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Evaluation of oral subchronic toxicity of Pu-erh green tea (*camellia sinensis var. assamica*) extract in Sprague Dawley rats

Di Wang [a,b,1], Jie Meng [c,1], Kunlong Xu [d], Rong Xiao [e], Mengjing Xu [a,b], Yang Liu [a,b], Yaowu Zhao [a,b], Ping Yao [a,b], Hong Yan [b], Liegang Liu [a,b,*]

[a] *Department of Nutrition and Food Hygiene, School of Public Health, Tongji Medical College, Huazhong University of Science & Technology, 13 Hangkong Road, Wuhan 430030, P.R. China*
[b] *MOE Key Lab of Environment and Health, School of Public Health, Tongji Medical College, Huazhong University of Science & Technology, 13 Hangkong Road, Wuhan 430030, P.R. China*
[c] *Reproductive Medicine Center, Tongji Hospital, Tongji Medical College, Huazhong University of Science & Technology, 13 Hangkong Road, Wuhan 430030, P.R. China*
[d] *College of Animal Science and Technology, Yunnan Agriculture University, Kunming, Yunnan 650201, P.R. China*
[e] *College of Food Science, Yunnan Agriculture University, Kunming, Yunnan 650201, P.R. China*

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ABSTRACT

Ethnopharmacological relevance: Pu-erh green tea, originally produced in the Yunnan province of P.R. China for about 1700 years, is believed to be beneficial to health in Asian countries. The potential toxicity of Pu-erh green tea when administered at high doses via concentrated extract, however, has not been completely investigated.
The aim of the study: The present study was aimed to evaluate the potential toxicity of Pu-erh green tea extract (PGTE) of sub-chronic administration to Sprague Dawley (SD) rats.
Materials and methods: Growing SD rats were administrated orally by gavage with PGTE at doses of 0, 1250, 2500, and 5000 mg/kg/day for 91 consecutive days. Clinical observations, including survival, hematology, serum biochemistry, urinalysis and histopathological examination were measured to monitor treatment-related adverse effects in rats.
Results: The results showed that oral administration of high dose of PGTE led to body weight gain suppression, liver and calcium deposition dysfunctions.
Conclusions: In conclusion, the no-observed-adverse-effect level for Pu-erh green tea extract derived from the results of the present study was 2500 mg/kg/day for both genders.

1. Introduction

Tea is traditionally used as a medication based on experience, and the physiological activities of components of tea have been extensively described in Asian countries, mainly in P.R. China and Japan. Data on over 7000 adults from the UK National Diet and Nutrition Survey (NDNS) (Henderson et al., 2002), indicate that 77% of people drink tea, with a mean consumption of 2.3 mugs (540 mL) per day. It is believed to have potential medicinal efficacy in the prevention and treatment of many diseases, and so longevity is often associated with the habit of drinking tea (Crespy and Williamson, 2004; Kao et al., 2006; Nagao et al., 2007).

On the basis of the processing procedures, tea can be generally divided into three types: nonfermented (green tea), semifermented (oolong tea), and fully fermented (black tea) (Lin et al., 1998a). Pu-erh teas, originally produced in the Yunnan province of P.R. China for about 1700 years, were recorded by *Compendium of Materia Medica* that Pu-erh tea can expel wind-evil, clear away heat and aid in losing weight. Usually, Pu-erh teas can be further differentiated into raw/green (Sheng) and ripened/black (Shou) teas, depending on the processing method or amount of aging.

Pu-erh green tea is obtained by first parching crude green tea leaves (*Camellia sinensis var. assamica (L.) O. Kuntze*; Theaceae), while Pu-erh black tea undergoes secondary fermentation with microorganisms such as *Aspergillus* sp. (postfermented) (Jeng et al., 2007). According to different procedure, the main ingredients between Pu-erh green tea and Pu-erh black tea are not the same. Our prior study found that catechins are the most abundant constituents in the Pu-erh green tea such as (—)- epigallocatechin 3-gallate (EGCG), (—)- epigallocatechin (EGC), (—)- epicatechin 3-gallate (ECG), (—)- epicatechin (EC), (+)- gallocatechin (GC)

* Corresponding author. Tel.: +86 27 8369 2711; fax: +86 27 83650522.
E-mail address: lgliu@mails.tjmu.edu.cn (L. Liu).
[1] These authors contributed equally to this work.

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and (+)- catechin (C), but the catechins are easily oxidized by polyphenol oxidase, and further polymerizations lead to theaflavins, thearubigins, and theabrownins in Pu-erh black tea during the special fermentation (Wang et al., 2010). Additionally, theogallin, which is rich in Indian and Ceylonese black teas (Hashimoto et al., 1992), was only detected in the Pu-erh green tea in our prior study (Wang et al., 2010). This is a significant characteristic of *Camellia sinensis var. assamica (L.) O. Kuntze*; Theaceae cultivated in the Yunnan Highlands of P.R. China. Caffeine, which is an important bioactive compound in tea, is also higher in the Pu-erh green tea. The Pu-erh green tea that has longer history is now mainly used as an important material to make Pu-erh black tea, also exhibits favorable function, i.e. antioxidation and hypocholesterolemia (Hou et al., 2009).

Though the health benefits of Pu-erh teas are accepted by the people all over the world, the safety should not be neglected when administered at a high dose as concentrated extracts or products. The systemic oral toxicity of Pu-erh black tea extract (PBTE) was evaluated in prior studies, and the subacute and subchronic toxicity in rodents showed the comprehensive safety profile of PBTE (Wang et al., 2010,2011). However, few studies have been conducted to investigate the toxicity of Pu-erh green tea extract (PGTE). The catechins, which are rich in the Pu-erh green tea, exhibit slight toxicities both in vitro and in vivo studies. For example, high dose of catechins intake could alter hepatic (Bonkovsky, 2006; Galati et al., 2006; Schmidt et al., 2005) and thyroid function adversely (Chandra and De, 2010). The EGCG mainly contained in Pu-erh green tea has shown weak embryotoxicity during early embryogenesis exposure (Wang et al., 2007). Besides, whether the theogallin also plays an important role in the toxicity of Pu-erh green tea remains unknown. Above all, although the Pu-erh green tea has been used as a functional drink for a long history, fewer data about its potential toxicity when administered at a high dose as concentrated extracts or products, are available. Following the suggestion given by "Food Safety in Europe (FOSIE): Risk Assessment of Chemicals in Food and Diet", it is necessary to assess the safety of new foods and food ingredients such as macronutrients and whole foods as a part of risk assessment (Smith, 2002). Therefore, safety evaluation of Pu-erh green tea extract is necessary.

In this experiment, Pu-erh green tea samples were collected from Yunnan high land of P.R. China, and the toxicity of its extract was evaluated with dietary administration to Sprague Dawley (SD) rats for consecutive 91 days as part of a safety assessment according to the internationally acceptable guidelines for Pu-erh green tea consumption.

2. Materials and methods

2.1. Tea extracts preparation

The commercial Pu-erh green tea samples were collected from Yunnan Highland of P.R. China. Voucher specimens of tea (*Camellia sinensis var. assamica (L.) O. Kuntze*; Theaceae) is collected from agro-forests and deposited at the Kunming Institute of Botany, Chinese Academy of Sciences. The crude green tea leaves cultivated in the Yunnan Highlands of China was used as the raw material. Leaves were collected and heated, dried at $<60\ ^{\circ}C$, and molded to make unfermented Pu-erh tea. The tea sample (100 g) was cut into small pieces, and soaked in boiled distilled water for three times (2 L, 1.5 L and 1.5 L of each bulk; 20 min, 15 min and 15 min of each time, respectively). After separating from leaves by filtration, the whole extracts were evaporated by a rotary evaporator at 65 °C (Yarong Biochemical Instrument, Shanghai, China) to 100 mL. The final beverages were sterilized at 121 °C for 20 min and kept at 4 °C in a closed container and were designated as 1 g/mL (tea/water) Pu-erh green tea extract (PGTE) for later experiments. The contents of biochemical ingredients including total polyphenols, total flavonoid, catechins and free amino acid in Pu-erh green tea were analyzed in our prior study (Wang et al., 2010).

2.2. Experimental animals

A total of 40 male and 40 female 5-week-old SD rats were purchased from Sino-British Sippr/BK (Shanghai, China) and acclimated for 1 week before studies were performed. During this period, to ensure the health of the animals, they underwent detailed physical examinations. Each animal was housed in a suspended wire mesh cage and given free access to commercial laboratory food and tap water. Animals were kept in an isolated room with barrier system, light–dark cycle controlled (12–12 h, lights on 7:00-19:00), ventilation (air-exchange rate of 18 times per hour), temperature ($23\pm2\ ^{\circ}C$) and relative humidity ($55\pm5\%$) during the study. The cages and the chip bedding were exchanged twice a week. The study was performed in accordance with the guide for the care and use of laboratory animals, prepared by National Institute of Health, USA (Guide for the Care and Use of Laboratory Animals, 1996).

2.3. Experimental design

The subchronic 91-day oral toxicity study was evaluated following the recommendations by OECD 408 (OECD, 1998). Groups of ten male and ten female received doses of 0, 1250, 2500 or 5000 mg/kg/day of PGTE at daily gavage of 1 ml/100 g · bw for 91 consecutive days. Twice a day throughout the study, changes in appearance, behavior and mortality of animals were recorded. In order to keep constant the doses administered adjustments in dosage were made based on weekly animals body weight gain. In addition, detailed clinical examination and food consumption were preformed weekly. The animals were sacrificed by exsanguinations from the abdominal aorta at the 92-day following fasting for 12–16 h. A sample of fresh blood (approximately 20 μL) was treated with DTA-2K to analyze hematological indexes. Serum from blood samples collected in separator tubes was stored at $-20\ ^{\circ}C$. During necropsy, the following organs were removed: liver, spleen, thymus, heart, lungs, stomach, ovaries, uterus, kidney, adrenals, trachea/thyroid gland, brain, pituitary gland, pancreas, perirenal fat, testes, and epididymis. All organs were visually inspected and weighed directly after dissection. Defined samples of the liver, brain, pancreas, stomach, kidney, adrenals, testes and ovaries were placed in 10% neutral buffered formalin for pathological examination.

2.4. Hematology and Blood chemistry

The fresh blood sample (approximately 20 μL) was treated with EDTA-2K for white blood cells (WBC), red blood cells (RBC), hemoglobin (Hb), platelets (PLT), percent of lymphocytes (LY), percent of monocytes (MO), percent of granulocyte (GR), hematocrit (HCT), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), mean platelet volume (MPV), Platelet hematocrit (PCT), platelet distribution width (PDW) and red blood cell distribution width (RDW) measurement using a hematology analyzer MEK-6318K (Nihon Kohden Co., Ltd.). Serum from blood samples collected in separator tubes were measured using a BS-200 automatic biochemistry analyzer (Mindary Co., Ltd.) including aspartate aminotransferase (AST), alanine aminotransferase (ALT), blood urea nitrogen (BUN), creatinine (Cre), total