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28-Day oral (gavage) toxicity studies of green tea catechins prepared for beverages in rats

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Abstract

The beneficial health effects associated with drinking green tea are widely considered to be due primarily to tea catechins. Heat treatment of marketed green tea beverages for sterilization causes epimerization and/or polymerization of tea catechins. Safety studies on heat-treated tea catechins are limited. The objective of the present study was to evaluate potential adverse effects, if any, of two standardized green tea catechin (GTC) preparations: one that underwent heat sterilization (GTC-H) and one that was not heat-sterilized (GTC-UH). A decaffeinated preparation of the GTC-H (GTC-HDC) was also evaluated to ascertain if any effects were due to caffeine. The GTC preparations were administered to rats once daily at levels up to 2000 mg/kg/day for 28 days. There were no deaths attributable to the GTC preparations. The clinical condition of the animals, functional observational battery, motor activity, clinical pathology evaluations, organ weights, and gross necropsy findings were unaffected by any of the GTC preparations. GTC-HDC or GTC-UH dosing had no effects on body weights or microscopic findings, whereas lower body weights and food consumption were observed in the 1000 and 2000 mg/kg/day GTC-H group males. The no observed-adverse-effect level (NOAEL) for localized gastric effects for GTC-H was 1000 mg/kg/day. No other target organs were identified. Thus, the NOAEL for systemic toxicity following oral administration was 2000 mg/kg/day for GTC-H, GTC HDC, and GTC-UH under the conditions of this study.

Keywords: Green tea extract; Green tea catechins; Rat; Systemic toxicity; Functional observational battery

1. Introduction

Green tea, a beverage made from natural tea leaves (*Camellia sinensis*), has been consumed for centuries, particularly in Japan and China. In recent years, green tea is gaining importance around the world because of its potential health benefits. There have been no reports of clinical toxicity following consumption of green tea as a beverage throughout the day in a traditional setting. Daily consumption of high volumes of home brewed green tea (~20 cups) is not uncommon in certain populations. In recent years, products containing green tea extracts, particularly with high levels of catechins and heat sterilization, are marketed commercially as dietary supplements for health benefits. Green tea contains volatile oils, vitamins, minerals, and caffeine, but the primary constituents of interest appear to be the polyphenols, particularly a group of catechins that includes catechin, epicatechin, gallocatechin, epigallocatechin, catechin gallate, epicatechin gallate, gallocatechin gallate, and epigallocatechin gallate (Fig. 1). Research aimed at finding the active compounds in green tea indicates that its beneficial effects appear to be related to catechins. Various physiological actions of tea catechins have been reported, such as antioxidative properties (Yoshino et al., 1994), antiviral properties (Nakayama et al., 1993), the inhibition of plaque formation (Hattori et al., 1990),

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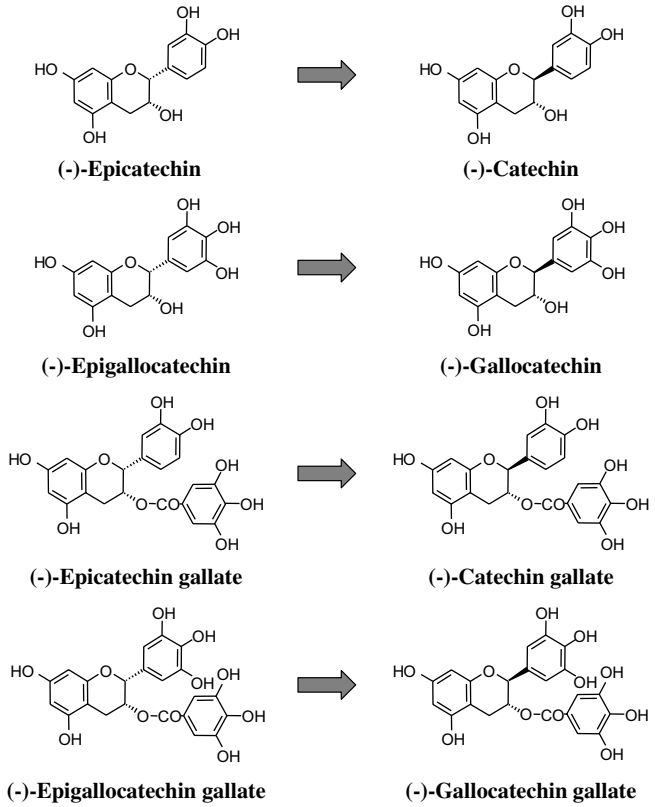

Fig. 1. Chemical structures of catechins and their corresponding epimers.

antiallergic properties (Kakegawa et al., 1985), the prevention of certain types of cancer (Katiyar and Mukhtar, 1996), radioprotector properties (Uchida et al., 1992), hypotensive properties (Henry and Stephen-Larson, 1984), and blood glucose-lowering effects (Matsumoto et al., 1993). In addition, studies in animals and humans have clearly demonstrated effects of tea catechins on lipid metabolism, including a reduction in triglycerides and total cholesterol (Chan et al., 1999), inhibition of liver fat accumulation (Chaudhari and Hatwalne, 1977) and body fat accumulation (Nagao et al., 2001, 2005, 2007), stimulation of lipid catabolism in the liver (Murase et al., 2002), and enhanced energy consumption (Dulloo et al., 2000; Osaki et al., 2001). Based on some of these findings, catechin-enriched green tea beverages and sports drinks that claim a preventive effect on body fat accumulation have been marketed in Japan as a "Food for Specified Health Use".

Conventional green tea drinks with high levels of tea catechins have a very bitter taste. Additionally, the qualitative and quantitative composition of catechin isomers in beverages can vary according to the heat-sterilization conditions as epimerization of tea catechins occurs under heating conditions (Seto et al., 1997). Approximately 50% of the tea catechins in the marketed green tea beverages are epimerized by heat treatment (Chen et al., 2001; Seto et al., 1997; Kim et al., 2007). Kim et al. (2007) reported that heating pasteurization-induced changes in the flavor of green tea liquor is a technical barrier in ready-to-drink tea production. The conditions and extent of heat sterilization are determined based on features of the beverage, including pH and packaging, such that significant epimerization of catechins occurs in certain types of green tea beverages and not in others (e.g., because of acidic pH of sports drinks, these types of beverages do not require high temperature sterilization). Kao Corporation has developed a unique extraction and refinement method to produce green tea beverages containing high levels of beneficial catechins and acceptable flavor to consumers. From a safety perspective, very few studies have explored the toxicity potentials of green tea preparations that contain high levels of catechins or its epimers resulting from heat sterilization.

The objective of the present study was to investigate the potential toxicity of two green tea catechin preparations that are representative of the catechin composition in marketed beverages that either have undergone heat sterilization (GTC-H) as is used for green tea beverages or have not been heat-sterilized (GTC-UH) as is used for sports drinks. As a result of the heat sterilization process, GTC-H contains the most epimerized catechins, whereas GTC-UH has no significant epimerization. We also evaluated a decaffeinated catechin preparation that also had heat sterilization (GTC-HDC) in order to identify any potential toxicity due to caffeine. The effects of GTC-H and GTC-UH were investigated in a dose–response study and were compared. This study was designed to be in general accordance with the Organisation for Economic Cooperation and Development (OECD) Guidelines for Testing of Chemicals, Health Effects Guidelines, Section 407, July 1995 and was conducted under Good Laboratory Practices.

2. Materials and methods

2.1. Experimental design overview

Green tea catechin preparations, GTC-H, GTC-HDC, or GTC-UH, in a vehicle (deionized water) were administered by oral gavage daily, 7 days per week, for at least 28 days (the first week of administration was week 0) to groups of five rats per sex per dose group. The study was conducted in two parts. In Study A, effects of GTC-H and GTC-HDC were investigated, while in Study B, the effects of GTC-UH were studied. The dose levels used for the animals in groups treated with GTC-H and GTC-UH were 0, 500, 1000, and 2000 mg/kg/day, while animals in the GTC-HDC group received only 2000 mg/kg/day. Animals that were administered only vehicle served as controls in both Study A and Study B. Clinical observations were recorded daily, and detailed physical examinations and food consumption were recorded weekly. Clinical pathology parameters (hematology, serum chemistry, and urinalysis) were evaluated at week 4. Evaluations of potential neurotoxicity by functional observational battery (FOB) and motor activity (MA) assessment were conducted prior to GTC-H, GTC-HDC, or GTC-UH administration (week −1) and during week 3. Necropsies were performed on all animals at week 4. Selected organs were weighed and selected tissues were examined microscopically from all animals (see specific methods sections for details).

2.2. Test articles

The test articles, GTC-H, GTC-HDC, and GTC-UH, were provided by Kao Corporation, who confirmed the purity and stability. GTC-H,