Ameliorative effects of *Bacopa monnieri* on olfactory bulbectomy-induced cognitive deficits in mice

Name of dispatched researcher （派遣研究者名）	Pham Thi Nguyet Hang
Affiliation of Instructor （所属）	Department of Pharmacology and Biochemistry, National Institute of Medicinal Materials, Hanoi, Vietnam
Host Collaborator （受入研究者）	Professor Kinzo Matsumoto Division of Medicinal Pharmacology Institute of Natural Medicine, University of Toyama

1. Abstract:

Aim: *Bacopa monnieri (L.) Wettst. (BM)* is a well-known medicinal herb for treatment of epilepsy, insomnia, etc.. This plant has been known to be useful for improving intelligence and memory for a long time. This study aimed to clarify the effects of alcoholic extract of BM (BM-E) on cognitive deficits in olfactory bulbectomized (OBX) mice, one of the animal models of Alzheimer's disease (AD) and its mechanisms underlying the action.

Methods: OBX ddY mice were treated with BM-E at a daily dose of 50 mg/kg via feeding needle for 1 week before and 2 weeks after 3 days of OBX. Administration of the reference drug tacrine (2.5 mg/kg/day, i.p.) to OBX mice was started 3 days after OBX. Cognitive performances of the animals was analyzed using the novel object recognition test, modified Y maze test, and fear conditioning test to elucidate non-spatial short term memory, spatial working memory, and long-term memory, respectively. After completing the behavioral experiments, the expression level of cholinergic marker proteins and the activity of acetylchoniesterase (AChE) in the brain were analyzed by Western blotting and Ellman's method, respectively.

Results: OBX caused impairment of non-spatial short term memory, spatial working memory, and long-term memory. OBX-induced memory deficits were reversed by BM-E and tacrine. The effect of BM-E on the spatial working memory deficit was reversed by scopolamine, a muscarinic receptor antagonist. BM-E attenuated OBX-induced down-regulation of choline acetyltransferase without affecting muscarinic M1 and M3 receptor expression or AChE activity.

Discussion: These finding demonstrate that BM-E improves memory deficits caused by OBX and that the effect of BM-E is at least in part mediated by stimulation/normalization of central cholinergic systems. Our findings further support the idea that BM is beneficial for treatment of cognitive deficits such as AD.

2. Introduction

Alzheimer's disease (AD) is a neurodegenerative disease related to cognitive and memory impairments which are affecting many elderly people worldwide. Although some drugs are available for AD treatment, the outcomes are often unsatisfactory. Therefore, the medicinal herbs are promising strategy to development of effective drugs for AD.

Bacopa monnieri (L.) Wettst. (BM) a well-known traditional Ayuredic medicinal plant and is used in India as a brain tonic. In Vietnam, BM grows throughout form from Northern to Southern and usually uses as daily vegetable not only for high income people but also for low income people. Some lines of evidence show that BM improves cognitive and memory impairment on animal experiments and healthy volunteer. However, it remains unclear the mechanism(s) of action of BM as well as to promote more possible usages of BM in cognitive deficit therapy. Therefore, this study aims to elucidate the anti-dementia effects of BM using olfactory bulbectomy (OBX) model. For this aim, we elucidate the non-spatial short term memory, spatial working memory, and long-term memory by using three different behavioral testes, including novel object recognition test, modified Y maze test, and fear conditioning test, respectively. To clarify the mechanism of the action of BM, we investigate the effect of BM on the central cholinergic systems in OBX animal.

3. Materials and Methods

3.1. Plant materials and preparation of crude extract:

BM was collected from Ho Chi Minh city (Southern of Vietnam). It was identified by Dr. Pham Thanh Huyen, Department of Medicinal Plant Resources, National Institute of Medicinal Materials (NIMM, Vietnam). The number code of voucher specimen is 9687 that was kept in the Herbarium, Department of Medicinal Plant Resources, NIMM. The aerial part of BM was cutted into small pieces, dried, and percolated with circulating 95% ethanol for 2 days at the ratio of 1 g : 12 ml. The residue was extracted again twice using 95% ethanol at the ratio of 1 g : 10 ml. The combined extract was dried under reduced pressure.

3.2. Animal

Male ddY mice (Japan SLC Inc., Shizuoka, Japan) were obtained at the age of 9 weeks old and housed with a 12-h light/dark cycle (light on: 07:30-19:30) at 22 ± 1oC. Food and water were available *ad libitum*. The animal were habituated to the laboratory animal room for at least 1 week before surgery. The behavioral experiments were performed during the light phase from 9:00 to 18:00. The present studies were conducted in accordance with the Guiding Principles for the Care and Use of Aninals and were approved by the Institutional Animal Use and Care Committee in the University of Toyama.

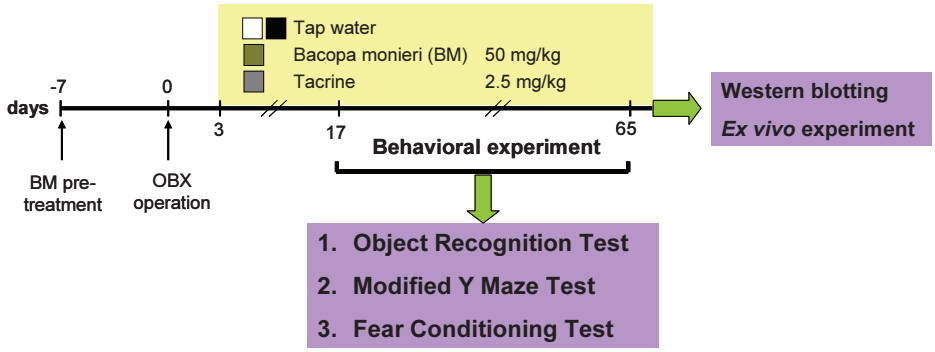
3.3. Surgical operation:

OBX of mice was conducted according to protocol of Prof. Kinzo Matsumoto (Division of Medicinal

Pharmacology, Institute of Natural Medicine, University of Toyama.

3.4. Drug administration:

Drug treatments and experiment schedule were done according to the schematic below:



3.5. Behavioral study

Behavioral experiment was conducted according to the protocol of Prof. Kinzo Matsumoto (Division of Medicinal Pharmacology, Institute of Natural Medicine, University of Toyama, including:

- Object recognition test
- Modified Y maze test
- Fear conditioning test

3.6. Neurochemical study:

- Ex vivo measurement of cholinesterase activity in the brain was conducted by a modified version of the colorimetric method of Ellman et al.

3.7. Western blotting

-ChAT protein, M1 and M3 proteins were detected by using the protocols of Prof. Kinzo Matsumoto (Division of Medicinal Pharmacology, Institute of Natural Medicine, University of Toyama.

4. Results and discussion

4.1. BM improves OBX-induced non- spatial memory deficit in the novel object recognition test

Using the novel object recognition test, we elucidate the effect of BM on the non- spatial cognitive performance of OBX mice. The result showed as the figure below:

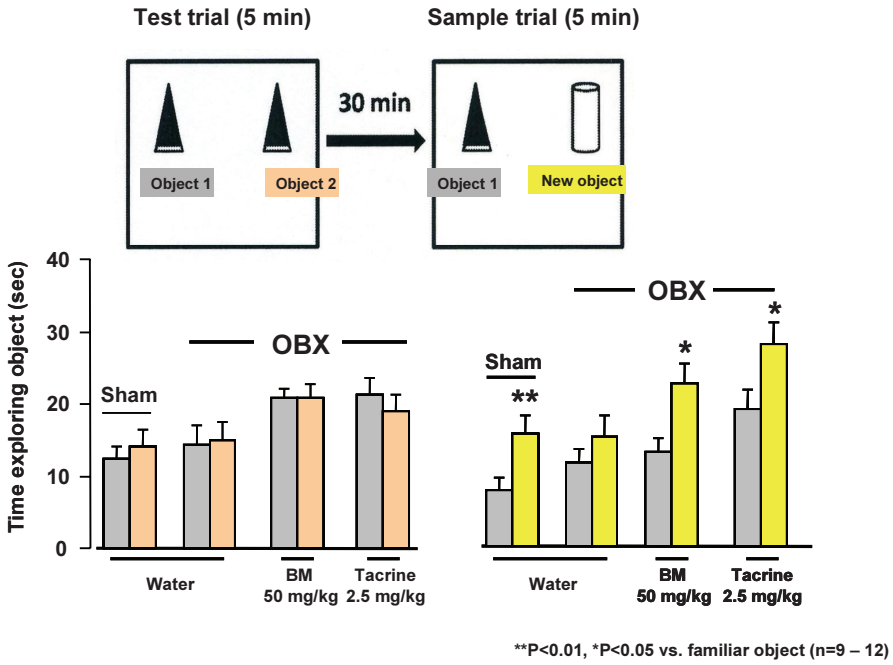
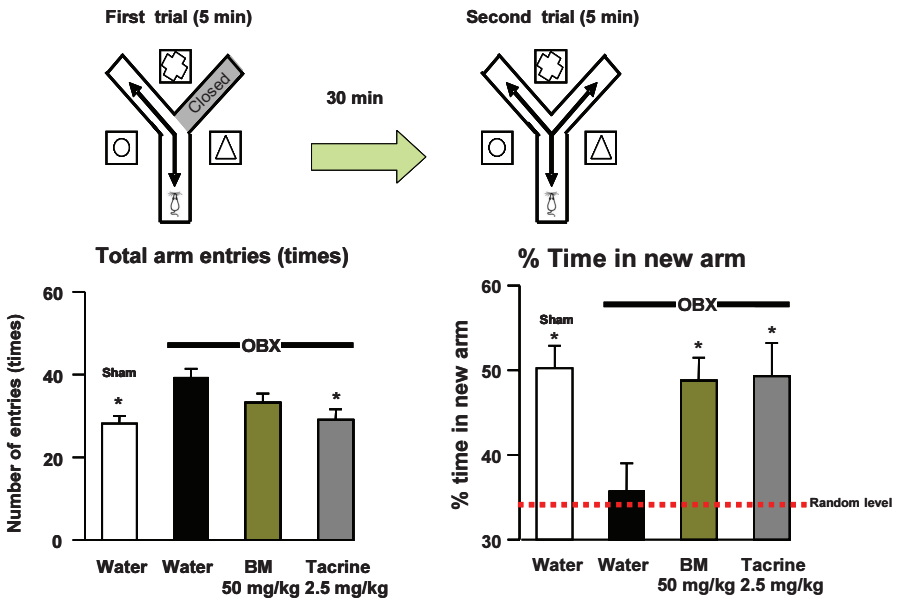


Fig. 1 Effects of BM on object recognition deficit in OBX mice

In the sample phase, none of the animal groups showed significant differences in the time spent exploring each identical object. However, in the test phase, the sham group spent a significantly longer time exploring the novel object than exploring the familiar one. In contrast, tacrine (2.5 mg/kg) and BM (50 mg/kg) treated OBX mice spent significantly longer time exploring the novel object than exploring the familiar one.

4.2. BM improved OBX induced spatial working memory deficits in the modified Y maze test.

Using the modified Y maze test, we evaluate the effect of BM on spatial working memory on OBX mice. The results showed as the figure below:



Figures 2: Effect of BM on spatial working memory deficit in the modified Y maze test

As shown in the Fig.2, the spatial working memory deficit cause by OBX was significantly improved by BM treatment.

After finishing the first modified Y maze test, OBX mice were treated with 1 mg/kg scopolamine (SPC i.p) or Sham group treated with tap water 30 min before the second modified Y maze test

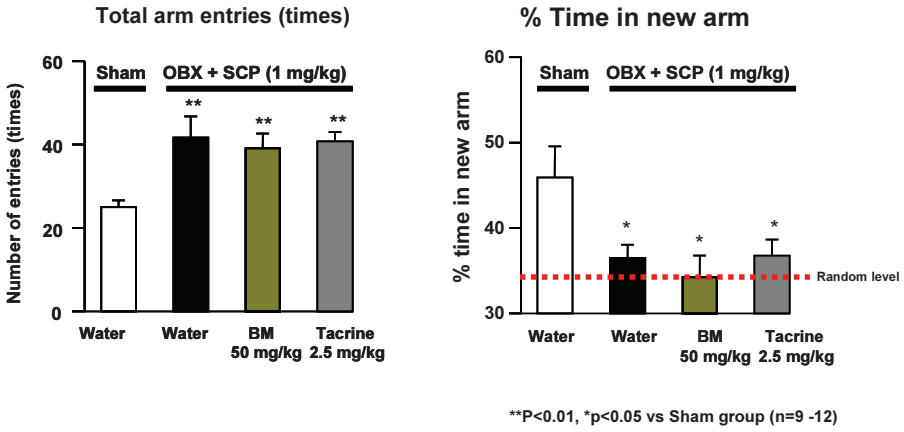


Fig. 3 Scopolamine (SCP: 1 mg/kg) abolished the ameliorative effects of BM in OBX mice

As shown in the fig. 3, the ameliorative effects of BM in the second trials were completely abolished by SCP (1mg/kg).

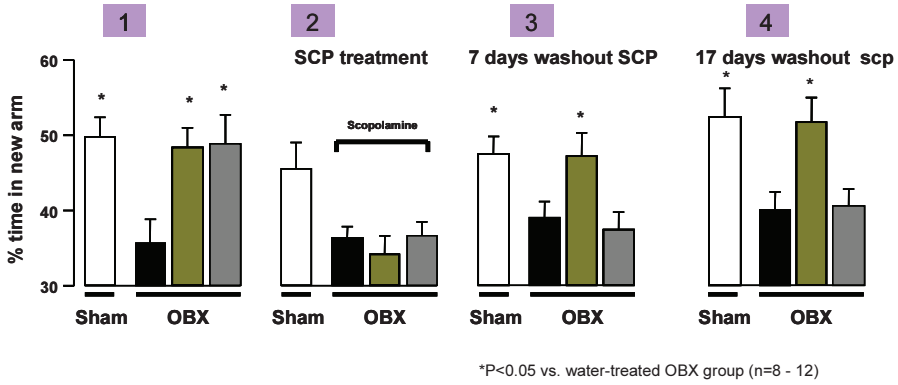


Fig. 4 BM again ameliorated the spatial working memory deficit in OBX mice

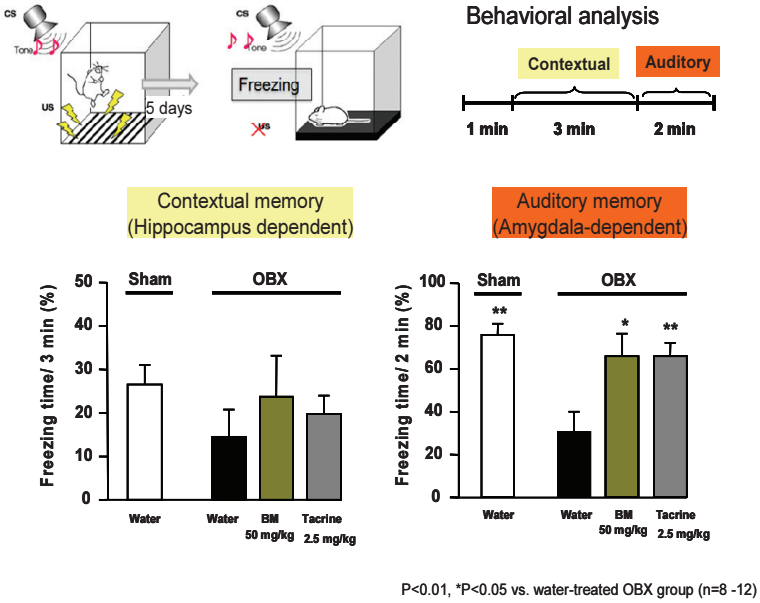
After the 7 days and 17 days of the second modified Y maze test, the third and fourth of modified Y maze testes were performed. The result showed as the figure below:

After 1 week washout period of SCP, the ameliorative effects of BM were again observed.

4.3. BM ameliorated OBX induced long term memory deficit in the fear conditioning test

We next used the Pavlovian fear conditioning test to determine the effects of BM on long-term memory deficits induced by OBX. One week after finishing the 4th of modified Y maze test, the fear conditioning test was performed. Mice were fear-conditioned to the context and auditory stimuli by electrical foot shocks as unconditioned stimuli. After a further 5 administration period

of BM for 5 days, freezing responses to the contextual and auditory stimuli were recorded. The results showed as the figure below:



P<0.01, *P<0.05 vs. water-treated OBX group (n=8 -12)

Fig 5 Effect of BM on long-term memory deficit in the fear conditioning test

As shown in fig.5, BM increased freezing to the auditory stimuli in the test session, that indicating long-term memory improvement.

4.4. Effect of BM on the cholinesterase activity

After completing the behavioral experiments, the cholinesterase activity in the cerebral cortex was measured to understand the mechanisms underlying the action of BM. The results indicated as table below:

Table 1

Operation	Daily treatment	acetylcholine esterase activity (nmol/mg tissue/min) mean ± S.E.M.		
sham	water	44.51	±	5.37
OBX	water	42.65	±	3.40
OBX	Ba (50 mg/kg, p.o.)	33.92	±	4.13
OBX	THA (2.5 mg/kg, i.p.)	29.87	±	2.57 *

*P<0.05 vs. water-treated OBX group and sham group (n=5 – 6)

The results showed that tacrine (2.5 mg/kg) significantly inhibited cholinesterase activity, but BM had no effect on this activity. Therefore, the mechanism of BM on cognitive deficits in OBX mice may different from those induced by tacrine.

4.5. Effects of BM on cholinergic markers protein expressions

To clarify the mechanism(s) underlying the ameliorative effects of BM on OBX mice induced cognitive deficit, we investigated expression changes of cholinergic marker protein, including choline acetyltransferase (ChAT), muscarinic M_1 and M_3 receptor in the hippocampus by Western Blotting method.

The results showed that BM significantly elevated the expression level of ChAT in the hippocampus without affecting on the expression of muscarinic M_1 or M_3 receptors or βactin. These finding suggested that the ameliorative effects of BM on OBX induced cognitive deficits are atributable at least in part mediated by stimulation/normalization of central cholinergic systems. Base on these results suggested that the impairment of learning and memory induced by OBX is caused by degeneration of cholinergic neurons and ChAT is an important role in the improvement process of cognitive deficits. Our findings further support the idea that BM is beneficial for treatment of cognitive deficits such as AD.