

Braintropia Co. Ltd.

Project Name(Materials Name): BT-11(the powder of root extracts of *Polygala tenuifolia* Willdenow)

1. Functional information: Memory improvement

"It can help adults improve memory." (KFDA acknowledged: No. 2009-12)

2. Anti-stress effects

The scientific name of the active substance: *Polygala tenuifolia* Willdenow

Submission Dates: 10 April 2010

Product Summary

As concentrated and spray-dried natural active ingredient extracted from the roots of *Polygala*
We commercially produced 1g BT-11 (100%) containing 0.3~0.9mg TMCA(Trimethoxy cinnamic acid).

- Functional information: The individual approval No. 2009-12 ("It can help adults improve memory."(Other grade II))

-Additional functional information: Tested an inhibitory effect of BT-11 on stress in animal models

-Functional components(Or indicator components): TMCA/BT-11=0.3~0.9mg/g

1. Proof of Effect and Function

○ Mechanism

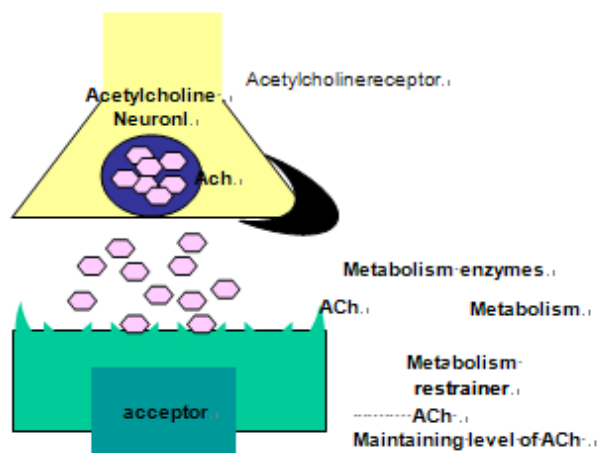
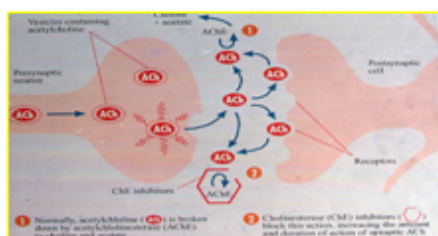
1) Confirmation of the reaction mechanism of BT-11(the powder of root extracts of *Polygala tenuifolia* Willdenow)

(1) Inhibitory effect of BT-11 on memory impairment(Anticholinesterase)

Many researchers recently have made efforts to investigate the cause of memory loss in the molecular genetics as well as in many different fields. But they have been having considerable difficulties because the cause is not exactly identified. Nevertheless they have been having active progress to inhibit primarily the main symptom of memory loss recently. In the lately 1970, as it was revealed that the dysfunction of cholinergic nervous system was closely associated with cognitive impairments(Selkoe, 1994 Bierer et al., 1995), they have been interested to develop the materials which improve learning and memory by maintaining Ach(Acetylcholine) levels in the brain though inhibition of AchE (Acetylcholinesterase).

Inhibitory effect of BT-11 on AChE

Role of Acetylcholinesterase in the Brain.



This study was reported that BT-11 could improve memory by maintaining levels of ACh though inhibition of AChE activity.

(2) Protective effect of BT-11 in brain cells

There was a neurotoxicity induced by glutamate, an excited amino acid and a cause of the cell death causes, commonly occurred in the Alzheimer's diseases(AD) and other neurodegenerative diseases. This study was greatly showed that BT-11 inhibited the neurotoxicity and reduced the brain cell death induced by glutamate. Furthermore the study demonstrated that it inhibited a toxicity of the amyloid β protein(A β) and C-terminal fragment plays a crucial role in the pathogenesis of AD.

(3) Inhibitory effect of TMCA on stress

This study showed the anti-stressful effects of TMCA(3,4,5-trimethoxycinnamic acid) on stress induced by cold or injection of corticotrophin-releasing hormone (CRH).

In two stress conditions, stress treated animals had low sleeping time induced by pentobarbital. Whereas when TMCA was injected in stress-treated animals, Sleeping time was significantly continued. In other case, When CRH was injected, norepinephrine(NE) contents on locus coeruleus(LC) is significantly increased. When TMCA was injected in CRH-treated animals its contents is significantly reduced. The study was showed that there was a sedative effect of TMCA inhibited contents of NE in LC.

(4) Conclusion

Although this study was carried out in vitro by using the cell culture purified from brain of the rat, it was suggested that BT-11 had inhibitory effects on neurotoxicity of Glu A β and CT. Therefore it was thought that the BT-11 was available for new active functional food extracted from natural plants that had effects of learning and memory enhancement. TMCA may be a functional candidate of BT-11 compounds.

○ *In vitro* Bio-assay , *In vivo* testing

Our company demonstrated effects of BT-11 on anticholinesterase, neuroprotection and anti-stress in

vitro, in vivo testing. Moreover we reported two papers regarding the functional action.

- 1) Subject: Novel Cognitive Improving and Neuroprotective Activities of Polygala tenuifolia Willdenow Extract, BT-11.
- Journal name: Journal of Neuroscience Research 70:484–492 (2002)
- 2) Subject: BT-11 Improves Stress-Induced Memory Impairments Through Increment of Glucose Utilization and Total Neural Cell Adhesion Molecule Levels in Rat Brains
- Journal name: Journal of Neuroscience Research 87:260–268 (2009)

[1] Abstract of the paper

- 1) Subject: Novel Cognitive Improving and Neuroprotective Activities of Polygala tenuifolia Willdenow Extract, BT-11. (Cf. Attachment 1.)
- Journal name: Journal of Neuroscience Research 70:484–492 (2002)

(1) Abstract:

We carried out this study to search a new active constituent that had cognitive enhancing activity and low side effects from natural source. We found that the extract of dried root of Polygala tenuifolia Willdenow (BT-11, 10 mg/kg, i.p.) could significantly reverse scopolamine-induced cognitive impairments in rat, using a passive avoidance and a water maze test. We also investigated the effects of BT-11 on neurotoxicity induced by glutamate (Glu) and toxic metabolites of amyloid precursor protein (APP) such as amyloid protein (A β) and C-terminal fragment of APP(CT) in primary cultured neurons of rat. The pre-treatment of BT-11 (0.5, 3, and 5 μ g/ml) significantly reduced cell death induced by Glu (1 mM), A (10 μ M) and CT105 (10 μ M) in a dose-dependent manner. In addition, BT-11 inhibited acetylcholinesterase(AChE) activity in a dose-dependent and noncompetitive manner (IC₅₀ value; 263.7 μ g/ml). Our novel findings suggest the possibility that this extract may have some protective effects against neuronal death and cognitive impairments in Alzheimer's disease (AD), or other neurodegenerative diseases related to excitotoxicity and central cholinergic dysfunction.

- 2) Subject: Subject: BT-11 Improves Stress-Induced Memory Impairments Through Increment of Glucose Utilization and Total Neural Cell Adhesion Molecule Levels in Rat Brains. (Cf. Attachment 2.)
- Journal name: Journal of Neuroscience Research 87:260–268 (2009)

(1) Abstract:

We previously reported that BT-11, extracted from those roots, improved scopolamine-induced amnesia in rats and inhibited acetylcholinesterase activities in vitro. Therefore, we proposed that BT-11 could remedy stress-induced memory deficits in rats. In this study, the stress-induced memory impairments in rats were significantly reversed almost to the control level by BT-11 treatment. To seek an active component of BT-11 that plays an important role in antipsychotic effects, we compared BT-11 with 3,4,5-trimethoxycinnamic acid(TMCA), which is a constituent of those root extracts. However, the effects of TMCA were less or were not consistent with those of BT-11 in some of tests. In particular, BT-11 reversed the stress-induced reduction of glucose utilization by [18fluorodeoxy glucose]FDG-PET and the levels of neural cell adhesion molecule (NCAM) in rat brains to the control levels, whereas TMCA did not. Therefore, BT-11 improved stress-induced memory impairments through increment of glucose utilization and total NCAM levels in rat brains. In conclusion, BT-11 may be strongly effective against stress-induced amnesia in rats, through the combined effects of TMCA and other active components of BT-11.

○ Clinical Trials

We carried out 3 times clinical trials and then demonstrated for human regarding efficacy(memory improvement, anti-stress effect) and safety. All tests were carried out through rightful methods approved by Institution Review Board(IRB). Two of 3 times trials was reported in papers and then the other is ongoing.

1)The first clinical test(IRB approval)

Abstract:

(1)Subject: Effects of BT-11 on memory in healthy humans

- Journal name: Neuroscience Letters 454 (2009) 111–114 (Cf. Attachment 3.)
- Recommended formulation: capsule(hard, soft), tablet, liquefied product etc.
- Recommended dosage(mg per day): 300mg/day

(2)Test Agency: Seoul National University Boramae Hospital/Neuropsychiatry/ Jun- Young, Lee Assis tant Professor

(3) Concentration and shape type used in test

- Concentration: 300mg/day as BT-11
- Formulation: Hard capsule(No.0 capsule)

(4)Study period: 06/01/2005 – 10/30/2005 follow-up survey for 2 month

(5)Clinical test design: Effects of BT-11 as a Functional Food: Randomized, Placebo-controlled, Double blind and Parellel-group study

(6)Research overview: In this randomized, double-blind and parallel-group study of BT-11 in health adult appealing to memory deficits, We assessed cognitive function involving memory 2 month later. This assessment was investigated by using Korean version of Consortium to Establish a Registry for Alzheimer's Disease Assessment Packet (CERAD-K).

(7)Overview of Study Result : This study was recruited by a total of 60 normal adults and only 53 of all recruiters finished this study. The total scores of CERAD-K were significantly increased more in the BT-11 treated group than in the placebo-treated group($P=0.003$). In Word list recall, constructional praxis, constructional praxis recall, Boston naming and trail making test A of subordinate scores, the scores were significantly increased more in the BT-11 treated group than in the placebo-treated group. In this test there was no side effect except a slight dyspepsia.

(8)Conclusion: This result suggested that BT-11 improved cognitive function in normal adult and it could be closely related to effect of memory improvement. BT-11 may be nutraceuticals to enhance or prevent cognitive dysfunction such as AD.

2) Second clinical test (IRB approval)

Abstract:

(1) Subject: BT-11 is effective for enhancing cognitive functions in the elderly humans

- Journal name: Neuroscience Letters 465 (2009) 157–159 (Cf. Attachment 4.)
- Recommended formulation: Capsule (Hard, Soft), Tablet, liquefied product etc.
- Recommended dosage (mg per day): 300mg/day

(2) Test Agency: Seoul National University Boramae Hospital/Neuropsychiatry/ Jun- Young, Lee Assistant Professor

(3) Study period: 10/30/2006 ~ 01/30/2007 follow-up survey for 1 month

(4) Clinical test design: Effects of BT-11 as a Functional Food: Randomized, Placebo-controlled, Double blind and Parallel-group study

(5) Study Overview: It has been previously reported that BT-11, the extract of dried roots of *Polygala tenuifolia* Willdenow, inhibited activity of acetylcholinesterase, neurotoxicity of Glutamate (Glu) and amyloid β protein. Additionally In clinical test of the elderly aged 60 and over, there were much more improvement of cognitive function in the BT-11-treated group. Especially, word list recognition was markedly improved in BT-11-treated group than in placebo-treated group. This study could be examined whether BT-11 could enhance memory in healthy humans like in the elderly aged 60 and over.

This study for those aged from 16 to 64 was a randomized, double-blind. The participants were given capsules of BT-11 or placebo 3 times daily for 4 weeks. And then we assessed any changes of cognitive functions including memory. The Korean version of the California Verbal Learning Test (K-CVLT) and the Self-Ordered Pointing Test (SOPT) were used to assess them.

(6) Overview of study result: 55 (BT-11-treated group=26, placebo-treated group=29) subjects participated in this study. 48 (BT-11-treated group=23, placebo-treated group=25) participants completed the 4 week study. In entire K-CVLT, the BT-11-treated group (12.96 ± 5.59) showed statistically significant increase in a comparison to the placebo-treated groups ($p=0.042$). And although scores in free recall and long-delay recall and recognition were remarkably increased, there were no statistically significant differences. There were no serious treatment-related side effects for all the 48 participants.

(7) Conclusion: This study suggested that BT-11 significantly improved working memory in a comparison to placebo.

3) Third clinical test (IRB approval):

Abstract:

(1) Subject: Effect on BT-11 for enhancing cognitive functions and anti-stress

- name: **IRB Report**
- Recommended formulation: Capsule (Hard, Soft), Tablet, liquefied product etc.
- Recommended dosage (mg per day): 180mg/day

(2) Test Agency: Gachon University of medicine and science/psychiatry/ Suk- Ju, Kim Professor

(3) Study period: 04/01/2008 ~ 08/ 31/2008 follow-up survey for 3 month

(4) Clinical test design: Effects of BT-11 as a Functional Food: Randomized, Placebo-controlled, Double blind and Parellel-group study

(5) Overview of the study: BT-11 is natural oriental medicine which has effect of enhancing cognitive function and anti-stress. We previously reported that BT-11, the extracts of polygala tenuifolia Willdenow enhanced cognitive functions involving safety in the elderly humans.

This study was to investigate effect of cognitive function and anti-stress after BT-11-treated for those aged 60 and below.

This study for those aged from 18 to 60 was a randomized, double-blind. The participants were given capsules of BT-11 or placebo for 12 weeks. And then we carried out Neuropsychological test battery and Stress test. Neuropsychological test battery made use of CANTAB(Cambridge neuropsychological test automated battery) and Stress test made use of HRV(Heart Rate Variability).

(6) Overview of study result: 60(BT-11-treated group=30, placebo-treated group=30) people participated in this study. 55(BT-11-treated group=28, placebo-treated group=27) participants completed the 12 week study. In the test result, there was no significant changes in placebo-treated group whereas Long-delay recall scores($p=0.0001$) Of PRM(pattern recognition memory) scores and RVIP(Rapid visual information processing) scores($p=0.0004$) were significantly increased in BT-11-treated group after BT-11 was given for 12 weeks. There were no significant difference between two groups in HRV test. There were no serious treatment-related side effects for all the 60 participants.

(7) Conclusion: This study suggested that BT-11 significantly enhanced the long-term visuospatial memory and working memory of healthy adults(60 aged and below) in a comparison to placebo.

2. Intellectual Property Right details

○ Patent information

■ Originality of the patent

Method for extraction and purification of effective component from *Polygala tenuifolia* and crude drug composition containing extract thereof for treatment of dementia.

■ The number of the patent: 1

■ Type of the patent: Usage patent

■ Patent content:

-Application Number: 10-2000-0023767

-Name: Method for extraction and purification of effective component from *Polygala tenuifolia* and crude drug composition containing extract thereof for treatment of dementia.

-Abstract: A method for effectively extracting *Polygala tenuifolia* Willdenow and purifying and crude drug composition containing the extract are provided. Whereby, the extract is effective in inhibition of acetylcholinesterase activity and enhancement of decreased memory.

-Right:

1:

Methods for solvent-extracting *Polygala tenuifolia* with alcohol aqueous

2:

Methods for extraction and purification of *Polygala tenuifolia* using solution such as methanol aqueous solution or ethanol aqueous solution.

3:

The crude drug composition for treatment of dementia containing the extracts of *Polygala tenuifolia*

4:

The extracts are obtained by solvent-extracting with alcohol aqueous solution.

○ Please explain the exclusive right

Answer: In this present, on domestic market Il-Yang Pharmaceutical Co. has leadingly tried to sell product and there is no discussion on right. Therefore we can go ahead with discussion regarding contract and exclusive right and first of all we seem global launching to be valid of global