Inhibitory Potential of Acephate and Ethanol Extract of
Bacopa Monnieri on AChE in Rat Cortex and Hippocampus

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Abstract: The objective of present study was to evaluate the in vivo effect of Bacopa monnieri leaf extract (100 mg/kg) and acephate (37.8 mg/kg) on Acetylcholinesterase (AChE) activity, kinetics and histology of cerebral cortex and hippocampus of brain of male albino rat. AChE activity was measured spectrophotometrically according to the method of Ellman et al.. The acephate inhibited AChE activity strongly in cerebral cortex (72.5%; P < 0.02) than in hippocampus (55.6%; P < 0.02). Bacopa monnieri similarly elicited higher AChE inhibition in cerebral cortex (51.6%; P < 0.05) than in hippocampus (38%; P < 0.01). However, synergistic inhibitory effect was recorded in rats pretreated with Bacopa monnieri followed by acephate exposure. Kinetic study of treated groups demonstrated competitive AChE inhibition in the brain regions, which was evident by increased K<sub>m</sub> and unchanged V<sub>max</sub> values against the control group. The histopathological examinations showed degeneration of pyramidal cells, deformity of neurocytes and vacuole formation in cytoplasm. These changes were pronounced with acephate. Our result shows that Bacopa monnieri has anti-cholinesterase potential hence it could provide novel pharmacological leads of potential benefit to the treatment of Alzheimer’s disease and therefore warrant further investigation.

Keywords: AChE, acephate, Bacopa monnieri, cerebral cortex, hippocampus.

1. Introduction

The levels of acetylcholine and acetylcholinesterase play very important role in the proper function of the nervous system. It’s imbalance in the neurons and synaptic junction leads to a number of neural diseases, particularly Alzheimer’s disease (AD), Myasthenia gravis (MG), and anti-cholinesterase poisoning. It has been proved that cholinesterase inhibitors boost the signal transmission in nerve synapses by prolonging acetylcholine and are useful for curing CNS degenerative diseases [1]. Some AChE inhibitors like galantamine and donezepil are presently in practice to treat AD patients, but they exhibit moderate side effects. Nature is a rich source of biological and chemical diversity. The history of drug discovery shows that plants are highly rich sources in the search for new active compounds [2]. Many synthetic drugs owe their origin to the plant-based complementary medicine. Since Alzheimer’s disease is one of the most common causes of death worldwide, new treatment strategies based on medicinal plants have been focused. Recently many medicinal plants have been screened to know for their anti-cholinesterase property [3]. The extracts prepared from cinnamon [Cinnamomum cassia Presl (Lauraceae)] [4], Iris suaveolens [5], Ficus carica [6], Tutin, (Umbelliferae), [7] and Datura stramonium [8] have shown anticholinesterase potential. Alzheimer’s
disease is associated with impairment in cholinergic transmission. A number of AChE inhibitors have been considered as candidates for the treatment of Alzheimer’s disease as the most useful strategy [9], [10]. In this disease there is deficit of acetylcholine which causes a progressive degenerative neurologic disorder resulting in impaired memory and behavior. Currently, reversible inhibitors of AChE are used in treatment of Alzheimer’s disease [11]. The alkaloids are the major compounds isolated from various species of plants showing inhibitory activity for AChE. Therefore, the present study was undertaken to explore the comparative effect of action of acephate and Bacopa monnieri in search of promising treatment for Alzheimer’s disease.

2. Materials and Methods

2.1. Preparation of Extract

Fresh Bacopa monnieri were procured from Jawaharlal Nehru Agricultural University, Jabalpur (M.P.) India. Leaves and twigs were thoroughly washed in water; shade dried and was ground to powder. Ethanol extract of Bacopa monnieri was prepared using Soxhlet apparatus. Filtrate was frozen, freeze-dried and re-constituted in de-ionized water prior to assy.

2.2. Animals and Treatment

20 male albino rats of 125-150 g were housed separately in polypropylene cages, under controlled laboratory condition at 30°C with 12/12 hours light and dark cycle for 15 days. They were allowed free access to commercial feed and water ad libitum. The animals were grouped in five and treated orally as: Group-I: Ground nut oil 1ml/kg, served as control (vehicle); Group-II: 37.8 mg/kg acephate (1/25th of LD50) in ground nut oil daily for 4 days; Group-III: Ethanol extract of Bacopa monnieri 100 mg/kg in deionized water for 7 days; Group-IV: Pretreatment of ethanol extract of Bacopa monnieri (100mg/kg) for 7 days daily followed by 37.8 mg/kg acephate for 4 days.

2.3. Tissue Preparation

Animals were euthanized by decapitation and brains were dissected out according to Zeman and Innes [12]. Cerebral cortex and Hippocampus were rapidly removed, weighed and thoroughly washed with isotonic saline. Tissues were homogenized in buffer followed by centrifugation at 4°C and supernatant was used as the source of enzyme.

2.4. Determination of AChE Activity

AChE activity was measured spectrophotometrically according to Ellman et al. [13] in the Brain regions. Homogenates were diluted with 2.6 ml 0.1 M Sodium phosphate buffer (pH 7.4) to which 100 mM DTNB and 75 mM ATChI was added. The rate of color production was measured at 412 nm in SL 164 UV-VIS spectrophotometer. All measurements were done in duplicate. Specific activity was expressed in µ mol/min/mg protein.

2.5. Protein Estimation

Protein was estimated following the method of Lowry et al. [14] using BSA as the standard. Samples of homogenate were diluted with reagents then 0.5 ml Folin’s reagent was added, and after 20 min read at 620 nm against a reagent blank. Measurements were done in duplicate.

2.6. AChE Kinetics

Lineweaver-Burk plots were drawn from assays using acephate (37.8 mg/kg) and Bacopa monnieri extract (100 mg/kg) at various substrate concentrations (0.66 mM, 0.44 mM, 0.33 mM, 0.26 mM ATCl). From these kinetic parameters (Km & Vmax) were determined for each assay by plotting the reciprocals of
velocity and substrate concentrations.

2.7. Histological Examination

Tissues from each group of rats were fixed in aqueous Bouin’s fluid, dehydrated and embedded in paraffin wax. Serial sections were cut at 7 micron and stained with Haematoxylin and Eosin. The sections were microphotographed at 100× and 400× with computer-aided microscope (Leica).

2.8. Statistical Analysis

For the data of statistical comparison between different treatments and control, data were analyzed by student’s t-test to determine the effect of the treatment. The level for the accepted statistical significance was $P>0.05$.

3. Results

3.1. AChE Inhibition

As shown in Table 1, the AChE activity in control rat was higher in the hippocampus (1.81 ± 0.54) than in the cerebral cortex (1.35 ± 0.51). The acephate produced higher AChE inhibition in the cerebral cortex (72.5 %) as compared to hippocampus (55.6 %). Ethanol extract of *Bacopa monnieri* demonstrated 51.6 % AChE inhibition in the cerebral cortex while it was 38.1 % in the hippocampus. However, combined group showed synergistic AChE inhibition in the cerebral cortex (83.5 %) and hippocampus (83.7 %).

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Control Group</th>
<th>Acephate</th>
<th>Bacopa monnieri</th>
<th>Bacopa monnieri and Acephate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebral cortex</td>
<td>1.35 ± 0.51</td>
<td>0.37 ± 0.22**</td>
<td>0.65 ± 0.5***</td>
<td>0.22 ± 0.08*</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>1.81 ± 0.54</td>
<td>0.80 ± 0.56***</td>
<td>1.12 ± 0.52*</td>
<td>0.29 ± 0.04</td>
</tr>
</tbody>
</table>

The AChE specific activity is expressed in μ moles of Acetylcholine (ACTI) hydrolyzed / mg protein / min. Each value is mean ± S.D. of 5 individual observations. $P<0.01$; $P<0.02$; $P<0.05$. AChE % inhibition given in parenthesis.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Control</th>
<th>Acephate</th>
<th>Bacopa monnieri</th>
<th>Bacopa monnieri and Acephate</th>
<th>$K_m \times 10^{-6}$M of Treated groups</th>
<th>$V_{max}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebral cortex</td>
<td>1 ± 0.5</td>
<td>1.60 ± 0.8** (+60 %)</td>
<td>1.11 ± 0.54** (+11 %)</td>
<td>1.80 ± 0.54* (+80%)</td>
<td>4.4</td>
<td></td>
</tr>
<tr>
<td>Hippocampus</td>
<td>0.67 ± 0.61</td>
<td>0.84 ± 0.50* (+25 %)</td>
<td>0.76 ± 0.62*** (+13 %)</td>
<td>1.25 ± 0.52* (+86 %)</td>
<td>0.25</td>
<td></td>
</tr>
</tbody>
</table>

Each value is mean ± S.D. of 5 individual observations. $P<0.01$; $P<0.02$; $P<0.05$. (% Per cent elevation in $K_m$ values given in parenthesis.)
3.2. AChE Inhibitory Kinetics

AChE inhibition kinetics of acephat, *Bacopa monnieri* ethanol extract and combined treatment were determined from Lineweaver-Burk plots (Fig. 1). The $K_m$ and $V_{max}$ values were derived from the trend line equation of the graphs (Table 2). The acephat and *Bacopa monnieri* both showed competitive AChE inhibition. This is evident from the series of lines crossing the $y (1/v)$ axis at the same point - i.e. $V_{max}$ is unchanged, but with a decreasing value of $1/K_m$ (and hence a higher $K_m$) in the presence of the inhibitor.

The result as shown in Table 2 revealed that ethanol extract of *Bacopa monnieri* followed by oral dose of acephate to rats, caused remarkable increase in $K_m$ in cerebral cortex and hippocampus.

![Fig. 1. Line Weaver–Burk plot of in vivo inhibition of AChE by acephate (37.8 mg/kg), *Bacopa monnieri* (100 mg/kg) and pretreatment of *Bacopa monnieri* (100 mg/kg) followed by acephate (37.8 mg/kg) treatment in cerebral cortex and hippocampus of rat brain. S is the concentration of ATCI, each point is mean of five assays.](image)

3.3. Histopathology

Exposure of acephate (37.8 mg/kg), ethanol extract of *Bacopa monnieri* (100 mg/kg), pretreatment of ethanol extract of *Bacopa monnieri* (100 mg/kg) for seven days followed exposure of acephate (37.8 mg/kg) for four days caused histological alterations in the Brain regions of rats.

Pyknosis and degeneration of pyramidal cells were observed in the cerebral cortex due to acephate (Fig. 2). We also noticed gradual concentrations of neurocytes. However, *Bacopa monnieri* showed deformity in the pyramidal cells and neurocytes in cerebral cortex. The changes were prominent in the cerebral cortex with the formation of vacuoles in rats treated with Bacopa followed by acephate.

The histopathological changes in the hippocampus of rat brain (Fig. 3) exposed to acephate, *Bacopa monnieri* and pre-treated with *Bacopa monnieri* followed by acephate indicated bifocal concentration of cytoplasm of CA1 layer and pyknosis in pyramidal cells in CA1 layer. These changes were not clearly seen in the hippocampus with treatment of *Bacopa monnieri*.

4. Discussion

A number of experimental studies have indicated that OP pesticides inhibit AChE activity in various organisms and they show different sensitivity to these pesticides [15]-[18]. The present study demonstrates significant inhibition in AChE in both the brain regions of rat exposed to 37.8 mg/kg acephate (Table 1). The inhibition was higher in the cortex than in hippocampus. Progressive inhibition of brain AChE activity
of rat with various pesticides was reported [19]-[22]. Swamy et al. [23] found higher AChE inhibition initially in the cortex and later in the striatum due to Monocrotophos. Similar observations were reported with Dichlorvos [24]. Malathion also elicits higher AChE inhibition in the striatum [25].

![Photomicrograph of T. S. of cerebral cortex of rat brain.](image1)

(A) Control, showing the normal structure. (B) Acephate (37.8 mg/kg), showing degeneration of neurocytes and pyknosis in the pyramidal cell. (C) *Bacopa monnieri* (100 mg/kg), showing gradual concentration of neurocytes. (D) Pretreatment of *Bacopa monnieri* (100 mg/kg) followed by Acephate (37.8 mg/kg), showing vacuoles due to cytoplasm destruction. H.E. × 400.

![Photomicrographs of T S of hippocampus of Rat brain](image2)

(A) Control, showing the normal structure. (B) Exposed to Acephate (37.8 mg/kg), showing pyknosis in pyramidal cells in CAI layer. (C) Exposed to *Bacopa monnieri* (100 mg/kg), shows no clear changes. (D) Exposed to pretreatment of *Bacopa monnieri* (100 mg/kg) followed by acephate (37.8 mg/kg) showing pyknosis in pyramidal cells in CAI layer. H. E. × 400.

We prepared *Bacopa monnieri* extract in ethanol as we got high yield of bacoside, this was also earlier
confirmed by Das et al. [26] and Chandel et al. [27]. Different species of Narcissus has been recently investigated and for the first time reported for the co-relation of plants alkaloids and AChE inhibition [9]. This presents a new approach drug about discovery. Previously, 26 species of Narcissus were found having many-fold AChE inhibitory potential [11]. Anticholinesterase property of extract containing heptyl-physotigmine was studied by De Sarno et al. [28]. 5 mg/kg extract produced 82 % inhibition in AChE of whole brain of rat. However, AChE inhibition was 40 % in cerebellum and 82 % in frontal cortex and medial septum. AChE inhibitory potency of huperzine-B, chines moss in rat brain was reported by Liu et al. [29]. It has been demonstrated that huperzine-A, an alkaloid isolated from chines club moss, \textit{Huperzine serreta}, was a reversible inhibitor of AChE. The dose of huperzine-A produces 54% AChE inhibition and increases the acetylcholine level to 230-time base line value. Hence they strongly recommended the huperzine-A promising compound for the pharmacological use in dementia [2]. The ethanol extract of cinnamon exhibited 63.02% AChE inhibition [4]. The petroleum ether extract of \textit{Iris suaveolens} indicated moderate anticholinesterase activity [5]. It has been revealed that the n-hexane and acetone extracts of \textit{Ficus carica} exerted a notable AChE inhibition [6]. Our study reveals anticholinesterase potential of \textit{Bacopa monnieri}.

Our enzyme kinetic study of control group of rat showed the highest $K_m$ value of Cerebral cortex (1.0 $\times$ 10$^{-3}$ M). Inhibitory kinetic of AChE of brain regions was investigated by few workers’ [30]. The in vivo inhibitory effect of acephate and \textit{Bacopa monnieri} on the AChE kinetic of the brain regions rat has not been reported so far. The all treatments yield a competitive inhibition in AChE of cerebral cortex and hippocampus of rat.

Our histopathological study shows changes due to treatment of acephate. Gradual concentrations of neurocytes, pyknosis and degeneration of pyramidal cells in cerebral cortex and CA1 or CA3 layer in Hippocampus were observed with acephate. Changes were more pronounced in the cerebral cortex with the formation of vacuoles due to the aggregation of cytoplasm in rat pretreated with \textit{Bacopa monnieri} followed by acephate. Kedar et al. [31] observed lesions with dose of sarin in the hippocampus, pyriform cortex and thalamus of rat. They further, observed significant decline in the area of CA1 and CA3 hippocampal cells. However, the damage was prominent in frontal cortex due to soman poising. Similar histopathological alterations due to soman were reported by various investigators [32]-[34]. We can conclude that this study indicates that the ethanol extract of \textit{Bacopa moneiri} may be a new potential resource of natural anticholinesterase compounds. The results of this study indicate that there is a great potential to search for novel usage of this medicinal herbs for the treatment of AD.

References


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