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Alterations in the Glutamate metabolism and Aminotransferases (AAT, ALAT) during PTZ - induced Epilepsy: Protective role of *Bacopa monnieri*

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ABSTRACT

Epilepsy, a common chronic neurological disorder characterized by repeated, spontaneous seizures, also known as seizure disorder. Seizure related neuronal injury has been assumed to be mediated by glutamate, the excitatory amino acid, in the central nervous system that causes a sudden imbalance between the inhibitory and excitatory signals in the brain with glutamate, γ-aminobutyric acid (GABA), noradrenaline, serotonin, and dopamine. Since long term usage of antiepileptic drugs cause high incidence of pharmacoresistence and untoward side effects, attention has been paid in recent years to screen bioactive compounds from natural medicinal plants for treatment of several neurological disorders including Epilepsy. Keeping in view of relative importance of natural medicinal plants, the present study is mainly focused to characterize the anti-convulsant effect of *Bacopa monnieri* (BM), an Indian herb which is being extensively used in Ayurvedic treatments related to neurological complications. The present study is designed to assess the neurotoxicity of Pentylene tetrazole (PTZ), an epileptic compounds, on the Glutamate metabolism and Amino transferases in different brain regions (Cerebral cortex, Cerebellum, Pons medulla and Hippocampus) of rat and to explore the possible antiepileptic effect of different extracts (Ethanol, n-Hexane, Chloroform, Ethyl acetate, n-Butanol and Aqueous extracts) of BM in comparison with Diazepam (DZ) (Reference control). The activities of glutamate dehydrogenase (GDH), glutamine synthetase (GS) and glutamine content were decreased in different regions of brain during PTZ induced epilepsy which were increased in epileptic rats pretreated with different extracts of *Bacopa monnieri* except EAE and

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AE. Glutaminase activity was increased in PTZ induced epilepsy and decreased on pretreatment with all the extracts of BM except AE. In addition aspartate (AAT) and alanine aminotransferase (ALAT) activity levels were increased during PTZ induced epilepsy when compared with normal control and levels were reversed on pretreatment with different extracts of BM. Recoveries of these parameters during antiepileptic treatment suggest that the bioactive factors present in the extracts offer neuroprotection by interrupting the pathological cascade of glutamatergic hyperexcitation that occurs during epileptogenesis**.**

Key words: Epilepsy, *Bacopa monnieri,* Pentylene tetrazole, Glutamate metabolism, AAT (Aspartate aminotransferase), ALAT (Alanine aminotransferase).

Introduction

Epilepsy is a disorder of the central nervous system (CNS) that is characterized by the magnified response of a group of neurons to an intense stimulus due to an imbalance predominantly among stimulating and inhibiting synapses mediated by glutamic acid (Glu) and γ-amino butyric acid (GABA) respectively (Fernandez ,1992). Several findings suggest that glutamate-glutamine cycle may be impaired in epilepsy. Some studies have provided convincing evidence to prove that antagonists of NMDA receptors and AMPA receptors as the powerful anticonvulsants in many epileptic models (Chapman, 1998). It has also have been demonstrated that participation of NMDA receptors plays a pivatol role in the generation of spontaneous hyperactivity which characterizes the chronic epileptic state (Bradford, 1995). Elevated glutamate levels in epileptic human brain (Petroff et al., 1995) and in cerebrospinal fluid of epileptic patients further support the role of slower glutamate clearance in the development of epilepsy. Excitatory glutamatergic mechanism is involved in learning, memory, and cellular plasticity and imbalance of this mechanism causes spontaneous, recurring seizures (Chapman, 2000).

Glutamate serves as an important metabolite in the central nervous system (Berl et al., 1975) and is present in high concentrations in the brain. In contrast, chronic hyperammonemia induces adaptive responses resulting in impairment of the signal transduction associated with NMDA receptors (Llansola et al., 2007). Since, glutamate is the major excitatory neurotransmitter in the mammalian central nervous system (CNS) (Coyle et al., 1993), it has the potential to be involved in the pathogenesis of many CNS diseases either due to excessive release, reduced uptake or alteration of receptor function (Platt, 2007). Glutamate is released from damaged axons and glia under hypoxic/ ischemic conditions (Back et al., 2007) and glutamate receptor-mediated excitotoxicity has been described as a predominant mechanism of hypoxic injury to the developing cerebral white matter (Volpe et al., 2004; Johnston, 2005; Sivakumr et al., 2010). GABA is known to be widely distributed in vertebrate brain and present in higher concentrations than many other neurotransmitters. The NMDA receptors are also proposed to be involved in the development of susceptibility to epileptic seizures. Thus, modulation of glutamate content is proposed to be beneficial in such conditions. New possibilities for the role of inhibition and interneurons in epilepsy should be considered, as GABAergic interneurons can effectively synchronize neuronal activity. Glutamine synthetase (GS) is one of the key enzymes in glutamate metabolism and its decreased activity leads to augmentation of glutamate in the extracellular space. The

synthesis of glutamine from glutamate and ammonia in the brain is an important step for detoxification of ammonia in hepatic failure with hyperammonemia (Dejong et al., 1996). Transaminases are important enzymes in animal metabolism which are intimately associated with amino acid synthesis and lysis. Among these, aspartate and alanine transaminases (AAT and ALAT) are widely distributed in the cells of all animals. The AAT catalyses the interconversion of aspartic acid and α-ketoglutaric acid to oxaloacetic acid and glutamic acid. While ALAT catalyses the interconversion of alanine and α-ketoglutaric acid to pyruvic acid and glutamic acid. There is much evidence for the alteration in the activities of these enzymes in a variety of environmental and physiological conditions (Knox and Greengard, 1965).

Herbs have been highly valued and used regularly for thousands of years for the treatment of different kinds of human ailments. During the past few years, there is growing interest to screen bioactive factors isolated from the medicinally important plants for treating several neurodegenerative disorders including Parkinson' s disease, Alzheimer's Disease, Schizophrenia and Epilepsy etc. *Bacopa monnieri*, commonly known as Brahmi, has been used for a long time in Ayurvedic medicine as nerve tonic for promoting mental health and improving memory. Recently, several studies have been carried out on the biological effects of this plant, especially for a therapeutic potential in the treatment or prevention of neurological diseases and cognitive processes. Ayurvedic medicine classifies Bacopa as belonging to a group of plant medicines known as medhya rasayana that improves mental health, intellect and memory (medhya) and promotes longevity and rejuvenation (rasayana) (Singh and Singh, 1980). The plant is an aphrodisiac, effective in treating scabies and syphilis, purification of the blood and having proven to be useful for the treatment of diarrhea and pyresis. The powdered dried leaf yielded satisfactory results in clinically tested cases of asthma, nervous breakdown and other low dynamic conditions (Singh and Dhawan, 1997). It also mediates GABAergic system (Shukia et al., 1987). Keeping in view the pivatol role of medicinal plants, the present study has been taken up to examine the anticonvulsant effect of different extracts of BM with particular reference to the glutamate metabolism and transaminases (AAT and ALAT) in different regions of brain during PTZ-induced epilepsy.

Material and methods

Plant material and preparation of extract

Bacopa Monnieri (BM) plant was collected from Thalakona forest and indentified by a botanist, Department of Botany, S.V.University, Tirupati. A voucher specimen was deposited in the herbarium of the Department of Botany, S.V.University, Tirupati (Voucher no. 428).The whole plant was shade dried and reduced to coarse powder. The extraction was carried out as specified by Watoo Phrompittayara et al., (2007). The whole plant powder was soaked in ethanol for 2 days at room temperature and the solvent was filtered. This was repeated 3-4 times until the extract gave no coloration. The extract was distilled and concentrated under reduced pressure in the Hahn vapor Rotary Evaporator HS-2005V yielding a gum-like residue, which was then suspended in water and extracted with various organic solvents of increasing polarity (starting with the lipophilic solvent n-Hexane, ending with the more hydrophilic n-Butanol). The solvent from each extract was distilled and concentrated under reduced pressure in the Hahn vapor Rotary Evaporator. The individual extracts were freeze dried and used for further use.

Animals

Male adult wistar rats weighing 150±25 grams were used as the experimental animals in the present investigation. The rats were maintained according to the ethical guidelines for animal protection and welfare bearing the CPCSEA 438/01/a/cpcsea/dt 17.07.2001 in its resolution

No/07/a / (i) /CPCSCA /IAEC /08-09/SVU/ZOOL/WR-EK/ dt.27.09.2009.

Drugs and dosing schedule

Pentylenetetrazole (PTZ), an anticonvulsant drug, was selected for the present study. It was obtained as commercial grade chemical from Sigma chemicals, USA. All other chemicals were of analytical grade. Each fraction of BM extract (180mg/Kg body weight) was dissolved in water and given to the animals for one week prior to the injection of PTZ. A gavage tube was used to deliver the substance by the oral route, which is the clinically expected route of administration of BM. The volume of administration was kept at 1ml to the animal.

Induction of Epilepsy

Epilepsy was induced by an intraperitoneal (i.p.) injection of Pentylenetetrazole (60mg/Kg body weight) dissolved in saline (Ray and Poddar, 1985; Gupta et al., 1999; Santos et al., 2002; Rizwan et al., 2003).

Administration of the test substance

Each fraction of BM extract (180mg/Kg body weight) was dissolved in water and given to the animals for one week prior to the injection of PTZ.

Isolation of Tissues

The animals were sacrificed after the treatment by cervical dislocation. The brain was 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, frozen in liquid nitrogen and stored at – 40^{o} C until further use.

Estimation of Enzymes associated with Glutamate metabolism

The activity levels of Glutamate dehydrogenase (GDH), Glutamine Synthetase (GS) , Glutaminase (Gln.ase) and Glutamine (Gln) content were estimated by the method of Lee and Lardy (1965), Wu (1963), Meister (1955), Colowick and Kaplan (1967) respectively. The activity levels of Aspartate aminotransferase (AAT) and Alanine aminotransferase (ALAT) was estimated by the method of Reitman and Frankel (1957).

Statistical analysis

The data were expressed as mean, standard deviation (SD) of six individual observations. The normal distributed data were subjected to Analyses of Variance (ANOVA) followed by Dunnets test. P values < 0.05 were considered significant.

Result

The activity levels of the enzymes associated with of Glutamate metabolism and such as GDH, GS, Gln.ase, Aminotransferases such as AAT, ALAT and glutamine content were estimated in different brain regions of rat during PTZ- induced epilepsy and on pretreatment with different extracts of BM and reference drug (DZ), and compared with saline control. The GDH activity was decreased in all the areas of brain during PTZ induced epilepsy when compared to the controls, and increased in all the brain regions of PTZ induced epileptic rats pretreated with different extracts of BM except for the treatment with EAE and AE (Table- 1). Glutamine synthetase activity was decreased in all areas of the brain during PTZ induced epilepsy when compared to the controls. GS activity was increased in all the brain regions of epileptic rats pretreated with different extracts of BM except for the treatment with EAE in PM and HC and AE (Table- 2). The activity levels of glutaminase was increased in all areas of the brain during PTZ induced epilepsy when compared to the controls, and decreased in all the brain regions of PTZ induced epileptic rats pretreated with different extracts of BM except for the treatment with AE (Table- 3). Glutamine content was decreased in all areas of the brain during PTZ induced epilepsy when compared to the controls, and increased in all the brain regions of rat during PTZ induced epilepsy pretreated with different extracts of BM except for the treatment with CE in CC and HC and AE (Table- 4). The levels of transaminases such as AAT, ALAT were increased in all the regions of

Table 1: Changes in the activity levels of Glutamate Dehydrogenase (GDH) in different brain regions of rats during PTZ-induced epilepsy and on Pre-treatment with different extracts of *Bacopa monnieri*

(Values are expressed in μ moles formazan formed/mg protein/hr)

S.No.	Brain area	SC	PTZ	EE+ PTZ	nHE+ PTZ	CE+ PTZ	EAE+ PTZ	nBE+ PTZ	AE+ PTZ	DZ+PTZ
1	CC	$3.517^{\{a\}}$ ± 0.319	$2.579^{\{b\}}$ ± 0.197 (-38.78)	$3.236^{\{ca\}}$ ± 0.055 (-7.989) [25.47]	$4.507^{\{d\}}$ ± 0.111 (28.14) [74.75]	$2.249^{\{fd\}}$ ± 0.256 (-36.05) [-12.79]	$4.391^{\{eb\}}$ ± 0.106 (33.63) [70.25]	$4.933^{\{gde\}}$ ± 0.417 (40.26) [91.27]	$2.335^{\{hbf\}}$ ± 0.490 (35.08) [-9.461]	$4.775^{\{ideg\}}$ ±0.383 (35.74) [85.14]
2	CB	$3.670^{\{a\}}$ ± 0.134	$3.249^{\{ba\}}$ ± 0.041 [-11.47]	$3.852^{\{ca\}}$ ±0.369 (4.959) [18.55]	$4.175^{\{dac\}}$ ± 0.028 (13.73) [28.60]	$2.870^{\{ed\}}$ ± 0.334 (-21.79) [-11.66]	$4.484^{\{eb\}}$ ± 0.028 (28.66) [38.01]	$4.975^{\{fe\}}$ ± 0.440 (35.55) [53.12]	$2.722^{\{gb\}}$ ± 0.160 (25.83) [-16.22]	$4.891^{\{ieg\}}$ ± 0.434 (33.26) [50.53]
3	PM	$2.625^{\{a\}}$ ±0.297	$2.097^{\{ba\}}$ [20.11]	$2.176^{\{cab\}}$ ± 0.407 (17.104) [3.767]	$4.490^{\{d\}}$ ± 0.262 (71.04) [114.11]	$1.975^{\{ed\}}$ ± 0.447 (-12.30) [-5.817]	$4.498^{\{fabc\}}$ ± 0.254 (32.76) [114.49]	$5.096^{\{gde\}}$ ± 0.427 (94.13) [143.01]	$2.085^{\{habcf\}}$ ± 0.129 (71.35) [-0.572]	$4.882^{\{ideg\}}$ ± 0.445 (85.98) [132.80]
4	HC	$3.526^{\{a\}}$ ± 0.205	$2.663^{\{b\}}$ ± 0.255 ([24.50]	$3.600^{\{ca\}}$ ± 0.101 (2.098) [35.18]	$4.951^{\{d\}}$ ± 0.504 (40.41) [85.91]	$3.054^{\{ed\}}$ ± 0.475 (-13.41) [-22.86]	$4.525^{\{fb\}}$ ± 0.215 (16.36) [69.92]	$5.978^{\{g\}}$ ± 0.512 (69.54) [124.48]	$2.326^{\{gbf\}}$ ±0.4899 (28.33) [-12.65]	$5.945^{\{ig\}}$ ± 0.520 (68.60) [123.24]

All the values are mean, ±SD of six individual observations.

Values with same Superscript are significant at P < 0.05. Values with different Superscript are non- significant at P < 0.05.

Values in '( )'parentheses are % change over saline control and values in '[ ]' are % change over PTZ treatment

Table 2: Changes in the activity levels of Glutamine synthetase (GS) in different brain regions of rats during PTZ-induced epilepsy and on Pre-treatment with different extracts *of Bacopa monnieri*

(Values are expressed in μ moles of γ-glutamyl hydroxymate formed/mg protein/hr)

S.No.	Brain area	SC	PTZ	EE+ PTZ	nHE+ PTZ	CE+ PTZ	EAE+ PTZ	nBE+ PTZ	AE+ PTZ	DZ+ PTZ
1	CC	$5.338^{\{a\}}$ ± 0.137	$4.165^{\{b\}}$ ± 0.200 (-21.97)	$7.943^{\{c\}}$ ± 0.138 (48.80) [90.70]	$7.904^{\{dc\}}$ ± 0.077 (48.07) [89.77]	$6.971^{\{e\}}$ ± 0.031 (30.59) [67.37]	$8.533^{\{f\}}$ ± 0.262 (59.85) [104.87]	$8.987^{\{g\}}$ ± 0.139 (68.35) [115.77]	$3.526^{\{h\}}$ ± 0.194 (-33.94) [-15.34]	$8.676^{\{if\}}$ ± 0.067 (62.53) [108.30]
2	CB	$4.784^{\{a\}}$ ± 0.295	$4.060^{\{b\}}$ ± 0.033 (-15.13)	$7.892^{\{c\}}$ ± 0.160 (64.96) [94.38]	$7.733^{\{dc\}}$ ± 0.082 (61.64) [90.46]	$6.933^{\{e\}}$ ± 0.062 (44.40) [70.76]	$7.642^{\{fcd\}}$ ± 0.192 (59.74) [88.22]	$8.304^{\{g\}}$ ± 0.073 (73.57) [104.53]	$4.057^{\{hb\}}$ ± 0.046 (-15.19) [-0.073]	$8.069^{\{icg\}}$ ±0.287 (68.66) [98.74]
3	PM	$3.492^{\{a\}}$ ± 0.088	$2.572^{\{b\}}$ ± 0.157 (-26.34)	$5.635^{\{c\}}$ ± 0.132 (61.36) [119.09]	$4.746^{\{d\}}$ ± 0.154 (35.91) [84.52]	$4.683^{\{ed\}}$ ± 0.158 (34.10) [82.07]	$1.731^{\{f\}}$ ± 0.203 (-50.42) [-32.69]	$6.026^{\{g\}}$ ± 0.017 (72.56) [134.29]	$2.139^{\{h\}}$ ± 0.125 (-38.74) [-16.83]	$5.911^{\{ig\}}$ ± 0.056 (69.27) [129.82]
4	HC	$5.406^{\{a\}}$ ±0.939	$4.944^{\{ba\}}$ ± 0.039 (-8.546)	$8.102^{\{c]}$ ± 0.290 (49.87) [63.95]	$7.911^{\{dc\}}$ ± 0.150 (46.34) [60.012]	$6.747^{\{e\}}$ ± 0.072 (24.80) [36.46]	$8.152^{\{fcd\}}$ ± 0.091 (50.79) [64.88]	$8.377^{\{gcdf\}}$ ± 0.290 (54.98) [69.43]	$4.538^{\{hb\}}$ ± 0.385 (-16.05) [-8.210]	$8.276^{\{icdfg\}}$ ± 0.323 (53.08) [67.39]

All the values are mean, ±SD of six individual observations.

Values with same Superscript are significant at $P < 0.05$. Values with different Superscript are non- significant at $P < 0.05$.

Values in '( )' are % change over saline control and values in '[ ]' are % change over PTZ treatment.

Table 3: Changes in the activity levels of Glutaminase (Gln.ase) in different brain regions of rats during PTZ-induced epilepsy and on Pre-treatment with different extracts of *Bacopa monnieri*

(Values are expressed in μ moles of ammonia formed/mg protein/hr)

S.No.	Brain area	SC	PTZ	EE+ PTZ	nHE+ PTZ	CE+ PTZ	EAE+ PTZ	nBE+ PTZ	AE+ PTZ	DZ+ PTZ
1	CC	$4.365^{\{a\}}$ ± 0.368	$5.835^{[b]}$ ± 0.069 (33.64)	$3.674^{\{c\}}$ ± 0.298 (15.83) [-37.03]	$3.414^{\{dc\}}$ ± 0.401 (-21.78) [-41.49]	$4.450^{\{ea\}}$ ± 0.271 (1.947) [-23.73]	$3.930^{\{faced\}}$ ± 0.207 (-9.965) [-32.64]	$2.531^{\{g\}}$ ± 0.338 (-42.01) [-56.62]	$6.850^{\{h\}}$ ± 0.355 (56.93) [17.39]	$4.796^{\{iae\}}$ ± 0.219 (55.69) [16.46]
2	CB	$3.479^{\{a\}}$ ± 0.285	$4.901^{\{b\}}$ ± 0.086 (40.87)	$2.605^{\{c\}}$ ± 0.103 (-25.12) [-46.84]	$2.093^{\{d\}}$ ± 0.058 (-39.83) [-57.29]	$3.974^{\{e\}}$ ± 0.025 (14.22) [-18.91]	$3.146^{\{f\}}$ ± 0.059 (-9.571) [-35.80]	$3.213^{\{gaf\}}$ ± 0.075 (-7.645) [(-34.44]	$5.929^{\{h\}}$ ± 0.042 (70.42) [20.97]	$4.448^{\{i\}}$ ± 0.288 (85.34) [31.56]
3	PM	$4.954^{\{a\}}$ ± 0.085	$5.955^{\{b\}}$ ± 0.015 (20.20)	$3.332^{\{c\}}$ ± 0.164 (-32.74) [-44.04]	$3.486^{\{dc\}}$ ± 0.259 (-29.63) [(-41.46]	$5.101^{\{ea\}}$ ± 0.004 (2.670) [-14.74]	$4.048^{\{f\}}$ ± 0.029 (-18.28) [-32.02]	$3.421^{\{gce\}}$ ± 0.444 (-34.57) [-42.55]	$6.655^{\{h\}}$ ± 0.134 (34.33) [11.75]	$5.474^{\{if\}}$ ±0.094 (30.68) [8.715]
4	HC	$3.346^{\{a\}}$ ± 0.210	$4.571^{\{b\}}$ ± 0.213 (36.61)	$3.146^{\{c\}}$ ± 0.087 (-5.977) [-31.17]	$2.713^{\{d\}}$ ± 0.151 (-18.91) [-40.64]	$3.970^{\{e\}}$ ± 0.059 1(8.64) [-13.14]	$2.115^{\{f\}}$ ± 0.094 (-36.79) [-53.73]	$2.671^{\{gd\}}$ ± 0.032 (-20.17) [-41.56]	$5.198^{\{h\}}$ ± 0.137 (55.34) [13.71]	$4.048^{\{ie\}}$ ± 0.020 (80.75) [32.31]

All the values are mean, ±SD of six individual observations.

Values with same Superscript are significant at $P < 0.05$. Values with different Superscript are non- significant at $P < 0.05$.

Values in '( )'parentheses are % change over saline control and values in '[ ]' are % change over PTZ treatment.

Table 4: Changes in the Glutamine (Gln) content in different brain regions of rats during PTZ-induced epilepsy and on Pre-treatment with different extracts *Bacopa monnieri*

(Values are expressed in μg of glutamine/g wet wt of tissue)

S.No.	Brain area	SC	PTZ	EE+ PTZ	nHE+ PTZ	CE+ PTZ	EAE+ PTZ	nBE+ PTZ	AE+ PTZ	DZ+ PTZ
1	CC	$4.448^{\{a\}}$ ± 0.067	$3.616^{\{b\}}$ ± 0.043 (-18.70)	$5.455^{\{c\}}$ ± 0.053 (22.63) [50.85]	$5.930^{\{d\}}$ ± 0.042 (33.31) [63.99]	$3.463^{\{e\}}$ ± 0.108 (-22.14) [-4.231]	$5.172^{\{f\}}$ ± 0.040 (16.27) [43.03]	$7.652^{\{g\}}$ ± 0.127 (72.03) [111.61]	$3.440^{\{he\}}$ ± 0.073 (-22.66) [-4.867]	$7.550^{\{ig\}}$ ± 0.023 (69.71) [108.79]
2	CB	$4.151^{\{a\}}$ ± 0.436	$3.958^{\{ba\}}$ ± 0.008 (-4.649)	$5.812^{\{c\}}$ ± 0.007 (40.01) [46.84]	$6.047^{\{dc\}}$ ± 0.235 (45.67) [52.77]	$3.981^{\{eb\}}$ ± 0.011 (-4.095) [0.581]	$6.525^{\{fa\}}$ ± 0.015 (57.16) [64.85]	$7.197^{\{g\}}$ ± 0.125 (73.37) [81.83]	$3.749^{\{hbe\}}$ ± 0.056 (-9.684) [-5.280]	$7.056^{\{ig\}}$ ± 0.034 (69.98) [78.27]
3	PM	$4.982^{\{a\}}$ ± 0.007	$3.987^{\{b\}}$ ± 0.010 (-19.97)	$5.988^{\{c\}}$ ± 0.009 (20.19) [50.18]	$6.404^{\{d\}}$ ± 0.163 (28.54) [60.62]	$4.878^{\{ea\}}$ ± 0.127 (-2.087) [22.34]	$6.572^{\{fd\}}$ ± 0.014 (31.91) [64.83]	$7.818^{\{g\}}$ ± 0.286 (56.92) [96.08]	$2.497^{\{h\}}$ ± 0.257 (-49.87) [-37.37]	$7.751^{\{ig\}}$ ± 0.010 (55.580 [94.40]
4	HC	$5.337^{\{a\}}$ ± 0.029	$4.861^{\{b\}}$ ± 0.137 (-8.918)	$5.870^{\{c\}}$ ± 0.009 (9.986) [20.75]	$6.346^{\{d\}}$ ± 0.181 (18.90) [30.54]	$3.345^{\{e\}}$ ± 0.015 (-37.32) [-31.18]	$6.907^{\{f\}}$ ± 0.106 (29.41) [42.09]	$6.911^{\{gf\}}$ ± 0.070 (29.49) [42.17]	$4.327^{\{h\}}$ ± 0.515 (-18.92) [-10.98]	$7.136^{\{ifg\}}$ ± 0.051 (33.70) [46.80]

All the values are mean, ±SD of six individual observations.

Values with same Superscript are significant at $P < 0.05$. Values with different Superscript are non- significant at $P < 0.05$.

Values in '( )' are % change over saline control and values in '[ ]' are % change over PTZ treatment.

Table 5: Changes in the activity levels of Aspartate aminotransferase (AAT) in different regions of rat brain during PTZ- induced epilepsy and on Pre- treatment with different extracts of *Bacopa monnieri.*

(Values are expressed as µ moles of pyruvate formed /mg protein / hr.

S.No.	Brain area	SC	PTZ	EE+ PTZ	nHE+ PTZ	CE+ PTZ	EAE+ PTZ	nBE+ PTZ	AE+ PTZ	DZ+ PTZ
1	CC	$0.786^{\{a\}}$ ±0.123	$0.971^{\{b\}}$ ±0.020 (23.53)	$0.616^{\{c\}}$ ±0.078 (-21.62) [-36.56]	$0.663^{\{dac\}}$ ±0.080 (-15.64) [-31.71]	$0.748^{\{acde\}}$ ±0.091 (-4.834) [-22.96]	$0.692^{\{aced\}}$ ±0.081 (-11.95) [-28.73]	$0.657^{\{acdef\}}$ ±0.048 (-16.41) [-32.33]	$0.659^{\{acdefg\}}$ ±0.050 (-16.15) [-32.13]	$0.695^{\{acdefgh\}}$ ±0.060 (-11.57) [-28.42]
2	CB	$1.493^{\{a\}}$ ±0.119	$1.806^{\{ba\}}$ ±0.045 (20.96)	$1.356^{\{ca\}}$ ±0.042 (-9.176) [-24.91]	$1.274^{\{dac\}}$ ±0.103 (-14.66) [-29.45]	$1.307^{\{acd\}}$ ±0.081 (-12.45) [-27.63]	$1.402^{\{abcde\}}$ ±0.137 (-6.095) [-22.36]	$1.297^{\{acdef\}}$ ±0.080 (-13.12) [-28.18]	$1.189^{\{acdefg\}}$ ±0.056 (20.36) [-34.16]	$1.098^{\{acdefgh\}}$ ±0.337 (-26.45 [-39.20]
3	PM	$1.629^{\{a\}}$ ±0.120	$2.153^{\{b\}}$ ±0.331 (36.16)	$1.598^{\{ca\}}$ ±0.079 (-1.903) [-25.77]	$1.520^{\{dac\}}$ ±0.132 (-6.691) [-29.40]	$1.493^{\{acd\}}$ ±0.119 (-8.348) [-30.65]	$1.394^{\{acde\}}$ ±0.046 (-14.42) [-35.25]	$1.328^{\{acdef\}}$ ±0.062 (-18.47) [-38.31]	$1.527^{\{acdefg\}}$ ±0.135 (-6.261) [-29.07]	$2.096^{\{ib\}}$ ±0.272 28.66) [-2.647]
4	HC	$2.018^{\{a\}}$ ±0.183	$2.264^{\{ba\}}$ ±0.502 (12.19)	$1.803^{\{ca\}}$ ±0.038 (10.65) [-20.36]	$1.928\{^{dabc\}}$ ±0.049 (-4.459) [14.84]	$1.834^{\{acd\}}$ ±0.033 (-9.117) [-18.99]	$1.915^{\{abcde\}}$ ±0.068 (5.104) [-15.41]	$1.890^{\{abcdef\}}$ ±0.080 (-6.342) [-16.51]	$1.792^{\{abcdefg\}}$ ±0.170 (-11.19) [-20.84]	$2.109^{\{abcdefgh\}}$ ±0.264 (4.509) [-6.846]

All the values are mean, ±SD of six individual observations.

Values with same Superscript are significant at $P < 0.05$. Values with different Superscript are non- significant at $P < 0.05$.

Values in '( )' are % change over saline control and values in '[ ]' are % change over PTZ treatment.

Table 6: Changes in the activity levels of Alanine aminotransferase in different regions of rat brain during PTZ-induced epilepsy and on Pre- treatment with different extracts of *Bacopa monnieri.*

(Values are expressed as µ moles of pyruvate formed /mg protein / hr.)

S.No.	Brain area	SC	PTZ	EE+ PTZ	nHE+ PTZ	CE+ PTZ	EAE+ PTZ	nBE+ PTZ	AE+ PTZ	DZ+ PTZ
1	CC	$1.029^{\{a\}}$ ±0.009	$1.429^{\{b\}}$ ±0.175 (38.87)	$1.025^{\{ca\}}$ ±0.010 (-0.388) [-28.27]	$1.017\{^{dac\}}$ ±0.008 (-1.166) [-28.83]	$0.936^{\{acd\}}$ ±0.082 (-9.037) [-34.49]	$1.024^{\{acde\}}$ ±0.007 (-0.485) [28.34]	$0.958^{\{acdef\}}$ ±0.041 (-6.899) [-32.96]	$0.876^{\{heg\}}$ ±0.041 (-14.86) [-38.69]	$1.028^{\{acdefg\}}$ ±0.012 (-0.097) [-28.06]
2	CB	$1.537^{\{a\}}$ ±0.123	$1.835^{\{b\}}$ ±0.033 (19.38)	$1.407^{\{ca\}}$ ±0.063 (-8.458) [-23.32]	$1.382^{\{dc\}}$ ±0.029 (-10.08) [-24.68]	$1.427^{\{acd\}}$ ±0.046 (-7.156) [-22.23]	$1.032^{\{f\}}$ ±0.020 (-32.46) [43.43]	$0.984^{\{gf\}}$ ±0.010 (-35.97) [-46.37]	$1.438^{\{acd\}}$ ±0.161 (-6.441) [-21.63]	$1.016^{\{ifg\}}$ ±0.009 (-33.89) [-44.63]
3	PM	$2.145^{\{a\}}$ ±0.307	$2.361^{\{ba\}}$ ±0.324 (10.06)	$2.094^{\{cab\}}$ ±0.274 (-2.377) [-11.30]	$1.930^{\{abc\}}$ ±0.043 (-10.02) [-18.25]	$2.018^{\{abcd\}}$ ±0.202 (-5.920) [-14.52]	$1.705^{\{adec\}}$ ±0.244 (-20.51) [-27.78]	$2.148^{\{abcdef\}}$ ±0.296 (0.139) [-9.021]	$1.535^{\{def\}}$ ±0.124 (-28.43) [-34.98]	$2.293^{\{abcde\}}$ ±0.397 (6.899) [-2.880]
4	HC	$2.738^{\{a]}$ ±0.143	$2.984^{\{ba\}}$ ±0.011 (8.984)	$2.729^{\{cab]}$ ±0.134 (-0.328) [-8.545]	$2.519^{\{dac\}}$ ±0.171 (-7.998) [-15.58]	$2.862^{\{abc\}}$ ±0.186 (4.528) [-4.088]	$2.823^{\{abce\}}$ ±0.167 (3.104) [-5.395]	$2.791^{\{abcdef\}}$ ±0.169 (1.935) [-6.467]	$2.897^{\{abcdefg\}}$ ±0.200 (5.807) [-2.915]	$2.957^{\{abcdefgh\}}$ ±0.078 (7.998) [-0.904]

All the values are mean, ±SD of six individual observations.

Values with same Superscript are significant at $P < 0.05$. Values with different Superscript are non- significant at $P < 0.05$.

Values in '( )' are % change over saline control and values in '[ ]' are % change over PTZ treatment

brain during PTZ induced epilepsy and on pre-treatment with different extracts of *Bacopa monnieri* and the levels were reversed on par with reference Drug (DZ) (Table 5 & 6).

Discussion

Glutamine and glutamate are known to play an important role in cerebral metabolism. Glutamate is required for normal brain function while excess amount leads to neuronal death due to the destructive effect mediated by glutamate receptors, particularly NMDA and non- NMDA receptors. However, pathological situations such as hypoxia and ischemia may lead to excessive release of glutamate and its accumulation in the extracellular space, which initiates the apoptotic pathway that leads to neuronal death (Rothman and Olney, 1986, Dienel and Hertz, 2005). Neuronal excitation involving the excitatory glutamate receptors is recognized as an important underlying mechanism in neurodegenerative disorders (Dong et al., 2009). Compelling evidence over the past few decades firmly implicating the possible involvement of glutamate as an excitatory putative neurotransmitter in the central nervous system which acts on N-methyl-D-aspartate (NMDA), α–amino-3-hydroxy-5-methyl-isoxazoli-4-Propionic acid (AMPA), kainite and metabotropic receptor mediated mechanisms during epileptic seizures (Akiyama et al., 1992; Hudspith, 1997). Since excitatory glutamatergic mechanisms are involved in learning, memory, and cellular plasticity and imbalance of this mechanism causes spontaneous, recurring seizures (Chapman, 2000). Since a wide number of glutamatergic molecular mechanisms provoke seizure, the present investigation is focused to study the alterations in glutamate and associated metabolism with particular reference to epilepsy and anti-epileptic treatment with different extracts of *Bacopa monnieri.* NAD dependent Glutamate dehydrogenase (GDH) was decreased in all the brain regions during PTZ-induced epilepsy suggesting that oxidative deamination of glutamate was inhibited and reductive amination of α- ketoglutarate was favored thus causing upsurge in the glutamate levels and subsequent excitotoxicity. Similarly the activity levels of glucogenic aminotransferases (AAT and ALAT) and glutaminase were elevated and glutamine levels were decreased in all the brain regions during PTZ-induced epilepsy.

Several lines of evidence indicate that glutamate and aspartate and their analogues as well as selective agonists such as NMDA, AMPA, kainate, ibotenic acid and domoic acid may induce convulsions when administer focally or systemically (Chapman, 2000). Wilson et al. (1996) have reported similar elevation in extracellular hippocampal release of glutamate during spontaneous seizures in ambulatory patients. Enhanced release in glutamate during seizures was also observed in chronic epilepsy models in rodents such as amygdale-kindled rats, genetically epilepsy prone rats, rats with spontaneous, recurrent seizures after kainate-induced status epilepticus (Chapman, 1998). Elevated glutamate levels in epileptic human brain (Petroff et al., 1995) and in cerebrospinal fluid of epileptic patients further support the role of slower glutamate clearance in the development of epilepsy. Several findings suggest that glutamate-glutamine cycle may be impaired in epilepsy. It has also been well documented that elevated cellular glutamate and impaired clearance by the glia could contribute to the increased excitability and ongoing excitotoxicity (Van Gelder, 1986; Bradford, 1995; Sherwin, 1999). In addition, the endogenous production of glutamate through $NADH_2$ dependent glutamate dehydrogenase and glutaminase reactions plays a prominent role in brain, since the transport of circulating glutamate to brain normally plays a minor role in regulating neuronal glutamate levels. The contribution of glutamate through the elevation of the activities of aminotransferases, and glutaminase adds credence to the above contention. Studies have shown that the rate of glutamate-glutamine cycle is an important factor in the maintaince of

glutamate homeostasis in the CNS. It has been observed that the rate of “glutamate-glutamine cycle” was found to be very low, despite the normal glutamate content and lowered glutamine content in epileptogenic human hippocampus (Petroff et al., 2002) and the low rates of neuron-glia cycling are caused by glial dysfunction, thus down regulating glutamine synthetase. Such failure of glutamine synthesis would obviously result in increased glial glutamate content which could contribute to slower glutamate uptake and enhanced glutamate transporter reversal. In support of this Mathern et al., (1998) have reported increased expression of glutamate transporter. The dysregulation of phosphate activated glutaminase, coupled with the alteration in glutamate dehydrogenase, glutamine synthetase and aminotransferase would possibly alter the glutamate (neuronal) and glutamine (glial) ratio. This hypothesis was further supported by the observation of Sellinger et al., (1986) who reported that inhibitors of glutamine synthesis (eg. Methioninesulfoximine) result in convulsions. Similarly, the glutamine synthetase activity was reduced in the seizure focus during ferric chloride model of focal epilepsy (Tiffany-Castiglioni et al., 1989). Hence, inhibition of glutamine synthesis would be expected to markedly slow glutamate-glutamine cycling, decrease in tissue glutamine content and efflux, which possibly promote epileptogenesis (Sellinger et al., 1986). Further, the declined glutamine synthetase has also been demonstrated during seizures in many species which suggest lesser mobilization of glutamate for the synthesis of glutamine and also signify the failure of the brain to opt a protective mechanism for maintaing low concentrations of glutamate.

The activity levels of NAD-Glutamate dehydrogenase, glutamine synthetase were elevated and glutaminase and aminotransferases were decreased in all the regions of brain of epileptic rats pre-treated with different extracts of *Bacopa monnieri* except aqueous extract. The elevation in GDH, GS and glutamine content signify lowered *de novo* synthesis and release of glutamate and thus offering neuroprotection by interrupting pathological cascade of glutamatergic excitotoxicity that occur during epileptogenesis. Although glutamate levels were not estimated under PTZ-administration and on antiepileptic treatment in the present investigation, the observed alteration in glutamate-glutamine interconversion seem to play a major role in regulating the glutamate levels during the seizure threshold and recovery. It has been reported that BM extract has been reported to show anti-oxidant property in brain (Bhattacharya et al., 2000) and enhance protein kinase activity and 5-HT levels in the hippocampus (Singh et al., 1997) which is the most vulnerable area in the epileptic brain damage. Several pharmacological studies have attributed the nootropic activity to the two major saponins of BM extract such as Bacoside A and B (Singh et al., 1988; Dhawan and Singh, 1997). Of these two, Bacoside A has been reported to chiefly facilitate memory (Rastogi et al., 1994) and has anxiolytic activity (Sairam et al., 2002). It has been reported that the mode of action of neuroprotective effects of Brahmi was attributed to the antioxidant activity and inhibition of acetylcholinesterase activity. Paulose et al., (2008) have shown that the neuroprotective role of BM extract in glutamate-mediated excitotoxicity during seizures and cognitive damage that occur during pilocorpine-induced epilepsy. It has also been demonstrated that BM treatment in epileptic rats significantly brought the reversal of the down regulated metabotrapic glutamate receptor (mglu R8) gene expression toward control level. Earlier findings suggested that BM plays a significant role in the down regulation of NMDA RI gene expression and glutamate receptor binding without any change in its affinity (Zhou et al., 2009). Several lines of evidence indicate that BM is recommended in the formulations for the management of a range of mental conditions, and epilepsy. Anti-

epileptic property of leaf extracts of BM showed their regulatory role through the muscarinic, glutamate and serotonin receptor sub-types (Amee et al., 2009). Hence, treatment with different BM extracts except AE as evident from our results, has immense clinical significance in the therapeutic management of epilepsy. It is well established that glutamate and other endogenous excitatory amino acids can cause extensive neuronal damage if the extracellular concentration in the brain becomes elevated (Rothman and Olney, 1986; Choi, 1988; Schousboe et al., 1991). Such increase in extracellular brain glutamate and aspartate levels are known to occur during pathological states such as ischemia, hypoxia and hypoglycemia (Benveniste et al., 1984). One important factor contributing to this increase in extracellular glutamate is likely to be a failure in high affinity glutamate uptake present primarily in astrocytes but also significantly in glutamatergic neurons (Schousboe et al., 1988). It is presumed from the present study that, while epilepsy and antiepileptic treatment could affect the glutamate release, reuptake and the activation of NMDA receptors (Hudspith, 1997) and these changes probably result from or at least associated with alterations in the levels of other parameters related to glutamate metabolism.

Thus, alterations of glutamate-glutamine interconversions could well be part of antiepileptic treatment, besides its effects on the cholinergic system, energy metabolism and biogenic amines. The activation or inactivation of these enzymes observed in the present study may be taken as an index of altered turnover of amino acids particularly glutamate during antileptic treatment.

Conclusion

The present investigation revealed that n-Butanol, Ethyl acetate, Chloroform, n-Hexane and Ethanol extracts of BM quench the alterations that occur in glutamate metabolism and aminotransferases during PTZ – induced epilepsy. Thus these extracts or bioactive compounds of these extracts can be used to treat epilepsy or to control seizure generation. Isolation and characterization of bioactive compounds from these extracts are in progress in order to explore the antiepileptic mechanism of the bioactive compounds.

Conflict of interest

The authors declare that they have no conflict of interest.

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References

Akiyama, K, Daigen, A, Yamada, N, Itoh, T, Kohira, I, Ujike, H, Otsuki, S. (1992). Long-lasting enhancement of metabotropic excitatory amino acid receptor mediated polyphosphoinositide hydrolysis in the amygdale/pyriform cortex of deep Prepiriform cortical kindled rats. Brain Res. Vol 569, pp. 71-77

Amee, K, Pretty, M.A, Jes, P, Paulose, C.S. (2009). Down-regulation of cerebellar 5-HT2C receptors in pilocarpine-induced epilepsy in rats: Therapeutic role of *Bacopa monnieri* extract. Journal of Neurological Sciences. Vol 284, pp. 124-128

Back, S.A, Craig, A, Kayton, R.J, Luo, N.L. (2007). Hypoxia-ischemia preferentially triggers glutamate depletion from oligodendroglia and axons in perinatal cerebral white matter. J Cereb Blood Flow Metab. Vol 27, pp. 334–347

Benveniste, H, Drejer, J, Schousboe, A, Diemer, N.H. (1984). Elevation of the extracellular concentrations of glutamate and aspartate in rat hippocampus during transient cerebral ischemia monitored by intracerebral microdialysis. J. Neurochem. Vol 43, pp. 1369-1374

Berl, S, Nicklas, W.J, Clarke, D.D. (1975). Compartmentation of citric acid metabolism in brain: Labelling of glutamate, glutamine, aspartate and GABA by several radioactive tracer metabolites. J. Neurochem. Vol 17, pp. 1009-1015

Bhattacharya, S.K, Bhattacharya, A, Kumar, A. (2000). Antioxidant activity of *Bacopa monniera* in rat frontal cortex, striatum and hippocampus. Phytother. Res. Vol 14, pp. 174–179

Bradford, H.F. (1995). Glutamate, GABA and epilepsy. Prog. Neurobiol. Vol 47, pp. 477-511

Chapman, A.G, Meldrum, B.S. (2000). Glutamate and glutamate receptors in status Epilepticus. In: Status Epilepticus: Mechanisms and Management (Wasterlain, C., Treiman, D.M. Eds.) Lippincott Raven Publishers. Philadelphia, pp. 1043S- 1045S

Chapman, A.G. (1998). Glutamate receptors in epilepsy. Ottersen, O. P, Langmoen, I. ., Gjerstad, L. (Eds.) Progress in Brain Research. Elsevier. Amsterdam. The Netherlands. Vol 116, pp. 348-360

Choi, D.W. (1988). Glutamate neurotoxicity and diseases of the nervous system. Neuron. Pp. 1623-1634

Colowick, S.P, Kaplan, N.O. (1967). Determination of amide residues by chemical methods. In: Methods in Enzymology (C.H.W. Hirsh, Ed.). vol 11, pp. 63-65

Coyle, J.T, Puttfarcken, P. (1993). Oxidative stress, glutamate, and neurodegenerative Disorders. Science vol 262, pp. 689-695

Dejong, C.H, Deutz, N.E, Soeters, P.B, (1996). Ammonia and glutamine metabolism during liver insufficiency: the role of kidney and brain in interorgan nitrogen exchange. Second J Gastroenterol Suppl. Vol 218, pp. 61-77

Dhawan, B.N, Singh, H.K, (1996). Pharmalogical studies on *Bacopa monnieri*, an ayurvedic Nootropic agent. Eur Neuropsychopharmacol. Vol 6, pp. 144

Dienel, G, Hertz, L. (2005). Astrocytic contributions to bioenergetics of cerebral ischemia. Vol 50, pp. 362-388

Dong, X.X, Wang, Y, Qin, Z.H. (2009). Molecular mechanisms of excitotoxicity and their relevance to pathogenisis of neurodegenerative diseases. Acta Pharmocol. Sin. Vol 30, pp. 379-387

Fernandez, A.G. (1992) .G. Med (Mexico) vol 128, pp. 443

Gupta, M, Mazumder, U.K, Bhawal, S.R. (1999). CNS activity of *Vitex negundo* Linn. In mice. Ind. J. Exp. Biol. Vol 37, pp. 143-146

Hudspith, M. J. (1997). Glutamate: A role in normal brain function, anesthesia, analgesia and CNS injury. Br J Anaesth. Vol 78, pp. 731–747

Johnston, M.W. (2005). Excitotoxicity in perinatal brain injury. Brain Pathol. Vol 15, pp. 234–240

Knox, W.E, Greengard, P. (1965). Regulation of some enzymes of nitrogen metabolism. In: Weber, G., (Ed.) Advan. Enzyme. Regul Pergamon press. Oxford. Vol 2, pp. 247

Lee, Y.L, Lardy, A.A, (1965). Influence of thyroid hormones on L-glycerophosphate dehydrogenases in various organs of the rat. J. Biol. Chem. Vol 240, pp. 1427-1430

Llansola, M, Rodrigo, R, Monfort, P, Montoliu, C, Kosenko, E, Cauli, O. (2007). NMDA receptors in hyperammonemia and hepatic encephalopathy. Metab Brain Dis. Vol 22, pp. 321-335

Mathern, G.W, Mendoza, D, Lozada, A. (1998). Hippocampal GABA and glutamate transporter immunoreactivity in patients with temporal lobe epilepsy. Neurology. Vol 52, pp. 453-72

Meister, A. (1955). In: Methods in Enzymology (Colowick, S.P, Kaplan, N.O. Eds.) Academic Press. New York. Vol 113, pp. 185-189

Petroff, C, Ognen, A. (2002). GABA and Glutamate in the Human Brain, Neurosci. Vol 8 issue 6, pp. 562-73

Paulose, C.S, Chathu, F, Khan, S.R, Krishnakumar, A. (2008). Neuroprotective role of *Bacopa monnieri* extract in epilepsy and effect of glucose supplementation during hypoxia: glutamate receptor gene expression. Neurochem. Res. Vol 33 issue 9, pp. 1663-1671

Petroff, O.A.C, Pleban, L.A, Spencer, D.D. (1995). Symbiosis between *in vivo* and *in vitro* NMR Spectroscopy: the creatine, N-acetylaspartate, glutmate, and GABA content of epileptic human brain. Magn Reson Imaging. Vol 13, pp. 1197-211

Platt, S.R. (2007). The role of glutamate in central nervous system health and disease - A review. Vet J. vol 173, pp. 278–286

Rastogi, S, Pal, R, Kulshreshtha, K. (1994). Bacoside A3- a triterpenoid saponin from *B. monniera*. Phytochemistry. Vol 36, pp. 133

Ray, S.K, Poddar, M.K. (1985). Effect of pentylenetetrazol on carbaryl-induced changes in striatal catecholamines. Biochem. Pharmacol. Vol 34, pp. 553-557

Reitman, S, Frankel, S, (1957). A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic-pyruvic transaminases. Am.J. Clin. Pathol. Vol 28, pp. 56

Rizwan, A.N, Ali, A, Dua, Y, Pal, S.N, Pillai, K.K. (2003). Effects of gabapentin and antidepressant drug combinations on convulsions and memoryin mice. Pol. J. Pharmacol. Vol 55, pp. 965–971

Rothman, S.M, Olney, J.W. (1986). Glutamate and the pathophysiology of hypoxic-ischemic brain damage. Ann Neurol. Vol 19, pp. 105–111

Sairam, K, Dorababu, M, Goel, R.K, Bhattacharya, S.K. (2002). Antidepressant activity of *Bacopa monniera* in exprerimental models of depresson in rats. Phytomedicine. Vol 9, pp. 207–211

Santos Junior, J.G, Do Monte, F.H.M, Russi, M, Agustine, P.E, Lanziotti, V.M.N.B. (2002). Proconvulsant effects of high doses of venlafaxine in Pentylenetetrazole-convulsive rats. Brazilian Journal of Medical and Biological Research. Vol 35, pp. 469-472

Schousboe, A, Drejer, J, Hertz, L, (1988). Uptake and release of glutamate and glutamine in neurons and

astrocytes in primary cultures. In: Kvamme, E., (Ed.) Glutamine and Glutamate in Mammals. Vol. II. CRC Press. Boca Raton. FL. Pp. 21-39

Schousboe, A, Frandsen, A, Wahl, P, Krogsgaard-Larsen, P, (1991). Excitatory amino acid induced cytotoxicity in cultured neurons: role of intracellular Ca2 homeostasis. In: Ascher P, Choi, D, Christen, Y, (Eds.) Glutamate. Cell Death and Memory. Springer. Berlin, pp. 137-152

Sellinger, O.Z, Schatz, R.A, Gregor, P. (1986). Cerebral methylations in epileptogenesis. Adv Neurol. Vol 44, pp. 465-73

Singh, K.H, Dhawan N.B. (1997). Neuropsychopharmacological effects of the Ayurvedic, nootropic *Bacopa monniera* Linn (Brahmi) Indian Journal of Pharmacology. Vol 29, pp. 359 -365

Sherwin, A.L. (1999). Neuroactive amino acids in focally epileptic human brain: a review. Neurochem Res. Vol 124, pp. 1387-95

Shukia, B, Khanna, N.K, Godhwani, J.L, (1987). Effect of Brahmi Rasayan on the central Nervous system. J Ethnopharmacol. Vol 21, pp. 65-74

Singh, H.K, Dhawan, B.N, (1997). Effect of *Bacopa monnieri Linn* (brahmi) extract on avoidance responses in rats. Journal of Ethnopharmacol. Vol 5, pp. 205-214

Singh, H.K, Rastogi, R.P, Srimal, R.C, Dhawan, B.N. (1998). Effect of bacosides A and B on avoidance responses in rats. Phytother. Res. Vol 2, pp. 70-75

Singh, R.H, Singh, L. (1980). Studies on the anti-anxiety effect of the Medyha Rasayana drug, Brahmi (*Bacopa monniera*Wettst.) – Part 1. J Res Ayur Siddha. Vol 1, pp. 133-148

Sivakumr, V, Ling, E.A, Lu, J, Kaur, C. (2010). Role of glutamate and its receptors and insulin-like Growth factors in hypoxia induced periventricular white matter injury. Glia. Vol 58, pp. 507– 523

Tiffany-Castiglioni, E, Roberst, J.A, Sheeler-gough, L, (1989). Reduction of glutamine synthetase specific activity in cultured astroglia by ferrous chloride. J Neurosci. Res. Vol 24, pp. 508-16

Van Gelder, N.M, Sherwin, A.L, (1986). Amino acid and catecholamine markers of metabolic abnormalities in human focal epilepsy. Adv Neurol. Vol 44, pp. 1011-32

Volpe, J.J. (2004). Neurobiology of periventricular leukomalacia in premature infant. Pediatr. Res. Vol 50, pp. 553–562

Wilson, C. L, Maidment, N. T, Shomer, M. H, Behnke, E. J, Ackerson, L, Fried, I, Engel, J. (1996). Comparison of seizure related amino acid release in human epileptic hippocampus versus a chronic, kainate rat model of hippocampal Epilepsy. Epilepsy Res. vol 265, pp. 245–254

Wu, C. (1963). Glutamine synthetase-I. A comparative study of its distribution in animals and its inhibition by DL-allo-σ-hydroxylysine. Comparative Biochemistry and Physiology. Vol. 8 issue 4, pp.335-351

Zhou, Y, Peng, L, Zhang, W.D, Kong, D.Y. (2009). Effect of Triterpenoid Saponins from *Bacopa monniera* on Scopolamine – Induced Memory Impairment in Mice. Planta Med. vol. 75 issue 6, pp. 568-574