and to that end; investigation of children with new onset epilepsy contribute to this literature. To date, a modest number of studies have examined cognition in children with new onset epilepsy (Bourgeois *et al.*, 1983; Williams *et al.*, 1998; Stores *et al.*, 1992; Kolk *et al.*, 2002; Oostrom *et al.*, 2003). Three of the five studies identified cognitive impairments at epilepsy onset and these mixed results are attributable, at least in part, to the variable age ranges, test batteries and epilepsy characteristics across studies. Also, interesting and very pertinent to this topic are reports of academic underachievement prior to and/or at the onset of idiopathic/cryptogenic epilepsy (Oostrom *et al.*, 2003; Holmes & Lenck-Santini, 2006; Shatskikh *et al.*, 2006) suggestive of an antecedent neurobiological insult of uncertain etiology.

Various animal models of epilepsy showed cognitive, learning and memory impairments. Holmes and his co-workers have studied the role of interictal epileptiform abnormalities in cognitive impairment. Most of the studies were performed in adult animals and suggest that epileptiform discharges in older age groups can impair cognitive abilities through interference with awake learning and memory, as well as memory consolidation during sleep (Williams *et al.*, 1998; Oostrom *et al.*, 2003; Holmes & Lenck-Santini, 2006). The effects appear to be more pronounced if the spikes are very frequent and widespread. In a series of elegant studies, Holmes and co-workers showed that the electrical induction of spikes in the hippocampus impairs recognition and spatial memory in rats by disrupting the ability

of hippocampal place cells to recognize the position of the animal (Shatskikh *et al.*, 2006; Zhou *et al.*, 2007). Genetically epileptic model rats, Ihara epileptic rat (IER/F substrain), have neuropathologic abnormalities and develop generalized convulsive seizures when they reach the age of ~5 months (Okaichi *et al.*, 2006). Young IER/F rats experiences showed severe learning impairments, genetically programmed microdysgenesis in the hippocampus (Okaichi *et al.*, 2006). Pilocarpine induced temporal lobe epileptic rats showed deficit in learning tests like Morris water maze demonstrated the impairment in spatial learning during epilepsy (Reas *et al.*, 2007) and rotarod performance demonstrated the impairment in the motor function and coordination (Amee *et al.*, 2009).

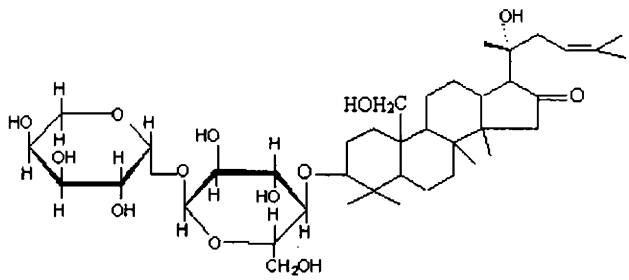
Some anti-epileptic medications can slow down processing of information in some children, while other anti-epileptic medications can induce fatigue that decreases the child's ability to learn (Sung & Kwon, 2008). These are transient behavioural abnormalities and falling numbers of patients continuing to take epileptic drugs during long-term treatment. Psychotic reactions have been carefully documented both in patients receiving standard and new anti-epilepsy drugs, and in up to 6% of patients after epilepsy surgery (Jeffrey *et al.*, 2009). As many psychotic episodes are associated with a dramatic cessation of seizures, it is not surprising that behavioural abnormalities are more often seen in patients receiving higher, more effective doses of drugs (Schmidt, 2009). Because of the side effects of chemical drugs, drugs of plant origin are gaining importance and are being investigated for remedies of a number of disorders. Since the introduction of adaptogen concept, several plants have been investigated, which were used earlier as tonics due to their adaptogenic and rejuvenating properties in traditional medicine. *Bacopa monnieri* has been reported to possess anxiolytic, antidepressant and memory enhancing activity (Bhattacharya & Ghosal, 1998; Sairam *et al.*, 2002; Das *et al.*, 2002).

***Bacopa monnieri* (Linn.) Pennel**

Bacopa monnieri (Linn.) belongs to the family Scrophulariaceae. It is a prostrate, juicy, succulent, glabrous annual herb rooting at the nodes with numerous ascending branches. Leaves are simple, opposite, decussate, sessile, obovate-oblong or spatulate, entire, fleshy, obscurely veined and punctate. Flowers are pale blue or whitish, axillary, solitary, arranged on long slender pedicels. Fruits are ovoid, acute, 2-celled, 2-valved capsules and tipped with style base. Seeds are minute and numerous (Warrier *et al.*, 1993). This plant are growing in grasslands occurring in aquatic sites, sand and wet soil occupying in the edges of freshwater or brackish pools and streams and lake beds. Distributed in the major part of the plains of India, Pakistan, Afghanistan, Nepal, Sri Lanka, subtropical US, tropical Asia, Africa and Australia (Russo & Borrelli, 2005). *Bacopa monnieri* is commercially cultivated for medicinal purpose.

Active Constituents

Earlier workers have isolated a number of chemical compounds from *Baopa monnieri*. *Baopa monnieri* contains alkaloids such as hydrocotyline, brahmine and herpestine (Dutta & Basu, 1963). Glycoside such as asiaticoside and thanakunicide. Flavonoids such as apigenin and luteonin. Saponins such as D-mannitol, Acid A, Monnierin, and Bacosides. Bacoside A is considered the major active component, first identified by Chatterji *et al* (1963), with Bacoside B being an optical isomer of Bacoside A (Singh *et al* 1998). Chemical structure of Bacoside A, B and C are represented as 3-0-α-L-arabinopyranosyl-20-0-α-L- arabinopyranosyl-jujubogenin, 3-0-[α-L-arabinopyranosyl (1-2)α-L- arabinopyranosy] pseudojujubogenin and 3-0-[β-D-glucopyranosyl (1-3){α-L- arabinofuranosyl (1-2)})α-L- arabinopyranosy] pseudojujubogenin respectively (Saraswati *et al.*, 1996).



Structure of Bacoside A

Ethnopharmacology

It is used in traditional Indian medicine, the Ayurveda, for the treatment of anxiety and in improving intellect and memory for several centuries (Singh & Dhawan, 1997). In addition to memory boosting activity, it is also claimed to be useful in the treatment of cardiac, respiratory and neuropharmacological disorders like insomnia, insanity, depression, psychosis, epilepsy and stress (Russo & Borrelli, 2005). It was reported to possess anti-inflammatory, analgesic, antipyretic, sedative, free radical scavenging and anti-lipid peroxidative activities (Kishore & Singh, 2005; Anbarasi, *et al.*, 2005) The plant is reported to have shown barbiturate hypnosis potentiation effect. The plant is anticancerous and improves learning ability. It is used as a tranquilliser. The plant is astringent, bitter, sweet, cooling, laxative, intellect promoting, anodyne, carminative, digestive, antiinflammatory, anticonvulsant, depurative, cardiotonic, bronchodialator, diuretic, emmenagogue, sudorfic, febrifuge and tonic (Basu & Lamsal, 1947; Rastogi, *et al.*, 1994). 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 (Singh & Dhawan, 1997). In animal studies, both purified Bacosides and extracts of *Bacopa monnieri* standardized for Bacosides have been found to enhance several aspects of mental function and learning ability (Singh & Dhawan, 1982; Singh, 1988; Singh & Dhawan, 1997) Additional brain effects of *Bacopa monnieri* demonstrated in animal research include reduction of both anxiety and depression (Bhattacharya & Ghosal, 1998; Sairam *et al.*, 2002). Biochemically, these nervous-system effects have been attributed to an enhancement of the effects of the neurotransmitters acetylcholine and (Bhattacharya, *et al.*, 2000; Stough *et al.*, 2001) possibly, serotonin or GABA (Dey & Datta, 1966; Ganguly & Malhtora 1967). *Bacopa monnieri* extracts also appear to have significant antioxidant activity in the brain (Bhattacharya, *et al.*, 2000) and other effects that help protect brain cells (Russo *et al.*, 2003). Animal research has also reported that *Bacopa monnieri* extracts can relax the muscles that control the blood vessels, the intestine and the airways of the respiratory system (Dar & Channa, 1997; Channa *et al.*, 2003; Dar & Channa, 1999) and can help both prevent and heal ulcers in the stomach (Sairam, *et al.*, 2001). Traditional herbal references recommend 5 to 10 grams per day of the powdered herb. Human research has used 300 to 450 mg per day of an extract standardized to contain 55% Bacosides. *Bacopa monnieri* appears to be well tolerated when taken in typical amounts (Singh & Dhawan, 1997), although one double-blind study reported significantly more symptoms of dry mouth, nausea, and muscle fatigue in participants taking *Bacopa monnieri* (Stough *et al.*, 2001).

Biological Activity

The herb has been described in Ayurvedic texts since around 800 BC and recorded as a treatment for a range of mental disorders in the 'Carak Samhita' (Singh & Dhawan, 1997), which, according to the literature, was written in the 6th century AD (Russo & Borrelli, 2005). Ayurvedic medicine classifies *Bacopa monnieri* as

belonging to a group of plant medicines known as medhya rasayana that improve mental health, intellect and memory (medhya) and promote longevity and rejuvenation (rasayana) (Singh & Singh, 1980). Hence *Bacopa monnieri* shares its Sanskrit name, Brahmi, with another herbal nervous system restorative *Centella asiatica*. Learning ability in rats has been significantly enhanced by *Bacopa monnieri* extract as it facilitated acquisition, consolidation and retention of three newly learned behavioural responses at an oral dosage of 40mg/kg three times daily (Singh & Dhawan, 1982). In this study, effects on cognitive function were measured by foot shock motivated brightness discrimination reaction, active conditioned flight reaction (jump to avoid shock) and continuous avoidance response (shock avoidance by lever pulling) tests. *Bacopa monnieri* facilitated all parameters of memory acquisition and retention. In a subsequent study the same authors investigated the constituents responsible for Bacopa's effect and demonstrated that the isolated Bacosides A and B were effective in enhancing memory in rats in learning tasks involving both positive and negative reinforcement (Singh & Dhawan, 1997). Additionally, this study demonstrated that the Bacosides produced changes in the hippocampus, cerebral cortex (areas critical to memory function) and hypothalamus regions of the brain and caused enhanced levels of protein kinase activity and increases in protein levels in these regions. This indicated positive implications for improved neurotransmission and repair of damaged neurons *via* enhanced regeneration of nerve synapses (Singh & Dhawan, 1997). The neuroprotective effects of *Bacopa monnieri* extract, was tested, its protection against the beta-amyloid protein and glutamate-induced neurotoxicity in primary cortical cultured neurons. From this study, the mode of action of neuroprotective effects of *Bacopa monnieri* appeared to be the results of its antioxidant effect to suppress neuronal oxidative stress and the acetylcholinesterase inhibitory activities. Singh and Dhawan observed the effects of isolated *Bacopa monnieri* saponins on memory. Administration of Bacosides to mice attenuated experimentally induced anterograde amnesia and improved memory as measured by a

well validated learning task- the Morris Water maze test (Kishore & Singh, 2005). The triterpenoid saponins and their Bacosides are responsible for *Bacopa monnieri*'s ability to enhance nerve impulse transmission. The Bacosides aid in repair of damaged neurons by enhancing kinase activity, neuronal synthesis and restoration of synaptic activity and ultimately nerve impulse transmission. Loss of cholinergic neuronal activity in the hippocampus is the primary feature of Alzheimer's disease. Based on animal study results, Bacosides appear to have antioxidant activity in the hippocampus, frontal cortex and striatum. Animal research has shown *Bacopa monnieri* extracts modulate the expression of certain enzymes involved in generation and scavenging of reactive oxygen species in the brain.

Antioxidant Capacity

The antioxidant activity of *Bacopa monnieri* has been reported in a number of laboratory studies (Singh & Singh, 1980; Negi *et al.*, 2000; Bhattacharya, *et al.*, 2000; Sairam, *et al.*, 2001; Sumathy, *et al.*, 2002; Russo *et al.*, 2003b; Russo *et al.*, 2003a). Antioxidant effects of *Bacopa monnieri* in areas of the brain that are key memory areas such as hippocampus, frontal cortex and striatum have been documented by Bhattacharya *et al.* (Bhattacharya, *et al.*, 2000) in rat brain. *Bacopa monnieri* was shown to protect the brain (Sumathy, *et al.*, 2002) and liver (Sumathy, *et al.*, 2001), from morphine induced inhibition of antioxidant enzyme systems. Russo and Borrelli (2005) demonstrated a free radical scavenging activity which protected against cytotoxicity and DNA damage in human fibroblasts. Further research by Russo et al (2003a) also demonstrated that *Bacopa monnieri* significantly reduced oxidation and DNA damage in cultured rat astrocytes induced by a nitric oxide donor. Furthermore, Anbarasi *et al.* (2005) demonstrated that isolated Bacoside A protected rat brain tissue from various parameters of oxidative stress caused by chronic cigarette smoke exposure. One of the foremost theories of brain ageing asserts that free radical damage results in both ageing-related changes in healthy brains (Trollor & Valenzuela, 2001)

and in neurodegenerative pathology, such as Alzheimer's disease (Singh *et al.*, 2004). Good antioxidant status is associated with better memory performance in the aged (Perrig *et al.*, 1997) and antioxidant therapy has been targeted as a promising dementia strategy (Jorm, 2002). Thus, the demonstrated antioxidant effects of *Bacopa monnieri*, particularly in brain tissue, support its potential as a therapy in neurodegenerative pathologies and age-related cognitive decline. Stress elicits a defensive response in living organisms. The defense response involves several mechanisms including stress gene expression, enhanced antioxidant protection and enhanced toxin clearance. *Bacopa monnieri* has been shown to facilitate each of these adaptive resources by modulation of Hsp 70 expression and enhancement of activity of both superoxide dismutase and cytochrome P450 enzymes in stressor exposed rat brain. Thus, *Bacopa monnieri* facilitate the capacity of the brain to withstand stress and help the brain to function under adverse conditions. These findings support the afore-mentioned medhya rasayana classification of *Bacopa monnieri* in ancient Ayurveda in that they imply a brain tonic and adaptogenic effect (adaptogenic meaning improved resistance to stress). This indicate some similarities with Panax ginseng, which is considered to be a major adaptogen and tonic, enhancing resistance to stress in numerous experimental situations as well as clinical trials (Mills & Bone, 2000; Blumenthal, 2003).

Epilepsy and *Bacopa monnieri*

The mechanism of action behind the memory and cognition enhancing effects of *Bacopa monnieri* is still uncertain, as its multiple active constituents have multifunctional properties, making its pharmacology complex. But the antioxidant properties of *Bacopa monnieri* have been well documented (Basu *et al.*, 1967). Glutamate is one of the chief excitatory amino acids that mediates excitotoxic neuronal degeneration. Treatment with *Bacopa monnieri* extract reduced the increase in glutamate dehydrogenase activity to near-control levels. Hence, it is suggested that

Bacopa monnieri has a definite role in decreasing glutamate excitotoxicity. *Bacopa monnieri* treatment induce membrane dephosphorylation and a concomitant increase in mRNA turnover and protein synthesis. It can also enhance protein kinase activity in the hippocampus, which is critically involved in learning and memory (Sumathy *et al.*, 2002). The water maze experiment conducted by Reas *et al.* (2007) to study the neurobiological mechanisms that underlie spatial learning and memory function in epileptic rats. The hippocampal formation is critical for computing place representations. The Morris water maze experiment demonstrated the impairment in spatial learning during epilepsy. Escape latency was increased in epileptic rats as compared with control rats. Treatment using *Bacopa monnieri* and Bacoside A increase the performance in Morris water maze (Reas *et al.*, 2008).

Amee *et al.* (2009) found increased, period of immobility in the forced swim test in epileptic rats compared to control. In the forced swim test, the space for rat's movement was restricted from which they cannot escape. Immobility in rats is considered to be a state of lower mood or hopelessness which the rodent experience when they are forced to swim in a constrained space from they cannot escape. This is believed to indicate a failure or reduced attempts towards escape directed behaviour from persistent stress. It also causes the development of passive behaviour that disengages the animal from coping up with stressful stimuli. Studies by Mazrati *et al.* (2008) also support that rats afflicted with pilocarpine induced epilepsy exhibited increased immobility time under forced swim test. This form of immobility which is a state of despair is reported to be reduced by a broad spectrum of anti-depressant drugs (Menard & Treit, 1999). *Bacopa monnieri* treatment once daily over a period of 15 days decreased the period of immobility in the epileptic rats which is indicative of its anti-depressant property. But studies by other workers revealed that *Bacopa monnieri* administration also acts as an anti-depressant (Bhattacharya & Ghosal, 1998; Bhattacharya *et al.*, 1999; Sairam *et al.*, 2002; Das *et al.*, 2002). Blumenthal (2003) have suggested shared neurobiological processes leading both to seizures and to

behavioral, emotional and cognitive disturbance which could possibly explain the how *Bacopa monnieri* is effective as an anti-convulsant and an anti-depressant. It is reported that the isolated Bacosides A and B were effective in enhancing memory in rats in learning tasks involving both positive and negative reinforcement (Singh & Dhawan, 1997; Singh *et al.*, 1998). Additionally Bacosides produced changes in the hippocampus, cerebral cortex (areas critical to memory function) and hypothalamus regions of the brain and caused enhanced levels of protein kinase activity and increases in protein levels in these regions. This indicated positive implications for improved neurotransmission and repair of damaged neurons *via* enhanced regeneration of nerve synapses (Singh & Dhawan, 1997). Previous studies showed decreased Rotarod performance demonstrated the impairment in the motor function and coordination in the epileptic rats, suggesting impairment in their ability to integrate sensory input with appropriate motor commands to balance their posture and at the same time adjust their limb movements on the metallic rod and is indicative of cerebellar dysfunction (Blumer *et al.*, 2004; Amee *et al.*, 2009). Treatment using *Bacopa monnieri* and its active component Bacoside A improved cerebellar function (Amee *et al.*, 2009). Study from our laboratory implicated a role for serotonin and 5-HT_{2C} receptors in the modulation of neuronal network excitability and seizure propagation. These neurofunctional deficits are one of the key contributors to motor deficits and stress associated with epilepsy. It is suggested that *Bacopa monnieri* extract treatment reverses the 5-HT_{2C} receptor mediated motor dysfunction in epilepsy (Amee *et al.*, 2009). Activation of mGlu in pilocarpine induced epileptic rats (Kral *et al.*, 2003) has been reported to have anticonvulsant effect (Attwell *et al.*, 1998; Gasparini *et al.*, 1999; Jiang *et al.*, 2007). Consistent with these reports study from our lab also showed a down-regulation of the mGlu gene expression during epileptic condition. Treatment with *Bacopa monnieri* extract caused a reversal to near control level giving protection against epilepsy (Paulose *et al.*, 2008).

The present work is to understand the alterations of total GABA, $GABA_A$ and $GABA_B$ 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 of the GABA receptors in the epileptic model administered with *Bacopa monnieri*, Bacoside A and Carbamazepine were also studied. These studies will help us to elucidate the functional role of GABA receptors in epilepsy.

CHEMICALS USED AND THEIR SOURCES

Biochemicals

Mesulergine, ethylene diamine tetra acetic acid (EDTA), Tris HCl, calcium chloride, atropine methyl bromide, carbamazepine and pilocarpine were purchased from Sigma Chemical Co., St. Louis, USA. All other reagents were of analytical grade purchased locally. HPLC solvents and chemicals were of HPLC grade obtained from SRL, India and Sigma Chemical Co, St. Louis, USA.

Radiochemicals

4-amino-n-[2, 3-^{3}H] butyric acid (Specific activity-84.0 Ci/mmol) was purchased from Amersham Bioscience, USA. Baclofen, (-)-[butyl-4-^{3}H (N)] (Specific activity-42.9 Ci/mmol) and Bicuculline methyl chloride, (-)-[methyl-^{3}H] (Specific activity-82.9 Ci/mmol) were purchased from NEN Life and Analytical Sciences, Boston, MA, USA.

Molecular Biology Chemicals

Tri-reagent kit was purchased from Sigma Chemical Co., St. Louis, USA. ABI PRISM High Capacity cDNA Archive kit, Primers and Taqman probes for Real-Time PCR were purchased from Applied Biosystems, Foster City, CA, USA. $GABA_{A\alpha 1}$ (Rn00788315_m1), $GABA_{A\alpha 5}$ (Rn00568803_m1), $GABA_{A\gamma 3}$ (Rn00577639_m1), $GABA_{A\delta}$ (Rn00568740_m1), $GABA_B$ (Rn00578911_m1), and GAD (Rn00562748_m1) primers were used for the study.

Confocal Dyes

Rat primary antibody for $GABA_{A\alpha 1}$ (No-31775) and rhodamine coated secondary antibody (No-AP307R) were purchased from Chemicon, USA.

Animals

Adult male Wistar rats of 250-300g body weight purchased from Amrita Institute of Medical Sciences, Cochin were used for all the 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.

Preparation of *Bacopa monnieri* Plant Extract

Crude whole plant extract was used to study the anti-epileptic effect in pilocarpine induced temporal lobe epilepsy. *Bacopa monnieri* plant extract was prepared by the procedure of Paulose *et al.* (2008). Fresh, whole *Bacopa monnieri* plant (6–8 months old) was collected (in the month of March) and washed. Leaves, roots and stems of *Bacopa monnieri* plant were cut into small pieces and dried in shade. About 100 g fresh plant dried in shade yielded 15 g powder. Homogenate was extracted at required concentration (300 mg fresh plant/Kg body weight) and used to study the anti-epileptic effect in pilocarpine induced temporal lobe epilepsy (Amee *et al.*, 2009).

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 powder. The extract of *Bacopa monnieri* contained 40-50% bacoside estimated as bacoside A. The estimation method involves acid hydrolysis of bacoside, 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 methyl bromide (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). Behavioural 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, stage 3- unilateral forelimb clonus, stage 4- bilateral forelimb clonus, stage- 5, rearing, falling and generalized convulsions. The occurrence of stage 3-5 was considered as one complete seizure. 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 to evaluate the development of seizure discharge. 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 (Reas *et al.*, 2007; Amee *et al.*, 2009; Jobin *et al.*, 2010a).

Determination of Anti-Epileptic Potential of *Bacopa monnieri*

Experimental Animals were Divided into Following Groups

a) Group 1: Control

b) Group 2: Epileptic

c) Group 3: Epileptic rats treated with *Bacopa monnieri*

d) Group 4: Epileptic rats treated with Bacoside A

e) Group 5: Epileptic rats treated with Carbamazepine

Animal Groups

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 (Reas *et al.*, 2008). The control group received saline instead of pilocarpine. The epileptic group showed 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 behaviour 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 second group that did not receive the treatment was epileptic. Extract of *Bacopa monnieri* was given orally to the third group of epileptic rats in the dosage of 300 mg/ Kg body weight/day. bacoside A extract was given orally to the fourth group of epileptic rats in the dosage

of 150mg/ Kg body/ day for 15 days. Carbamazepine was given orally to the fifth group of epileptic rats in the dosage of 150 mg /Kg body weight/ day for 15 days. After 15 days of treatment rats were sacrificed (Jobin *et al.*, 2010a).

Tissue Preparation

Control and experimental rats were sacrificed by decapitation. The brain regions (cerebral cortex, cerebellum and brainstem) and body parts were dissected out instantly over ice according to the procedure of Glowinski and Iversen (1966). Hippocampus was dissected out immediately over ice according to the procedure of Heffner *et al.*, (1980). The tissues were stored at -80°C for various experiments. All animal care and procedures were in accordance with Institutional and National Institute of Health guidelines.

Acetylcholine Esterase Assay

Acetylcholine esterase asssay was done using the spectrophotometric method of Ellman *et al.*, (1961). The homogenate (10%) was prepared in 30mM sodium phosphate buffer pH-7.0. One ml of 1% Triton x 100 was added to the homogenate to release the membrane bound enzyme and centrifuged at 10,000xg 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 (Jobin *et al.*, 2010b).

Estimation of Circulating Insulin by Radioimmunoassay

Principle of the Assay

The assay was done according to the procedure of BARC radioimmunoassay kit. The radioimmunoassay method was based on the competition of unlabelled insulin in the standard or samples and [^{125}I] insulin for the limited binding sites on a

specific antibody. At the end of incubation, the antibody bound and free insulin were separated by the second antibody-polyethylene glycol (PEG) aided separation method. Measuring the radioactivity associated with bound fraction of sample and standards quantitates insulin concentration of samples.

Assay Protocol

Standards, ranging from 0 to 200μU/ml, insulin free serum and insulin antiserum (50μl each) were added together and the volume was made up to 250μl with assay buffer. Samples of appropriate concentration from experiments were used for the assay. They were incubated overnight at 2°C. Then 50μl [^{125}I]insulin was added and incubated at room temperature for 3 hrs. 50μl second antibody was added along with 500μl of PEG. The tubes were then vortexed and incubated for 20 minutes and they were centrifuged at 1500xg for 20 minutes. The supernatant was aspirated out and the radioactivity in the pellet was determined in a gamma counter.

A standard curve was plotted with $\%B/B_0$ on the Y-axis and insulin concentration/ml on the X-axis of a log-logit graph. $\%B/B_0$ was calculated as:

$$\frac{\text{Corrected average count of standard or sample}}{\text{Corrected average count of zero standard}} \times 100$$

Insulin concentration in the samples was determined from the standard curve plotted using MultiCalc™ software (Wallac, Finland).

Estimation of Circulating Triiodothyronine (T3) by Radioimmunoassay

Principle of the Assay

The assay was done according to the procedure of BARC radioimmunoassay kit. The radioimmunoassay method was based on the competition of unlabelled endogenous T3 with [^{125}I]T3 for the limited binding sites on the antibody (Ab1) made specially for T3. The antibody was in the form of a complex with second antibody (Ab2). At the end of incubation, the T3 (Ag) bound to the antibody- second antibody complex (Ag-Ab1-Ab2) and free T3 was separated by the addition of PEG. The amount bound to the antibody complex in the assay tubes were compared with values of known T3 standards and the T3 concentration in the samples were calculated.

Assay Protocol

Standards, ranging from 0.15 to 2.5ng, T3 free serum, [^{125}I]T3 and antiserum complex were added together and the volume was made up to 275µl with assay buffer. Samples of appropriate concentration from experiments were used for the assay. They were incubated at 37°C for 45 minutes. The PEG was added to all tubes and they were centrifuged at 1500xg for 20 minutes. The supernatant was aspirated out and the radioactivity in the pellet was determined in a gamma counter.

A standard curve was plotted with %B/B$_0$ on the Y-axis and T3 concentration (ng /ml) on the X-axis of a log-logit graph. %B/B$_0$ was calculated as:

$$\frac{\text{Corrected average count of standard or sample}}{\text{Corrected average count of zero standard}} \times 100$$

T3 concentrations in the samples were determined from the standard curve plotted using MultiCalc™ software (Wallac, Finland).

Malate Dehydrogenase Activity

Malate Dehydrogenase was assayed according to Mehelar *et al.* (1948). Crude sample was prepared by making a 5% homogenate of the muscle in phosphate buffer, pH 7.4 using polytron homogenizer. The homogenate was centrifuge at 1000xg for 10 minutes. The supernatant was collected and centrifuged at 10,000xg for 20 minutes. The reaction mixture contained phosphate buffer, pH 7.4, NADH, oxaloactate and enzyme. The reaction mixture of 1ml was assayed at 340nm in the spectrophotometer by measuring the decrease in optical density due to oxidation of NADH measured at 15 second interval for 1 minute at room temperature. One unit of enzyme activity was equal to the change in OD of 0.1 for 100 seconds at 334nm. Kinetic parameters such as Vmax and Km were calculated from the data of MDH activity measured at substrate concentration 0.0125 - 0.2 mM (Jobin *et al.*, 2010b).

Quantification of Epinephrine and Norepinephrine in the Experimental Groups of Rats

The epinephrine and norepinephrine were assayed according to the modified procedure of Paulose *et al.* (1988). The heart of experimental groups of rats was homogenised in 0.4N perchloric acid. The homogenate was then centrifuged at 5000xg for 10 minutes at 4°C in a Sigma 3K30 refrigerated centrifuge and the clear supernatant was filtered through 0.22 μm HPLC grade filters and used for HPLC analysis.

Epinephrine and norepinephrine contents were determined in high performance liquid chromatography (HPLC) with electrochemical detector (ECD) (Waters, USA) fitted with CLC-ODS reverse phase column of 5 μm particle size. The mobile phase consisted of 50mM sodium phosphate dibasic, 0.03M citric acid, 0.1mM

EDTA, 0.6mM sodium octyl sulfonate, 15% methanol. The pH was adjusted to 3.25 with orthophosphoric acid, filtered through 0.22 μm filter (Millipore) and degassed. Waters model 515, Milford, USA, pump was used to deliver the solvent at a rate of 1 ml/minute. The neurotransmitters and their metabolites were identified by amperometric detection using an electrochemical detector (Waters, model 2465) with a reduction potential of +0.80 V. Twenty microlitre aliquots of the acidified supernatant were injected into the system for quantification. The peaks were identified by relative retention times compared with external standards and quantitatively estimated using an integrator (Empower software) interfaced with the detector. Data of the experimental and control rats were statistically analysed and tabulated.

Quantification of GABA

GABA content in the brain regions of control and experimental rats were quantified by displacement method using the procedure of Kurioka *et al.*(1981) where the incubation mixture contained 1nM [^{3}H]GABA with and without GABA at a concentration range of 10^{-9} M to 10^{-4} M. The unknown concentrations were determined from the standard displacement curve using appropriate dilutions and calculated for μmoles/g wt. of the tissue.

GABA RECEPTOR STUDIES USING [^{3}H] RADIOLIGANDS

GABA Receptor Binding Assays

[^{3}H]GABA binding to GABA receptor was assayed in Triton X-100 treated synaptic membranes according to the procedure of Kurioka *et al.* (1981). Crude synaptic membranes were prepared using sodium free 10mM Tris buffer pH 7.4. Each assay tube contained a protein concentration of 100μg. In saturation binding experiments, 5-40 nM of [^{3}H]GABA was incubated with and without excess of

unlabelled 100μM GABA for 20 min at 5°C and terminated by centrifugation at 35000xg for 20 min. [^{3}H]GABA in the pellet was determined by liquid scintillation spectrometry. Specific binding was determined by subtracting non-specific binding from the total.

$GABA_A$ Receptor Binding Assays

[^{3}H]bicuculline binding to $GABA_A$ receptor was assayed in Triton X-100 treated synaptic membranes according to the procedure of Kurioka et al (1981). Crude synaptic membranes were prepared using sodium free 10mM Tris buffer pH 7.4. Each assay tube contained a protein concentration of 100μg. In saturation binding experiments, 5-40 nM [^{3}H]bicuculline was incubated with and without excess of unlabelled 100μM bicuculline for 20 min at 5°C and terminated by centrifugation at 35000xg for 20 min. [^{3}H]bicuculline in the pellet was determined by liquid scintillation spectrometry. Specific binding was determined by subtracting non-specific binding from the total.

$GABA_B$ Receptor Binding Assays

[^{3}H]baclofen binding to $GABA_B$ receptors were assayed in Triton X-100 treated synaptic membranes according to the procedure of Kurioka *et al.* (1981). Crude synaptic membranes were prepared using sodium free 10mM Tris buffer (pH 7.4). Each assay tube contained a protein concentration of 100μg. In saturation binding experiments, 5-40 nM of [^{3}H]baclofen was incubated with and without excess of unlabelled 100μM baclofen for 20 min at 5°C and terminated by centrifugation at 35000xg for 20 min. [^{3}H]baclofen in the pellet was determined by liquid scintillation spectrometry. Specific binding was determined by subtracting non-specific binding from the total.

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 Spectrophotometer at 660nm.

Linear Regression Analysis for Scatchard plots

The data was 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. The K_d is inversely related to receptor affinity.

GENE EXPRESSION STUDIES

Preparation of RNA

RNA was isolated from the different brain regions such as cerebral cortex, hippocampus, cerebellum, corpus striatum and brain stem of control and experimental rats using the Tri reagent from Sigma Aldrich.

Isolation of RNA

Tissue (25-50 mg) homogenates were made in 0.5 ml Tri Reagent and was centrifuged at 12,000x*g* for 10 minutes at 4^0C. The clear supernatant was transferred to a fresh tube and it was allowed to stand at room temperature for 5 minutes. 100µl of chloroform was added to it, mixed vigorously for 15 seconds and allowed to stand at room temperature for 15 minutes. The tubes were then centrifuged at 12,000x*g* for 15 minutes at 4°C. Three distinct phases appear after centrifugation. The bottom red

organic phase contained protein, interphase contained DNA and a colourless upper aqueous phase contained RNA. The upper aqueous phase was transferred to a fresh tube and 250µl of isopropanol was added and the tubes were allowed to stand at room temperature for 10 minutes. The tubes were centrifuged at 12,000x*g* for 10 min at 4°C. RNA precipitate forms a pellet on the sides and bottom of the tube. The supernatants were removed and the RNA pellet was washed with 500µl of 75% ethanol, vortexed and centrifuged at 12,000x*g* for 5 min 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 in spectrophotometer (Shimadzu UV-1700 pharmaSPEC). For pure RNA preparation the ratio of absorbance at 260/280 was 2. The concentration of RNA was calculated as one absorbance 260 = 40µg.

cDNA Synthesis

Total cDNA synthesis was performed using ABI PRISM cDNA Archive kit in 0.2ml microfuge tubes. The reaction mixture of 20µl contained 0.2µg total RNA, 10X RT buffer, 25X dNTP mixture, 10X 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 designed using Primer Express Software Version (3.0).

Real -Time PCR Assay

Real Time PCR assays were performed in 96-well plates in a ABI 7300 Real Time PCR instrument (Applied Biosystems). To detect gene expression, a TaqMan quantitative 2-step reverse transcriptase polymerase chain reaction (RT-PCR) was used to quantify mRNA levels. First-strand cDNA was synthesized with use of a

TaqMan RT reagent kit, as recommended by the manufacturer. PCR analyses were conducted with gene-specific primers and fluorescently labeled TaqMan probe, designed by Applied Biosystems. Endogenous control, β-actin, was labelled with a reporter dye (VIC). All reagents were purchased from Applied Biosystems. The probes for specific gene of interest were labeled with FAM at the 5' end and a quencher (Minor Groove Binding Protein - MGB) at the 3' end. The Real-Time data was analyzed with Sequence Detection Systems software version 1.7. All reactions were performed in duplicate.

The TaqMan reaction mixture of 20μl contained 25ng of total RNA-derived cDNAs, 200nM each of the forward primer, reverse primer and PCR analyses were conducted with gene-specific primers and fluorescently labelled Taqman probes of $GABA_{A\alpha 1}$ (Rn00788315_m1), $GABA_{A\alpha 5}$ (Rn00568803_m1), $GABA_{A\gamma}$ (Rn00577639_m1), $GABA_{A\delta}$ (Rn00568740_m1), $GABA_{B}$ (Rn00578911_m1), and GAD (Rn00562748_m1). Endogenous control (β-actin) was labeled with a reporter dye (VIC). 12.5μl of TaqMan 2X Universal PCR Master Mix was taken and the volume was made up with RNAse free water. Each run contained both negative (no template) and positive controls.

The thermocycling profile conditions were as follows:

50°C -- 2 minutes --- Activation

95°C -- 10 minutes --- Initial Denaturation

95°C -- 15 seconds --- Denaturation 40 cycles

50°C -- 30 seconds --- Annealing

60°C -- 1 minutes --- Final Extension

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 ($2^{-\Delta\Delta CT}$).

Confocal Studies

Control and experimental rats were deeply anesthetized with chloral hydrate. The rat was transcardially perfused with PBS (pH- 7.4) followed by 4% paraformaldehyde in PBS (Chen *et al.*, 2007). After perfusion the brains were dissected and immersion fixed in 4% paraformaldehyde for 1 hr and then equilibrated with 30% sucrose solution in PBS (0.1 M). 10 μm sections were cut using Cryostat (Leica, CM1510 S). The sections were treated with PBST (PBS in 0.05% Triton X-100) for 20 min. Brain slices were incubated overnight at 4 °C with rat primary antibody for $GABA_{A\alpha1}$ (No- 31775, diluted in PBST at 1: 1000 dilution). After overnight incubation, the brain slices were rinsed with PBST and then incubated with rhodamine coated secondary antibody (No-AP307R, diluted in PBST at 1: 1000 dilution) or Rhodamine dye (No:AP307R Chemicon, diluted in PBST at 1: 1000 dilution). The sections were observed and photographed using confocal imaging system (Leica SP 5). The Pixel intensity of each image was calculated using LAS software from Lyca (Savitha *et al.*, 2009a, b). The specificity of the immunocytochemical procedure is validated by negative controls to ensure that the labeling method accurately identifies the antibody bound to the specific $GABA_{A\alpha1}$ receptor subunit. The given pixel value is the net value which is deducted from the negative control pixel values.

BEHAVIOURAL STUDIES

Radial Maze Test

Radial maze behavioral testing was conducted under normal room lighting using an eight armed radial maze elevated 100 cm from the floor. Each arm of the maze (11.5 cm wide) was extended 68.5cm from an octagonally shaped central platform (40 cm across). Black Plexiglas walls (11.5 cm high) were placed for the first 20 cm of each arm to prevent the rat crossing from one arm to another without returning to the central platform. Circular food wells (1.3 cm deep, 3.2 cm diameter) were located 2.5cm from the end of each arm. The maze was centered in an enclosed room where lighting and spatial cues (e.g., posters, door and boxes) remained constant throughout the course of the experiment. Arms were baited by placing one raisin in each food well.

Rats were placed on the maze 3 days prior to the start of formal acquisition testing in order to habituate them to the apparatus. On the first day of habituation, 4 food pellets were scattered along the length of each arm. The rats were then systematically confined to each arm for 1 min to ensure their exposure to the entire maze. On the second day of habituation, the previous day's procedure was repeated except that the animals were not confined to each arm following 5 min of exploration. On the third day, one food pellet was placed in the food well at the end of each arm and a second was placed halfway down each arm. Once the rats were habituated to the maze, testing began. Trials began by placing a single rat in the center of the maze facing away from the experimenter. The trial ended when the rat had obtained all 4 pellets or 5min had elapsed, whichever occurred first. Rats were run until they achieved criterion performance for task acquisition. Criterion was attained when the rat collected 3 out of the 4 food pellets within their first 4 arm entries within a trial (while still completing the trial) with this level of performance being maintained for 5 consecutive criterion performance. The number of trials up to and including the last of

these 5 criterion performance formed the "number of trials to criterion" measure. Experimental subjects were tested under blind conditions. The time of testing was consistent from day to day for each subject but testing of the various treatment groups was distributed randomly throughout the day. Performance was recorded during daily behavioral trials according to the terminology in previous studies (Jarrard, 1980; Lopes da Silva et al., 1986; Leung et al., 1990). Entry into an unbaited arm was scored as a reference error and reentry into a baited arm was scored as a working error.

Y-Maze Test

The Y-maze was made of grey wood, covered with black paper and consisted of three arms with an angle of 120 degrees between each of the arms. Each arm was 8 cm width ×30 cm length ×15 cm height. The three identical arms were randomly designated: Start arm, in which the rat started to explore (always open); Novel arm, which was blocked at the 1st trial, but open at the 2nd trial; and the other arm (always open). The maze was placed in a separate room with enough light. The floor of the maze was covered with sawdust, which was mixed after each individual trial in order to eliminate olfactory stimuli. Visual cues were placed on the walls of the maze.

The Y-maze test consisted of two trials separated by an inter-trial interval (ITI). The first trial (training) was of 10 minutes duration and allowed the rat to explore only two arms (start arm and the other arm) of the maze, with the third arm (novel arm) blocked. After a 1 hour ITI (Ma *et al.*, 2007), the second trial (retention) was conducted, during which all three arms were accessible and novelty vs. familiarity was analyzed through comparing behavior in all three arms. For the second trial, the rat was placed back in the maze in the same starting arm, with free access to all three arms for 5 minutes. The time spent in each arm was analyzed. Data was expressed as percentage of performance in all three arms during the five minutes of test (Akwa *et al.*, 2001, Jobin, *et al.*, 2010).

Grid Walk Test

Deficits in descending motor control were examined by assessing the ability to navigate across a 1 m long runway with irregularly assigned gaps (0.5–5 cm) between round metal bars. Crossing this runway requires that animals accurately place their limbs on the bars. In baseline training and postoperative testing, every animal had to cross the grid for at least three times. The number of footfalls (errors) was counted in each crossing for 3 minute and a mean error rate was calculated (Z'Graggen *.et al.*, 1998).

Narrow Beam Test

The narrow beam test was performed according to the descriptions of Haydn and Jasmine (1975). A rectangular 1.2-cm wide beam, 1.05m long and elevated 30 cm from the ground was used for the study. After training, normal rats were able to traverse the horizontal beams with less than three footfalls. When occasionally their feet slipped off the beam, they were retrieved and repositioned precisely. The time the rats could remain balanced on the beam was counted.

Open-field Behavior Test

Evaluation of open-field behavior was done according to the procedure of Laurie and Gary (1982). Extraneous noise was minimized during testing, and the observer was the only person present. The observer hand carried the rat from its cage to the open field. At the beginning of testing, the rat was placed in the center of an open field enclosure constructed of corrugated cardboard 1 m X 0.75 m X 0.75 m with 4 walls and floor but no ceiling. The rat was observed for 2.5 minutes after being placed in the open field and its behavior was evaluated and recorded every 6 seconds. Each 6-second period was scored according to the predominant behavior seen during that period, even if more than one behavior was observed. During testing the observer sat as far from the open field as possible so as not to disturb the rat and made no sudden movements or noises. Between trials, fecal pellets and urine were removed

from the open field. Behaviors are classified into 4 mutually exclusive categories: grooming, sniffing, resting, and staring. Grooming is self directed behavior in which the rat scratches itself, bites its fur, or cleans its whiskers. Sniffing is exploratory behavior in which the rat changes location, with rapid movement of its nose and whiskers. Resting is a passive behavior in which the rat does not move and has a relaxed body posture. Staring is a behavior in which the rat has a tense body posture and seems to be looking at something.

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). Sigma Plot software (version 2.0, Jandel GmbH, Erkrath, Germany). Competitive binding data were analysed using non-linear regression curve-fitting procedure (GraphPad PRISM™, San Diego, USA). Empower software were used for HPLC analysis. Relative Quantification Software was used for analyzing Real-Time PCR results.

Results

Seizure frequency per 4 hours interval over the 72 hours video recording period of control and experimental rats

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

Acetylcholine esterase activity in the muscle of control and experimental rats

V_{max} of acetylcholine esterase significantly increased ($p<0.01$) in the muscle of epileptic rats compared to control. *Bacopa monnieri*, Bacoside A and Carbamazepine treatment significantly reversed the V_{max} ($p<0.01$) to near control. K_m showed no significant change in the experimental groups (Table-2).

Acetylcholine esterase activity in the heart of control and experimental rats

V_{max} acetylcholine esterase significantly decreased ($p<0.01$) in the heart of epileptic group compared to control. *Bacopa monnieri*, Bacoside A and Carbamazepine treatment significantly reversed the V_{max} ($p<0.01$) to near control. K_m showed no significant change in the experimental groups (Table-3).

Malate dehydrogenase activity in the muscle of control and experimental rats

V_{max} of malate dehydrogenase significantly increased ($p<0.01$) in the muscle of epileptic group compared to control. *Bacopa monnieri*, Bacoside A and Carbamazepine treatment significantly reversed the V_{max} ($p<0.01$) to near control. Km significantly decreased ($p<0.01$) in the epileptic rats compared to control. *Bacopa monnieri* ($p<0.01$) and Bacoside- A ($p<0.05$) treatment significantly reversed the Km ($p<0.01$) to near control (Table-4).

Epinephrine and Norepinephrine content in the heart of control and experimental rats

Epinephrine and norepinephrine content showed no significant changes in the serum of epileptic, *Bacopa monnieri*, Bacoside A and Carbamazepine treated rats (Table-5).

T3 content in the serum of control and experimental groups of rats

T3 content in the serum of the epileptic rats was significantly ($p<0.001$) increased compared to control. *Bacopa monnieri*, Bacoside A ($p<0.001$) and Carbamazepine ($p<0.01$) treatment significantly reversed the T3 content to near control (Table-6, Figure-5).

Circulating insulin level in the serum of control and experimental rats

Insulin content in the serum was significantly ($p<0.001$) increased in the epileptic rats compared to control. *Bacopa monnieri*, Bacoside A ($p<0.001$) and Carbamazepine ($p<0.01$) treatment significantly reversed the insulin content to near control (Table-7, Figure-6).

I. GABA CONTENT IN DIFFERENT BRAIN REGIONS OF CONTROL AND EXPERIMENTAL RATS

1. Cerebral cortex

GABA content in the Cerebral Cortex was significantly ($p<0.001$) decreased in epileptic rats compared to control. GABA content was significantly reversed to near control by the treatment using *Bacopa monnieri* ($p<0.01$), Bacoside A ($p<0.01$) and Carbamazepine ($p<0.05$) (Table-8, Figure-7).

2. Hippocampus

GABA content in the hippocampus was significantly ($p<0.001$) decreased in epileptic rats compared to control. GABA content was significantly ($p<0.01$), reversed to near control by the treatment using *Bacopa monnieri,* Bacoside A and Carbamazepine (Table-9, Figure-8).

3. Cerebellum

GABA content in the Cerebellum was significantly ($p<0.001$) decreased in epileptic rats compared to control. GABA content was significantly ($p<0.01$), reversed to near control by the treatment using *Bacopa monnieri,* Bacoside A and Carbamazepine (Table-10, Figure-9).

4. Corpus striatum

GABA content in the corpus striatum was significantly ($p<0.001$) decreased in epileptic rats compared to control. GABA content was significantly reversed to near control by the treatment using *Bacopa monnieri* ($p<0.01$), Bacoside A ($p<0.01$) and Carbamazepine ($p<0.05$) (Table-11, Figure-10).

5. Brain stem

GABA content in the brain stem was significantly ($p<0.001$) decreased in epileptic rats compared to control. GABA content was significantly reversed to near control by the treatment using *Bacopa monnieri* ($p<0.001$), Bacoside A ($p<0.001$) and Carbamazepine ($p<0.01$) (Table-12, Figure-11).

II. BRAIN GABA RECEPTOR ALTERATIONS IN THE CONTROL AND EXPERIMENTAL RATS

A. CEREBRAL CORTEX

1. Scatchard analysis of [^{3}H]GABA binding against GABA in the control and experimental rats

Scatchard analysis of [3H]GABA binding against GABA in the cerebral cortex showed a significant decrease ($P<0.001$) in B_{max} and K_d ($P<0.01$) of epileptic group compared to control rats. Treatment using *Bacopa monnieri,* Bacoside A and Carbamazepine reversed the B_{max} to near control. K_d showed significant increase in *Bacopa monnieri,* ($P<0.01$) Bacoside A ($P<0.05$) and Carbamazepine ($P<0.01$) treated group compared to epileptic rats (Table-13, Figure-12).

2. Scatchard analysis of [^{3}H]bicuculline against bicuculline in the control and experimental rats

Scatchard analysis of [^{3}H]bicuculline against bicuculline in the cerebral cortex showed a significant decrease ($P<0.001$) in B_{max} and K_d ($P<0.01$) of epileptic rats compared to control rats. Treatment using *Bacopa monnieri,* Bacoside A and Carbamazepine reversed the B_{max} to near control. K_d showed significant increased in *Bacopa monnieri,* ($P<0.01$) and Bacoside A ($P<0.05$) treated group compared to epileptic rats (Table-14, Figure-13).

3. Scatchard analysis of [^{3}H]baclofen against baclofen in the control and experimental rats

Scatchard analysis of [^{3}H]baclofen against baclofen in the cerebral cortex showed a significant decrease ($P<0.001$) in the B_{max} of epileptic rats compared to controls. K_d showed significant increase ($P<0.05$) in epileptic group compared to

control. Treatment using *Bacopa monnieri*, Bacoside A and Carbamazepine reversed the B_{max} to near control significantly, without showing any significant change in K_d ($P<0.01$) (Table-15, Figure-14).

4. Real Time-PCR analysis of $GABA_{A\alpha1}$ in the control and experimental rats

Gene expression of $GABA_{A\alpha1}$ receptor subunit mRNA showed significant down regulation ($p<0.001$) in the cerebral cortex of epileptic rats compared to control. Treatment using *Bacopa monnieri* ($p<0.001$), Bacoside A ($p<0.001$) and Carbamazepine ($p<0.01$) were significantly up regulated $GABA_{A\alpha1}$ receptor subunit to near control (Table-16, Figure-15).

5. Real Time-PCR analysis of $GABA_{A\alpha5}$ in the control and experimental rats

Gene expression of $GABA_{A\alpha5}$ receptor subunit mRNA showed significant up regulation ($p<0.001$) in the cerebral cortex of epileptic rats compared to control. Treatment using *Bacopa monnieri* ($p<0.001$), Bacoside A ($p<0.001$) and Carbamazepine ($p<0.01$) were significantly down regulated $GABA_{A\alpha5}$ receptor subunit to near control (Table-17, Figure-16).

6. Real Time-PCR analysis of $GABA_{A\gamma3}$ in the control and experimental rats

Gene expression of $GABA_{A\gamma3}$ receptor subunit mRNA showed significant down regulation ($p<0.001$) in the cerebral cortex of epileptic rats compared to control. Treatment using *Bacopa monnieri* ($p<0.001$), Bacoside A ($p<0.001$) and Carbamazepine ($p<0.01$) were significantly reversed the $GABA_{A\gamma3}$ gene expression to near control (Table-18, Figure-17).

7. Real Time-PCR analysis of $GABA_{A\delta}$ in the control and experimental rats

Gene expression of $GABA_{A\delta}$ receptor subunit mRNA showed significant down regulation ($p<0.001$) in the cerebral cortex of epileptic rats compared to control. Treatment using *Bacopa monnieri,* Bacoside A and Carbamazepine were significantly ($p<0.001$) reversed the $GABA_{A\delta}$ gene expression to near control (Table-19, Figure-18).

8. Real Time-PCR analysis of $GABA_B$ in the control and experimental rats

Gene expression of $GABA_B$ receptor mRNA showed significant down regulation ($p<0.001$) in the cerebral cortex of epileptic rats compared to control. Treatment using *Bacopa monnieri* ($p<0.001$), Bacoside-A ($p<0.001$) and Carbamazepine ($p<0.01$) were significantly reversed the $GABA_B$ gene expression to near control (Table-20, Figure-19).

9. Real Time-PCR analysis of GAD in the control and experimental rats

Gene expression of GAD mRNA showed significant down regulation ($p<0.001$) in the cerebral cortex of epileptic rats compared to control. Treatment using *Bacopa monnieri* ($p<0.001$), Bacoside A ($p<0.001$) and Carbamazepine ($p<0.01$) were significantly reversed the GAD gene expression to near control (Table-21, Figure-20).

B. HIPPOCAMPUS

1. Scatchard analysis of [^{3}H]GABA binding against GABA in the control and experimental rats

Scatchard analysis of [^{3}H]GABA binding against GABA in the hippocampus showed a significant decrease ($P<0.001$) in B_{max} and K_d ($P<0.01$) of epileptic rats compared to control rats. Treatment using *Bacopa monnieri,* Bacoside A and Carbamazepine reversed the B_{max} to near control. K_d showed significant ($P<0.01$)

reversal in *Bacopa monnieri,* Bacoside A and Carbamazepine treated group compared to epileptic rats (Table-22, Figure-21).

2. Scatchard analysis of [^{3}H]bicuculline against bicuculline in the control and experimental rats

Scatchard analysis of [^{3}H]bicuculline against bicuculline in the hippocampus showed a significant decrease ($P<0.001$) in B_{max} and K_d ($P<0.01$) of epileptic rats compared to control rats. Treatment using *Bacopa monnieri,* Bacoside A and Carbamazepine reversed the B_{max} to near control. K_d showed significant ($P<0.01$) reversal in *Bacopa monnieri,* Bacoside A and Carbamazepine treated groups compared to epileptic rats (Table-23, Figure-22).

3. Scatchard analysis of [^{3}H]baclofen against baclofen in the control and experimental rats

Scatchard analysis of [^{3}H]baclofen against baclofen in the hippocampus showed a significant decrease ($P<0.001$) in B_{max} and K_d ($P<0.01$) of epileptic group compared to control rats. Treatment using *Bacopa monnieri,* Bacoside A and Carbamazepine reversed the B_{max} to near control. K_d showed significant reversal in *Bacopa monnieri,* ($P<0.05$) and Bacoside A ($P<0.01$) treated groups compared to epileptic rats (Table-24, Figure-23).

4. Real Time-PCR analysis of $GABA_{A\alpha 1}$ in the control and experimental rats

Gene expression of $GABA_{A\alpha 1}$ mRNA showed significant down regulation ($p<0.001$) in the hippocampus of epileptic rats compared to control. Treatment using *Bacopa monnieri* ($p<0.001$), Bacoside A ($p<0.01$) and Carbamazepine ($p<0.01$) were significantly reversed the $GABA_{A\alpha 1}$ gene expression to near control (Table-25, Figure-24).

5. Real Time-PCR analysis of $GABA_{A\hat{a}5}$ in the control and experimental rats

Gene expression of $GABA_{A\hat{a}5}$ mRNA showed significant down regulation ($p<0.001$) in the hippocampus of epileptic rats compared to control. Treatment using *Bacopa monnieri*, Bacoside A and Carbamazepine were significantly ($p<0.001$), reversed the $GABA_{A\hat{a}5}$ gene expression to near control (Table-26, Figure-25).

6. Real Time-PCR analysis of $GABA_{A\gamma3}$ in the control and experimental rats

Gene expression of $GABA_{A\gamma3}$ mRNA showed significant down regulation ($p<0.001$) in the hippocampus of epileptic rats compared to control. Treatment using *Bacopa monnieri* ($p<0.001$), Bacoside A ($p<0.001$) and Carbamazepine ($p<0.01$) were significantly reversed the $GABA_{A\gamma3}$ gene expression to near control (Table-27, Figure-26).

7. Real Time-PCR analysis of $GABA_{A\delta}$ in the control and experimental rats

Gene expression of $GABA_{A\delta}$ mRNA showed significant up regulation ($p<0.001$) in the hippocampus of epileptic rats compared to control. Treatment using *Bacopa monnieri* ($p<0.001$), Bacoside A ($p<0.001$) and Carbamazepine ($p<0.01$) were significantly reversed the $GABA_{A\delta}$ gene expression to near control (Table-28, Figure-27).

8. Real Time-PCR analysis of $GABA_B$ in the control and experimental rats

Gene expression of $GABA_B$ mRNA showed significant down regulation ($p<0.001$) in the hippocampus of epileptic rats compared to control. Treatment using *Bacopa monnieri* ($p<0.001$), Bacoside A ($p<0.001$) and Carbamazepine ($p<0.01$) were significantly reversed $GABA_B$ gene expression to near control (Table-29, Figure-28).

9. Real Time-PCR analysis of GAD in the control and experimental rats

Gene expression of GAD mRNA showed significant down regulation ($p<0.001$) in the hippocampus of epileptic rats compared to control. Treatment using *Bacopa monnieri,* Bacoside A and Carbamazepine were significantly ($p<0.001$), reversed GAD gene expression to near control (Table-30, Figure-29).

C. CEREBELLUM

1. Scatchard analysis of [^{3}H]GABA binding against GABA in the control and experimental rats

Scatchard analysis of [^{3}H]GABA binding against GABA in the cerebellum showed a significant decrease ($P<0.001$) in B_{max} and K_d ($P<0.001$) of epileptic group compared to control rats. Treatment using *Bacopa monnieri,* Bacoside A and Carbamazepine reversed the B_{max} to near control. K_d showed significant reversal in *Bacopa monnieri* ($P<0.001$), Bacoside A ($P<0.01$) and Carbamazepine ($P<0.01$) treated group compared to epileptic rats (Table-31, Figure-30).

2. Scatchard analysis of [^{3}H]bicuculline against bicuculline in the control and experimental rats

Scatchard analysis of [^{3}H]bicuculline against bicuculline in the cerebellum showed a significant decrease ($P<0.001$) in B_{max} ($P<0.01$) of epileptic rats compared to control. Treatment using *Bacopa monnieri,* Bacoside A and Carbamazepine reversed the B_{max} to near control. K_d showed no significant change in the experimental rats (Table-32, Figure-31).

3. Scatchard analysis of [^{3}H]baclofen against baclofen in the control and experimental rats

Scatchard analysis of [^{3}H]baclofen against baclofen in the cerebellum showed a significant decrease ($P<0.001$) in B_{max} and of epileptic rats compared to control. Treatment using *Bacopa monnieri*, Bacoside A and Carbamazepine reversed the B_{max} to near control. K_d showed no significant change in the experimental rats (Table-33, Figure-32).

4. Real Time-PCR analysis of $GABA_{A\alpha1}$ in the control and experimental rats

Gene expression of $GABA_{A\alpha1}$ mRNA showed significant down regulation ($p<0.001$) in the cerebellum of epileptic rats compared to control. Treatment using *Bacopa monnieri* ($p<0.001$), Bacocide A ($p<0.01$) and Carbamazepine ($p<0.01$) were significantly reversed $GABA_{A\alpha1}$ gene expression to near control (Table-34, Figure-33).

5. Real Time-PCR analysis of $GABA_{A\alpha5}$ in the control and experimental rats

Gene expression of $GABA_{A\alpha5}$ mRNA showed significant down regulation ($p<0.001$) in the cerebellum of epileptic rats compared to control. Treatment using *Bacopa monnieri* ($p<0.001$), Bacoside A ($p<0.001$) and Carbamazepine ($p<0.01$) were significantly reversed $GABA_{A\alpha5}$ gene expression to near control (Table-35, Figure-34).

6. Real Time-PCR analysis of $GABA_{A\gamma3}$ in the control and experimental rats

Gene expression of $GABA_{A\gamma3}$ mRNA showed significant up regulation ($p<0.001$) in the cerebellum of epileptic rats compared to control. Treatment using *Bacopa monnieri* ($p<0.001$), Bacoside A ($p<0.001$) and Carbamazepine ($p<0.01$) were significantly reversed $GABA_{A\gamma3}$ gene expression to near control (Table-36, Figure-35).

7. Real Time-PCR analysis of $GABA_{A\delta}$ in the control and experimental rats

Gene expression of $GABA_{A\delta}$ mRNA showed significant down regulation ($p<0.001$) in the cerebellum of epileptic rats compared to control. Treatment using *Bacopa monnieri,* Bacocide A and Carbamazepine were significantly ($p<0.001$), reversed $GABA_{A\delta}$ gene expression to near control (Table-37, Figure-36).

8. Real Time-PCR analysis of $GABA_B$ in the control and experimental rats

Gene expression of $GABA_B$ mRNA showed significant down regulation ($p<0.001$) in the cerebellum of epileptic rats compared to control. Treatment using *Bacopa monnieri,* Bacoside A and Carbamazepine were significantly ($p<0.001$), reversed $GABA_B$ gene expression to near control (Table-38, Figure-37).

9. Real Time-PCR analysis of GAD in the control and experimental rats

Gene expression of GAD mRNA showed significant down regulation ($p<0.001$) in the cerebellum of epileptic rats compared to control. Treatment using *Bacopa monnieri,* Bacoside A and Carbamazepine were significantly ($p<0.001$), reversed GAD gene expression to near control (Table-39, Figure-38).

D. CORPUS STRIATUM

1. Scatchard analysis of [^{3}H]GABA binding against GABA in the control and experimental rats

Scatchard analysis of [^{3}H]GABA binding against GABA in the corpus striatum showed a significant decrease ($P<0.001$) in B_{max} and K_d ($P<0.01$) of epileptic rats compared to control. Treatment using *Bacopa monnieri,* Bacoside A and Carbamazepine reversed the B_{max} to near control. K_d showed significant ($P<0.01$) reversal in *Bacopa monnieri,* Bacoside A and Carbamazepine treated group compared to epileptic rats (Table-40, Figure-39).

2. Scatchard analysis of [^{3}H]bicuculline against bicuculline in the control and experimental rats

Scatchard analysis of [^{3}H]bicuculline against bicuculline in the corpus striatum showed a significant decrease ($P<0.001$) in B_{max} and K_d of epileptic rats compared to control. Treatment using *Bacopa monnieri,* Bacoside A and Carbamazepine reversed the B_{max} to near control. K_d showed significant ($P<0.01$) increase in *Bacopa monnieri,* Bacoside A and Carbamazepine treated group compared to epileptic rats (Table-41, Figure-40).

3. Scatchard analysis of [^{3}H]baclofen against baclofen in the control and experimental rats

Scatchard analysis of [^{3}H]baclofen against baclofen in the corpus striatum showed a significant decrease ($P<0.001$) in of epileptic rats compared to control. Treatment using *Bacopa monnieri,* Bacoside A and Carbamazepine reversed the B_{max} to near control. K_d showed significant increase in *Bacopa monnieri* ($P<0.05$), Bacoside A ($P<0.05$), and Carbamazepine ($P<0.01$) treated group compared to control and epileptic rats (Table-42, Figure-41).

4. Real Time-PCR analysis of $GABA_{A\alpha1}$ in the control and experimental rats

Gene expression of $GABA_{A\alpha1}$ mRNA showed significant down regulation ($p<0.001$) in the corpus striatum of epileptic rats compared to control. Treatment using *Bacopa monnieri* ($p<0.001$), Bacoside A ($p<0.001$) and Carbamazepine ($p<0.01$) were significantly reversed $GABA_{A\alpha1}$ gene expression to near control (Table-43, Figure-42).

5. Real Time-PCR analysis of $GABA_{A\hat{a}5}$ in the control and experimental rats

Gene expression of $GABA_{A\hat{a}5}$ mRNA showed significant up regulation ($p<0.001$) in the corpus striatum of epileptic rats compared to control. Treatment using *Bacopa monnieri* ($p<0.001$), Bacoside A ($p<0.001$) and Carbamazepine ($p<0.01$) were significantly reversed $GABA_{A\hat{a}5}$ gene expression to near control (Table-44, Figure-43).

6. Real Time-PCR analysis of $GABA_{A\gamma3}$ in the control and experimental rats

Gene expression of $GABA_{A\gamma3}$ mRNA showed significant down regulation ($p<0.001$) in the corpus striatum of epileptic rats compared to control. Treatment using *Bacopa monnieri* ($p<0.001$), Bacoside A ($p<0.001$) and Carbamazepine ($p<0.01$) were significantly reversed $GABA_{A\gamma3}$ gene expression to near control (Table-45, Figure-44).

7. Real Time-PCR analysis of $GABA_{A\delta}$ in the control and experimental rats

Gene expression of $GABA_{A\delta}$ mRNA showed significant down regulation ($p<0.001$) in the corpus striatum of epileptic rats compared to control. Treatment using *Bacopa monnieri,* Bacoside A and Carbamazepine were significantly ($p<0.001$) reversed $GABA_{A\delta}$ gene expression to near control (Table-46, Figure-45).

8. Real Time-PCR analysis of $GABA_B$ in the control and experimental rats

Gene expression of $GABA_B$ mRNA showed significant down regulation ($p<0.001$) in the corpus striatum of epileptic rats compared to control. Treatment using *Bacopa monnieri,* Bacoside A and Carbamazepine were significantly ($p<0.001$) reversed $GABA_B$ gene expression to near control (Table-47, Figure-46).

9. Real Time-PCR analysis of GAD in the control and experimental rats

Gene expression of GAD mRNA showed significant down regulation ($p<0.001$) in the corpus striatum of epileptic rats compared to control. Treatment using *Bacopa monnieri,* Bacoside A and Carbamazepine were significantly ($p<0.001$), reversed GAD gene expression to near control (Table-48, Figure-47).

E. BRAIN STEM

1. Scatchard analysis of [^{3}H]GABA binding against GABA in the control and experimental rats

Scatchard analysis of [^{3}H]GABA binding against GABA in the brain stem showed a significant decrease ($P<0.001$) in B_{max} and K_d ($P<0.05$) of epileptic rats compared to control rats. Treatment using *Bacopa monnieri,* Bacoside A and Carbamazepine reversed the B_{max} to near control. K_d showed significant ($P<0.01$) reversal in Bacoside A treated group compared to epileptic rats (Table-49, Figure-48).

2. Scatchard analysis of [^{3}H]bicuculline against bicuculline in the control and experimental rats

Scatchard analysis of [^{3}H]bicuculline against bicuculline in the brain stem showed a significant decrease ($P<0.001$) in B_{max} and K_d of epileptic rats compared to control rats. Treatment using *Bacopa monnieri,* Bacoside A and Carbamazepine reversed the B_{max} to near control. K_d showed significant reversal in *Bacopa monnieri,* ($P<0.001$) *Bacoside* A ($P<0.01$) and Carbamazepine ($P<0.001$) treated group compared to epileptic rats (Table-50, Figure-49).

3. Scatchard analysis of [^{3}H]baclofen against baclofen in the control and experimental rats

Scatchard analysis of [^{3}H]baclofen against baclofen in the brain stem showed a significant decrease ($P<0.001$) in B_{max} of epileptic rats compared to control rats. Treatment using *Bacopa monnieri*, Bacoside A and Carbamazepine reversed the B_{max} to near control. K_d showed significant ($P<0.01$) reversal in *Bacopa monnieri* Bacoside A and Carbamazepine treated group compared to control and epileptic rats (Table-51, Figure-50).

4. Real Time-PCR analysis of $GABA_{A\alpha1}$ in the control and experimental rats

Gene expression of $GABA_{A\alpha1}$ mRNA showed significant down regulation ($p<0.001$) in the brain stem of epileptic rats compared to control. Treatment using *Bacopa monnieri* ($p<0.001$), Bacoside A ($p<0.001$) and Carbamazepine ($p<0.01$) were significantly reversed $GABA_{A\alpha1}$ gene expression to near control (Table-52, Figure-51).

5. Real Time-PCR analysis of $GABA_{A\alpha5}$ in the control and experimental rats

Gene expression of $GABA_{A\alpha5}$ mRNA showed significant down regulation ($p<0.001$) in the brain stem of epileptic rats compared to control. Treatment using *Bacopa monnieri* ($p<0.001$), Bacoside A ($p<0.001$) and Carbamazepine ($p<0.01$) were significantly reversed $GABA_{A\alpha5}$ gene expression to near control (Table-53, Figure-52).

6. Real Time-PCR analysis of $GABA_{A\gamma3}$ in the control and experimental rats

Gene expression of $GABA_{A\gamma3}$ mRNA showed significant up regulation ($p<0.001$) in the brain stem of epileptic rats compared to control. Treatment using *Bacopa monnieri* ($p<0.001$), Bacoside A ($p<0.001$) and Carbamazepine ($p<0.01$) were significantly reversed $GABA_{A\gamma3}$ gene expression to near control (Table-54, Figure-53).

7. Real Time-PCR analysis of $GABA_{A\delta}$ in the control and experimental rats

Gene expression of $GABA_{A\delta}$ mRNA showed significant down regulation ($p<0.001$) in the brain stem of epileptic rats compared to control. Treatment using *Bacopa monnieri* ($p<0.001$), Bacoside A ($p<0.001$) and Carbamazepine ($p<0.01$) were significantly reversed $GABA_{A\delta}$ gene expression to near control (Table-55, Figure-54).

8. Real Time-PCR analysis of $GABA_B$ in the control and experimental rats

Gene expression of $GABA_B$ mRNA showed significant down regulation ($p<0.001$) in the brain stem of epileptic rats compared to control. Treatment using *Bacopa monnieri,* Bacoside A and Carbamazepine were significantly ($p<0.001$ reversed $GABA_B$ gene expression to near control (Table-56, Figure-55).

9. Real Time-PCR analysis of GAD in the control and experimental rats

Gene expression of GAD mRNA showed significant down regulation ($p<0.001$) in the brain stem of epileptic rats compared to control. Treatment using *Bacopa monnieri* ($p<0.001$), Bacoside A ($p<0.001$ and Carbamazepine ($p<0.01$) were significantly reversed GAD gene expression to near control (Table-57, Figure-56).

III. BEHAVIORAL STUDY

1. Behavioral response of control and experimental rats on Y-Maze performance

Number of visits and time spent in the novel arm decreased significantly ($p<0.001$) in the epileptic group compared to control. Lower percentage of arm visits between the novel arm and the start arm and decreased time spent in the novel arm compared to the other two arms within the epileptic rats showed their decreased exploratory behaviour which have considerable role in the motor learning. Time spends in the novel arm and number of visit to the novel arm was increased in the

epileptic rats treated with Bacopa *monnieri* and Bacoside A (Table-58, 59; Figure-57, 58).

2. Behavioral response of control and experimental rats on criterion performance in radial arm maze

There was significant increase ($p<0.001$) in the number of trials required to achieve five consecutive criterion performances in the epileptic rats compared to control. The number of trials up to and including the last of these 5 criterion performance formed the "number of trials to criterion" measure. Increased numbers of trials to criterion performance was indicating the learning and memory deficit in epileptic rats. Treatment using *Bacopa monnieri* and Bacoside A reversed these changes to near control (Table-60, Figure-59).

3. Behavioral response of control and experimental rats on reference errors in radial arm maze

There was significant increase ($p<0.001$) in the number of reference errors to achieve five consecutive criterion performances in the epileptic rats compared to control. Number of reference errors to achieve five consecutive criterion performances was significantly decreased in epileptic rats administered with *Bacopa monnieri* ($p<0.01$), Bacoside A ($p<0.01$) and Carbamazepine ($p<0.05$) (Table-61, Figure-60).

4. Behavioral response of control and experimental rats on working errors in radial arm maze

There was significant increase ($p<0.001$) in the number of working errors to achieve five consecutive criterion performances in the epileptic rats compared to control. Number of working errors to achieve five consecutive criterion performances was significantly decreased in epileptic rats administered with *Bacopa monnieri* ($p<0.01$), Bacoside A ($p<0.01$) and Carbamazepine ($p<0.05$) (Table-62, Figure-61).

5. Behavioral response of control and experimental rats on grid walk test

There was significant increase ($p<0.001$) in the foot falls in epileptic rats compared to control. Foot falls were significantly decreased in epileptic rats administered with *Bacopa monnieri* ($p<0.01$), Bacoside A ($p<0.01$) and Carbamazepine ($p<0.05$) (Table-63, Figure-62).

6. Behavioral response of control and experimental rats on narrow beam test

There was significant decrease in the retention of balance on the narrow beam ($p<0.001$) in epileptic rats compared to control. Balance on the narrow beam was significantly increased in epileptic rats treated with *Bacopa monnieri* ($p<0.01$), Bacoside A ($p<0.01$) and Carbamazepine ($p<0.05$) (Table-64, Figure-63).

7. Behavioral response of control and experimental rats on open field test

In epileptic rats resting behavior significantly increased ($p<0.01$) and sniffing behavior decreased ($p<0.01$) compared to control. Treatment using *Bacopa monnieri*, Bacoside A and Carbamazepine significantly increased the sniffing and decreased the resting behaviour (Table-65).

IV. CONFOCAL STUDIES

1. $GABA_{A\alpha1}$ receptor antibody staining in the Cerebral cortex of Control and experimental rats

$GABA_{A\alpha1}$ receptor subunit antibody staining in the Cerebral cortex showed a significant decrease ($p<0.01$) in the pixel intensity of $GABA_{A\alpha1}$ receptor subunit in epileptic rats compared to control. *Bacopa monnieri*, Bacoside A and Carbamazepine treated epileptic rats showed a significant reversal ($p<0.01$) of decreased $GABA_{A\alpha1}$ receptor subunit staining in the cerebral cortex compared to epileptic rats (Table-66, Figure-64).

2. $GABA_{A\alpha1}$ receptor antibody staining in the Hippocampus of Control and experimental rats

$GABA_{A\alpha1}$ receptor subunit antibody staining in the hippocampus showed a significant decrease ($p<0.01$) in the pixel intensity of $GABA_{A\alpha1}$ receptor subunit in epileptic rat compared to control. *Bacopa monnieri,* Bacoside A and Carbamazepine treated epileptic rats showed a significant reversal ($p<0.01$) of the decreased $GABA_{A\alpha1}$ receptor subunit staining in the hippocampus compared to epileptic rats (Table-66, Figure-65).

3. $GABA_{A\alpha1}$ receptor antibody staining in the cerebellum of Control and experimental rats

$GABA_{A\alpha1}$ receptor subunit antibody staining in the cerebellum showed a significant decrease ($p<0.01$) in the pixel intensity of $GABA_{A\alpha1}$ receptor subunit in epileptic rat compared to control. *Bacopa monnieri,* Bacoside A and Carbamazepine treated epileptic rats showed a significant reversal ($p<0.01$) of the decrease in $GABA_{A\alpha1}$ receptor subunit staining in the cerebellum compared to epileptic rats (Table-66, Figure-66).

4. $GABA_{A\alpha1}$ receptor antibody staining in the corpus striatum of Control and experimental rats

$GABA_{A\alpha1}$ receptor subunit antibody staining in the corpus striatum showed a significant decrease ($p<0.01$) in the pixel intensity of $GABA_{A\alpha1}$ receptor subunit in epileptic rat compared to control. *Bacopa monnieri,* Bacoside A and Carbamazepine treated epileptic rats showed a significant reversal ($p<0.01$) of the decreased $GABA_{A\alpha1}$ receptor subunit staining in the corpus striatum compared to epileptic rats (Table-66, Figure-67).

5. $GABA_{A\alpha 1}$ receptor antibody staining in the brain stem of Control and experimental rats

$GABA_{A\alpha 1}$ receptor subunit antibody staining in the brain stem showed a significant decrease ($p<0.01$) in the pixel intensity of $GABA_{A\alpha 1}$ receptor subunit in epileptic rat compared to control. *Bacopa monnieri,* Bacoside A and Carbamazepine treated epileptic rats showed a significant reversal ($p<0.01$) of the decreased $GABA_{A\alpha 1}$ receptor subunit staining in the brain stem compared to epileptic rats (Table-66, Figure-68).

DISCUSSION

SEIZURE LATENCY AND MAGNITUDE OF DRUG EFFECT

Increase in the seizure onset latency and decrease in the duration of seizures of various antiepileptic drugs were reported earlier (Eric *et al.*, 2002). In our study epileptic rats showed increased seizure frequency. Treatment using *Bacopa monnieri*, Bacoside A and Carbamazepine, reduced the number of seizures per hour compared to the epileptic rat groups. The severities of the seizures in the treated rat groups were also decreased. These results are suggestive evidence of the ability of the *Bacopa monnieri* and Bacoside A in reducing the spontaneous seizures, which showed their antiepileptic property.

PERIPHERAL ACETYLCHOLINE ESTERASE, MALATE DEHYDOGENASE, T3 AND INSULIN CHANGES DURING TEMPORAL LOBE EPILEPSY

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). Certain brain areas such as temporal and frontal lobes are more susceptible to produce epileptic seizure activity than the other regions. However, there are also patients with unresolved etiology of epilepsy (Hauser, 1997). Epilepsy is a seizure disorder. A seizure is an event that involves loss of consciousness and motor (muscular) control. A person with a seizure disorder often experiences repetitive muscle jerking called convulsions. The condition is caused by a sudden change in electrical activity in the brain. Generalized tonic-clonic seizure is a seizure involving the entire body, also called a grand mal seizure. Such seizures frequently involve muscle rigidity, violent muscle contractions and loss of consciousness (Martinelli *et al.*, 1978). Epilepsy typically cause myoclonic twitches, usually caused by sudden muscle contractions; they also can result from

brief lapses of contraction (Fumisuke, 2009). We have investigated acetylcholine esterase (AChE) activity in the heart and muscle, malate dehydrogenase (MDH) activity in the muscle, T3, insulin content in the serum and epinephrine and norepinephrine in the heart of the control and experimental rats. Our results showed that AChE and MDH activity increased in the muscle and decreased in the heart of the epileptic rats compared to control. Circulating Insulin and triiodothyronine (T3) content were increased in the serum of the epileptic rats.

Acetylcholine induced seizure-like activity in chronically epileptic rats (Wonnacott, 1997). Nicotinic acetylcholine receptors are widespread ligand-gated ion channels that mediate fast cholinergic transmission at both the peripheral and central nervous systems. In addition to their involvement in neuromuscular and autonomic ganglia synaptic transmission, they play an important role in cognitive and addictive processes (Lena & Changeux, 1998). Furthermore, their dysfunction has been linked to a number of human diseases, including congenital myasthenia, schizophrenia, epilepsy and neurodegenerative disorders such as Alzheimer's and Parkinson's diseases (Lena & Changeux, 1998; Hogg *et al.*, 2003). Nicotinic acetylcholine receptors are present in many tissues in the body and are the best studied of the ionotropic receptors. The neuronal receptors are found in the central nervous system and the peripheral nervous system. The neuromuscular receptors are found in the neuromuscular junctions of somatic muscles; stimulation of these receptors causes muscular contraction (Lorkovi *et al.*, 1995). Seizures are initiated by the excessive neuronal activity in the brain which is transmitted through the peripheral nervous system and stimulate the release of ACh into the neuromuscular junction. Acetylcholine released into the neuromuscular junction from the presynaptic neurons initiate the muscle contraction. On the other hand ACh inhibit heart beat and epinephrine and norepinephrine initiate it (Zaugg & Schaub, 2005). AChE activity has been used as a marker for cholinergic activity (Finla *et al.*, 2008). The enzyme acetylcholine esterase converts acetylcholine into the inactive metabolites choline and

acetate. AChE is abundant in the synaptic cleft, and its role in rapidly clearing free acetylcholine from the synapse is essential for proper muscle function. AChE plays a very important role in the ACh-cycle, including the release of ACh (Kouniniotou-Krontiri & Tsakiris 1989). The duration of ACh at the synaptic cleft is critically dependent on AChE activity (Cooper *et al.*, 2003). Our study showed increased AChE activity in the muscle and decreased activity in the heart of the epileptic rats. Increased cholinergic activity in the neuromuscular junction makes the muscle more susceptible to seizures.

Malate dehydrogenase is an enzyme in the citric acid cycle that catalyzes the conversion of malate into oxaloacetate using NAD^+ and *vice versa*. Malate dehydrogenase MDH is also involved in gluconeogenesis, the synthesis of glucose from smaller molecules. The activity of lactate dehydrogenase (LDH) and MDH was estimated as indicators of anaerobic metabolism (Renato *et al.*, 2008). As previously reported, mitochondrial MDH binds to purified complex I of the electron transport system and mitochondrial function is a key determinant of both excitability and viability of neurons (Achmunjal *et al.*, 2005). Brain mitochondrial membrane was more fluidized in epileptic animals with a possible consequence of mitochondrial respiratory chain (MRC) dysfunction. Studies showed that impairment of MRC function along with structural alterations suggest novel pathophysiological mechanisms important for chronic epileptic condition (Achmunjal *et al.*, 2005). Alcoholic extract of *Bacopa monnieri* was tested for its protective role on morphine-induced brain mitochondrial enzyme status in rats. The level of the brain mitochondrial enzymes was significantly lower in the morphine-treated group when compared with control animals. These enzymes were maintained at normal level when *Bacopa monnieri* extract was administered before the administration of morphine (Sumathy *et al.*, 2002). Our study showed increased MDH activity in the muscle of the epileptic rats indicative of the increased metabolic rate in the muscle of the

epileptic rats. Administration of *Bacopa monnieri* and Bacoside A decreased the enzyme activity to near control level.

Insulin facilitates the entrance of glucose into the muscle cells (Park *et al.*, 1955). The only mechanism by which cells take up glucose is by facilitated diffusion through a family of hexose transporters. In muscle tissue the major transporter used for uptake of glucose is glucose transporter-4 (GLUT4) which was made available in the plasma membrane through the action of insulin (Duehlmeier *et al.*, 2007). Insulin regulates glucose uptake into these cells by recruiting membrane vesicles containing the GLUT4 glucose transporters from the interior of the cells to the cell surface, where it allows glucose to enter cells by facilitated diffusion (Heidi *et al.*, 2002). Once in the cytoplasm, the glucose is phosphorylated and thereby trapped inside cells. The effect of insulin on GLUT4 distribution is reversible. Within an hour of insulin exclusion, GLUT4 was removed from the membrane and restored intracellular in vesicles were ready to be re-recruited to the surface by insulin (Chandrasagar *et al.*, 2008). Our study showed increased insulin content in the serum of the epileptic rats. Increased insulin content in the serum of epileptic rats facilitates the rapid intake of glucose into the muscle cells.

T3 is the major metabolic hormone which has effect on basal metabolic rate (BMR) (Acheson *et al.*, 1984). The thyroid gland secretes mostly thyroxine (T4) and very little T3. Most of the T3 that drives cell metabolism is produced by action of the enzyme named 5'-deiodinase, which converts T4 to T3. T3 is the metabolically active thyroid hormone. In adult life, it exerts a profound effect on basal metabolic rate, increasing respiration rate and simultaneously lowering metabolic efficiency (Jin *et al.*, 2006). It mainly acts through the coordinated and synergistic modulation of both nuclear and mitochondrial genome expressions giving rise to a complex network of factors and cellular events (Silvestri *et al.*, 2008). Increased plasma T3 level is an indicator of increased BMR (Bernt *et al.*, 2008). In our study epileptic rats showed increased T3 content in the serum compared to control and treatment using *Bacopa*

monnieri and Bacoside A decreased the T3 content to near control. Treatment of rats with T3 resulted in a significant decrease in body weight (Lloyd *et al.*, 1983), while the heart weight increased which indicated that T3 and T4 thyrotoxicosis results in impaired energy metabolism in heart mitochondria (Katyare & Billimoria, 1989). T3 has the ability to uncouple oxidation of substrates from ATP production, resulting in reduced ATP production and an astounding production of heat (Lanni *et al.*, 2003, 1999). Muscle glycogen is also more rapidly depleted and less efficiently stored during hyperthyroidism, which create muscle weakness.

Thus our study showed increased excitability and energy metabolism in epileptic rats. It is suggested that repetitive seizures resulted in increased metabolism and excitability in epileptic rats. *Bacopa monnieri* and Bacoside A treatment decreased the seizures frequency in the epileptic rats there by reducing the impairment on peripheral nervous system (Jobin *et al.*, 2010b).

GABA RECEPTOR ALTERATIONS IN THE CEREBRAL CORTEX

GABA neurons constitute 20–30% of all neurons in the cerebral cortex and perform critical roles in modulating cortical functional output (Cherubini & Conti, 2001; Krimer & Goldman-Rakic, 2001). During development, GABA neurons modulate neocortical neuroepithelial cell proliferation, neuronal migration and circuit formation (Owens & Kriegstein, 2002; Ben-Ari *et al.*, 2004). Abnormalities in GABA neuron function occur in a variety of developmental disorders including epilepsy, schizophrenia, anxiety and autism (Benes, 2000; Treiman, 2001; Levitt *et al.*, 2004). GABA, the principal inhibitory neurotransmitter in the cerebral cortex, maintains the inhibitory tones that counter balances neuronal excitation. When this balance is perturbed, seizures ensue (Gregory & Mathews, 2007). GABA is formed within GABAergic axon terminals and released into the synapse, where it acts at one of two types of receptor $GABA_A$ and $GABA_B$ (Labrakakis *et al.*, 1997). $GABA_A$ receptor binding influences the early portion of the GABA mediated inhibitory postsynaptic

potential, whereas $GABA_B$ binding influences the late portion. $GABA_A$ receptor activation in neurons induced a complex physiological response, namely the activation of a Cl^- conductance in concert with a blockade of the resting K^+ outward conductance results in hyperpolarisation. Both responses were mediated by the activation of $GABA_A$ receptors, since they were both mimicked by the $GABA_A$ receptor agonist muscimol and antagonized by picrotoxin and bicuculline (Labrakakis *et al.*, 1997). A balance of excitatory and inhibitory neurotransmission is required for normal functioning of the central nervous system. GABAergic neurons provide inhibitory control in the brain and have an important role in selective neuronal degeneration following ischemia and epilepsy (Mileson *et al.*, 1992; Sperk *et al.*, 2004). In addition to augmentation of endogenous protective mechanisms following different pathophysiological conditions, alterations in the kinetics and pharmacology of $GABA_A$ receptors is associated with the development of spontaneous seizure activity (Coulter, 2001; Treiman, 2001; Nishimura *et al.*, 2005). Our results showed decreased GABA receptor binding and gene expression in the cerebral cortex of the epileptic rats showing the decreased GABA receptor distribution. Imbalance between excitatory and inhibitory synaptic transmission in key brain areas are implicated in the pathophysiology of TLE, in which there is a decrease in the GABA mediated inhibition. TLE seizures reflect excess excitation, which result from local inhibitory circuit dysfunction. Reduction of GABA mediated inhibition and decreased activity of GAD has been reported in studies of human epileptic brain tissue. Impairment of GABA functions produces seizures, whereas enhancement results in an anticonvulsant effect (MacDonald & Barker, 1979; Schulz & Macdonald, 1981). Support for a chronic loss of GABAergic function in epileptogenic human cortex has been based on biochemical assays of tissue resected for relief of focal seizure (Lloyd *et al.*, 1981, 1984). Altered $GABA_A$ receptor function has been described in the cerebral cortex and thalamus of two inbred strains of rats that exhibit spontaneous spike and wave

seizures and are recognized as genetic models of absence epilepsy (Coenen *et al.*, 2003).

GAD is an enzyme that catalyzes the decarboxylation of glutamate to GABA. GAD is the rate limiting enzyme of GABA synthesis and it is used as a marker for GABAergic activity (Sophie *et al.*, 1990). It is reported that in both epileptic and histologically damaged cortex, there are significant decreases in GAD and GABA binding (Lloyd *et al.*, 1981). Our study showed down regulation of GAD mRNA in the cerebral cortex of the epileptic rats compared to control. GAD plays a very important role in maintaining excitatory inhibitory balance of the central nervous system (Quan *et al.*, 2003). Analysis of GAD activity was used as a marker of over all GABAergic activity and was found to be lower in epileptic mice. The enzymatic activity of GAD is the rate limiting step in the production of GABA and GAD serve as a marker of inhibitory neurons. Moreover, preliminary findings indicate that the decrease in GABA is associated with reduced GABA synthesis rather than increased degradation (Mason *et al.*, 2001).

The incidence of psychiatric diseases in epileptics is significantly higher than in the general population (Robertson *et al.*, 1986; Mendez *et al.*, 1986; Kanner & Soto, 1998; Mendez *et al.*, 1993). Depressive and anxiety disorders are the most common psychiatric diseases in these patient groups. Recent studies employing magnetic resonance spectroscopy (MRS) suggest that unipolar depression is associated with reductions in cortical GABA levels (Sanacora *et al.*, 1999, 2000). Antidepressant and mood-stabilizing treatments also appear to raise cortical GABA levels and to ameliorate GABA deficits in patients with mood disorders (Krystal *et al.*, 2002). Anxiety disorders have long been associated with disturbances of GABA function because of the ability of the benzodiazepine anxiolytics to facilitate brain GABA neurotransmission (Honig, *et al.*, 1988). Interestingly, as with plasma studies MRS also reveals lowered concentrations of GABA in occipital cortex in panic disorder (Goddard, *et al.*, 2001) and in subjects with alcohol dependence (Behar, *et*

al., 1999). We observed a significant decrease in the B_{max} of GABA receptors and GABA content in the cerebral cortex of epileptic rats compared to control. GABA receptors and GAD gene expression patterns were similar to the receptor binding studies. Treatment using *Bacopa monnieri* and Bacoside A increased the receptor binding and up regulated the receptor subunit gene expression in the epileptic rats. Our studies and previous studies from our lab showed that *Bacopa monnieri* treatment to epileptic rats reduced the number of seizures per hour which is suggestive of its anticonvulsant property (Reas *et al.*, 2008). The increasing brain GABA content and administration of centrally active GABA-mimetic agents have been used as an effective therapeutic approach for the treatment of epilepsy (Olsen & Avoli, 1997). However, it has also been suggested that the use of drugs that continually alter synaptic transmission does not represent the best strategy to control seizure expression (Gale 1992). Prolonged administration of drugs enhancing GABA action lead to reduced GABAergic function (Gyenes *et al.*, 1988) and alterations in $GABA_A$ receptors (Calkin & Barnes, 1994; Yu & Ticku, 1995) as well as tolerance and dependence, especially to sedative and anticonvulsant effects of drugs such as benzodiazepines. Therefore instead of using the drugs like benzodiazepines, administration of herbal medicine like *Bacopa monnieri* have significant role in the management of epilepsy.

GABA RECEPTOR ALTERATIONS IN THE HIPPOCAMPUS

TLE is a common form of drug-refractory epilepsy (Engel, 1998). Hippocampal sclerosis, the main neuropathological feature of TLE is characterized by massive neuronal loss and gliosis in CA1, CA3 and hilus, while most granule cells of the dentate gyrus are preserved. It is associated with axon and synaptic reorganization of surviving neurons. Reorganized axons include sprouting of excitatory mossy fibres, inhibitory GABAergic neurons and fibres with other neurotransmitters such as neuropeptide Y and somatostatin (Mathern *et al.*, 1997). Occurrence of epileptic

seizures is the consequence of an imbalance in the neurotransmission systems in favour of neuronal hyperexcitation. In our study GABA receptor binding and gene expression were considerably decreased in the hippocampus of the epileptic rats. *Bacopa monnieri* and Bacoside A treatment helped to enhance the GABA receptor in the epileptic rats. The plasticity of inhibitory $GABA_A$ receptor-mediated synaptic inhibition is considered to contribute to hyper-excitability in the dentate gyrus granule cells layer in TLE patients. Indeed, the dentate gyrus critically regulates seizures by making synchronous activities from entorhinal cortex were able to invade the hippocampus. However, a failure in the gating function of the dentate gyrus can generate robust paroxysmal discharges (Lothman *et al.*, 1992; Behr *et al.*, 1998). The presence of the markers of the GABAergic phenotype in the glutamate-releasing granule cells (Crawford & Connor, 1973) of the dentate gyrus suggests that GABA could be synthesized, vesiculated and released from the same neurons (Gutiérrez, 2002). These markers are highly expressed during development, down-regulated and barely detectable in the adult rat, but reappear after strong excitation, especially after seizures (Sandler & Smith, 1991; Schwarzer & Sperk, 1995; Sloviter *et al.*, 1996; Lehman *et al.*, 1996;). Accordingly, cellular electrophysiological studies have shown that mossy fibre activation produces, in addition to monosynaptic glutamate receptor-mediated responses, monosynaptic $GABA_AR$-mediated synaptic potentials/currents in their target cells, pyramidal cells and interneurons of CA3, when the GABAergic markers are highly expressed (Walker *et al.*, 2001; Gutiérrez *et al.*, 2003; Bergersen *et al.*, 2003).

Degeneration of CA3-pyramidal neurons in hippocampus of rat model of temporal lobe epilepsy leads to persistent reductions in hippocampal GAD interneuron numbers indicating loss of GAD expression in a majority of interneurons (Ashok *et al.*, 2000). There could be a direct link between the loss of functional inhibition and reduction in GAD positive interneuron numbers. Therefore, strategies that restore GAD interneuron numbers to levels observed in intact hippocampus is beneficial for

both restoring the functional inhibition and ameliorating hyperexcitability (Ashok *et al.*, 2000). Our results showed a significant down regulation of GAD gene expression in the hippocampus of the epileptic rats and the administration of *Bacopa monnieri* and Bacoside A reversed this change to near control. GAD plays a very important role in maintaining excitatory-inhibitory balance of the central nervous system (Quan *et al.*, 2003). Analysis of GAD activity was used as a marker of over all GABAergic activity and was found to be lower in epileptic mice. Failure of GABergic inhibition or enhancement of excitatory neurotransmission contributed to seizure initiation, especially at or near the site of seizure initiation (Balters & Francesco, 2002). Thus it is clear that the decreased GABA receptors along with decreased GAD gene expression in the hippocampus have an important role in TLE.

Thus our results suggest that decreased GABA content, GABA receptors and GAD activity in the hippocampus encompass an important role during seizure initiation. We conclude from our studies that *Bacopa monnieri* and Bacoside A treatment potentiates a beneficial effect against epilepsy by reversing the alterations in general GABA, $GABA_A$ and $GABA_B$ receptor binding, $GABA_A$ receptor subunits and GAD gene expression that occur during epilepsy, resulting in an increased GABA mediated inhibition in the over stimulated hippocampal neurons. Increased GABA receptors along with increased GAD activity prevent the seizure by ensuring hyper polarisation in the hippocampal neurons.

GABA RECEPTOR ALTERATIONS IN THE CEREBELLUM

Cerebellum is a region of the brain that plays an important role in the integration of sensory perception, memory consolidation, coordination and motor control. In order to coordinate motor control, there are many neural pathways linking the cerebellum with the cerebral motor cortex and the spinocerebellar tract (Roberta & Peter, 2003). There is currently enough anatomical, physiological and theoretical evidence to support the hypothesis that cerebellum is the region of the brain for

learning, basal ganglia for reinforcement learning and cerebral cortex for unsupervised learning (Doya, 1999). The cellular basis of motor learning has been mostly attributed to long term depression (LTD) at excitatory parallel fiber (PF)-Purkinje cell (PC) synapses. LTD is induced when PFs are activated in conjunction with a climbing fiber (CF), the other excitatory input to PCs. Recently, by using whole-cell patch-clamp recording from PCs in cerebellar slices, a new form of synaptic plasticity was discovered. Stimulation of excitatory CFs induced a long-lasting rebound potentiation (RP) of γ-amino-butyric acid A ($GABA_A$) receptor mediated inhibitory postsynaptic currents (IPSCs) (Peer *et al.*, 2009; Bibiana *et al.*, 2008). As in LTD, induction of RP requires transient elevation of intracellular calcium concentration due to activation of voltage-gated Ca^{2+} channels (Kano, 1996; Chitoshi & Yoshiro, 2004; Siqiong *et al.*, 2008). Activity of inhibitory synapses also seems to be necessary for RP to occur. Memory deficit is a major problem in TLE patients (Isabelle, 2006; Indre, 2000).

In our study, [^{3}H]GABA, [^{3}H]bicuculline and [^{3}H]baclofen binding were significantly decreased in the cerebellum of epileptic rats. $GABA_A$ receptor subunits $GABA_{A\alpha1}$, $GABA_{A\alpha5}$ and $GABA_{A\delta}$, were down regulated in the cerebellum of the epileptic rats compared to control. Treatment using *Bacopa monnieri* and Bacoside A were increasing the GABA receptor binding and GABA receptor subunit gene expression. Alterations in $GABA_A$ receptor subunit expression and composition in epilepsy are well documented in human (Loup *et al.*, 2000, 2006) and in animal models (Peng *et al.*, 2004; Gilby *et al.*, 2005; Nishimura *et al.*, 2005; Roberts *et al.*, 2005; Li *et al.*, 2006). The changes in $GABA_A$ receptor subunit contribute to the changes in inhibitory function that underlie epileptogenesis and occurrence of chronic recurrent seizures (Jean-Marc, 2008). In the mouse pilocarpine model of TLE, a profound decrease in δ subunit immunoreactivity was observed, correlating with a redistribution of the γ2 subunit from synaptic to perisynaptic sites, where it assembled with the α4 subunit, which is normally associated with the δ subunit (Zhang *et al.*, 2007). A down-regulation of the α5 subunit also occurs in CA1 pyramidal cells of

pilocarpine-treated rats (Houser & Esclapez, 2003), resulting in a loss of diazepam-sensitive tonic inhibition seen upon blockade of GABA reuptake. Despite this change, tonic inhibition is enhanced in pyramidal cells, suggesting compensatory up regulation of other extrasynaptic $GABA_A$ receptors, possibly containing the α4 subunit (Jean-Marc, 2008). These studies showed that during epilepsy the subunits of $GABA_A$ receptors showed different subunit combination with varying inhibitory potential.

Thus it is suggested that decreased GABA receptor in the cerebellum plays an important role in the motor and memory deficit and the GABA receptor enhancing property of *Bacopa monnieri* and Bacoside A which ameliorate this abnormalities. The receptor analysis and gene expression studies implicated a role for GABA receptors in the modulation of neuronal network excitability and cerebellar motor learning. Our results put forward that changes in the GABAergic activity, motor learning and memory deficit are induced by the occurrence of repetitive seizures. *Bacopa monnieri* and Bacoside A treatment prevents the occurrence of seizures there by reducing the impairment on GABAergic activity, motor learning and memory deficit (Jobin *et al.*, 2010a).

GABA RECEPTOR ALTERATIONS IN THE CORPUS STRIATUM

Striatum is anatomically the most prominent nucleus of the basal ganglia and many of the proposed functions of the basal ganglia have been linked to synaptic processing among cells in the striatum. These functions include possible roles in focusing cortical activity (Mallet *et al.*, 2005; Ouyang et al., 2007) and procedural and habit learning (Hikosaka *et al.*, 2002; Morris *et al.*, 2004; Graybiel, 2005; Frank & Claus, 2006). The striatum is thought to play an important role in the spreading of epilepsy from cortical areas to deeper brain structures. Cortical neurons generate synchronous electrical activity under a variety of physiological and pathological conditions (Gray *et al.*, 1989; Nicolelis *et al.*, 1995; Bragin *et al.*, 1999; Grenier *et al.*, 2003). In particular, epilepsy is a heterogeneous disorder that includes multiple

conditions, the common trait of which is paroxysmal cortical synchronization (Amzica & Steriade, 1999, 2000; McKeown & McNamara, 2001; McCormick, 2002). Such a synchronous activity, which is characterized by repetitive action potential firing of cortical neurons, will synaptically affect striatal neurons and, as a consequence, will influence the downstream nuclei of the basal ganglia and the thalamus, which impinge back on the cortex. Epileptiform activity in the cortex evoke synchronized depolarizing bursts accompanied by action potentials in striatal projection neurons. All classes of striatal neurons receive prominent inhibitory GABAergic inputs. These inhibitory interactions are likely to be essential for striatal processing. GABA receptors are found at pre or postsynaptic locations in almost all neuronal elements in the striatum (Fujiyama, *et al.*, 2000; Waldvogel *et al.*, 2004). In our study GABA receptor binding and gene expression were decreased in the epileptic rats. It is found that $GABA_A$ receptor blockade, not only increased neuronal spiking in the striatum, as was expected in light of the earlier studies in rodents, but also induced the development of striking changes in striatal local field potentials (LFPs) and in the simultaneously recorded motor cortical EEG activity (Olivier & Thomas, 2008). Studies demonstrated that ambient GABA in the monkey striatum strongly inhibits firing activity of striatal neurons and limits synchronous striatal and cortical activity. GABAergic inputs to striatal interneurons also originate from several sources and GABA receptor blockade at these sites, counteracting an ambient GABAergic tone that contributed to the changes in neuronal spiking. Cholinergic interneurons receive GABAergic input predominately from Medium Spiny Neurons collaterals (Martone *et al.*, 1992). It is suggested that GABAergic inhibition in the striatum is one of the mechanisms that normally prevent excessive movements (Marsden *et al.*, 1975; Crossman *et al.*, 1988; Nakamura *et al.*, 1990; Yoshida *et al.*, 1991). It is possible that reduced inhibition in the striatum contribute to movement disorders such as some forms of dystonia (Fredow & Loscher, 1991; Loscher & Horstermann, 1992; Nobrega *et al.*, 1995; Kreil & Richter, 2005) or myoclonus (Darbin *et al.*, 2000). GABAergic

inhibition in the striatum also act to inhibit seizure generation (Dematteis *et al.*, 2003) or propagation (Sasaki *et al.*, 2000; Benedek *et al.*, 2004; Biraben *et al.*, 2004; Bouilleret *et al.*, 2005). Modulations of striatal GABA levels therefore represent a pharmacological target of interest in the treatment of movement disorders and seizures. Our results showed decreased GABA receptors in the striatum of the epileptic rats. This decreased GABA receptors facilitate the rapid spread of seizure originate in the temporal lobe to different part of the brain which leads to seizure generation. Evidence has accumulated that GABA is involved in the regulation of other neuronal systems, such as dopaminergic (Dray, 1979; O'Neill, 1986) and cholinergic (Ferkany & Enna, 1980; Scatton & Bartholini, 1982) neurons. Local injection of GABA agonists into the midbrain provided seizure protection without a widespread augmentation of GABA-mediated activity throughout the brain and without impairing either alertness or motor function. Synapses in the striatum appear to represent an important control mechanism for inhibiting the propagation of generalized convulsions (Iadarola & Gale, 1982). *Bacopa monnieri* and Bacoside A treatment were found to enhance the GABA receptor binding and gene expression in the striatum, giving a protection against the spreading of epileptic seizure through this root. *Bacopa monnieri* extract appeared to promote cell survival compared to neuronal cells growing in regular culture medium. Further study showed that *Bacopa monnieri* treated neurons expressed lower level of reactive oxygen species suggesting that *Bacopa monnieri* restrained intracellular oxidative stress which in turn prolonged the lifespan of the culture neurons. *Bacopa monnieri* extract also exhibited both reducing and lipid peroxidation inhibitory activities (Limpeanchob *et al.*, 2008). The data indicated that *Bacopa monnieri* has potential to modulate the activities of heat shock protein, Hsp70, P450 and SOD thereby possibly allowing the brain to be prepared to act under adverse conditions such as stress (Kar *et al.*, 2002).

Our results suggest that decreased GABA receptors in the striatum encompass an important role in the spreading of seizure through the striatum during epilepsy. We

conclude from our studies that *Bacopa monnieri* and Bacoside A treatment potentiates a therapeutic effect against epilepsy by reversing the alterations in general GABA, $GABA_A$ receptor binding and $GABA_A$ receptor subunits gene expression that occur during epilepsy, resulting an increased GABA mediated inhibition in the over stimulated striatal neurons.

GABA RECEPTOR ALTERATIONS IN THE BRAIN STEM

Neurological functions located in the brainstem include those necessary for survival. The brainstem is the pathway for all fiber tracts passing up and down from peripheral nerves and spinal cord to the highest parts of the brain. GABA is a major inhibitory neurotransmitter in the mammalian brain stem. In our study GABA content was significantly decreased in the brain stem of the epileptic rats compared to control. Scatchard analysis of [^{3}H]GABA, [^{3}H]bicuculline and [^{3}H]baclofen in the brain stem of the epileptic rat showed significant decrease in B_{max} ($P<0.001$) compared to control. Real Time PCR amplification of $GABA_A$ receptor subunits such as $GABA_{A\alpha 1}$, $GABA_{A\alpha 5}$ and $GABA_{A\delta}$ were down regulated in the brain stem of the epileptic rats compared to control. $GABA_B$ and GAD were also down regulated in the epileptic rats. Confocal imaging study confirmed the decreased $GABA_A$ receptor in the epileptic rats. *Bacopa monnieri* and Bacoside A treatment reversed these changes near to control.

Synaptically released GABA activates postsynaptic $GABA_A$ receptors, which increase the membrane permeability to chloride, evoking a hyperpolarizing inhibitory postsynaptic current (IPSC). Synaptic release increases the concentration of GABA to a relatively high level (millimolar range) within the synapse (Farrant & Nusser, 2005). The brief current evoked by synaptic release of GABA from presynaptic terminals is referred to as "phasic" inhibition. In monkeys made epileptic by cortical application of alumina gel, a highly significant numerical decrease of GAD-positive nerve terminals occurred at sites of seizure foci indicating a functional loss of GABAergic inhibitory synapses (Ribak *et al.*, 1979). Loss of such inhibition at seizure foci could lead to

epileptic activity of cortical pyramidal neurons. Long-term $GABA_A$ receptor alterations occur in hippocampal dentate granule neurons of rats that develop epilepsy after status epilepticus in adulthood (Zhang *et al.*, 2004). Abnormalities of GABAergic function have been observed in genetic and acquired animal models of epilepsy. Reductions of GABA mediated inhibition and decreased activity of glutamate decarboxylase has been reported in studies of human epileptic brain tissue. Our study showed that GABA receptors were decreased in the brain stem of the epileptic rats and treatment using *Bacopa monnieri* and Bacoside A increased the GABA receptor density providing an antiepileptic effect.

Thus our results support that decreased GABA receptors and GAD activity in the brain stem comprise an important role in the TLE seizures. We concluded that *Bacopa monnieri* and Bacoside A treatment giving a beneficial effect by reversing the alterations in general GABA, $GABA_A$, $GABA_B$ receptor binding, $GABA_A$ receptor subunits and GAD gene expression.

DIFFERENTIAL REGULATION OF $GABA_A$ SUBUNITS IN THE BRAIN REGIONS

We have investigated the gene expression of $GABA_A$ receptor subunits such as $GABA_{A\alpha 1}$, $GABA_{A\alpha 5}$, $GABA_{A\gamma}$ and $GABA_{A\delta}$ in the cerebral cortex, hippocampus, cerebellum, corpus striatum and brain stem of control and experimental rats. $GABA_{A\alpha 5}$ receptor subunit in the cerebral cortex and corpus striatum, $GABA_{A\delta}$ receptor subunit in the hippocampus, $GABA_{A\gamma}$ receptor subunit in the cerebellum and brain stem were up regulated in the epileptic rats compared to control. Other receptor subunits were down regulated in the brain regions of the epileptic rats compared to control. This showed the differential expression of $GABA_A$ receptor subunits in the brain regions of the epileptic rats.

Differential expression of $GABA_A$ receptor subunit is a common phenomenon in epileptic condition (Paolo et al., 1999; Porter et al., 2005) and they represent a

major aspect of homeostatic synaptic plasticity and contribute to the excitation/inhibition (E/I) balance under physiological conditions and upon pathological challenges (Fritschy, 2008). The kinetics and pharmacological properties of $GABA_A$ receptors depend on subunit composition (Fritschy, 2008) and where subunits are assembled on plasma membranes of neurons. In the CNS, a functional $GABA_A$ receptor requires a combination of two α, two β and one γ subunit, but the γ subunit can be replaced by δ, ε, or π (Gilby *et al.*, 2005; Nishimura *et al.*, 2005). Inclusion of specific subunits can affect not only channel kinetics but also localization of $GABA_A$ receptor. $GABA_A$ receptor, in the combination α6β2/3δ are located on nonsynaptic plasma membranes, have high affinity for GABA, do not desensitize in the presence of GABA and contribute mainly to tonic inhibition of neurons. α1β2/3γ2 receptors have low affinity for GABA, show more pronounced desensitization to GABA and are concentrated at synaptic junctions where they mediate strong, phasic inhibition, because a high concentration of GABA is present only briefly (Jean-Marc, 2008).

Alterations in $GABA_A$ receptor subunit expression and composition in epilepsy are well documented in human (Loup *et al.*, 2000, 2006) and in animal models (Peng *et al.*, 2004; Gilby *et al.*, 2005; Nishimura *et al.*, 2005; Roberts *et al.*, 2005; Li *et al.*, 2006).The changes in $GABA_A$ receptor subunit contribute to the changes in inhibitory function that underlie epileptogenesis and occurrence of chronic recurrent seizures (Jean-Marc, 2008). In the mouse pilocarpine model of TLE, a profound decrease in δ subunit immunoreactivity was observed, correlating with a redistribution of the γ2 subunit from synaptic to perisynaptic sites, where it assembled with the α4 subunit, which is normally associated with the δ subunit (Zhang *et al.*, 2007). A down-regulation of the α5 subunit also occurs in CA1 pyramidal cells of pilocarpine-treated rats (Houser & Esclapez, 2003), resulting in a loss of diazepam-sensitive tonic inhibition seen upon blockade of GABA reuptake (Jean-Marc, 2008). Despite this change, tonic inhibition is enhanced in pyramidal cells, suggesting

compensatory up regulation of other extrasynaptic $GABA_A$ receptors, possibly containing the α4 subunit (Jean-Marc, 2008). These studies showed that during epilepsy the subunits of $GABA_A$ receptors showed different subunit combination with varying inhibitory potential. Our study showed that differential expression of $GABA_A$ receptor subunits in the brain regions of epileptic rats plays an important role in the excitation/inhibition balance and the affinity to the GABA.

BEHAVIOURAL DEFICITS IN EPILEPTIC RATS

We have evaluated the behavioural response of epileptic rats in Radial arm maze, Y-maze and memory enhancing property of *Bacopa monnieri* and Bacoside A (Katz & Chudler, 1980; Woo *et al.*, 2008). Also, we have investigated the performance of control and experimental rats on narrow beam test, grid walk test and open field test. Radial arm maze is a tool to examine the neural influence of pharmacological compounds on memory (Indre *et al.*, 2000; Caterina *et al.*, 2003). Y-maze is used to evaluate the spatial learning in different rat models (Murugesan, 2005). The performance of epileptic rats in the radial arm maze and Y-maze were impaired. The number of trials to attain five consecutive criterion performances increased significantly in the epileptic rats. Increased numbers of trials to criterion performance was indicated the learning and memory deficit in epileptic rats. Treatment using *Bacopa monnieri* and Bacoside A considerably reduced the number of trials to attain the criterion performance. Lower percentage of arm visits between the novel arm and the start arm and decreased time spend in the novel arm compared to the other two arm within the epileptic rats showed their decreased exploratory behaviour which have considerable role in the motor learning (Jobin *et al.*, 2010a). Also, in open field behavioural test epileptic rats showed increased resting behaviour and decreased sniffing behaviour. This is related to the decreased GABA receptors in the brain regions which plays an important role in the motor learning (Yuji *et al.*, 2007). Time spent in the novel arm and number of visit to the novel arm was

increased in the epileptic rats treated with *Bacopa monnieri* and Bacoside A. Epileptic rats showed increased number of foot slips in grid walk test and decreased time spent in narrow beam test compared to control. This indicated the motor dysfunction in the epileptic rats. Moreover *Bacopa monnieri* and Bacoside A treatment improved the motor performance of the epileptic rats (Jobin *et al.*, 2010a).

Hippocampal theta rhythm contributes to learning and memory by promoting plasticity at excitatory synapses. IPSCs have been shown to be depressed during the expression of excitatory LTP in CA1 pyramidal cells (Stelzer *et al.*, 1994) and this LTD of GABAergic inhibition underlie the EPSP-to-spike coupling associated with LTP (Lu *et al.*, 2000). Studies showed that theta-patterned activity also induces plasticity at GABAergic synapses. Physiological relevance of LTP at GABA synapses is an activity-dependent regulation of excitability of pyramidal cells. In addition, since inhibitory interneurons contribute to pacing of theta oscillations in the hippocampus (Chapman, 1999a, b) theta-dependent strengthening of GABA synapses serve to reinforce their role in hippocampal theta activity. Finally, rhythmic activation of excitatory and inhibitory inputs to pyramidal cells appears necessary to cooperatively promote plasticity of GABA synapses. Such cooperative induction of plasticity at inhibitory synapses provide a novel mechanism through which hippocampal excitability is modulated adaptively in an activity-dependent manner and thus participate in the processes mediating learning and memory. Long-term potentiation is a widely accepted model of synaptic plasticity that is thought to underlie in learning and memory processes (Bliss & Collingridge, 1993; Martin & Morris, 2002).

Long term depression of excitatory transmission at cerebellar parallel fibre Purkinje cell synapses is a form of synaptic plasticity crucial for cerebellar motor learning (Ito, 2002; Jean *et al.*, 2002). The efficacy of transmission at certain parallel fibre Purkinje cell synapses undergoes LTD following correlated transmission at these synapses and climbing fibre–Purkinje cell synapses (Ito, 2002). Purkinje cells express a high density of $GABA_B$ receptor, a Gi/o protein coupled receptor for the inhibitory

neurotransmitter GABA (Jones *et al.*, 1998; Kaupmann *et al.*, 1998; Kuner *et al.*, 1999). Interestingly, $GABA_B$ receptors are concentrated on the annuli of parallel fibre-innervated dendritic spines (Ige *et al.*, 2000; Kulik *et al.*, 2002) where LTD occurs. When the neighbouring interneurons are stimulated at a relatively high frequency, $GABA_B$ receptors in the dendritic spines receive 5–10 μm GABA spilt over from the synapses of the interneurons (Dittman & Regehr, 1997). $GABA_B$ receptors have enough high affinity to sense this level of GABA (Sodickson & Bean, 1996). $GABA_B$ receptors activated by spilt-over GABA could possibly influence the signalling of mGluR1, a Gq/11 protein-coupled receptor, because these receptors colocalize at the annuli of Purkinje cell dendritic spines (Kulik *et al.*, 2002; Lujan *et al.*, 2007) and are likely to form complexes (Tabata et al., 2004). mGluR1 signalling is an essential factor for inducing LTD (Conquet *et al.*, 1994; Shigemoto *et al.*, 1994; Ichise *et al.*, 2000; Ito, 2002); this signalling leads to the facilitated endocytosis of AMPA receptor at parallel fibre–Purkinje cell synapses (Matsuda *et al.*, 2000; Wang & Linden, 2000; Chung *et al.*, 2003). Therefore, possible influence of $GABA_B$ receptor on mGluR1 could modulate the induction of LTD.

The administration of a crude extract of *Bacopa monnieri* to epileptic rats decreased the trial to attain the criterion performance in the radial arm maze to control levels. These results indicated the memory enhancing property of *Bacopa monnieri* in epileptic rats. It is also reported to facilitate the acquisition, consolidation, retention and recall of learned tasks (Glosser *et al.*, 1997) and improve the speed at which visual information is processed. *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 neuroprotective action (Basu & Lamsal, 1947; Sairam, *et al.*, 2002). Preliminary studies established that the treatment with crude extract and with the alcoholic extract of *Bacopa monnieri* plant (Singh *et al.*, 1988) enhanced learning ability in rats. Nigel *et al.*, (2003) have suggested shared neurobiological processes leading both to seizures and to

behavioural, emotional and cognitive disturbance which could possibly explain how *Bacopa monnieri* is effective as an anti-convulsant and an anti-depressant. It is reported that the isolated Bacosides A and B were effective in enhancing memory in rats in learning tasks involving both positive and negative reinforcement (Singh & Dhawan, 1997; Singh, *et al.*, 1988). Additionally Bacosides produced changes in the hippocampus, cerebral cortex (areas critical to memory function) and hypothalamus regions of the brain and caused enhanced levels of protein kinase activity and increases in protein levels in these regions. This indicated positive implications for improved neurotransmission and repair of damaged neurons *via* enhanced regeneration of nerve synapses (Singh & Dhawan, 1997).

Previous studies showed decreased rotarod performance which demonstrated the impairment in the motor function and coordination in the epileptic rats, suggesting impairment in their ability to integrate sensory input with appropriate motor commands to balance their posture and at the same time adjust their limb movements on the metallic rod and is indicative of cerebellar dysfunction (Blumer *et al.*, 2004; Amee *et al.*, 2009). Treatment using *Bacopa monnieri* and its active component Bacoside A improving cerebellar function (Amee *et al.*, 2009). Our study showed impaired performance of epileptic rats in radial arm maze, Y-maze, narrow beam test, grid walk test and open field behavioural response demonstrated decreased motor learning and memory. This is correlated to the decreased GABA receptor mediated long term potentiation in the brain regions. Long term potentiation plays an important role in motor learning and memory. The GABA receptor analysis and gene expression along with the behavioural studies implicated a role for these receptors in the modulation of neuronal network excitability and behaviour. Thus it is suggested that decreased GABA receptor in the brain regions plays an important role in the motor and memory deficit and the GABA receptor enhancing property of *Bacopa monnieri* and Bacoside A ameliorate this abnormalities.

Thus our results suggest that GABAergic system is impaired during epilepsy. We observed a decreased GABA content, GABA receptor binding and gene expression in the brain regions of the epileptic rats which showed the deficit in GABA mediated inhibition. Decreased GABA receptor functional regulation leads to seizures in epileptic rats. Epileptic rats show impairment in the behavioral tests, for assessing the motor learning and memory. Treatment using *Bacopa monnieri* and bacoside A decreased the seizure frequency in the epileptic rats. *Bacopa monnieri* and bacoside A increased the GABA content, GABA receptor binding and gene expression. Increased GABA receptors in the brain regions is suggested to prevent the occurrence of seizures in epileptic rats. The memory enhancing property of *Bacopa monnieri* and bacoside A is suggested to be acting through GABA receptors.

Summary

1) Pilocarpine induced temporal lobe epileptic rats were used as a model to study the alterations of total GABA, $GABA_A$ subunits and $GABA_B$ receptor and their functional regulation by *Bacopa monnieri* and Bacoside A.

2) Antiepileptic activity of whole plant extract of *Bacopa monnieri*, Bacoside A and Carbamazepine were evaluated for seizure frequency over 72 hours video recording.

3) Acetylcholine esterase activity has been used as a marker for cholinergic activity. In epileptic rats the acetylcholine esterase activity was increased in the muscle and decreased in the heart. *Bacopa monnieri*, Bacoside A and Carbamazepine treatment reversed activity of acetylcholine esterase to near control.

4) Malate dehydrogenase activity in the muscle, circulating insulin and T3 level in the serum were measured to analyze the metabolic changes due to recurring seizures in epileptic rats. These were increased in the epileptic rats. *Bacopa monnieri*, Bacoside A and Carbamazepine treatment reversed these changes to near control.

5) GABA content was decreased in the cerebral cortex, hippocampus, cerebellum, corpus striatum and brainstem. Treatment with, *Bacopa monnieri*, Bacoside A and Carbamazepine reversed GABA content to near control.

6) GABA receptor functional status was analyzed by Scatchard analysis using [^{3}H]GABA. During epilepsy total GABA receptor binding was decreased in cerebral cortex, hippocampus, cerebellum, corpus striatum and brainstem. Treatment with *Bacopa monnieri*, Bacoside A and Carbamazepine reversed the receptors to near control.

7) $GABA_A$ receptor functional status was analyzed by Scatchard analysis using [^{3}H]bicuculline. During epilepsy $GABA_A$ receptors binding was decreased in cerebral cortex, hippocampus, cerebellum, corpus striatum and brainstem. Treatment with *Bacopa monnieri*, Bacoside A and Carbamazepine reversed the receptors to near control.

8) $GABA_B$ receptor functional status was analyzed by Scatchard analysis using [^{3}H]baclofen. During epilepsy $GABA_B$ receptors binding was decreased in cerebral cortex, hippocampus, cerebellum, corpus striatum and brainstem. Treatment with *Bacopa monnieri*, Bacoside A and Carbamazepine reversed the receptors to near control.

9) Real Time PCR studies using the mRNA of $GABA_A$ subunits, $GABA_B$ receptors and GAD were used to confirm the receptor binding studies. In cerebral cortex and corpus striatum $GABA_A$ receptor subunits $GABA_{A\alpha 1}$, $GABA_{A\gamma 3}$ and $GABA_{A\delta}$, were down regulated; $GABA_{A\alpha 5}$ receptor subunit was up regulated and $GABA_B$ receptor and GAD were down regulated in epileptic rats. *Bacopa monnieri*, Bacoside A and Carbamazepine treatment to epileptic rats reversed these changes to near control.

10) Real Time PCR studies in the hippocampus showed that the $GABA_A$ receptor subunits $GABA_{A\alpha 1}$, $GABA_{A\alpha 5}$ and $GABA_{A\gamma 3}$ were down regulated; $GABA_{A\delta}$ receptor subunit was up regulated; $GABA_B$ receptor and GAD were down regulated in epileptic rats. *Bacopa monnieri*, Bacoside A and Carbamazepine treatment to epileptic rats reversed these changes to near control.

11) Real Time PCR studies in the cerebellum and brain stem showed that the $GABA_A$ receptor subunits $GABA_{A\alpha 1}$, $GABA_{A\alpha 5}$ and $GABA_{A\delta}$, were down regulated; $GABA_{A\gamma 3}$ receptor subunit was up regulated; $GABA_B$ receptor and GAD were down regulated in epileptic rats. *Bacopa monnieri*, Bacoside A and Carbamazepine treatment to epileptic rats reversed the changes to near control.

12) Behavioural tests were done using Y-maze, radial arm maze, grid walk test, narrow beam test and open field test to assess the motor learning and memory in epileptic rats. Epileptic rats showed impaired behavioral response. There was deficit in cognition, memory and motor learning in epileptic rats. *Bacopa monnieri*, Bacoside A and Carbamazepine treatment to epileptic rats reversed the behavioural performance to near control.

13) Confocal study of the $GABA_{A\alpha 1}$ in cerebral cortex, hippocampus, cerebellum, corpus striatum and brain stem of the epileptic rats showed decreased expression. *Bacopa monnieri*, Bacoside A and Carbamazepine treatment to epileptic rats reversed the changes to near control.

Thus from our results, we conclude that GABA receptor functional balance play an important role in the pathophysiology of temporal lobe epilepsy. Decreased GABA receptors status in the brain regions has an important role in motor learning and memory deficit of pilocarpine induced temporal lobe epilepsy. *Bacopa monnieri* and Bacoside A treatment to epileptic rats was effective in reversing the GABA receptor functional regulation to near control. Our results suggested that motor function and cognition enhancing property of *Bacopa monnieri* and Bacoside A in epileptic rats mediated through GABA receptors. This has immense clinical significance in the therapeutic management of epilepsy.

Conclusion

GABAergic system has an important regulatory role in the pathophysiology of pilocarpine induced temporal lobe epilepsy in rats. Acetylcholine esterase activity was increased in the muscle and decreased in the heart of epileptic rats. Serum insulin and T3 were increased in epileptic rats. Increase in malate dehydrogenase activity in the muscle showed increased excitability and metabolism in epileptic rats due to repetitive seizures. Administration of *Bacopa monnieri* and Bacoside A decreased the seizure frequency of the epileptic rats and improved the peripheral functional status. GABA content was decreased in cerebral cortex, hippocampus, cerebellum, corpus striatum and brain stem of the epileptic rats. Receptor binding studies showed decreased total GABA, $GABA_A$ and $GABA_B$ receptors in the brain regions of the epileptic rats. Real Time PCR amplification of the $GABA_A$ receptor subunits and confocal study of the $GABA_{A\alpha 1}$ in cerebral cortex, hippocampus, cerebellum, corpus striatum and brain stem of the epileptic rats confirmed the receptor data. Treatment using *Bacopa monnieri* and Bacoside A reversed the decreased GABA binding in the epileptic rats to near control reducing the seizure occurrence. Behavioural studies by radial arm maze test, Y maze test, grid walk test, narrow beam test and open field test showed deficit in spatial learning, motor control and memory. *Bacopa monnieri* and Bacoside A administration improved the behavioural performance of the epileptic rats. GABA receptor subtypes kinetic parameters and their gene expression along with behavioural studies showed their functional role in motor learning, cognition and memory. Thus, we conclude from our studies that GABA receptor subtypes have a functional regulatory role in epileptic seizures. *Bacopa monnieri* and Bacoside A treatment to epileptic rats showed functional reversal of GABA receptors and the metabolism in the management of epilepsy.

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Abstract Presented

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Table-1
Mean seizure frequency per 4 hours interval over the 72 hours video recording period of control and experimental rats

Animal status	Mean seizure frequency/4 hourse
Control	0
Epileptic	0.34 ± 0.04**
Epileptic + *Bacopa monnieri*	0.14 ± 0.03@@*
Epileptic + Bacoside A	0.13 ±0.01@@*
Epileptic + Carbamazepine	0.11 ± 0.03@@*

Values are mean ± SEM of 4-6 separate experiments. Each group consists of 6-8 rats **p<0.01, *p<0.05 when compared to control, @@p<0.01 when compared to epileptic group.

Table-2
Acetylcholine esterase activity in the muscle of control and experimental rats

Animal status	Vmax (µmoles/min/mg protein)	Km (µM)
Control	0.81 ± 0.03	0.025 ± 0.02
Epileptic	1.29 ± 0.05**	0.023 ± 0.025
Epileptic + *Bacopa monnieri*	1.01 ± 0.09@@*	0.022 ± 0.015
Epileptic + Bacoside A	0.98 ±0.04 @@	0.025 ± 0.02
Epileptic + Carbamazepine	0.96 ± 0.06@@	0.026 ± 0.018

Values are mean ± SEM of 4-6 separate experiments. Each group consists of 6-8 rats. **p<0.01, *p<0.05 when compared to control, @@p<0.01 when compared to epileptic group.

Table-3
Acetylcholine esterase activity in the heart of control and experimental rats

Animal status	Vmax (μmoles/min/mg protein)	Km (μM)
Control	2.01 ± 0.05	1.34 ± 0.07
Epileptic	1.53 ± 0.05**	1.23 ± 0.065
Epileptic + *Bacopa monnieri*	1.85 ± 0.04$^{@@}$	1.25 ± 0.025
Epileptic + Bacoside A	1.98 ±0.07 $^{@@}$	1.45 ± 0.062$^{@}$
Epileptic + Carbamazepine	1.76 ± 0.02$^{@@}$*	1.16 ± 0.078

Values are mean ± SEM of 4-6 separate experiments. Each group consists of 6-8 rats. **p <0.01, *p<0.05 when compared to control, $^{@@}$p<0.01, $^{@}$p<0.015 when compared to epileptic group.

Table-4
Malate dehydrogenase activity in the muscle of control and experimental rats

Animal status	Vmax (μmoles/min/mgprotein)	Km (μM)
Control	28.7 ± 0.03	0.12 ± 0.020
Epileptic	39.3 ± 0.05**	0.06 ± 0.025**
Epileptic + *Bacopa monnieri*	30.9 ± 0.09$^{@@}$	0.13 ± 0.015$^{@@}$
Epileptic + Bacoside A	32.5 ±0.04 $^{@@}$	0.10 ± 0.02$^{@}$
Epileptic + Carbamazepine	34.4 ± 0.06$^{@@}$	0.08 ± 0.018

Values are mean ± SEM of 4-6 separate experiments. Each group consists of 6-8 rats. **p <0.01 when compared to control, $^{@@}$p<0.01, $^{@}$p<0.05 when compared to epileptic group.

Figure-1
Seizure frequency per 4 hours interval over the 72 hours video recording period of epileptic rats

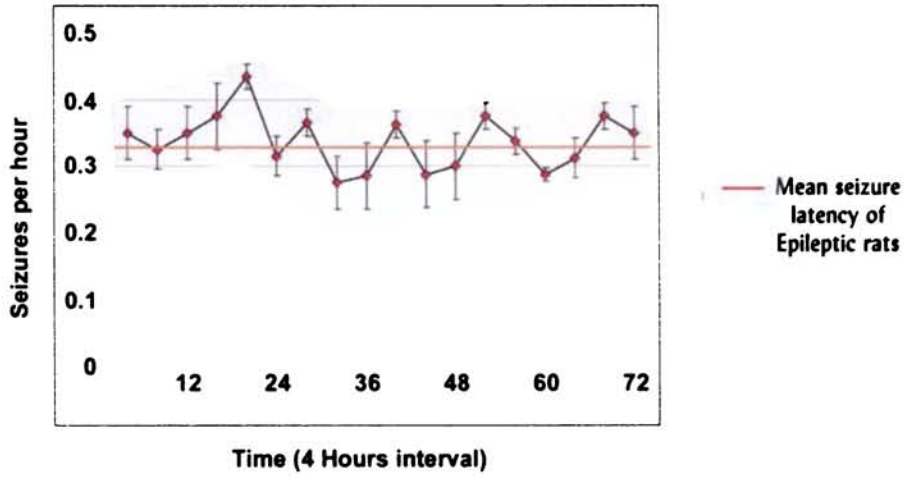


Figure-2
Seizure frequency per 4 hours interval over the 72 hours video recording period of epileptic rats treated with *Bacopa monnieri*

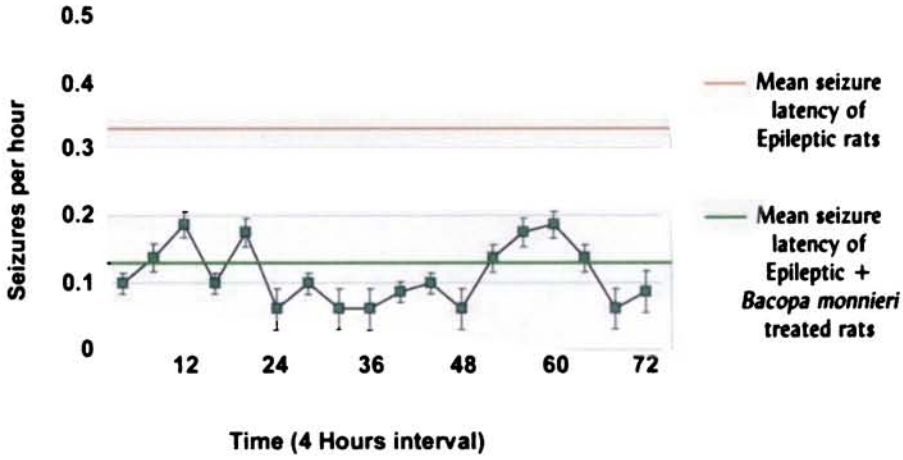


Figure-3
Seizure frequency per 4 hours interval over the 72 hours video recording period of epileptic rats treated with Bacoside A

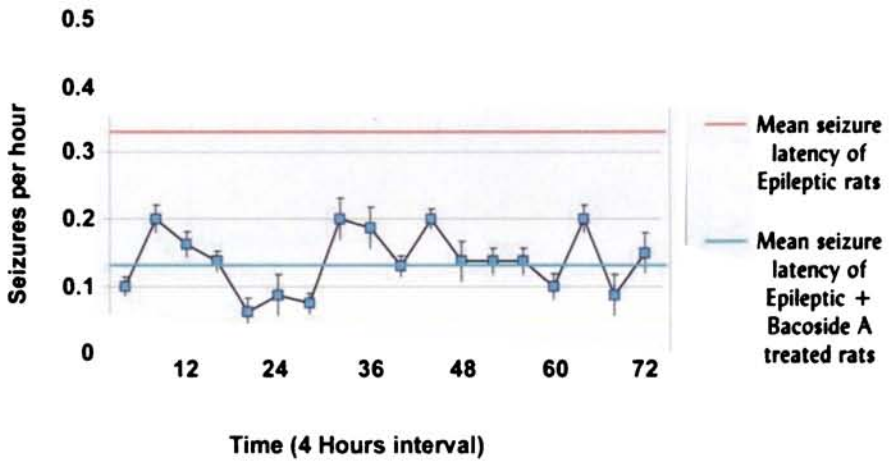


Figure-4
Seizure frequency per 4 hours interval over the 72 hours video recording period of epileptic rats treated with carbamazepine

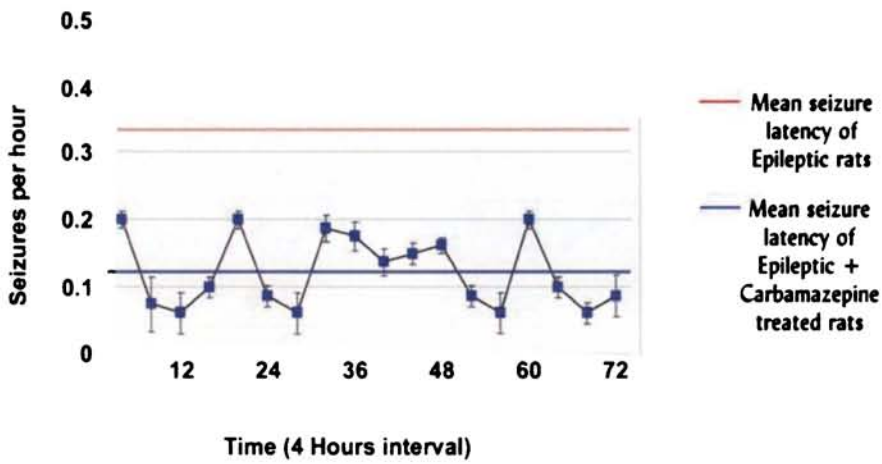
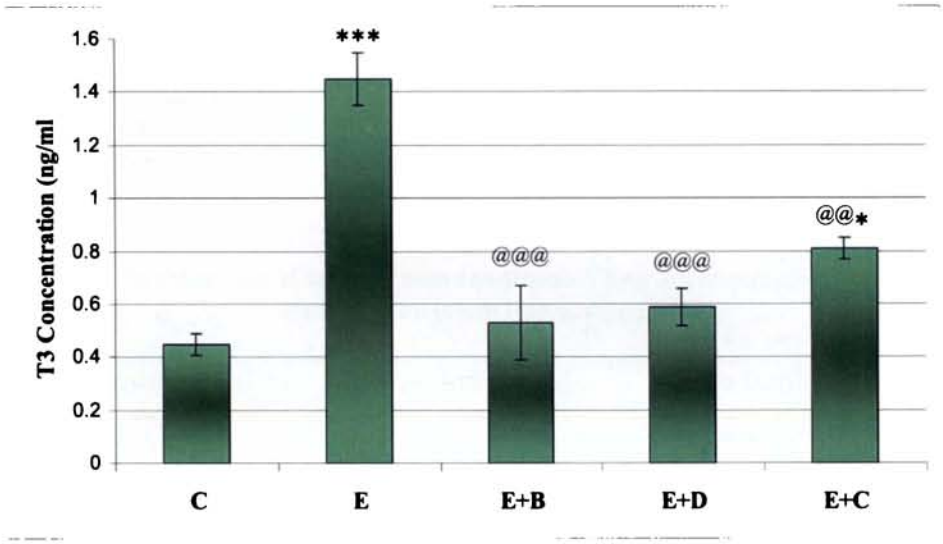


Table-5
Epinephrine and Norepinephrine content in the heart of control and experimental rats

Animal status	Epinephrine (nmoles/g wet wt)	Norepinephrine (nmoles/g wet wt)
Control	19.54 ± 0.13	13..34 ± 0.77
Epileptic	18.37 ± 0.51	12.23 ± 0.65
Epileptic + *Bacopa monnieri*	17.85 ± 0.43	11.25 ± 0.25
Epileptic + Bacoside A	20.98 ±0.70	14.45 ± 0.62
Epileptic + Carbamazepine	18.76 ± 0.25	12.16 ± 0.78

Values are mean ± SEM of 4-6 separate experiments. Each group consists of 6-8 rats.

Figure-5
Triiodothyronine (T3) content in the serum of control and experimental rats



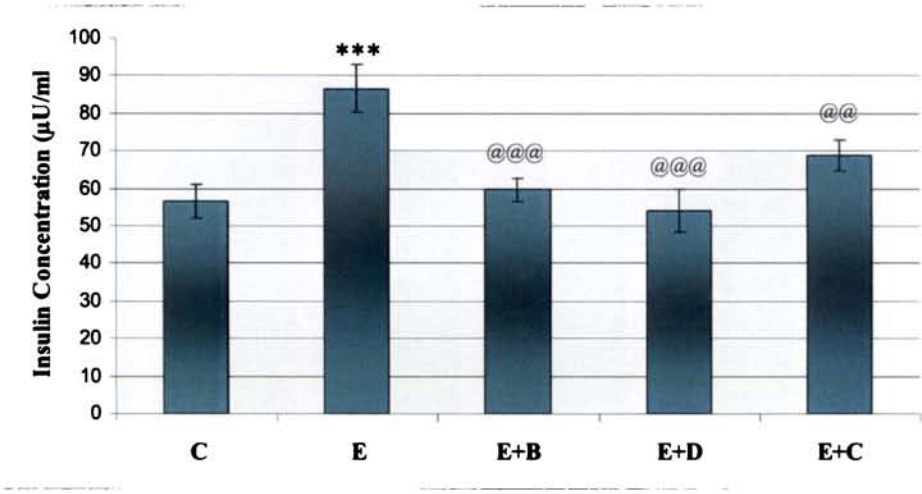
C-Control, E-Epileptic, E+B-Epileptic rats treated with *Bacopa monnieri,* E+D-Epileptic rats treated with Bacoside A and E+C-Epileptic rats treated with Carbamazepine.

Table-6
Triiodothyronine (T3) content in the serum of control and experimental rats

Animal status	T3 Concentration (ng/ml)
Control	0.45 ± 0.04
Epileptic	1.42 ± 0.10***
Epileptic + *Bacopa monnieri*	0.53 ± 0.14@@@
Epileptic + Bacoside A	0.59 ± 0.07 @@@
Epileptic + Carbamazepine	0.81 ± 0.02@@*

Values are mean ± SEM of 4-6 separate experiments. Each group consist of 6-8 rats ***p<0.001 when compared to control, @@@p<0.001, @@p<0.01 when compared to epileptic group.

Figure-6
Circulating insulin level in the serum of control and experimental rats



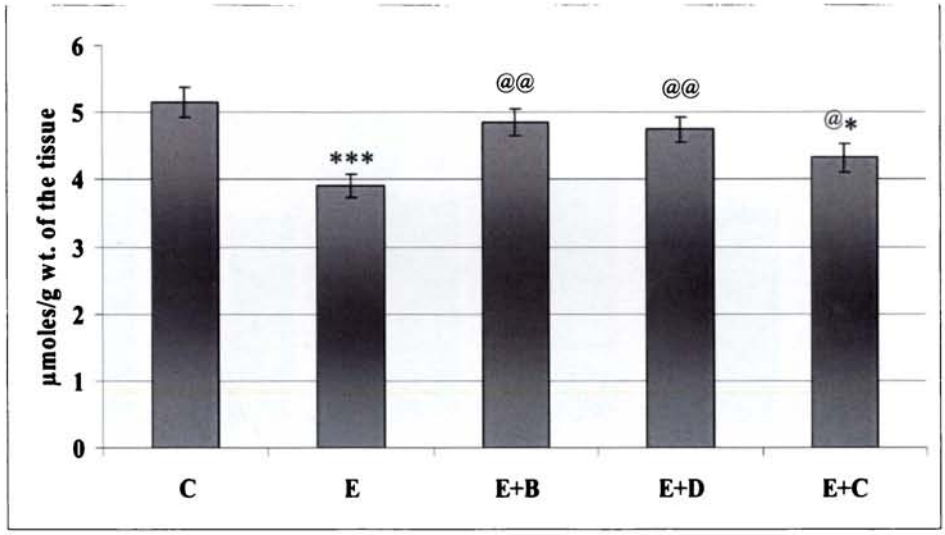
C-Control, E-Epileptic, E+B-Epileptic rats treated with *Bacopa monnieri*, E+D-Epileptic rats treated with Bacoside A and E+C-Epileptic rats treated with Carbamazepine.

Table-7
Insulin content in the serum of control and experimental rats

Animal status	Insulin Concentration (µU/ml)
Control	56.4 ± 4.5
Epileptic	86.6 ± 6.4***
Epileptic + *Bacopa monnieri*	59.7 ± 3.1$^{@@@}$
Epileptic + Bacoside A	54.2 ± 5.8 $^{@@@}$
Epileptic + Carbamazepine	68.9 ± 4.2$^{@@}$

Values are mean ± SEM of 4-6 separate experiments. Each group consist of 6-8 rats ***$p<0.001$ when compared to control, $^{@@@}p<0.001$, $^{@@}p<0.01$ when compared to epileptic group.

Figure-7
GABA content in the cerebral cortex of control and experimental rats



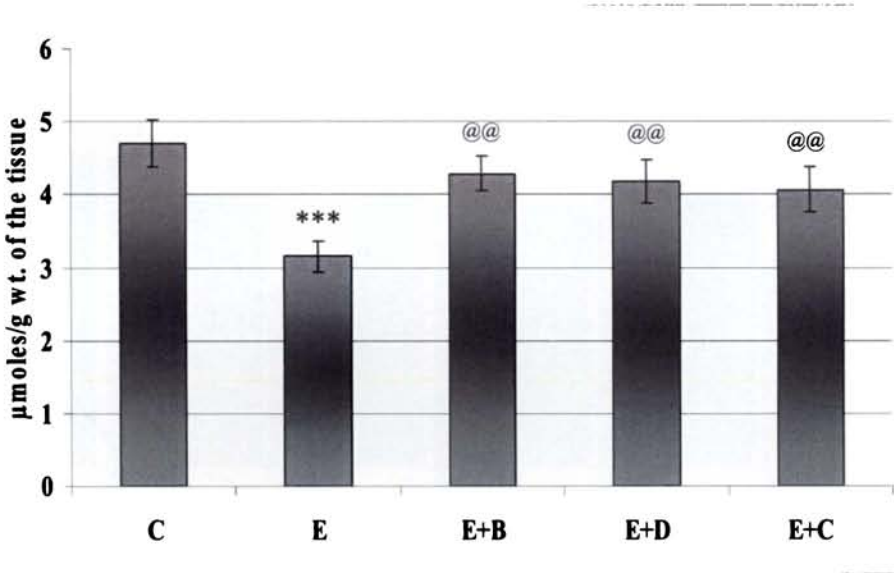
C-Control, E-Epileptic, E+B-Epileptic rats treated with *Bacopa monnieri,* E+D-Epileptic rats treated with Bacoside A and E+C-Epileptic rats treated with Carbamazepine.

Table-8
GABA content in the cerebral cortex of control and experimental rats

Animal status	GABA content (µmoles/g wt. of the tissue)
Control	5.16 ± 0.23
Epileptic	3.91 ± 0.17***
Epileptic + *Bacopa monnieri*	4.86 ± 0.20@@
Epileptic + Bacoside A	4.75 ± 0.19@@
Epileptic + Carbamazepine	4.32 ± 0.21@*

Values are mean ± SEM of 4-6 separate experiments. Each group consist of 6-8 rats ***$p<0.001$ when compared to control, @@<0.001, @$p<0.05$ when compared to epileptic group.

Figure-8
GABA content in the hippocampus of control and experimental rats



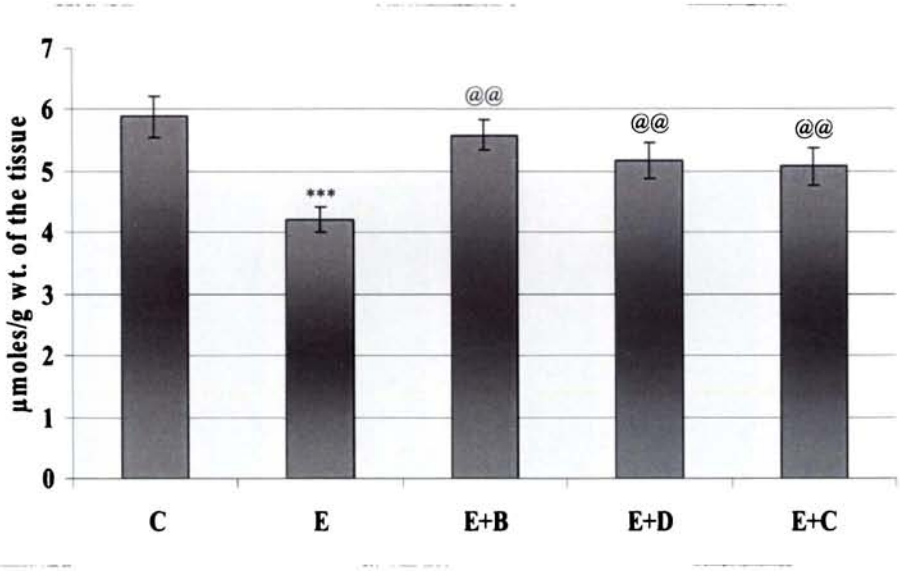
C-Control, E-Epileptic, E+B-Epileptic rats treated with *Bacopa monnieri,* E+D-Epileptic rats treated with Bacoside A and E+C-Epileptic rats treated with Carbamazepine.

Table-9
GABA content in the hippocampus of control and experimental rats

Animal status	GABA content (µmoles/g wt. of the tissue)
Control	4.71 ± 0.33
Epileptic	3.10 ± 0.21***
Epileptic + *Bacopa monnieri*	4.29 ± 0.24@@
Epileptic + Bacoside A	4.18 ± 0.29@@
Epileptic + Carbamazepine	4.07 ± 0.31@@

Values are mean ± SEM of 4-6 separate experiments. Each group consist of 6-8 rats ***p<0.001 when compared to control and @@p<0.001 when compared to epileptic group.

Figure-9
GABA content in the cerebellum of control and experimental rats



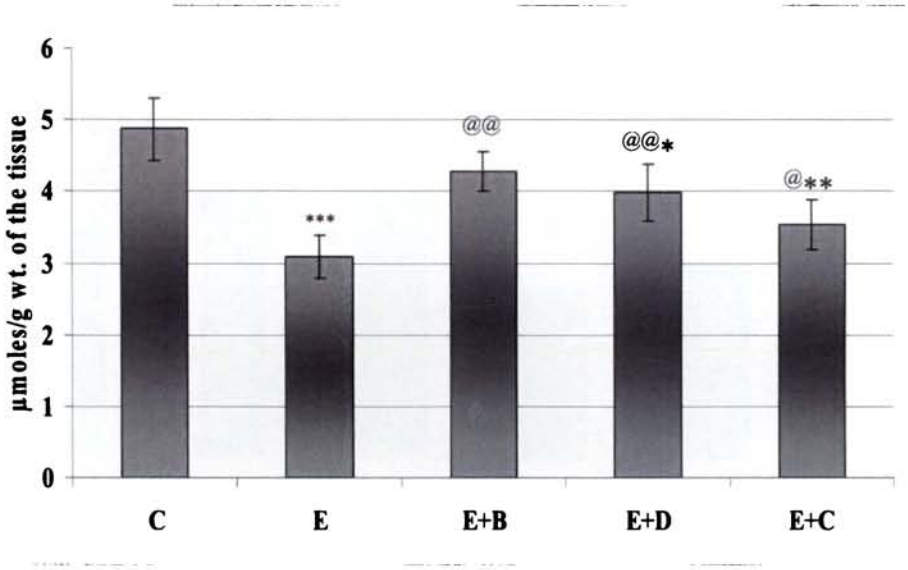
C-Control, E-Epileptic, E+B-Epileptic rats treated with *Bacopa monnieri,* E+D-Epileptic rats treated with Bacoside A and E+C-Epileptic rats treated with Carbamazepine.

Table-10
GABA content in the cerebellum of control and experimental rats

Animal status	GABA content (µmoles/g wt. of the tissue)
Control	5.89 ± 0.34
Epileptic	4.21 ± 0.18***
Epileptic + *Bacopa monnieri*	5.59 ± 0.23$^{@@}$
Epileptic + Bacoside A	5.17 ± 0.25$^{@@}$
Epileptic + Carbamazepine	5.08 ± 0.32$^{@@}$

Values are mean ± SEM of 4-6 separate experiments. Each group consist of 6-8 rats ***$p<0.001$ when compared to control, $^{@@}p<0.001$ when compared to epileptic group.

Figure-10
GABA content in the corpus striatum of control and experimental rats



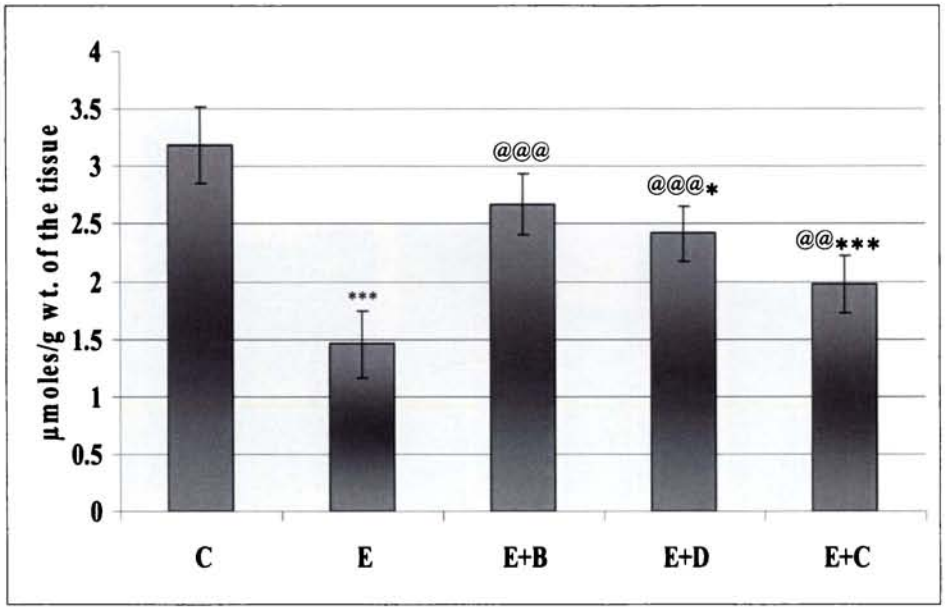
C- Control, E-Epileptic, E+B-Epileptic rats treated with *Bacopa monnieri*, E+D-Epileptic rats treated with Bacoside A and E+C-Epileptic rats treated with Carbamazepine.

Table-11
GABA content in the corpus striatum of control and experimental rats

Animal status	GABA content (µmoles/g wt. of the tissue)
Control	4.87 ± 0.43
Epileptic	3.09 ± 0.29***
Epileptic + *Bacopa monnieri*	4.29 ± 0.27$^{@@}$
Epileptic + Bacoside A	3.98 ± 0.39$^{@@}$*
Epileptic + Carbamazepine	3.53 ± 0.35$^{@}$**

Values are mean ± SEM of 4-6 separate experiments. Each group consist of 6-8 rats ***p<0.001, **p<0.01, *p<0.05 when compared to control, $^{@@}$p<0.01, $^{@}$p<0.05 when compared to epileptic group.

Figure-11
GABA content in the brain stem of control and experimental rats



C-Control, E-Epileptic, E+B-Epileptic rats treated with *Bacopa monnieri,* E+D-Epileptic rats treated with Bacoside A and E+C-Epileptic rats treated with Carbamazepine.

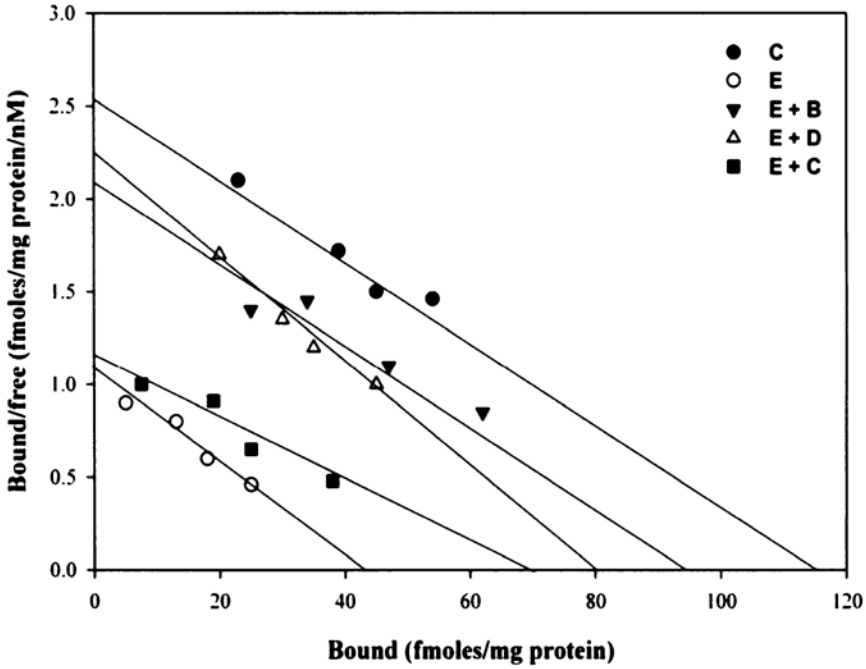
Table-12
GABA content in the brain stem of control and experimental rats

Animal status	GABA content (µmoles/g wt. of the tissue)
Control	3.19 ± 0.33
Epileptic	1.46 ± 0.29***
Epileptic + *Bacopa monnieri*	2.67 ± 0.27$^{@@@}$
Epileptic + Bacoside A	2.42 ± 0.24$^{@@@*}$
Epileptic + Carbamazepine	1.98 ± 0.25$^{@@***}$

Values are mean ± SEM of 4-6 separate experiments. Each group consist of 6-8 rats ***p<0.001, *p<0.05 when compared to control, $^{@@@}$p<0.001, $^{@@}$<0.01 when compared to epileptic group.

Figure-12

Scatchard analysis of [^{3}H] GABA binding against GABA in the cerebral cortex of control and experimental rats



C-Control, E-Epileptic, E+B-Epileptic rats treated with *Bacopa monnieri,* E+D-Epileptic rats treated with Bacoside A and E+C-Epileptic rats treated with Carbamazepine.

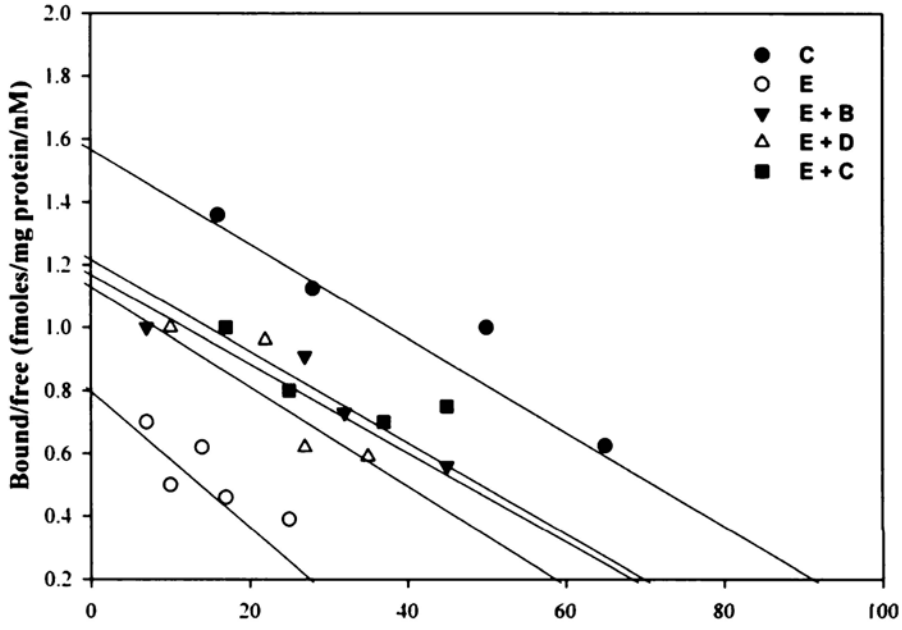
Table-13
Scatchard analysis of [^{3}H]GABA binding against GABA in the cerebral cortex of control and experimental rats

Animal status	B_{max} (fmoles/mg protein)	K_d (nM)
Control	112.3 ± 6.3	43.1 ± 2.3
Epileptic	44.0 ± 3.5***	29.3 ± 1.1**
Epileptic + *Bacopa monnieri*	89.8 ± 5.6$^{@@@}$	39.0 ± 3.2$^{@@}$
Epileptic + Bacoside A	93.2 ± 4.7 $^{@@@}$	19.8 ± 1.2$^{@}$**
Epileptic + Carbamazepine	80.7 ± 5.2$^{@@@}$	38.4 ± 2.9$^{@@}$

Values are mean ± SEM of 4-6 separate experiments. Each group consist of 6-8 rats ***p<0.001, **p<0.01 when compared to control, $^{@@@}$p<0.001, $^{@@}$p<0.01, $^{@}$p<0.05 when compared to epileptic group.

Figure-13

Scatchard analysis of [^{3}H]bicuculline binding against bicuculline in the cerebral cortex of control and experimental rats



C-Control, E-Epileptic, E+B-Epileptic rats treated with *Bacopa monnieri,* E+D-Epileptic rats treated with Bacoside A and E+C-Epileptic rats treated with Carbamazepine.

Table-14

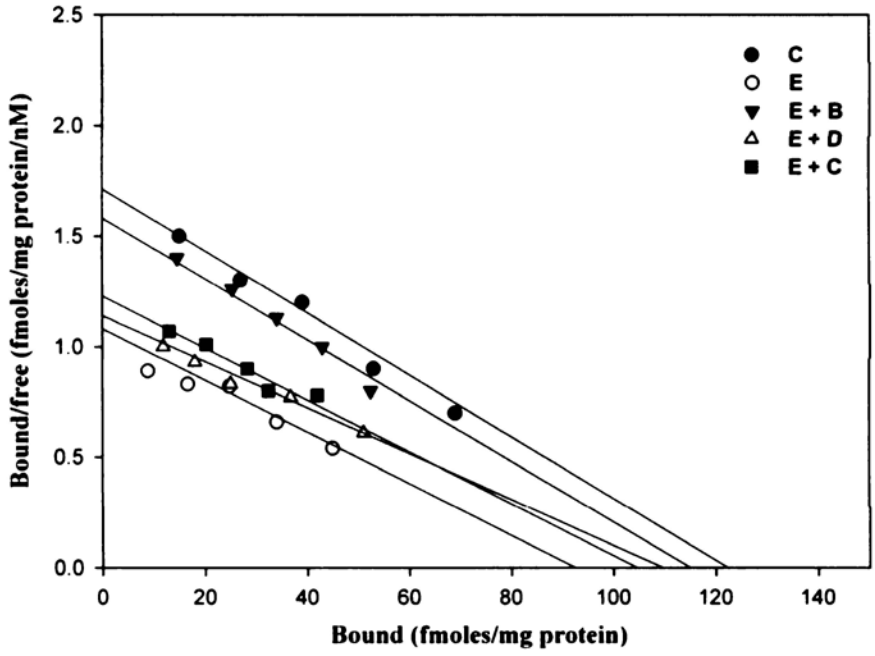
Scatchard analysis of [^{3}H]bicuculline binding against bicuculline in the cerebral cortex of control and experimental rats

Animal status	B_{max} (fmoles/mg protein)	K_d (nM)
Control	92.5 ± 4.5	60.0 ± 4.2
Epileptic	29.7 ± 3.9***	37.1 ± 2.5**
Epileptic + *Bacopa monnieri*	58.9 ± 4.5$^{@@@}$*	53.5 ± 3.7$^{@@}$
Epileptic + Bacoside A	70.7 ± 5.1$^{@@@}$	58.9 ± 4.3$^{@}$
Epileptic + Carbamazepine	68.5 ± 4.7$^{@@@}$	62.2 ± 3.9

Values are mean ± SEM of 4-6 separate experiments. Each group consist of 6-8 rats ***p<0.001, **p<0.01, *p<0.05, when compared to control, $^{@@@}$p<0.001, $^{@@}$p<0.01, $^{@}$p<0.05 when compared to epileptic group.

Figure-14

Scatchard analysis of [^{3}H]baclofen binding against baclofen in the cerebral cortex of control and experimental rats



C-Control, E-Epileptic, E+B-Epileptic rats treated with *Bacopa monnieri,* E+D-Epileptic rats treated with Bacoside A and E+C-Epileptic rats treated with Carbamazepine.

Table-15

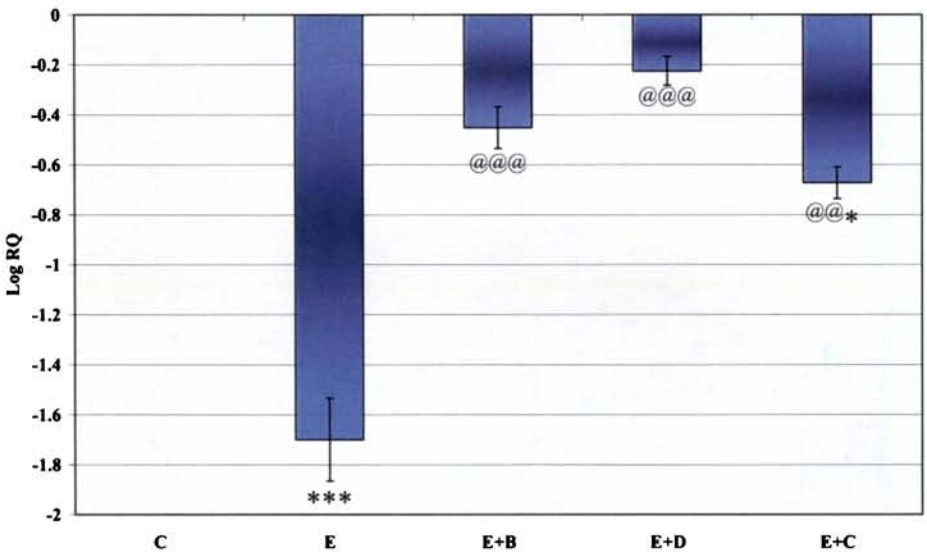
Scatchard analysis of [^{3}H]baclofen binding against baclofen in the cerebral cortex of control and experimental rats

Animal status	B_{max} (fmoles/mg protein)	K_d (nM)
Control	124.3 ± 8.5	68.8 ± 5.2
Epileptic	91.9 ± 6.9***	76.5 ± 4.1*
Epileptic + *Bacopa monnieri*	115.0 ± 7.5$^{@@@}$	71.6 ± 6.3
Epileptic + Bacoside A	111.7 ± 5.1$^{@@@}$	85.9 ± 5.0
Epileptic + Carbamazepine	107.1 ± 7.7$^{@@@}$	76.4 ± 4.7

Values are mean ± SEM of 4-6 separate experiments. Each group consist of 6-8 rats ***$p<0.001$, *$p<0.05$ when compared to control, $^{@@@}p<0.001$ when compared to epileptic group.

Figure-15

Real Time PCR amplification of $GABA_{A\acute{a}1}$ receptor subunit mRNA from the cerebral cortex of control and experimental rats



C-Control, E-Epileptic, E+B-Epileptic rats treated with *Bacopa monnieri,* E+D-Epileptic rats treated with Bacoside A and E+C-Epileptic rats treated with Carbamazepine.

Table-16

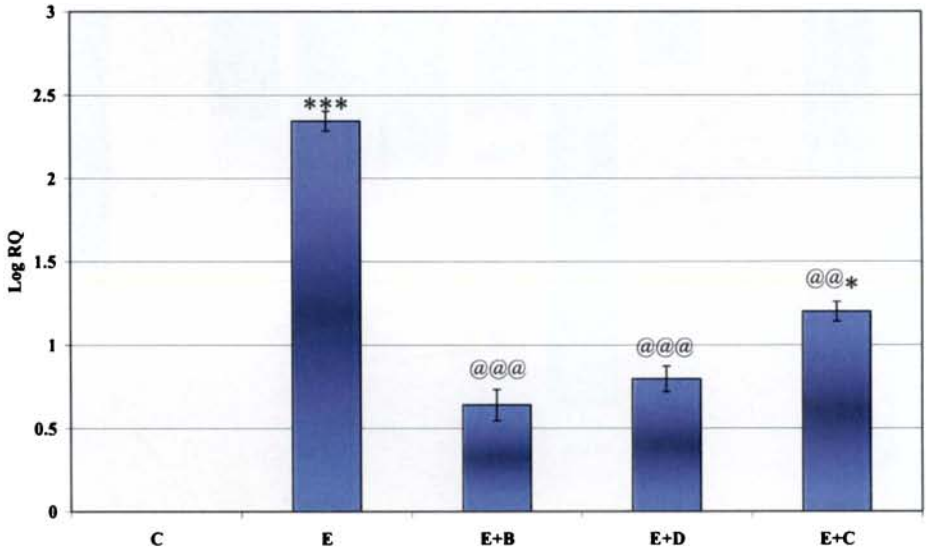
Real Time PCR amplification of $GABA_{A\acute{a}1}$ receptor subunit mRNA from the cerebral cortex of control and experimental rats

Animal status	Log RQ value
Control	0
Epileptic	-1.68 ± 0.16 ***
Epileptic + *Bacopa monnieri*	-0.49 ± 0.082 @@@
Epileptic + Bacoside A	-0.22 ± 0.056 @@@
Epileptic + Carbamazepine	-0.68 ± 0.063 @@*

Values are mean ± SEM of 4-6 separate experiments. Each group consist of 6-8 rats ***p<0.001, *p<0.05 when compared to control, @@@p<0.001, @@p<0.01 when compared to epileptic group.

Figure-16

Real Time PCR amplification of $GABA_{A\alpha5}$ receptor subunit mRNA from the cerebral cortex of control and experimental rats



C-Control, E-Epileptic, E+B-Epileptic rats treated with *Bacopa monnieri,* E+D-Epileptic rats treated with Bacoside A and E+C-Epileptic rats treated with Carbamazepine.

Table-17

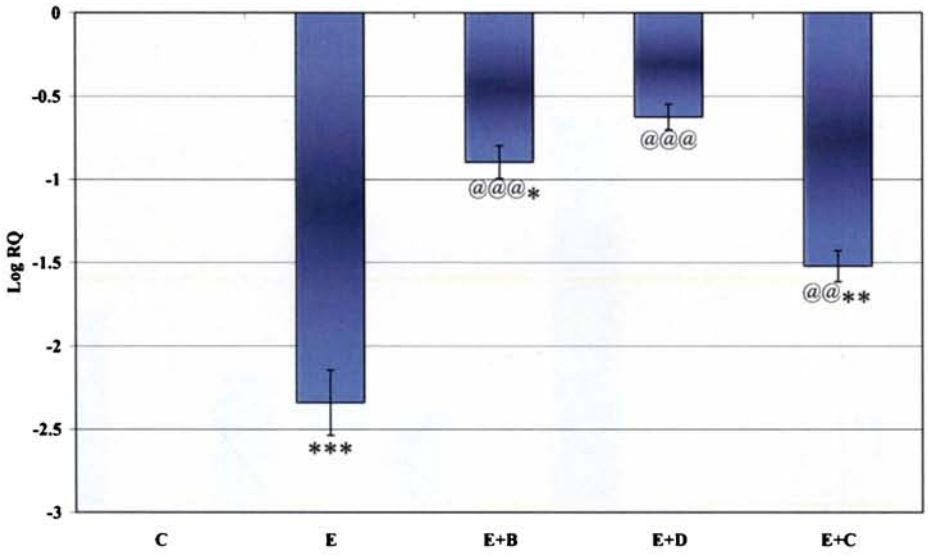
Real Time PCR amplification of $GABA_{A\alpha5}$ receptor subunit mRNA from the cerebral cortex of control and experimental rats

Animal status	Log RQ value
Control	0
Epileptic	2.34 ± 0.060 ***
Epileptic + *Bacopa monnieri*	$0.63 \pm 0.092^{@@@}$
Epileptic + Bacoside A	0.79 ± 0.076 $^{@@@}$
Epileptic + Carbamazepine	1.19 ± 0.059 $^{@@}$*

Values are mean ± SEM of 4-6 separate experiments. Each group consist of 6-8 rats ***p<0.001, *p<0.05, when compared to control, $^{@@@}$p<0.001, $^{@@}$p<0.01 when compared to epileptic group.

Figure-17

Real Time PCR amplification of $GABA_{A\gamma3}$ Receptor subunit mRNA from the cerebral cortex of control and experimental rats



C-Control, E-Epileptic, E+B-Epileptic rats treated with *Bacopa monnieri,* E+D-Epileptic rats treated with Bacoside A and E+C-Epileptic rats treated with Carbamazepine.

Table-18

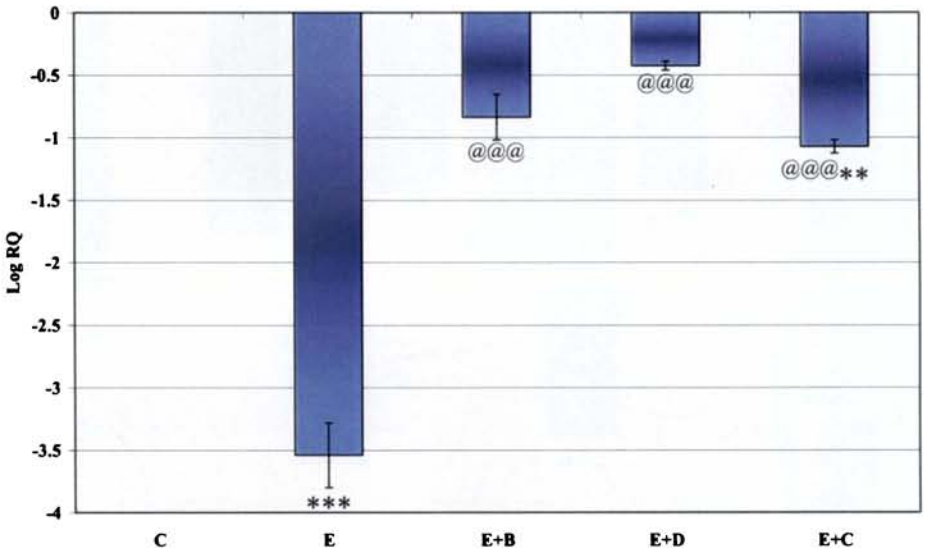
Real Time PCR amplification of $GABA_{A\gamma3}$ Receptor subunit mRNA from the cerebral cortex of control and experimental rats

Animal status	Log RQ value
Control	0
Epileptic	-2.33 ± 0.190 ***
Epileptic + *Bacopa monnieri*	-0.89 ± 0.098 @@@*
Epileptic + Bacoside A	-0.63 ± 0.076 @@@
Epileptic + Carbamazepine	-1.53 ± 0.093 @@**

Values are mean ± SEM of 4-6 separate experiments. Each group consist of 6-8 rats ***p<0.001, **p<0.01, *p<0.05 when compared to control, @@@p<0.001, @@p<0.01 when compared to epileptic group.

Figure-18

Real Time PCR amplification of $GABA_{A\delta}$ receptor subunit mRNA from the cerebral cortex of control and experimental rats



C-Control, E-Epileptic, E+B-Epileptic rats treated with *Bacopa monnieri,* E+D-Epileptic rats treated with Bacoside A and E+C-Epileptic rats treated with Carbamazepine.

Table-19

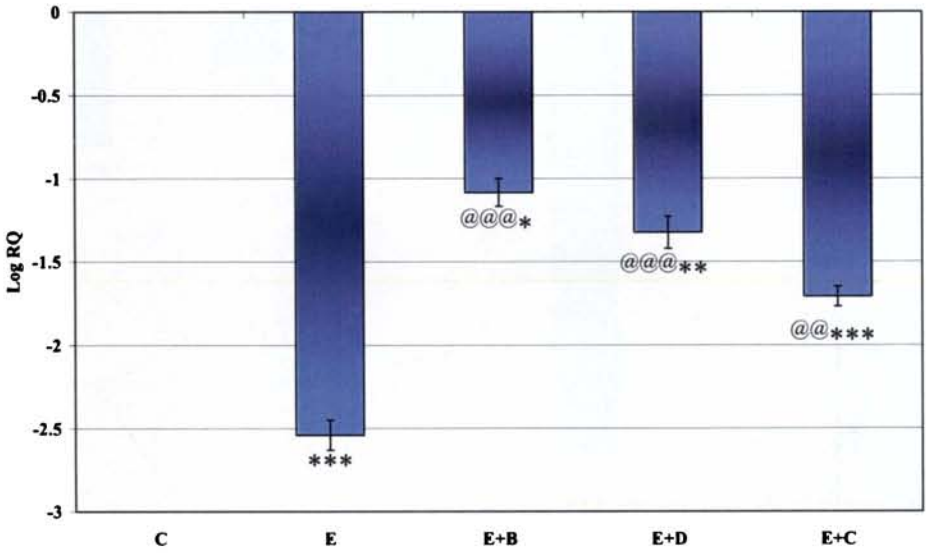
Real Time PCR amplification of $GABA_{A\delta}$ receptor subunit mRNA from the cerebral cortex of control and experimental rats

Animal status	Log RQ value
Control	0
Epileptic	-3.56 ± 0.260 ***
Epileptic + *Bacopa monnieri*	-0.83 ± 0.181 @@@
Epileptic + Bacoside A	-0.42 ± 0.036 @@@
Epileptic + Carbamazepine	-1.07 ± 0.053 @@@**

Values are mean ± SEM of 4-6 separate experiments. Each group consist of 6-8 rats ***p<0.001, **p<0.01 when compared to control, @@@p<0.001 when compared to epileptic group.

Figure-19

Real Time PCR amplification of $GABA_B$ receptor mRNA from the cerebral cortex of control and experimental rats



C-Control, E-Epileptic, E+B-Epileptic rats treated with *Bacopa monnieri,* E+D-Epileptic rats treated with Bacoside A and E+C-Epileptic rats treated with Carbamazepine.

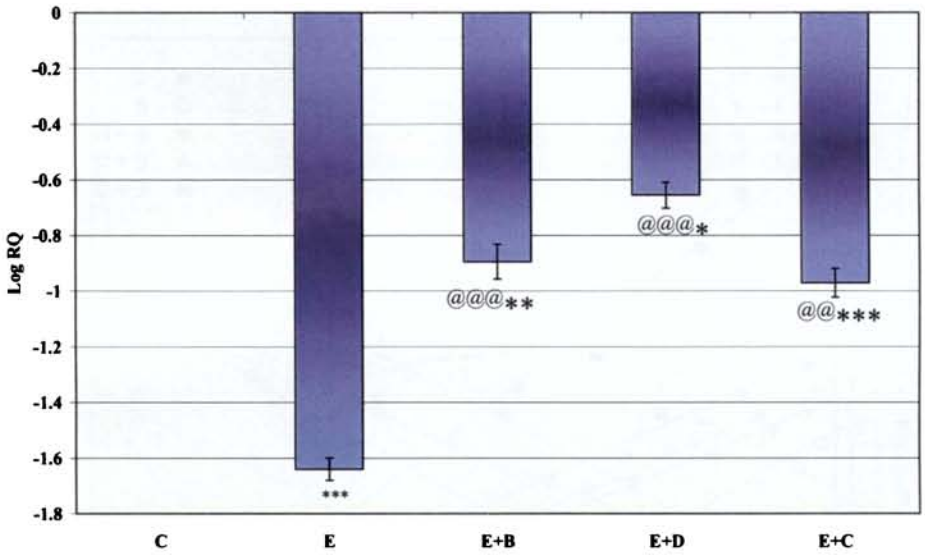
Table-20

Real Time PCR amplification of $GABA_B$ receptor mRNA from the cerebral cortex of control and experimental rats

Animal status	Log RQ value
Control	0
Epileptic	-2.52 ± 0.090 ***
Epileptic + *Bacopa monnieri*	$-1.04 \pm 0.082^{@@@*}$
Epileptic + Bacoside A	$-1.35 \pm 0.096^{@@@**}$
Epileptic + Carbamazepine	$-1.71 \pm 0.059^{@@***}$

Values are mean ± SEM of 4-6 separate experiments. Each group consist of 6-8 rats ***p<0.001, **p<0.01, *p<0.05 when compared to control, $^{@@@}$p<0.001, $^{@@}$p<0.01 when compared to epileptic group.

Figure-20
Real Time PCR amplification of GAD mRNA from the cerebral cortex of control and experimental rats



C-Control, E-Epileptic, E+B-Epileptic rats treated with *Bacopa monnieri,* E+D-Epileptic rats treated with Bacoside A and E+C-Epileptic rats treated with Carbamazepine.

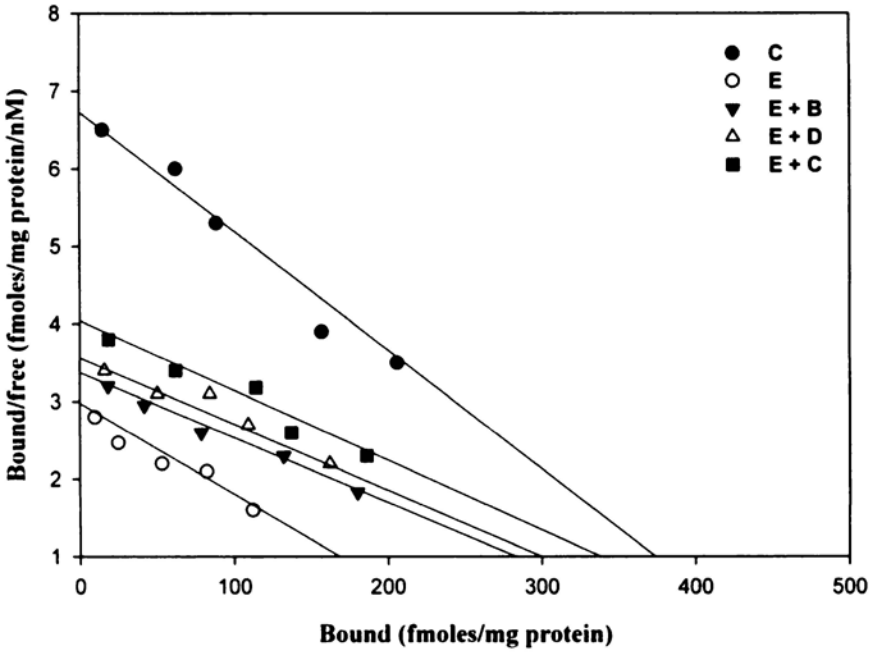
Table-21
Real Time PCR amplification of GAD mRNA from the cerebral cortex of control and experimental rats

Animal status	Log RQ value
Control	0
Epileptic	-1.67 ± 0.040 ***
Epileptic + *Bacopa monnieri*	-0.89 ± 0.062 @@@**
Epileptic + Bacoside A	-0.65 ± 0.039 @@@*
Epileptic + Carbamazepine	-0.97 ± 0.052 @@***

Values are mean ± SEM of 4-6 separate experiments. Each group consist of 6-8 rats ***p<0.001, **p<0.01, *p<0.05 when compared to control, @@@p<0.001, @@p<0.01 when compared to epileptic group.

Figure-21

Scatchard analysis of [^{3}H] GABA binding against GABA in the hippocampus of control and experimental rats



C-Control, E-Epileptic, E+B-Epileptic rats treated with *Bacopa monnieri,* E+D-Epileptic rats treated with Bacoside A and E+C-Epileptic rats treated with Carbamazepine.

Table-22

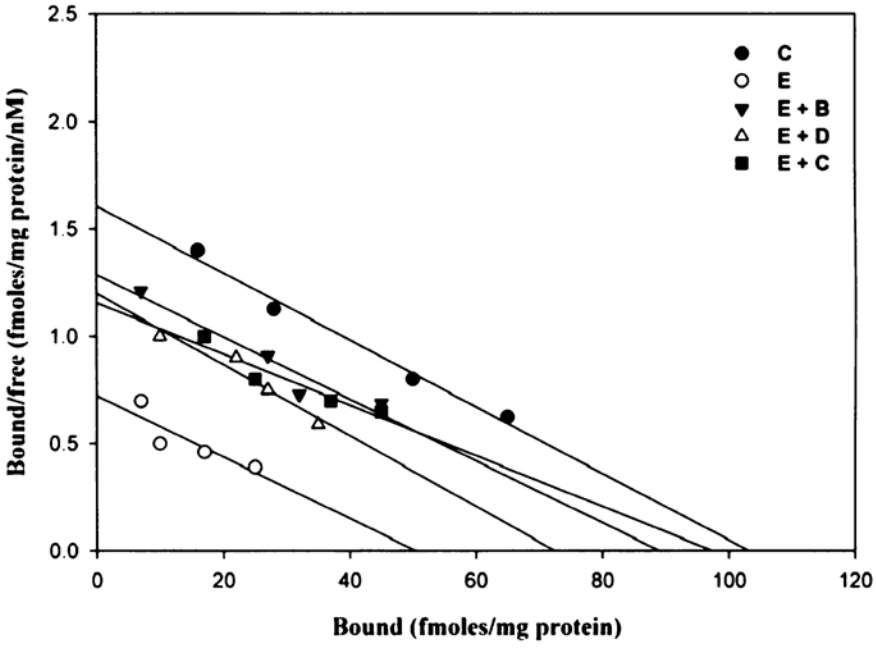
Scatchard analysis of [^{3}H]GABA binding against GABA in the hippocampus of control and experimental rats

Animal status	B_{max} (fmoles/mg protein)	K_d (nM)
Control	370.6 ± 14.5	85.2 ± 4.2
Epileptic	168.8 ± 3.9***	56.2 ± 4.1**
Epileptic + *Bacopa monnieri*	287.6 ± 12.5@@@	84.5 ± 5.8@@
Epileptic + Bacoside A	297.7 ± 10.1@@@	82.6 ± 5.4@@
Epileptic + Carbamazepine	338.9 ± 9.7@@@	82.6 ± 5.7@@

Values are mean ± SEM of 4-6 separate experiments. Each group consist of 6-8 rats ***$p<0.001$, **$p<0.01$ when compared to control, @@@$p<0.001$, @@$p<0.01$ when compared to epileptic group.

Figure-22

Scatchard analysis of [^{3}H]bicuculline binding against bicuculline in the hippocampus of control and experimental rats



C-Control, E-Epileptic, E+B-Epileptic rats treated with *Bacopa monnieri,* E+D-Epileptic rats treated with Bacoside A and E+C-Epileptic rats treated with Carbamazepine.

Table-23

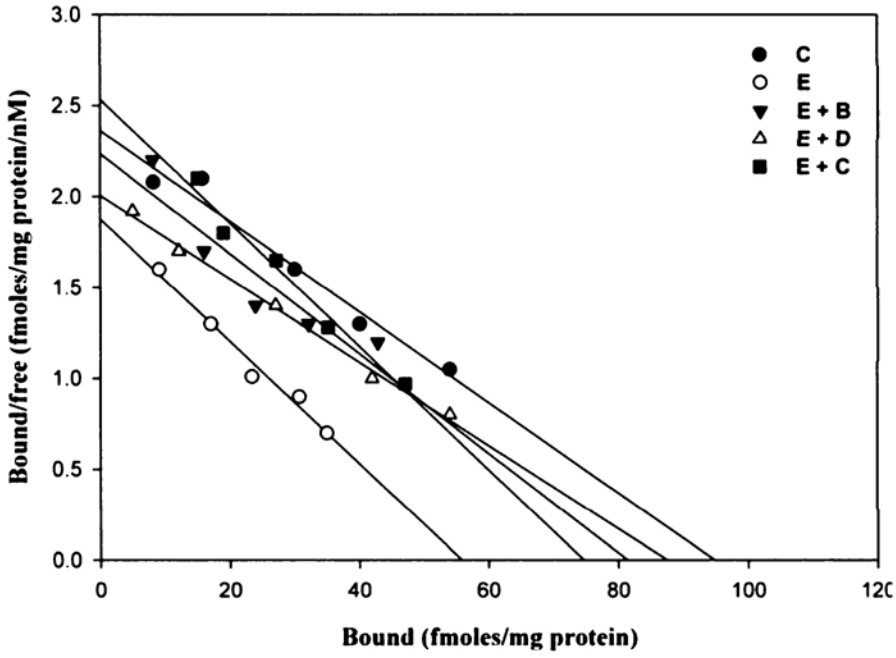
Scatchard analysis of [^{3}H]bicuculline binding against bicuculline in the hippocampus of control and experimental rats

Animal status	B_{max} (fmoles/mg protein)	K_d (nM)
Control	103.3 ± 6.3	67.5 ± 4.5
Epileptic	51.2 ± 3.9***	32.0 ± 2.1**
Epileptic + *Bacopa monnieri*	88.6 ± 5.6$^{@@@}$	63.4 ± 4.6$^{@@}$
Epileptic + Bacoside A	73.9 ± 4.8$^{@@@}$	49.3 ± 2.4$^{@@}$
Epileptic + Carbamazepine	97.5 ± 6.7$^{@@@}$	88.1 ± 04.0$^{@@}$

Values are mean ± SEM of 4-6 separate experiments. Each group consist of 6-8 rats ***p<0.001, **p<0.01 when compared to control, $^{@@@}$p<0.001, $^{@@}$p<0.01 when compared to epileptic group.

Figure-23

Scatchard analysis of [^{3}H]baclofen binding against baclofen in the hippocampus of control and experimental rats



C-Control, E-Epileptic, E+B-Epileptic rats treated with *Bacopa monnieri,* E+D-Epileptic rats treated with Bacoside A and E+C-Epileptic rats treated with Carbamazepine.

Table-24

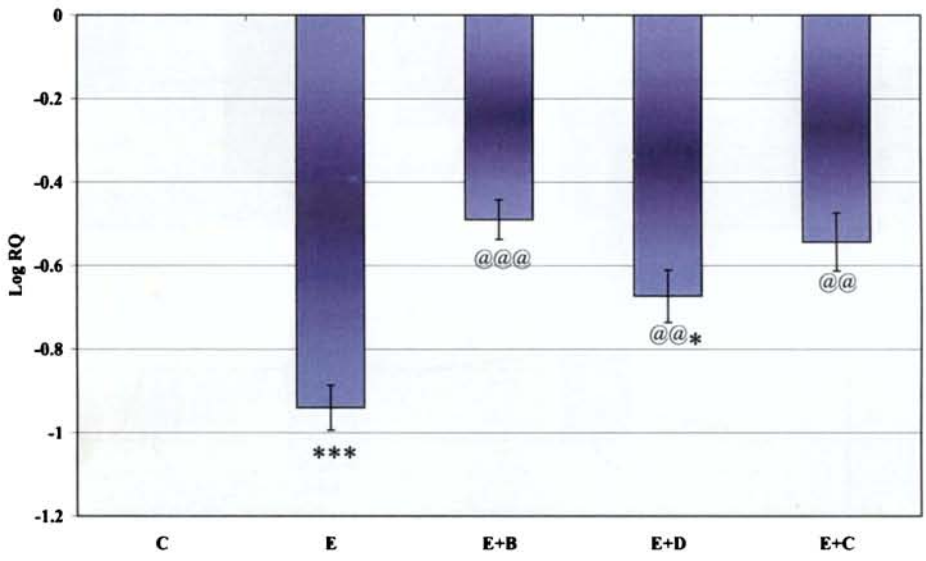
Scatchard analysis of [^{3}H]baclofen binding against baclofen in the hippocampus of control and experimental rats

Animal status	B_{max} (fmoles/mg protein)	K_d (nM)
Control	96.3 ± 5.7	40.1 ± 3.2
Epileptic	56.2 ± 3.2***	31.8 ± 2.5**
Epileptic + *Bacopa monnieri*	81.6 ± 5.6$^{@@@}$	35.4 ± 2.7$^{@}$
Epileptic + Bacoside A	84.3 ± 4.8$^{@@@}$	40.1 ± 3.6$^{@@}$
Epileptic + Carbamazepine	74.9 ± 6.7$^{@@@}$	29.9 ± 2.2

Values are mean ± SEM of 4-6 separate experiments. Each group consist of 6-8 rats ***p<0.001, **p<0.01 when compared to control, $^{@@@}$p<0.001, $^{@@}$p<0.01, $^{@}$p<0.05 when compared to epileptic group.

Figure-24

Real Time PCR amplification of $GABA_{A\alpha 1}$ receptor subunit mRNA from the hippocampus of control and experimental rats



C-Control, E-Epileptic, E+B-Epileptic rats treated with *Bacopa monnieri,* E+D-Epileptic rats treated with Bacoside A and E+C-Epileptic rats treated with Carbamazepine.

Table-25

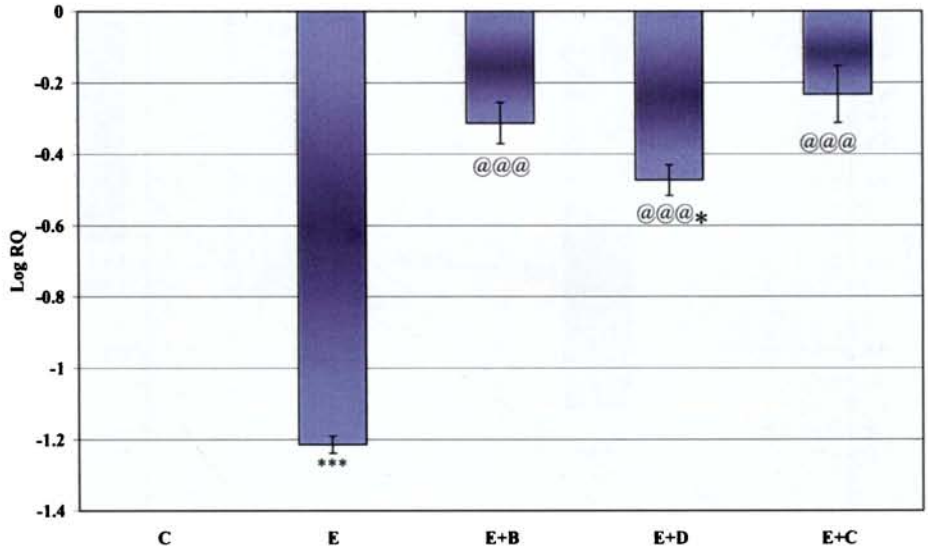
Real Time PCR amplification of $GABA_{A\alpha 1}$ receptor subunit mRNA from the hippocampus of control and experimental rats

Animal status	Log RQ value
Control	0
Epileptic	-0.93 ± 0.053 ***
Epileptic + *Bacopa monnieri*	$-0.483 \pm 0.047^{@@@}$
Epileptic + Bacoside A	$-0.67 \pm 0.062^{@@}$
Epileptic + Carbamazepine	$-0.54 \pm 0.069^{@@*}$

Values are mean ± SEM of 4-6 separate experiments. Each group consist of 6-8 rats ***p<0.001, *p<0.05 when compared to control, $^{@@@}$p<0.001, $^{@@}$p<0.01, when compared to epileptic group.

Figure-25

Real Time PCR amplification of $GABA_{A\delta5}$ receptor subunit mRNA from the hippocampus of control and experimental rats



C-Control, E-Epileptic, E+B-Epileptic rats treated with *Bacopa monnieri,* E+D-Epileptic rats treated with Bacoside A and E+C-Epileptic rats treated with Carbamazepine.

Table-26

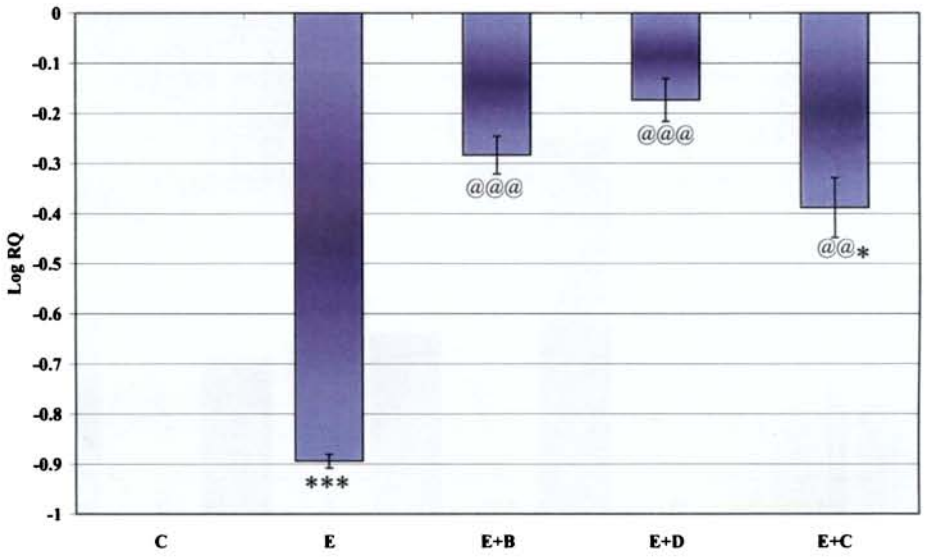
Real Time PCR amplification of $GABA_{A\delta5}$ receptor subunit mRNA from the hippocampus of control and experimental rats

Animal status	Log RQ value
Control	0
Epileptic	$-1.21 \pm 0.023^{***}$
Epileptic + *Bacopa monnieri*	$-0.31 \pm 0.057^{@@@}$
Epileptic + Bacoside A	$-0.68 \pm 0.042^{@@@*}$
Epileptic + Carbamazepine	$-0.23 \pm 0.079^{@@@}$

Values are mean ± SEM of 4-6 separate experiments. Each group consist of 6-8 rats ***$p<0.001$, *$p<0.05$ when compared to control, $^{@@@}p<0.001$ when compared to epileptic group.

Figure-26

Real Time PCR amplification of $GABA_{A\gamma3}$ Receptor subunit mRNA from the hippocampus of control and experimental rats



C-Control, E-Epileptic, E+B-Epileptic rats treated with *Bacopa monnieri,* E+D-Epileptic rats treated with Bacoside A and E+C-Epileptic rats treated with Carbamazepine.

Table-27

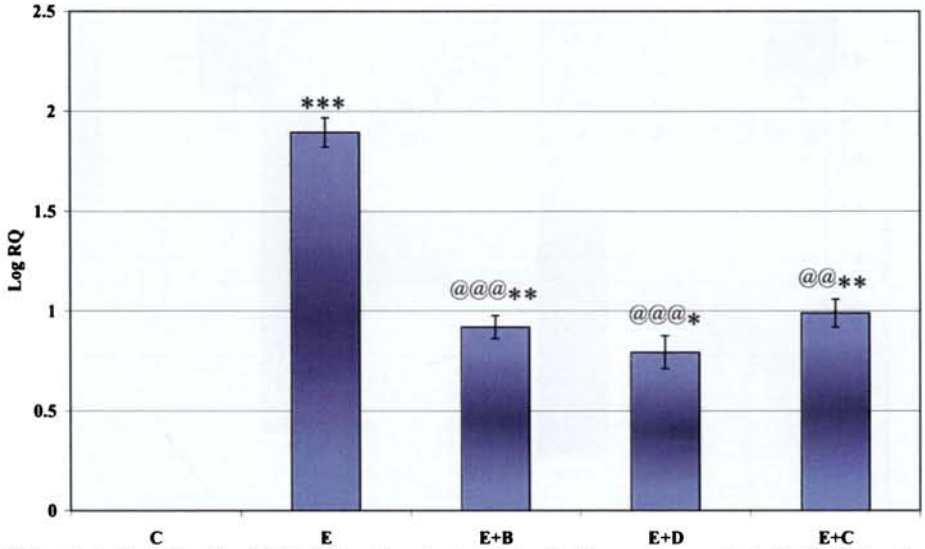
Real Time PCR amplification of $GABA_{A\gamma3}$ Receptor subunit mRNA from the hippocampus of control and experimental rats

Animal status	Log RQ value
Control	0
Epileptic	-0.89 ± 0.016 ***
Epileptic + *Bacopa monnieri*	-0.18 ± 0.038$^{@@@}$
Epileptic + Bacoside A	-0.17 ± 0.047$^{@@@}$
Epileptic + Carbamazepine	-0.38 ± 0.054$^{@@*}$

Values are mean ± SEM of 4-6 separate experiments. Each group consist of 6-8 rats ***p<0.001, *p<0.05 when compared to control, $^{@@@}$p<0.001, $^{@@}$p<0.01 when compared to epileptic group.

Figure-27

Real Time PCR amplification of $GABA_{A\delta}$ receptor subunit mRNA from the hippocampus of control and experimental rats



C-Control, E-Epileptic, E+B-Epileptic rats treated with *Bacopa monnieri,* E+D-Epileptic rats treated with Bacoside A and E+C-Epileptic rats treated with Carbamazepine.

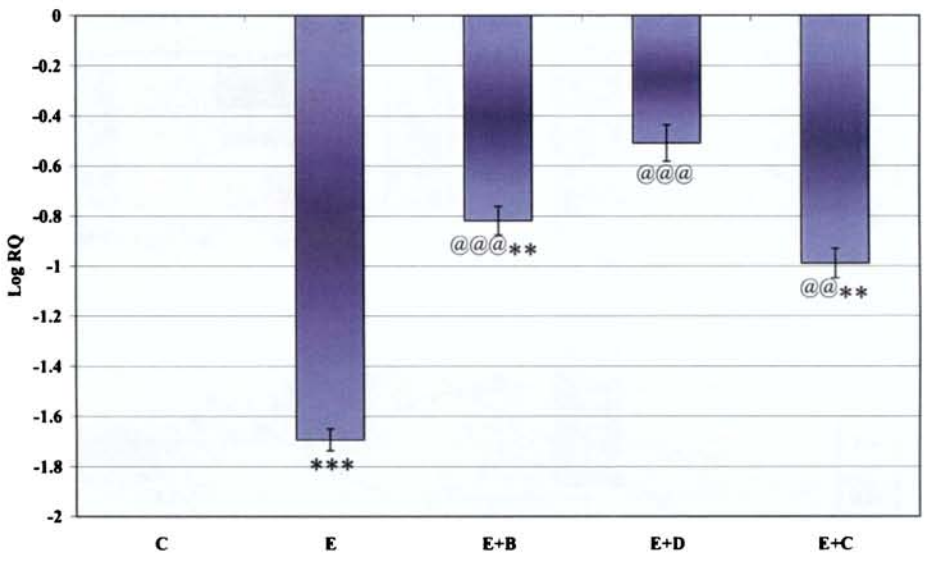
Table-28

Real Time PCR amplification of $GABA_{A\delta}$ receptor subunit mRNA from the hippocampus of control and experimental rats

Animal status	Log RQ value
Control	0
Epileptic	$1.86 \pm 0.073^{***}$
Epileptic + *Bacopa monnieri*	$0.917 \pm 0.057^{@@@**}$
Epileptic + Bacoside A	$0.795 \pm 0.082^{@@@*}$
Epileptic + Carbamazepine	$0.956 \pm 0.069^{@@**}$

Values are mean ± SEM of 4-6 separate experiments. Each group consist of 6-8 rats ***p<0.001, **p<0.01, *p<0.05 when compared to control, $^{@@@}$p<0.001, $^{@@}$p<0.01 when compared to epileptic group.

Figure-28
Real Time PCR amplification of $GABA_B$ receptor mRNA from the hippocampus of control and experimental rats



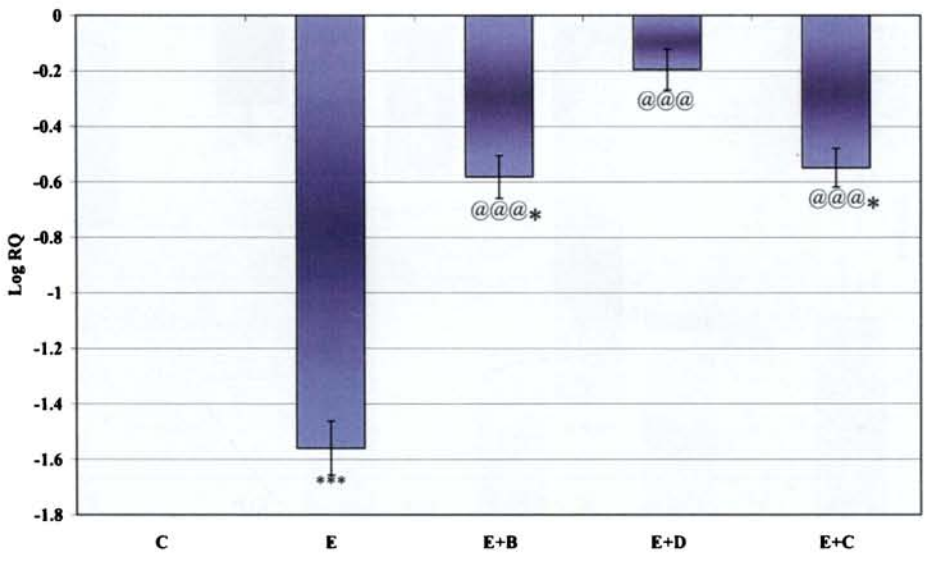
C-Control, E-Epileptic, E+B-Epileptic rats treated with *Bacopa monnieri,* E+D-Epileptic rats treated with Bacoside A and E+C-Epileptic rats treated with Carbamazepine.

Table-29
Real Time PCR amplification of $GABA_B$ receptor mRNA from the cerebral cortex of control and experimental rats

Animal status	Log RQ value
Control	0
Epileptic	-1.69 ± 0.043 ***
Epileptic + *Bacopa monnieri*	-0.81 ± 0.057 $^{@@@}$**
Epileptic + Bacoside A	-0.50 ± 0.072 $^{@@@}$
Epileptic + Carbamazepine	-0.981 ± 0.059 $^{@@}$**

Values are mean ± SEM of 4-6 separate experiments. Each group consist of 6-8 rats ***p<0.001, **p<0.01 when compared to control, $^{@@@}$p<0.001, $^{@@}$p<0.01 when compared to epileptic group.

Figuer-29
Real Time PCR amplification of GAD mRNA from the hippocampus of control and experimental rats



C-Control, E-Epileptic, E+B-Epileptic rats treated with *Bacopa monnieri,* E+D-Epileptic rats treated with Bacoside A and E+C-Epileptic rats treated with Carbamazepine.

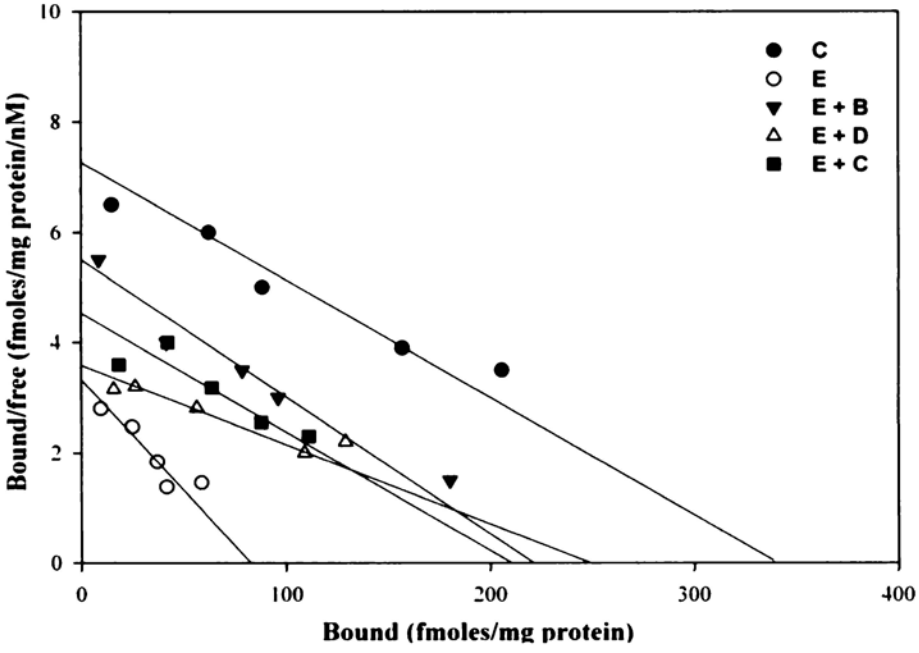
Table-30
Real Time PCR amplification of GAD mRNA from the hippocampus of control and experimental rats

Animal status	Log RQ value
Control	0
Epileptic	-1.55 ± 0.09***
Epileptic + *Bacopa monnieri*	-0.58 ± 0.07$^{@@@}$*
Epileptic + Bacoside A	-0.19 ± 0.07$^{@@@}$
Epileptic + Carbamazepine	-0.54 ± 0.06$^{@@@}$*

Values are mean ± SEM of 4-6 separate experiments. Each group consist of 6-8 rats ***p<0.001, *p<0.05 when compared to control, $^{@@@}$p<0.001 when compared to epileptic group.

Figure-30

Scatchard analysis of [^{3}H]GABA binding against GABA in the cerebellum of control and experimental rats



C-Control, E-Epileptic, E+B-Epileptic rats treated with *Bacopa monnieri,* E+D-Epileptic rats treated with Bacoside A and E+C-Epileptic rats treated with *Carbamazepine.*

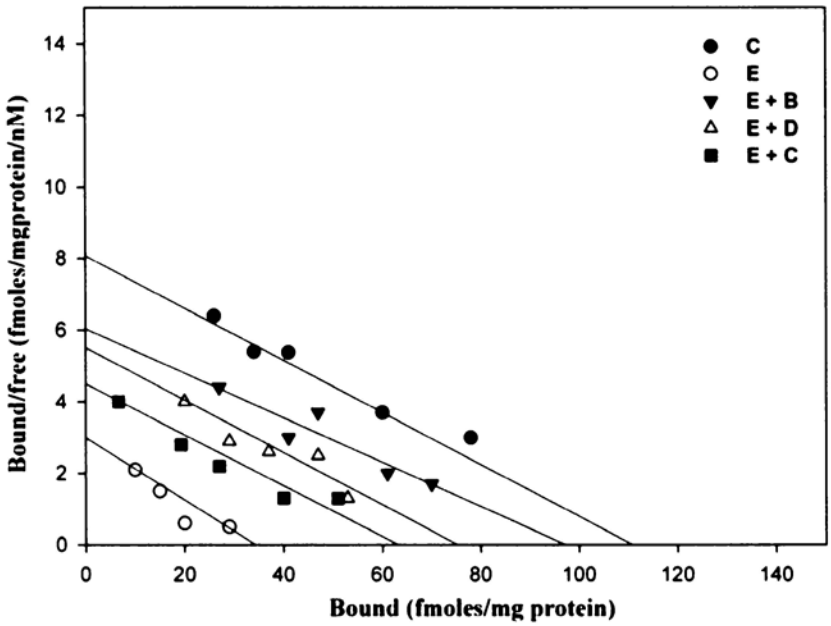
Table-31
Scatchard analysis of [^{3}H]GABA binding against GABA in the cerebellum of control and experimental rats

Animal status	B_{max} (fmoles/mg protein)	K_d (nM)
Control	340.5 ± 14.3	46.6 ± 3.3
Epileptic	76.2 ± 12.5***	21.1 ± 1.4***
Epileptic + *Bacopa monnieri*	224.8 ± 9.6$^{@@@}$*	97.7 ± 5.2$^{@@@}$**
Epileptic + Bacoside A	250.2 ± 11.7$^{@@@}$	69.4 ± 3.7$^{@@}$*
Epileptic + Carbamazepine	207.4 ± 15.2$^{@@@}$*	49.3 ± 2.9$^{@@}$

Values are mean ± SEM of 4-6 separate experiments. Each group consist of 6-8 rats ***p<0.001, **p<0.01, *p<0.05 when compared to control, $^{@@@}$p<0.001, $^{@@}$p<0.01 when compared to epileptic group.

Figure-31

Scatchard analysis of [^{3}H]bicuculline binding against bicuculline in the cerebellum of Control and experimental rats



C-Control, E-Epileptic, E+B-Epileptic rats treated with *Bacopa monnieri,* E+D-Epileptic rats treated with Bacoside A and E+C-Epileptic rats treated with Carbamazepine.

Table-32

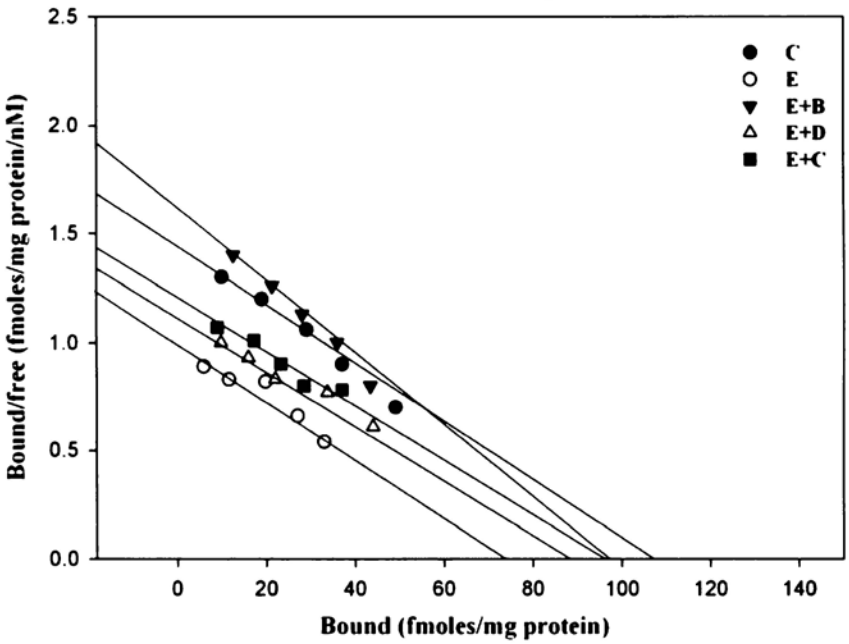
Scatchard analysis of [^{3}H]bicuculline binding against bicuculline in the cerebellum of control and experimental rats

Animal status	B_{max} (fmoles/mg protein)	K_d (nM)
Control	110.8 ± 5.7	13.8 ± 0.9
Epileptic	36.7 ± 3.2***	12.9 ± 0.7
Epileptic + *Bacopa monnieri*	96.2 ± 5.6@@@	16.0 ± 1.0
Epileptic + Bacoside A	76.5 ± 4.8@@@*	13.4 ± 0.8
Epileptic + Carbamazepine	64.9 ± 6.7@@@**	15.2 ± 1.1

Values are mean ± SEM of 4-6 separate experiments. Each group consist of 6-8 rats ***p<0.001, **p<0.01, *p<0.05 when compared to control, @@@p<0.001 when compared to epileptic group.

Figure-32

Scatchard analysis of [^{3}H]baclofen binding against baclofen in the cerebellum of control and experimental rats



C-Control, E-Epileptic, E+B-Epileptic rats treated with *Bacopa monnieri,* E+D-Epileptic rats treated with Bacoside A and E+C-Epileptic rats treated with Carbamazepine

Table-33

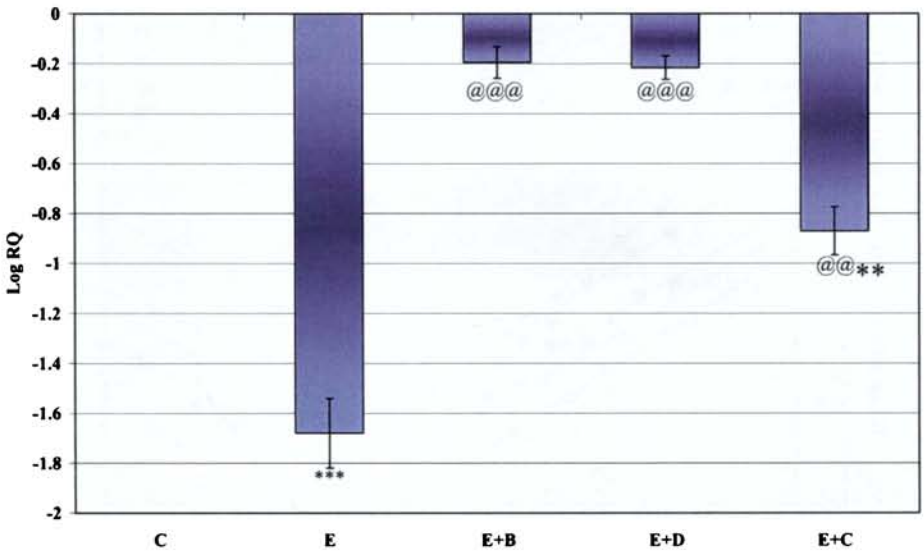
Scatchard analysis of [^{3}H]baclofen binding against baclofen in the cerebellum of control and experimental rats

Animal status	B_{max} (fmoles/mg protein)	K_d (nM)
Control	108.8 ± 5.7	63.8 ± 0.9
Epileptic	72.7 ± 3.2***	58.5 ± 0.7
Epileptic + *Bacopa monnieri*	95.2 ± 5.6$^{@@@}$	50.1 ± 1.0
Epileptic + Bacoside A	88.3 ± 4.8$^{@@@}$	65.4 ± 0.8
Epileptic + Carbamazepine	94.9 ± 6.7$^{@@@}$	65.0 ± 1.1

Values are mean ± SEM of 4-6 separate experiments. Each group consist of 6-8 rats ***$p<0.001$ when compared to control, $^{@@@}p<0.001$ when compared to epileptic group.

Figure-33

Real Time PCR amplification of $GABA_{A\acute{a}1}$ receptor subunit mRNA from the cerebellum of control and experimental rats



C-Control, E-Epileptic, E+B-Epileptic rats treated with *Bacopa monnieri,* E+D-Epileptic rats treated with Bacoside A and E+C-Epileptic rats treated with Carbamazepine.

Table-34

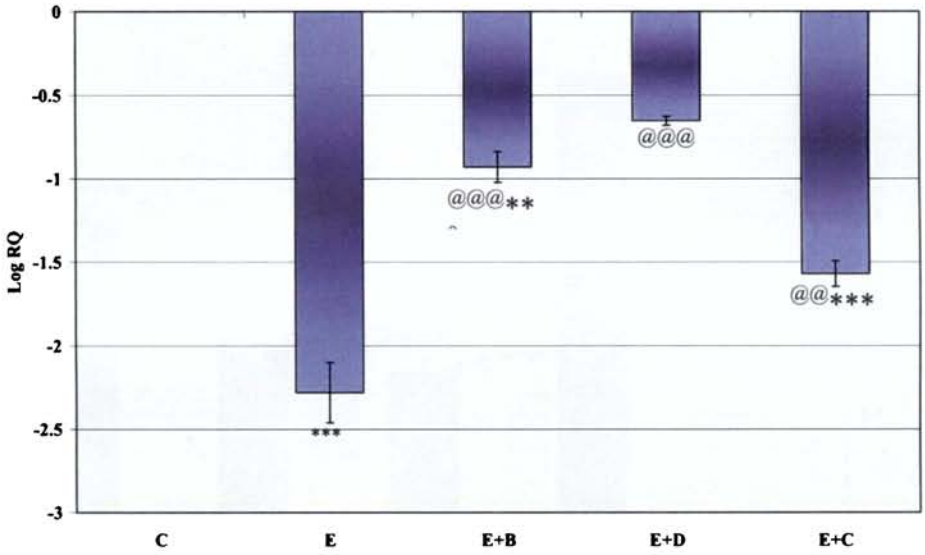
Real Time PCR amplification of $GABA_{A\acute{a}1}$ receptor subunit mRNA from the cerebellum of control and experimental rats

Animal status	Log RQ value
Control	0
Epileptic	$-1.67 \pm 0.14^{***}$
Epileptic + *Bacopa monnieri*	$-0.14 \pm 0.06^{@@@}$
Epileptic + Bacoside A	$-0.21 \pm 0.04^{@@@}$
Epileptic + Carbamazepine	$-0.86 \pm 0.09^{@@**}$

Values are mean ± SEM of 4-6 separate experiments. Each group consist of 6-8 rats ***p<0.001, **p<0.01 when compared to control, $^{@@@}$p<0.001, $^{@@}$p<0.01 when compared to epileptic group.

Figure-34

Real Time PCR amplification of $GABA_{A\delta5}$ receptor subunit mRNA from the cerebellum of control and experimental rats



C-Control, E-Epileptic, E+B-Epileptic rats treated with *Bacopa monnieri,* E+D-Epileptic rats treated with Bacoside A and E+C-Epileptic rats treated with Carbamazepine.

Table-35

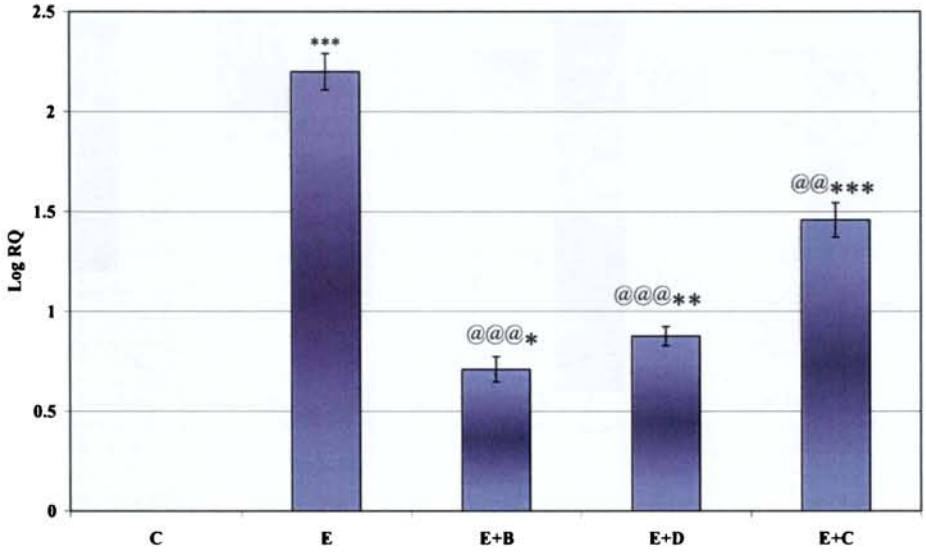
Real Time PCR amplification of $GABA_{A\delta5}$ receptor subunit mRNA from the cerebellum of control and experimental rats

Animal status	Log RQ value
Control	0
Epileptic	$-2.26 \pm 0.18^{***}$
Epileptic + *Bacopa monnieri*	$-0.93 \pm 0.09^{@@@}$
Epileptic + Bacoside A	$-0.65 \pm 0.02^{@@@}$
Epileptic + Carbamazepine	$-1.51 \pm 0.07^{@@}$

Values are mean ± SEM of 4-6 separate experiments. Each group consist of 6-8 rats ***p<0.001, **p<0.01 when compared to control, $^{@@@}$p<0.001, $^{@@}$p<0.01 when compared to epileptic group.

Figure-35

Real Time PCR amplification of $GABA_{A\gamma3}$ Receptor subunit mRNA from the cerebellum of control and experimental rats



C-Control, E-Epileptic, E+B-Epileptic rats treated with *Bacopa monnieri,* E+D-Epileptic rats treated with Bacoside A and E+C-Epileptic rats treated with Carbamazepine.

Table-36

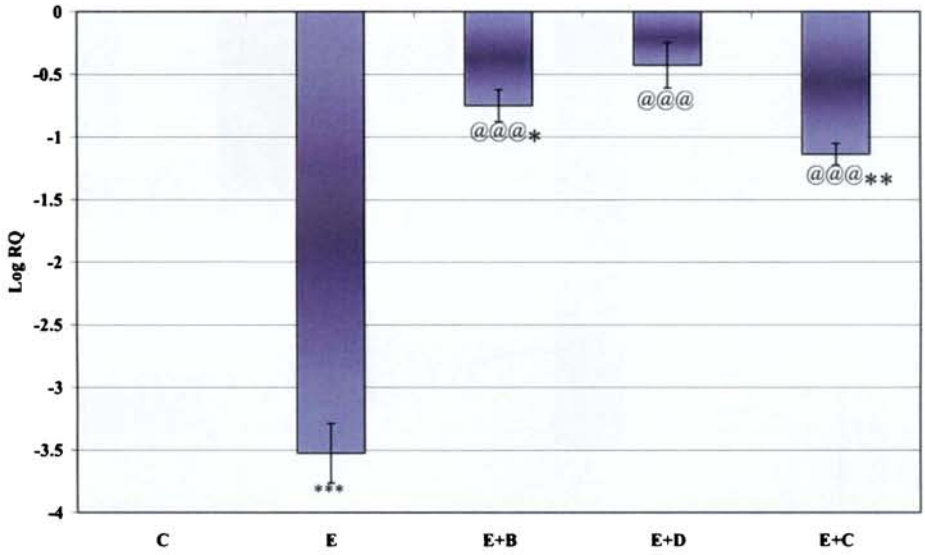
Real Time PCR amplification of $GABA_{A\gamma3}$ Receptor subunit mRNA from the cerebellum of control and experimental rats

Animal status	Log RQ value
Control	0
Epileptic	$2.19 \pm 0.090^{***}$
Epileptic + *Bacopa monnieri*	$0.71 \pm 0.062^{@@@*}$
Epileptic + Bacoside A	$0.87 \pm 0.046^{@@@**}$
Epileptic + Carbamazepine	$1.14 \pm 0.086^{@@***}$

Values are mean ± SEM of 4-6 separate experiments. Each group consist of 6-8 rats ***p<0.001, **p<0.01, *p<0.05 when compared to control, $^{@@@}$p<0.001, $^{@@}$p<0.01 when compared to epileptic group.

Figure-36

Real Time PCR amplification of $GABA_{A\delta}$ receptor subunit mRNA from the cerebellum of control and experimental rats



C-Control, E-Epileptic, E+B-Epileptic rats treated with *Bacopa monnieri,* E+D-Epileptic rats treated with Bacoside A and E+C-Epileptic rats treated with Carbamazepine.

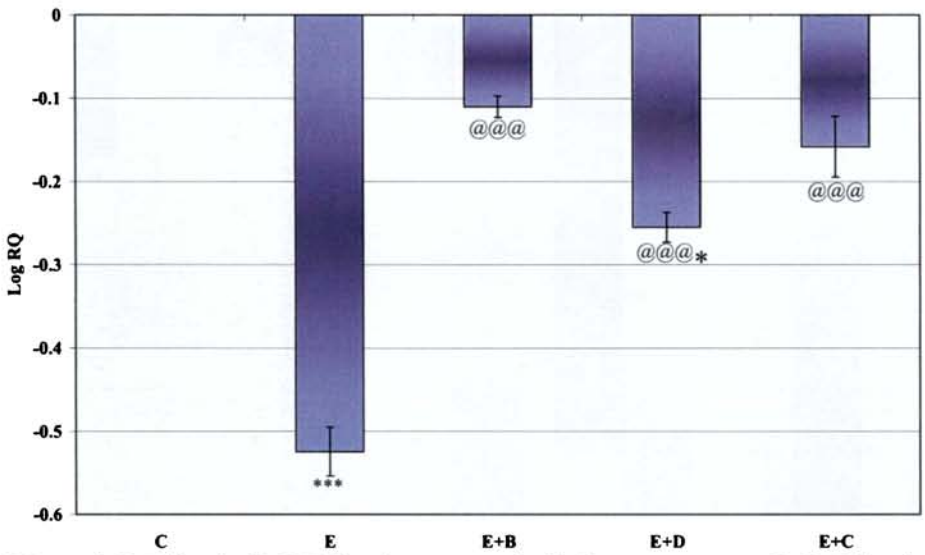
Table-37

Real Time PCR amplification of $GABA_{A\delta}$ receptor subunit mRNA from the cerebellum of control and experimental rats

Animal status	Log RQ value
Control	0
Epileptic	-3.52 ± 0.23 ***
Epileptic + *Bacopa monnieri*	-0.75 ± 0.12 @@@*
Epileptic + Bacoside A	-0.42 ± 0.18 @@@
Epileptic + Carbamazepine	1.13 ± 0.08 @@@**

Values are mean ± SEM of 4-6 separate experiments. Each group consist of 6-8 rats ***p<0.001, **p<0.01, *p<0.05 when compared to control, @@@p<0.001 when compared to epileptic group.

Figure-37
Real Time PCR amplification of $GABA_B$ receptor mRNA from the cerebellum of control and experimental rats



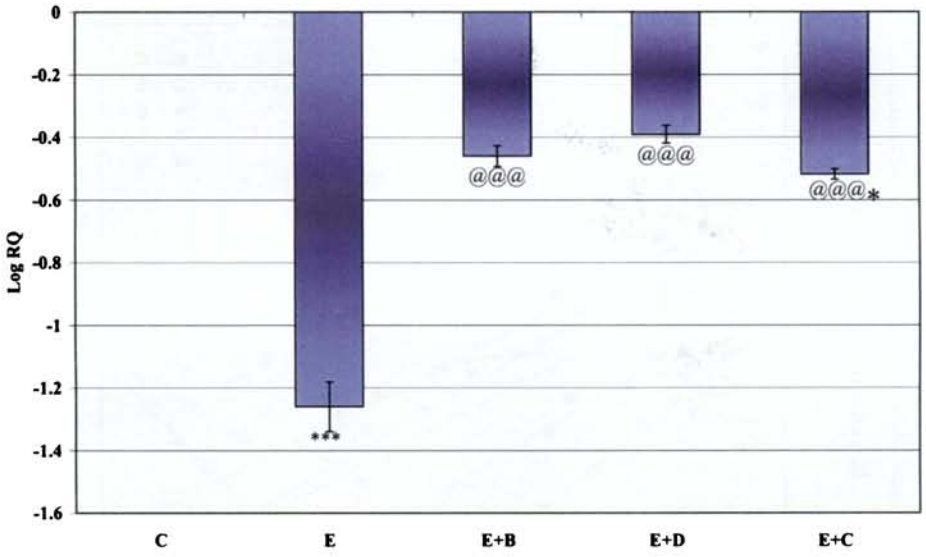
C-Control, E-Epileptic, E+B-Epileptic rats treated with *Bacopa monnieri,* E+D-Epileptic rats treated with Bacoside A and E+C-Epileptic rats treated with Carbamazepine.

Table-38
Real Time PCR amplification of $GABA_B$ receptor mRNA from the cerebellum of control and experimental rats

Animal status	Log RQ value
Control	0
Epileptic	$-0.53 \pm 0.02^{***}$
Epileptic + *Bacopa monnieri*	$-0.11 \pm 0.01^{@@@}$
Epileptic + Bacoside A	$-0.25 \pm 0.01^{@@@*}$
Epileptic + Carbamazepine	$-0.15 \pm 0.03^{@@@}$

Values are mean ± SEM of 4-6 separate experiments. Each group consist of 6-8 rats ***p<0.001, *p<0.05 when compared to control, @@@p<0.001 when compared to epileptic group.

Figure-38
Real Time PCR amplification of GAD mRNA from the cerebellum of control and experimental rats



C-Control, E-Epileptic, E+B-Epileptic rats treated with *Bacopa monnieri,* E+D-Epileptic rats treated with Bacoside A and E+C-Epileptic rats treated with Carbamazepine.

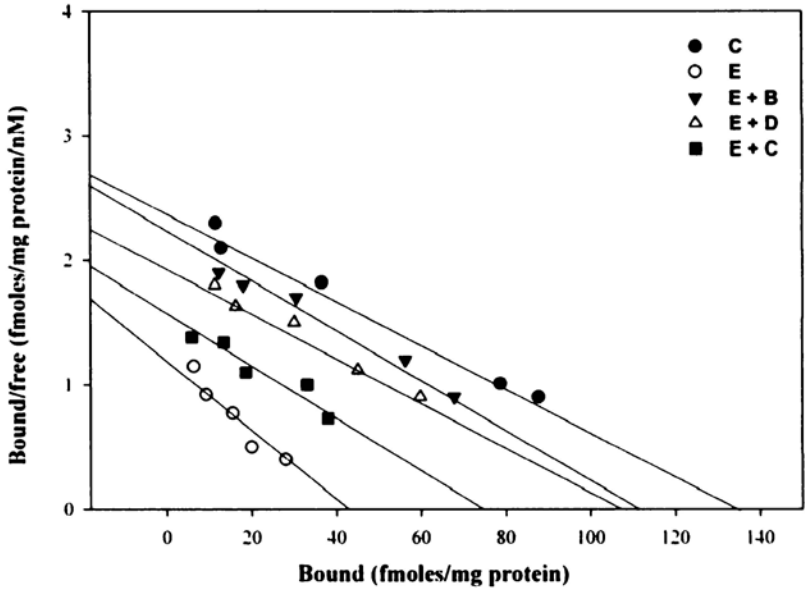
Table-39
Real Time PCR amplification of GAD mRNA from the cerebellum of control and experimental rats

Animal status	Log RQ value
Control	0
Epileptic	$-1.23 \pm 0.07^{***}$
Epileptic + *Bacopa monnieri*	$-0.46 \pm 0.03^{@@@}$
Epileptic + Bacoside A	$-0.39 \pm 0.02^{@@@}$
Epileptic + Carbamazepine	$-0.58 \pm 0.01^{@@@*}$

Values are mean ± SEM of 4-6 separate experiments. Each group consist of 6-8 rats ***p<0.001, *p<0.05 when compared to control, @@@p<0.001 when compared to epileptic group.

Figure-39

Scatchard analysis of [^{3}H]GABA binding against GABA in the corpus striatum of control and experimental rats



C-Control, E-Epileptic, E+B-Epileptic rats treated with *Bacopa monnieri,* E+D-Epileptic rats treated with Bacoside A and E+C-Epileptic rats treated with Carbamazepine.

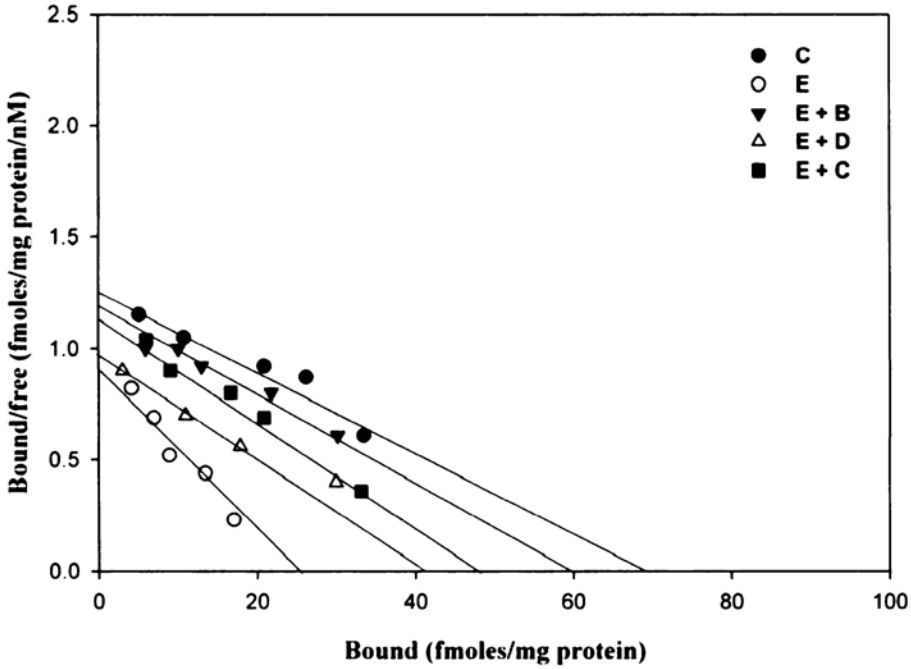
Table-40
Scatchard analysis of [^{3}H]GABA binding against GABA in the corpus striatum of control and experimental rats

Animal status	B_{max} (fmoles/mg protein)	K_d (nM)
Control	137.9 ± 9.1	51.0 ± 3.2
Epileptic	41.7 ± 6.9***	26.0 ± 1.9**
Epileptic + *Bacopa monnieri*	113 ± 8.3$^{@@@}$	43.4 ± 3.3$^{@@}$
Epileptic + Bacoside A	108.8 ± 4.1$^{@@@}$	47.3 ± 3.6$^{@@}$
Epileptic + Carbamazepine	77.3 ± 4.7$^{@@@}$**	40.9 ± 3.4$^{@@}$

Values are mean ± SEM of 4-6 separate experiments. Each group consist of 6-8 rats ***p<0.001, **p<0.01 when compared to control, $^{@@@}$p<0.001, $^{@@}$p<0.01 when compared to epileptic group.

Figure40

Scatchard analysis of [^{3}H]bicuculline against bicuculline in the corpus striatum of control and experimental rats



Control, E-Epileptic, E+B-Epileptic rats treated with *Bacopa monnieri,* E+D-Epileptic rats treated with Bacoside A and E+C-Epileptic rats treated with Carbamazepine.

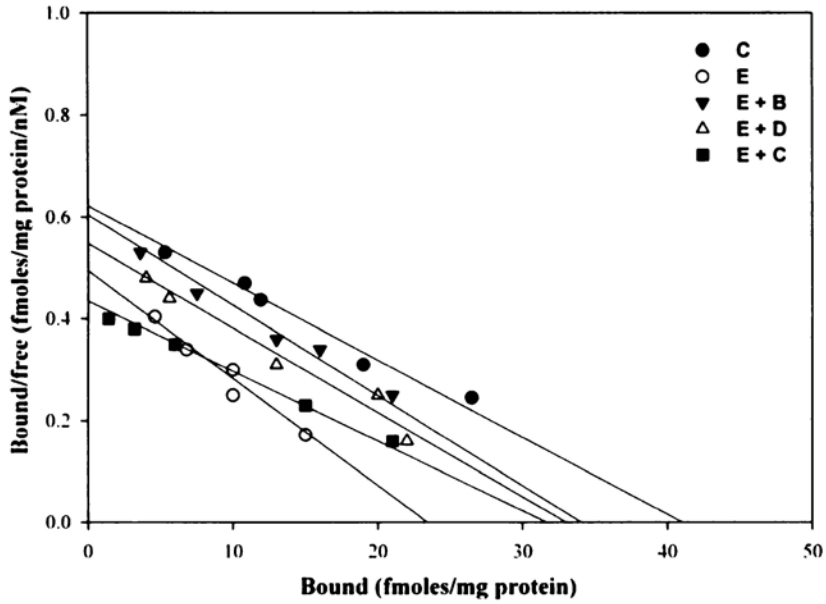
Table-41
Scatchard analysis of [^{3}H]bicuculline binding against bicuculline in the corpus striatum of control and experimental rats

Animal status	B_{max} (fmoles/mg protein)	K_d (nM)
Control	69.8 ± 9.1	77.5 ± 4.8
Epileptic	24.6 ± 6.9***	15.3 ± 0.8***
Epileptic + *Bacopa monnieri*	59.8 ± 8.3$^{@@@}$	49.8± 2.6$^{@@}$
Epileptic + Bacoside A	41.5 ± 4.1$^{@@@}$*	46.1 ± 2.5$^{@@}$
Epileptic + Carbamazepine	44.9 ± 4.7$^{@@@}$*	34.5 ± 2.1$^{@@}$

Values are mean ± SEM of 4-6 separate experiments. Each group consist of 6-8 rats., *p<0.05, ***p<0.001 when compared to control, $^{@@@}$p<0.001, $^{@@}$p<0.01 when compared to epileptic group.

Figure-41

Scatchard analysis of [^{3}H]baclofen binding against baclofen in the corpus striatum of control and experimental rats



Control, E-Epileptic, E+B-Epileptic rats treated with *Bacopa monnieri,* E+D-Epileptic rats treated with Bacoside A and E+C-Epileptic rats treated with Carbamazepine.

Table-42

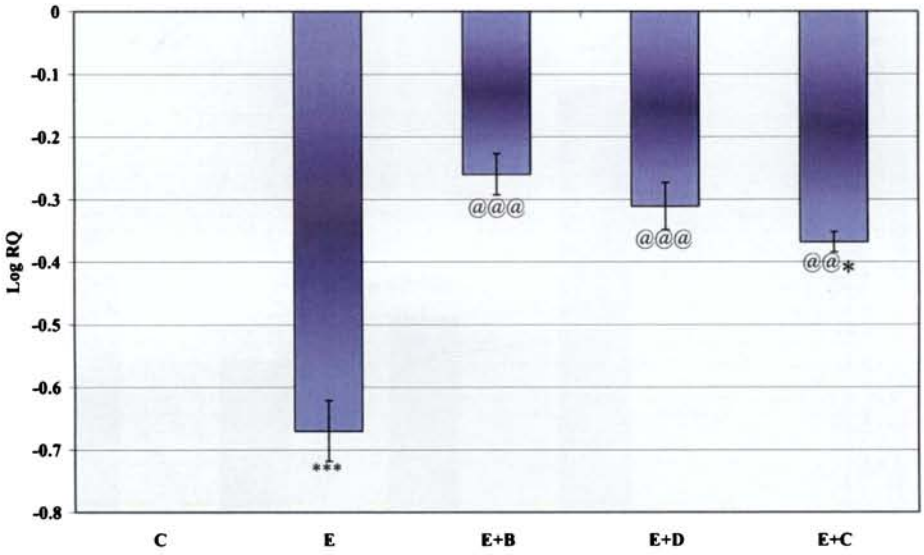
Scatchard analysis of [^{3}H]baclofen binding against baclofen in the corpus striatum of control and experimental rats

Animal status	B_{max} (fmoles/mg protein)	K_d (nM)
Control	41.3 ± 2.7	45.8 ± 3.1
Epileptic	23.6 ± 1.9***	47.2 ± 3.3
Epileptic + *Bacopa monnieri*	33.8 ± 1.3$^{@@@}$	56.3 ± 3.6*
Epileptic + Bacoside A	32.5 ± 2.1$^{@@@}$	65.0 ± 4.1*
Epileptic + Carbamazepine	31.8 ± 2.7$^{@@@}$	79.5 ± 4.6**

Values are mean ± SEM of 4-6 separate experiments. Each group consist of 6-8 rats ***p<0.001, **p<0.01, *p<0.05 when compared to control, $^{@@@}$p<0.001 when compared to epileptic group.

Figure-42

Real Time PCR amplification of $GABA_{A\alpha 1}$ receptor subunit mRNA from the corpus striatum of control and experimental rats



C-Control, E-Epileptic, E+B-Epileptic rats treated with *Bacopa monnieri,* E+D-Epileptic rats treated with Bacoside A and E+C-Epileptic rats treated with Carbamazepine.

Table-43

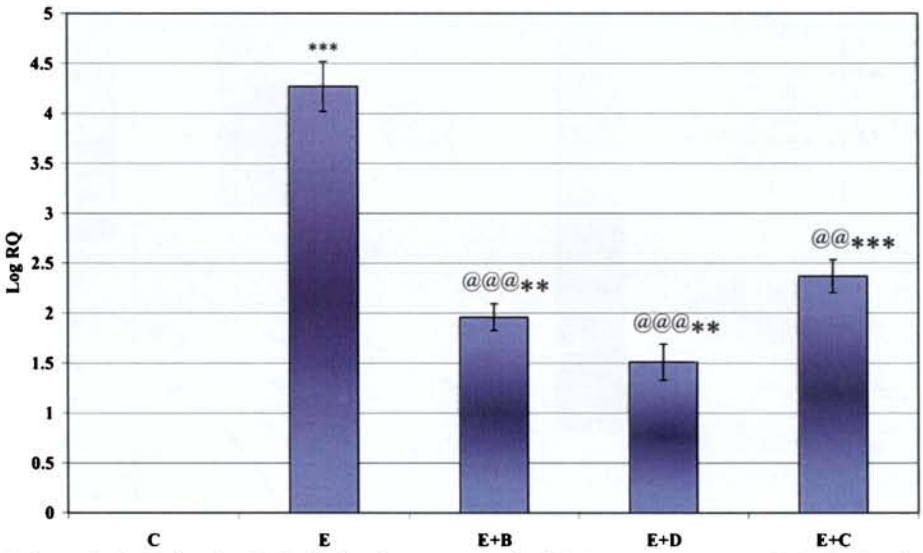
Real Time PCR amplification of $GABA_{A\alpha 1}$ receptor subunit mRNA from the corpus striatum of control and experimental rats

Animal status	Log RQ value
Control	0
Epileptic	$-0.67 \pm 0.04^{***}$
Epileptic + *Bacopa monnieri*	$-0.26 \pm 0.03^{@@@}$
Epileptic + Bacoside A	$-0.31 \pm 0.03^{@@@}$
Epileptic + Carbamazepine	$-1.36 \pm 0.01^{@@*}$

Values are mean ± SEM of 4-6 separate experiments. Each group consist of 6-8 rats ***p<0.001, *p<0.05 when compared to control, @@@p<0.001, @@p<0.01 when compared to epileptic group.

Figure-43

Real Time PCR amplification of $GABA_{A\delta5}$ receptor subunit mRNA from the corpus striatum of control and experimental rats



C-Control, E-Epileptic, E+B-Epileptic rats treated with *Bacopa monnieri,* E+D-Epileptic rats treated with Bacoside A and E+C-Epileptic rats treated with Carbamazepine.

Table-44

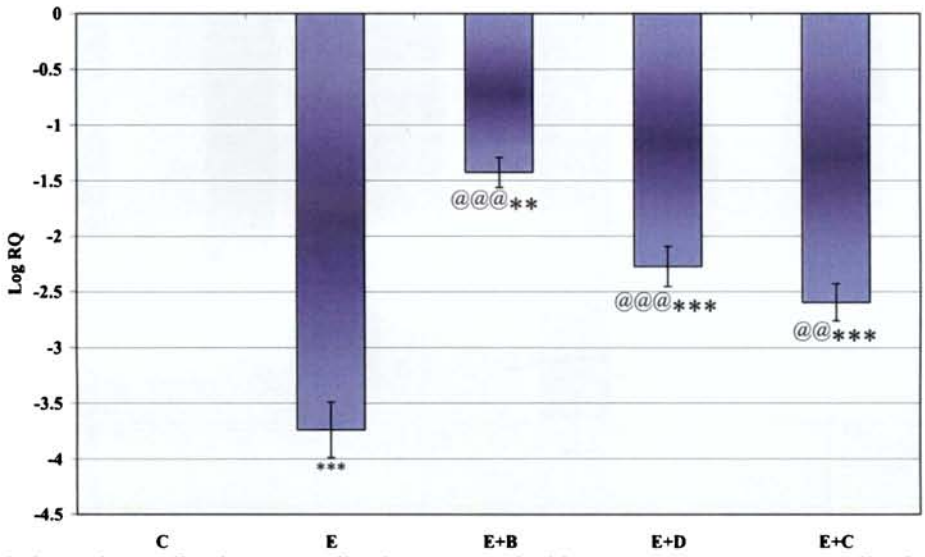
Real Time PCR amplification of $GABA_{A\delta5}$ receptor subunit mRNA from the corpus striatum of control and experimental rats

Animal status	Log RQ value
Control	0
Epileptic	$4.23 \pm 0.24^{***}$
Epileptic + *Bacopa monnieri*	$1.92 \pm 0.13^{@@@**}$
Epileptic + Bacoside A	$1.59 \pm 0.18^{@@@**}$
Epileptic + Carbamazepine	$2.31 \pm 0.16^{@@***}$

Values are mean ± SEM of 4-6 separate experiments. Each group consist of 6-8 rats ***p<0.001, **p<0.01 when compared to control, @@@p<0.001, @@p<0.01 when compared to epileptic group.

Figure-44

Real Time PCR amplification of $GABA_{A\gamma3}$ Receptor subunit mRNA from the corpus striatum of control and experimental rats



C-Control, E-Epileptic, E+B-Epileptic rats treated with *Bacopa monnieri,* E+D-Epileptic rats treated with Bacoside A and E+C-Epileptic rats treated with Carbamazepine.

Table-45

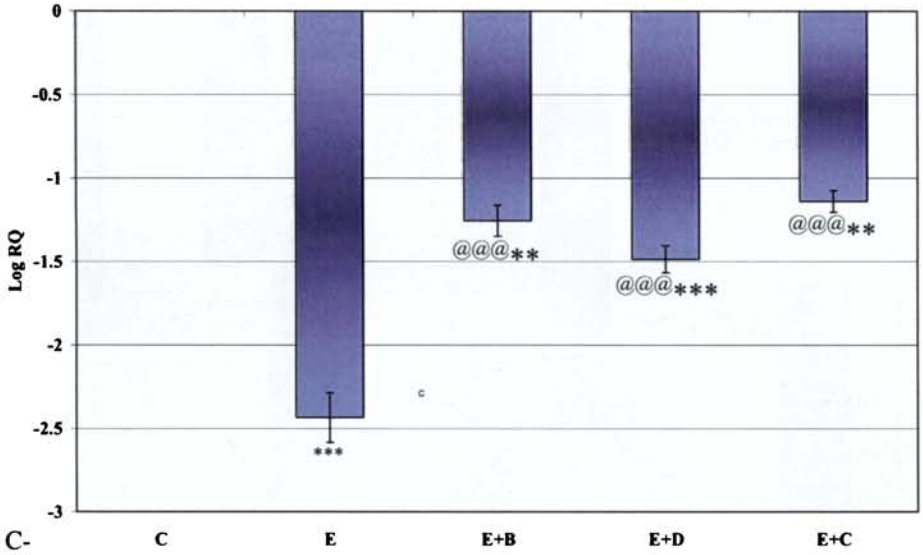
Real Time PCR amplification of $GABA_{A\gamma3}$ Receptor subunit mRNA from the corpus striatum of control and experimental rats

Animal status	Log RQ value
Control	0
Epileptic	$-3.74 \pm 0.24^{***}$
Epileptic + *Bacopa monnieri*	$-1.47 \pm 0.13^{@@@**}$
Epileptic + Bacoside A	$-2.26 \pm 0.18^{@@@***}$
Epileptic + Carbamazepine	$-2.53 \pm 0.16^{@@***}$

Values are mean ± SEM of 4-6 separate experiments. Each group consist of 6-8 rats ***p<0.001, **p<0.01 when compared to control, $^{@@@}$p<0.001, $^{@@}$p<0.01 when compared to epileptic group.

Figure-45

Real Time PCR amplification of $GABA_{A\delta}$ receptor subunit mRNA from the corpus striatum of control and experimental rats



Control, E-Epileptic, E+B-Epileptic rats treated with *Bacopa monnieri,* E+D-Epileptic rats treated with Bacoside A and E+C-Epileptic rats treated with Carbamazepine.

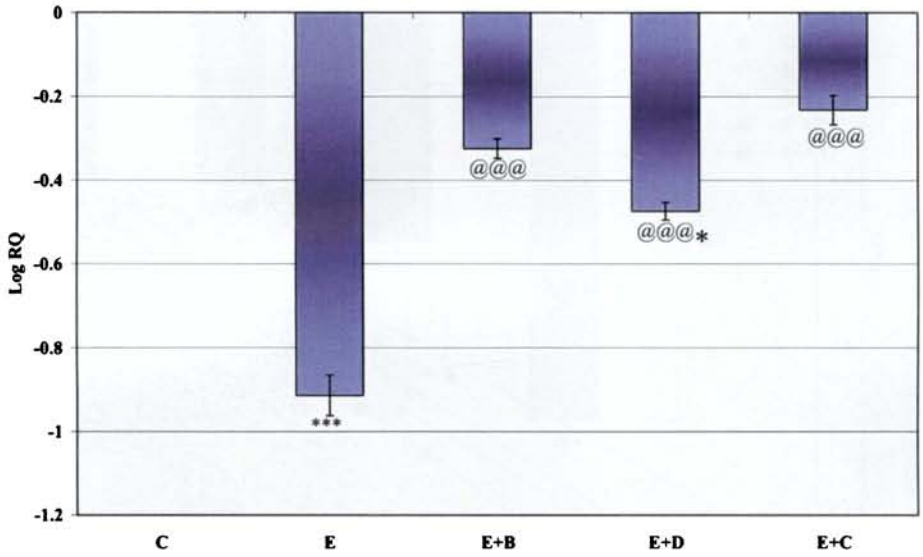
Table-46

Real Time PCR amplification of $GABA_{A\delta}$ receptor subunit mRNA from the corpus striatum of control and experimental rats

Animal status	Log RQ value
Control	0
Epileptic	$-2.35 \pm 0.14^{***}$
Epileptic + *Bacopa monnieri*	$-1.24 \pm 0.09^{@@@**}$
Epileptic + Bacoside A	$-1.49 \pm 0.08^{@@@***}$
Epileptic + Carbamazepine	$-1.12 \pm 0.06^{@@**}$

Values are mean ± SEM of 4-6 separate experiments. Each group consist of 6-8 rats ***p<0.001, **p<0.01 when compared to control, $^{@@@}$p<0.001, $^{@@}$p<0.01 when compared to epileptic group.

Figure-46
Real Time PCR amplification of $GABA_B$ mRNA from the corpus striatum of control and experimental rats



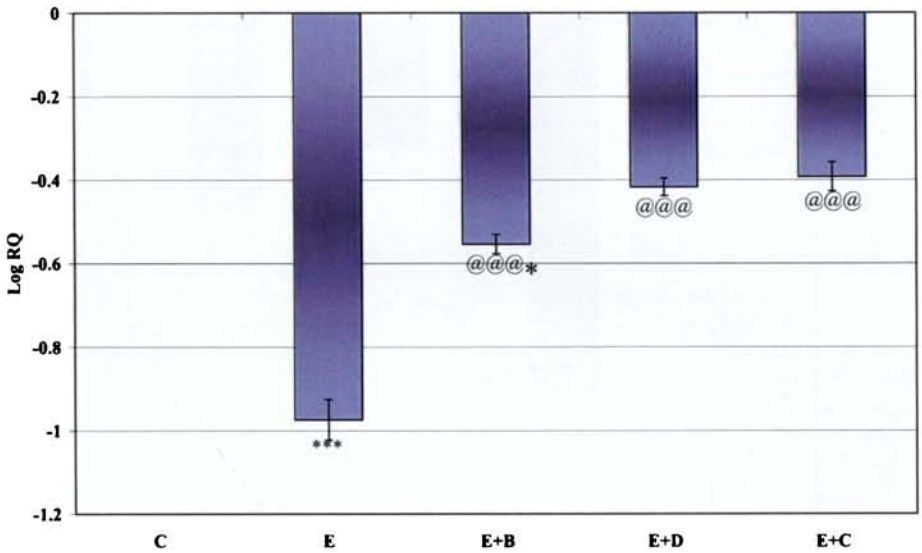
C-Control, E-Epileptic, E+B-Epileptic rats treated with *Bacopa monnieri,* E+D-Epileptic rats treated with Bacoside A and E+C-Epileptic rats treated with Carbamazepine.

Table-47
Real Time PCR amplification of $GABA_B$ mRNA from the corpus striatum of control and experimental rats

Animal status	Log RQ value
Control	0
Epileptic	-0.91 ± 0.04***
Epileptic + *Bacopa monnieri*	-0.32 ± 0.02@@@
Epileptic + Bacoside A	-0.47 ± 0.02@@@*
Epileptic + Carbamazepine	-0.23 ± 0.03@@@

Values are mean ± SEM of 4-6 separate experiments. Each group consist of 6-8 rats ***$p<0.001$, *$p<0.05$ when compared to control, @@@$p<0.001$ when compared to epileptic group.

Figure-47
Real Time PCR amplification of GAD mRNA from the corpus striatum of control and experimental rats



C-Control, E-Epileptic, E+B-Epileptic rats treated with *Bacopa monnieri,* E+D-Epileptic rats treated with Bacoside A and E+C-Epileptic rats treated with Carbamazepine.

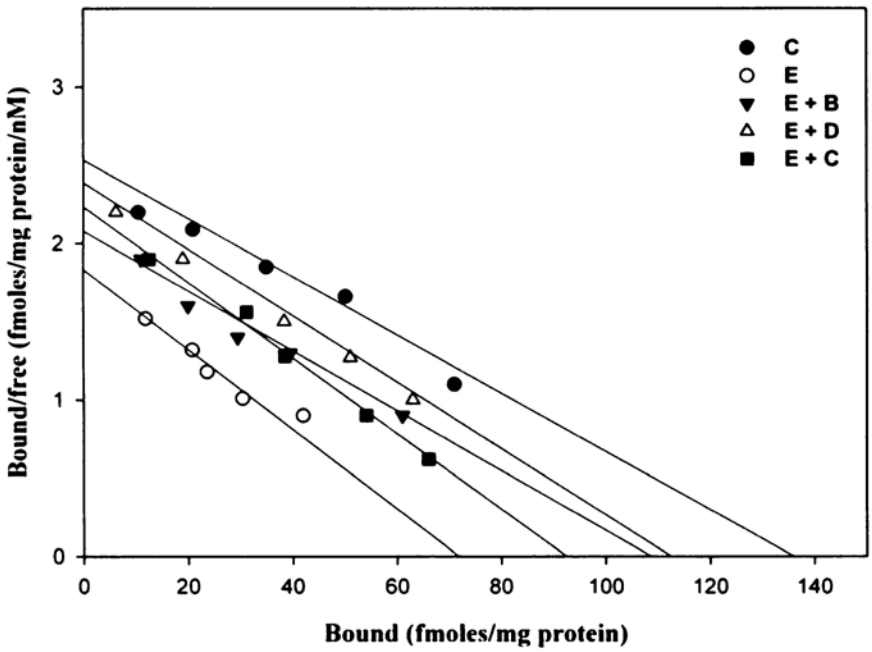
Table-48
Real Time PCR amplification of GAD mRNA from the corpus striatum of control and experimental rats

Animal status	Log RQ value
Control	0
Epileptic	-0.97 ± 0.04***
Epileptic + *Bacopa monnieri*	-0.57 ± 0.01$^{@@@*}$
Epileptic + Bacoside A	-0.48 ± 0.02$^{@@@}$
Epileptic + Carbamazepine	-0.39 ± 0.04$^{@@@}$

Values are mean ± SEM of 4-6 separate experiments. Each group consist of 6-8 rats ***p<0.001, *p<0.05 when compared to control, $^{@@@}$p<0.001 when compared to epileptic group.

Figure-48

Scatchard analysis of [^{3}H]GABA binding against GABA in the brain stem of control and experimental rats



C-Control, E-Epileptic, E+B-Epileptic rats treated with *Bacopa monnieri,* E+D-Epileptic rats treated with Bacoside A and E+C-Epileptic rats treated with Carbamazepine.

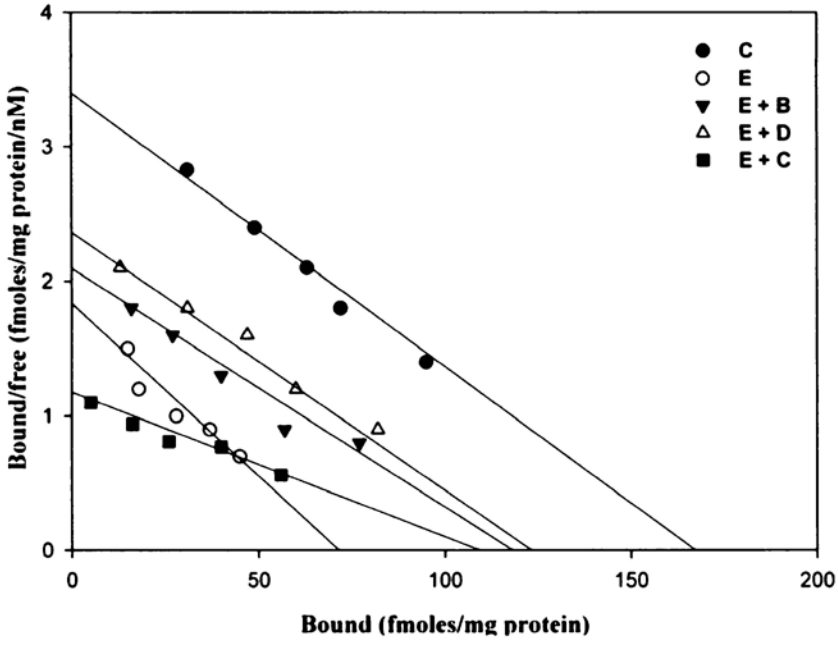
Table-49
Scatchard analysis of [^{3}H]GABA binding against GABA in the brain stem of control and experimental rats

Animal status	B_{max} (fmoles/mg protein)	K_d (nM)
Control	136.8 ± 5.9	52.6 ± 3.9
Epileptic	72.0 ± 4.7***	40 ± 3.0*
Epileptic + *Bacopa monnieri*	109.8 ± 5.6$^{@@@}$	47.7 ± 2.7
Epileptic + Bacoside A	112.2 ± 4.3 $^{@@@}$	51.0 ± 3.6$^{@}$
Epileptic + Carbamazepine	92.4 ± 6.8$^{@@@}$*	44.0 ± 2.5

Values are mean ± SEM of 4-6 separate experiments. Each group consist of 6-8 rats ***p<0.001, *p<0.05 when compared to control, $^{@@@}$p<0.001, $^{@}$p<0.05 when compared to epileptic group.

Figure-49

Scatchard analysis of [^{3}H]bicuculline binding against bicuculline in the brain stem of control and experimental rats



C-Control, E-Epileptic, E+B-Epileptic rats treated with *Bacopa monnieri,* E+D-Epileptic rats treated with Bacoside A and E+C-Epileptic rats treated with Carbamazepine.

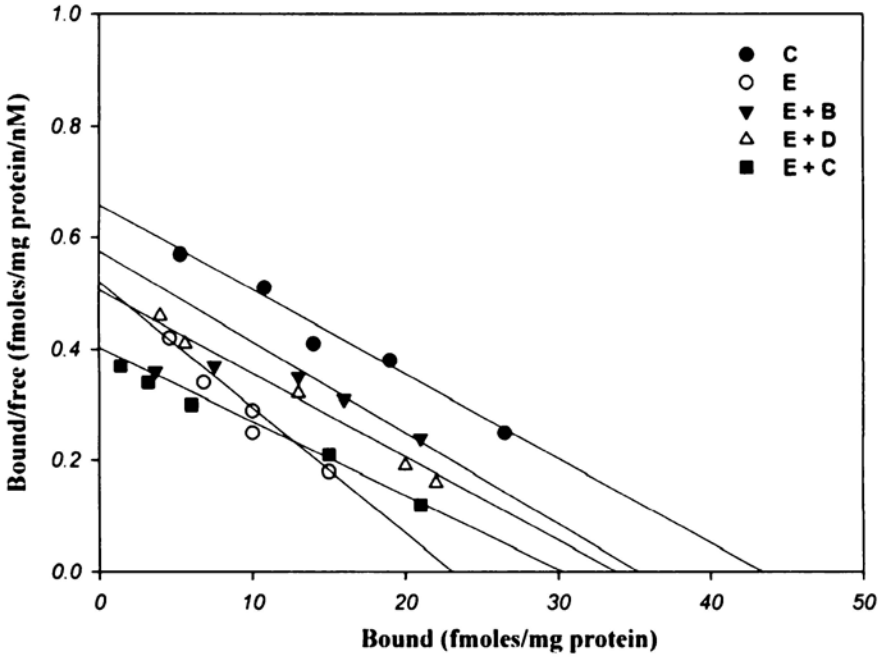
Table-50
Scatchard analysis of [^{3}H]bicuculline binding against bicuculline in the brain stem of control and experimental rats

Animal status	B_{max} (fmoles/mg protein)	K_d (nM)
Control	167.3 ± 7.4	64.3 ± 3.2
Epileptic	69.8 ± 4.1***	38.7 ± 1.4***
Epileptic + *Bacopa monnieri*	123.3 ± 5.9$^{@@@}$*	61.6.0 ± 3.4$^{@@@}$
Epileptic + Bacoside A	126.9 ± 4.4 $^{@@@}$*	57.6 ± 2.5$^{@@}$
Epileptic + Carbamazepine	110.5 ± 6.7$^{@@@}$**	100.4 ± 4.6$^{@@@}$**

Values are mean ± SEM of 4-6 separate experiments. Each group consist of 6-8 rats ***p<0.001, **p<0.01, *p<0.05 when compared to control, $^{@@@}$p<0.001, $^{@@}$p<0.01 when compared to epileptic group.

Figure-50

Scatchard analysis of [^{3}H]baclofen binding against baclofen in the brain stem of control and experimental rats



C-Control, E-Epileptic, E+B-Epileptic rats treated with *Bacopa monnieri,* E+D-Epileptic rats treated with Bacoside A and E+C-Epileptic rats treated with Carbamazepine.

Table-51

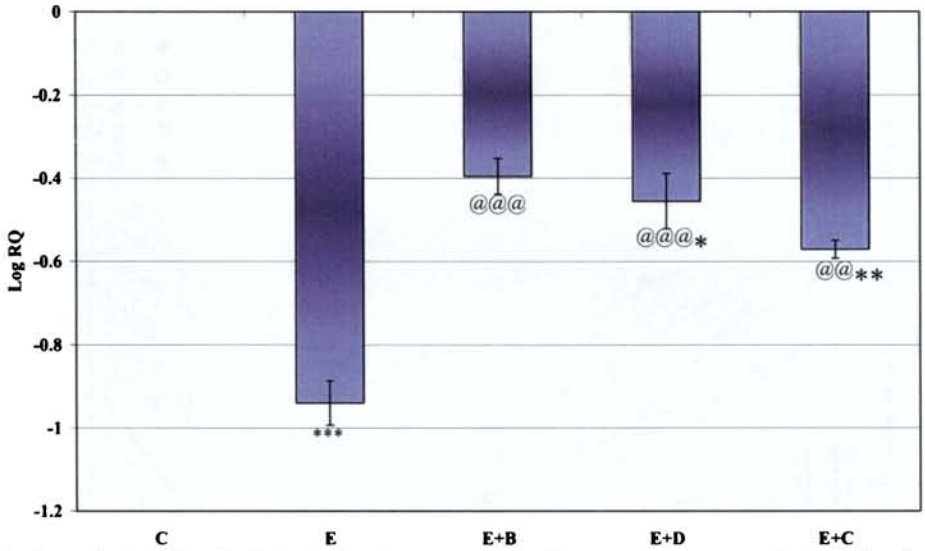
Scatchard analysis of [^{3}H]baclofen binding against baclofen in the brain stem of control and experimental rats

Animal status	B_{max} (fmoles/mg protein)	K_d (nM)
Control	43.9 ± 2.8	73.1 ± 0.03
Epileptic	23.6 ± 2.1***	47.2 ± 0.01**
Epileptic + *Bacopa monnieri*	33.8 ± 5.6$^{@@@}$	67.6 ± 0.02$^{@@}$
Epileptic + Bacoside A	34.7 ± 4.7 $^{@@@}$	69.4 ± 0.02$^{@@}$
Epileptic + Carbamazepine	30.6 ± 3.2$^{@@@}$	76.5 ± 0.01$^{@@}$

Values are mean ± SEM of 4-6 separate experiments. Each group consist of 6-8 rats ***p<0.001, **p<0.01 when compared to control, $^{@@@}$p<0.001, $^{@@}$p<0.01 when compared to epileptic group.

Figure-51

Real Time PCR amplification of $GABA_{A\acute{a}1}$ receptor subunit mRNA from the brain stem of control and experimental rats



C-Control, E-Epileptic, E+B-Epileptic rats treated with *Bacopa monnieri,* E+D-Epileptic rats treated with Bacoside A and E+C-Epileptic rats treated with Carbamazepine.

Table-52

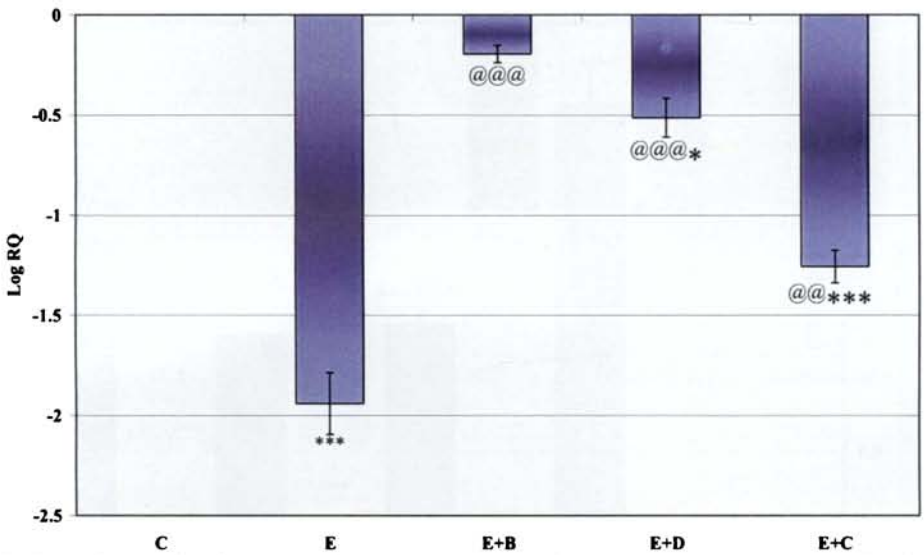
Real Time PCR amplification of $GABA_{A\acute{a}1}$ receptor subunit mRNA from the brain stem of control and experimental rats

Animal status	Log RQ value
Control	0
Epileptic	$-0.93 \pm 0.053^{***}$
Epileptic + *Bacopa monnieri*	$-0.39 \pm 0.042^{@@@}$
Epileptic + *Bacoside A*	$-0.45 \pm 0.066^{@@@*}$
Epileptic + Carbamazepine	$-0.57 \pm 0.021^{@@**}$

Values are mean ± SEM of 4-6 separate experiments. Each group consist of 6-8 rats ***p<0.001 when compared to control, @@@p<0.001, @@p<0.01 when compared to epileptic group.

Figure-52

Real Time PCR amplification of $GABA_{A\acute{a}5}$ receptor subunit mRNA from the brain stem of control and experimental rats



C-Control, E-Epileptic, E+B-Epileptic rats treated with *Bacopa monnieri,* E+D-Epileptic rats treated with Bacoside A and E+C-Epileptic rats treated with Carbamazepine.

Table-53

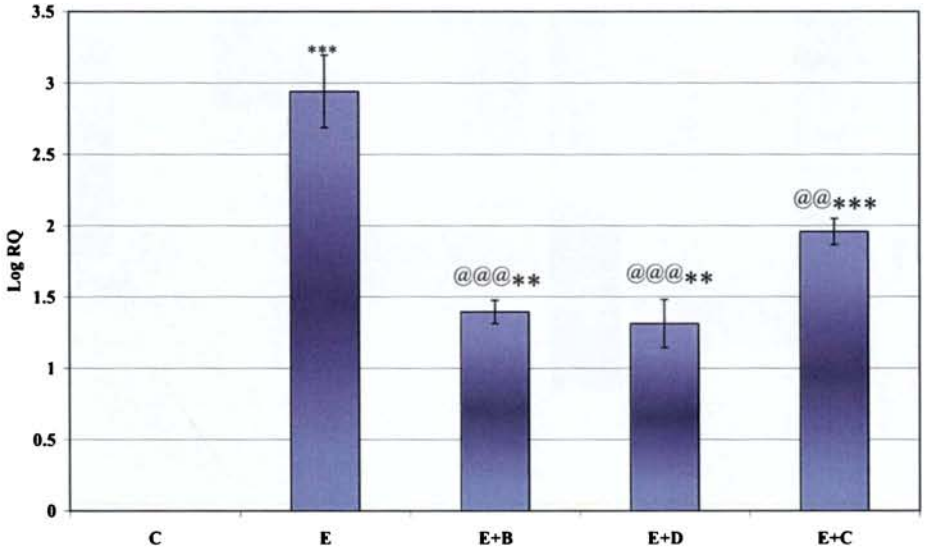
Real Time PCR amplification of $GABA_{A\acute{a}5}$ receptor subunit mRNA from the brain stem of control and experimental rats

Animal status	Log RQ value
Control	0
Epileptic	$-1.93 \pm 0.15^{***}$
Epileptic + *Bacopa monnieri*	$-0.19 \pm 0.042^{@@@}$
Epileptic + *Bacoside A*	$-0.51 \pm 0.096^{@@@*}$
Epileptic + Carbamazepine	$-1.25 \pm 0.081^{@@***}$

Values are mean ± SEM of 4-6 separate experiments. Each group consist of 6-8 rats ***p<0.001, *p<0.05 when compared to control, $^{@@@}$p<0.001, $^{@@}$p<0.01 when compared to epileptic group.

Figure-53

Real Time PCR amplification of $GABA_{A\gamma3}$ Receptor subunit mRNA from the brain stem of control and experimental rats



C-Control, E-Epileptic, E+B-Epileptic rats treated with *Bacopa monnieri,* E+D-Epileptic rats treated with Bacoside A and E+C-Epileptic rats treated with Carbamazepine.

Table-54

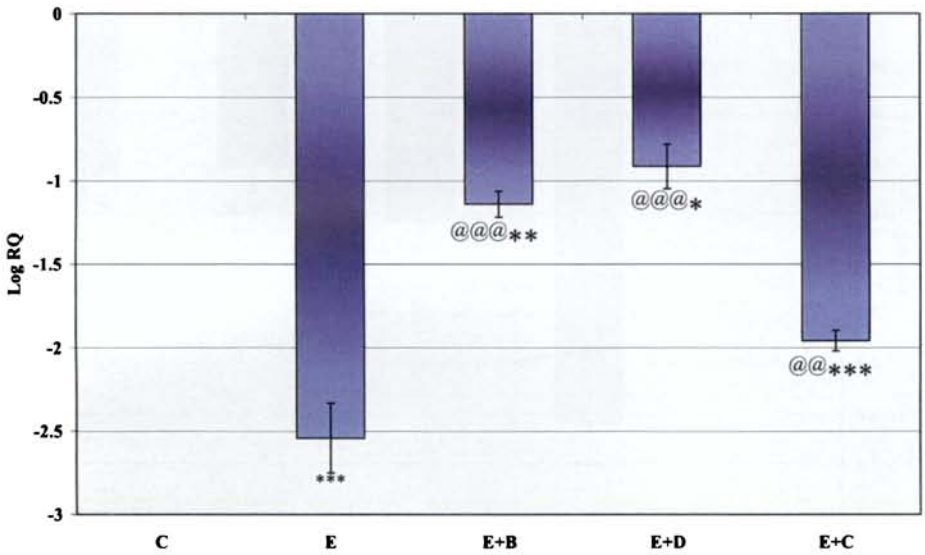
Real Time PCR amplification of $GABA_{A\gamma3}$ Receptor subunit mRNA from the brain stem of control and experimental rats

Animal status	Log RQ value
Control	0
Epileptic	2.93 ± 0.25 ***
Epileptic + *Bacopa monnieri*	$1.39 \pm 0.082^{@@@}$**
Epileptic + *Bacoside A*	$1.31 \pm 0.16^{@@@}$**
Epileptic + Carbamazepine	$1.95 \pm 0.091^{@@}$***

Values are mean ± SEM of 4-6 separate experiments. Each group consist of 6-8 rats ***p<0.001, **p<0.01 when compared to control, $^{@@@}$p<0.001, $^{@@}$p<0.01 when compared to epileptic group.

Figure-54

Real Time PCR amplification of $GABA_{A\delta}$ receptor subunit mRNA from the brain stem of control and experimental rats



C-Control, E-Epileptic, E+B-Epileptic rats treated with *Bacopa monnieri,* E+D-Epileptic rats treated with Bacoside A and E+C-Epileptic rats treated with Carbamazepine.

Table-55

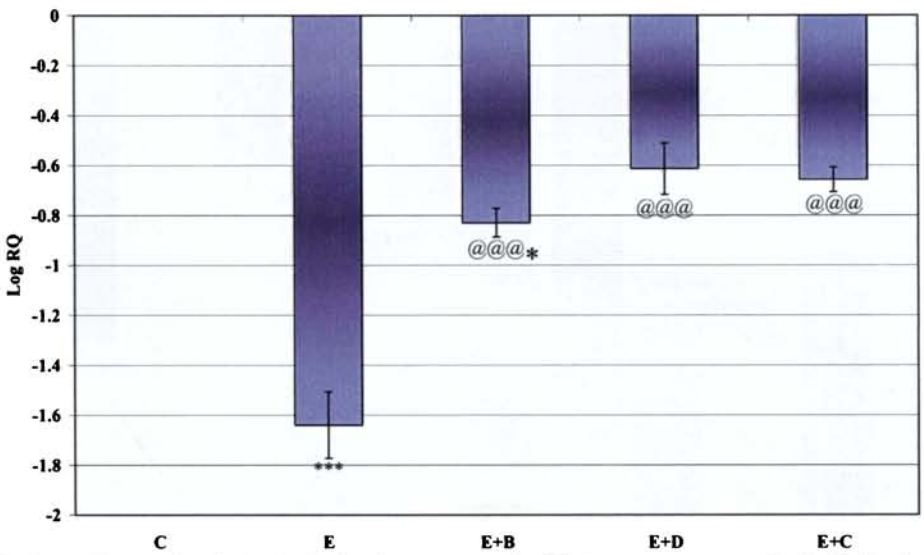
Real Time PCR amplification of $GABA_{A\delta}$ receptor subunit mRNA from the brain stem of control and experimental rats

Animal status	Log RQ value
Control	0
Epileptic	$-2.53 \pm 0.20^{***}$
Epileptic + *Bacopa monnieri*	$-1.13 \pm 0.07^{@@@**}$
Epileptic + *Bacoside A*	$-0.91 \pm 0.13^{@@@*}$
Epileptic + Carbamazepine	$-1.95 \pm 0.06^{@@***}$

Values are mean ± SEM of 4-6 separate experiments. Each group consist of 6-8 rats ***p<0.001, **p<0.01, *p<0.05 when compared to control, $^{@@@}$p<0.001, $^{@@}$p<0.01 when compared to epileptic group.

Figure-55

Real Time PCR amplification of $GABA_B$ receptor mRNA from the brain stem of control and experimental rats



C-Control, E-Epileptic, E+B-Epileptic rats treated with *Bacopa monnieri,* E+D-Epileptic rats treated with Bacoside A and E+C-Epileptic rats treated with Carbamazepine.

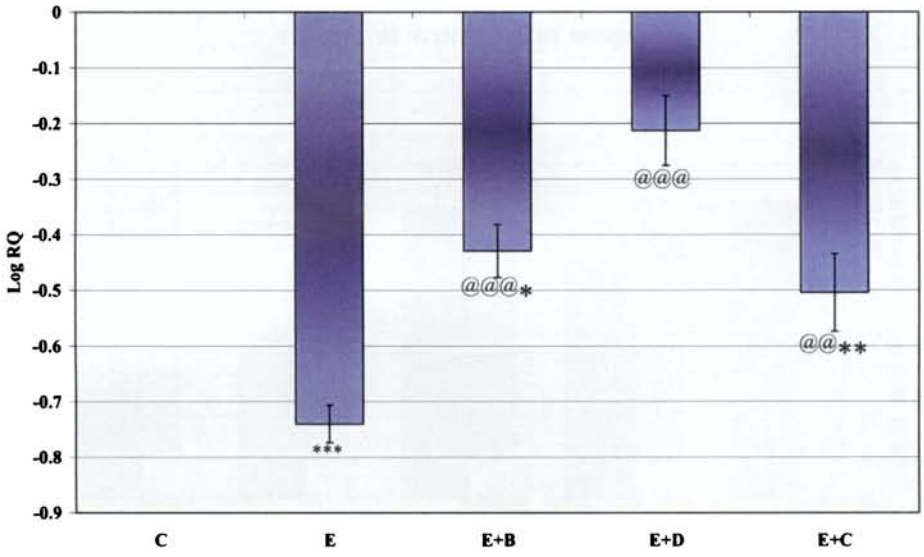
Table-56

Real Time PCR amplification of $GABA_B$ receptor mRNA from the brain stem of control and experimental rats

Animal status	Log RQ value
Control	0
Epileptic	$-1.63 \pm 0.13^{***}$
Epileptic + *Bacopa monnieri*	$-0.82 \pm 0.05^{@@@*}$
Epileptic + *Bacoside A*	$-0.61 \pm 0.10^{@@@}$
Epileptic + Carbamazepine	$-0.65 \pm 0.04^{@@@}$

Values are mean ± SEM of 4-6 separate experiments. Each group consist of 6-8 rats ***p<0.001, *p<0.05 when compared to control, @@@p<0.001 when compared to epileptic group.

Figuer-56
Real Time PCR amplification of GAD mRNA from the brain stem of control and experimental rats



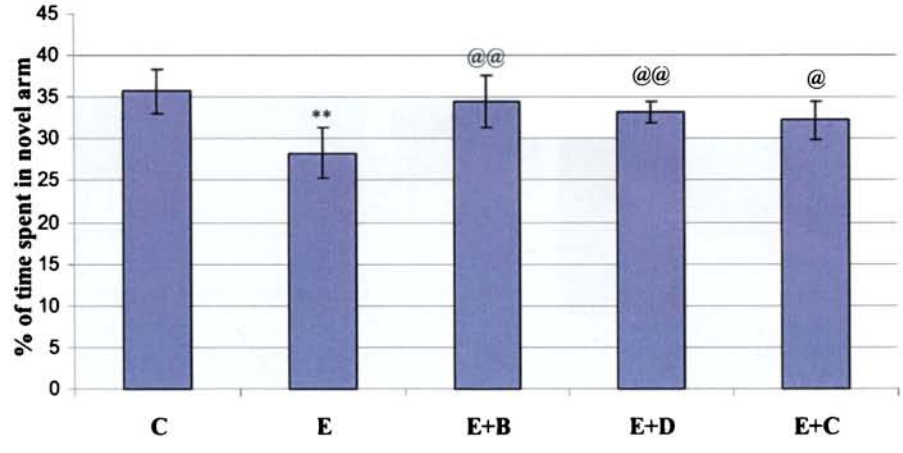
C-Control, E-Epileptic, E+B-Epileptic rats treated with *Bacopa monnieri,* E+D-Epileptic rats treated with Bacoside A and E+C-Epileptic rats treated with Carbamazepine.

Table-57
Real Time PCR amplification of GAD mRNA from the brain stem of control and experimental rats

Animal status	Log RQ value
Control	0
Epileptic	-0.73 ± 0.033 ***
Epileptic + *Bacopa monnieri*	-0.42 ± 0.047$^{@@@*}$
Epileptic + *Bacoside A*	-0.21 ± 0.062$^{@@@}$
Epileptic + Carbamazepine	-0.50 ± 0.069$^{@@**}$

Values are mean ± SEM of 4-6 separate experiments. Each group consist of 6-8 rats ***p<0.001, **p<0.01, *p<0.05 when compared to control, $^{@@@}$p<0.001, $^{@@}$p<0.01 when compared to epileptic group.

Figure-57
Behavioral response of control and experimental rats on time spent in novel arm in Y maze



C-Control, E-Epileptic, E+B-Epileptic rats treated with *Bacopa monnieri,* E+D-Epileptic rats treated with Bacoside A and E+C-Epileptic rats treated with Carbamazepine.

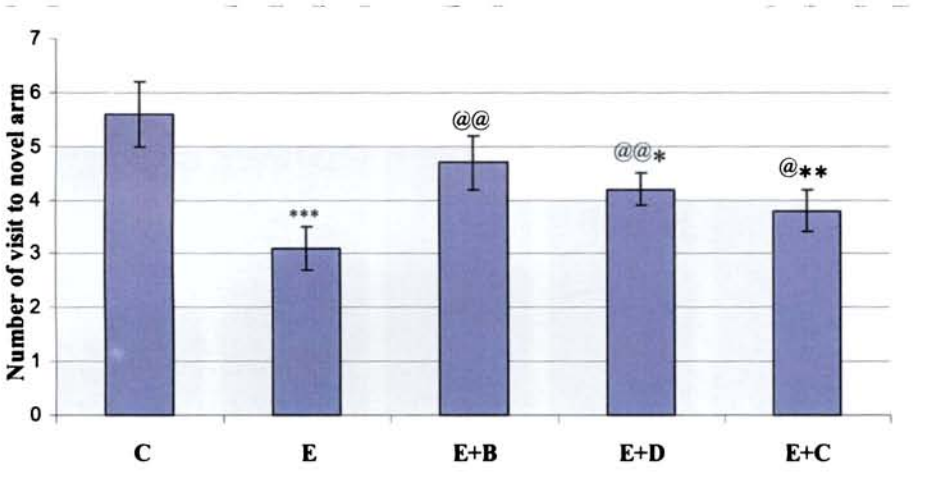
Table-58
Behavioral response of control and experimental rats on time spent in novel arm in Y maze

Animal status	% of time spent in noval arm
Control	35.7 ± 2.6
Epileptic	28.3 ± 3.1**
Epileptic + *Bacopa monnieri*	34.5 ± 3.2$^{@@}$
Epileptic + *Bacoside A*	33.2 ± 1.3$^{@@}$
Epileptic + Carbamazepine	32.2 ± 2.3$^{@}$

Values are mean ± SEM of 4-6 separate experiments. Each group consist of 6-8 rats **$p<0.01$ when compared to control, $^{@@}p<0.01$, $^{@}p<0.05$ when compared to epileptic group.

Figure-58

Behavioral response of control and experimental rats on number of visit to novel arm (count/5 minutes) in Y maze



C-Control, E-Epileptic, E+B-Epileptic rats treated with *Bacopa monnieri,* E+D-Epileptic rats treated with Bacoside A and E+C-Epileptic rats treated with Carbamazepine.

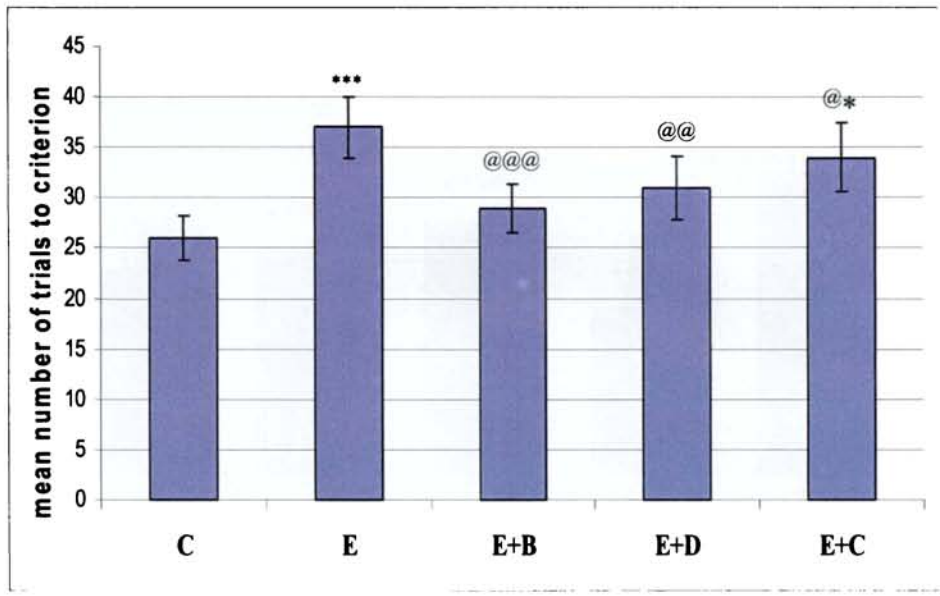
Table-59

Behavioral response of control and experimental rats on number of visit to novel arm (count/5 minutes) in Y maze

Animal status	Number of visit to novel arm
Control	5.6 ± 0.6
Epileptic	3.1 ± 0.4***
Epileptic + *Bacopa monnieri*	4.7 ± 0.5$^{@@}$
Epileptic + *Bacoside A*	4.2 ± 0.3$^{@@}$*
Epileptic + Carbamazepine	3.8 ± 0.4$^{@}$**

Values are mean ± SEM of 4-6 separate experiments. Each group consist of 6-8 rats ***p<0.001, **p<0.01, *p<0.05 when compared to control, $^{@@}$p<0.01, $^{@}$p<0.05 when compared to epileptic group.

Figure-59
Behavioral response of control and experimental rats on criterion performance in radial arm maze



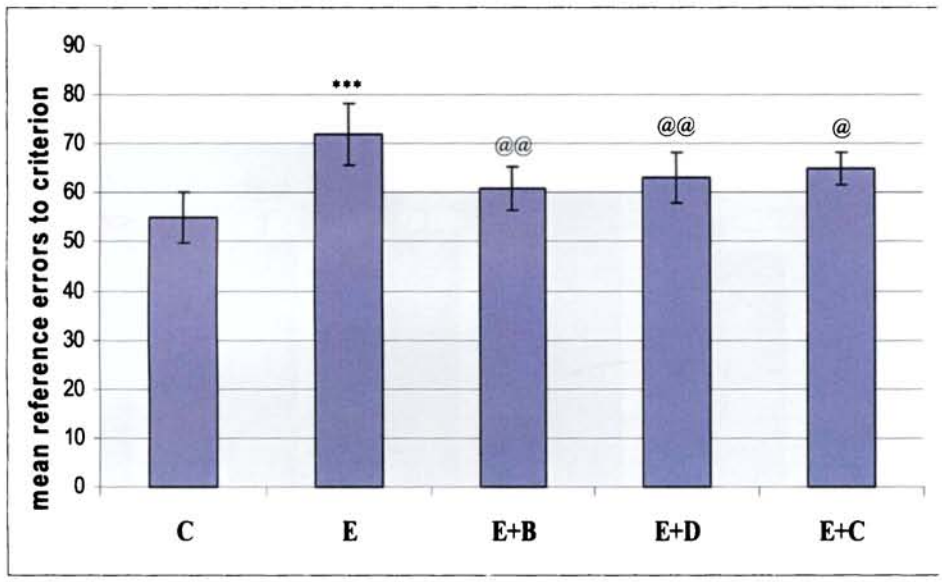
C-Control, E-Epileptic, E+B-Epileptic rats treated with *Bacopa monnieri,* E+D-Epileptic rats treated with Bacoside A and E+C-Epileptic rats treated with Carbamazepine.

Table-60
Behavioral response of control and experimental rats on criterion performance in radial arm maze

Animal status	Mean number of trials to criterion
Control	26.3 ± 2.3
Epileptic	37.7 ± 2.9***
Epileptic + *Bacopa monnieri*	31.4 ± 2.4$^{@@@}$
Epileptic + *Bacoside A*	32.9 ± 2.1$^{@@}$
Epileptic + Carbamazepine	34.8 ± 2.7$^{@}$*

Values are mean ± SEM of 4-6 separate experiments. Each group consist of 6-8 rats ***p<0.001 when compared to control, $^{@@@}$p<0.001, $^{@@}$p<0.01, $^{@}$p<0.05 when compared to epileptic group.

Figure-60
Behavioral response of control and experimental rats on reference errors in radial arm maze



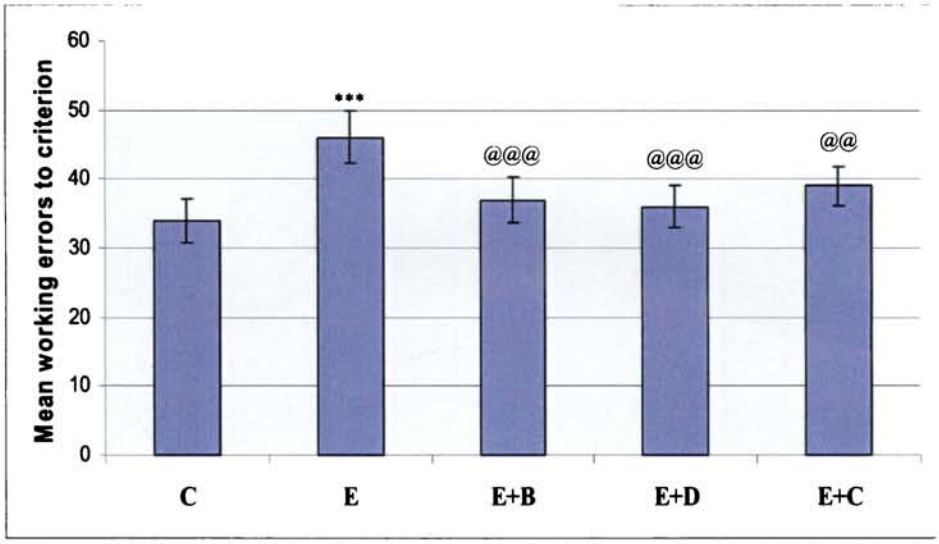
C-Control, E-Epileptic, E+B-Epileptic rats treated with *Bacopa monnieri,* E+D-Epileptic rats treated with Bacoside A and E+C-Epileptic rats treated with Carbamazepine.

Table-61
Behavioral response of control and experimental rats on reference errors in radial arm maze

Animal status	Mean reference errors to criterion
Control	55.6 ± 3.3
Epileptic	72.2 ± 4.1***
Epileptic + *Bacopa monnieri*	61.8 ± 3.2$^{@@}$
Epileptic + *Bacoside A*	63.4 ± 3.1$^{@@}$
Epileptic + Carbamazepine	65.5 ± 3.4$^{@}$

Values are mean ± SEM of 4-6 separate experiments. Each group consist of 6-8 rats ***p<0.001 when compared to control, $^{@@}$p<0.01, $^{@}$p<0.05 when compared to epileptic group.

Figure-61
Behavioral response of control and experimental rats on working errors in radial arm maze



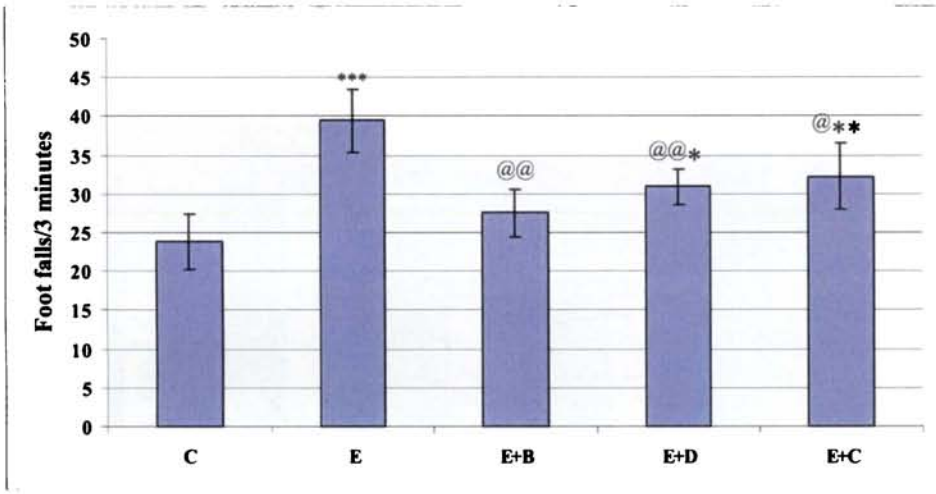
C-Control, E-Epileptic, E+B-Epileptic rats treated with *Bacopa monnieri,* E+D-Epileptic rats treated with Bacoside A and E+C-Epileptic rats treated with Carbamazepine.

Table-62
Behavioral response of control and experimental rats on working errors in radial arm maze

Animal status	Mean working errors to criterion
Control	34.5 ± 3.2
Epileptic	46.9 ± 3.8***
Epileptic + *Bacopa monnieri*	37.3 ± 3.4$^{@@@}$
Epileptic + *Bacoside A*	36.7 ± 3.1$^{@@@}$
Epileptic + Carbamazepine	39.4 ± 2.9$^{@@}$

Values are mean ± SEM of 4-6 separate experiments. Each group consist of 6-8 rats ***$p<0.001$ when compared to control, $^{@@@}p<0.001$, $^{@@}p<0.01$ when compared to epileptic group.

Figure-62
Behavioral response of control and experimental rats on grid walk test



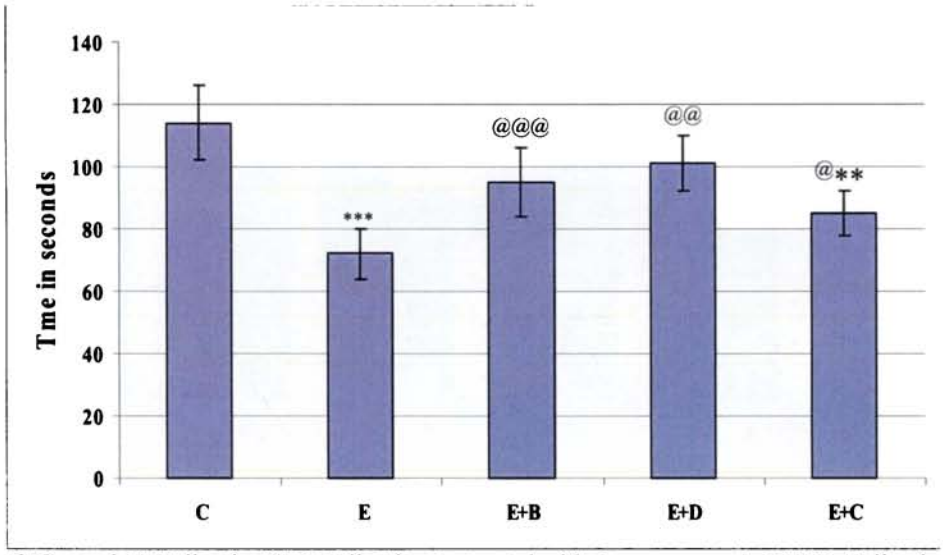
C-Control, E-Epileptic, E+B-Epileptic rats treated with *Bacopa monnieri,* E+D-Epileptic rats treated with Bacoside A and E+C-Epileptic rats treated with Carbamazepine.

Table-63
Behavioral response of control and experimental rats on grid walk test

Animal status	Foot slips/3 minutes
Control	23.8 ± 3.6
Epileptic	39.4 ± 4.1***
Epileptic + *Bacopa monnieri*	27.5 ± 3.1$^{@@}$
Epileptic + *Bacoside A*	30.9 ± 2.3$^{@@}$*
Epileptic + Carbamazepine	32.2 ± 4.3$^{@}$**

Values are mean ± SEM of 4-6 separate experiments. Each group consist of 6-8 rats ***p<0.001, **p<0.01, *p<0.05 when compared to control, $^{@@}$p<0.01 and $^{@}$p<0.05 when compared to epileptic group.

Figure-63
Behavioral response of control and experimental rats on narrow beam test



C-Control, E-Epileptic, E+B-Epileptic rats treated with *Bacopa monnieri,* E+D-Epileptic rats treated with Bacoside A and E+C-Epileptic rats treated with Carbamazepine.

Table-64
Behavioral response of control and experimental rats on narrow beam test

Animal status	Time in seconds
Control	114.8± 12.0
Epileptic	72.6 ± 8.1***
Epileptic + *Bacopa monnieri*	95.2 ± 11.6$^{@@@}$
Epileptic + Bacoside A	101.8 ± 9.3$^{@@}$
Epileptic + Carbamazepine	85.5 ± 7.5$^{@}$**

Values are mean ± SEM of 4-6 separate experiments. Each group consist of 6-8 rats ***p<0.01, **p<0.01 when compared to control, $^{@@@}$p<0.001, $^{@@}$p<0.01, $^{@}$p<0.05 when compared to epileptic group.

Table-65

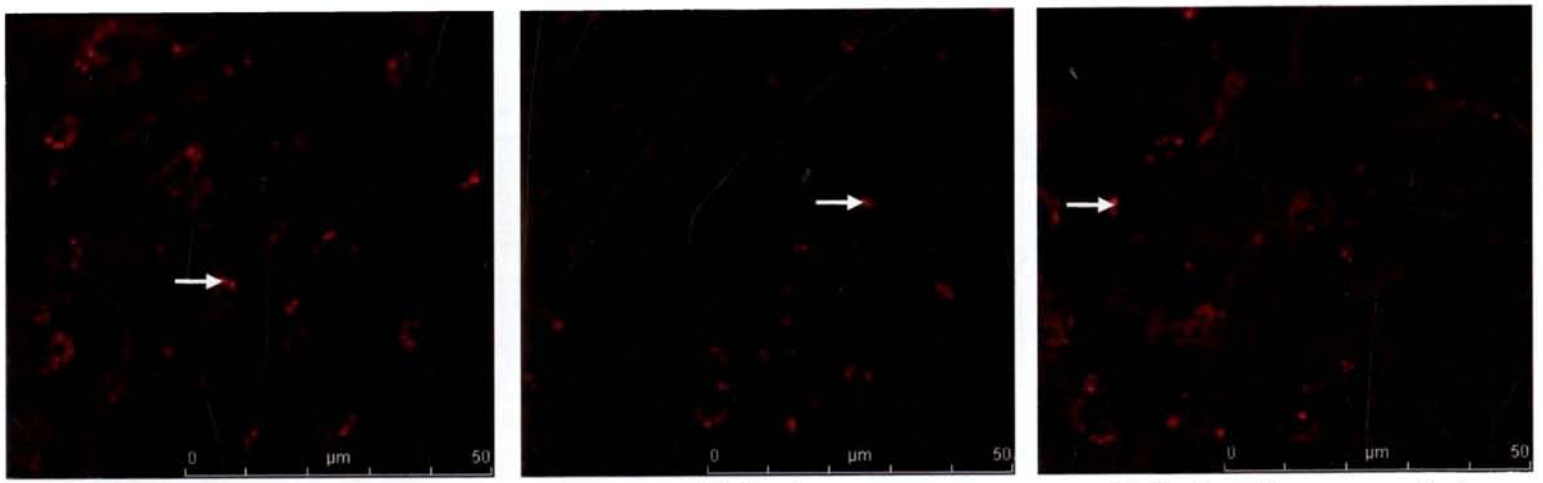
Behavioral response of control and experimental rats on open field test

Animal status	Grooming	Sniffing	Resting	Staring
C	3.32±0.29	13.04±1.0	5.19±0.42	3.55±0.27
E	2.92±0.14	7.18±0.68**	12.65±1.1***	2.33±0.19
E+B	5.25±0.61@@	11.32±0.93@@	6.04±0.87@@	2.41±0.21
E+D	4.74±0.36	12.17±0.84@@	3.29±0.27@@	3.84±0.29
E+C	3.46±0.23@	8.02±0.64@	8.69±0.63@	4.84±0.38@@

Values are mean ± SEM of 4-6 separate experiments. Each group consist of 6-8 rats ***$p<0.001$, **$p<0.01$ when compared to control, @@$p<0.01$ and @$p<0.05$ when compared to epileptic group. C-Control, E-Epileptic, E+B-Epileptic rats treated with *Bacopa monnieri,* E+D-Epileptic rats treated with Bacoside A and E+C-Epileptic rats treated with Carbamazepine.

Figure-64

$GABA_{A\alpha 1}$ receptor expression in the cerebral cortex of control and experimental rats

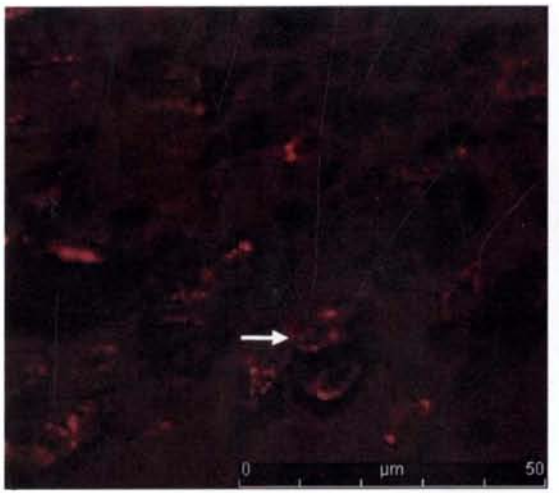


Control **Epileptic** **Epileptic + *Bacopa monnieri***

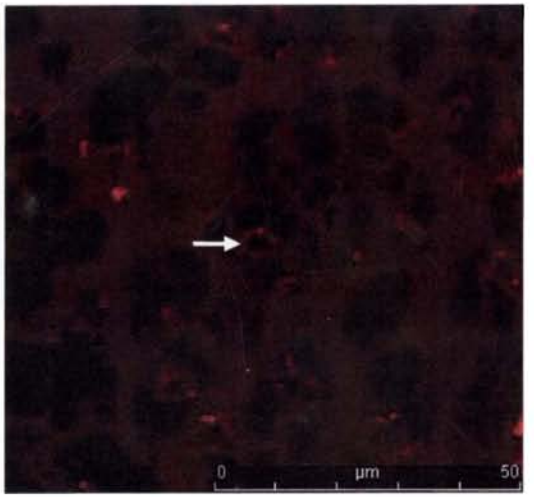
→ **$GABA_{A\alpha 1}$ receptor subunit**

Figure-64

$GABA_{A\alpha1}$ receptor expression in the cerebral cortex of control and experimental rats



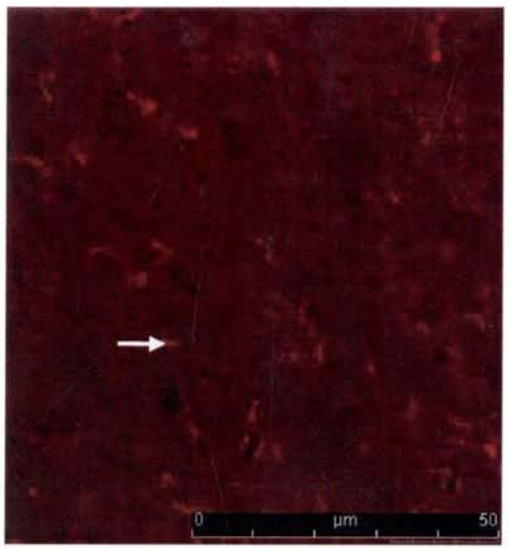
Epileptic + Bacoside A



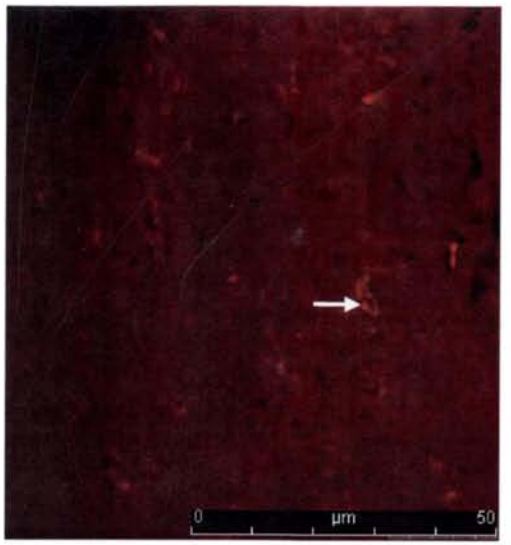
Epileptic + Carbamazepine

Figure-65

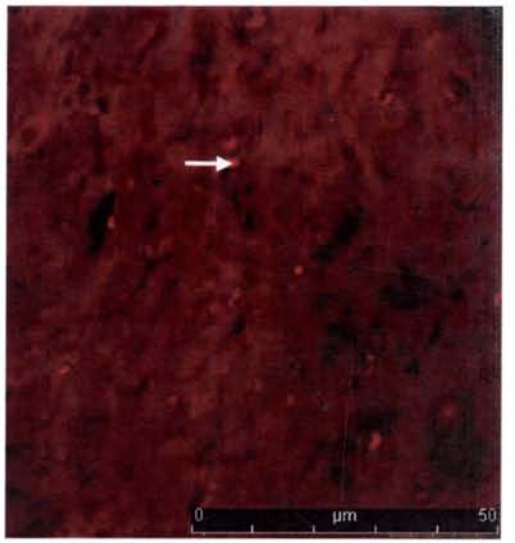
$GABA_{A\alpha1}$ receptor expression in the hippocampus of control and experimental rats



Control



Epileptic

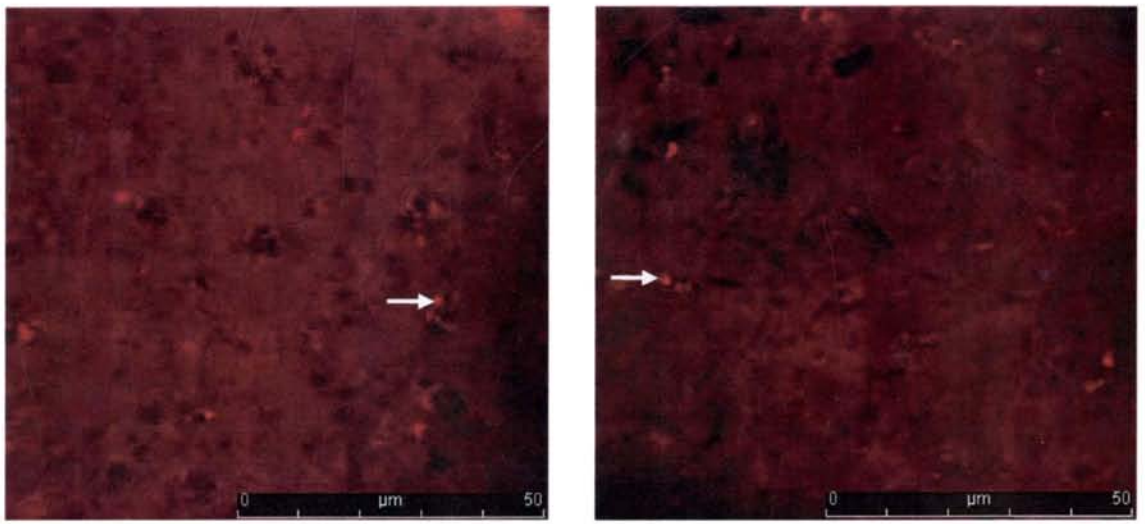


Epileptic + *Bacopa monnieri*

→ **GABA $_{A\alpha1}$ receptor subunit**

Figure-65

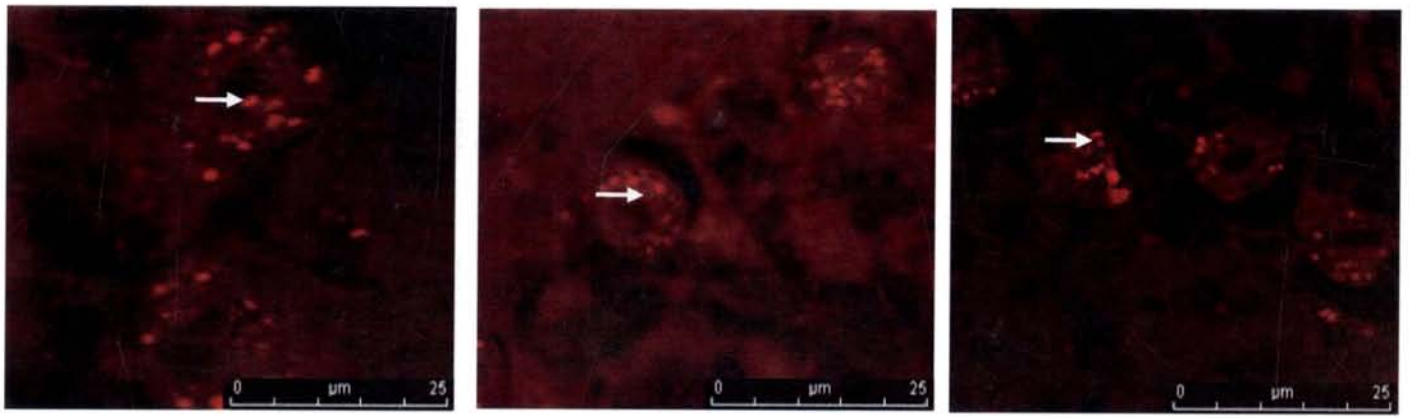
$GABA_{A\alpha1}$ receptor expression in the hippocampus of control and experimental rats



Epileptic + Bacoside A **Epileptic + Carbamazepine**

Figure-66

$GABA_{A\alpha 1}$ receptor expression in the cerebellum of control and experimental rats

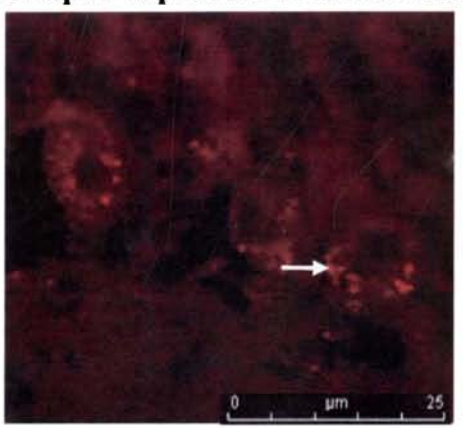


Control **Epileptic** **Epileptic + *Bacopa monnieri***

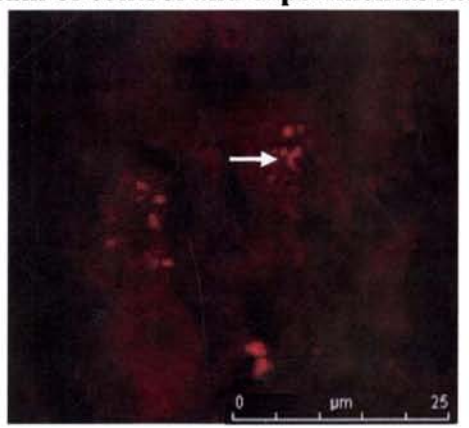
→ **$GABA_{A\alpha 1}$ receptor subunit**

Figure-66

$GABA_{A\alpha 1}$ receptor expression in the cerebellum of control and experimental rats



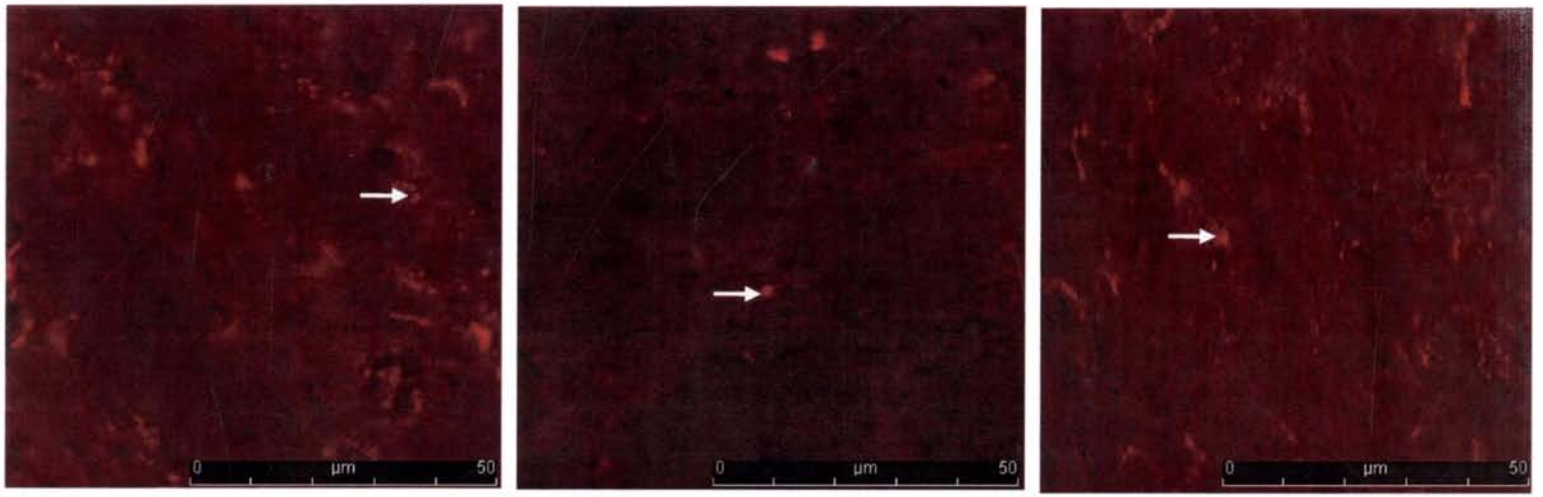
Epileptic + Bacoside A



Epileptic + Carbamazepine

Figure-67

$GABA_{A\alpha1}$ receptor expression in the corpus striatum of control and experimental rats

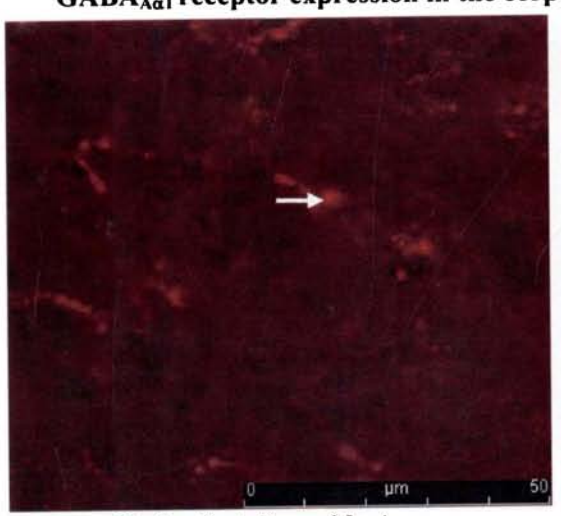


Control **Epileptic** **Epileptic + *Bacopa monnieri***

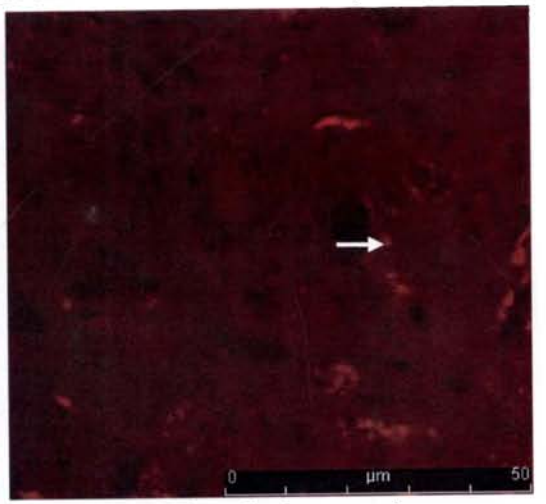
→ **$GABA_{A\alpha1}$ receptor subunit**

Figure-67

$GABA_{A\alpha1}$ receptor expression in the corpus striatum of control and experimental rats



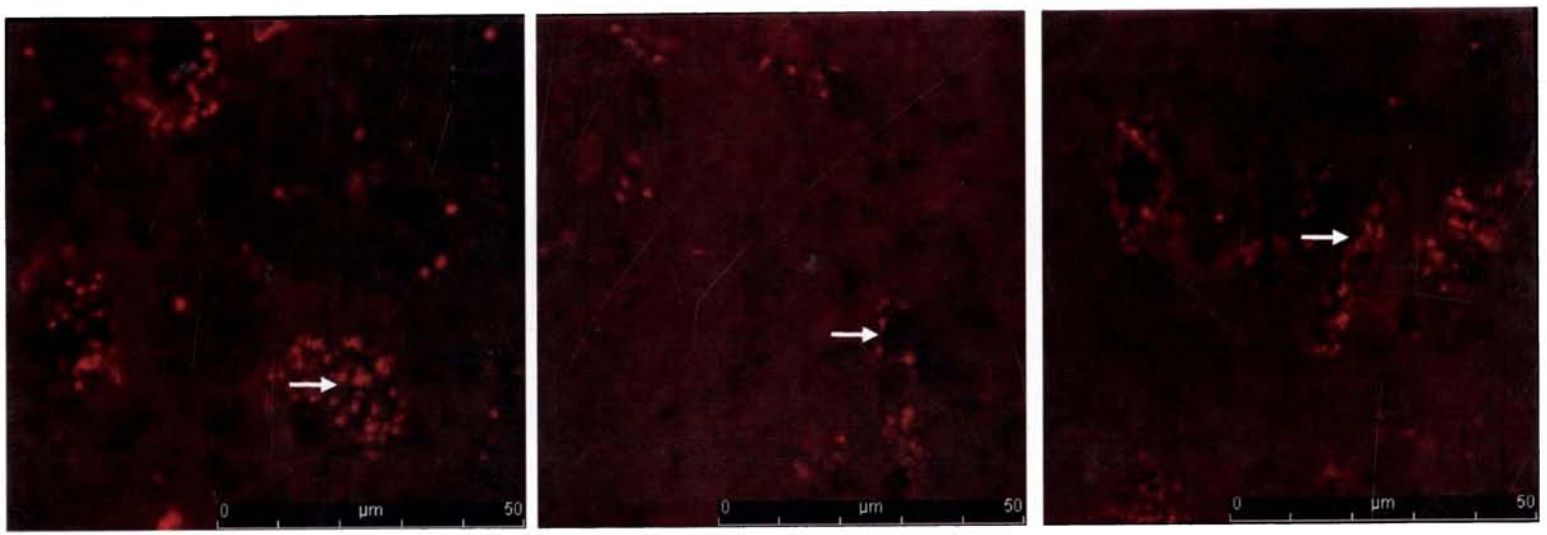
Epileptic + Bacoside A



Epileptic + Carbamazepine

Figure-08

$GABA_{A\alpha1}$ receptor expression in the corpus striatum of control and experimental rats

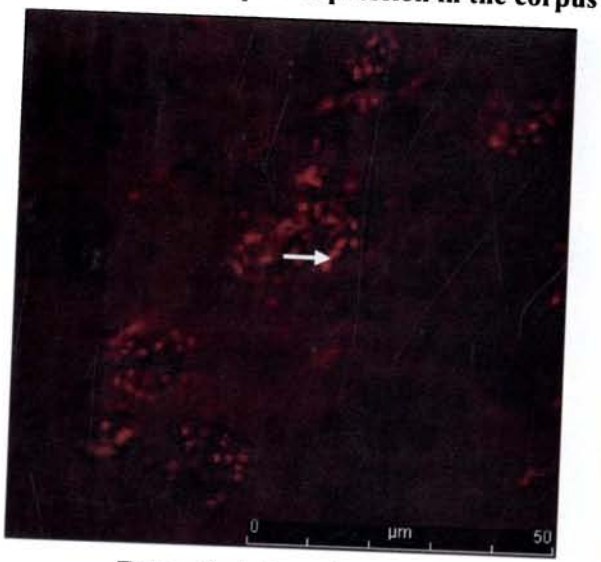


Control **Epileptic** **Epileptic + *Bacopa monnieri***

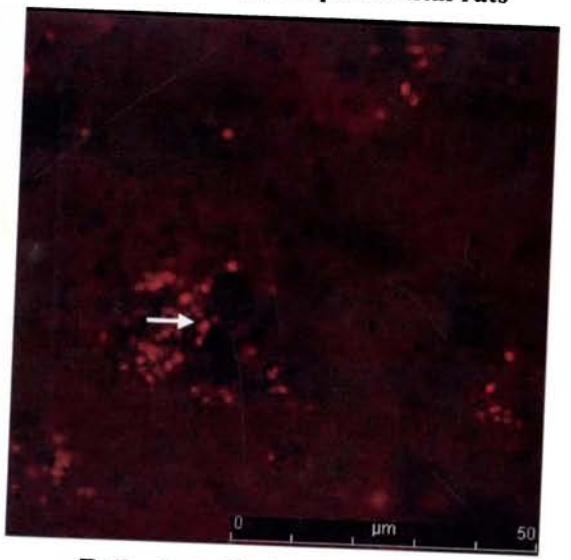
→ **$GABA_{A\alpha1}$ receptor subunit**

Figure-68

$GABA_{A\alpha1}$ receptor expression in the corpus striatum of control and experimental rats



Epileptic + Bacoside A



Epileptic + Carbamazepine

Table-66

$GABA_{A\alpha1}$ receptor expression in the brain regions of control and experimental rats

Cerebral cortex		Hippocampus	Cerebellum	Corpus striatum	Brain stem
Animal status	**Pixel intensity**				
C	103465 ± 343	138545 ± 425	98545 ± 945	128545 ± 1135	128545 ± 1034
E	76456 ± 393***	80545 ± 397***	80182 ± 619**	82182 ± 1267***	79182 ± 974***
E + B	95835 ± 235@@	121045± 387@@@	921745 ± 724@@	109745 ± 1312@@	109745 ± 952@@@
E + D	90564 ± 253@@	123785± 376@@@	919785 ± 865@@	103785 ± 1342@@	103785 ± 872@@@
E + C	87593 ± 293@@	119164± 369@@	880164 ± 794@@	110164 ± 1039@	110164 ± 794@@

Values are mean ± SEM of 4-6 separate experiments. Each group consist of 6-8 rats ***p<0.001, **p<0.01 when compared to control, @@@p<0.001 and @@p<0.01 when compared to epileptic group. C-Control, E-Epileptic, E+B-Epileptic rats treated with *Bacopa monnieri,* E+D-Epileptic rats treated with Bacoside A and E+C-Epileptic rats treated with Carbamazepine.

Figure Legends

Figure 64

Confocal image of $GABA_{A\alpha 1}$ receptor subunit in the cerebral cortex of control and experimental rats using receptor specific primary antibody and rhodamine coated secondary antibody. There was a significant decrease of $GABA_{A\alpha 1}$ receptor subunit in the epileptic rats which was reversed to near control with the administration of *Bacopa monnieri* Bacoside-A and Carbamazepine

Figure 65

Confocal image of $GABA_{A\alpha 1}$ receptor subunit in the hippocampus of control and experimental rats using receptor specific primary antibody and rhodamine coated secondary antibody. There was a significant decrease of $GABA_{A\alpha 1}$ receptor subunit in the epileptic rats which was reversed to near control with the administration of *Bacopa monnieri* Bacoside-A and Carbamazepine

Figure 66

Confocal image of $GABA_{A\alpha 1}$ receptor subunit in the cerebellum of control and experimental rats using receptor specific primary antibody and rhodamine coated secondary antibody. There was a significant decrease of $GABA_{A\alpha 1}$ receptor subunit in the epileptic rats which was reversed to near control with the administration of *Bacopa monnieri* Bacoside-A and Carbamazepine

Figure 67

Confocal image of $GABA_{A\alpha 1}$ receptor subunit in the corpus striatum of control and experimental rats using receptor specific primary antibody and rhodamine coated secondary antibody. There was a significant decrease of $GABA_{A\alpha 1}$ receptor

subunit in the epileptic rats which was reversed to near control with the administration of *Bacopa monnieri* Bacoside-A and Carbamazepine

Figure 68

Confocal image of $GABA_{A\alpha 1}$ receptor subunit in the brain stem of control and experimental rats using receptor specific primary antibody and rhodamine coated secondary antibody. There was a significant decrease of $GABA_{A\alpha 1}$ receptor subunit in the epileptic rats which was reversed to near control with the administration of *Bacopa monnieri* Bacoside-A and Carbamazepine