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Safety assessment of 4-week oral intake of proanthocyanidin-rich grape seed extract in healthy subjects

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ABSTRACT

A clinical study was conducted to assess the safety and tolerability of oral doses of proanthocyanidin-rich grape seed extract (GSE) in healthy Japanese adult volunteers. In an open-label, 4-week toxicity test, 29 subjects daily received 1000, 1500, or 2500 mg GSE orally. Serum Fe levels of two subjects in the 2500 mg GSE group decreased to 61 and 60 μg/100 mL from 205 and 182 μg/100 mL at baseline respectively, at second week of GSE consumption; these values are low but within the normal range for the Japanese population. Two weeks after completing the 4-week course of GSE ingestion, the serum Fe levels of both subjects returned to near baseline levels (210 and 189 μg/100 mL). No subject discontinued the study. Oral intake of GSE up to 2500 mg for 4 weeks was found to be generally safe and well tolerated in humans. Research with a larger number of subjects is required to confirm these findings.

1. Introduction

Proanthocyanidins (PAs) are a class of polyphenols that naturally occur as oligomers or polymers of polyhydroxy flavan-3-ol units, such as (+)-catechin and (−)-epicatechin (Porter, 1988). They are typically present in red wine, fruits, vegetables, nuts, seeds, and barks. As natural antioxidants, PAs have been reported to exhibit various *in vitro* and *in vivo* biological effects. Grape seeds are a particularly rich source of PA, consisting almost solely of the procyanidin type partly esterified by gallic acid (Fuleki and Ricardo da Silva, 1997; Santos-Buelga et al., 1995). For this reason, PA derived from grape seeds is chemically different from that derived from other sources, such as pine bark (Pycnogenol®), which contains highly polymerized PAs without gallic acid esters, or lychee fruit (Oligonol®), which contains less polymerized PAs. Proanthocyanidin-rich extract prepared from grape seeds has potent antioxidant activity (Ricardo da Silva et al., 1991), and has been shown to have pharmacological effects in animal models, including attenuation of atherosclerosis (Yamakoshi et al., 1999), improvement of chloasma (Yamakoshi et al., 2004), and prevention of osteoarthritis (Aini et al., 2012). As these effects are considered to be beneficial to human health, PA-rich grape seed extract (GSE) has appeared on the market as a nutritional supplement in many countries, including the United States, European Union nations, Australia, China, and Japan. In Japan, it is also used as an additive in various food applications. Although safety assessments of GSE have been conducted using animal models (Ray et al., 2001; Yamakoshi et al., 2002), studies in human subjects are limited and mostly consist of incident reports in effective intervention trials.

The lethal dose 50 (LD_{50}) of GSE is higher than 5000 mg/kg in rats (Lluís et al., 2011). The no-observed-adverse-effect level (NOAEL) of GSE in a subchronic toxicity study in rats was 1410 mg/(kg body weight • day) in males and 1501 mg/(kg body weight • day) in females (Yamakoshi et al., 2002). Clinical studies have shown that GSE has antioxidant activity against malondialdehyde-modified low-density lipoprotein (555 mg/day for 12 weeks; Sano et al., 2007), and therapeutic and protective effects in hypertension (1000 mg/day for 6 weeks; Ward et al., 2004) and leg swelling (150 mg/day for 2 weeks; Sano et al., 2013). Although no abnormal events were reported in these studies, the existing studies do not constitute a systematic safety assessment of orally dosed GSE in humans.

Abbreviations: AE, adverse event; A/G ratio, albumin-globulin ratio; ALT, alanine aminotransferase; ALP, alkaline phosphatase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; CPK, creatinine phosphokinase; γ-GTP, gamma-glutamyl transpeptidase; GSE, grape seed extract; Hb, hemoglobin; HbA1c, hemoglobin A1c; HDL, high-density lipoprotein; Ht, hematocrit; LDH, lactate dehydrogenase; LDL, low-density lipoprotein; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; PA, proanthocyanidin; Plt, blood platelet count; RBC, red blood cells; UA, uric acid; WBC, white blood cells.

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This paucity of information led us to conduct our own clinical study to evaluate the safety and tolerability of continuous intake of oral GSE. We conducted a 4-week toxicity test with daily doses of 1000, 1500, and 2500 mg proanthocyanidin-rich GSE in healthy Japanese volunteers.

2. Material and methods

2.1. Grape seed extract (GSE) preparation and administration

GSE (Gravinol-SY™, Kikkoman Co., Japan) was prepared from grape seeds (*Vitis vinifera* L.) and its PA content was measured as described previously (Yamakoshi et al., 2002). Briefly, grape seeds were washed with water at 60 °C for 2 h and then extracted with hydrous ethanol at 90 °C for 2 h. The extract solvent was separated from the grape seeds and a spray dry process was applied to obtain a powdery, PA-rich GSE. The GSE used in this study was composed of (all percentages dry weight to dry weight) 80% total PAs, including 6.9% dimers, 5.2% trimers, 3.0% tetramers, 64.9% oligomers and polymers larger than pentamer, 12.0% monomeric flavonols [5.4% (+)-catechin, 6.4% (−)-epicatechin, 0.1% (−)-epicatechin gallate, and 0.05% (−)-catechin gallate], 0.05% gallic acid, 6.1% carbohydrate, 1.1% protein, and 0.7% ash. Tablets were prepared containing 100 ± 5 mg PA derived from the previously described GSE. Our previous stability tests determined that the quality of the PAs in the tablets did not change over a period of three years; therefore, the tablets have a three-year shelf life.

Subjects ingested tablets daily before breakfast in accordance with instructions printed on the tablet sachet, which listed the date of administration. If the subject forgot to take the tablets or did not eat breakfast, they were advised to take the tablets before lunch or supper on the same day. If the subject took too many tablets, they decreased the number of tablets they took the next day accordingly. If the subject forgot to take the tablets for an entire day, they resumed taking the usual dose the following day without adjustment. The participants' progress in taking the tablets was monitored weekly.

2.2. Study design

This open-label study was sequentially conducted in accordance with study protocols approved by independent ethics committees at the study sites (Yahata Clinic, Tokyo and Akatsuka Ekimae Clinic, Tokyo). Written informed consent was obtained from all participants.

People with any health problems were excluded from the study (Table 1). Thirty healthy Japanese male and female volunteers aged 20–64 years signed the consent document and were enrolled in the study.

A total population of 30 male and female subjects were randomized into three groups (n = 10 in each group). The groups received GSE tablets at a daily dose of 1000, 1500, or 2500 mg for 4 consecutive weeks. These dosages correspond to 800, 1200, and 2000 mg PA, respectively. All subjects were followed for 2 weeks after the 4-week food intervention period. Since serious adverse events (AEs) were not reported in previous human studies on GSE (Sano et al., 2007; Ward et al., 2004; Yamakoshi et al., 2004), changes of parameters only in the GSE ingestion groups are evaluated for over months.

2.3. Measurement of physical and biological parameters in study subjects

The study subjects were examined at the start of the intervention (Week 0), 2 and 4 weeks after the start of intervention (Weeks 2 and 4, respectively), and 2 weeks after the last intake of GSE tablets (Post-week 2). At each of these visits, physical parameters such as body weight, blood pressure, and pulse rate were measured and clinical symptoms and signs were examined by physicians. Fasting venous blood samples in the morning were also collected in Weeks 0, 2 and 4, as well as in Post-week 2. Within 2 h of collection, the blood samples were centrifuged at the study sites. The serum samples were analyzed at Shin-Akasaka Medical Laboratories, Tokyo. Table 2 lists tests that were performed.

2.4. Safety assessments

Adverse events are defined as any untoward or unexpected medical occurrence in a participant; they do not necessarily have a causal relationship with GSE tablet ingestion. All AEs that occurred during or following the intervention were recorded and documented according to their severity, time of onset and duration, and the investigator's assessment of their relationship to the GSE intervention. Events involving adverse food reactions or illness occurring during the study were also recorded. All AEs were followed up, even after GSE intervention was discontinued, until their sequelae had resolved or been stabilized. Investigators (in-charge physicians) were also requested to immediately report any serious AEs occurring during the study period, regardless of whether a relationship to the GSE was suspected.

Biological parameter values outside of the Japanese normal range were defined as abnormal, and the possibility that GSE tablet ingestion had caused the change was assessed. Physical parameters (body weight, blood pressure, and pulse rate) were also used for safety assessment.

Table 1
Exclusion criteria.

Acute or chronic infection
Clinically significant allergies to medicine or food
Diabetes mellitus
Familial hyperlipidemia
Hepatic, cardiorespiratory, or renal dysfunction
History of serious illness
Intake of antibiotics
Intake of antihypertensive or antihyperlipoproteinemic medication, and/or other medicine or food that may potentially affect lipid metabolism
Intake of antioxidative food, including red wine
Participation in another clinical study
Physical abnormalities or abnormal laboratory test results
Pregnancy or lactation
Any other health problem that an in-charge medical doctor believes would make participation in the study inappropriate for a given subject