

## The Improvement of Learning and Memory Ability of Normal Persons by BF-7

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To investigate whether BF-7, extracted from *Bombyx mori*, improved learning and memory of ordinary people, Rey-Kim Memory Test was performed with placebo group (32 persons), 200 mg BF-7 treated group (33 persons) and 400 mg BF-7 treated group (34 persons). BF-7 enhanced significantly learning and memory function in a dose-dependent manner. To know how BF-7 plays such a positive role, we measured the concentration of acetylcholine in the brain from memory impairment animal model. Treatment of BF-7 significantly increased the concentration of acetylcholine. So, it is supposed that the protection of cholinergic neuron and/or keeping proper concentration of acetylcholine might be one of the mechanisms by which BF-7 improve brain function. So, our results suggest that the BF-7 is effective material for improving learning and memory ability.

**Key Words:** Brain, Learning and memory, BF-7, Acetylcholine, Neuroprotection

### INTRODUCTION

Over 100 billion neurons are integrated into the structural fabric, that is the brain. The neuron is the basic unit of the nervous system. Each neuron is in synapse contact through its processes with other neurons, so that each neuron is a segment in the network of which the nervous system is composed. Functional or physiological damage of each neuron induces impairment of brain functions like learning, memory, and so on (Chklovskii et al, 2004; Montgomery & Madison, 2004). There are lots of causes making neuron damaged, for example, many endogenous factors like as, oxidative stress generated naturally during energy metabolism (Carney et al, 1994; Serrano & Klann, 2004), abnormally regulated calcium (Sato et al, 2003; Sullivan, 2000), accumulated toxic substances as well as exogenous toxic materials, and so on (Cristina et al, 2003; Swain et al, 2004; Yilmazer-Hanke et al, 2003). Thus, brain ordinary meet with chemical, environmental and psychological stress, which leads to decline of brain function (Friedrich et al, 2004; Sandi, 2004; Sui and Rose, 1997).

It has been suggested that cholinergic neurons are closely related with cognitive function like as learning and memory (Gibbs et al, 2004; Riekkinen et al, 1998; Seeger et al, 2004). So, the protection of neurons and/or maintaining acetyl-

choline concentration can be used as a method for improving cognitive function (Zhang et al, 2004).

Lots of studies pursuing neuroprotection and anti-aging have been carried on. Furthermore, the ways for improving brain function have been studied. However, so far, there is no effective strategy improving learning and memory or protecting nervous system. So, finding effective materials of strategy making brain function better is very important in many ways. Recently, it has been reported that the BF-7, natural extract from *Bombyx mori*, exerted significant neuroprotection (Chea et al, 2004).

So, in this study we tried to find out whether the BF-7 is effective for improving learning and memory function of ordinary people, clinically. If then, we also tried to estimate how the BF-7 plays such a positive role on the brain function.

### METHODS

#### Material

BF-7 used in this study was extracted and purified from *Bombyx mori*. And BF-7 was presented from National Institute of Agricultural Science and Technology.

#### Experimental animal

Male Sprague-Dawley rats, 8 weeks old (around 200~

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**ABBREVIATIONS:** MQ, memory quotient; KAVLT, K-auditory verbal learning test; KCFT, K-complex figure test; PBS, phosphate buffered saline; TCA, trichloroacetic acid.

250 g), were purchased from Korean BioLink Co. After 1 week for adaptation, total 28 rats were divided into 4 groups with 7 rats each. Four groups were as follows: (1) vehicle control group treated with PBS, (2) beta amyloid (2 nmole) treated group to bring cognitive function down, (3) beta amyloid (2 nmole) treated with BF-7 (5 mg/kg) group, (4) beta amyloid (2 nmole) treated with BF-7 (10 mg/kg) group.

### Examinees

Volunteers covered all adult ages from 19 to 64. It included 20~30 s who were required high memory and learning ability, 30~40 s who start to decrease in memory ability, 50~60 s who had a certain degree of decline in memory. So this volunteer group would be representatively of the general population of people. Volunteers, the one of cases as follow was excluded from the test. (1) in case who had experience of intaking medicine and/or functional food, (2) in case who had neuropsychiatric problem, (3) in case who had any kind of serious disease (4) in case of who had neurodegenerative disease, (5) in case whom was determined not suitable to participate in this test.

119 examinees were randomly divided into 3 groups, 39 of placebo group, 40 of BF-7 200 mg group, and 40 of BF-7 400 mg group. And finally 32 of placebo group, 33 of BF-7 200 mg group, and 34 of BF-7 400 mg group, total 99 examinees finished the test.

### Clinical experiments

Examinees were randomly distributed into placebo and test groups in sequence. The test was performed in double-blind manner. Before intaking test material (BF-7), baseline assessment (vital sign, weight, checking sickness) and Rey-Kim Memory Test were performed. Indication of BF-7 was two capsules b.i.d., p.o., three weeks. Baseline assessment and Rey-Kim test were performed again to evaluate changes after administration. During it was required that examinees should take meal and smoking as usual, but drinking was prohibited.

### Rey-Kim memory test

All examinees were administered Rey-Kim Memory Test (Kim, 1999) to evaluate enhancement after administration of BF-7. In Rey-Kim Memory Test, verbal memory performance is assessed by K-Auditory Verbal Learning Test (KAVLT), a Korean version of Rey Auditory Verbal Learning Test (Rey, 1964), and nonverbal memory performance by K-Complex Figure Test (KCFT), a Korean version of Rey Complex Figure Test (Rey, 1941). KAVLT required serial learning of a list of 15 unrelated words over 5 consecutive trials, each trial followed by immediate recall. After a delay period of 20 min, the examinee was again required to recall the 15 words. Following completion of the delayed recall, the examinee was presented with a list of 50 words and required to choose a total of 15 words that were in the original list spoken by the examiner. KCFT was essentially identical to a standard version of Rey Complex Figure Test (Lezak, 1983). The examinee was required to copy the figure as accurately as possible with no time limit imposed. An immediate recall trial was administered following completion of the figure copy. After a delay period of 20 min, the examinee was again required to recall the

figure. All KCFT productions were scored according to a standard version of 36-point scoring system (Lezak, 1983).

### Animal model for cognitive malfunction

2 nmole of beta amyloid was injected into the region of hippocampus. Treatments of each group were as follow: (1) vehicle control group : PBS was injected into ventricle and PBS was fed orally for 2 weeks, (2) beta amyloid treated group: 2 nmole of beta amyloid was injected into ventricle and PBS was fed orally for 2 weeks, (3) 2 nmole of beta amyloid was injected into ventricle and 5 mg/kg of BF-7 was fed orally for 2 weeks, (4) 2 nmole of beta amyloid was injected into ventricle and 10 mg/kg of BF-7 was fed orally for 2 weeks.

### Determination of acetylcholine concentration

The acetylcholine concentration of brain was determined by the chemiluminescence method (Israel & Lesbats). This method was based on the reaction of hydrolysis of acetylcholine by acetylcholinesterase and conversion reaction of choline with H<sub>2</sub>O<sub>2</sub> into betaine. The composition of reaction reagent was as follows, 10 ml of 0.2 M sodium phosphate buffer (pH8.6), 25µl of choline oxidase (100 units/ml in water), 10µl of peroxidase type II (2 mg/ml in water), 50µl of luminol. 100 mg of brain tissue was immersed and extracted in 5% TCA solution. After centrifugation, it was extracted with 5~10 ml of ether, and ether was discarded. This ether extraction was performed 5-6 times more. 20µl of 0.5% sodium metaperiodate was added to 200µl of aqueous layer. 200µl of sample was mixed with 285µl of reaction reagent, and the intensity of chemiluminescence was measured.

### Statistical analysis

The data is expressed as a mean S.E.M. values. The paired t-test was used to analyze the relationship of difference of scores between scores of before and after administration. One-way ANOVA (Tukey Post Hoc comparison test) was used to analyze the relationship of difference of scores among scores of placebo, BF-7 200 mg, and BF-7 400 mg groups.

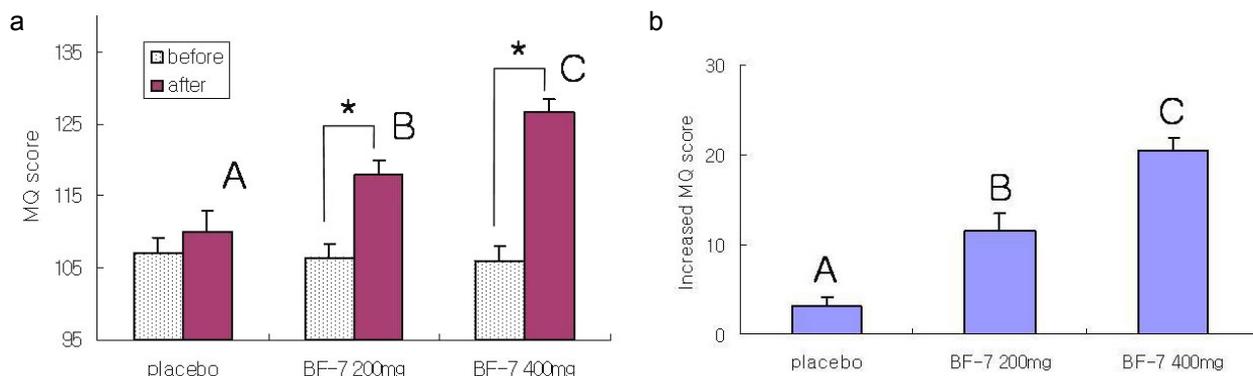
## RESULTS

### Increase of memory quotient (MQ)

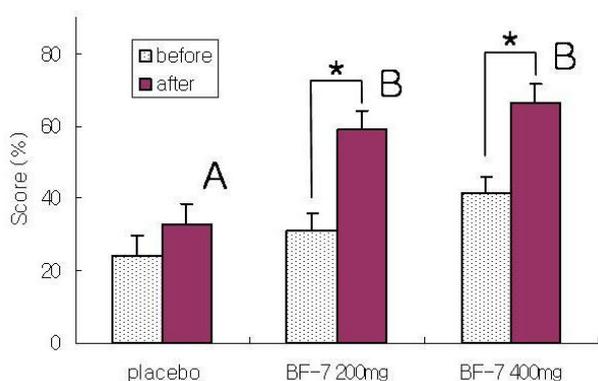
The memory index MQ, which is the most direct reflective index of memorizing ability. The average MQ of all 98 examinees was around 105. Interestingly, the average MQ was significantly increased to about 126.6 after intake of BF-7 for 3 weeks, but not in placebo group (Fig. 1a). Also, to further investigate if BF-7 can help the enhancement of memorizing ability, we examined that the improved MQ score differences. The increased memory index MQ score was significantly increased from 3.1 to 20.6 in a dose-dependent manner (Fig. 1b). This results represent that BF-7 enhances memorizing ability effectively.

### Enhancement of memory recall efficiency

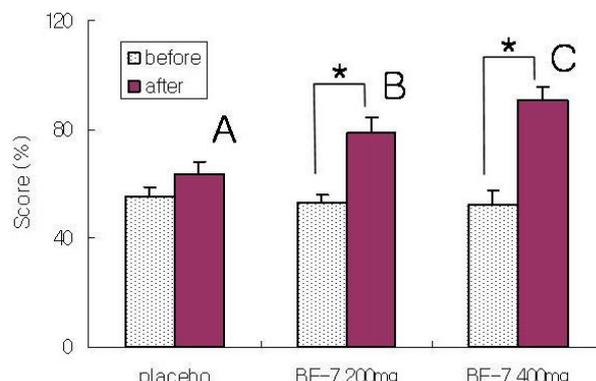
Memory recall efficiency, which is the reflective index of



**Fig. 1.** Enhanced memory quotient (MQ) by BF-7. MQ scores (a) and improved score differences (b) were represented as mean±S.E.M. The each scores before and after administration of BF-7 were compared using paired t-tests. And significant differences are shown as \*(p<0.05). Relationship among placebo, 200 mg of BF-7, and 400 mg of BF-7 group were analyzed using one-way ANOVA (Tukey's multiple comparison test), and their statistical significant differences are shown as different characters (A, B, and C) (p<0.05).



**Fig. 2.** Increased memory recall efficiency by BF-7. The values were represented as mean±S.E.M. The scores before and after administration of BF-7 were compared using paired t-tests. And significant differences are shown as \*(p<0.05). Relationship among placebo, 200 mg of BF-7, and 400 mg of BF-7 group were analyzed using one-way ANOVA (Tukey's multiple comparison test), and their statistical significant differences are shown as different characters (A and B) (p<0.05).



**Fig. 3.** Positive role of BF-7 on intelligence quotient/memory relationship. The values were represented as mean±S.E.M. The scores before and after administration of BF-7 were compared using paired t-tests. And significant differences are shown as \*(p<0.05). Relationship among placebo, 200 mg of BF-7, and 400 mg of BF-7 group were analyzed using one-way ANOVA (Tukey's multiple comparison test), and their statistical significant differences are shown as different characters (A, B and C) (p<0.05).

efficiency to memorize something, indicates how much and how precisely recall preserved memory. The higher ranked percentage represents better efficiency. As shown in figure, the memory recall efficiency score was significantly increased from 31% to 58.9% and 41.5% to 66.5% by intaking 200 mg/day and 400 mg/day of BF-7 for 3 weeks, respectively, but not in placebo case (Fig. 2). The results represent that the memory recall efficiency was greatly improved by BF-7.

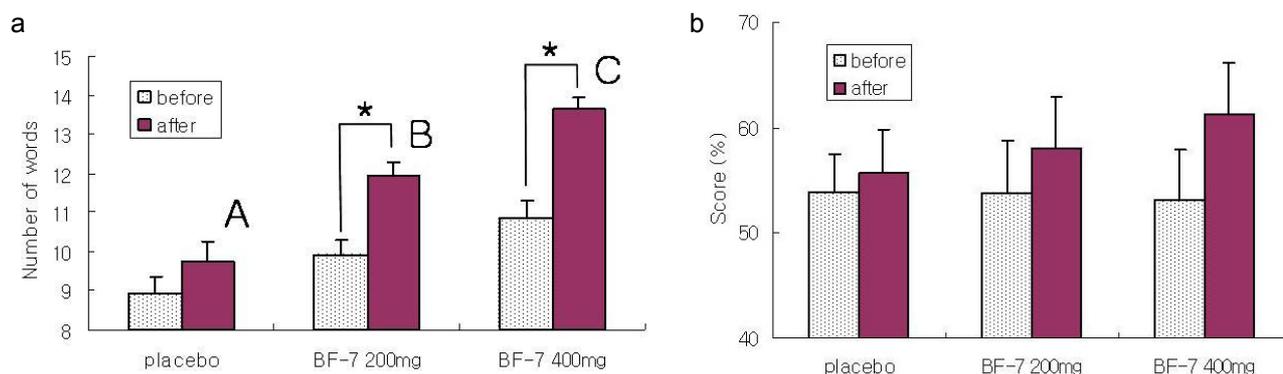
**Enhancement of the intelligence quotient and memory quotient relationship**

The intelligence quotient and Memory quotient consensus score is the direct reflective index of relationship between intelligence quotient and memory quotient. The higher ranked percentage represents better memory ability compared with intelligence quotient. As shown in figure,

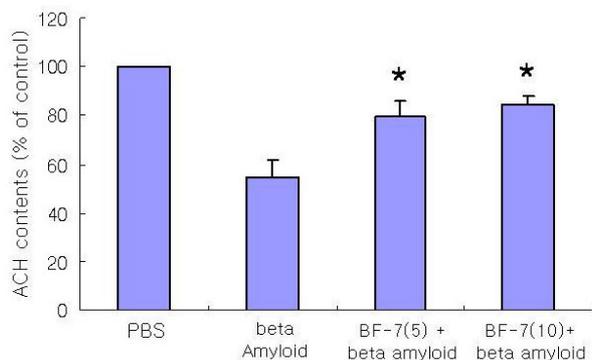
The intelligence quotient/Memory consensus score was significantly enhanced from 52.9% to 78.9% and 52.5% to 91.1% after intake of BF-7 200 mg/day and 400 mg/day for 3 weeks, respectively (Fig. 3). The scores were increased in a dose-dependent manner and there is no change in placebo group. These results represent that memory quotient compared with intelligence quotient is effectively strengthened by BF-7.

**Improvement of memory maintenance**

The memory maintenance is the index of how much and how well the memorized matters can be kept precisely. This index can be represented as score of memory maintenance and the number of memorized words. As shown in figure, the numbers of memorized words were improved by BF-7, interestingly. Administration of 200 mg BF-7 and 400 mg BF-7 significantly increased the numbers of memorized



**Fig. 4.** Improvement of memory maintenance by BF-7. The numbers of memorized words (a) and scores of memory maintenance (b) were represented as mean±S.E.M. The scores before and after administration of BF-7 were compared using paired t-tests. And significant differences are shown as \*( $p < 0.05$ ). Relationship among placebo, 200 mg of BF-7, and 400 mg of BF-7 group were analyzed using one-way ANOVA (Tukey's multiple comparison test), and their statistical significant differences are shown as different characters (A, B and C) ( $p < 0.05$ ).



**Fig. 5.** Positive role of BF-7 upkeeping the concentration of acetylcholine in brain. The concentration of acetylcholine is shown as relative percentage against PBS treated group. The values were represented as mean±S.E.M. Relationship among PBS, 5 mg/kg of BF-7, and 10 mg/kg of BF-7 group were analyzed using one-way ANOVA test. Statistically significant differences by Tukey post Hoc comparison test are shown as different character (\*) ( $p < 0.05$ ).

words compared to placebo in a dose-dependent manner (Fig. 4a). In the score of memory maintenance, the higher ranked percentage represents better memory maintenance. It was shown that the BF-7 was effective for keeping of memory, as the memory maintenance index was increased from 53.2% to 61.3% after intake of BF-7 for 3 weeks, but not in placebo group (Fig. 4b).

#### Up-keeping of acetylcholine concentration

As cognitive function is decreased, the concentration of acetylcholine is reduced. Our result showed that the acetylcholine concentration, in the brain from the rat treated with beta amyloid, was reduced to 45% compared with no treated control. Interestingly, the concentration was recovered by BF-7. Treatment with 5 mg/kg and 10 mg/kg of BF-7 increase the acetylcholine level to 78% and 80% compared to control, respectively (Fig. 5).

## DISCUSSION

There are many factors leading decline of brain function or brain damages. Among them, severe stress or aging also possibly induce learning memory impairment (Diamond et al, 2004; Sandi, 2004). Beyond transient functional declines, severe physiological, chemical, environmental stress give rise to an anatomical, physiological brain damages (Bartolomucci et al, 2002; Takahashi et al, 2004). To find ways or materials protecting brain is very meaningful. Our study was based on the facts that the BF-7, extract from *Bombyx Moti*, effectively protected neuron against either beta amyloid or reactive oxygen species (Chae et al, 2004, Yeo et al, 2004). By the way, normal ordinary people meet various kind of causes leading to brain damage consistently, which would be negative to normal brain activities (Friedrich et al, 2004). So, the BF-7 was examined to check whether it enhanced learning and memory of ordinary people. For this study, Rey-Kim Memory Test which is authorized and frequently used methods to check learning and memory (Choi et al, 2004; Kang et al, 2003; Min et al, 2001). As describe in "Methods", to know if BF-7 is working to ordinary people, volunteers were collected from various ages and circumstances.

Significantly increased MQ in a dose-dependent manner represented that the BF-7 was very effective on the enhancing memory ability. The result, showing significantly increased score in memory recall test, suggested that BF-7 might be helpful to take out and use specific memories precisely. Also our results concerning the intelligence quotient and memory quotient relationship implicated that BF-7 assisted person to get upgrade memory power, since the index means that how much someone efficiently use memory ability compared with others having similar level of intelligence quotient. Furthermore, even though number of memorized words is larger than placebo group, the group intake BF-7 maintained the memory better. The results represent that BF-7 plays a pivotal role on keeping memories better.

The positive role of BF-7 would be raised from various molecular mechanisms, including neuroprotection from various insults. Since BF-7 protected neuron against neurotoxicity of beta amyloid and oxidative stress, such

protective role might be a set of reasons enhancing brain function. Along with this protection, it could be suggested that there might be another mechanisms by which improve learning and memory. Hypothetically several mechanisms could be suggested as follows. At first, BF-7 possibly induces spins on neurite, by which neurons connect others more, which results in reinforcement of neural network.

Another possibility is that BF-7 is of help on neurogenesis. Generally, it has been known that severely damaged neuron is not supposed to recovered. However several line of report showed that there is somehow neurogenesis. For example, neurogenesis in hippocampus, which is induced by estrogen (Tanapat et al, 1999). If some neurons were damaged, then other neurons should compensate functionally, so at least it could be suggested that the BF-7 assist the compensation. This hypothesis is supported by our previous results showing that BF-7 protected neurons very efficiently against various kind of insults. The other possibility is that the BF-7 aids keeping homeostasis of various signal molecules like as calcium, cAMP, and so on, as well as neurotransmitters, like as acetylcholine.

Among above possible mechanism, in this study here, the maintenance of proper concentration of acetylcholine was examined, taking advantage of beta amyloid induced memory impaired rat model (Dodart et al, 2002; Li et al, 2004). Our result showed that BF-7 was of help to up-keep the concentration of acetylcholine, consequently the concentration was close to normal level. So, it is suggested that the ability of BF-7 maintaining acetylcholine proper concentration would be one of the mechanism for the positive role.

When the neuroprotective and neuropromoting materials were screened, the another important thing is safety, since it should be intake for a long time relatively. Since, the BF-7 is derived from safe natural product, together with is promising positive roles, it is very useful material for protecting nervous system and improving learning and memory.

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