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Original Article

Cognitive Improvement Effect of Resplex Alpha A in the Scopolamine-induced Mouse Model

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ABSTRACT

Administration of Scopolamine can be considered a psychopharmacological model of Alzheimer's disease (AD). We made an animal model of Alzheimer's disease (AD) by administering Scopolamine to Blab/c mice. In this study, we investigated the effects of Resplex Alpha on memory impairment and cognitive function in mice in a mouse animal model of Scopolamine-induced memory impairment. Through Y-mazed and passive avoidance behavioral assays, we observed that Resplex Alpha recovered Scopolamine-induced short-term memory and cognitive functions. The results of our study imply that Resplex Alpha may be beneficial in the prevention of Alzheimer's disease (AD).

Keywords Resveratrol-alginate nano-complex (RANCP), Ginkgo biloba extract (EGb231), Cognitive function, Memory dysfunction, Alzheimer's disease (AD), dementia

INTRODUCTION

Dementia is a disease in which learning and memory deteriorate due to various factors. Among these, Alzheimer's disease (AD) is the most common neurodegenerative disease with the manifestation of numerous cognitive and neuropsychiatric symptoms. AD is age-related and clinically induces cognitive impairment, linguistic and motor functions, and behavioral changes[1]. Neuropathological characteristics include plaque composed of amyloid-β (Aβ) peptide, extensive loss of neurons and synapses accompanied by neurofibrillary tangles, altered neurotransmitter systems, etc. In particular, the loss of cholinergic cells in the basal forebrain leads to the loss of neurotransmitters such as acetylcholine (ACh) thereby acting as a critical factor in the progression of AD[2, 3]. Based on this cholinergic hypothesis, efforts are being made to treat AD by inhibiting acetylcholinesterase (AChE), which is the enzyme responsible for the breakdown of ACh at neuronal junctions. Moreover, various AChE inhibitors such as physostigmine, tacrine, Donepezil, galanthamine, huperzine A and rivastigmine, increase the utilization of ACh at cholinergic junctions in the central nervous system. It is actually being used to treat AD, and new effective AChE inhibitors are still being developed at the moment[1, 2]. Although tacrine (CognexTM), a representative AChE inhibitor for AD is selected first and used, effectively inhibits the loss of cholinergic neurons in the cerebral cortex and hippocampus, its clinical

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use is limited by the fact that it is known to induce severe increases in blood alanine aminotransferase levels, that is, liver toxicity, in approximately 30% of patients[4-6]. Therefore, efforts have been made for the attempt to develop AChE inhibitors derived from natural substances with relatively lower side effects[7, 8], and it has been known that various antioxidants improve memory in dementia[9, 10].

Resplex Alpha is a complex natural substance with resveratrol, ginkgo biloba extract (EGb 761) and vitamin E as the key ingredients that help with restoration of cognitive function. Resveratrol has been reported to reduce Aβ levels, improve brain volume and enhance cognitive functions in AD patients[11] EGb 761 has been shown to display a variety of anti-AD effects in preclinical and clinical studies[12-14]. It has also been reported that long-term treatment with EGb 761 can reduce Aβ pathology in the brain by activating b-secretase and inhibiting Ab aggregation[15]. Vitamin E has been reported to slow the functional deterioration in AD[16].

Functional food (Product A from company M), contains extracts of a mixture of Dimocarpus longan, Angelica tenuissima Nakai and Polygala tenuifolia Willd, as well as ginkgo biloba extract and vitamin, and has been shown to reduce Aβ pathology in the brain. The active constituents of Polygala tenuifolia have been reported to have multiple neuroprotective potentials related to AD, such as anti-Aβ aggregation, anti-inflammatory, antioxidant, neuroprotective, and neuro proliferation[17]. Angelica tenuissima Nakai has been reported to improve cognitive impairment, reduce the overexpression of $A\beta_{1-42}$, and inhibit neuronal loss and neuroinflammatory responses in an AD mouse model[18]. Dimocarpus longan also has neuroprotective and autophagic actions[19].

Scopolamine competitively inhibits muscarinic receptors for acetylcholine and acts as a nonselective muscarinic antagonist, with effects on the central sedative, antiemetic and memory removal[20]. Administration of scopolamine can be considered a psychopharmacological model for AD.

MATERIALS AND METHODS

Test Materials

Resplex Alpha used in the experiment was provided by JBKLAB Co., Ltd.. It is a complex of natural substances with resveratrol-alginate complex (RANCP), ginkgo biloba leaf extract and vitamin E as the key ingredients. Product A from company M is known to be a functional product that helps with cognitive function and is composed of mixed extracts including Dimocarpus longan, Angelica tenuissima Nakai and Polygala tenuifolia Willd along with ginkgo biloba leaf extract and vitamin E as the main ingredients, was purchased from a pharmacy.

Test Animals

6-weeks old female Balb/c mice were purchased from Samtako BioKorea. After acclimatization to the laboratory environment for 7 days, the mice were randomly divided into normal control, scopolamine hydrobromide trihydrate (Sigma-Aldrich, St. Louis, MO, USA) treated, Donepezil (Sigma-Aldrich, St. Louis, MO, USA) treated, Resplex Alpha treated, and Product A treated groups based on an average body weight of 16.2 ± 0.45g as the reference for the experiment. Mice were housed for 1 week in an animal room with temperature and humidity maintained at 20-25°C and 40-45%, respectively, and a 12-hour light/dark cycle. (Table 1.) This animal

experiment was conducted after having been approved by the Institutional Animal Care and Use Committee (IACUC) on animal experiments of JBKLAB.

Administration of Test Substances

Resplex Alpha and Product A were diluted with saline solution to a concentration of 200 mg/kg and administered orally at the dose of 100 μl per day in the morning every day for 2 weeks. The administration concentration of Resplex Alpha and Product A was judged to be 200 mg/kg when converting the daily human intake to mouse. 3 mg/kg of Donepezil was administered orally at the dose of 100 μl per administration 1 hour before the behavioral experiment and scopolamine was diluted with saline solution to a concentration of 2mg/kg and intraperitoneal (i.p) was administered 30 minutes before the behavioral experiment. The normal control, scopolamine and dopezil groups were orally administered 100 μl of saline solution instead of the experimental substance to impart the same administration and compensatory stress. (Table 1.)

Production of Mouse Model of Cognitive Dysfunction Using Scopolamine

Scopolamine hydrobromide trihydrate was dissolved in saline solution to a concentration of 2 mg/kg. Scopolamine was i.p administered at the dose of 100 μl 30 min before the behavioral experiment to induce cognitive dysfunction. The normal control group was also IP administered with 100 μl of saline to impart the same stress.

Measurement of the Body Weight

Body weight was measured using an electronic scale at 3-day intervals from the start of the experiment.

Y-maze test

Y-maze test was performed in an asymmetrical black Plexiglass Y-maze with 3 arms (35 cm long × 3 cm wide × 12 cm high) at an angle of 120° designated A, B, and C. Mouse was placed in the middle of the Y-maze and acclimated to the maze for 5 min. After removing the mouse from the Y-maze, all odors and dirt were removed with 70 % ethanol. The mouse was placed in the center of the Y-maze and allowed to explore for 8 min. A video camera mounted over the Y-maze was used to record mouse movement and the video image carried out with a record of 6 min excluding the first 2 min was analyzed. The memory function was analyzed by calculating the alteration rate as the following formula: Alterations/(ArmEntries−2)x100. If the mouse chooses a different arm than the one it arrived at, this choice is called alteration. This is considered the correct response but returned to the previous arm is an error.

Table 1. In vivo experimental test groups used in this study

Group	Inducer	Treatment drug	Dose (mg/kg/d)
Control	Saline (vehicle)	Saline (vehicle)	-
	Scopolamine (2 mg/kg)	Scopolamine	2 mg/kg
Control drug	Scopolamine (2 mg/kg)	Donepezile	3 mg/kg
Active	Scopolamine (2 mg/kg)	Resplex Alpha	200 mg/kg
	Scopolamine (2 mg/kg)	Product A (company M)	200 mg/kg

Passive Avoidance Test

Passive avoidance test is a short and long-term memory in an associative manner. It's an experiment that makes use of the mouse's habit of avoiding bright rooms and going to dark rooms. The experiment was started using a shuttle box device consisting of two compartments separated by a retractable door. One compartment is made of dark opaque walls, roofs and another compartment is made of bright lighting. The floor in both compartments was made of shocking metal grids, except that the floor was wired to receive a mild electric shock of 0.15 mA intensity for 1-sec duration on the unsafe side.

The experiment was conducted for a total of 2 days. The mouse was allowed to adapt while freely moving from a box separated into two compartments for 300 sec. After adaptation, the mouse was removed from the box and returned to the cage. The box was cleaned with 70% ethanol after changing the mice. On the first day, the mouse was placed in a bright room, and after 30 sec, the door to the darkroom was opened, and the initial latency to move to the dark room was measured for up to 300 sec. When the mouse entered the dark room, the door was closed, and after 5 seconds, an electric shock of 0.15 mA was applied through the floor grid for 1 sec followed by 1 min waiting. The mouse was removed from the box. On the second day, the mouse was placed in a bright room, and the door to the darkroom was opened 30 seconds later. The time for the mouse entering the darkroom was measured. It was observed for up to 300 seconds.

Statistical Analysis

Data were quantified using Graph Pad Prism (version 5, Graph Pad Software, Inc., La Jolla, California). Significant differences, between the two groups were analyzed using the student's t-test and One-way analysis of the variance (ANOVA) with Tukey's multiple comparison test. Significant differences were indicated as *p < 0.05, **p < 0.01, ***p < 0.001, #p < 0.05, ###p < 0.001. *p vs Saline, #p vs Scopolamine.

RESULTS

Changes in Body Weight

We acclimatized Balb/c mice for 7 days and orally administered the experimental substance for 14 days before conducted behavioral experiments for 3 days from the 15th day (Fig. 1). Compared with the normal control, there was no significant or meaningful change in the body weight of mice in the scopolamine, donepezil and Resplex Alpha treatment groups. However, in the Product A group, an entity with a sharp change in body weight on day 15th day was confirmed (Fig. 2)

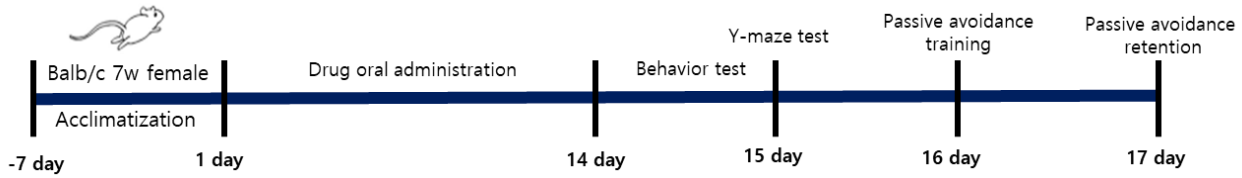
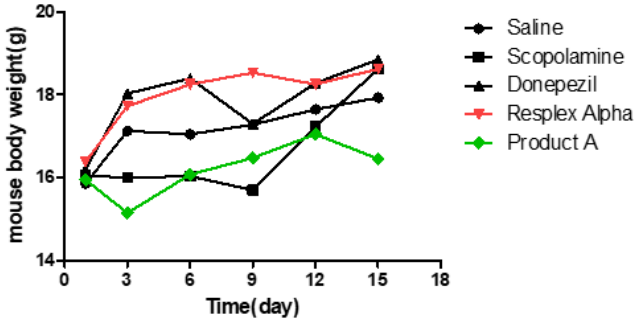


Fig. 1. In vivo experimental designs used in this study. Female Balb/c mice were selected into five groups (4 mice in each group, a total of 20 mice). Resplex Alpha and Product A were orally administered to mice once a day at concentrations of 200 mg/kg for 14 days. Memory was impaired by scopolamine i.p treatment (2 mg/kg). Additionally, as a positive control, Donepezil was orally administered at a concentration of 3 mg/kg 30 minutes before scopolamine administration



Group	Body weights (g) after initial test substance treatment					
	1 day	3 day	6 day	9 day	12 day	15 day
Control	15.85±0.77	17.16±0.45	17.05±0.48	17.28±0.38	17.65±0.45	17.93±0.39
Scopolamine control	16.05±0.31	16.00±2.43	16.03±1.51	15.70±0.62	17.23±0.85	18.63±0.15
Donepezil	16.20±0.46	18.03±0.67	18.40±0.47	17.30±0.55	18.28±0.64	18.85±0.58
Resplex Alpha	16.38±0.31	17.76±0.38	18.25±0.44	18.53±0.28	18.25±0.84	18.63±0.80
Product A	15.95±0.33	15.15±1.74	16.08±1.54	16.48±0.78	17.05±0.45	16.45±1.98

Fig. 2. Body weight changes in mice. Changes were measured from day 1 to day 15. During the experiment, no significant changes were found in the Resplex Alpha treatment group. The Product A treatment group showed significant changes on day 15.

Y-maze test

Y-maze test was conducted as the first behavioral experiment. As illustrated in Fig. 3, it can be confirmed that there is a significant decrease in cognitive function in the scopolamine-alone group compared to the normal control group. It is also confirmed that administration of Donepezil, a drug for the control group, blocked the scopolamine-induced cognitive deterioration and significantly recovered it to the level of the normal control group. Resplex Alpha administered group also displayed significant restoration to the level of the normal control group through blockage of the cognitive deterioration. However, although the Product A group appeared to recover like the normal control group, the results were not significant due to large differences between each of the mice in the group. (Fig. 3.) These results imply that Resplex Alpha could be helpful in recovering cognitive function.

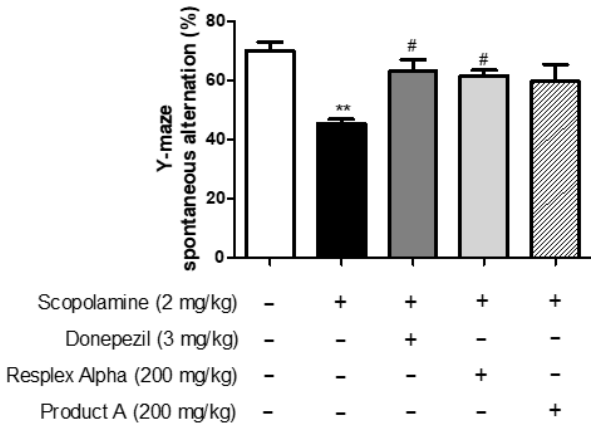


Fig. 3. Y-maze test. The alteration rate was measured in all groups. Calculated the formula of `alterations/(ArmEntries−2)x100. Statistical difference was determined by student t-test, and the data are presented as the mean ± SD. **p < 0.01, #p < 0.05, ###p < 0.001. **p > 0.01 vs Saline, #p < 0.05 vs Scopolamine.

Passive Avoidance Test

Memory capability was measured using the passive avoidance test. On day 1, the animals were electrically shocked into training and full-scale evaluation was conducted 24 hours thereafter. As illustrated in Fig. 4, the time taken to enter the dark area prior to the training was not significantly different in all the groups. In contrast, the scopolamine administration following the application of electric shock significantly increased the time taken compared to that of the normal control group. This signifies that memory has been impaired due to scopolamine administration. In the Donepezil group, which is the drug control group, and the Resplex Alpha group, it was possible to confirm that the memory deterioration caused by scopolamine was significantly restored. However, it was confirmed that the scopolamine-induced memory deterioration did not significantly recover the Product A treated group. These results imply that Resplex Alpha acts more effectively than product A in restoring memory.

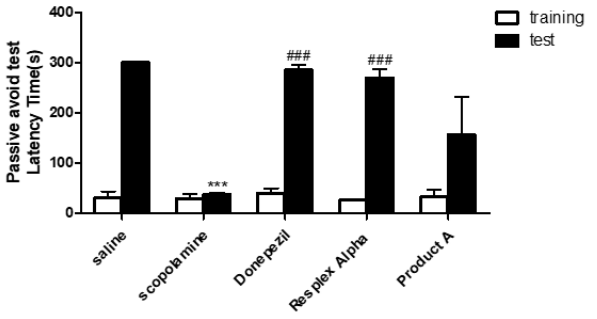


Fig. 4. Passive avoidance test. Latency refers to the time spent in the bright room before mice enter the darkroom. The time to enter the darkroom before applied the mild electric shock was measured. After applied a mild electric shock, the time to enter the darkroom from the lightroom was measured 24 hours later. Statistical difference was determined by student t-test, and the data are presented as the mean ± SD. ***P < 0.001, $^{###}$P < 0.001, ***P < 0.001 vs Saline, $^{###}$P < 0.001 vs Scopolamine.

Discussion

AD is a representative disease for which the level of risk increases exponentially with age. With the growing number of elderly people in modern society, there is an increase in interest and concern for the discovery of medicine for the prevention or treatment of AD. Currently, available medicines for the treatment of AD are aimed only at keeping the disease from aggravating rather than curing it. Moreover, some of these treatment medicines have been associated with side effects such as brain hemorrhages, thereby resulting in their use with the burden of such risks. AD is a disease that can start as early as the 50s, although it can also occur as late as the 60s due to the accumulation of Aβ in the brain as early as 30 years earlier than the manifestation of the symptoms of AD. There is an extensive range of research being conducted on highly

diversified natural substances that are known to be effective in treating AD, and there are also many foods that have been shown to be effective against dementia. Therefore, in this study, as part of the development of natural substance-derived functional foods to improve cognitive function, resveratrol-alginate nanocomplex (RANCP)-based natural substance developed by JBKLAB was used for the evaluation of its effect on the improvement of memory and cognitive function by creating a cognitive dysfunction mouse model. Donepezil, a known AD treatment medicine, was used for the drug control group and one of the functional foods (Product A from company M), which is currently sold in pharmacies that help with the improvement of cognitive function, was used for the comparison group. Our study illustrated that Resplex Alpha recovered cognitive deterioration to the level same as that of Donepezil and significantly improved memory dysfunction. However, Product A did not significantly improve cognitive and memory dysfunction. Resplex Alpha developed by JBKLAB is composed of its in-house developed RANCP, ginkgo biloba extract and vitamin E, etc., which are natural substances known to have efficacy on the improvement of cognitive function and symptoms of dementia. As such, it is thought that Resplex Alpha will be able to remove reactive oxygen in the brain, improve cognitive function through anti-inflammatory and anti-Ab actions and prevent memory dysfunction in order to ultimately prevent AD. The results of such evaluation lead to the expectation that Resplex Alpha would be highly effective as a functional food or medicine ingredient for effective treatment and/or improvement of diversified memory dysfunctions such as AD and cognitive function.

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None.

CONFLICT OF INTEREST

The authors have no competing interests to declare.

Reference

1. Chuong, N. N.; Trung, B. H.; Luan, T. C.; Hung, T. M.; Dang, N. H.; Dat, N. T. Anti-amnesic effect of alkaloid fraction from Lycopodiella cernua (L.) Pic. Serm. on scopolamine-induced memory impairment in mice. *Neurosci Lett* **2014**, *575*, 42-46. DOI: 10.1016/j.neulet.2014.05.031 From NLM.
2. Becker, R.; Giacobini, E.; Elble, R.; McIlhany, M.; Sherman, K. Potential pharmacotherapy of Alzheimer disease. A comparison of various forms of physostigmine administration. *Acta Neurol Scand Suppl* **1988**, *116*, 19-32. DOI: 10.1111/j.1600-0404.1988.tb07983.x From NLM.
3. Taraschenko, O. D.; Barnes, W. G.; Herrick-Davis, K.; Yokoyama, Y.; Boyd, D. L.; Hough, L. B. Actions of tacrine and galanthamine on histamine-N-methyltransferase. *Methods Find Exp Clin Pharmacol* **2005**, *27* (3), 161-165. DOI: 10.1358/mf.2005.27.3.890872 From NLM.
4. Farlow, M.; Gracon, S. I.; Hershey, L. A.; Lewis, K. W.; Sadowsky, C. H.; Dolan-Ureno, J. A controlled trial of tacrine in Alzheimer's disease. The Tacrine Study Group. *Jama* **1992**, *268* (18), 2523-2529. From NLM.
5. Watkins, P. B.; Zimmerman, H. J.; Knapp, M. J.; Gracon, S. I.; Lewis, K. W. Hepatotoxic effects of tacrine

administration in patients with Alzheimer's disease. *Jama* **1994**, *271* (13), 992-998. From NLM.

6. Park, S. M.; Ki, S. H.; Han, N. R.; Cho, I. J.; Ku, S. K.; Kim, S. C.; Zhao, R. J.; Kim, Y. W. Tacrine, an oral acetylcholinesterase inhibitor, induced hepatic oxidative damage, which was blocked by liquiritigenin through GSK3-beta inhibition. *Biol Pharm Bull* **2015**, *38* (2), 184-192. DOI: 10.1248/bpb.b14-00430 From NLM.
7. Houghton, P. J.; Ren, Y.; Howes, M. J. Acetylcholinesterase inhibitors from plants and fungi. *Nat Prod Rep* **2006**, *23* (2), 181-199. DOI: 10.1039/b508966m From NLM.
8. Oh, M. H.; Houghton, P. J.; Whang, W. K.; Cho, J. H. Screening of Korean herbal medicines used to improve cognitive function for anti-cholinesterase activity. *Phytomedicine* **2004**, *11* (6), 544-548. DOI: 10.1016/j.phymed.2004.03.001 From NLM.
9. Hasanein, P.; Mahtaj, A. K. Ameliorative effect of rosmarinic acid on scopolamine-induced memory impairment in rats. *Neurosci Lett* **2015**, *585*, 23-27. DOI: 10.1016/j.neulet.2014.11.027 From NLM.
10. Palit, P.; Mukherjee, D.; Mandal, S. C. Reconstituted mother tinctures of Gelsemium sempervirens L. improve memory and cognitive impairment in mice scopolamine-induced dementia model. *J Ethnopharmacol* **2015**, *159*, 274-284. DOI: 10.1016/j.jep.2014.09.008 From NLM.
11. Buglio, D. S.; Marton, L. T.; Laurindo, L. F.; Guiguer, E. L.; Araújo, A. C.; Buchaim, R. L.; Goulart, R. A.; Rubira, C. J.; Barbalho, S. M. The Role of Resveratrol in Mild Cognitive Impairment and Alzheimer's Disease: A Systematic Review. *J Med Food* **2022**, *25* (8), 797-806. DOI: 10.1089/jmf.2021.0084 From NLM.
12. Weinmann, S.; Roll, S.; Schwarzbach, C.; Vauth, C.; Willich, S. N. Effects of Ginkgo biloba in dementia: systematic review and meta-analysis. *BMC Geriatr* **2010**, *10*, 14. DOI: 10.1186/1471-2318-10-14 From NLM.
13. Tan, M. S.; Yu, J. T.; Tan, C. C.; Wang, H. F.; Meng, X. F.; Wang, C.; Jiang, T.; Zhu, X. C.; Tan, L. Efficacy and adverse effects of ginkgo biloba for cognitive impairment and dementia: a systematic review and meta-analysis. *J Alzheimers Dis* **2015**, *43* (2), 589-603. DOI: 10.3233/jad-140837 From NLM.
14. DeFeudis, F. V.; Drieu, K. Ginkgo biloba extract (EGb 761) and CNS functions: basic studies and clinical applications. *Curr Drug Targets* **2000**, *1* (1), 25-58. DOI: 10.2174/1389450003349380 From NLM.
15. Liu, X.; Hao, W.; Qin, Y.; Decker, Y.; Wang, X.; Burkart, M.; Schötz, K.; Menger, M. D.; Fassbender, K.; Liu, Y. Long-term treatment with Ginkgo biloba extract EGb 761 improves symptoms and pathology in a transgenic mouse model of Alzheimer's disease. *Brain Behav Immun* **2015**, *46*, 121-131. DOI: 10.1016/j.bbi.2015.01.011 From NLM.
16. Farina, N.; Llewellyn, D.; Isaac, M.; Tabet, N. Vitamin E for Alzheimer's dementia and mild cognitive impairment. *Cochrane Database Syst Rev* **2017**, *4* (4), Cd002854. DOI: 10.1002/14651858.CD002854.pub5 From NLM.
17. Deng, X.; Zhao, S.; Liu, X.; Han, L.; Wang, R.; Hao, H.; Jiao, Y.; Han, S.; Bai, C. Polygala tenuifolia: a source for anti-Alzheimer's disease drugs. *Pharm Biol* **2020**, *58* (1), 410-416. DOI: 10.1080/13880209.2020.1758732 From

NLM.

18. Choi, M.; Lee, Y.; Cho, S. H. Angelica tenuissima Nakai Ameliorates Cognitive Impairment and Promotes Neurogenesis in Mouse Model of Alzheimer's Disease. *Chin J Integr Med* **2018**, *24* (5), 378-384. DOI: 10.1007/s11655-017-2812-2 From NLM.
19. Li, H.; Kim, J.; Tran, H. N. K.; Lee, C. H.; Hur, J.; Kim, M. C.; Yang, H. O. Extract of Polygala tenuifolia, Angelica tenuissima, and Dimocarpus longan Reduces Behavioral Defect and Enhances Autophagy in Experimental Models of Parkinson's Disease. *Neuromolecular Med* **2021**, *23* (3), 428-443. DOI: 10.1007/s12017-020-08643-x From NLM.
20. Renner, U. D.; Oertel, R.; Kirch, W. Pharmacokinetics and pharmacodynamics in clinical use of scopolamine. *Ther Drug Monit* **2005**, *27* (5), 655-665. DOI: 10.1097/01.ftd.0000168293.48226.57