

## Anticholinesterase Efficacy of *Bacopa monnieri* against the Brain Regions of Rat - A novel approach to therapy for Alzheimer's disease



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**Abstract :** *Bacopa monnieri* (Neer Brahmi) is a well-known memory enhancer in Indian folklore. Ethanol extract of both leaves and stems of *Bacopa monnieri* was detected to have acetylcholinesterase inhibitory properties. The *in vivo* anticholinesterase effect of ethanol extract of *Bacopa monnieri* on discrete brain regions of male albino rat was studied. The oral dose (100 mg/kg body weight) of ethanol extract of *Bacopa monnieri* inhibited acetylcholinesterase (AChE) differentially in various brain regions viz. Cerebral cortex (51.6 %) > Cerebellum (51%) > Pons (44 %) > Thalamus (41.6 %) > Hippocampus (38.1 %) > Brain stem (34.3 %) > Striatum (24.9 %). *In vivo* enzyme kinetic study indicated enhancement of Km values for AChE in each brain region due to extract of *Bacopa monnieri*. The highest increase in Km value was observed in the cerebral cortex while lowest in the brain stem. However, Vmax was unchanged in treated group and remained same as that of control group. Thus, extract of *Bacopa monnieri* elicited competitive inhibition in AChE in all brain regions studied. *Bacopa monnieri* may provide a potential natural source of bioactive compounds and maybe beneficial to the treatment of Alzheimer's disease.

**Key Words :** Acetylcholinesterase (AChE), Alzheimer's disease, *Bacopa monnieri*, Rat, Brain regions, Km, Vmax.

### Introduction :

Acetylcholinesterase, a major component of the central and peripheral nervous system, is ubiquitous among multi-cellular animals, where its main function is to terminate synaptic transmission by hydrolyzing the neurotransmitter, acetylcholine.

Alzheimer's disease (AD) is one of the most common forms of dementia affecting approximately 10% of the population over the age of 65 years (Racchi *et al.*, 2004, Heinrich and Toeh, 2004, Alvarez and Inestrosa, 2005). In a field of several theoretical options, the best approach has been the use of AChE inhibitors (AChEIs), which led to the introduction of tacrine as the first AChEI specifically approved for the treatment of AD. Now, several kinds of AChEIs, such as donepezil, galantamine and rivastigmine are available for the symptomatic treatment of patients with mild to moderate AD (Racchi *et al.*, 2004). However, these compounds have been reported to have the problems associated with the gastrointestinal disturbances and bioavailability (Schulz, 2003). One of the most important approaches for treatment of this disease involves the enhancement of acetylcholine level in brain using AChE inhibitors. Several studies have reported anticholinesterase activity of the plant extracts and drugs (Whitehouse, 1993; Hacibekiroglu, 2011).

Recently, galanthamine, an alkaloid isolated

from different *Galanthus* species (Amaryllidaceae) has been found to be a potent and reversible acetylcholinesterase inhibitor (Orhan, 2009). *Salvia triloba* and *Teucrium polium* contain active components, which have potential inhibitory efficacy against Acetylcholinesterase (Orhan and Aslam, 2009). However, no reports are available on the *in vivo* anticholinesterase activity of *Bacopa monnieri*, hence the present investigation was undertaken to evaluate *in vivo* anticholinesterase activity of ethanol extracts of aerial parts of *Bacopa monnieri* against rat brain AChE.

### Materials And Methods :

#### Plant extract :

Fresh *Bacopa monnieri* plants were procured from Jawaharlal Nehru Agriculture University, Jabalpur (M.P.). It was authenticated by taxonomists. The fresh aerial parts of the plant i.e. leaves and twigs were thoroughly washed in water and shade dried then were ground to powder. The powdered plant material was extracted with ethanol (90%) in a soxhlet apparatus. The residue obtained after removing the solvent dried to semisolid mass.

#### Animals:

Adult male rats weighing 225-250 gms were housed in polypropylene cages under controlled environmental conditions (room temp. 30 ± 2°C with

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12:12 h. light/dark cycle) with free standard rat feed and tap water *ad libitum* were available. Rats were acclimatized for 7 days before experimentation. Rats were dosed daily for 15 days using an animal feeding canula.

#### Treatment:

The treatment groups were: (I) – Control: Administered double distilled water vehicle 1ml/kg body weight. (II): Administered 100 mg/kg body weight ethanol extract of *Bacopa monnieri* in double distilled water, daily for 15 days.

After administration of last dose of experiment, rats were euthanized with ether, brain was quickly removed. Various brain regions viz. cerebral cortex, striatum, cerebellum, thalamus, pons, hippocampus, and brain stem (medulla) were separated following method of Zeman and Innes, (1963).

Tissues were washed separately in 0.9% pre chilled saline. 10 % homogenates (w/v) were prepared in chilled 0.1 M phosphate buffer (pH 7.4) using Potter-Elvehgem homogenizer followed by centrifugation at 2000 rpm at 4°C for 10 min. in cooling centrifuge. Homogenates were kept in deep freeze after preparation and analyzed for AChE activity, inhibition and enzyme kinetic assays.

#### Enzyme Assay:

AChE activity was determined spectrophotometrically at 412 nm following method of Ellman *et al.* (1961). Acetylthiocholine iodide was used as substrate. Enzyme kinetic constants like Michaelis Menten constants ( $K_m$ ) and maximum velocity ( $V_{max}$ ) were determined by Lineweaver Burk plots. Protein contents in tissues were determined according to method of Lowry *et al.* (1951), using Bovine serum albumin as standard.

#### Statistical Analysis :

Mean  $\pm$  S.D was calculated for specific activity of AChE in the brain regions of each group of rat. Student's t-Test was employed to find out significance values of AChE activity and AChE kinetics of different groups of animals.

#### Results :

##### ***In vivo* effect of ethanol extract of *Bacopa monnieri* on Acetylcholinesterase activity of the Brain regions of rat (Table- 1, Fig- 1)**

The result of oral dose (100 mg/kg body weight) of ethanol extract of *Bacopa monnieri* on AChE activity in Brain areas is given in table - 1, which, demonstrate that the highest AChE inhibition i.e. 51.6 % occurred in the cerebral cortex and cerebellum and lowest inhibition

i.e. 24.9% was found in the striatum. The AChE inhibition in these tissues were observed as : Cerebral cortex (51.6%) > Cerebellum (51%) > Pons (44%) > Thalamus (41.6 %) > Hippocampus (38.1%) > Brain stem (34.3 %) > Striatum (24.9%).

##### ***In vivo* effect of ethanol extract of *Bacopa monnieri* on Acetylcholinesterase kinetics of brain regions of rat (Table - 1, Fig-2)**

The enzyme kinetic study indicated enhancement of  $K_m$  values for AChE in each tissue of rat. The highest increase in  $K_m$  value was observed in the cerebral cortex while lowest in the brain stem. The gradual increasing  $K_m$  was seemed to be as follows: Cerebral cortex ( $1.11 \times 10^{-3}$  M) > Striatum ( $1.10 \times 10^{-3}$  M) > Thalamus ( $1.0 \times 10^{-3}$  M) > Pons ( $0.89 \times 10^{-3}$  M) > Hippocampus ( $0.76 \times 10^{-3}$  M) > Cerebellum ( $0.54 \times 10^{-3}$  M) > Brain stem ( $0.26 \times 10^{-3}$  M). The maximum velocity i.e.  $V_{max}$  was found to be constant in each tissue of both control and treated group of rat. The result demonstrated that the ethanol extract of *Bacopa monnieri* yields competitive inhibition in AChE kinetics in each Brain region of the rat.

#### Discussion :

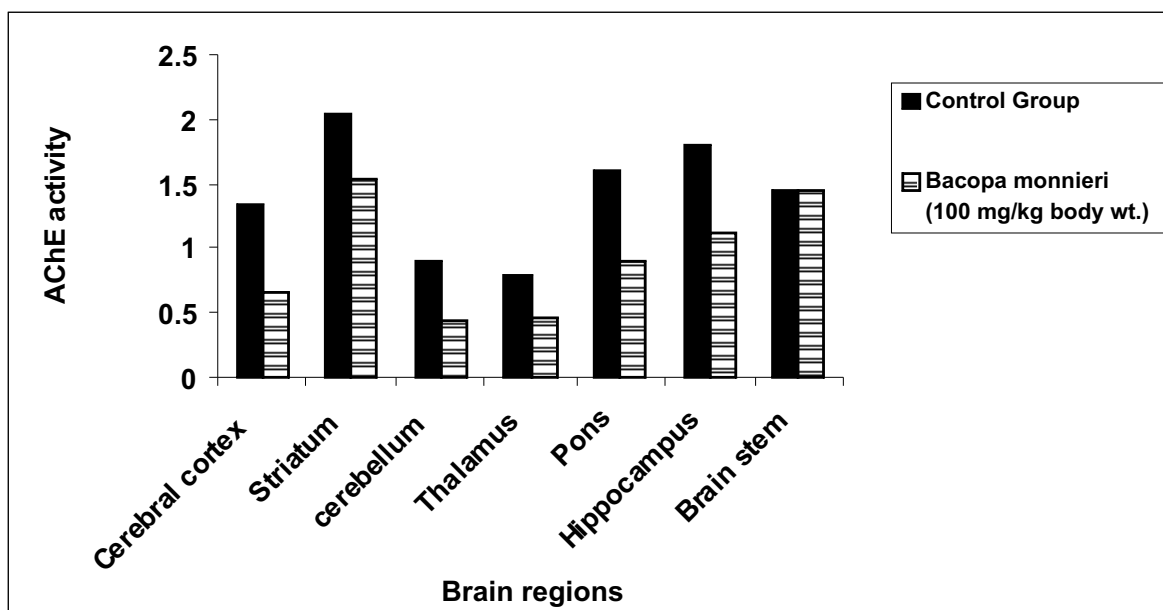
In the present investigation significant AChE inhibition was observed due to 100 mg/kg body weight oral dose of ethanol extract of *Bacopa monnieri* in all brain regions of albino rats. A significant inhibition was observed in each brain region showing highest inhibition in the cerebral cortex. The extract of *Bacopa monnieri* contained 45-50% bacosides measured according to the method of Pal and Sarin (1992). The bacoside is an alkaloid, which might be responsible for the AChE inhibition.

Das *et al.* (2002) compared the effect of ethanolic extracts of *Bacopa monnieri* and *Ginkgo biloba* on AChE activity of mice brain. 30 mg/kg; 100 mg/kg and 300 mg/kg *Bacopa monnieri* and *Ginkgo biloba* showed a concentration dependent *in vitro* AChE inhibition in mice brain. However, AChE was inhibited more in presence of *Ginkgo biloba* than *Bacopa monnieri*. Ronsted *et al.* (2008) recently investigated the inhibitory effect of all species of Narcissus. He presented a new approach drug discovery and for the first time reported co-relation of plants alkaloids and AChE inhibition. Previously, similar attempt has been made by Lopez *et al.* (2002) to find other AChE inhibitor of plant origin. They investigated 26 species of Narcissus and found many-fold variation in AChE inhibitory activity by different Narcissus species. Anticholinesterase property of extract contain heptyl-physostigmine was studied by De Sarno *et al.* (1989). 5 mg/kg extract produced 82% inhibition in AChE activity of whole brain of rat. However, differential

**Table – 1.** *In vivo* Acetylcholinesterase activity (Activity/mg protein/min.), inhibition, Km x10<sup>-3</sup> M and Vmax of AChE of Brain regions of Rat of control and exposed group to *Bacopa monnieri* (100 mg/kg body wt.). The AChE specific activity was expressed in μ moles of ACTI hydrolyzed/ mg protein /min.

Brain regions	AChE activity		Km x10 <sup>-3</sup> M		Vmax of both groups
	Control	<i>Bacopa monnieri</i> (100 mg/kg body wt.)	Control	<i>Bacopa monnieri</i> (100 mg/kg body wt.)	
Cerebral cortex	1.35 ± 0.51 -	0.65 ± 0.5*** (-51.6)	1±0.5**	1.11±0.54** +11%	0.4
Striatum	2.04 ± 0.63 -	1.53 ± 0.7* (-24.9)	0.83±0.25*	1.1±0.41* +50%	0.23
Cerebellum	0.89 ± 0.61 -	0.43 ± 0.25** (-51)	0.46±0.32***	0.54±0.45* +17%	0.27
Thalamus	0.79 ± 0.60 -	0.46 ± 0.35*** (-41.6)	0.68±0.51***	1±0.45* +47%	0.33
Pons	1.60 ± 0.65 -	0.88 ± 0.55*** (-44.8)	0.50±0.35***	0.89±0.61*** +78%	0.25
Hippocampus	1.81 ± 0.54 -	1.12 ± 0.52* (-38.1)	0.67±0.61***	0.76±0.62*** +13%	0.25
Brain stem	1.44 ± 0.67 -	0.95 ± 0.42* (-34.3)	0.20±0.12**	0.26±0.23*** +30%	0.20

Values are expressed as mean ± SD of 5 animals in each group, analyzed by student's t-test  
\*\*\*;p<0.001, \*\*;p<0.01,\*;p<0.05, compared with control vs. treated.



**Fig. - 1.** *In vivo* Acetylcholinesterase activity (Activity/mg protein/min.) in Brain regions of Rat of control and exposed group to *Bacopa monnieri* (100 mg/kg body wt.). The AChE specific activity was expressed in μ moles of ACTI hydrolyzed/ mg protein /min.

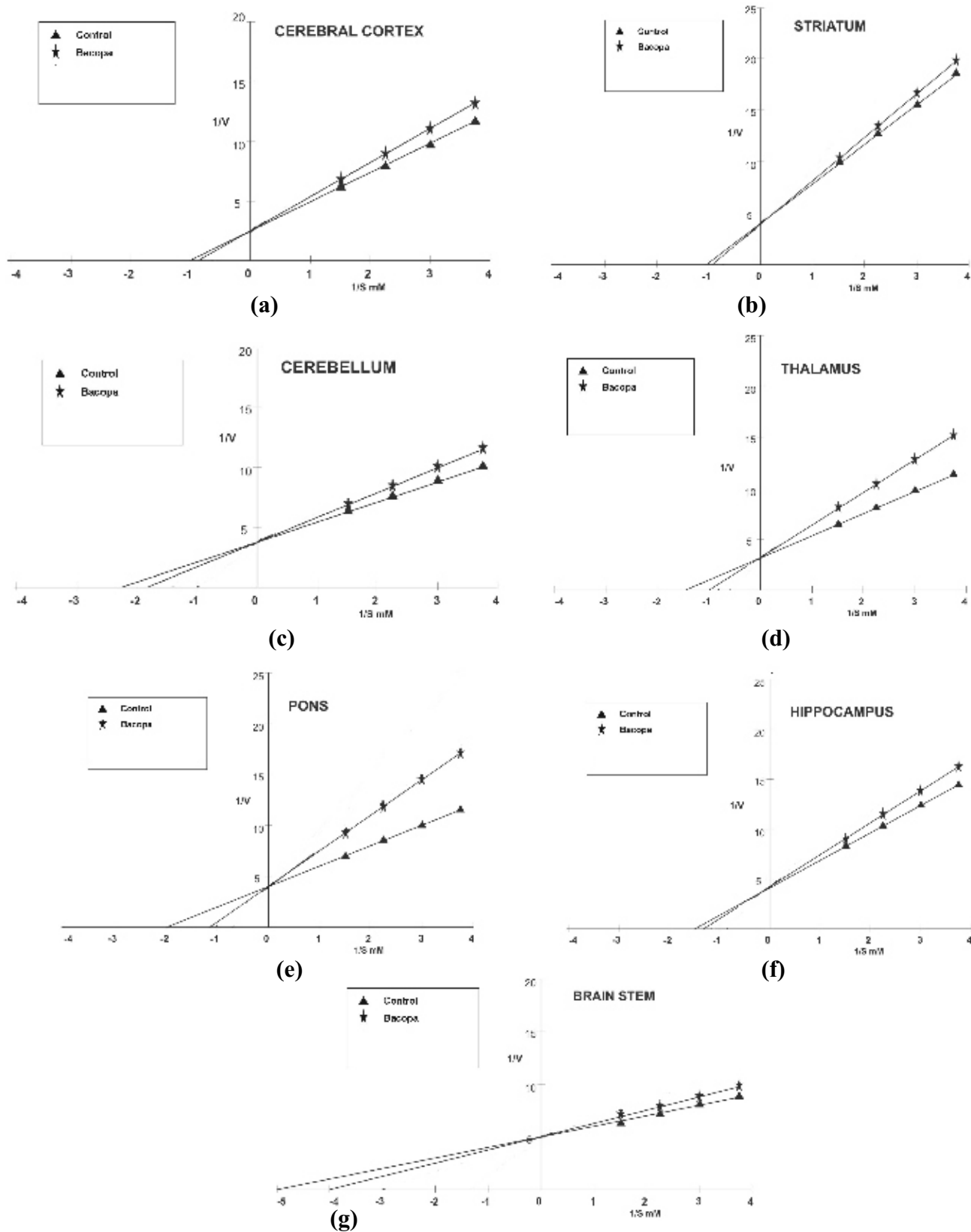


Fig-2 : Lineweaver Burk plots of *in vivo* inhibition of AChE by ethanol extract of *Bacopa monnieri* (100 mg/kg body wt.) in discrete brain regions of Rat. S is the concentration of ATChI, Each point is mean of five assays.

inhibition in AChE was observed in various brain regions viz. 40% in cerebellum and 82% in frontal cortex and medial septum. Similar inhibitory potency by huperzine-B, Chinese moss was reported by Liu *et al.* (1999). They found huperzine-B as potent inhibitor of rat brain AChE. Later, study of Tonduli *et al.* (2000) demonstrated that huperzine-A, an alkaloid isolated from Chinese club moss, *Huperzine serreta*, was a reversible inhibitor of AChE. The dose of huperzine-A reduced AChE inhibition to 54% and increases the acetylcholine level to 230-time base line value. Hence they strongly recommended the huperzine-A as promising compound to protect ACh subjected against organophosphate chemicals.

Ingkaninan *et al.* (2003) screened a large number of medicinal plants to determine their AChE inhibitory power. They reported 90% AChE inhibition by methanolic extracts from roots of *Stephania suberosa* and *Tabernaemontana divaricata*. They further observed 50-60% AChE inhibition by methanolic extracts of stem of *Piper interruptum* Opiz., seeds of *Piper nigrum* L., root and barks of *Butea superba* Roxb. and roots of *Cassia fistula* L. However, 50% AChE inhibition was reported due to hydroalcohol extracts of *Centella asiatica*, *Nardostachys jatamansi*, *Myristica fragrans*, *Evalvulus alsinoides* (Mukherjee *et al.*, 2007). Methanolic Leaf extracts of *Rhizophora lamareckii*, *Suaeda monica*, *Avicennia officinalis* and *Sesuvium portulacastrum* also found to produce 50% AChE inhibition (Suganthi *et al.*, 2009). Recently a dose dependent AChE inhibition was reported due to carbazole alkaloid, mahanimbine isolated by *Murraya koenigii* (Kumar *et al.*, 2010). Plants extract containing essential oils from *Pinus nigra* sub-sps. *nigra*, *P. nigra* var. *calabrica*, and *P. heldreichii* sub-sp. *Leucodermis* have been reported to be potent AChE inhibitor (Bonesi *et al.*, 2010). Aqueous extract of stem bark of *F. racemosa* proved acetylcholinesterase inhibition (Ahmed and Urooj, 2010). Our results also indicated that ethanol extract of *Bacopa monnieri* produced significant AChE inhibition in rat brain regions, which ranged from 24-51%.

The data and plots (Table-1 & fig. 2) of *Bacopa monnieri* extract indicated the competitive inhibition in brain areas of rat. The inhibition revealed that the extract might compete with ATCI for binding at substrate binding site of AChE. For this study, it was first made in effort to screen the *Bacopa monnieri* for inhibition of acetylcholinesterase in different brain regions.

Central cholinergic system plays very important role in regulation of cognitive functions. Loss of cholinergic neuronal in brain regions especially in hippocampal area, is the major cause of Alzheimer's disease. The use of anticholinesterase in enhancement

of central cholinergic activity is presently the basis of treatment of dementia of Alzheimer's type (Enz *et al.*, 1993). In the present days Alzheimer's disease (AD) is one of the most prevailing diseases of cognitive dysfunction in the elderly people. There is currently no cure for this disease. In the patient of AD, the ACh gets deficient and thus an important approach to manage is inhibition of AChE.

The present study suggests that the ethanol extract of *Bacopa monnieri* may be a new potential resource of natural anticholinesterase compounds as a herbal alternative for AD treatment. However, these findings of anti-AChE activities from ethanol extracts of *Bacopa monnieri* need to be carried out further so as to isolate and identify the bioactive components.

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