Food and Chemical Toxicology 49 (2011) 2624–2630

Contents lists available at ScienceDirect

Food and Chemical Toxicology

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

A subacute toxicity evaluation of green tea (*Camellia sinensis*) extract in mice

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ARTICLE INFO

Article history:
Received 16 November 2010
Accepted 7 July 2011
Available online 13 July 2011

Keywords:
Catechins
Green tea extract
Mice
Subacute toxicity

ABSTRACT

Green tea is believed to be beneficial to health because it possesses antioxidant, antiviral and anticancer properties. The potential toxicity of green tea when administered at high doses via concentrated extracts, however, has not been completely investigated. The objective of the present study was to evaluate the safety of green tea extract in ICR mice using a subacute exposure paradigm. In this study, mice were orally administered (gavage) green tea extract at doses of 0 (as normal group), 625, 1250 and 2500 mg/kg body weight/day for 28 days. The results showed that oral administration of green tea extract did not cause adverse effects on body weight, organ weights, hematology, serum biochemistry, urinalysis or histopathology. Additionally, administering green tea extract via gavage significantly reduced triglyceride and cholesterol levels. These observed effects could be attributed to the high levels of catechins present in green tea as these compounds have been reported to have beneficial health effects. The no-observed-adverse-effect level for green tea extract derived from the results of the present study was 2500 mg/kg body weight/day.

1. Introduction

Green tea (*Camellia sinensis*, Theaceae) is one of the most popular beverages in the world and is deeply rooted in the cultures of China and Japan. Due to the widespread consumption of green tea, the potential biological effects have been studied both *in vitro* and *in vivo*. Most of the beneficial effects of green tea are attributed to the presence of polyphenols. These polyphenols are mainly comprised of catechins and catechin derivatives, including (−)-epigallocatechin-3-gallate (EGCG), (−)-epicatechin (EC), (−)-epigallocatechin (EGC), (−)-epicatechin gallate (ECG) and (−)-gallocatechin gallate (GCG) (Wang et al., 2003). Green tea catechins have been the subject of a considerable amount of research as they are believed to have beneficial effects on health due to their antioxidant (Higdon and Frei, 2003), antifungal, antibacterial (Friedman et al., 2006) and anticancer properties (Bode and Dong, 2009). Green tea catechins have also been shown to protect against 2-nitropropane-induced hepatotoxicity and cisplatin-induced nephrotoxicity in mice (Khan et al., 2009a; Sai et al., 1998).

In particular, EGCG has been the focus of research in recent years due to its relatively high levels in green tea and higher antioxidant activity. Indeed, a considerable body of literature has shown that EGCG arrests the progression of hepatic fibrosis (Zhen et al., 2007) and prevents carbon tetrachloride (CCl_4)-induced liver injury in animal models by inhibiting oxidative damage (Chen et al., 2004). EGCG has also been shown to inhibit lipopolysaccharide-induced tumor necrosis factor-α and inducible nitric oxide synthase production in mice (Yang et al., 1998; Lin and Lin, 1997). Although EGCG is the most plentiful of the green tea catechins and exhibits a high level of antioxidant activity, preventive effects appear to be stronger when a mixture of tea catechins, such as polyphenon E, a decaffeinated green tea catechin mixture, or a green tea extract, are administered (Bode and Dong, 2009; Fu et al., 2009).

A recent study has suggested that green tea extract is safe as a dietary supplement and has many properties that are beneficial for human health (Frank et al., 2009). However, laboratory studies of

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; ANOVA, analysis of variance; AST, aspartate aminotransferase; BUN, blood urea nitrogen; C, (+)-catechin; CCl_4, carbon tetrachloride; EC, (−)-epicatechin; ECG, (−)-epicatechin gallate; EGC, (−)-epigallocatechin; EGCG, (−)-epigallocatechin gallate; GA, gallic acid; GC, (+)-gallocatechin; GCG, (−)-gallocatechin gallate; Hb, hemoglobin; Ht, hematocrit; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; MPV, mean platelet volume; OECD, Organization for Economic Cooperation and Development; RBC, red blood cell; SD, standard deviation; WBC, white blood cell.

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doi:10.1016/j.fct.2011.07.007

green tea-derived preparations such as Teavigo, a commercially available green tea polyphenol preparation containing greater than 90% EGCG and isolating from the initial hot water extract with ethyl acetate and subjected to chromatographic separation of EGCG followed by spray drying, in rodents have revealed toxic effects when high doses (2000 mg/kg) were administered intragastrically (*i.g.*) (Isbrucker et al., 2006a). Additionally, *in vitro* studies reported that administration of rat hepatocytes with high concentrations of EGCG resulted in reduced cell viability (Schmidt et al., 2005; Galati et al., 2006). *In vivo* studies also suggest that administration with a single dose of 1500 mg/kg, *i.g.* EGCG in mice may result in hepatotoxicity (Lambert et al., 2010). Due to numerous interactions and synergisms, it is difficult to study the effects of natural dietary supplements on human health when administered in complex mixtures as opposed to a purified compound (Vitaglione et al., 2004). Thus, a conscientious and careful safety evaluation of green tea extract is necessary. The aim of the present study was to evaluate the safety of green tea extract using a subacute toxicity study design. Female and male ICR mice were administered green tea extract orally at doses of 625, 1250 or 2500 mg/kg/day for 28 consecutive days. Clinical observations, including survival, urinalysis, hematology and serum biochemistry, were measured to monitor treatment-related adverse effects in mice. The extent of treatment-related changes in organ tissues was assessed with histopathology.

2. Materials and methods

2.1. Materials and dosing

Gallic acid (GA), (+)-gallocatechin (GC), (−)-epigallocatechin (EGC), (+)-catechin (C), (−)-epigallocatechin gallate (EGCG), (−)-epicatechin (EC), (−)-gallocatechin gallate (GCG) and (−)-epicatechin gallate (ECG) standards were purchased from Sigma Chemical Company (St. Louis, MO, USA) and the purity of all standards are greater than 95%. Orthophosphoric acid and methanol (MeOH) were analytical grade and purchased from Merck (Darmstadt, Germany). Deionized water was prepared using a Mill-RQ and Milli Q-UV water purification system (Millipore Co. Ltd., Taipei City, Taiwan).

Aqueous green tea extract made from natural tea leaves (*C. sinensis*) was obtained from AGV Co. Ltd., Chiayi City, Taiwan. According to the manufacturer's information, green tea extract was prepared by adding tea leaves 5 g to 500 mL of boiling water, steeped for 30 min. The extraction solution was cooled to room temperature and then filtered. The tea leaves were extracted a second time with 500 mL of boiling water and filtered, and the two extraction solution were combined to obtain the green tea extract solution. The green tea extracts solution preparation currently used in fresh green tea drinks in Taiwan and similar to tea brews consumed by humans. In accordance with the company-provided general analysis, the green tea extract was comprised of 70.05% water, 0.84% protein, 0.36% lipid, 28.26% carbohydrate and 0.49% ash.

The dosages selecting in this study were in accordance with the Guidelines of Health Food Safety Assessment set forth by the Health Food Control Act (Department of Health of the Executive Yuan of the Republic of China, 1999). These regulations conform to the OECD Guidelines for Testing of Chemicals, Section 407 (1995). Generally, at least three test groups and a control group should be used. The high dose was selected with the expectation that it would induce observable toxicity but not death or severe suffering. Thereafter, the moderate and low doses were selected to elucidate dose response effects. Two- to fourfold intervals are frequently optimal for setting the descending dose levels. According to the rationale and desirable green tea intake from previous studies, the highest dose level was 2500 mg/kg body weight/day and a descending sequence of dose levels should be selected at 1250 and 625 mg/kg body weight/day. The dose volume for all treatment groups was 1 mL/100 g body weight. The commercial extract was stored at 4 °C and dosing solutions were freshly prepared with distilled water prior to administration. Dosing solutions were prepared based on the most recently recorded body weights to provide an accurate dosage.

2.2. Animals

Male and female ICR mice (20 ± 2 g; 5 weeks old) were obtained from the Animal Department of BioLASCO Taiwan Co. Ltd., Taipei City, Taiwan. Animals were quarantined and allowed to acclimate for 1 week prior to beginning experimentation. Animals were separated by sex and housed 3–4 per cage under standard laboratory conditions with a 12 h light/dark cycle. The animal room temperature was maintained at 25 ± 2 °C with a relative humidity of 55 ± 5%. Air handling units in the animal rooms were set to provide approximately 12 fresh air changes per hour. A standard rodent diet (Rodent LabDiet 5001; PMI Nutrition International, LLC, Richmond, IN, USA) was used for these studies. Appropriate analyses for the constituents and nutrients were performed by the manufacturer and provided to Laboratory Animal Center, Chung Shan Medical University (Taichung City, Taiwan). Food and water were provided *ad libitum*. The experimental protocols for this study were approved by the Institutional Animal Care and Use Committee and the animals were cared for in accordance with the institutional ethical guidelines.

2.3. Experimental design

Animals were randomly divided into four groups consisting of 10 mice of each gender. Group I animals (control) were administered distilled water by gavage throughout the course of the study. Animals in Groups II (625 mg/kg body weight/day), III (1250 mg/kg body weight/day) and IV (2500 mg/kg body weight/day) were orally administered green tea extract dissolved in deionized water by gastric intubation for a period of 28 days. Urinalyses were conducted during the last 4 days of the administration period. Each group animals were collected urine for 24 h and the volume of urine was measured. Animals were individually placed in metabolic cages in batches for a period of 24 h and provided with water but not food. The animals were fasted only in metabolic cages for a period of 24 h. Food and water were provided *ad libitum* during the other 3 days of other animal groups sampling. At the end of the experiment, animals were anesthetized with phenobarbital sodium (6.0 mg/100 g body weight, intraperitoneal injection) and then cut open for blood sampling from the abdominal aorta. After animals had been cut open and the blood was already withdrawn, animals were put in a CO_2 box for euthanasia. This study was in accordance with the Guidelines of Health Food Safety Assessment set forth by the Health Food Control Act (Department of Health of the Executive Yuan of the Republic of China, 1999). These regulations conform to the OECD Guidelines for Testing of Chemicals, Section 407 (1995).

2.4. Clinical observations and survival

Animals were observed twice daily (morning and afternoon) for signs of clinical toxicity and mortality. Body weights were recorded weekly throughout the study period. Mean daily food consumption was calculated twice a week by subtracting the weight of the remaining food from the weight of the supplied food. Clinical examinations were performed twice daily; first at the time of dose administration and approximately 1–2 h following dose adminis-