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Research Article
Eriobotrya japonica Improves Cognitive Function in Healthy Adolescents: A 12-week, Randomized Double-blind, Placebo-controlled Clinical Trial

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Abstract

Eriobotrya japonica Lindley, a subtropical plant in the family Rosaceae, has been used as a folk medicine in Asia, which is known to contain various phytochemicals and have neuroprotective and anti-oxidant effects. In this study, the efficacy and safety of ethanol extract from leaves of *Eriobotrya japonica* Lindley (ELEJ) were investigated for improvement of cognitive function in healthy Korean adolescents by conducting a randomized clinical trial. Eighty subjects from 16-19 years of age with normal cognitive function were randomly assigned to receive either ELEJ (750 mg, twice a day) or placebo in this 12 week, double-blind and placebo-controlled trial. Neuropsychological assessments including Korean-Mini Mental State Examination (K-MMSE) as primary outcome, Rey-kim memory test, brief cognitive rating scale, prospective and retrospective memory questionnaire, spielberger state-trait anxiety inventory and blood brain-derived neurotrophic factor (BDNF) as secondary outcomes were used to assess the cognitive function. Intake of ELEJ for 12 week significantly increased both the total score and the score of the K-MMSE compared with placebo ($p = 0.043$, $p = 0.014$, respectively). There were no significant differences in the changes of other neuropsychological assessment scores between the two groups. There was no observed adverse event related to the ingestion of ELEJ. This result suggest that ELEJ supplementation safely improves cognitive function, especially memory, in healthy Korean adolescents in a safe manner as shown by the increase in the score of K-MMSE. Further studies exploring whether ELEJ is effective for the treatment of cognitive impairment are warranted.

Key words: *Eriobotyra japonica*, cognitive function, memory, adolesent, MMSE

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Eriobotrya japonica Lindley (EJ) is a subtropical woodland plant in the family Rosaceae that is native to the Himalayas and East Asia. The EJ has been cultivated and used as a folk medicine in Asia for more than 2,000 years. The EJ is commercially cultivated worldwide and the number of scientific studies exploring its efficacy is increasing (Lin *et al*., 2010).

The EJ is recognized as a rich source of phytochemicals. The bioactive components of EJ include flavonoids (Jung *et al*., 1999), phenolics (Ding *et al*., 2001), amygdalin (Zhou *et al*., 2007), triterpenic acids (Liang *et al*., 1990) and carotenoids (Godoy and Amaya, 1995). Previous studies suggest that extract of EJ seed exhibits anti-oxidative activity in mice (Hamada *et al*., 2004; Yokota *et al*., 2006; Yoshioka *et al*., 2010). Methanol extracts of EJ stems exhibit anti-microbial and antioxidant activities *in vitro* (Rashed and Butnariu, 2014). In mouse models, EJ leaves exhibit hypoglycemic (Chen *et al*., 2008; Shih *et al*., 2010; Wu *et al*., 2014), anti-lipidemic (Shih *et al*., 2010), anti-inflammatory and anti-tumor (Banno *et al*., 2005; Cha *et al*., 2011a), anti-obesity effects (Tanaka *et al*., 2010) and anti-metastatic property (Cha *et al*., 2011b). Also, extracts from EJ leaves have antimutagenic, antigenotoxic and antioxidant activities *in vitro* (Mokdad-Bzeouich *et al*., 2015), inhibitory effects on bone mineral density loss *in vitro* (Tan *et al*., 2014), anti-inflammatory effect *in vitro* test in the human gingival fibroblast stimulated by lipopolysaccharide (Choi *et al*., 2011), cytotoxicity against human oral tumor cell lines (Ito *et al*., 2000) and apoptotic cell death in human leukemia cell line (Kikuchi *et al*., 2011). Among these evidences from *in vitro* and *in vivo* experiments, a recent study report that extracts from EJ leaves have neuroprotective effects: Treatment with EJ extract protected neuronal cells from β-amyloid ($A\beta_{1-42}$) induced neuronal cell death via potent free radical scavenging activity and improved memory impairment in $A\beta_{1-42}$ treated mice (Kim *et al*., 2011).

Previous study that investigated the effect of anti-hyperglycemic effect of EJ in diabetic mice model also studied the toxicity of EJ and reported that EJ was safe (360 g kg^{-1} as maximum dose, 400.1 g kg^{-1} as median lethal dose) (Li *et al*., 2007). Before starting the present research, toxicity and safety of oral administration of EJ were tested in rats and no toxicity was observed. Therefore, EJ was considered to be safe to study its effects of the oral administration in human.

Based upon the neuroprotective effects of EJ that was tested in animal model, this study was aimed if EJ is safe and effective for improving cognition in humans. However, the effects of EJ extract on cognition and especially on memory function have not been studied in humans. Therefore, the effects of ethanol extract from leaves of EJ (ELEJ) on cognitive function as measured by various neuropsychological assessments of healthy Korean adolescents were investigated and also the safety of ELEJ was assessed.

MATERIALS AND METHODS

Ethics statements: The study was conducted at the Clinical Trial Center for Functional Foods (CTCF2), Chonbuk National University Hospital, according to the principles of the Helsinki declaration and guidelines for good clinical practice. The study protocol and informed consent form were approved by the Functional Foods Institutional Review Board of Chonbuk National University Hospital. The participants were recruited through local advertisements during 2012 and 2013. All participants gave written informed consent before the study began. The protocol is registered at www.ClinicalTrials.gov (NCT01734200).

Subjects: The participants were Korean male and female adolescents ranging from 16-19 years of age who were mentally and physically healthy. The exclusion criteria were as follows: (1) Taking medication or supplements for cognitive improvement, (2) Difficulty in common communication, (3) Inability to write or see images due to visual impairment, (4) Inability to produce handwriting due to motor disabilities of the hands or upper limbs, (5) Current illnesses or history of chronic diseases within the prior three years, (6) Participation in any other clinical trials with investigative medicinal products within the past 2 months, (7) Allergies or hypersensitivities to any of the ingredients in the investigational products, (8) Drug or alcohol dependency, (9) Pregnancy or breast feeding and (10) Conditions, which in the opinion of the investigators, could interfere with successful participation in the study or affect subject safety.

Study design: This study was a 12 week, randomized, double-blind, placebo-controlled and parallel trial. Treatment arms were comprised of a test group (ELEJ group, n = 40) and a control group (placebo group, n = 40). Random allocation sequence was generated by the permuted block randomization method (Excel, Microsoft Office 2007) with an allocation ration of 1:1 for a test group and a control group. Randomization codes were produced by an independent contract research organization and were concealed until the

Table 1: Composition of investigational products per capsule

Content (mg)	ELEJ	Placebo
Extract from leaves of *Eriobotrya japonica*	250.0	0.0
Microcrystalline cellulose	195.0	411.6
Caramel color	4.2	33.0
Cochineal extract	0.0	4.6
Pine flavor	0.35	0.35
Magnesium stearate	0.45	0.45
Total	450.0	450.0

ELEJ: Ethanol extract from leaves of *Eriobotrya japonica*

study database was locked. All investigators, coordinators and participants were blinded to treatment assignment throughout the study. The subjects visited the CTCF2 four times during the 12-week intervention period: A screening visit (visit 0), randomization day (visit 1) and after 6 (visit 2) and 12 weeks (visit 3, end of the study). Visit 0 and 1 were considered as baseline. The primary outcome measure was the K-MMSE score (measured at visits 0, 2 and 3). Secondary outcome measures were the rey-kim memory test (visits 1 and 3), Brief Cognitive Rating Scale (BCRS), Prospective and Retrospective Memory Questionnaire (PRMQ), spielberger state-trait anxiety inventory (STAI) (visits 0 and 3) and blood BDNF level (visits 1 and 3). To assess safety, adverse events, vital signs and physical examinations were evaluated at visits 1, 2 and 3 and ECG and laboratory tests at visits 0 and 3. During the 12-week intervention period, participants were asked to maintain their usual lifestyle (e.g., diet, physical activities, work and sleeping habits) and a detailed dietary assessment was performed by the 3-day record method just before the beginning and at the end of the intervention. Participants were prohibited from taking any medications or dietary supplements known to be related to cognitive function. Medications that participants had taken 4 or more weeks prior to the initiation of the study and believed not to affect study reliability were permitted under the physician's supervision. Participants also were asked to report any adverse events and any changes in lifestyle, recent illness, or medical treatment to the investigators immediately.

Investigational product: The test supplement and placebo were produced and supplied under Good Manufacturing Practice (GMP) by Korea Inspham, Inc. (Hwasoon, Jeonnam, South Korea). Standardization of ELEJ was ensured during the manufacturing process. The ELEJ powder was obtained from leaves of EJ through three main phases, i.e., extraction by 5% ethanol, concentration and drying using a 5 t extractor (Hanyeon Machinery Technology, Korea) and a spray-dryer (NiroKorea, Korea). The index components were chlorogenic acid and quercetin. Average contents of chlorogenic acid and quercetin in ELEJ were 4.0 and 0.7 mg g^{-1}, respectively, as identified using High Performance Liquid Chromatography (HPLC) at Korea health supplement institute. Two Hundred and fifty milligram of ELEJ per capsule was dispensed. Placebo was manufactured and matched for size, color, opacity and odor with the test supplement. The compositional analysis of ELEJ is presented in Table 1. The test group consumed 1.5 g of ELEJ daily (750 mg of ELEJ, twice a day) for 12 weeks and the control group consumed the same number of placebo pills. The products were provided to participants at visits 1 and 2 and any remaining doses were returned at subsequent visits to assess compliance.

Outcome measurements for cognitive function

Korean mini-mental state examination (K-MMSE): The mini-mental state examination (MMSE) developed by Folstein *et al*. (1975) is the most widely used measure to screen cognitive status and examines orientation, memory, attention, calculation, recall and language abilities. The Korean version of the MMSE (K-MMSE), which was validated in a previous study of dementia patients (Kang *et al*., 1997) was used in the present study. This technique is reliable and valid, with total scores ranging from 0-30. A total score greater than 23 is classified as normal, 18-23 as mild cognitive impairment and below 18 as severe impairment.

Rey-Kim memory test: The Rey-Kim memory test (Kim, 2001) consists of the Rey-Kim Auditory Verbal Learning Test (K-AVLT) and the Rey-Kim Complex Figure Test (K-CFT). The K-AVLT is a modified version of the rey auditory verbal learning test (Rey, 1964) translated into Korean. The K-CFT is a standardized Korean version of the Rey-Osterrieth Complex Figure Test (ROCFT) (Shin *et al*., 2006) to assess visuo-constructional and visual memory skills. The K-AVLT consists of three steps, which are the immediate recall of 15 words five times (K-AVLT: Trials 1-5), delayed recall of 15 words 20 min later (K-AVLT: Delayed recall) and delayed retrieval to select 15 of 50 words (K-AVLT: Delayed recognition). The K-CFT consists of three steps, which are to copy a complex figure (K-CFT: Drawing), to recall and draw the complex figure (K-CFT: Immediate recall) and to draw the complex figure again after 30 min (K-CFT: Delayed recall). The Memory Quotient (MQ), memory retention, retrieval efficiency and drawing/memory consistency are also calculated.

Brief Cognitive Rating Scale (BCRS): The BCRS (Reisberg and Ferris, 1988; Allen, 2011) is used to assess functional and cognitive abilities in both normal aging and progressive dementia. The BCRS provides objective ratings of a number of

Table 2: Baseline demographic and anthropometric characteristics of the subjects

Parameters	ELEJ (n = 40)	Placebo (n = 40)	Total (n = 80)	p-value[1]
Sex (Male/female)	20/20	21/19	41/39	0.823[2]
Age (year)	18.25±0.84	18.20±0.76	18.23±0.80	0.781
Height (cm)	168.25±10.12	168.95±7.97	168.60±9.06	0.732
Weight (kg)	60.36±12.07	59.92±7.55	60.14±10.01	0.847
BMI (kg m^{-2})	21.17±2.80	20.96±1.92	21.06±2.39	0.700
Alcohol consumption (persons)	25 (62.50%)	27 (67.50%)	52 (65.00%)	0.639[2]
Drinking (units/week)	3.66±6.13	2.76±2.79	3.20±4.68	0.507
Smoker (persons)	1 (2.50%)	0 (0.00%)	1 (1.25%)	1.000[2]

Values are presented as Mean±SD or number (percentage), [1]: Analyzed by independent t-test, [2]: Analyzed by chi-square test or fisher's exact test, ELEJ: Ethanol extract from leaves of *Eriobotrya japonica* and BMI: Body mass index

domains including concentration, recent memory, remote memory, orientation and functioning and self-care. In this study, a modified Korean version of the BCRS was used. Each domain is scored from 1-7 and higher scores indicate greater impairment.

Prospective and retrospective memory questionnaire (PRMQ): The PRMQ is a 16-item self-report measure of prospective and retrospective failures in everyday life. Eight of the items address prospective memory and eight retrospective memories. Each item is rated on a 5-point scale and higher scores indicate memory impairment (Smith *et al*., 2000).

Spielberger state-trait anxiety inventory (STAI): The STAI (Spielberger, 2010) is comprised of two scales, state anxiety (current) and trait (general) anxiety. Each scale contains 20 sentences. Subjects are asked to rate each sentence on a four-point scale. The sums of ratings for each of the two scales can range from 20-80, with higher ratings indicating greater anxiety.

Brain-derived neurotrophic factor (BDNF): Venous blood samples for serum BDNF tests were taken after overnight fasting at visits 1 and 3. Serum BDNF levels were measured by enzyme-linked immunosorbent assay (ELISA) using the Human BDNF Immunoassay kit (Quantikine R and D Systems, Minneapolis, MN, USA) and their absorbance was read on a VERSA Max microplate reader (Molecular Devices Corp., Sunnyvale, California, USA).

Laboratory examinations: Laboratory tests included WBC, RBC, hemoglobin, hematocrit, platelet count, total protein, albumin, ALP, γ-GTP, AST, ALT, BUN, creatinine, glucose, creatine kinase, total cholesterol, triglyceride and urinalysis. Venous blood samples were drawn after a 12 h overnight fast at visits 0 and 3.

Statistical analysis: The primary analyses of efficacy were predetermined with the Per Protocol (PP) analysis set and safety analyses with the safety analysis set. For determining sample size, a mean difference of K-MMSE scores of 2.05 points in the test group and 1.04 points in the control group and Standard Deviations (SD) of 1.63 points in both groups with 80% power and a two-tailed α of 0.05 was assumed. Therefore, a total of 80 subjects were needed, allowing a 20% dropout rate. Statistical analyses were performed using SAS® version 9.2 (SAS Institute, USA). Differences between the two groups after 12 weeks were analyzed using linear mixed model and t-tests. Differences among baseline, 6 and 12 week in each group were analyzed using paired t-tests. Chi-square tests were performed to determine differences in frequencies of categorized variables between the groups at baseline. Data are presented as Mean±SD. A difference was considered statistically significant when the p-value was less than 0.05.

RESULTS

Subjects: Among the 83 volunteers initially screened, 80 subjects who fulfilled the inclusion criteria were registered and randomly assigned to either a test group (n = 40) or a placebo group (n = 40) between October, 2012 and July, 2013. A total of 75 participants completed the study. During the study, five participants (three in the ELEJ group, two in the placebo group) were dropped due to consent withdrawal. Three participants in the placebo group lacking pill compliance (below 70%) were excluded. The flow of participants through the study is depicted in Fig. 1. There were no significant differences between the ELEJ and placebo groups in any of the baseline demographic data and anthropometric parameters (Table 2).

Safety profiles: Laboratory tests, vital signs (blood pressure, heart rate) and anthropometric parameters (body weight, BMI) showed no significant change from baseline to the end of the

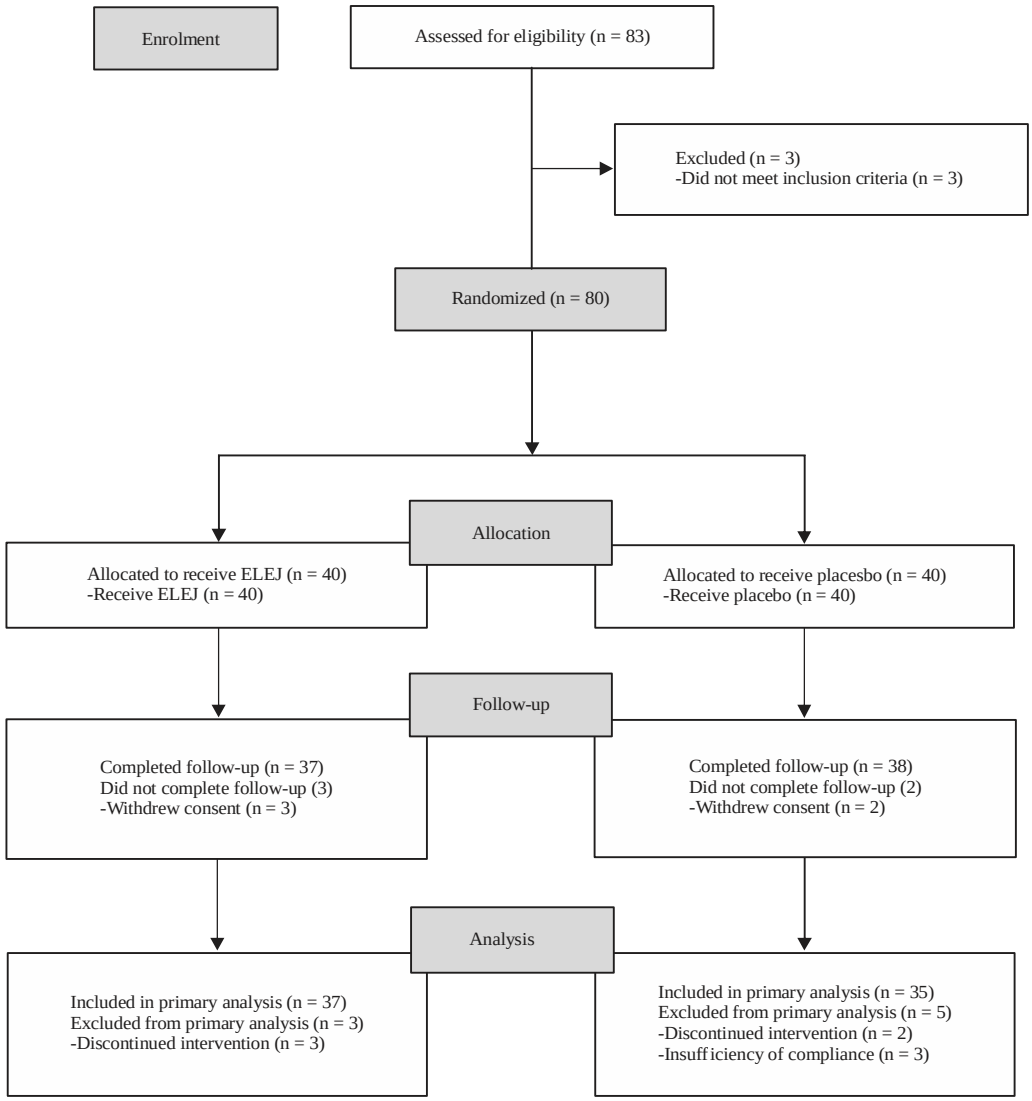

Fig. 1: Consort flowchart to track participants through randomized controlled trial

study in both groups. There were significant differences in changes of creatinine and Creatinine Kinase (CK) levels from baseline to the end of the study between the ELEJ and placebo group, which were determined to have no clinical significance because their values were within normal reference ranges. There were 40 cases of mild adverse events, including 21 cases among 14 participants in the ELEJ group and 19 cases among 14 participants in the placebo group. There were no significant differences in occurrence of adverse events between groups. These adverse events were determined to be unrelated to consumption of the test supplement.

Efficacy evaluations: There was no significant difference in baseline total score of K-MMSE between the ELEJ (n = 40) and placebo group (n = 40) (29.33±0.92 in the ELEJ group, 29.53±0.72 in the placebo group, p = 0.280). After the 12 week of intervention, the total K-MMSE score significantly increased in the ELEJ group (p = 0.003) while that of the placebo group did not change (p = 0.869). Comparisons between the two groups showed a significant difference in changes of the total K-MMSE scores (p = 0.043) (Table 3). The score of a memory recall item also significantly increased in the ELEJ group (p = 0.023) with a significant difference between the two groups (p = 0.014). The scores of attention and calculation items showed an increasing tendency in the ELEJ group (p = 0.051) (Table 3). The scores of individual items of the rey-kim memory test increased after the 12 week intervention in both groups and there were no significant differences in score changes between the two groups

Table 3: Comparisons of K-MMSE items between the ELEJ group and placebo group after 12 week administration of ELEJ

	ELEJ (n = 37)			Placebo (n = 35)			
Parameters	Baseline	Endpoint	p-value[1]	Baseline	Endpoint	p-value[1]	p-value[2]
Time orientation	4.86±0.35	4.97±0.16	0.091	4.86±0.36	4.91±0.28	0.46	0.607
Place orientation	5.00±0.00	4.97±0.16	0.324	4.94±0.24	5.00±0.00	0.16	0.082
Memory registration	3.00±0.00	3.00±0.00	-	3.00±0.00	3.00±0.00	-	-
Attention and calculation	4.57±0.87	4.76±0.72	0.051	4.8±0.53	4.71±0.75	0.539	0.101
Memory recall	2.86±0.35	3.00±0.00	0.023*	3.00±0.00	2.97±0.17	0.324	0.014*
Language ability	8.00±0.00	8.00±0.00	-	7.97±0.17	7.94±0.24	0.562	0.55
Drawing	1.00±0.00	1.00±0.00	-	1.00±0.00	1.00±0.00	-	-
Total	29.3±0.94	29.7±0.74	0.003**	29.57±0.65	29.54±0.85	0.869	0.043*

Values are presented as Mean±SD, [1]: Analyzed by paired t-test, [2]: Analyzed by linear mixed model for repeated measures data, *p<0.05, **p<0.01, ELEJ: Ethanol extract from leaves of *Eriobotrya japonica* and K-MMSE: Korean-mini mental state examination

Table 4: Comparisons of Rey-Kim memory test between the ELEJ group and placebo group after 12 week administration of ELEJ

	ELEJ (n = 37)			Placebo (n = 35)			
Parameters	Baseline	Endpoint	p-value[1]	Baseline	Endpoint	p-value[1]	p-value[2]
MQ	96.92±14.46	111.73±11.06	<.0001***	101.89±14.99	112.71±14.80	<.0001***	0.061
K-AVLT: Trial 1	10.27±2.87	14.41±1.99	<.0001***	10.54±2.78	13.40±1.94	<.0001***	0.061
K-AVLT: Trial 2	9.95±3.43	13.68±2.47	<.0001***	9.74±3.08	12.51±3.46	<.0001***	0.241
K-AVLT: Trial 3	9.32±3.18	12.68±2.06	<.0001***	9.49±3.54	11.86±3.03	<.0001***	0.167
K-AVLT: Trial 4	9.70±3.55	12.32±2.43	<.0001***	10.09±3.25	12.00±2.46	0.0011**	0.356
K-AVLT: Trial 5	9.08±2.68	10.84±1.72	<.0001***	9.00±3.15	10.66±2.48	0.0003***	0.858
K-AVLT: Delayed recall	9.76±2.94	12.30±2.32	<.0001***	10.20±3.39	11.71±3.49	0.003**	0.098
K-AVLT: Delayed recognition	8.68±1.80	9.46±1.12	0.003**	8.89±2.07	9.09±1.80	0.445	0.104
K-CFT: Drawing	8.03±3.06	8.57±3.64	0.283	8.11±2.47	8.83±2.96	0.102	0.792
K-CFT: Immediate recall	9.57±2.72	10.65±2.79	0.005**	10.57±2.87	11.77±2.85	0.002**	0.814
K-CFT: Delayed recall	9.92±2.85	11.19±2.65	0.002**	10.91±2.54	12.57±3.09	<.0001***	0.449
Memory retention	61.5±26.82	76.44±22.69	0.004**	68.21±27.13	72.95±25.97	0.380	0.157
Retrieval efficiency	61.04±25.51	83.58±20.69	<.0001***	62.06±30.61	80.41±27.95	0.0012**	0.524
Drawing/memory consistency	59.88±23.37	71.21±26.31	0.013*	69.78±23.27	79.61±18.82	0.009**	0.792

Values are presented as Mean±SD, [1]: Analyzed by paired t-test, [2]: Analyzed by linear mixed model for repeated measure data, *p<0.05, **p<0.01, ***p<0.001, ELEJ: Ethanol extract from leaves of *Eriobotrya japonica*, MQ: Memory quotient, K-AVLT: Korean version of Rey-Kim auditory verbal learning test and K-CFT: Korean version of Rey-Kim complex figure test

Table 5: Comparisons of BCRS, PRMQ, STA and BDNF between the ELEJ group and placebo group after 12 week administration of ELEJ

	ELEJ (n = 37)			Placebo (n = 35)			
Parameters	Baseline	Endpoint	p-value[1]	Baseline	Endpoint	p-value[1]	p-value[2]
BCRS	10.59±8.34	7.00±7.38	0.028*	8.69±6.55	5.54±5.05	0.005**	0.813
PRMQ	29.67±6.56	28.73±6.35	0.268	31.00±8.03	28.26±5.66	0.015*	0.212
STAI X-1	39.32±7.49	38.03±6.89	0.227	38.31±7.12	37.66±8.59	0.683	0.736
STAI X-2	41.32±7.77	39.65±6.69	0.126	39.34±8.01	39.91±9.26	0.683	0.201
Total STAI	80.65±14.75	77.68±12.86	0.145	77.66±13.85	77.57±17.22	0.976	0.398
BDNF (pg mL^{-1})	25596.76±8481.76	27816.19±7825.60	0.058	25007.94±8087.15	24445.23±8043.60	0.604	0.080

Values are presented as Mean±SD, [1]: Analyzed by paired t-test, [2]: Analyzed by linear mixed model for repeated measure data, *p<0.05, **p<0.01, ELEJ: Ethanol extract from leaves of *Eriobotrya japonica*, BCRS: Brief cognitive rating scale, PRMQ: Prospective and retrospective memory questionnaire, STAI: State-trait anxiety inventory, STAI-X1: State anxiety scale of state-trait anxiety inventory, STAI-X2: Trait anxiety scale of State-trait anxiety inventory and BDNF: Brain-derived neurotrophic factor

(Table 4). The level of serum BDNF showed an increasing tendency in the ELEJ group (p = 0.058) without significant differences between the ELEJ and placebo group (p = 0.080). There were no significant differences in the changes of BCRS, PRMQ and STAI between the two groups (Table 5).

DISCUSSION

This study was designed to investigate the efficacy and safety of extract from leaves of *Eriobotrya japonica* Lindley (ELEJ) when used to improve cognitive function in healthy Korean adolescents. The K-MMSE was the primary outcome measure used for detecting changes of cognition and memory in this study. The total score of the K-MMSE and the score of a memory recall item showed significant improvements in the ELEJ group compared to the placebo group after the 12 week intervention. The scores of attention and calculation items showed an increasing trend in the ELEJ group compared to baseline. The MMSE has been used to detect cognitive impairment and to screen for cognitive disorders in

epidemiologic studies and to follow cognitive change in clinical trials (Folstein *et al*., 1975). It is a brief and simple method used to grade cognitive status and is known to have high correlations with other comprehensive standardized instruments such as the wechsler adult intelligence scale (Horton *et al*., 1987). The total score of the MMSE measures overall cognitive function and the score of a memory recall item measures verbal memory. There are three steps in memory function including registration, retention and retrieval. Registration refers to the acquisition of information, retention to the ability to store and consolidate information over time and retrieval to the ability to retrieve the stored information (Morris and Kopelman, 1986; Erickson, 1990). The 3-words recall item in the MMSE reflects the process of verbal memory retention. Therefore, this data suggests that the administration of ELEJ may induce the enhancement of verbal memory with general cognitive improvement. However, this suggestion has a few limitations. The embedded memory test in the MMSE is a brief delayed recall of 3 words without the assessment of long-term delayed recall or any recognition memory test, executive ability test, or spatial function assessment (Malloy *et al*., 1997). The 3-words recall item has been reported to be one of the best discriminators of dementia (Galasko *et al*., 1990) but it is problematic to use it to address delayed recall since the interval from registration to recall is too short. Therefore, the improvements in these scores was observed might not represent long-term memory retention or spatial memory. In addition, whereas MMSE is mainly intended to detect cognitive impairment and monitor response to therapeutic intervention in geriatric patients, the participants in this study were healthy young adolescents with mean age of 18 years and baseline total scores of 29. Therefore, the scale may not have been appropriate to reflect cognitive enhancement after intervention, because the baseline scores were already close to full marks.

There have been many studies focusing on cognition and memory function using animal and human models of dementia. The suggested pathogenesis of cognitive deficits in alzheimer's disease includes brain inflammation, mediated through cytokines and other secretory products of activated glial cells, on neurotransmission (nitric oxide, glutamate, acetylcholine), amyloidosis, proteolysis and oxidative stress (Gahtan and Overmier, 1999). Memory deficits and cognitive dysfunction are known to be associated with inflammatory processes, which cause abnormal protein aggregation and result in neurodegeneration (Rogers *et al*., 1996; Gahtan and Overmier, 1999). A rat model of dementia illustrates the impairment of learning and memory associated with altered brain oxidative stress (El-Sherbiny *et al*., 2003). Thus, oxidative stress and inflammatory process may play important roles in cognitive impairment. Several studies have reported anti-inflammatory (Banno *et al*., 2005; Cha *et al*., 2011a) and antioxidant effects of ELEJ (Hamada *et al*., 2004; Yokota *et al*., 2006; Rashed and Butnariu, 2014). Kim *et al*. (2011) demonstrated the neuroprotective effects of ELEJ in a mouse model, showing the ability of ELEJ to suppress oxidative stress in the brain. Therefore, protection against oxidative stress and cognitive deficits may be expected in humans and the present study suggests that cognitive and memory improvements result from the administration of ELEJ in humans.

The results of Rey-Kim memory testing suggested significant improvement in all items except the K-CFT: Drawing in the ELEJ group after the 12-week intervention, as well as significant improvement in the placebo group except for in the K-AVLT: Delayed recognition, K-CFT: Drawing and memory retention. However, there were no significant differences in the changes of these values after intervention between groups. Learning effects after repeated exposure to the same test might explain the results of the present study. However, the scores of MQ and K-AVLT: Trial 1 in the ELEJ group showed increasing tendencies compared to placebo ($p = 0.061$ and 0.061, respectively). The MQ is the most direct index reflecting memorization ability and the item of K-AVLT: Trial 1 to trial 5 indicates immediate memory that reflects acquisition of the memory process (Vakil and Blachstein, 1993). By repeating the trials, items from trial 5 indicate long-term memory function in the process of memory registration (Kim, 2001). The results of this study illustrate a trend of enhancing verbal memory function and overall memorization ability, which may demonstrate the cognitive and memory enhancing effects of ELEJ.

Other parameters such as BCRS, PRMQ, STAI and BNDF that were examined in this study did not significantly differ between the two groups. It could be concluded that ELEJ did not influence participants mood or change their anxiety levels, based on the results of the STAI. The BDNF levels were elevated in the ELEJ group after the 12 week administration of ELEJ with an increasing tendency compared to the placebo group. The BDNF has been considered to play an essential role in neuronal survival and differentiation, as well as synaptic plasticity relevant to learning and memory (McAllister *et al*., 1999; Yamada *et al*., 2002). The BDNF is thought to be involved in memory formation as well as consolidation via synaptic change (Bekinschtein *et al*., 2008). Serum BDNF levels were measured to investigate the mechanisms of ELEJ influencing cognitive function, but it was unable to verify that the administration of ELEJ brings about improvements in cognition and memory function induced by the neuronal plasticity of adult brains.

The safety profile of ELEJ was also investigated before and after administration. The biochemical markers and vital signs did not change significantly in either group, except for creatinine and creatinine kinase, which exhibited changes that remained within normal values. Forty cases of adverse events were reported, but were not related to ELEJ administration. No clinically significant adverse events occurred during the intervention period. These results suggest that ELEJ exerted no significant toxicity during the study period of 12 weeks.

CONCLUSION

Supplementation with ELEJ for 12 weeks improved cognition function, especially memory in healthy Korean adolescents according to K-MMSE scores. There were no changes in other neuropsychological assessment. The ELEJ caused no significant harmful events, suggesting its safety for use in humans. In conclusion, materials extracted from *Eriobotrya japonica* Lindley will be researched further as potential therapeutics that can induce beneficial effects on cognition through dietary intervention.

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