SUMMARY AND CONCLUSIONS
SUMMARY AND CONCLUSIONS

Epilepsy is a common chronic neurological condition characterized by recurrent spontaneous seizures also known as seizure disorder, affecting 1-2% of population worldwide. Epilepsy begins anywhere between the ages of 3 and 14 years, and continues indefinitely (AllReferHealth-seizures (convulsions).html). The epilepsies are a complex group of disorders whose common feature is a tendency for hyper excitability to develop in one or other regions of central nervous system. The epileptic seizures are thought to occur via alterations in the behavior of neural networks in the brain that induce spontaneous and synchronized burst firing interspersed by periods of normal electrical activity. Potential causes of epilepsy include brain tumors, central nervous system infections, traumatic brain injury, stroke, immune disorders, chemical imbalance, toxic chemicals or drugs, prenatal insults, degenerative disorders (Alzheimer's, Parkinsonism) and cerebrovascular diseases. The common ictogenesis-related characteristics of epilepsy are imbalance between excitatory and inhibitory neurotransmission, alterations in neurotransmitter expression and function, development of "epileptic ion channels" (Channelopathies), functional changes in neurons, morphological and physiological changes such as hippocampal sclerosis and axonal sprouting leading to aberrant neuronal synchronization. Hippocampal necrosis and/or neocortical cell loss and axonal and dendritic plasticity are the most striking changes during epileptic seizures.

It has been well established that impaired GABAergic activity and/or exaggerated activity of glutamatergic neurotransmission are thought to contribute to the various types of epilepsies. Alterations in voltage and receptor gated ion channels; neurotransmitter release, uptake and receptor functions have also been implicated in epileptic models (Martin et al., 2001). Despite the varied primary pathology of epileptic seizures, the
mechanisms involving in generating and spreading epileptic seizures converge on a common cellular pathology in which the excitatory glutamatergic system plays a key role.

Considering the multifactor neurochemical and neurophysiologic malfunctions consequent to epileptic seizures, attempts have been made to design antiepileptic drugs (AEDs) in order to mitigate the debilitating aspects of epilepsy. The classical antiepileptic drugs (AED) such as carbamazepine, Phenobarbital, phenytoin, valproate, felbamate, gabapentin, lamotrigine, tiagabine, vigabatrin have been used in the clinical trials which can alleviate neuronal damage and delay the development of epileptogenesis. Many AEDs employed in ameliorating seizures generally met with partial success and suffer from problems such as pharmacoresistance (development of tolerance) (Boggs et al 2000), neurotoxic effects and idiosyncratic reactions such as skin rashes etc. (Loscher and Schmidt 2002). Chronic toxicity was observed with some of the older AEDs such as osteoporosis, gingival hyperplasia, and alterations in reproductive endocrine function. Hence, there is an imperative need to screen therapeutically important compounds from natural medicinal plants which show less side effects and greater potential in restoring the epileptic neurons to normalcy.

Medicinal plants are of great interest to the researchers in the field of biotechnology as most of the drug industries depend, in part, on plants for the production of pharmaceutical compounds (Chand et al. 1997). Among the World's 25 best selling pharmaceutical medicines, 12 are plant derived (O'Neill and Lewis 1993).

Today, herbal products are being employed worldwide in a variety of healthcare settings and as home remedies. In developing countries like India, communities rely heavily upon traditional practitioners and herbal medicines to meet primary health care needs, and in industrialized countries plant products are gaining popularity as an
alternative and complimentary therapies. Keeping in view of the relative importance of the medicinal plants, the principal focus of the present project is aimed at evaluation of antiepileptic and neuroprotective effect of different fractions of indigenous medicinal plant, *Bacopa monnieri*.

*Bacopa monnieri* belonging to the Scrophulariaceae family, which grows in wetlands, shallow water, and muddy shores throughout the country, was selected for the present study. Traditionally, in ayurveda, brahmi is considered astringent, diuretic, laxative and a tonic for the heart and nerves. It is used to improve memory and intelligence, and to treat anemia, anorexia, arthritis, cough, dermatitis, diabetes, dropsy, dyspepsia, emaciation, fever and insanity. In other Indian traditional medicine systems, the drug is also used to treat anxiety, asthma, epilepsy, as a potent nerve tonic, cardiotonic and diuretic.

Recently, many studies have revealed its pharmacological roles as cognition-enhancer (Singh & Dhawan, 1997; Stough et al., 2001; Das et al., 2002; Sumathi et al., 2002), antidepressant (Sairam et al., 2002), antioxidant (Pawar et al., 2001; Russo et al., 2003), antiulcerogenic agent (Sairam et al., 2001), and calcium antagonist (Dar & Channa, 1999). It has been used for a long time in Ayurvedic medicine as a nerve tonic for promoting mental health and improving memory. Recently, several studies have been published on the therapeutic potential of this plant extracts in the treatment or prevention of neurological diseases and cognitive processes. Although bramhi is indicated as an anti-epileptic in ayurveda, animal research shows anticonvulsant activity only at high doses over extended periods of time.

*Bacopa* contains a wide variety of medically active substances, including stigma sterol, sapogenins, and flavonoids. Other compounds include triterpenoid saponins. Plant
contains two saponins, bacoside A and B. The Plant contains five major saponins such as bacoside A3 bacopaside II, bacopasaponin C isomer, bacopasaponin C and bacopaside I. Bacopa also contains betulic acid, beta-sitosterol, nicotine, and amino acids such as alpha-alanine, aspartic acid, glutamic acid, and serine. The heavy metals / elements include as Al, Cd. Cr, Cu, Fe, Hg. Mn, Ni, Pb and Zn.

The present study is undertaken to examine the anticonvulsant or antiepileptic effect of different extracts of Bacopa monnieri on selected neurochemical parameters in different target and non-target areas of the rat brain during induced epilepsy.

- Male adult wistar rats weighing 150±25 grams were used as the experimental animals. The rats were maintained under laboratory conditions of 28±2°C temperature with photoperiod of 12 hours light and 12 hours dark and 75% relative humidity, and fed with standard pellet diet and water ad libitum. The rats were maintained according to the ethical guidelines for animal protection and welfare bearing the CPCSEA 438/01/a/epesea/dt 17.07.2001 in its resolution No/07/a / (i)/CPCSCSA/IAEC/08-09/SVU/ZOOL/WR-EK/ dt 27.09.2009.

- Pentylenetetrazole, an epileptic drug, was selected for the present study. It was obtained as a commercial grade chemical from Sigma chemicals, USA. This drug was selected because of its reportedly reasonable potency to seizure generation. PTZ (60mg/Kg body weight) was given intraperitoneally which is sufficient to induce seizures.

- Bacopa monnieri, an anticonvulsant herb, was selected for the present study. It was extracted with different solvents viz. Ethanol, n-Hexane, Chloroform, Ethyl acetate, n-Butanol and Water. These extracts were tested for their antiepileptic
property. Each extract was administered orally of 180mg/kg/bw, a single dose/day for one week to study the antiepileptic activity as per the standard protocol.

- Rats were divided into 9 groups. 1st group was referred as saline-control; Group-2: Normal rats treated with PTZ (Epileptic); Group-3: Epileptic rats pre-treated with Ethanol Extract (PTZ+ EE); Group-4: Epileptic rats pre-treated with n-hexane extract (PTZ +n-HE); Group-5: Epileptic rats pre-treated with Chloroform extract (PTZ+ CE); Group-6: Epileptic rats pre-treated with Ethyl acetate extract (PTZ E+AE); Group-7: Epileptic rats pre-treated with n-Butanol extract (PTZ +n-BE); Group-8: Epileptic rats pre-treated with Aqueous extract (PTZ +AE); Group-9: Epileptic rats pre-treated with Diazepam (Reference control) (DZ +PTZ)

- After specific treatment regimen the rats were sacrificed and the brains were isolated immediately and placed on a chilled glass plate. Different brain areas, viz. cerebral cortex (CC), cerebellum (CB), pons-medulla (PM) and hippocampus (HC) were isolated and immediately frozen in liquid nitrogen (-180°C) and stored at -40°C until further use. At the time of analyses the tissues were thawed and used.

- The present study has been taken up to examine the effect of different extracts like Ethanol, n-Hexane (nHE), Chloroform (CE), Ethylacetate (EAE), n-Butanol (nBE) and Aqueous Extracts (AE) of Bacopa monniera (BM) on selected biochemical parameters in different areas of rat brain during PTZ-induced epilepsy and on pretreatment with different extracts of Bacopa monnieri.

- The aspects chosen for biochemical analyses were I. Cholinergic system represented by acetylcholine (ACh) and acetylcholinesterase (AChE); II. Parameters related to biogenic amines viz. Norepinephrine (NE), epinephrine
(EP), dopamine (DA), 5-Hydroxytryptamine (5-HT), and Monoamine oxidase (MAO); III. Parameters related to glutamate metabolism, viz. glutamate dehydrogenase (GDH), glutamine synthetase (GtS), glutaminase, glutamine; IV. Transaminations represented by Aspartate aminotransferase (AAT), Alanine aminotransferase (ALAT); and Branched chain (Val-VAT; Leucine-LAT and Isoleucine-IIAT) amino transferases. ATPases viz. Na\(^+\)/K\(^+\), Mg\(^2+\), Ca\(^{2+}\) and adenosine triphosphatases.

- Acetylcholinesterase (AChE) activity was inhibited in all the four different brain regions during PTZ administration. The AChE activity showed decrease in all areas of the brain with a highest decrease in CB followed by HC, CC and PM following the PTZ administration. It was elevated in all the brain regions of epileptic animals pre-treated with all the extracts except EE and AE.

- On par with AChE activity the levels of Acetylcholine (ACh) showed significant elevation during PTZ administration in all the brain regions. The ACh content showed an increase in all areas of the brain with a highest increase in CB followed by HC, CC and PM during PTZ –induced epilepsy.

- ACh content returned to normal levels with the treatment of different extracts of BM except EE and AE. ACh content and AChE activity recorded maximal recovery in nHE, nBE and EAE treated rats. Although a direct role of cholinergic pathways during BM treatment has not been demonstrated, it may be stated that different extracts of BM cause perceptible changes in the cholinergic system, at least as part of its antiepileptic effect. The role of the cholinergic system during seizure activity, if there is one, is an intriguing problem that has been subjected to intensive but not particularly systematic investigation. The changes in parameters related to the cholinergic system suggest that BM may exercise its effects through
changes in ACh levels as one of the facets of its antiepileptic activity. Different extracts of BM was found to have a challenging role in quenching PTZ-induced cholinergic system during antiepileptic treatment.

- The levels of monoamines such as Norpinephrine (NE), Epinephrine (EP), Dopamine (DA), Serotonin (5-HT) were decreased in all the brain areas during PTZ administration. The levels of biogenic amines were recovered in all the areas of brain of epileptic rats pre-treated with all the extracts of *Bacopa* except with AE.

- Pre-treatment with different extracts of BM except AE to the epileptic animals caused significant elevation in NE content in all the areas of brain. EP showed elevation with all the extracts except AE in all the regions except CB. DA showed elevation with all the extracts in all the brain regions except PM where it showed negligible decrease with AE during pre-treatment with different extracts to the epileptic animals. 5-HT levels were also increased in different regions of brain of rat in epileptic animals during pre-treatment with different extracts except AE. MAO showed elevation in different brain regions of rat during PTZ-induced epilepsy which was reversed in epileptic animals pre-treated with different extracts except AE and DZ.

- The results obtained on parameters related to monoamines and their metabolizing enzyme MAO activity supports the involvement of catecholamines in the epileptic seizures. The decline in the catecholamine levels coupled with elevation in MAO activity concludes that catecholaminergic system has an inhibitory influence on PTZ-induced convulsions. Pre-treatment with different extracts of *Bacopa monnieri* prior to the induction of epilepsy exhibited antiepileptic effect by modulating monoamine neurotransmitter system in the different areas of the brain.
Hence, it is obvious that alterations in monoamine neurotransmitter system in CNS might partly contribute to the impairments associated with seizure generation and the BM extracts except for AE compensates the monoaminergic abnormalities associated with neurological dysfunction.

- Glutamate and the associated metabolism showed differential changes upon induction of epilepsy and during antiepileptic treatment with different extracts of BM. GDH, GS and Glutamine were decreased with PTZ administration in all the brain regions. However, the GDH activity levels were increased in all the regions of brain of rats pre-treated with different extracts of BM except EAE and AE. GS activity was decreased in all the regions with PTZ administration and increased with all the extracts except EAE in PM and AE. Glutamine content was decreased in PTZ-treated rats and increased with the treatment of different extracts of *Bacopa monnieri*. Glutaminase activity was increased with PTZ administration and reversed to the normal levels in epileptic animals during pretreatment with all the extracts except AE and DZ.

- The decrease in the activity levels of GDH and GS and glutamine content suggests lowered oxidative deamination, lowered mobilization of glutamate towards glutamine formation during PTZ administration. Recoveries of these parameters during antiepileptic treatment suggest that administration of BM extracts probably could antagonize the *de novo* synthesis and release of glutamate and offers neuroprotection by interrupting the pathological cascade of glutamatergic hyper-excitation that occur during epileptogenesis.

- AAT and ALAT activities were increased in different regions of brain with PTZ administration and decreased during pre-treatment with all the extracts of BM. The elevation in the activity levels of glucogenic aminotransferases (AAT and
ALA'I (in all the brain regions during PTZ-induced epilepsy suggests mobilization of free amino acids towards gluconeogenesis to meet the energy demands. Elevation in the activity levels of branched chain aminotransferases in all the regions of brain during PTZ-induced epilepsy connotes reconstitution of carbon skeleton of these free amino acids forming respective ketoacids. The elevated branched chain aminotransferases may also be responsible for the generation of series of products essential for fatty acid bio-synthesis and TCA cycle operation. Elevation in the activity levels of all the aminotransferases may also addend glutamate to the already existing glutamate excitotoxicity. Reduced activities of all aminotransferases during treatment with different BM extracts suggest possible conversion of amino acids and accretion of nitrogen homeostasis and also reduce glutamate overload that occur during induced epilepsy.

- ATPases have been classified based on the requirement of specific cations such as Na\(^+/\) K\(^+\)-ATPase, Mg\(^{2+}\)-ATPase and Ca\(^{2+}\)-ATPase and these enzymes are significantly involved in cerebral energy exchange, ion-transport and synaptic function. In the present study, all the three ATPases (Na\(^+/\)K\(^+\), Mg\(^{2+}\) and Ca\(^{2+}\)-ATPases) recorded decreased activity levels upon PTZ administration in different brain regions. The activity levels of Na\(^+/\)K\(^+\) and Mg\(^{2+}\)-ATPases, in general, were increased in epileptic rats pre-treated with different extracts of BM except AE. The decreased activity levels of ATPases indicate that the administration of PTZ caused lowered energy metabolism and alteration in membrane transport functions which leads to higher seizure susceptibility and also explains the cascade of neuropathological events during induced epilepsy. Further, the recovery of these ATPase activities upon the treatment with selected Bacopa monnieri extracts
suggests that antiseizure activity of different extracts of BM and/or increased seizure threshold.

In conclusion, the results obtained in the present investigation reveal that there is a significant positive regulation of all the selected neurochemical parameters during pre-treatment with Ethanol extract (FE), n-Hexane extract (nHE), Ethyl acetate extract (CE) and n-Butanol extract (nBE) in all the brain regions studied. These four extracts of Bacopa monnieri considerably increased the seizure threshold in the experimental model of generalized tonic-clonic seizures and elicit perceptible changes in different facets of neurotransmitter systems and the associated metabolic profiles, at least, as part of its antiepileptic effect. These extracts were found to have a challenging role in quenching the PTZ-induced abnormalities that occur in cholinergic system, monoamine neurotransmitter system, glutamate metabolism, transamination and energy metabolisms in different brain regions of rat. Hence, the information gained from the present study can be used for proposing a better pharmacological tool for the treatment of epilepsy and related neurological disorders. The present study also helps in the discovery of new neuroprotective and anticonvulsant bioactive factors from the medicinal plants. Thus, these four extracts may be beneficial in antiepileptic treatment or the bioactive compounds present in these extracts can be used in the formulation of herbal drugs which can be used in the treatment of epilepsy or to control the seizure generation. Since Bacopa monnieri exhibited anti-seizure activity as evidenced from the present investigation, it might be clinically useful in the control of human epilepsies.