Journal of Ethnopharmacology 175 (2015) 14–20

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

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

Green tea polyphenols decreases uric acid level through xanthine oxidase and renal urate transporters in hyperuricemic mice

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ARTICLE INFO

Article history:
Received 12 May 2015
Received in revised form
26 July 2015
Accepted 26 August 2015
Available online 3 September 2015

Keywords:
Green tea polyphenols
Xanthine oxidase
Urate transporters
Hyperuricemia

ABSTRACT

Ethnopharmacological relevance: Green tea is a Chinese materia medica with the main functions of "inducing urination and quenching thirst". Green tea polyphenols (GTP) are generally acknowledged as the main active fraction with multiple pharmacological functions in green tea. However, the effect of GTP on hyperuricemia is not clear till now.

Aim of study: The present study was carried out to investigate the effect of GTP on serum level of uric acid in potassium oxonate (PO)-induced hyperuricemic mice, and explore the underlying mechanisms from two aspects of production and excretion of uric acid.

Materials and methods: PO and GTP were intragastricly administered to mice for consecutive 7 days. Serum level of uric acid, and xanthine oxidase (XOD) activity in serum and liver were examined. Simultaneously, expression of XOD protein in liver was analyzed by Western blot assay. Expressions of urate transporters including urate-anion transporter (URAT) 1, organic anion transporter (OAT) 1 and 3 in kidney were analyzed by immunohistochemistry staining method.

Results: 300 and 600 mg/kg GTP significantly decreased serum level of uric acid of hyperuricemic mice in a dose-dependent manner ($p < 0.05$ or $p < 0.01$). Besides, 300 and 600 mg/kg GTP markedly reduced XOD activity in serum and liver of hyperuricemic mice (both $p < 0.01$). Furthermore, 300 and 600 mg/kg GTP clearly reduced XOD expression in liver, as well as reduced URAT1 expression and increased OAT1 and OAT3 expressions in kidney of hyperuricemic mice ($p < 0.05$ or $p < 0.01$).

Conclusions: These results demonstrated that GTP had the effect of lowering uric acid through decreasing the uric acid production and increasing uric acid excretion. Our study suggested that GTP would be a promising candidate as a novel hypouricaemic agent for further investigation.

1. Introduction

Tea, made from the dried leaves of the plant *Camellia sinensis* Theaceae, is the second most popular beverage in the world after water. There are three types of tea called green, oolong, and black tea, which differ in terms of machining process and chemical constituents (Cheng, 2006). Green tea is dominantly consumed in Asian but is becoming increasingly common globally. Green tea is produced by steaming or panfrying tea leaves, and this process inactivates the polyphenol oxidases and preserves the product by stabilizing the chemical constituents. Green tea is characterized by the presence of a high concentration of polyphenolic constituents known as catechins. The major green tea polyphenols (GTP) are (−)-epigallocatechin-3-gallate (EGCG), (−)-epigallocatechin (EGC), (−)-epicatechin-3-gallate (ECG) and (−)-epicatechin (EC), (−)-gallocatechingallate (GCG) and (+)-Catechin (C), and EGCG is the most abundant green tea catechin and accounts for about 30–50% of the catechin content (Sang et al., 2011).

Green tea was first used as a Chinese material medica in ancient China thousands of years ago. In the theory of traditional Chinese medicine, green tea has the functions of "inducing urination, quenching thirst, detoxification and healing sore and ulcer of skin", and is used to treat gouty foot attack and foot ulceration

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http://dx.doi.org/10.1016/j.jep.2015.08.043
0378-8741/

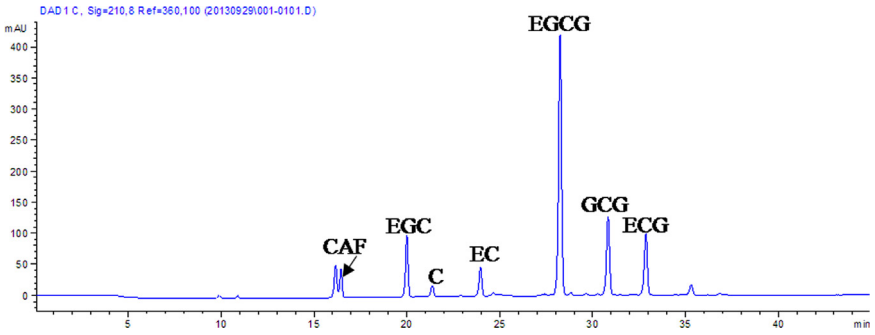

Fig. 1. The chromatogram of GTP using a HPLC system.

(Yang et al., 2014). In modern society the health benefits of green tea are becoming increasingly recognized (Khan and Mukhtar, 2013), and GTP is considered as the main active fraction of green tea (Khan and Mukhtar, 2007). GTP has been documented to have preventive effects against many chronic diseases including obesity (Huang et al., 2013), atherosclerosis (Rosenblat et al., 2008), neurodegenerative disease (Weinreb et al., 2009) and cancer (Forester and Lambert, 2011).

Uric acid is the terminal product of the metabolism of purine nucleotides. Xanthine oxidase (XOD) in liver is the key enzyme to catalyze uric acid production (George and Struthers, 2009), and urate transporters including urate-anion transporter (URAT) l, organic anion transporter (OAT) 1 and 3 in kidneys are the main transporters for uric acid clearance (So and Thorens, 2010). Hyperuricemia (HUA), characterized by the high level of uric acid in blood, is a pathological condition which may originate from the increase in purine metabolism (excessive production of uric acid) and/or impairment of renal clearance of uric acid (Merriman and Dalbeth, 2011). Chronic HUA may result in the deposition in joint tissues of monosodium urate crystals, which promotes inflammation and causes gout, a form of arthritis accompanying intense pain. Plenty of evidence suggests that HUA is also closely associated with obesity (Wang et al., 2013), hypertension (Krishnan, 2014), metabolic syndrome (You et al., 2014) and cardiovascular disease (Gustafsson and Unwin, 2013).

Study has found that black tea consumption could significantly reduce plasma uric acid and C-reactive protein levels in humans susceptible to cardiovascular diseases (Bahorun et al., 2010). However, the effect of GTP on HUA is not clear up to now. In this study, to figure out whether GTP could improve HUA, we investigated the effects of GTP on serum level of uric acid, activity and expression of XOD, as well as expressions of URATl, OAT1 and OAT 3 in potassium oxonate (PO)-induced hyperuricemic mice.

2. Materials and methods

2.1. Chemicals and reagents

All chemicals and reagents were purchased from Sigma-Aldrich (USA) unless otherwise specified. Allopurinol was purchased from Chongqing Qingyang Pharmaceutical Co., Ltd. (China). Antibodies against XOD, URATl, OAT1, OAT3 and β-actin were purchased from Santa Cruz (USA). Polyvinylidene fluoride (PVDF) membrane and enhanced chemiluminescence (ECL) reagents were purchased from Milipore (USA). Enhanced bicinchoninic acid (BCA) protein assay kit was purchased from Beyotime Biotech (China). XOD activity detection kit and uric acid detection kit were purchased from Nanjing Jiancheng Biotech (China). Two-step polymer (nonbiotin) detection kit was purchased from Beijing Zhongshan Golden Bridge Biotechnology Co., Ltd. (China).

2.2. GTP preparation

Green tea leaves (100 g) were brewed three times, each with 1 L of hot distilled water (95 °C) for 30 min. The infusion was cooled to room temperature, and then filtered with cellulose filter paper (0.45 μm, Millipore, USA). The filtrate was then concentrated using a vacuum rotary evaporator. Then the extract was extracted with 1.5 fold chloroform for four times to remove chlorophyll and caffeine. After that, aqueous phase were extracted with 1.5-fold ethyl acetate for four times, and the ethyl acetate phase was evaporated with a vacuum rotary evaporator. The extraction yield of GTP was 15.6% (w/w) and GTP was kept at −20 °C until used.

2.3. High-performance liquid chromatography analysis (HPLC) of GTP

HPLC analysis was performed using Hewlett Packard Agilent 1100 series HPLC System. Sample solution was injected onto a Supelco Discovery RP Amide C16 guard column (15 cm × 4.6 mm, 5 μm) (Sigma-Aldrich, USA). A gradient elution was carried out using the following solvent systems: mobile phase A-double distilled water/phosphoric acid (99.95/0.05; v/v); mobile phase B-acetonitrile. The elution was performed with a gradient procedure as follows: 0–1 min, 2% B; 2–60 min, from 2% B to 50% B. The column heater was kept at 35 °C. The flow rate used was 0.8 ml/min and detection was performed at 210 nm. Each sample (10 μl) was injected into the column after filtration through a 0.45 μm filter disk. Identification of the tea polyphenols was carried out by comparing the retention times and the UV absorbance of the unknown peaks to those of the standards. The components content of GTP were showed in Fig. 1 and Table 1. In this study, the content of total polyphenols in the extract was 81.41%.

2.4. Experimental animals

Male Kunming mice (20 ± 2 g) were purchased from Chongqing Medical University (China). The mice were housed in a temperature-controlled room (23 ± 2 °C) under a 12-h light/dark cycle with available food and water ad libitum. The mice were allowed one week to adapt to their environment before initiation of the experiments. All animal procedures were approved by the institutional animal care and use committee of Chongqing Technology and Business University (Ethics No. CTBU20131119).

2.5. Induction of hyperuricemia and drugs treatment

In this study, PO was used to induce HUA in mice as previously described (Wang et al., 2011). Mice were divided into 6 groups, which were (1) Control group, (2) Model group, (3) 5 mg/kg allopurinol (API, as a positive control drug) group, (4) 150 mg/kg GTP