Journal of Ethnopharmacology 224 (2018) 91–99

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jethpharm

# The enhancing effect of Aubang Gahl Soo on the hippocampal synaptic plasticity and memory through enhancing cholinergic system in mice

Jihye Lee[a,1], Huiyoung Kwon[b,1], Jimin Yu[b,1], Eunbi Cho[b], Jieun Jeon[b], Seungheon Lee[c], Jong Hoon Ryu[d,e,f], Young Choon Lee[b,g], Dong Hyun Kim[b,g,*], Ji Wook Jung[h,**]

[a] Division of Endocrinology, School of Medicine, Kyungpook National University, Daegu 41944, Republic of Korea
[b] Department of Medicinal Biotechnology, College of Health Sciences, Dong-A University, Busan 49315, Republic of Korea
[c] Department of Aquatic Biomedical Sciences, School of Marine Biomedical Science, College of Ocean Science, Jeju National University, Jeju 63243, Republic of Korea
[d] Department of Life and Nanopharmaceutical Science, Kyung Hee University, Hoeki-dong, Dongdaemoon-Ku, Seoul 02447, Republic of Korea
[e] Department of Oriental Pharmaceutical Science, College of Pharmacy, Kyung Hee University, Seoul 02447, Republic of Korea
[f] Kyung Hee East-West Pharmaceutical Research Institute, College of Pharmacy, Kyung Hee University, Seoul 02447, Republic of Korea
[g] Institute of Convergence Bio-Health, Dong-A University, Busan 49315, Republic of Korea
[h] Division of Bio-technology and Convergence, College of Bio-industry, Daegu Haany University, Kyungsan 38578, Republic of Korea

ARTICLE INFO

*List of compound:*
Scopolamine
Donepezil

*Keywords:*
Aubang Gahl Soo
Long-term potentiation
Learning and memory
Acetylcholine

ABSTRACT

*Ethnopharmacological relevance:* Aubang Gahl Soo (AGS) is a Korean traditional drink manufactured from medicinal plants and fruits using sugar or honey. Although traditional old book stated its effects on body, there is no scientific evidence yet. Therefore, in the present study, we tested AGS on brain functions.

*Aim of this study:* In this study, we tried to uncover the effect of on brain functions. To do this we examined the action of AGS on the hippocampal synaptic function and memory in mice.

*Materials and methods:* To examine the effect of AGS on synaptic plasticity, we observed input-output curves (I/O curve), paired-pulse facilitation (PPF), and long-term potentiation (LTP) using mouse hippocampal slices. Moreover, to investigate the functional relevance of the effect of AGS on synaptic plasticity, we conducted passive avoidance, Y-maze and Morris water maze tests. To examine relevant mechanism, acetylcholinesterase (AChE) activity and acetylcholine (ACh) level assay were also conducted.

*Results:* In the basal synaptic transmission study, we found that AGS did not affect I/O curves and PPF. However, AGS facilitated hippocampal LTP in a concentration-dependent manner. Moreover, AGS blocked AChE activity ($IC_{50}$ = 485 μg/ml). Moreover, ACh level was increased by AGS (100 μg/ml) treatment. Along with this, facilitating effect of AGS on hippocampal LTP also blocked by scopolamine, a muscarinic acetylcholine receptor antagonist. Moreover, AGS also ameliorated memory impairments induced by scopolamine in passive avoidance, Y-maze, and Morris water maze tests.

*Conclusions:* These results suggest that AGS facilitates hippocampal LTP through activating cholinergic system and ameliorates cholinergic dysfunction-induced memory deficit.

## 1. Introduction

Aubang Gahl Soo (AGS, thirst water) is a non-alcoholic traditional Korean beverage (ref wikifidia). AGS is described in an old book, 『Imwonshibyukji』 written in 1827, the Joseon Dynasty period. This book indicates that AGS has effects on body including improving body strength, immunity, and clear mind (Lee et al., 2001). However, scientific evidence of effects of AGS has never tested before. AGS is composed with Cinnamomi Cortex Spissus (*Cinnamomum cassia* Blume), Citri Pericarpium (*Citrus unshiu* Markovich), Amomi Rotundus Fructus (*Amomum kravanh* Pierre ex Cagnep), Syzygii Flos (*Syzygium aromaticum* Merr et Perry), Amomi Fructus (*Amomum xanthioides* Wall),

*Abbreviations:* AGS, Aubang Gahl Soo; I/O curves, input-output curves; PPF, paired-pulse facilitation; LTP, long-term potentiation; AChE, acetylcholinesterase; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; NMDA, N-methyl-D-aspartic acid; HPLC, high-performance liquid chromatography; fEPSP, field excitatory postsynaptic potential; DNPZ, Donepezil; SCO, scopolamine; mAChR, muscarinic acetylcholine receptors; AD, Alzheimer's disease

* Corresponding author at: Department of Medicinal Biotechnology, College of Health Sciences, Dong-A University, Busan 49315, Republic of Korea.
** Corresponding author.
*E-mail addresses:* lghlovely@naver.com (J. Lee), kwonhuiyoung@naver.com (H. Kwon), wlals2463@naver.com (J. Yu), bee2634@naver.com (E. Cho), ji6785@naver.com (J. Jeon), slee76@jejunu.ac.kr (S. Lee), jhryu63@khu.ac.kr (J.H. Ryu), yclee@dau.ac.kr (Y.C. Lee), mose79@dau.ac.kr (D.H. Kim), jwjung@dhu.ac.kr (J.W. Jung).
[1] These authors equally contributed in this study.

https://doi.org/10.1016/j.jep.2018.05.017
Received 11 January 2018; Received in revised form 13 April 2018; Accepted 13 May 2018
Available online 26 May 2018
0378-8741/ 

yeast, malt, and honey. Previous reports suggested that *Cinnamomum cassia, Citrus unshiu* and *Syzygium aromaticum* have regulatory effects on brain functions (Ihara et al., 2012; Okuyama et al., 2012; Yu et al., 2007). Therefore, AGS can be speculated to have beneficial effects on various brain disorders.

Synaptic plasticity is the ability of the synapse to strengthen or weaken, changing the number of AMPA receptor and NMDA receptor on synapse, also is recognized to the significant mechanism involved in strengthening memory (Martin et al., 2000; Neves et al., 2008). LTP (long-term potentiation) is the phenomenon that enhances persistently the signal transmission between two neurons. The neuron performs the signal transmission through synapse connection and the memory is considered to be accumulated in this synapse connection (Martin et al., 2000; Tsien et al., 1996). LTP is significantly considered as the cytological mechanism of the learning, and making and strengthening the memory (Neves et al., 2008). LTP increases the ability of the signal transmission between presynaptic neuron and postsynaptic neuron through synapse because LTP increases the number of AMPA receptor and NMDA receptor on postsynaptic neuron (Lau and Zukin, 2007; Malinow and Malenka, 2002). The present study concerning LTP is mostly focusing on the cause-and-effect relationship between the basic biological comprehension and the behavioral study, also is developing the pharmacological method, etc. that induces LTP.

The decline of the cholinergic nerve is recognized to be a principal factor to cause the dementia symptom in disease such as Alzheimer's disease (Cummings and Kaufer, 1996; Fotiou et al., 2015; Francis et al., 1999). Actually, drug such as donepezil used for the present clinical trial increases the activity of declined cholinergic nerve by preventing the breakdown of acetylcholine and improves the memory (Anand and Singh, 2013). In addition, cholinergic nerve is reported to be an important factor in synaptic plasticity of the hippocampus (Dennis et al., 2016; Freund et al., 2016). Therefore, we suggest that drug to regulate synaptic plasticity through the cholinergic nerve in the hippocampus is a good method for Alzheimer's disease.

## 2. Materials and methods

### 2.1. Animal

Male ICR mice (4 weeks old) were purchased from Daehan Biolink (Choongbook, Korea). Mice were housed animal room (light/dark cycle = 12 h, temperature = 23 ± 2 °C, humidity = 50 ± 10%). Animal allowed to freely accessing water and food. Mice were used for animal experiments at 2 weeks after. All animal experiments in this study were approved by Institutional Animal Care and Use Committee of Daegu Haany University, Korea (DHU2016-056).

### 2.2. Preparation of AGS

Dried Cinnamomi Cortex Spissus [*Cinnamomum cassia* (L.) J.Presl], Citri Pericarpium [*Citrus unshiu* (Yu. Tanake ex Swingle) Marcow], Amomi Rotundus Fructus (*Amomum verum Blackw*), Syzygii Flos (*Syzygium aromaticum* (L.) Merr. & L.M.Perry) and Amomi Fructus [*Amomum villosum* var. *xanthioides* (Wall. ex Baker) T.L.Wu & S.J.Chen] were purchased from Gamchodang yakupsa (Kyungbook, Korea) (Table 1). Voucher specimens (Cinnamomi Cortex Spissus, DGHU-00101; Citri Pericarpium, DGHU-00102; Amomi Rotundus Fructus, DGHU-00103; Syzygii Flos, DGHU-00104; Amomi Fructus, DGHU-00105) were deposited at the herbarium of the College of Bio-industry, Daegu Haany University. These was crushed with blend and mixed with distilled water (10 x volume). This mixture was extracted for 2 h (80 °C) and filtered. The extract lyophilized for experiment (yield = 15.895%).

**Table 1**
Prescription of AGS.

| Herbal name | Weight (g) |
|---|---|
| Cinnamomi Cortex Spissus | 40 |
| Citri Pericarpium | 20 |
| Amomi Rotundus Fructus | 20 |
| Syzygii Flos | 20 |
| Amomi Fructus | 20 |
| Total amount | 120 |

The stock solution of cinnamic acid, rutin or eugenol was dissolved in methanol (100 μg/ml). Sample was filtered through a 0.2 μm membrane filter (Chromdisc®, Korea) and an aliquot of 10 μl sample was injected into the high-performance liquid chromatography (HPLC) system. HPLC analysis was performed using an Alliance 2795 system (Waters, USA). Separation was performed at 25 °C on a YMC-Triart $C_{18}$ column (4.6 × 250 mm, 5 μm). The mobile phase consisted of 31% (v/v) acetonitrile with 1% acetic acid for cinnamic acid and was operated at a flow rate of 1.0 ml/min. The UV detection wavelength was carried out at 280 nm (Fig. 1A). For rutin, the mobile phase A consisted of 0.1% formic acid in water and B consisted of 0.1% formic acid in acetonitrile. A gradient elution program was applied as follows : A was linearly decreased from 80% to 75% in 5 min, and from 75% to 55% in 20 min, and then returned to 80% A. The mobile phase flow rate was 1.0 ml/min and UV detection wavelength was carried out at 355 nm (Fig. 1B). For eugenol, the mobile phase consisted of 80%(v/v) methanol. The mobile phases were operated at a flow rate of 0.5 ml/min. The UV detection wavelength was carried out at 280 nm (Fig. 1C). The concentrations of each compound in AGS are presented: cinnamic acid, 0.07%; rutin, 11.14%; eugenol, 15.58%.

### 2.3. Preparation of the hippocampal slices

Mice brain was removed and incubated in ice-cold artificial cerebrospinal fluid (ACSF) (NaCl 124 mM, KCl 3 mM, NaHCO3 26 mM, $NaH_2PO_4$ 1.25 mM, $CaCl_2$ 2 mM, $MgSO_4$ 1 mM, D-glucose 10 mM, bubbled with 95% $O_2$/5% $CO_2$). Using vibratome, coronal brain slices (400 μm thickness) were made. The brain slices were recovered for more than 1 h in ACSF (20–25 °C).

### 2.4. Electrophysiology

Hippocampal field excitatory postsynaptic potential (fEPSP) was recorded in stratum pyramidale of the hippocampus. Stimulating electrode was located on Shaffer collateral-commissural pathway. Stimulation maintained continuously with 30 s interval. Input-output (I/O) curve obtained from recording the serial responses induced by 0, 2, 4, 8, 10, 15, 20 V of stimulation. Paired pulse facilitation (PPF) was measured from the ratio of responses induced by 5 V stimulation with 25, 50, 100, 200, and 500 ms interval. To induce LTP, high frequency stimulation (100 Hz, 100 pulses, 2 trains) was introduced at 20 min of stable baseline.

### 2.5. Acetylcholinesterase assay

A mixture including 134 μl of sodium phosphate buffer (0.1 M), 1 μl of acetylthiocholine iodide (75 mM), 5 μl of 5,5-dithiobis-2-nitrobenzoate (10 mM), and 50 μl of various concentration of AGS was incubated for 10 min at 25 °C. Ten μl of naïve mouse brain homogenate was added to the mixture and then absorbance was measured at 405 nm. For *ex vivo* study, AGS (800 mg/kg) was orally administered to naïve mice and the brain homogenate was obtained 1 h after the administration.

### 2.6. Acetylcholine assay

To measure acetylcholine level, acetylcholine assay kit (Cell Biolabs, San Diego, CA) was used. Hippocampal slices were incubated with AGS for 2 h for in vitro assay. For in vivo assay, mice were administered AGS