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ARTICLE

Comparative Safety Evaluation of Chinese Pu-erh Green Tea Extract and Pu-erh Black Tea Extract in Wistar Rats

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Pu-erh teas are believed to be beneficial beverages for health since they possess several pharmacological properties such as antioxidation, hypocholesterolemia, and antiobesity properties, but their potential toxicities when administered at a high dose as concentrated extracts have not been completely investigated. In this study, the chemical components in Pu-erh green tea and Pu-erh black tea were analyzed and compared, and the safety of tea extracts was evaluated in Wistar rats. The polysaccharide, tea pigment, and flavonoid levels were substantially increased in the Pu-erh black tea, while the polyphenol and free amino acid levels were higher in unfermented green tea. Low toxicities of Pu-erh green tea extract (GTE) were observed at doses of 2500 and 5000 mg/kg/day with a 28-day subacute study. Serum biochemical data including alanine aminotransferase increased to 5000 mg/kg/day GTE males, and creatinine (Cr) increased in all 5000 mg/kg/day GTE groups and 2500 mg/kg/day GTE males. Slight bile duct hyperplasia in the liver was also observed. The target organs of GTE were considered to be the liver and kidney. Comparatively, no adverse effects were observed in Pu-erh black tea extract (BTE)-treated rats. In conclusion, a dose of 1250 mg/kg/day for GTE and 5000 mg/kg/day for BTE following oral administration could be considered safe under the conditions of this study.

KEYWORDS: Chinese Pu-erh teas; components analysis; safety evaluation

INTRODUCTION

Tea is traditionally used as a medication based on experience, and the physiological activities of the components of tea have been extensively described in Asian countries, mainly in Japan and China (*1*). According to the processing procedures, tea can be generally divided into three types: nonfermented (green tea), semifermented (oolong tea), and fully fermented (black tea and Pu-erh-fermented tea) (*2*). Chinese black tea (Pu-erh-fermented tea), originally produced in the Yunnan province of China, is obtained by first parching crude green tea leaves and then fermenting them with microorganisms such as *Aspergillus* sp. (*3*). On the basis of the processing method, Pu-erh teas can be further differentiated into raw/green (Sheng) and ripened/black (Shou) tea. Shou Pu-erh is the fully fermented tea; during the fermentation process, polyphenol oxidase in tea oxidizes catechin into quinine, which then condenses to form bisflavanol, theaflavin (TF), thearubigen, and other high molecular components (*4*). In addition, the Pu-erh black tea is relatively rich in natural statins (*3*). Conversely, Sheng Pu-erh tea lacks this special fermentation process, so more polyphenol and caffeine can be preserved. Certain catechins are the most biologically active group of the polyphenols in tea components. The major catechins including (−)-epigallocatechin 3-gallate (EGCG), (−)-epigallocatechin (EGC), (−)-epicatechin 3-gallate (ECG), (−)-epicatechin (EC), and (+)-gallocatechin gallate (GCG) were found in Paka Pu-erh green tea in a previous study (*5*).

Despite favorable evidence supporting the benefits of a diet rich in tea and its associated bioactive components, such as antioxidation, hypocholesterolemia, and antiobesity effects (*6*−*8*), from a safety perspective, few researchers have investigated their potential toxicity when they were administered at a high dose as concentrated extracts or products. Clinical studies found that rare cases of hepatic necrosis were associated with ingestion of large quantities of tea extracts by humans (*9*). In addition, weak hepatotoxicity and renal toxicity were observed in animals when given a high dose of tea extracts (*10*, *11*), and EGCG, the main catechins in green tea, showed a cellular toxicity on experimental animals at high doses (*12*). Other studies showed that green tea catechins have enhanced hepatocarcinogenesis and intestinal carcinogenesis in rat models (*13*) and have been shown to promote DNA cleavage in the presence of Cu^{2+} in vitro (*14*).

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Another related study reported that EGCG had a protective effect on DNA at low concentrations, but it enhanced the DNA oxidative damage at higher concentrations, exhibiting a prooxidant effect on DNA (*15*). Comparatively, most catechins in Pu-erh black tea were oxidized into other high molecular components, and EGCG was almost not detected (*5*). Whether the toxicity of BTE is different from Pu-erh green tea extracts (GTE) is unknown, and the related safety evaluation is not systematic. For example, a study showed that a single bolus dose (10-fold the daily intake) for mice and repeated dosing of BTE at five times the daily intake (i.e., 5 g/day) over a 5 week period for volunteers were safe (*8*), but the dosage level and method were not sufficient to prove the safety of BTE for consumers.

The composition of tea varies with species, climate, and horticultural practices (*16*). Pu-erh teas, as a special product series, are becoming a favorable choice for people. However, not enough attention has been given to their potential toxicity. In this experiment, six kinds of Pu-erh teas were selected from China, the chemical components of concentrated GTE and BTE were analyzed, and the toxicities of two teas were evaluated with dietary administration to male and female Wistar rats for 28 days as part of a safety assessment according to the internationally acceptable guidelines for Pu-erh tea consumption.

MATERIALS AND METHODS

Tea Extracts Preparation. Six commercial tea samples including Pu-erh green tea (three samples) and Pu-erh black tea (three samples) from Yunnan of China were collected. These tea samples were collected from three tea factories located in different fields (Qizi brick tea, Xishuangbanna; Paka brick tea, Simao; and Longrun brick tea, Lincang, respectively). Of the arbitrary two kinds of Pu-erh teas, for the different processing technologies, there could be great differences among the tea constituents even if they were manufactured by the same fresh tea leaves material (*5*). In brief, *Camellia sinensis* (Linn.) var. assamica (Masters) Katamura cultivated in the Yunnan Highlands of China was used as the raw material. Leaves were collected and heated, dried at < 60 °C, and molded to make unfermented Pu-erh tea. To make fermented Pu-erh tea, the unfermented Pu-erh tea was dampened and fermented with a pure culture of *Aspergillus niger* for 50 days at controlled temperature and humidity. Fermented Pu-erh tea was then dried at < 60 °C and packed. In our study, the Pu-erh green tea sample and Pu-erh black tea sample were composed of similar commercial tea samples, respectively. Each mixed tea sample (100 g) was cut into small pieces and soaked in boiled distilled water for three deferent time periods (2, 1.5, and 1.5 L of each bulk, respectively; 20, 15, and 15 min for each time, respectively). After the leaves were separated 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) GTE and BTE for later experiments, respectively.

Contents of Regular Ingredients in Pu-erh Teas. The total tea polyphenols were determined according to Folin−Ciocalteu's test using GA as the standard (*17*). Polysaccharides were quantitated using the anthrone−sulfuric acid method with modifications as described (*18*). Three kinds of tea pigments, including TFs, thearubigins (TRs), and theabrownins (TBs), were analyzed using spectrophotometry (*19*). The flavonoid content was calculated using the aluminum trichloride colorimetric method (*20*). Additionally, free amino acid was analyzed by ninhydrin colorimetric method as described by William (*21*).

HPLC Analysis of Polyphenol in Pu-erh Tea Extracts. The composition of catechins, gallic acid (GA), theogallin, and caffeine in Pu-erh tea extract was determined by high-performance liquid chromatography (HPLC) analysis using a Waters 2695 system controller. The Waters 484 turnable absorbance detector was used to detect tea constituents at 280 nm, and all peaks were plotted and integrated by a waters 2996 detector. The HPLC method used an Agillent ZORBAX SB-C18 packed column (250 mm × 4.6 mm, 5 μm) (Waters, Inc., United States). The tea extract was filtered through a 0.45 μm filter disk, and then, 10 μL was injected into the column. The concentrations of authentic catechins, GA, theogallin, and caffeine working solutions were 15 μg/mL. The amount of each authentic standard compound injected was 300 ng. The mobile phase was acetonitrile/phosphate buffer (4:96, v/v) and run by an isocratical elution at a flow rate of 1.0 mL/min.

Experimental Animals. A total of 70 male and 70 female 5-week old Wistar rats were purchased from experimental animal central of Hubei Centers for Disease Control and Prevention (Wuhan, China) and used after 1 week of acclimatization. Individual body weights were recorded, and detailed physical examinations were performed twice during the acclimation period to ensure the use of healthy animals. Each animal was housed in a cage-suspended wiremesh and given free access to commercial laboratory feed and tap water during the nonexposure periods. Animals were fed in a specific pathogen-free level room with a barrier system controlled for the light−dark cycle (12−12 h, lights on 7:00−19:00), ventilation (air exchange rate of 18 times per hour), temperature (23 ± 2 °C), and relative humidity (55 ± 5%) during the study. The cages and the chip bedding were exchanged twice a week. The study was performed in accordance with the guidelines for the care and use of laboratory animals (*22*), prepared by the National Institute of Health (United States).

Experimental Design. Groups of 10 males and 10 females received doses of 0, 1250, 2500, or 5000 mg/kg bw of GET and BET at a daily gavage of 1 mL/100 g bw for 28 consecutive days. Observations were made twice daily for mortality and changes in general appearance or behavior. Body weights were recorded twice every week, and doses were adjusted for body weights. In addition, detailed clinical examination and measurement of food consumption were preformed once weekly. At the end of the exposure period, animals were necropsied at the 29th day and placed on a 12−16 h fast. The animals were then anesthetized with ether, and blood samples were collected from the abdominal aorta. A sample of blood (approximately 20 μL) was treated with EDTA-2K to analyze hematological indexes. Serum from blood samples collected in separator tubes was stored at −20 °C for biochemistry tests. During necropsy, dissected were the following organs: liver, spleen, thymus, heart, lungs, stomach, ovaries, uterus, kidney, adrenals, trachea/thyroid gland, brain, pituitary gland, testes, and epididymis. All organs were visually inspected and weighed directly after dissection to reduce mechanical damage. Defined samples of the liver, brain, pancreas, stomach, kidney, adrenals, testes, and ovaries were placed in 10% neutral-buffered formalin.

Hematology and Blood Chemistry. One blood sample (approximately 20 μL) was treated with EDTA-2K for red blood cell count (RBC), hematocrit (Hct), hemoglobin concentration (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count, and white blood cell count (WBC) using a hematology analyzer MEK-6318K (Nihon Kohden Co., Ltd.). Serum from blood samples collected in separator tubes was measured using a BS-200 automatic biochemistry analyzer (Mindary Co., Ltd.) for biochemistry tests, including aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea nitrogen, creatinine (Cr), total cholesterol (TC), triglyceride (TG), total protein (TP), albumin (Alb), and glucose. Ionized calcium (iCa) and total calcium (TCa) were analyzed using the 7020 automatic biochemistry analyzer (Hitachi Co., Ltd.) and for sodium (Na), potassium (K), chloride (Cl), and pH value analyses.

Histopathological Examination. Samples of the liver, brain, pancreas, stomach, kidney, adrenals, testes, and ovaries were placed in 10% neutral-buffered formalin, sectioned, and stained with hematoxylin and eosin. The histological preparations from animals in the control and high dose groups were examined.

Statistical Analysis. Variance in data for body and organ weights as well as the results of hematology and serum biochemistry were checked for homogeneity by Bartlett's procedure. For homogeneous data, one-way analysis of variance for homogeneity was used. In the heterogeneous cases, the Kruskal−Wallis test was applied. When statistically significant differences were indicated, Dunnett's multiple tests were employed for comparisons between control and treated groups.

RESULTS

Contents of Regular Ingredients in Pu-erh Teas. The active ingredients, including tea polyphenol, polysaccharides, tea

Table 1. Contents of Regular Ingredients in Pu-erh Teas

ingredients	Pu-erh green tea[a]	Pu-erh black tea[a]
liquid	9.08 ± 0.12	9.97 ± 0.13
dry	4.98 ± 0.73	8.12 ± 0.11
water-soluble	48.83 ± 0.08	30.82 ± 0.77
polysaccharides	0.44 ± 0.06	0.59 ± 0.05
TFs	0.07 ± 0.02	0.17 ± 0.01
TRs	4.31 ± 1.24	5.88 ± 0.88
TBs	2.85 ± 0.11	9.73 ± 0.11
polyphenol	21.97 ± 0.48	10.31 ± 0.16
free amino acid	2.13 ± 0.13	1.56 ± 0.27
flavonoid	10.90 ± 0.73	12.37 ± 0.08

[a] Unit, % (w/w).

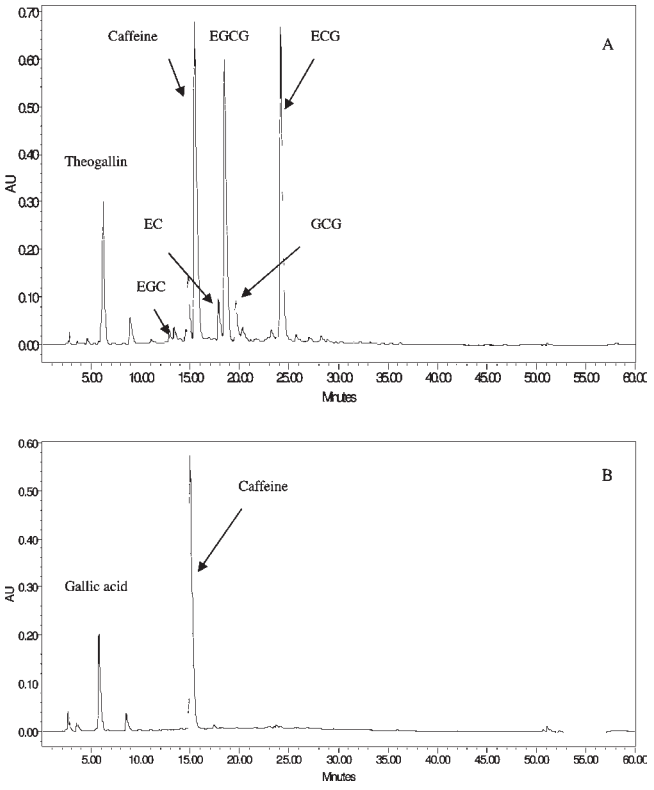

Figure 1. Isocratic HPLC separation of catechins, GA, theogallin, and caffeine in Pu-erh raw tea extract (**A**) and BTE (**B**) at 280 nm.

pigments, flavonoids, and free amino acid in Pu-erh green tea and Pu-erh black tea were analyzed and compared in our study (**Table 1**). Total polyphenols in Pu-erh black tea were significantly decreased during the fermentation processing (*23*). In addition, the polysaccharide, tea pigments, and flavonoid levels were substantially increased in the fermented tea, while the free amino acid level increased as compared to unfermented green tea.

HPLC Separation of Chemical Composition of Pu-erh Raw Tea and Pu-erh Black Tea Extracts (BTE). The representative HPLC patterns of Yun-Nan GTE and BTE are illustrated in **Figure 1**. Catechins, theogallin, and caffeine were detected in GTE (22.189, 3.225, and 3.085%, respectively) (**Table 2**). A mixture of five kinds of catechins including EGC, EC, EGCG, ECG, and GCG (2.149, 1.225, 7.689, 9.890, and 1.236%, respectively) was separated by a gradient HPLC in GTE. Comparatively, BTE contains only a scarce amount of catechins (**Figure 1B**) and relatively high levels of GA (0.620%). The levels of catechins, GA, theogallin, and caffeine are summarized in **Table 2**. Otherwise, low levels of caffeine were detected in BTE as compared with GTE.

Table 2. Composition of Catechins, GA, Theogallin, and Caffeine Used on Oral (Gavage) Toxicity Studies with Pu-Erh Green Tea and Pu-Erh Black Tea in Wistar Rats

	composition of GA, caffeine, catechins, and theogallin[a]							
samples	GA	caffeine	EGC	EC	EGCG	GCG	ECG	theogallin
Pu-erh green tea	ND[b]	3.085	2.149	1.225	7.689	1.236	9.890	3.225
Pu-erh black tea	0.620	2.583	ND	ND	ND	ND	ND	ND

[a] Unit, %. [b] Not detectable.

Clinical Signs and Mortality. There was no mortality attributed to any effect of GTE and BTE. One female and one male rat given BTE at doses of 1250 and 5000 mg/kg/day, respectively, died within the first 2 weeks of the study due to inadvertent gavage accidents. In life observations, all animals given tea beverage show a highly active version as compared to control rats. Moreover, there were no treatment-related changes at autopsy in the GTE group, BTE group, or control group in either sex.

Body Weights and Food Consumption. Changes in body weight during the study are illustrated in **Table 3**. Statistically significant, the body weights of three groups of male rats given GTE are lower than those in the control group, and the BTE groups with all doses in either sex are lower than the control group at the fourth week ($P < 0.05$ or $P < 0.01$). As a result, the mean body weight of GTE group with males given a dose of 5000 mg/kg/day is only 250.8 ± 16.7 g (18.4 and 7.6% lower than the control and corresponding BTE group, respectively), which is the lowest in all groups.

During the 4 week study, the mean food consumption of the males at the high-dose BTE (19.7 g/day food) was lower than the controls at the second week (21.0 g/day food), and middle-dose BTE (26.3 g/day food) were higher than the controls at the third week (23.5 g/day food). In addition, there were no differences in mean food consumption at any other observation points in any group (**Figure 2**).

Organ Weights. The results for relative organ weights are shown in the **Tables 4** and **5**. No treatment-dependent variation of absolute organ weights was observed in all groups. There were no test article-related macroscopic findings at the scheduled or unscheduled necropsies. However, in males, the organ coefficient of brain was increased in the 5000 and 2500 mg/kg/day GTE groups, and the relative testes weight in the 5000 mg/kg/day GTE group was increased as compared to controls (**Table 4**).

Hematological and Serum Biochemical Data. In hematology parameters, the RBC, HGB, and HCT were increased in the 5000 mg/kg/day GTE males as compared to the controls. The MCHC was increased in the 2500 and 1250 mg/kg/day GTE males. Besides, MO was decreased and MCH was increased in the 2500 mg/kg/day GTE group females. The RBC and MCHC in the 5000 mg/kg/day BTE females were significantly different from the control group, and the MCHC was significantly higher in the 2500 mg/kg/day BTE males when compared to the controls at study week 5 (**Tables 6** and **7**). In blood serum biochemistry of males, ALT and Cr in the 5000 mg/kg/day GTE group and Cr in the 2500 mg/kg/day GTE group were increased as compared to the controls. There were no significant changes in serum biochemistry parameters of BTE gavage rats (**Table 8**). In females, Cl, TCa, and iCa were decreased and Cr was increased in the 5000 mg/kg/day GTE group; TC in the 5000 and 1250 mg/kg/day BTE groups was increased; and TCa and iCa in the 5000 mg/kg/day BTE group were decreased after the 28 days of tea extracts exposure (**Table 9**).

Histopathological Examination. In liver, minimal bile duct hyperplasia, vacuolation, and inflammation were observed in 5000 mg/kg/day GTE rats (**Figure 3**). The incidence of bile duct

Table 3. 28 Day Repeated Dose Toxicity Study of Pu-erh Tea Extracts in Rats—Body Weight Values (Mean ± SD)

day	group A1[a] N = 10	group A2[a] N = 10	group A3[a] N = 10	group B1[b] N = 9	group B2[b] N = 10	group B3[b] N = 10	control N = 10
				males			
0[c]	95.7 ± 6.8	93.3 ± 3.2	94.2 ± 7.3	98.2 ± 5.6	92.6 ± 6.6	95.1 ± 8.1	94.2 ± 4.9
7[c]	135.0 ± 9.9[d]	132.9 ± 4.9[d]	138.4 ± 7.4[d]	138.6 ± 7.6	130.5 ± 11.3[d]	138.5 ± 11.0	145.7 ± 6.8
14[c]	173.1 ± 12.8[d]	175.7 ± 10.6[d]	178.8 ± 7.8	175.9 ± 8.4	172.3 ± 16.0[d]	180.7 ± 13.5	188.9 ± 13.3
21[c]	215.6 ± 18.6[e]	225.3 ± 12.2[d]	229.9 ± 15.3[d]	216.9 ± 13.7[e]	216.1 ± 20.9[d]	229.5 ± 13.4[d]	245.8 ± 15.5
28[c]	250.8 ± 16.7[e]	278.3 ± 14.8[e]	285.9 ± 14.8[d]	271.3 ± 14.3[e]	276.9 ± 20.5[d]	284.6 ± 12.0[d]	307.4 ± 18.3
				females			
0[c]	89.3 ± 8.4	96.9 ± 8.1	92.7 ± 10.1	97.7 ± 7.5	95.0 ± 6.4	96.7 ± 9.1	95.1 ± 8.5
7[c]	123.7 ± 10.6	128 ± 10.2	127.0 ± 13.5	126.7 ± 9.2	126.5 ± 5.7	127.8 ± 7.1	133.7 ± 8.9
14[c]	153.0 ± 12.7	159.5 ± 11.9	159.8 ± 16.2	153.1 ± 8.3	162.5 ± 18.3	156.1 ± 11.0	162.4 ± 11.3
21[c]	181.9 ± 13.6	181.9 ± 14.9	184.1 ± 19.0	178.7 ± 8.8[d]	180.4 ± 10.4[d]	178.3 ± 14.1[d]	192.6 ± 12.3
28[c]	209.7 ± 12.4	210.8 ± 17.6	213.5 ± 19.8	203.0 ± 6.6[d]	205.5 ± 9.8[d]	207.5 ± 11.7[d]	223.7 ± 15.4

[a] A1, 5000 mg/kg/day GTE; A2, 2500 mg/kg/day GTE; and A3, 1250 mg/kg/day GTE. [b] B1, 5000 mg/kg/day BTE; B2, 2500 mg/kg/day BTE; and B3, 1250 mg/kg/day BTE. [c] Unit, g. [d] Statistically significant as compared to controls ($P < 0.05$). [e] Statistically significant as compared to controls ($P < 0.01$).

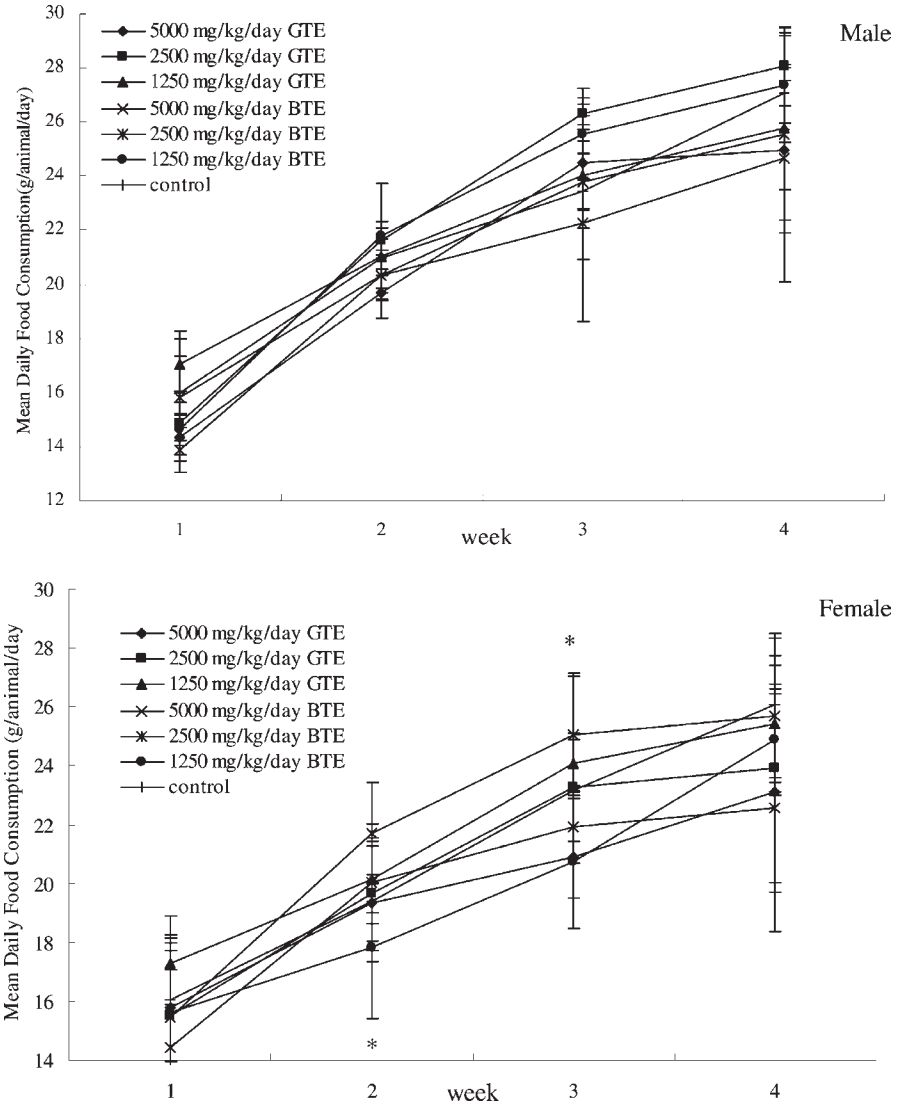

Figure 2. Mean food consumption for male rats during a 28 day oral (gavage) toxicity study with Pu-erh tea extracts. *Statistically significant as compared to controls ($P < 0.05$).

hyperplasia in the high-dose GTE group, which was not significantly different in both males and females, with or without vacuolation and inflammation, suggested a test article-related effect at the high GTE dose. The similar histopathological changes were rarely identified in BTE and control groups. In kidneys, a similar incidence of minimal tubular basophilia was noted in the experimental and control groups and thus not considered to be test article exposure-related. Otherwise, there were no obvious histopathological findings of other tissues in any groups and either sex.

Table 4. Relative Organ Weights of Male Rats (Mean ± SD)

organs	group A1[a] N = 10	group A2[a] N = 10	group A3[a] N = 10	group B1[b] N = 9	group B2[b] N = 10	group B3[b] N = 10	control N = 10
body weights[c]	250.8 ± 16.7	278.3 ± 14.8	285.9 ± 14.8	271.3 ± 14.3	276.9 ± 20.5	284.6 ± 12.0	307.4 ± 18.3
brain[d]	0.49 ± 0.03[f]	0.46 ± 0.02[e]	0.45 ± 0.03	0.45 ± 0.02	0.46 ± 0.04	0.44 ± 0.03	0.43 ± 0.03
pituitary[d]	0.0031 ± 0.00	0.0029 ± 0.00	0.0029 ± 0.00	0.0027 ± 0.00	0.0029 ± 0.00	0.0026 ± 0.00	0.0029 ± 0.00
lung[d]	0.65 ± 0.20	0.56 ± 0.10	0.57 ± 0.19	0.66 ± 0.20	0.61 ± 0.12	0.57 ± 0.13	0.50 ± 0.09
heart[d]	0.38 ± 0.04	0.35 ± 0.04	0.36 ± 0.06	0.35 ± 0.06	0.37 ± 0.09	0.37 ± 0.04	0.37 ± 0.06
thyroids/parathyroids[d]	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
thymus[d]	0.18 ± 0.05	0.22 ± 0.04	0.20 ± 0.04	0.14 ± 0.05	0.19 ± 0.03	0.18 ± 0.02	0.18 ± 0.06
liver[d]	3.43 ± 0.46	3.80 ± 0.42	3.81 ± 0.99	3.43 ± 0.50	3.56 ± 0.41	3.72 ± 0.37	3.64 ± 0.31
spleen[d]	0.29 ± 0.05	0.32 ± 0.07	0.27 ± 0.04	0.25 ± 0.05	0.27 ± 0.04	0.28 ± 0.06	0.27 ± 0.05
stomach[d]	0.66 ± 0.06	0.60 ± 0.08	0.58 ± 0.06	0.62 ± 0.09	0.68 ± 0.10	0.60 ± 0.04	0.58 ± 0.06
kidneys[d]	0.84 ± 0.12	0.85 ± 0.08	0.78 ± 0.09	0.75 ± 0.08	0.80 ± 0.11	0.87 ± 0.07	0.79 ± 0.08
adrenals[d]	0.03 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01
testes[d]	1.14 ± 0.07[e]	1.05 ± 0.11	1.07 ± 0.10	1.09 ± 0.09	1.08 ± 0.11	1.02 ± 0.15	0.98 ± 0.11
epididymides[d]	0.17 ± 0.06	0.22 ± 0.04	0.23 ± 0.03	0.22 ± 0.02	0.23 ± 0.03	0.23 ± 0.03	0.21 ± 0.03

[a] A1, 5000 mg/kg/day GTE; A2, 2500 mg/kg/day GTE; and A3, 1250 mg/kg/day GTE. [b] B1, 5000 mg/kg/day BTE; B2, 2500 mg/kg/day BTE; and B3, 1250 mg/kg/day BTE. [c] Unit, g. [d] Unit, % body weights. [e] Statistically significant as compared to controls ($P < 0.05$). [f] Statistically significant as compared to controls ($P < 0.01$).

Table 5. Relative Organ Weights of Female Rats (Mean ± SD)

organs	group A1[a] N = 10	group A2[a] N = 10	group A3[a] N = 10	group B1[b] N = 10	group B2[b] N = 10	group B3[b] N = 9	control N = 10
body weights[c]	209.7 ± 12.4	210.8 ± 17.6	213.5 ± 19.8	203.0 ± 6.6	205.5 ± 9.8	207.5 ± 11.7	223.7 ± 15.4
brain[d]	0.59 ± 0.05	0.58 ± 0.06	0.59 ± 0.04	0.58 ± 0.04	0.60 ± 0.03	0.61 ± 0.05	0.57 ± 0.04
pituitary[d]	0.0048 ± 0.00	0.0044 ± 0.00	0.0043 ± 0.00	0.0040 ± 0.00	0.0044 ± 0.00	0.0041 ± 0.00	0.0041 ± 0.00
lung[d]	0.57 ± 0.05	0.59 ± 0.06	0.55 ± 0.07	0.64 ± 0.09	0.62 ± 0.06	0.60 ± 0.14	0.54 ± 0.09
heart[d]	0.36 ± 0.04	0.39 ± 0.04	0.37 ± 0.03	0.45 ± 0.08	0.41 ± 0.04	0.42 ± 0.06	0.39 ± 0.04
thyroids/parathyroids[d]	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
thymus[d]	0.21 ± 0.06	0.22 ± 0.06	0.21 ± 0.03	0.21 ± 0.05	0.19 ± 0.03	0.24 ± 0.04	0.22 ± 0.04
liver[d]	4.10 ± 0.78	3.86 ± 0.49	3.80 ± 0.37	4.26 ± 0.53	4.03 ± 0.42	3.87 ± 0.56	3.63 ± 0.40
spleen[d]	0.29 ± 0.04	0.29 ± 0.05	0.28 ± 0.04	0.32 ± 0.06	0.30 ± 0.04	0.29 ± 0.05	0.27 ± 0.04
stomach[d]	0.75 ± 0.20	0.76 ± 0.10	0.72 ± 0.08	0.89 ± 0.13	0.86 ± 0.08	0.67 ± 0.09	0.62 ± 0.07
kidneys[d]	0.82 ± 0.07	0.81 ± 0.11	0.79 ± 0.09	0.85 ± 0.12	0.87 ± 0.11	0.83 ± 0.09	0.78 ± 0.05
adrenals[d]	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.02	0.04 ± 0.01
uterus[d]	0.20 ± 0.04	0.19 ± 0.03	0.23 ± 0.08	0.23 ± 0.16	0.19 ± 0.03	0.21 ± 0.08	0.20 ± 0.07
ovary[d]	0.06 ± 0.02	0.07 ± 0.02	0.06 ± 0.01	0.06 ± 0.01	0.07 ± 0.02	0.06 ± 0.01	0.05 ± 0.01

[a] A1, 5000 mg/kg/day GTE; A2, 2500 mg/kg/day GTE; and A3, 1250 mg/kg/day GTE. [b] B1, 5000 mg/kg/day BTE; B2, 2500 mg/kg/day BTE; and B3, 1250 mg/kg/day BTE. [c] Unit, g. [d] Unit, % body weights.

Table 6. Hematological Values for Male Rats (Mean ± SD)

parameter[a]	group A1[b] N = 10	group A2[b] N = 10	group A3[b] N = 10	group B1[c] N = 9	group B2[c] N = 10	group B3[c] N = 10	control N = 10
WBC[1]	17.22 ± 2.00	15.39 ± 5.69	15.74 ± 4.64	16.20 ± 7.23	16.59 ± 6.90	15.96 ± 5.63	13.22 ± 2.72
RBC[2]	9.11 ± 0.87[d]	7.93 ± 0.87	8.45 ± 0.84	8.37 ± 0.47	8.45 ± 0.69	8.39 ± 1.00	7.98 ± 0.85
HGB[3]	16.45 ± 1.79[d]	14.73 ± 1.65	15.49 ± 1.27	15.43 ± 0.92	15.88 ± 1.57	15.52 ± 1.58	14.45 ± 1.54
PLT[4]	326.00 ± 42.38	335.30 ± 59.99	320.40 ± 59.07	329.30 ± 57.37	373.30 ± 39.42	340.90 ± 35.33	342.10 ± 66.11
LY[5]	63.20 ± 12.93	71.73 ± 8.60	75.98 ± 9.59	63.79 ± 9.92	63.00 ± 12.77	72.79 ± 9.74	70.90 ± 10.28
MO[6]	6.11 ± 1.65	5.58 ± 1.89	5.69 ± 1.53[d]	6.51 ± 3.11	6.17 ± 3.31	6.26 ± 2.22	5.34 ± 1.37
GR[7]	30.69 ± 14.06	22.67 ± 8.02	19.41 ± 7.71	25.98 ± 13.06	27.55 ± 15.69	20.95 ± 8.52	23.76 ± 9.63
HCT[8]	45.78 ± 5.01[d]	40.50 ± 4.17	42.09 ± 3.60	42.77 ± 2.94	43.40 ± 3.38	43.07 ± 5.33	41.13 ± 4.40
MCH [9]	18.05 ± 0.66	18.57 ± 0.67	18.35 ± 0.65	18.43 ± 0.78	18.77 ± 0.84	18.55 ± 0.61	18.14 ± 0.72
MCHC[10]	35.950 ± 0.80	36.35 ± 0.78[d]	36.81 ± 0.58[d]	36.12 ± 1.35	36.53 ± 1.04[d]	36.13 ± 1.16	35.15 ± 1.08
MCV[11]	50.23 ± 2.11	51.15 ± 2.05	49.87 ± 1.67	51.06 ± 1.69	51.38 ± 1.41	51.28 ± 1.08	51.60 ± 1.46
MPV[12]	9.54 ± 0.59	9.20 ± 0.49	9.21 ± 0.69	9.50 ± 0.40	9.44 ± 0.44	9.45 ± 0.25	9.36 ± 0.33
PCT[13]	0.31 ± 0.05	0.30 ± 0.06	0.29 ± 0.08	0.31 ± 0.05	0.35 ± 0.05	0.32 ± 0.04	0.32 ± 0.06
PDW[14]	11.95 ± 1.04	11.83 ± 0.44	11.65 ± 1.15	12.42 ± 0.90	12.15 ± 0.71	11.99 ± 0.78	12.32 ± 0.79
RDW[15]	13.40 ± 0.86	12.97 ± 0.47	13.16 ± 0.63	13.44 ± 1.10	13.28 ± 0.98	13.28 ± 1.06	13.36 ± 1.20

[a] Parameters: 1, white blood cells (thousands/μL); 2, red blood cells (millions/μL); 3, hemoglobin (g/dL); 4, platelets (thousands/μL); 5, percent of lymphocytes (%); 6, percent of monocytes (%); 7, percent of granulocyte (%); 8, hematocrit (%); 9, mean corpuscular hemoglobin (pg); 10, mean corpuscular hemoglobin concentration (g/dL); 11, mean corpuscular volume (fL); 12, mean platelet volume (fL); 13, platelet hematocrit (%); 14, platelet distribution width (fL); and 15, red blood cell distribution width (fL). [b] A1, 5000 mg/kg/day GTE; A2, 2500 mg/kg/day GTE; and A3, 1250 mg/kg/day GTE. [c] B1, 5000 mg/kg/day BTE; B2, 2500 mg/kg/day BTE; and B3, 1250 mg/kg/day BTE. [d] Statistically significant as compared to controls ($P < 0.05$).

DISCUSSION

Pu-erh teas are consumed by most people living in southern China, the Taiwan area, and Japan. In this study, the chemical compounds in Pu-erh green tea and Pu-erh black tea from Yunnan Highland were analyzed and compared for the first time. Xishuangbanna, Simao, and Lingcang are three geographic regions that serve as a principal source of Pu-erh teas supply, so the results of this study could represent certain fundamental characteristics. It has been demonstrated that polyphenols are the most abundant group of constituents in the Pu-erh green tea leaf.

Table 7. Hematological Values for Female Rats (Mean ± SD)

parameter[a]	group A1[b] N = 10	group A2[b] N = 10	group A3[b] N = 10	group B1[c] N = 10	group B2[c] N = 10	group B3[c] N = 9	control N = 10
WBC[1]	13.93 ± 4.12	13.30 ± 4.87	15.63 ± 2.5	16.06 ± 2.93	16.52 ± 4.21	15.14 ± 5.08	13.17 ± 3.77
RBC[2]	8.33 ± 0.6	8.42 ± 0.65	8.42 ± 0.93	8.27 ± 0.54[d]	9.27 ± 0.90	9.01 ± 0.93	8.84 ± 0.41
HGB[3]	15.43 ± 0.98	15.91 ± 1.14	15.66 ± 1.34	15.42 ± 1.44	16.65 ± 1.66	16.36 ± 1.71	15.68 ± 0.91
PLT[4]	377.20 ± 51.14	392 ± 78.89	345.20 ± 83.86	359.50 ± 39.71	396.50 ± 80.13	356.56 ± 53.32	339.50 ± 46.71
LY[5]	66.17 ± 7.42	75.49 ± 9.82	75.56 ± 10.46	63.32 ± 10.43	70.33 ± 6.87	61.77 ± 11.89	70.44 ± 12.71
MO[6]	4.64 ± 0.75	4.42 ± 1.33[d]	4.65 ± 1.24	5.68 ± 1.68	4.37 ± 1.35	5.59 ± 1.17	5.78 ± 1.43
GR[7]	29.67 ± 7.95	21.75 ± 7.29	19.79 ± 10.19	21.00 ± 9.30	26.17 ± 6.59	32.64 ± 11.70	23.78 ± 12.38
HCT[8]	42.69 ± 3.77	43.39 ± 2.73	43.10 ± 4.2	43.58 ± 3.26	46.47 ± 4.56	45.58 ± 4.30	44.32 ± 2.32
MCH[9]	18.56 ± 0.90	18.96 ± 1.42[d]	18.65 ± 0.80	17.12 ± 0.92	17.97 ± 0.81	18.14 ± 0.99	17.74 ± 0.60
MCHC[10]	36.25 ± 1.65	36.67 ± 1.61	36.37 ± 0.74	37.06 ± 1.26[d]	35.85 ± 1.37	35.88 ± 11.83	35.39 ± 13.3
MCV[11]	51.23 ± 2.21	51.65 ± 2.28	51.26 ± 1.57	51.62 ± 1.90	50.11 ± 0.82	50.61 ± 1.49	50.14 ± 1.34
MPV[12]	9.68 ± 0.67	9.55 ± 0.33	9.44 ± 0.53	9.91 ± 0.78	9.55 ± 0.64	9.50 ± 0.71	9.70 ± 0.67
PCT[13]	0.33 ± 0.15	0.37 ± 0.08	0.32 ± 0.08	0.29 ± 0.133	0.37 ± 0.09	0.34 ± 0.07	0.32 ± 0.05
PDW[14]	12.29 ± 0.99	12.57 ± 0.77	12.26 ± 0.90	12.50 ± 0.59	12.16 ± 0.54	12.21 ± 0.93	12.22 ± 0.86
RDW[15]	13.21 ± 0.80	12.83 ± 1.01	12.83 ± 1.07	13.31 ± 0.75	12.93 ± 1.04	12.77 ± 0.59	12.69 ± 0.60

[a] Parameters: 1, white blood cells (thousands/μL); 2, red blood cells (millions/μL); 3, hemoglobin (g/dL); 4, platelets (thousands/μL); 5, percent of lymphocytes (%); 6, percent of monocytes (%); 7, percent of granulocyte (%); 8, hematocrit (%); 9, mean corpuscular hemoglobin (pg); 10, mean corpuscular hemoglobin concentration (g/dL); 11, mean corpuscular volume (fL); 12, mean platelet volume (fL); 13, platelet hematocrit (%); 14, platelet distribution width (fL); and 15, red blood cell distribution width (fL). [b] A1, 5000 mg/kg/day GTE; A2, 2500 mg/kg/day GTE; and A3, 1250 mg/kg/day GTE. [c] B1, 5000 mg/kg/day BTE; B2, 2500 mg/kg/day BTE; and B3, 1250 mg/kg/day BTE. [d] Statistically significant as compared to controls ($P < 0.05$).

Table 8. Serum Biochemistry Values for Male Rats (Mean ± SD)

parameter[a]	group A1[b] N = 10	group A2[b] N = 10	group A3[b] N = 10	group B1[c] N = 9	group B2[c] N = 10	group B3[c] N = 10	control N = 10
TC[1]	87.57 ± 31.87	83.28 ± 24.64	75.19 ± 30.14	78.12 ± 16.00	83.55 ± 14.37	76.67 ± 24.76	82.26 ± 38.28
TG[2]	58.26 ± 13.69	55.59 ± 15.54	58.56 ± 13.36	58.28 ± 10.79	54.54 ± 11.91	43.63 ± 10.83	56.02 ± 6.30
Glu[3]	112.39 ± 24.79	121.39 ± 31.35	104.41 ± 21.33	101.95 ± 35.11	93.84 ± 19.57	102.57 ± 21.19	119.35 ± 31.10
TP[4]	6.14 ± 1.17	7.10 ± 1.93	6.81 ± 1.46	5.25 ± 1.83	7.91 ± 2.68	7.71 ± 2.36	7.57 ± 3.35
Alb[5]	4.86 ± 1.33	4.89 ± 1.32	5.03 ± 1.03	3.93 ± 1.15	3.83 ± 1.29	4.44 ± 0.92	4.04 ± 1.05
ALT[6]	42.24 ± 11.39[d]	36.83 ± 11.33	25.03 ± 6.74	34.76 ± 4.04	29.81 ± 8.05	25.39 ± 12.29	29.44 ± 8.96
AST[7]	126.65 ± 18.31	116.28 ± 18.94	117.01 ± 31.63	127.21 ± 16.21	117.20 ± 19.99	111.09 ± 37.78	111.69 ± 36.56
Crea[8]	62.94 ± 14.61[e]	57.86 ± 9.37[d]	43.30 ± 11.44	47.97 ± 5.87	48.87 ± 6.36	43.87 ± 3.96	41.98 ± 8.20
Urea[9]	9.03 ± 2.31	10.31 ± 2.88	8.97 ± 2.94	10.11 ± 3.33	8.14 ± 1.48	8.25 ± 2.33	7.64 ± 1.14
K[10]	6.10 ± 0.69	6.19 ± 0.66	6.38 ± 0.55	6.64 ± 0.94	6.52 ± 0.84	6.68 ± 0.56	6.31 ± 0.84
Na[11]	135.03 ± 8.87	137.55 ± 9.87	139.79 ± 8.27	142.21 ± 8.42	139.47 ± 3.55	144.12 ± 4.80	138.72 ± 10.56
Cl[12]	101.04 ± 8.37	103.35 ± 8.78	105.37 ± 7.31	107.60 ± 6.77	104.88 ± 3.93	108.20 ± 4.09	103.85 ± 8.81
iCa[13]	1.33 ± 0.07	1.34 ± 0.07	1.37 ± 0.07	1.36 ± 0.05	1.36 ± 0.05	1.33 ± 0.05	1.37 ± 0.08
TCa[14]	2.59 ± 0.14	2.62 ± 0.13	2.68 ± 0.15	2.66 ± 0.09	2.66 ± 0.09	2.57 ± 0.16	2.68 ± 0.17
pH	7.58 ± 0.02	7.58 ± 0.03	7.58 ± 0.03	7.63 ± 0.40	7.64 ± 0.02	7.66 ± 0.03	7.61 ± 0.02

[a] Parameters: 1, total cholesterol (mg/dL); 2, triglycerides (mg/dL); 3, glucose (mg/dL); 4, total protein (g/L); 5, albumin (g/L); 6, alanine aminotransferase (U/L); 7, aspartate aminotransferase (U/L); 8, creatinine (mmol/L); 9, urea (mmol/L); 10, kalium (mmol/L); 11, natrium (mmol/L); 12, chlorine (mmol/L); 13, ionized calcium (mmol/L); and 14, total calcium (mmol/L). [b] A1, 5000 mg/kg/day GTE; A2, 2500 mg/kg/day GTE; and A3, 1250 mg/kg/day GTE. [c] B1, 5000 mg/kg/day BTE; B2, 2500 mg/kg/day BTE; and B3, 1250 mg/kg/day BTE. [d] Statistically significant as compared to controls ($P < 0.05$). [e] Statistically significant as compared to controls ($P < 0.01$).

Table 9. Serum Biochemistry Values for Female Rats (Mean ± SD)

parameter[a]	group A1[b] N = 10	group A2[b] N = 10	group A3[b] N = 10	group B1[c] N = 9	group B2[c] N = 10	group B3[c] N = 8	control N = 10
TC[1]	106.53 ± 28.48	74.86 ± 16.06	93.55 ± 18.66	67.54 ± 13.55[d]	80.08 ± 31.84	66.74 ± 22.00[d]	92.42 ± 24.60
TG[2]	55.40 ± 11.97	63.00 ± 10.48	59.18 ± 8.81	63.75 ± 17.46	58.41 ± 18.32	60.28 ± 14.13	52.04 ± 10.49
Glu[3]	102.11 ± 26.18	110.06 ± 24.96	124.02 ± 33.35	110.05 ± 40.39	94.71 ± 23.73	120.39 ± 8.5	98.97 ± 26.89
TP[4]	7.58 ± 1.84	7.24 ± 2.40	7.24 ± 1.47	5.81 ± 2.06	6.31 ± 2.51	5.26 ± 1.56	7.58 ± 2.11
Alb[5]	5.15 ± 1.14	5.33 ± 1.52	4.71 ± 1.17	5.33 ± 2.04	4.41 ± 1.48	4.56 ± 1.47	4.35 ± 1.57
ALT[6]	38.85 ± 6.95	32.58 ± 6.65	28.99 ± 3.89	29.49 ± 6.89	32.24 ± 4.56	38.36 ± 5.92	31.87 ± 9.60
AST[7]	124.58 ± 13.62	112.42 ± 18.84	106.21 ± 15.32	117.11 ± 15.44	119.66 ± 24.52	129.30 ± 27.78	124.39 ± 22.16
Crea[8]	58.58 ± 6.75[d]	51.65 ± 6.52	49.27 ± 6.49	52.85 ± 16.71	54.82 ± 10.92	55.80 ± 7.21	43.87 ± 10.88
Urea[9]	9.35 ± 1.90	7.80 ± 1.30	9.46 ± 1.19	8.94 ± 3.69	9.05 ± 2.21	7.94 ± 1.66	8.61 ± 1.05
K[10]	6.49 ± 0.42	6.75 ± 0.94	6.67 ± 0.71	6.82 ± 0.90	6.70 ± 0.63	6.49 ± 0.78	6.79 ± 0.65
Na[11]	133.62 ± 6.28	138.99 ± 6.02	137.50 ± 6.08	142.41 ± 6.33	138.50 ± 9.08	139.49 ± 8.55	140.57 ± 7.36
Cl[12]	101.78 ± 5.56[d]	108.55 ± 6.32	105.71 ± 6.37	106.87 ± 4.47	104.67 ± 7.73	105.01 ± 8.04	108.75 ± 5.71
iCa[13]	1.27 ± 0.09[d]	1.34 ± 0.04	1.33 ± 0.05	1.35 ± 0.05	1.30 ± 0.07[d]	1.32 ± 0.04	1.37 ± 0.05
TCa[14]	2.48 ± 0.17[d]	2.61 ± 0.07	2.60 ± 0.09	2.63 ± 0.09	2.63 ± 0.09[d]	2.57 ± 0.08	2.66 ± 0.09
pH	7.61 ± 0.04	7.59 ± 0.03	7.60 ± 0.04	7.60 ± 0.03	7.60 ± 0.03	7.58 ± 0.03	7.58 ± 0.02

[a] Parameters: 1, total cholesterol (mg/dL); 2, triglycerides (mg/dL); 3, glucose (mg/dL); 4, total protein (g/L); 5, albumin (g/L); 6, alanine aminotransferase (U/L); 7, aspartate aminotransferase (U/L); 8, creatinine (mmol/L); 9, urea (mmol/L); 10, kalium (mmol/L); 11, natrium (mmol/L); 12, chlorine (mmol/L); 13, ionized calcium (mmol/L); and 14, total calcium (mmol/L). [b] A1, 5000 mg/kg/day GTE; A2, 2500 mg/kg/day GTE; and A3, 1250 mg/kg/day GTE. [c] B1, 5000 mg/kg/day BTE; B2, 2500 mg/kg/day BTE; and B3, 1250 mg/kg/day BTE. [d] Statistically significant as compared to controls ($P < 0.05$).

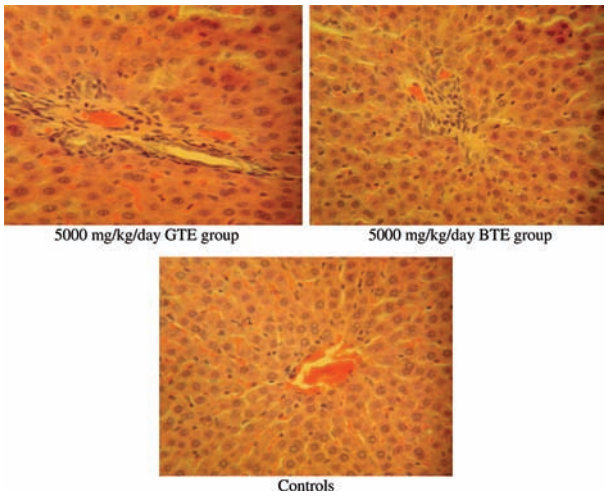

Figure 3. Histopathological changes in liver (40 × 10 times).

Among these, the catechins constitute the major components, up to 22.189% of the tea extracts, which have been identified as the major active principles of green tea. In addition, 3.259% of theogallin (**Figure 1**), which is rich in Indian and Ceylonese black teas (*24*), was detected in the Pu-erh green tea. This is a significant characteristic of *C. sinensis* (Linn.) var. assamica (Masters) Katamura cultivated in the Yunnan Highlands of China, but few reports about the biological activities and toxicity of theogallin have been available until now. It is worth noting that the tea pigment was also elevated in the Pu-erh green tea (**Table 1**). When dried at a low temperature (under 60 °C), Pu-erh green tea retained more water as compared to common green tea, which provided suitable conditions for fungus existing in the air. Therefore, a small amount of catechins in Pu-erh green tea could be oxidized to tea pigment after staying storage for a while (more than 3 months).

At the fermentation stage during the manufacture of Pu-erh black tea, the catechins are easily oxidized by polyphenol oxidase, and further polymerizations lead to TFs (*25*), TRs, and compounds of higher molecular mass (*4*). TFs contribute to the characteristic bright orange-red color of black teas, and TFs were considered to have equally effective functions with catechins in green tea (*26*). However, whether these higher molecular ingredients could have direct effects on the toxicities between GTE and BTE is unclear. In present study, GA in Pu-erh black tea was analyzed by HPLC, which is the most important phenolic acid in tea. The amount of GA increases during the fermentation because of its liberation from catechin gallates. In addition, lower levels of caffeine were detected in the BTE than GTE (3.037 vs 2.580%, respectively). Different levels of chemical constituents in GTE and BTE were the basal of varied toxicity discussed below.

In the present study, with daily oral administration of Pu-erh unfermented tea extract and fermented tea extract at dose levels up to 5000 mg/kg/day for 28 days, only limited evidence of toxicity was obtained. Reduced body weights throughout the experimental period were observed in 5000, 2500, and 1250 mg/kg/day GTE males and all groups given BTE. Similar results appeared in Jen-kun Lin's study (*23*); the body weights of rats placed on a basal diet had decreased from 5 to 30 weeks after they were given 4% green tea and 1.5 and 4% Pu-erh tea. Lower body weights were observed in the 5000, 2500, and 1250 mg/kg/day BET group males in the present study, although it is not clear to what extent the caffeine content was similar to EGCG or other kinds of tea. A study from Dulloo (*27*) suggested that there was a synergistic effect between caffeine and EGCG with regard to increased thermogenesis in brown fat tissue of rats. In addition, in vitro studies show that green tea extracts rich in catechin can inhibit the activity of digest enzymes, such as α-amylase (*28*) and the Na-dependent glucose transporter in intestinal mucosa (*29*). In the present study, however, the caffeine and catechin were lower in Pu-erh black tea (**Table 2**). There should be some special components in Pu-erh black tea that play the same role as EGCG in body weights. A study from Michael (*11*) showed that fecal fat was significantly increased to approximately 15% in rats and body weights were slightly increased when rats received 3.0 and 6.0% black and green tea and mulberry mixture extracts. Studies show that black tea is rich in TF, which inhibits pancreatic lipase in vitro (*30*), but the same conclusion has not been verified in vivo.

In the blood serum biochemistry data, an increase in ALT in 5000 mg/kg/day GTE males and Cr in 5000 mg/kg/day GTE groups and 2500 mg/kg/day males presumably reflects weak hepatotoxicity and renal toxicity of GTE. The ALT level in 5000 mg/kg/day was also increased, but the results were not significantly different with the controls. However, relative liver and kidney weights in these groups were normal as compared to the control groups. More polyphenols have been detected in GTE in the present study, of which toxic effects have been reported in rat hepatocytes and kidney (*10*, *11*). In the GTE group with a dose of 5000 mg/kg/day, this 28 day study showed a low hepatic functional disorder on the basis of the serum marker enzyme levels (ALT). In Michael Levitt's study (*11*), the ALT of rats receiving the 3.0 or 6.0% dietary concentration of a mixture of extracts of black and green teas and mulberry leaf was significantly increased relative to the controls. In addition, the increased Cr in 5000 and 2500 mg/kg/day GTE males and 5000 mg/kg/day GTE females was observed in this study, which could be related to the renal function impairment. A vitro study (*31*) suggested that EGCG was the major contributor to the observed cytotoxic effect of green tea extracts, since a similar toxicity was obtained with this constituent in a concentration range representative for the cytotoxic level of green tea extracts. A possible mechanism could be relevant to prooxidant properties of tea polyphenols or EGCG depending on the high concentration and free radical source (*15*). A related study showed that exposure of mammalian cells to polyphenols is accompanied by the increase in intracellular reactive oxygen species (ROS) levels, and the ROS-induced cell injuries could be one toxicological mechanism of tea polyphenols (*32*, *33*). Connected to this study, the EGCG content in GTE was significantly higher than the BTE, which suggests that EGCG and/or other catechins could be the major chemical compounds in GTE that lead to toxicities in rats. The relative mechanism will be discussed in a later study.

As to the decrease in TC in 5000 and 1250 mg/kg/day BTE males, a recent report proved that Pu-erh-fermented tea significantly decreased the serum cholesterol levels in a rat hyperlipidemia model (*5*) and slightly decreased the TC at the 15th week by the given Pu-erh tea leaves (*23*). The decreasing effect of TC level is widely accepted to be beneficial in lowering atherosclerotic risk. However, in this study, these effects were not observed in both sexes and the 2500 mg/kg/day BTE male group, so it is not considered to be toxicologically meaningful.

Other findings for clinical signs, hematology (**Tables 6** and **7**), serum biochemistry parameters (**Tables 8** and **9**), and organ weights (**Tables 4** and **5**) were without toxicological significance/dose relation. For example, increased RBC, HGB, and HCT levels appeared in the 5000 mg/kg/day GTE and BTE males, which could associate with immediate exercises after tea gavage (*34*). A decrease in the relative brain and testes weights in 5.0% GTE males was a reflection of lower body weights, and it was considered not to be toxicologically significant. The other

serum biochemistry changes including Cl, Na, and Ca decreasing in 5000 mg/kg/day GTE females and Ca decreasing in 2500 mg/kg/day BTE females without dose relation were not considered tea extract-related.

In conclusion, the present 28 day toxicity study of dietary Pu-erh tea extract in Wistar rats demonstrated reduced body weights in 5000 mg/kg/day GTE males, an increase in ALT and Cr in 5000 mg/kg/day GTE males, and an increased Cr in 5000 mg/kg/day GTE females and 2500 mg/kg/day GTE males. Moreover, bile duct hyperplasia in the liver was observed in pathological examination. The target organs of GTE were considered to be liver and kidney in the Wistar rats. Thus, the repeated dose 28 day toxicity tests can provide initial information to evaluate the toxic characteristics of a test compound. Most importantly, the quantified no observed adverse effect level (NOAEL) could not be achieved, so further toxicological tests including subchronic and chronic toxicity testing may be required.

SAFETY

All of the materials including Pu-erh tea, animals, reagents, and so on used in this study are harmless to humans, and the experimental methods have been proven to be safe.

ABBREVIATIONS USED

ALT, alanine aminotransferase; Alb, albumin; AST, aspartate aminotransferase; Cl, chloride; Cr, creatinine; EGCG, (−)-epigallocatechin 3-gallate; EGC, (−)-epigallocatechin; ECG, (−)-epicatechin 3-gallate; EC, (−)-epicatechin; GA, gallic acid; GCG, (+)-gallocatechin gallate; Hct, hematocrit; Hb, hemoglobin concentration; HPLC, high-performance liquid chromatography; iCa, ionized calcium; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; NOAEL, no observed-adverse-effect level; K, potassium; BTE, Pu-erh black tea extracts; GTE, Pu-erh green tea extracts; TFs, theaflavins; TRs, thearubigins; TBs, theabrownins; RBC, red blood cell count; ROS, reactive oxygen species; Na, sodium; TC, total cholesterol; TCa, total calcium; TG, triglyceride; TP, total protein; WBC, white blood cell count.

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