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Antiepileptic potential of *Bacopa monnieri* in the rat brain during PTZ-induced epilepsy with reference to cholinergic system and ATPases

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ABSTRACT

Epilepsy is a chronic central nervous system disorder that occurs not only with the imbalance of glutamatergic neurons and inhibitory gamma-aminobutyric acid (γ-GABA) neurons, but also with abnormal Central cholinergic neuronal regulation. Since long term usage of antiepileptic drugs cause high incidence of pharmacoresistance and untoward side effects, attention has been paid in recent years to screen bioactive compounds from natural medicinal plants for the 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 compound with particular reference to Cholinergic system and ATPases in different brain regions of rat to explore the possible antiepileptic effect of different extracts of BM in comparison with Diazepam (DZ) (Reference control). The activity levels of Acetyl cholinesterase (AChE) and ATPases 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 AE. In addition Acetylcholine (ACh), 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 suggest that the bioactive factors present in the extracts offer neuroprotection by interrupting the pathological cascade that occurs during epileptogenesis.

1. Introduction

Epilepsy is one of the most complex groups of neurological disorders resulting in hyper excitability with asymmetrical bursts of electrical activity in dierent regions of central nervous system. It has been well documented that inhibition of GABAergic activity and/or exaggerated activity of glutamatergic neurotransmission has been primarily implicated in various types of epilepsies.[1,2] Although a direct role of Cholinergic pathway has not been established, studies in several models of epilepsy have suggested a link between Cholinergic activation and epileptogenesis.[3–6] In addition it has been recognized that purinergic transmission has also been involved in the release of ATP, as a co transmitter along with glutamate noradrenalin, GABA, acetylcholine and dopamine in the CNS.[7,8] Accumulating evidence suggest that ATPases such as Na^+, K^+ - ATP ases Mg^{2+} - ATPase and Ca^{2+} - ATPase play a pivatol role in maintenance of ionic gradiant coupled with ATP hydrolysis[9] and modulation of the activities of these enzymes have been associated with epileptogenesis. Considering the multiacterial neurochemical malfunctions as a consequence of epileptic seizeires, attempts have been made to design novel antileptic drugs (AEDs) targeting dier-ent neurotransmitter mechanisms in order to ameliorate the neurological deficits that occur during epileptogenesis. However, clinical management of epilepsy is a very comlex task because of co-existing neuro psychiatric complications[10] and manifestation of idiosyncratic reactions.[2]

During the fast few years, focus on ethnopharmacology research has increased all over the world and a growing body of evidence has indicated immense potential of medicinal plants as alternative and

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complementary therapies for many human ailments. *Bacopa monnieri* (BM) is being used in traditional medicine in the treatment of several neurological disorders including epilepsy.[11] *Bacopa monnieri* (brahmi), a traditional ayurvedic medicine has been used for centuries as a memory enhancer, anti-inflammatory, analgesic, antipyretic, and anti-epileptic agent.[11] More recently, preclinical studies have reported cognitive enhancing effects with various extracts of *Bacopa monnieri.* The triterpenoid saponins and their bacosides are responsible for enhancement of nerve impulse transmission. Many studies have revealed its pharmacological roles as cognition-enhancer,[12–14] antidepressant,[15] antioxidant,[16,17] antiulcerogenic agent,[18] and calcium antagonist.[19]

Keeping in view the neuroprotictive role of this medicinel plant the present investigation is carried out to investigate the antiepileptic effect of this medicinal plant during PTZ-induced epilepsy with respect to cholinergic neurotransmission and ATPase activities.

2. Material and methods

2.1. Plant material and preparation of extract

Bacopa Monnieri (BM) plant was collected from Thalakona forest and identified 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).[20] 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.

2.2. Animals

Male adult wistar rats weighing 150 ± 25 g were divided into six groups of six animals each and used as the experimental animals in the present investigation. The rats were purchased from the Sri Venkateswara Traders Pvt. Limited, Bangalore, maintained in the animal house of the department in polypropylene cages under laboratory conditions of 28 ± 2 °C temperature with photoperiod of 12 h light and 12 h dark and 75% relative humidity. The rats were fed with standard pellet diet (Hindustan Lever Ltd., Mumbai) and water *ad libitum*. 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.

2.3. 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 (180 mg/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 1 ml to the animal.

2.4. Induction of epilepsy

Convulsions were induced by an intraperitoneal (i.p.) injection of Pentylenetetrazole (60 mg/kg body weight) dissolved in saline.[21,22]

2.5. Administration of the test and reference substance

Each fraction of BM extract (180 mg/kg body weight) was dissolved in water and given to the animals for one week prior to the injection of PTZ.[23] Each animal were administered with standard drug diazepam (4 mg/kg, i.p) for one week prior to the injection of PTZ.[24]

2.6. 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 and 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 for analysis.

2.7. Biochemical analyses

2.7.1. Estimation of acetyl cholinesterase (AchE) activity

Acetyl cholinesterase activity was assayed by the method of Ellmann et al. (1961) with slight modifications.[25] The reaction mixture contains 270 μ moles of sodium phosphate buffer (pH 8.0), 10 μ moles of DTNB, 1.5 μl of acetyl thiocholine iodide (AtChI) and 100 μl of 2% brain homogenate. The initial absorbance of the reaction mixture was recorded at 412 nm in a Hitachi U-2000 spectrophotometer prior to the addition of the substrate. The reaction was initiated by adding acetyl thiocholineiodide. After 15 min incubation at room temperature, the yellow colour developed was read at 412 nm. A molar extinction coefficient of 4.12×10^{-3} was used to calculate the enzyme activity. The true cholinesterase activity was estimated by running parallel sets with a cholinesterase inhibitor 1:5-bis- [N-allyl-N-methyl-4-aminophenyl] pental-3-one dibromide (BW 284 C51 dibromide). This strongly inhibits the acetylcholinesterase present in the reaction mixture. The pseudocholinesterase activity can be calculated from this set. The pseudocholinesterase activity was subtracted from total cholinesterase activity to get true cholinesterase activity. The enzyme activity was expressed as μ moles of acetyl thiocholine hydrolyzed/mg protein/hr.

2.7.2. Acetylcholine (ACh) content

Acetylcholine content was estimated by the method of Hestrin (1949)[26] as given by Augustinson (1957).[27] Each region of brain tissue was placed in a test tube and boiled in hot water-bath for 10 min to denature the AChE activity. The tissues were homogenized in 2.0 ml of distilled water. To the homogenate, 1.0 ml of alkaline hydroxylamine hydrochloride was added followed by 1.0 ml of 1:1 hydrochloric acid solution. The contents were mixed thoroughly and centrifuged. To the supernatant 0.5 ml of 0.37 M ferric chloride was added and the brown colour developed was read at 540 nm against the reagent blank. The acetylcholine content was expressed as μ moles of acetylcholine/gm wet weight of tissue.

2.7.3. ATPases (ATP phosphohydrolase)

(a) Mg^{2+} and Na^{+}, K^{+} - ATP ases activities were assayed by the method of Fritz and Hamrick (1966) as reported by Desaiah and Ho (1979) with slight modifications.[28]

Tissue homogenates were prepared in ice cold 0.32 M sucrose containing 1.0 mM EDTA and 10 mM imidazole (pH 7.5). The homogenates were centrifuged at 1000×*g* and the supernatant obtained was used as an enzyme source. The reaction mixture in a volume of 3.0 ml contained 3 mM ATP, 3 mM $MgCl_2$, 100 mM NaCl, 20 mM KCl, 135 mM imidazole hydrochloric acid buffer (pH 7.5) and 10 mg of protein. The reaction mixture was incubated at 37 °C for 30 min and stopped by the addition of 0.1 ml of 50% TCA. Samples were then assayed for inorganic phosphate using the method of Lowry and Lopez (1946) as modified by Phillips and Hayes (1977).[29,30] The colour was read at 800 nm in a spectrophotometer. Mg^{2+} - ATPase activity was measured in presence of 1 mM ouabain, a specific inhibitor of Na^{+}, K^{+} - ATPase (McIlwain, 1963). Ouabain sensitive Na^{2+}, K^{+} - ATPase activity was obtained by the difference between total ATPase and Mg^{2+} - ATPase activity. The enzyme activity was expressed as μ moles of inorganic phosphate formed/mg protein/hr.

(b) Ca^{2+} - ATPase activity was determined by measuring the inorganic phosphate liberated during the hydrolysis of ATP. The reaction mixture contained 135 mM imidazole – HCl buffer (pH 7.5), 5 mM $MgCl_2$, 0.05 mM $CaCl_2$, 4 mM ATP and 30 mg of protein. The mixture was incubated at 37 °C for 30 min and stopped by the addition of 0.1 ml of 50% TCA. The inorganic phosphate formed was estimated by the method of Lowry and Lopez (1946) as modified by Phillips and Hayes (1977).[29,30] Mg^{2+} - ATPase activity was measured in the presence of 0.5 mM EGTA and this value was subtracted from total ATPase activity to get Ca^{2+} - ATPase activity. Enzyme activity was expressed as μ moles of inorganic phosphate formed/mg protein/hr

2.8. Statistical analysis

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

3. Result

ACh content and AChE activity were studied in different brain regions of rat during PTZ-induced epilepsy and on pre-treatment with different extracts of *Bacopa monnieri.* The ACh content was increased and the AChE activity was decreased during PTZ induced epilepsy in all the areas of brain (CC, CB, HC and PM) when compared to saline control. On pre-treatment with different extracts of *Bacopa monnieri,* the ACh content and AChE activity recorded maximal recovery in nHE, CE, nBE and EAE treated rats when compared to the PTZ-induced epileptic group (Tables 1 and 2).

The activity levels of Na^{+}/K^{+} - ATPase, Mg^{2+} - ATPase and Ca^{2+} - ATPase were estimated in different areas of the brain during PTZ-induced epilepsy and during the anticonvulsant treatment. The three ATPases recorded decreased activity levels in different regions of rat brain upon administration of PTZ when compared to saline controls. The activity levels were increased in all the brain regions of epileptic rats pre-treated with different extracts of *Bacopa monnieri* except for the treatment with aqueous extract (AE) when compared to the respective PTZ-induced epileptic group (Tables 3–5).

4. Discussion

Studies on several models of epilepsy have suggested a direct link between cholinergic activation in the hippocampus of mice and epileptogenesis.[4] In support of this Turski et al. (1989) reported induction of generalized seizures using muscarinic acetylcholine receptor (mAChR) agonist Pilocarpine.[31] The present study demonstrates differential levels of ACh content and AChE activity in different areas of the rat brain. AChE is an enzyme that terminates the signal transmission at the cholinergic synapses of neurons by rapid hydrolysis of the neuro transmitter Ach. Generally brain has the highest AChE activity than the other tissues. The different areas of brain are known to subserve different functions and any change in the cholinergic system of these areas will reflect in the behavior. The present study manifests differential level of ACh content and AChE activity in different areas of the rat brain during induced epilepsy and anti-epileptic treatment. AChE is an enzyme that terminates the signal transmission at the cholinergic synapses of neurons by rapid hydrolysis of the neurotransmitter, ACh.

It is well established that excessive levels of ACh in the cerebral cortex can produce epileptiform activity.[32] In consonance with the ACh content, the AChE activity was found to be inhibited during PTZ administration in all the brain regions. The cholinergic excitation appears to be controlled by the interaction of ACh with its receptor, and also subsequent interaction with AChE[33] and hence AChE can be used as an index of Cholinergic function and changes in enzyme activity may indicate alterations in the availability of ACh at the level of its receptors.[34] Differential changes in AChE activity was observed with different extracts in different regions of brain in epileptic-treated rats. In general, the AChE activity levels showed an increase with all the extracts except EE and AE. However, decreased AChE activity levels with some extracts indicating the countervail actions of these extracts on cholinergic hyperexcitability.

The medicinal importance of Brahmi with particular reference to neuroprotection in epilepsy and insomnia has been well documented in Indian traditional literature such as Athar-ved, Charak Samhita, Susrutha Samhita.[35] It has also been reported that the extracts of BM improve the activities of antioxidant defense enzymes such as superoxide dismutase (SOD), Catalase (CAT)[36] and scavange the free radicals.[17] Although the exact mechanism of action of BM is not known, there is evidence that the neuroprotective action could be attributed to a combination of cholinergic modulation and antioxidant effects.

In contrast, the increased AChE and decreased ACh with some plant extracts in all the areas of the brain implicate anticonvulsant effect of *Bacopa monnieri* extracts. From these result it is obvious that selected extracts of *Bacopa monnieri* exhibited conspicuous effects on the recovery of PTZ-induced seizures. This indicates that the antiepileptic effect of *Bacopa monnieri* extracts proceeds through generalized changes in different areas of brain in addition to the specific impact on the target areas. The subsequent decrease and increase in the activities of enzymes in PTZ, and epileptic-treated rats, however, would tend to indicate that direct relationship exists with respect to activities of cholinergic pathway and seizure susceptibility.[37] Hence, the changes in the expression of key cholinergic proteins and the associated cholinergic dysfunction may be considered as the key factors in explaining the basic mechanisms underlying in the CNS related epilepsy.[38]

Although the direct role of cholinergic pathways during induced epilepsy and antiepileptic treatment has not been demonstrated, these results implicate a mechanism involving a summation of

Table 1
Changes in the Acetylcholine (ACh) content in different brain regions of rats during PTZ-induced epilepsy and on Pre-treatment with different extracts of *Bacopa monnieri*.

S.No.	Brain area	SC	PTZ	EE + PTZ	nHE + PTZ	CE + PTZ	EAE + PTZ	nBE + PTZ	AE + PTZ	DZ + PTZ
1	CC	$4.973^{\{a\}}$	$6.588^{\{b\}}$	$6.737^{\{cb\}}$	$3.407^{\{d\}}$	$5.769^{\{e\}}$	$3.327^{\{fd\}}$	$3.264^{\{gdf\}}$	$7.836^{\{h\}}$	$6.203^{\{i\}}$
		±0.024	±0.045	±0.012	±0.051	±0.159	±0.068	±0.079	±0.022	±0.341
			(32.47)	(35.47)	(-31.49)	(16.00)	(-33.11)	(-34.36)	(33.44)	(-24.733)
				[2.261]	[-48.28]	[-12.43]	[-49.49]	[-50.45]	[18.94]	[-5.843]
2	CB	$4.956^{\{a\}}$	$7.061^{\{b\}}$	$7.077^{\{cb\}}$	$3.059^{\{d\}}$	$6.641^{\{e\}}$	$4.009^{\{fc\}}$	$3.973^{\{gf\}}$	$7.928^{\{h\}}$	$6.741^{\{ie\}}$
		±0.039	±0.193	±0.034	±0.035	±0.358	±0.203	±0.010	±0.067	±0.061
			(42.47)	(42.47)	(-38.27)	(33.99)	(-19.12)	(-13.57)	(59.96)	(-36.01)
				[0.127]	[-56.67]	[-5.948]	[-43.22]	[-43.73]	[12.27]	[-4.531]
3	PM	$4.646^{[a]}$	$6.222^{\{b\}}$	$7.093^{\{cg\}}$	$5.062^{\{da\}}$	$5.481^{\{ed\}}$	$3.725^{\{f\}}$	$2.783^{\{g\}}$	$6.804^{\{hc\}}$	$5.440^{\{ide\}}$
		±0.458	±0.214	±0.034	±0.125	±0.413	±0.202	±0.021	±0.084	±0.187
			(33.92)	(52.66)	(-8.953)	(18.70)	(-19.82)	(-40.12)	(46.42)	(-17.08)
				[13.99]	[-18.64]	[-11.90]	[-40.13]	[-55.27]	[9.353]	[-12.56]
4	HC	$7.034^{\{a\}}$	$8.629^{\{b\}}$	$9.090^{\{c\}}$	$5.372^{\{d\}}$	$6.196^{\{e\}}$	$6.625^{\{f\}}$	$6.596^{\{gf\}}$	$9.122^{\{hc\}}$	$5.928^{\{i\}}$
		±0.012	±0.019	±0.025	±0.060	±0.088	±0.005	±0.004	±0.069	±0.003
			(34.49)	(29.23)	(-23.61)	(11.91)	(-5.814)	(-6.226)	(29.68)	(-15.72)
				[5.342]	[-37.74]	[-28.19]	[-23.22]	[-23.56]	[5.713]	[31.30]

All the values are Mean, ±SD of six individual observations. Values with same Superscript is 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 Acetylcholinesterase (AChE) in different brain regions of rats during PTZ-induced epilepsy and on Pre-treatment with different extracts of *Bacopa monnieri*.

S.No.	Brain area	SC	PTZ	EE + PTZ	nHE + PTZ	CE + PTZ	EAE + PTZ	nBE + PTZ	AE + PTZ	DZ + PTZ
1	CC	$4.869^{\{a\}}$	$3.218^{\{b\}}$	$2.064^{\{C\}}$	$6.207^{\{d\}}$	$3.716^{\{e\}}$	$6.338^{\{fd\}}$	$5.009^{\{ca\}}$	$2.583^{\{h\}}$	$7.181^{\{i\}}$
		±0.046	±0.417	±0.043	±0.096	±0.149	±0.086	±0.007	±0.273	±0.065
			(-33.90)	(-57.60)	(27.47)	(-23.68)	(30.19)	(2.875)	(-46.95)	(47.51)
				[-35.86]	[92.88]	[15.47]	[96.95]	[55.65]	[-19.73]	[123.15]
2	CB	$5.297^{\{a\}}$	$2.872^{\{b\}}$	$1.549^{\{gb\}}$	$6.396^{\{d\}}$	$3.233^{\{e\}}$	$6.537^{\{fd\}}$	$5.708^{\{fd\}}$	$1.826^{\{hc\}}$	$5.639^{\{iag\}}$
		±0.096	±0.332	±0.154	±0.210	±0.163	±0.188	±0.111	±0.123	±0.022
			(-45.78)	(-70.75)	(20.72)	(-38.98)	(23.40)	(7.759)	(-65.52)	(6.456)
				[-46.06]	[122.70]	[12.56]	[127.61]	[98.74]	[-36.42]	[96.34]
3	PM	$5.074^{\{a\}}$	$3.497^{\{b\}}$	$2.734^{\{g\}}$	$5.344^{\{da\}}$	$3.986^{\{e\}}$	$5.477^{\{fd\}}$	$5.603^{\{gdf\}}$	$2.595^{\{hc\}}$	$6.778^{\{i\}}$
		±0.065	±0.205	±0.396	±0.030	±0.017	±0.103	±0.092	±0.163	±0.132
			(-31.08)	(-46.11)	(25.03)	(-21.44)	(7.963)	(10.42)	(-48.850)	(33.60)
				[-21.81]	[52.81]	[13.98]	[56.61]	[60.22]	[-25.79]	[93.82]
4	HC	$4.973^{\{a\}}$	$3.017^{\{b\}}$	$2.135^{\{gb\}}$	$6.437^{\{d\}}$	$4.497^{\{e\}}$	$5.313^{\{fa\}}$	$5.997^{\{gd\}}$	$2.993^{\{hb\}}$	$6.935^{\{i\}}$
		±0.312	±0.004	±0.525	±0.156	±0.118	±0.050	±0.217	±0.012	±0.063
			(-39.33)	(-57.06)	(29.41)	(9.591)	(6.836)	(20.59)	(-39.81)	(39.43)
				[-29.23]	[113.35]	[49.05]	[76.10]	[98.77]	[-0.795]	[129.86]

All the values are Mean, ±SD of six individual observations. Values with same Superscript is 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 3
Changes in the activity levels of Sodium, Potassium – ATPase (Na^+/K^+ -ATPase) in different brain regions of rats during PTZ-induced epilepsy and on Pre-treatment with different of extracts *Bacopa monnieri*.

S.No.	Brain area	SC	PTZ	EE + PTZ	nHE + PTZ	CE + PTZ	EAE + PTZ	nBE + PTZ	AE + PTZ	DZ + PTZ
1	CC	$5.424^{\{a\}}$	$4.420^{[b]}$	$8.799^{[c]}$	$7.134^{[d]}$	$5.557^{[ea]}$	$5.690^{[fae]}$	$8.442^{[g]}$	$4.153^{[hb]}$	$9.118^{[ic]}$
		±0.334	±0.073	±0.214	±0.140	±0.248	±0.112	±0.118	±0.103	±0.070
			(-18.51)	(62.22)	(31.52)	(2.452)	(4.904)	(55.64)	(-23.43)	(68.10)
				[99.07]	[61.40]	[25.72]	[28.73]	(90.99]	[-6.040]	[106.28]
2	CB	$5.336^{[a]}$	$3.656^{[b]}$	$7.052^{[c]}$	$6.297^{[d]}$	$6.708^{[e]}$	$7.316^{[fc]}$	$8.327^{[g]}$	$4.855^{[h]}$	$8.466^{[ig]}$
		±0.057	±0.157	±0.062	±0.103	±0.278	±0.016	±0.158	±0.275	±0.088
			(-13.35)	(32.15)	(18.00)	(25.71)	(37.106)	(56.05)	(-9.014)	(58.65)
				[92.88]	[72.23]	[83.47]	[100.10]	(127.76]	[-21.90]	[131.56]
3	PM	$7.795^{[a]}$	$6.180^{[b]}$	$9.298^{[c]}$	$9.600^{[dc]}$	$8.661^{[e]}$	$8.879^{[fe]}$	$8.998^{[gcef]}$	$6.968^{[h]}$	$9.975^{[id]}$
		±0.348	±0.065	±0.262	±0.205	±0.185	±0.167	±0.327	±0.025	±0.012
			(-20.71)	(19.20)	(23.15)	(11.10)	(13.90)	(15.43)	(-10.60)	(27.96)
				[50.45]	[55.33]	[40.14]	[43.67]	(45.59]	[-3.430]	[61.40]
4	HC	$7.676^{[a]}$	$6.176^{[b]}$	$9.524^{[c]}$	$8.795^{[d]}$	$8.998^{[ed]}$	$9.298^{[fce]}$	$9.759^{[gc]}$	$5.614^{[h]}$	$9.965^{[ig]}$
		±0.060	±0.059	±0.312	±0 .172	±0.126	±0.047	±0.218	±0.242	±0.032
			(-19.54)	(24.07)	(14.57)	(17.22)	(21.13)	(27.13)	(-26.86)	(29.82)
				[54.20]	[42.40]	[45.69]	[50.55]	(58.01]	[-9.099]	[61.35]

All the values are Mean, ±SD of six individual observations. Values with same Superscript is 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 activity levels of Magnesium – ATPase (Mg^{2+} -ATPase) in different brain regions of rats during PTZ-induced epilepsy and on Pre-treatment with different extracts Bacopa *monnieri*.

S.No.	Brain area	SC	PTZ	EE + PTZ	nHE + PTZ	CE + PTZ	EAE + PTZ	nBE + PTZ	AE + PTZ	DZ + PTZ
1	CC	8.565[a] ±0.130	6.479[b] ±0.297 (-25.64)	9.659[c] ±0.089 (12.77) [49.08]	7.341[d] ±0.192 (14.29) [13.30]	7.219[ed] ±0.078 (4.063) [11.42]	8.984[fa] ±0.360 (4.892) [38.66]	9.615[gc] ±0.265 (12.25) [48.40]	5.695[h] ±0.096 (-33.50) [-12.10]	9.702[icg] ±0.246 (13.27) [49.74]
2	CB	7.899[a] ±0.127	5.944[b] ±0.053 (-24.74)	9.907[c] ±0.095 (25.42) [66.67]	9.149[dc] ±0.130 (15.82) [53.91]	9.538[ecd] ±0.380 (16.96) [60.46]	8.853[fd] ±0.117 (12.07) [48.94]	9.538[gcde] ±0.259 (16.951) [60.47]	5.492[h] ±0.389 (-30.47) [-7.604]	9.849[ieg] ±0.105 (24.68) [65.69]
3	PM	6.261[a] ±0.200	4.585[b] ±0.099 (-26.68)	7.984[c] ±0.009 (27.51) [74.13]	7.387[d] ±0.169 (17.98) [61.11]	6.624[e] ±0.157 (5.797) [44.47]	6.961[f] ±0.021 (11.21) [51.82]	7.782[gc] ±0.047 (16.30) [69.72]	4.251[h] ±0.165 (-32.10) [-7.284]	6.261[ia] ±0.200 (24.29) [36.55]
4	HC	7.912[a] ±0.119	5.864[b] ±0.140 (-25.88)	8.996[c] ±0.267 (13.70) [53.41]	8.600[d] ±0.327 (8.695) [46.65]	8.881[ecd] ±0.105 (12.24) [51.44]	8.502[fd] ±0.118 (7.457) [44.98]	8.391[gdf] ±0.081 (6.054) [43.09]	5.491[h] ±0.199 (-30.59) [-6.360]	9.041[ice] ±0.011 (14.26) [54.17]

All the values are Mean, ±SD of five individual observations. Values with same Superscript is 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 5
Changes in the activity levels of Calcium – ATPase (Ca^{2+} -ATPase) in different brain regions of rats during PTZ-induced epilepsy and on Pre-treatment with different extracts of *Bacopa monnieri*.

S.No.	Brain area	SC	PTZ	EE + PTZ	nHE + PTZ	CE + PTZ	EAE + PTZ	nBE + PTZ	AE + PTZ	DZ + PTZ
1	CC	8.596[a] ±0.161	7.504[b] ±0.219 (-12.705)	9.713[c] ±0.269 (13.00) [29.43]	8.990[da] ±0.009 (4.583) [19.80]	9.399[ec] ±0.164 (9.341) [25.25]	9.977[fc] ±0.015 (16.06) [32.95]	9.609[gcef] ±0.274 (11.79) [28.05	6.719[hb] ±0.293 (-10.19) [-10.46]	10.400[g] ±0.226 (20.98) [37.36]
2	CB	8.105[a] ±0.033	7.103[b] ±0.072 (-12.35)	9.626[c] ±0.145 (18.78) [35.52]	9.323[dc] ±0.088 (15.04) [31.25]	8.396[ea] ±0.125 (3.590) [18.20]	8.835[f] ±0.049 (9.006) [24.38]	9.440[gd] ±0.056 (16.47) [32.90]	6.126[hb] ±0.121 (-12.08) [-13.97]	9.836[g] ±0.048 (21.37) [38.47]
3	PM	7.349[a] ±0.309	6.713[b] ±0.309 (-8.65)	8.987[c] ±0.195 (22.29) [33.82]	8.079[d] ±0.147 (9.933) [20.34]	8.625[ec] ±0.229 (17.37) [28.48]	8.623[fce] ±0.099 (17.35) [28.45]	8.918[gcef] ±0.563 (21.36) [32.84]	6.094[h] ±0.129 (-17.06) [-9.220]	9.936[g] ±0.042 (35.22) [48.01]
4	HC	8.825[a] ±0.089	6.976[b] ±0.028 (-20.95)	9.894[c] ±0.093 (12.11) [41.82]	9.597[d] ±0.169 (8.747) [37.57]	9.938[ec] ±0.021 (12.61) [42.45]	9.892[fce] ±0.092 (12.07) [41.80]	9.842[gcdef] ±0.045 (11.52) [41.08]	6.325[h] ±0.299 (-28.32) [9.332]	9.986[gcefg] ±0.026 (13.15) [43.14]

All the values are Mean, ±SD of six individual observations. Values with same Superscript is 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.

excitatory neurotransmission in CNS during epilepsy. Different extracts of *Bacopa monnieri* cause perceptible changes in the cholinergic system, at least as part of its antiepileptic effect. In the present study, the ACh content and AChE activities recorded changes that were maximal with nHE, EAE, nBE and CE. After the antiepileptic treatment with different BM extracts, the ACh content vis-à-vis Cholinesterase activities started returning to the control levels reaching more or less to the control levels, suggesting that selected BM extracts offer neuroprotection by ameliorating the cholinergic activity during induced epilepsy.

The work in this study has also been taken up with the aim of examining the changes in the enzymes concerned with energy metabolism and membrane transport functions, viz. Na^+/K^+ -, Mg^{2+}- and Ca^{2+} -ATPase activities. The changes were recorded with different extracts of *Bacopa monnieri* extracts following the administration of epileptic drug PTZ. Sodium Potassium ATPase and Calcium ATPase are membrane bound enzymes and play a pivotal role in the homeostasis of Na^+, K^+ and Ca^{2+} in the cells. Apart from these active transport mechanisms, ionic balance is also affected by the activities of sodium and potassium channels whose activity (open or closed condition) is voltage dependent. It has been reported that alteration in the activities of different ATPases during stress might have broder biological effects such as alteration in the permeability property of nerve membrane to Ca^{2+} ions, the blockage of the membrane to the passage of Na^{2+} ions and inhibition of sodium-potassium pump. The activity levels of different ATPases were decreased in all the brain areas during PTZ-induced epilepsy. The declined activity of ATPases with PTZ administration is in agreement with earlier reports.[39,40] Parallel to the Na^{2+}, K^+ and Mg^{2+},-ATPases, the decreased activity of Ca^{2+}-ATPase suggests its involvement in Ca^{2+} dependent quantal release of neurotransmitters with particular reference to cholinergic and adrenergic neurons.

Although the mechanism of inhibition of ATPase in PTZ-induced epilepsy is not clear, it is presumed that PTZ, the GABA antagonist, induces oxidative stress as evidenced by significant enhancement of lipid peroxidation in different regions of rat brain which might cause alterations in the membrane architecture and activity levels of membrane bound enzymes including ATPases. In addition, it has also been reported that neurochemical alterations during PTZ-induced epilepsy might be implicated to the generation of free-radicals through altered antioxidant defense mechanisms.[41] Hence,

membrane lipid peroxidation associated with in an altered membrane fluidity, altered permeability to ions and changes in the activity of membrane bound enzymes have been implicated in the PTZ-induced epilepsy. Inhibition of the activities of all three ATPases, as observed in the present investigation, leads to uncontrolled dendritic discharges in Purkinje cells of rat cerebellum, and causes electrographically recorded seizures. Emerging evidence suggest that certain epilepsies may be a family of channelopathies with defects involving mutations in the Na^{+}, K^{+} or Ca^{2+} channels whose activities are related to their voltage dependent conditions or defects in the membrane-bound enzymes such as Na^{+}, K^{+} ATPase and Ca^{2+} ATPase that regulate the transport of ions across the cell membrane.[42] Defects in Na^{+} and K^{+} transport have also been implicated to the reduced clearance of extracellular K^{+} released from depolarized neurons during epilepsy. It has also been reported that the defect may be in the passive or active transport mechanisms where in Na^{+}, K^{+} -ATPase could be altered which has an indirect bearing on Ca^{2+}- transport mechanism involving Ca^{2+}ATPase activity. Lowered enzyme activities of ATPases in epilepsy might be due to this defect apart from possible defect in cell membrane.

The decrease in the activity levels of Na^{+}, K^{+} ATPase and Ca^{2+} ATPase observed in this study is similar to the results seen in the case of animal models also. Rapport et al. (1981)[39] have found reduced activity of Na^{+} K^{+} ATPase in human and monkey epileptic brain. Trams and Lauter (1978)[43] found a deficiency of ecto - Ca^{2+} ATPase in seizure prone mice. Indeed, various CNS disorders such as seizures, Parkinson, Huntington, and Alzheimer diseases have been associated with mitochondrial dysfunction, excitotoxicity, and generation of reactive oxygen species generation.[44,45] Since the ionic balance in the neurons are important in the conduction of action potentials, the decrease in the activities of ATPases possibly alter the flux of ions thus alter the membrane permeability properties during induced epilepsy. All the three ATPases showed increased activities in all the regions of brain of epileptic rats after pre-treatment with different extracts of BM except AE. The present findings are in agreement with the observations of Oliveira et al. (2004).[46] Our results indicate that GABAergic failure may underlie the currently observed inhibition of ATPase activity by PTZ, and that *Bacopa monniera* extracts may act on the GABAergic system, since it also prevented the convulsions and inhibition of ATPase activity induced by PTZ. Our results also suggest that the neuroprotective effect of DZ, EE, nBE, nHE, EAE, and CE extracts against PTZ induced seizures. Several authors have shown that *B. monniera* was able to prevent lipid peroxidation *in vitro* and *in vivo*,[14,47] and quench super oxide, hydroxyl and nitric oxide radicals effectively *in vitro*.[16,17] It is widely accepted that neuronal damage can be significantly minimized by free radical scavengers. *B. monniera* showed significant antioxidant effect per se and in stressed animals.[18,36] It has been shown that *B. monniera* protected morphine induced rat brain mitochondrial damage that could have favored the efficiency of ATP production.[14] This energy-promoting action may be responsible for the improved mitochondrial function and maintenance of ATPases in the present study.

Although not related to the present studies, it has been shown that *Curcumin* plays neuroprotective role by elevating Na^{+}, K^{+} -ATPase activity in all the brain regions of rat.[48] Similar elevation of ATPase activity levels have also been reported in brain microsomes of rats pre-treated with *Curcumin*.[49] Studies have also shown that Bacoside A, an active factor from *Bacopa monniera,* cause marked inhibition in lipid peroxidation, activation of the activities of ATPases, and also maintains ionic equilibrium.[50] Similarly, *Ginko biloba* extract (EGD761) pre-treatment exhibited conspicuous neuroprotective effect by activating the activities of ATPases and inhibiting the lipid peroxidation that were altered during ischemia.[51] The present findings in conjunction with the earlier reports suggest that the bioactive factors presented in selected extracts of BM exhibit neuroprotective and anticonvulsant effects by reversing the purinergic metabolism that has been inhibited during PTZ-induced epilepsy. These findings add to the growing list of channelopathies[52] and suggest that drugs that directly or indirectly modulate the activities of the ATPases will be helpful in the treatment of seizure disorders.

5. 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 (EE), 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, and ATPases 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. 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 e*xhibited anti-seizure activity as evidenced from the present investigation, it might be clinically useful in the control of human epilepsies.

Declaration of competing interest

The authors declare that they have no conflict of interest.

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