Antioxidant Activity of *Bacopa monniera* in Rat Frontal Cortex, Striatum and Hippocampus

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The effect of a standardized extract of *Bacopa monniera* Linn. was assessed on rat brain frontal cortical, striatal and hippocampal superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) activities, following administration for 7, 14 or 21 days. The effects induced by this extract (bacoside A content 82% ± 0.5%), administered in doses of 5 and 10 mg/kg, orally, were compared with the effects induced by (∼) deprenyl (2 mg/kg, p. o.) administered for the same time periods. *Bacopa monniera* (BM) induced a dose-related increase in SOD, CAT and GPX activities, in all the brain regions investigated, after 14 and 21 days of drug administration. On the contrary, deprenyl induced an increase in SOD, CAT and GPX activities in the frontal cortex and striatum, but not in the hippocampus, after treatment for 14 or 21 days. The results suggest that BM, like deprenyl, exhibits a significant antioxidant effect after subchronic administration which, unlike the latter, extends to the hippocampus as well. The results suggest that the increase in oxidative free radical scavenging activity by BM may explain, at least in part, the cognition-facilitating action of BM, recorded in Ayurvedic texts, and demonstrated experimentally and clinically. Copyright © 2000 John Wiley & Sons, Ltd.

Keywords: Bacopa monniera; Ayurvedic medhyarasayana (memory promoting drug); bacoside A; superoxide dismutase, catalase, glutathione peroxidase.

INTRODUCTION

*Bacopa monniera* Linn. (syn. *Herpestis monniera* Linn. H. B. & K), family Scrophulariaceae, (Sanskrit; Brahmi), is a creeping annual plant found throughout the Indian subcontinent in wet, damp and marshy areas. *B. monniera* (BM) is an important constituent of the Ayurvedic materia medica and is classified as a *medhyarasayana*, a drug used to improve memory and intellect (*medhya*) (Chunekar, 1960; Satyavati et al., 1976). BM finds extensive use in Ayurveda as a brain tonic, restorative in debilitated conditions and for the promotion of longevity (Chunekar, 1960). Extensive investigations (reviewed by Singh and Dhawan, 1992) indicate that the ethanol extract of BM facilitated learning acquisition, improved retention of learning (memory) and delayed the extinction of learned tasks in several experimental paradigms in rats. Subsequent studies indicated that the cognition-facilitating effect was due to two active saponins, bacosides A and B, present in the ethanol extract (Singh and Dhawan, 1992). These active principles, apart from facilitating learning and memory in normal rats, inhibited the amnesic effects of scopoline, electroshock and immobilization stress (Dhawan and Singh, 1996). The mechanism underlying the memory-facilitating, or nootropic, action of BM and its active constituents remains conjectural. It was suggested that the bacosides induce membrane dephosphorylation, with a concomitant increase in protein and RNA turnover in specific brain areas (Singh et al., 1990). A recent study (Bhattacharya et al., 1999) reports that a standardized bacoside rich (bacoside A content 82 ± 0.5) extract of BM, administered for 2 weeks in rats, reversed cognitive deficits induced by intracerebroventricularly administered colchicine and that induced by injecting ibotenic acid into the nucleus basalis magnocellularis. The drug also reversed the depletion of acetylcholine, the reduction in choline acetylase activity and the decrease in muscarinic cholinergic receptor binding in the frontal cortex and hippocampus, induced by the neurotoxin, colchicine, following administration for 2 weeks.

After clinical trials in human volunteers (Dhawan and Singh, 1996), a standardized extract of BM has been now been made available for clinical use by the Central Drug Research Institute, Lucknow, India.

The present study investigated the effect of subchronic administration of BM on the oxidative free radical scavenging enzymes, superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX), in order to assess the role of a possible antioxidant effect in the cognition-facilitating action of the drug. Memory deficits induced by the neurotoxins, colchicine and ibotenic acid, proposed as animal models of Alzheimer’s disease (Smith, 1988), are at least partly due to neurodegeneration induced by oxidative stress injury (Halliwell and Gutteridge, 1985; Bhattacharya et al., 1995a).
### Table 1. Effects of B. monniera (BM) and deprenyl on rat brain superoxide dismutase (SOD) activity (data are mean ± SEM)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Frontal cortex</th>
<th>Striatum</th>
<th>Hippocampus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 7</td>
<td>Day 14</td>
<td>Day 21</td>
</tr>
<tr>
<td>Vehicle</td>
<td>14.8 ± 1.2</td>
<td>14.1 ± 2.2</td>
<td>12.9 ± 1.6</td>
</tr>
<tr>
<td>BM (5)</td>
<td>16.2 ± 1.9</td>
<td>20.4 ± 1.8</td>
<td>23.4 ± 0.9</td>
</tr>
<tr>
<td>BM (10)</td>
<td>16.2 ± 2.3</td>
<td>25.9 ± 1.6</td>
<td>29.4 ± 1.2</td>
</tr>
<tr>
<td>Deprenyl (2)</td>
<td>16.2 ± 1.8</td>
<td>21.4 ± 1.6</td>
<td>28.2 ± 1.1</td>
</tr>
</tbody>
</table>

The drugs were administered once daily for 7, 14 or 21 days. Statistical significance:
- \( a_p < 0.05 \)
- \( b < 0.01 \)
- \( c < 0.001 \), different from vehicle-treated group (Mann–Whitney U-test).

### Table 2. Effects of B. monniera (BM) and deprenyl on rat brain catalase (CAT) activity (data are mean ± SEM)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Frontal cortex</th>
<th>Striatum</th>
<th>Hippocampus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 7</td>
<td>Day 14</td>
<td>Day 21</td>
</tr>
<tr>
<td>Vehicle</td>
<td>14.6 ± 2.6</td>
<td>15.2 ± 1.9</td>
<td>15.8 ± 1.6</td>
</tr>
<tr>
<td>BM (5)</td>
<td>17.2 ± 1.9</td>
<td>21.4 ± 1.4</td>
<td>25.2 ± 1.0</td>
</tr>
<tr>
<td>BM (10)</td>
<td>18.9 ± 2.2</td>
<td>24.6 ± 0.9</td>
<td>30.2 ± 0.8</td>
</tr>
<tr>
<td>Deprenyl (2)</td>
<td>16.8 ± 1.6</td>
<td>19.6 ± 1.8</td>
<td>26.8 ± 1.6</td>
</tr>
</tbody>
</table>

The drugs were administered once daily for 7, 14 or 21 days. Statistical significance:
- \( a_p < 0.05 \)
- \( b < 0.01 \)
- \( c < 0.001 \), different from vehicle-treated group (Mann–Whitney U-test).
Table 3. Effects of *B. monniera* (BM) and deprenyl on rat brain glutathione peroxide (GPX) activity (data are mean ± SEM)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Frontal cortex</th>
<th>Striatum</th>
<th>Hippocampus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Day 7</td>
<td>Day 14</td>
<td>Day 21</td>
</tr>
<tr>
<td>Vehicle</td>
<td>14</td>
<td>0.052 ± 0.01</td>
<td>0.049 ± 0.009</td>
<td>0.056 ± 0.012</td>
</tr>
<tr>
<td>BM (5)</td>
<td>8</td>
<td>0.058 ± 0.009</td>
<td>0.062 ± 0.01</td>
<td>0.082 ± 0.009a</td>
</tr>
<tr>
<td>BM (10)</td>
<td>8</td>
<td>0.064 ± 0.012</td>
<td>0.072 ± 0.008a</td>
<td>0.098 ± 0.007b</td>
</tr>
<tr>
<td>Deprenyl (2)</td>
<td>6</td>
<td>0.068 ± 0.01</td>
<td>0.074 ± 0.009a</td>
<td>0.16 ± 0.008c</td>
</tr>
</tbody>
</table>

The drugs were administered once daily for 7, 14 or 21 days.

Statistical significance:

- $a p < 0.05$
- $b p < 0.01$
- $c p < 0.001$, different from vehicle-treated group (Mann–Whitney U-test).
MATERIALS AND METHODS

Animals. Adult male Wistar rats (180–220 g) were used. The animals were housed in colony cages, with free access to standard pellet chow and drinking water, at an ambient temperature of 25 ± 2°C and 45%–55% relative humidity, with a 12 h light/12 h dark cycle. The Principles of Laboratory Animal Care (NIH publication no. 85-23, revised 1985) guidelines were followed.

Test compound. Stems and leaves of a cultured variety of BM (Ayurvedic Gardens, Banaras Hindu University) were extracted with 50% ethanol. The residue from this extract was subjected to Sephadex LH-20 chromatography using H₂O–MeOH as eluents. The ethanol extracts were subjected to HPTLC (CAMAG TLC evaluation software; CATS 3.16; Scanner II V 3.14) for estimation of the active saponin, bacoside A, using a standard curve. The following details were recorded: bacoside A: Rp 0.38 (n-BuOH–AcOH–H₂O, 4:1:2); Rp 0.15 (ethylacetate–acetic acid–formic acid–water, 100:11:11:27); mode of detection, quenching (wavelength 260 nm); staining reagent 2,4-dinitrophenylhydrazine sulphate; reflectance spectra: wavelength max 278 nm. Properties of the isolated bacoside A were MP 248–252 °C (decomposition; decomposition; a 22Δ + 5.5 ethyl alcohol, c = 0.88); yellow spot with DNP staining reagent. The percentage of bacoside A in the initial extract was 25.5 ± 0.8, whereas the percentage in the Sephadex LH-20 (H₂O–MeOH eluate) was 82 ± 0.5. The ‘enriched’ BM extractive was used.

Treatment protocol. BM (5 and 10 mg/kg) and (-) deprenyl (2 mg/kg), were suspended in 0.3% carboxymethyl cellulose in distilled water and administered orally once daily, in a volume of 2 mL/kg, for 7, 14 or 21 days. The control animals received an equivalent volume of the vehicle orally for the same time periods. Animals were killed 1 h after the last drug or vehicle administration on days 7, 14 or 21. All drug administrations and killing of the animals were done between 0900 and 1200 h.

Biochemical estimations. The brains were removed and the frontal cortex, striatum and hippocampus were dissected out (Glowinski and Iversen, 1966) and weighed. The tissues were homogenized in 2 mL of ice-cold triple distilled water and sonicated for 15 s. The homogenates were then centrifuged (10000 × g, 2 min) and the supernatants were used for enzyme estimation. The following methods were used.

Superoxide dismutase (SOD) activity. The assay was based on the ability of SOD to inhibit spontaneous oxidation of adrenaline to adrenochrome (Saggu et al., 1989). The results are expressed as units (U) of SOD activity/mg protein. One unit of SOD activity induced approximately 50% inhibition of auto-oxidation of adrenaline.

Catalase (CAT) activity. The assay was based on the ability of CAT to induce the disappearance of hydrogen peroxide, which was followed spectrophotometrically (Beers and Sizer, 1952). One unit of CAT was defined as the amount of enzyme required to decompose 1 μmol of hydrogen peroxide per min, at 25°C and pH 7.0. The results are expressed as units (U) of CAT activity/mg protein.

Glutathione peroxidase (GPX) activity. The technique of Carrillo et al. (1991) was followed, using hydrogen peroxide as the substrate. Sodium azide (1 mM) was added to the reaction mixture in order to inhibit remnant CAT activity. One unit of GPX was defined as the amount of the enzyme decomposing 1 μmol hydrogen peroxide at 25°C and pH 7.0. Results are expressed as units (U) of GPX activity/mg protein.

PROTEIN estimation. Protein estimation was done the method of Lowry et al. (1951).

RESULTS

BM (5 and 10 mg/kg, p.o.) induced a dose-related increase in SOD, CAT and GPX activities in the frontal cortex, striatum and hippocampus, which was evident only after 14 days of drug administration and was accentuated on day 21 in all the brain regions (Tables 1–3). On the contrary, deprenyl (2 mg/kg, p.o.) induced decreases in SOD, CAT and GPX activities in the frontal cortex and striatum, but not in the hippocampus, after treatment for 14 or 21 days (Tables 1–3).

DISCUSSION

BM has been subjected to extensive chemical investigations (Rastogi, 1990). The major chemical entity shown to be responsible for the memory-facilitating action of BM, bacoside A, was assigned as 3- (β-D-xylopyranosyl)-O-B-D-glucopyranoside-10,20-dihydroxy-16-keto-dammar-24-ene (Chatterjee et al., 1965). Bacoside A usually co-occurs with bacoside B, the latter differing only in optical rotation and is likely to be an artifact during the process of isolation of bacoside A (Rastogi, 1990).

Accumulation of neurotoxic free radicals, and consequent neurodegeneration in specific brain areas, has been proposed as the causal factor in Alzheimer’s disease, Parkinsonism and in the process of aging (Glover and Sandler, 1993). This accumulation of oxidative free radicals is due to defective antioxidant defence mechanisms resulting from decreased function of the free radical scavenging enzymes, including SOD, CAT and GPX (Harman, 1991). Potential therapy in these neurodegenerative conditions should, therefore, include agents capable of augmenting antioxidant defence systems (Maxwell, 1995). The choice of the areas in this study was based on the evidence that they are highly vulnerable to oxidative stress induced damage (Balin and Allen, 1986). In addition, Alzheimer’s disease has been associated with free radical-induced neurogeneration in the frontal cortex and hippocampus (Hall and Braughler, 1993), whereas Parkinsonism and the process of aging has been correlated with striatal free radical activity (Knoll, 1993).

The most abundant free radicals in living cells are the superoxide anion, hydrogen peroxide and the highly toxic hydroxyl radical, collectively known as reactive oxygen species (ROS). These radicals appear to function in
concert to induce cell degeneration via peroxidation of membrane lipids, breaking of DNA strands and denaturing cellular proteins (Maxwell, 1995). The natural cellular antioxidant enzymes include SOD, which removes superoxide radicals by speeding their dismutation, CAT, a haeme enzyme which removes hydrogen peroxide, and GPX, a selenium-containing enzyme which removes hydrogen peroxide and other peroxides (Halliwell and Gutteridge, 1989). The radical scavenging activity of SOD is effective only when it is followed by actions of CAT and GPX, since SOD generates hydrogen peroxide as a metabolite, which is more toxic than oxygen radicals and requires to be scavenged by CAT and/or GPX (Harman, 1991). Apart from toxicity, hydrogen peroxide, in the presence of transition metals like iron, leads to the generation of the highly toxic hydroxyl ions which are known to induce lipid peroxidation (Harman, 1991). As such, an effective antioxidant agent should be capable of augmenting intracellular concentrations of not only SOD but that of CAT and/or GPX as well in finally reducing lipid peroxidation (Halliwell and Gutteridge, 1989).

In the present study, a standardized extract of BM was found to increase SOD, CAT and GPX activities in all the brain regions investigated. However, the onset of action appears to be delayed and was evident only after subchronic administration. It is interesting to note that the nootropic, or memory-facilitating effect of BM, has a delayed onset of action as well (Singh and Dhawan, 1982). Likewise, the reversal of memory-deficits and decrease in cholinergic markers in the frontal cortex and hippocampus noted in laboratory models of Alzheimer’s disease in rats, was evident only after 7 to 14 days of BM administration (Bhattacharya et al., 2000). It was postulated that the memory deficits and decreases in the frontal cortex and hippocampus acetylcholine concentrations, choline acetylase activity and muscarinic cholinergic receptors, following ibotenic acid and colchicine administration into rat brain, could be due to oxidative-stress induced neurodegeneration. The reversal of this neurotoxin induced memory-dysfunction and deficits in the cholinergic markers by subchronic administration of BM was attributed to possible antioxidant activity. The present findings lend credence to that postulate. (-) Deprenyl, a selective MAO B inhibitor, has been reported to be effective in retarding the progression of Parkinson’s disease (Knoll, 1989) and to increase life span in male rats (Knoll, 1993) following prolonged administration. It was suggested that these effects were due to an increase in antioxidant activity in the striatum (Knoll, 1993). In the dose used in this study (2 mg/kg), deprenyl increased SOD and CAT in the rat striatum but not in the hippocampus (Carrillo et al., 1992), as was also noted in this study. The effect of deprenyl on rat brain GPX activity remains controversial. Whereas, Knoll (1990) reported an increase, a later report (Carrillo et al., 1992) indicated that deprenyl did not significantly effect rat brain striatal and hippocampal GPX activity. We found that deprenyl induced a significant increase in cortical and striatal, but not of hippocampal, GPX activity. This difference could be due to the different rat strains used by us (Wistar) and others (Charles River or Fischer-344). Deprenyl does not have significant nootropic activity, even on prolonged administration, and BM does not have MAO B inhibiting effect (unpublished data). In this context, the increase in hippocampal antioxidant activity by BM, and the ineffectiveness of deprenyl, assumes importance in explaining the memory-facilitating action of BM.

The present investigation may help in explaining, at least in part, the mechanism of nootropic action of BM demonstrated experimentally and clinically. It is of interest to note that another Ayurvedic drug, shilajit, used in cognitive dysfunctions, and shown to promote learning and memory (Ghosal et al., 1993), increases rat brain SOD, CAT and GPX activities (Bhattacharya et al., 1995b). Apart from its use in cognitive dysfunctions, BM has been utilized in Ayurveda for its anti-aging action (Chunekar, 1960). The deprenyl-like effect of BM on striatal antioxidant activity may explain this use. Since BM is now in clinical use, its antioxidant effects in other tissues require investigation. These studies are in progress.

REFERENCES


