【Original Article】

Safety Assessment of Eucalyptus Leaf Extract Oral Consumption for 4 Weeks in Human Subjects: A Pilot Study

Keiichiro SUGIMOTO^{1,*}, Kazuya NAKAGAWA¹, Suguru FUJIWARA²,
Katsuhisa SAKANO², Shukuko EBIHARA³

¹Research and Development Center, Nagaoka Co., Ltd.

²CPCC Co., Ltd.

³Chiyoda Paramedical Care Clinic

【ABSTRACT】

Eucalyptus (*Eucalyptus globulus* Labill.) is an evergreen tree found worldwide. The aqueous ethanolic extract of the leaves (eucalyptus leaf extract; ELE) is used as a functional food, and its active constituents are generally polyphenols. Toxicity and mutagenicity of ELE have been previously assessed using rodents, and no adverse effects were observed. Although clinical trials of low-dose ELE ingestion have been conducted on humans, safety evaluation of high-dose ELE has not yet been conducted. We conducted an open-label clinical trial as a pilot study to assess the safety of excessive oral intake of ELE in Japanese adult men. A powdery preparation of ELE, commercially sold as Eucagrandin[®], was prepared using spray drying method and contained approximately 80% ELE. Test capsules were packed with the powdery ELE. Six male subjects (aged 47.3 ± 12.4 years,

mean ± SD) orally consumed 2,592 mg of ELE per day for 4 consecutive weeks. The subjects were examined at the start of the intervention, 4 weeks after the start, and 2 weeks after the last intake of test capsules. During the study period, 2 adverse events were reported. However, the causal relationship with the consumption of ELE was denied in both events because one event was attributed to a bruise and the other, which was elevated CPK levels, was due to excessive exercise. No unusual changes related to ELE consumption were observed in physical examination and during medical interviews. Further, blood and urine tests were normal during the trial period. These results demonstrated that oral consumption of ELE at the indicated dose is safe for humans.

【Key words】

Eucalyptus leaf extract, tannin, polyphenol, clinical study, tolerability, toxicity

INTRODUCTION

Eucalyptus globulus Labill. (Myrtaceae), commonly known as eucalyptus or Tasmanian blue gum, is an evergreen tree widely distributed around the world. Eucalyptus leaves are used to make herbal tea in Europe and as a traditional remedy for diabetes mellitus in South America and Africa ¹⁾. The leaves are rich in volatile components ²⁾ and polyphenols ³⁻⁷⁾. The essential oil, which is made by steam distillation, is used in food and cosmetics industries as a raw material for developing flavors and fragrances. It is also used in aromatherapy. The eucalyptus leaf extract (ELE) is non-volatile and its active components are usually polyphenols. It has recently been used as a functional food against oxidation ^{3,4)}, caries ⁸⁾, and skin aging ^{9,10)}. We have previously shown that ELE inhibits fructose absorption in the intestine and suppresses the rise in the levels of hepatic triacylglycerols and accumulation of visceral fat induced by excessive ingestion of dietary fructose in rats ¹¹⁾.

In Japan, eucalyptus leaf is available as a food material. It has been included in the “list of raw materials not deemed pharmaceuticals unless claiming medicinal efficacy” under the section “Borderline of Pharmaceuticals to Non-pharmaceuticals” in the Pharmaceutical Affairs Act (Notification No. 476 of the Pharmaceutical Affairs Bureau [1 June 1971]; Ministry of Health and Welfare). This classification indicates that eucalyptus leaf is considered both food and drug. According to National Institute of Health and Nutrition, Japan ¹²⁾, the amount of eucalyptus leaf contained in regular food is probably safe but overdose should be avoided, because there is no reliable information available regarding its safety and effectiveness. ELE was included as an antioxidant in the “List of Existing Food Additives” (Notification No. 120 [16 April 1996]; Ministry of Health and Welfare). A chewing gum containing ELE is available in the market for the prevention of dental caries as Food for Specified Health Uses (FOSHU), and it is certified by the Consumer Affairs Agency ¹³⁾. In addition, eucalyptus leaf is recognized as Old Dietary Ingredient in the Dietary Supplement Health and Education Act of 1994 in the United States ¹⁴⁾. World Health Organization ¹⁵⁾ and the European Medicines Agency ¹⁶⁾ have issued monographs on eucalyptus leaf and its essential oils.

The safety data on ELE have been reported by the Ministry of Health and Welfare, Japan ¹⁷⁾. They conducted a 90-day repeated dose toxicity study using F344 rats at the doses of 100, 300, and 1,000

mg/kg of body weight/day and did not observe any adverse effect. ELE was tested negative in the following genotoxicity tests: reverse mutation test using *Salmonella typhimurium* TA98, TA100, TA1535, TA1537, and *Escherichia coli* WP2uvrA/pKM101 with or without metabolic activation; chromosome aberration test using cultured Chinese hamster lung cells; and mouse bone-marrow micronucleus assay when ICR male mice were administered ELE at the dose of 1,000 mg/kg body weight, 2 times at 24 h intervals. Similar results were reported by the Consumer Affairs Agency of Japan ¹³⁾. We have also confirmed no adverse effects of our ELE preparation using rodents. The lethal dose 50 (LD₅₀) of our ELE was higher than 2,000 mg/kg body weight in Wistar male rats (8 weeks old; *n* = 10, unpublished data). Our ELE preparation had a negative test result in mouse bone-marrow micronucleus assay when ICR male and female mice (7 weeks old; *n* = 10, respectively) were administered with ELE at the dose of 1,000 mg/kg of body weight, 2 times (unpublished data). During a trial of ELE on humans, a single dose of 3 g was orally administered to 18 adult volunteers (12 men and 6 women), and no adverse effect was observed ¹⁸⁾.

Despite having several safety studies, the data on meal experience of eucalyptus leaves are insufficient compared to those of general food items. Faulds ¹⁹⁾ reported in 1902 that eucalyptus leaf, from which tea is made, was used as a traditional remedy for diabetes in New Zealand and India. This is apparently the first report on meal experience of eucalyptus leaf. Eating experience of this plant is considered to be only over 100 years.

Although none of the abovementioned studies have reported abnormal events, they do not constitute a systematic safety assessment of orally dosed ELE in humans. This paucity of information led us to conduct our own clinical study to evaluate the safety of continuous oral consumption of ELE. The dose of ELE is expected to be approximately up to 500 mg per day as a functional food according to our studies previously conducted (unpublished data). In this study, we conducted a safety assessment test with daily a dose of 2,592 mg of ELE for 4 consecutive weeks in Japanese male volunteers to evaluate the effects of excessive consumption (approximately 5 times of the dose as functional food).

MATERIALS AND METHODS

1. Preparation of ELE capsules

Dried eucalyptus leaves, which were harvested in Spain, were extracted using 33% (w/w) aqueous ethanol at 65°C for 2 h. The extract was filtered and evaporated *in vacuo*. After mixing the concentrate with dextrin, a spray dry process was used to obtain powdery ELE (Commercial name, Eucagrandin® P20; Nagaoka Co., Osaka, Japan). The powdery ELE contained approximately 80% ELE and the rest was dextrin. The nutritional components of the powdery ELE were as follows: moisture (normal pressure at 105°C for 3 h), 2.8%; ash (produced by direct ashing), 4.2%; protein (measured as leucine equivalents using Kjeldahl method), 1.6%; and lipid (measured using Röse-Gottlieb method), 2.9%. The content of 1,8-cineole in the powdery ELE was less than 0.1%, as 0.1% is the minimum detectable quantity by gas chromatography and gas chromatography-mass spectrometry²⁰⁾. The total polyphenol content of the powdery ELE was approximately 23%, as measured in terms of gallic acid (Wako Pure Chemical Industries Co., Osaka, Japan) equivalents by Folin-Ciocalteu's method²¹⁾.

To conduct this trial, the powdery ELE was packed in gelatin-made capsules (120 mg of the powdery ELE per capsule; thus, 96 mg of ELE per capsule) by Aliment Industry Co. (Yamanashi, Japan).

2. Subjects and exclusion criteria

The subjects were employees of Nagaoka Co. and were >20 years old. The following exclusion criteria were applied: (1) weak digestive system (for example, discomfort or stomach astigmatism after intake of drinks containing tannins such as strong green tea, black tea, or coffee); (2) severe anemia or diagnosis of severe anemia by the doctor; (3) previous and/or current medical history of serious diseases; (4) heavy use of alcohol (as defined by National Health and Nutrition Survey of Japan); (5) extremely erratic lifestyle and/or dietary habits; (6) allergy to any food or medical substance; (7) participation in another clinical trial within 4 weeks prior to the current study; (8) pregnancy, possible pregnancy, or ongoing lactation; and (9) declaration by the doctor in charge of the study as inadequate for reasons other than above.

Eligible subjects in the study included people who were fully informed about the contents and methods of the study and anticipated adverse symptoms. Six Japanese male adults aged 30–68 (mean age, 47.3 ± 12.4) years were enrolled; they provided written

informed consent. Their mean height was 170.9 ± 6.9 cm.

3. Study design

This open-labeled trial was conducted in accordance with study protocols approved by an independent ethics committee at the study site (Chiyoda Paramedical Care Clinic, Tokyo, Japan) on April 20, 2017. The trial was supervised by doctors, and the principles of the Helsinki Declaration and the Good Clinical Practice Guidelines were followed.

The subjects consumed 9 capsules/meal three times a day along with a meal with a glass of plain water for 4 consecutive weeks. The subjects were examined at the start of the intervention (Week 0), 4 weeks after the start of intervention (Week 4), and 2 weeks after the last intake of capsules (post-week 2) as a post-intake observation.

All the subjects were given the following instructions: (1) Do not alter lifestyle habits and abstain from excessive eating, drinking, or exercise during the study period; (2) Inform the clinic staff and obtain prior permission from the principal doctor of this trial to use any medication, except in emergency situations; (3) Note down their food intake and amount of exercise in a diary for three consecutive days before each examination so that their nutritional (energy, proteins, lipids, and carbohydrates) and alcohol intake can be calculated; (4) Do not drink alcohol on the day before visiting the clinic; and (5) Have dinner by 21:00 on the evening before examination and visit the clinic on the examination day without having breakfast. In addition, the study subjects were requested to inform immediately the clinical staff on occurrence or feeling of any adverse events as soon as possible, even if it was tolerable. The study team noted the number of instances on which the subjects missed the dose by counting the number of capsules returned by the subjects. A subject was considered compliant if over 90% of the capsules were consumed according to the prescribed regimen.

The trials were conducted in Japan from April 2017 to June 2017. All subjects completed the study without any severe adverse event.

4. Measurement of physical and biological parameters

At each visit, the subjects went through physician examinations, physical tests, hematological and blood biochemical tests, and urine test to assess their safety status. These tests were performed at Chiyoda Paramedical Care Clinic. Height, body weight, and

body fat were measured by biochemical impedance analysis using a body composition analyzer Lookin' Body 3.2 (InBody Japan Inc., Tokyo, Japan). Blood pressure and pulse rate were measured using an automatic blood pressure monitor TM-2655P (A&D Co., Tokyo, Japan). Hematological and blood biochemical parameters were measured at the LSI Medience Co. (Tokyo, Japan). The following blood parameters were recorded: white blood cells (WBC), red blood cells (RBC), hemoglobin (Hb), hematocrit (Ht), platelets (PLT), total proteins (TP), albumin (ALB), aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), total bilirubin (T-BIL), alkaline phosphatase (ALP), γ -glutamyl transpeptidase (γ -GTP), creatine phosphokinase (CPK), urea nitrogen (BUN), creatinine (Cre), uric acid (UA), electrolytes (Na, Cl, K, and Ca), total cholesterol (T-Chol), high-density lipoprotein cholesterol (HDL-Chol), low-density lipoprotein cholesterol (LDL-Chol), triacylglycerols (TG), and glucose (GLC). Urinary protein, sugar, urobilinogen, and bilirubin were also evaluated.

5. Safety assessments

Adverse event was defined as any untoward medical occurrence in a subject that occurred after the administration of the test capsules regardless of a causal relationship with the treatment. Biological parameter values outside of the Japanese normal range were defined as abnormal, and the possibility that the test capsule ingestion had caused the change was assessed. Physical parameters were also used for safety assessment.

6. Statistical analyses

The values associated with evaluated parameters were expressed as mean $\pm$ standard deviation. Comparisons between week 0, week 4, and post-week 2 were conducted by the Dunnett's test. The Wilcoxon signed-rank test with Bonferroni correlation was used to compare urinalysis parameters at each examination point with those at week 0. Statistical analyses were conducted using SPSS 20.0 (SPSS Japan Inc., Tokyo, Japan) and Microsoft Excel (Microsoft Japan, Tokyo), with the significance level set at $<5\%$ in two-sided testing.

RESULTS

1. Consumption of test capsules

The mean compliance rates for the consumption of

test capsules by the subjects were $99.8 \pm 0.5\%$ (days) and $99.8 \pm 0.5\%$ (number of test capsules). All data were analyzed because no subject dropped out of the trial.

2. Adverse events

No serious adverse events were observed. Overall, two adverse events were reported during the trial period; thus, the incidence of adverse events was 33%. However, these events were determined by the principal investigator to be independent of test capsule intake for the following reasons. Both subjects were healthy persons who had no significant medical history at the start of the trial. One event was attributed to a bruise while riding a bicycle at 15 days after the start of intake. The subject consulted a doctor and took medication, following which healing was confirmed on day 38. The other event in which subject's CPK level elevated from 379 U/L at the start of the intervention to 752 U/L at week 4 and decreased to 252 U/L at post-week 2 was attributed to muscle pain due to excessive exercise.

3. Physical parameters and nutritional intake

The measurements of physical parameters are shown in Table 1. The data measured at the start of the intervention and 2 weeks after the last intake of test capsules are shown in the "Baseline" and "Post-week 2" columns. No significant difference was observed in all parameters during the trial period. The total consumption of energy derived from meals and alcohol recorded for 3 consecutive days before each examination showed no significant differences among all three examination days (data not shown).

4. Hematology, blood biochemistry, and urinalysis

The measurements of hematology and blood biochemistry parameters are shown in Tables 2 and 3. No significant changes were noted in these parameters. When individual changes in these parameters were examined, some parameters randomly showed transient values outside the reference range. However, all these variations were considered to be mild and not medically significant. The results of the urine tests are shown in Table 4. The protein grading changed from negative to positive (1+) in one subject at post-week 2. However, this change was not considered to be a significant adverse event because it was mild and transient. No significant changes were noted in other parameters of urinalysis.

Table 1 Changes in physical parameters during the study

Items	Unit	Baseline	Week 4	Post-week 2
Body weight	kg	70.3 ± 14.7	70.3 ± 14.8	69.9 ± 14.8
Body mass index	kg/m ²	24.0 ± 3.9	23.9 ± 3.9	23.8 ± 3.9
Body fat	%	24.7 ± 6.7	24.5 ± 6.6	24.5 ± 6.2
Systolic blood pressure	mmHg	115.8 ± 9.5	110.5 ± 14.9	109.3 ± 12.1
Diastolic blood pressure	mmHg	75.5 ± 10.7	72.0 ± 9.6	70.7 ± 4.2
Pulse rate	bpm	66.3 ± 5.1	63.8 ± 9.7	66.0 ± 9.1

Data are expressed as the mean ± standard deviation ($n = 6$). No significant changes were noted in the results calculated by the Dunnet's test.

Table 2 Changes in hematological parameters during the study

Items	Unit	Baseline	Week 4	Post-week 2
WBC	/μL	4750 ± 1048	4433 ± 799	4550 ± 740
RBC	10 ⁴ /μL	492 ± 44	486 ± 41	480 ± 44
Hb	g/dL	15.4 ± 0.9	15.1 ± 0.6	14.8 ± 0.8
Ht	%	47.1 ± 2.8	47.2 ± 2.6	46.6 ± 2.3
PLT	10 ⁴ /μL	23.6 ± 4.8	23.6 ± 4.9	25.1 ± 5.3

Data are expressed as the mean ± standard deviation ($n = 6$). No significant changes were noted in the results calculated by the Dunnet's test.

Table 3 Changes in biochemical parameters during the study

Items	Unit	Baseline	Week 2	Post-week 2
TP	g/dL	7.4 ± 0.3	7.2 ± 0.2	7.4 ± 0.4
ALB	g/dL	4.6 ± 0.5	4.6 ± 0.2	4.6 ± 0.3
AST (GOT)	U/L	24.7 ± 8.8	25.3 ± 10.8	25.8 ± 7.1
ALT (GPT)	U/L	30.5 ± 8.1	27.7 ± 10.6	30.2 ± 6.7
LDH (LD)	U/L	176 ± 34	182 ± 41	172 ± 37
T-BIL	mg/dL	0.80 ± 0.28	0.93 ± 0.19	0.72 ± 0.19
ALP	U/L	206 ± 37	197 ± 33	204 ± 39
γ-GTP (γ-GT)	U/L	57.0 ± 28.1	51.0 ± 23.4	56.5 ± 24.5
CPK (CK)	U/L	149 ± 116	226 ± 263	133 ± 72
BUN (UN)	mg/dL	12.4 ± 3.0	12.8 ± 2.7	11.5 ± 3.0
Cre	mg/dL	0.79 ± 0.08	0.80 ± 0.09	0.78 ± 0.10
UA	mg/dL	5.1 ± 1.7	4.9 ± 1.8	4.7 ± 1.7
Na	mEq/L	141 ± 1	141 ± 2	142 ± 1
Cl	mEq/L	105 ± 2	104 ± 2	105 ± 1
K	mEq/L	4.2 ± 0.1	4.2 ± 0.2	4.3 ± 0.1
Ca	mg/dL	9.5 ± 0.3	9.4 ± 0.3	9.4 ± 0.2
T-Cho	mg/dL	204 ± 17	205 ± 30	202 ± 17
LDL-Cho	mg/dL	118 ± 13	119 ± 18	119 ± 15
HDL-Cho	mg/dL	64 ± 23	63 ± 22	60 ± 20
TG	mg/dL	96 ± 27	108 ± 47	102 ± 27
GLC (GLU)	mg/dL	88.8 ± 7.9	91.0 ± 5.4	90.2 ± 7.3

Data are expressed as the mean ± standard deviation ($n = 6$). No significant changes were noted in the results calculated by the Dunnet's test.

Table 4 Parameters of the urine test during the study

Item	Grading	Baseline	Week 4	Post-week 2
Protein	(-)	6	6	5
	(+-)	0	0	1
	(1+)	0	0	0
Sugar	(-)	6	6	6
	(+-)	0	0	0
	(1+)	0	0	0
Urobilinogen	(-)	0	0	0
	(+-)	6	6	6
	(1+)	0	0	0
Bilirubin	(-)	6	6	6
	(+-)	0	0	0
	(1+)	0	0	0

Data are expressed as the number of cases. No significant changes were noted in the results calculated by the Wilcoxon signed-rank test with Bonferroni correlation.

DISCUSSION

In this trial, six Japanese adult males orally consumed the test capsules made up of powdery ELE at 3,240 mg/day (2,592 mg/day as ELE) for 4 weeks. Since all subjects showed consumption rates of 90%, they were all considered compliant, and statistical analyses were performed on all of them.

Based on our physician's evaluation of adverse events, physical examinations (Table 1), and clinical tests (Table 2–4), we confirmed that oral intake of ELE is safe.

During the study period, 2 adverse events were reported, and the occurrence rate was 33%. The subject presented with elevated CPK at week 4; however, it was considered to be owing to excessive exercise. Both incidents occurred accidentally and independently of the consumption of test capsules. In both events, the cause was identified, and the causal relationship with the consumption of powdery ELE was denied. No subjects showed abnormalities at the time of medical examinations. The physical, hematological, and blood biochemical parameters, as well as urinalyses, did not change significantly; thus, there was no adverse effect related to powdery ELE consumption.

The American Herbal Products Association (AHPA) classifies eucalyptus leaf safety as class 1 (herbs that can be safely consumed when used appropriately)²²⁾. However, it is known as toxic and Koala has a unique metabolic system for detoxification^{23,24)}. The leaves contain 1%–3% of volatile components, mainly 1,8-cineole²⁾. The

essential oil of eucalyptus does not have any effect on health when consumed in minute amounts as a flavor. However, the volatile components are considered toxic, because some adverse events due to accidental consumption in excess have been reported²⁵⁾. Especially, eucalyptus essential oil is contraindicated for children^{22,25)}. The safety of the consumption of the volatile components should be attended in this study, the powdery ELE contains trace amounts of volatile constituents (> 0.1% as 1,8-cineole), and therefore, there is no effect of volatile components.

AHPA alerts that products containing more than 10% tannin may have adverse effects due to overdose of tannin²²⁾. Eucalyptus leaf was on the list of herbs containing more than 10% tannins along with leaf and stem of the tea plant (*Camellia sinensis* (L.) Kuntze). Green tea, made from this plant without fermentation, is commonly consumed in Japan. The eucalyptus leaves are rich in polyphenols including hydrolysable tannins^{4–7)}, flavonoid glycosides^{4,5,7)}, and triterpene-phloroglucinol complexes^{3,4)}, and these can be regarded as eucalyptus-derived tannins²⁶⁾. Since the subjects ingested 2,592 mg of ELE per day, the polyphenol content consumed by the subjects was 596 mg/day. The powdery ELE showed no adverse effects at this dose in this trial. In addition, we reported that no adverse events were observed in 18 adult volunteers when they consumed 3 g of ELE in a single-dose study¹⁸⁾. However, large intake of tannic acid can cause vomiting by irritating the mucous membranes of

the gastrointestinal tract²⁷⁾. Eucalyptus leaf contains gallotannins^{4,7)}, which has constituents similar to tannic acid²⁸⁾. Therefore, we think that people with weak gastrointestinal tracts should avoid ingesting high amounts of ELE.

This trial demonstrated that the consumption of powdery ELE for 4 weeks at relatively high dose (3,240 mg/day, of which ELE is 2,592 mg/day) is safe for humans. This is the first report showing that ELE does not have any adverse effects at a high dose. However, this was a pilot study, and a major limitation of the study is that only men participated as subjects. In addition, the sample size was only six subjects. Small sample size affords poor statistical discrimination power. Further evaluation is necessary to confirm the safety of oral ELE intake with a larger sample size and with subjects including both men and women.

CONCLUSION

These results show that the consumption of the powdery ELE is safe and well-tolerated at a dose of 3,240 mg/day (2,592 mg/day of ELE) for 4 weeks in Japanese adult men. The present study is the first to systematically assess the safety of oral ELE intake at a high dose.

CONFLICT OF INTERESTS

This work was financially supported by Nagaoka Co., Ltd. S. Fujiwara, K. Sakano, and S. Ebihara were involved in data collection, analysis, and interpretation of results. K. Sugimoto and K. Nakagawa are employees of Nagaoka Co., Ltd and were not involved in data handling in any way. All six subjects were employees of Nagaoka Co., Ltd.

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要 旨

ヒトを対象としたユーカリ葉抽出物の 4 週間経口摂取における安全性の評価—予備試験

杉本圭一郎¹, 中川一弥¹, 藤原 優², 坂野克久², 海老原淑子³

¹長岡香料株式会社 技術開発研究所

²CPCC 株式会社

³チヨダパラメディカルケアクリニック

ユーカリ (*Eucalyptus globulus*) 葉水性エタノール抽出物 (ELE) は機能性食品として利用され、活性成分はポリフェノールであることが多い。ELE は、すでにげっ歯類を用いた 90 日間反復投与毒性確認試験および変異原性試験が評価された。臨床試験は、低用量は実施されたが高用量の報告がない。我々は予備試験として日本人成人男性を対象に、ELE の過剰摂取安全性評価オープン試験を実施した。ELE を 80% 含むスプレードライ粉末 (市販名: ユーカグランジン®) カプセルを試験食とした。6 名 (47.3 ± 12.4 歳) を対象に 4 週間、ELE を毎日 2,592 mg 摂取させた。試験開始時、4 週間後、摂取終了より 2 週間後に検査を実施した。試験期間中 2 件の有害事象が報告されたが、それらは ELE 摂取と関連性がないと判断された。1 件は打撲に起因し、他の 1 件は過剰な運動が原因で CPK 値が上昇したと結論付けられた。その他の項目では、ELE 摂取に関連する変化は認められなかった。以上、ヒトを対象に ELE の安全性を確認することができた。

キーワード: ユーカリ葉抽出物, タンニン, ポリフェノール, 臨床試験, 忍容性, 毒性