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Original article

The anti-hyperuricemic effect of epigallocatechin-3-gallate (EGCG) on hyperuricemic mice

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ABSTRACT

Epigallocatechin-3-gallate (EGCG), a major constituent of green tea catechin, has been used for antioxidant. This study aimed to evaluate the antihyperuricemic activity of EGCG on hyperuricemic mice. We demonstrated that serum uric acid (UA) level was decreased significantly with dose-dependence by EGCG treated with 10, 20, and 50 mg/kg. Compared with the model, data on blood urea nitrogen (BUN) supported that there was significance with high dose of EGCG (50 mg/kg). Levels of serum creatinine (Cr) in each EGCG-treated group were decreased but not significant; the activities of hepatic xanthine oxidase (XOD) and adenosine deaminase (ADA) in high dose groups' EGCG were notably lower than those of model group. EGCG could downregulate the renal mRNA expression levels of glucose transporter 9 (GLUT9) and urate transporter 1 (URAT1) on hyperuricemic mice. These results presented that EGCG had obvious hypouricemic and renal protective effects on hyperuricemic mice. Our data may have a potential value in clinical practice in the treatment of hyperuricemia.

1. Introduction

Hyperuricemia, a metabolic disease, is characterized by high uric acid levels in the blood that precipitate urate crystals in both the kidneys and joints. Hyperuricemia is a well-known risk factor for gout, hypertension and diabetes [1]. 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 [2].

Anti-hyperuricemic drugs such as allopurinol and probenecid are demonstrated to produce adverse effects such as hypersensitivity and agranulocytosis [3,4]. Therefore, it underlines much impetus for urgent need of safer and more effective anti-hyperuricemic agents [5,6].

Tea (*Camellia sinensis L.O. Kuntze*) is the most popular flavored and healthy beverage beyond water in the world [7] and it is a kind of Chinese materia medicine [8]. The major four catechins of green tea polyphenols (GTP) are (−)-epigallocatechin-3-gallate (EGCG), (−)-epigallocatechin (EGC), (−)-epicatechin-3-gallate (ECG) and (−)-epicatechin (EC). EGCG is the major catechin and account for 50–80% of the total catechins in tea [9]. In modern society, the healthy benefits of green tea are becoming increasingly recognized [10], and GTP is regard to the main active fraction of green tea, especially EGCG [11].

Study has found that black tea could significantly reduce uric acid and C-reactive protein levels in humans susceptible to cardiovascular diseases [12], and green tea polyphenols could decrease uric acid level through xanthine oxidase and renal urate transporters on hyperuricemic mice [13]. In this study, to figure out whether EGCG could improve hyperuricemia, we investigated the effects of EGCG on serum level of uric acid, enzyme expressions of XOD, ADA and relative renal mRNA expressions.

2. Materials and methods

2.1. Reagents and drugs

1.2 AP was obtained from Shanghai Sine Wanxiang Pharmaceutical Co., Ltd (China). Potassium oxonate (PO) was purchased from Nanjing Duly Biotech Co., Ltd (China). Yeast extract was the products of Beijing Aobox Biotechnology Co., Ltd (China). Green tea was purchased from Chinese market. Standard substances of EGCG was purchased from Shanghai Yuanye Bio-Technology Co., Ltd (China) (Fig. 1). Assay kits of UA, creatinine (Cr), blood urea nitrogen (BUN), adenosine deaminase (ADA) and xanthine oxidase (XOD) were purchased from Nanjing Jiancheng Biotech (China). The HiScriptII1 st Strand cDNA Synthesis

Abbreviations: UA, uric acid; BUN, blood urea nitrogen; Cr, creatinine; XOD, xanthine oxidase; ADA, adenosine deaminase; AP, allopurinol; PO, potassium oxonate; EGCG, epigallocatechin-3-gallate; GLUT9, glucose transporter 9; URAT1u, rate transporter 1

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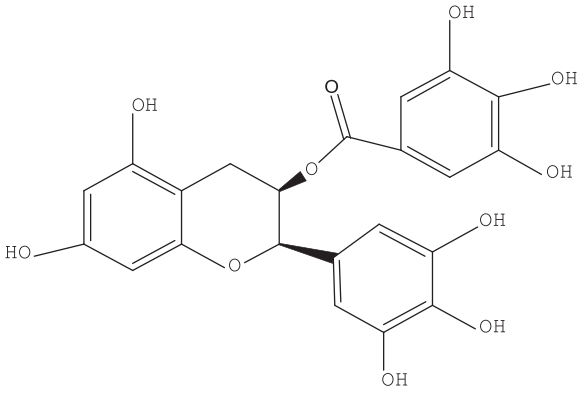

Fig. 1. Chemical structures of EGCG.

Kit and SYBR Green Master Mix Kit were obtained from Vazyme Biotech Co., Ltd (China).

2.2. Preparation of EGCG

Green tea leaves (50 g) were brewed three times, each with 500 mL of boiling distilled water for 30 min. The infusion was cooled to room temperature, and then filtered with cellulose filter paper (20 μm, Xinxing, China). The filtrate was evaporated with vacuum rotary evaporator and 1.5 fold dichloromethane was added into it to remove chlorophyll and caffeine four times. After that, the aqueous phase was evaporated with a vacuum rotary evaporator. After extracting with ethyl acetate, the extractive was purified by Preparative Medium Pressure Liquid Chromatography (MPLC). The purity of EGCG would be improved by silica gel chromatography. The gradient elution was carried out using the following: dichloromethane/methyl alcohol (15:1; v/v) and the aqueous phase was evaporated with vacuum rotary evaporator, then save the sample at −20 °C after freeze-drying.

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

HPLC analysis was performed using Waters 2489 series HPLC system, that was carried out using a Luna 5 u C18 (2) 100A column (250 × 4.60 mm, 5 μm) (Phenomenex,USA). Column temperature was kept at 35 °C. The gradient elution was composed of mobile phase A (water containing 0.17% acetic acid) and mobile phase B (acetonitrile). The gradient of mobile phase A was: 0–10 min, 100%, 10–25 min, from 100% to 68%, 25–35 min, from 68% to 100% and then at 100% for 10 min. The flow rate used was 1 mL/min and the detection was performed at 278 nm. Each sample (10 μL) was injected into the column after filtration through a 0.22 μm filter disk.

2.4. Test animals

Male Kunming mice (25 ± 2.0 g) used in the study were purchased from Shanghai SLAC Laboratory Animal Co. Ltd. (Shanghai, China). All mice were housed under the following conditions: temperature 22 ± 2 °C, humidity 50 ± 10%, and constant 12-h/12-h light/dark cycle. They were acclimatized for 1 week with free access to water and food before the experiments. The protocol was approved by the Laboratory Animal Center of SLAC, Shanghai, China (Permit Number: 2012-0002). All animals were humanly treated in accordance with the Guide for the Care and Use of Laboratory Animals (Ministry of Science and Technology of the People's Republic of China) and the animal protocols were reviewed and approved by the ethics committee of Anhui Agricultural University.

2.5. Building hyperuricemic model and drug administration

The mice were randomly divided into six groups (n = 6 each) and the experiment was performed as follows: (1) the mice in the normal control group were intragastrically administrated with 0.5 mL of saline consecutive 7 days; (2) the mice in the model group were intragastrically treated with 0.5 mL of saline for continuous 6 days and treated with PO (250 mg/kg) and yeast extract (7.5 g/kg) dissolved in distilled-water on the seventh day [14]; (3) the mice in the AP group were intraperitoneally injected with 5 mg/kg AP everyday, but on the seventh day, they were treated with AP 1 h after modeling; the mice in groups (4)–(6) group were intragastrically treated with 10, 20 and 50 mg/kg EGCG, respectively, for 6 days, and on the seventh day, they were treated with EGCG 1 h after modeling. Food, but not water, was withdrawn from the animals 12-h prior to drug administration. All drugs given were based on body weight measured immediately prior to each dose. No adverse events were observed during the 7-day experimental period.

2.6. Blood, liver and kidney samples collection

The mice were sacrificed under anesthesia 1 h after the last drug administration, and peripheral blood from the ophthalmic vein was collected into an Eppendorf tube. The blood was allowed to clot for approximately 1 h at room temperature and centrifuged at 3000 rpm for 10 min to obtain serum. The serum samples were stored at −20 °C until assayed. Serum uric acid levels, creatinine (Cr), blood urea nitrogen (BUN) were determined using the phosphotungstic acid method [15]. Livers were excised and rinsed in ice-cold saline, and pieces of hepatic tissue were stored at −80 °C. Before determination, the tissue was homogenized in nine volumes of saline. The homogenate was then centrifuged at 3000g for 10 min. The lipid layer was carefully removed and the final supernatant was used for assessing XOD and ADA activities. One kidney of each mice was quickly collected in an RNase-free Eppendorf tube filled with RNAstore reagent, the other was quickly collected in eppendorf tube filled with 10% formalin.

2.7. Renal histological analyses

Briefly, The renal samples were fixed in 10% formalin, dehydrated in gradual ethanol, embedded in paraffin, cut into 4 μm thick sections, and stained with hematoxylin and eosin staining (HE) [16].The renal sections were observed using a light microscope at magnification of 400×.

2.8. Analysis of mRNA expression by real-time RT- PCR

Total RNA was extracted from kidney tissue using RNA isolater (Vazyme Biotech Co., Ltd (China)). The HiScriptII1 st Strand cDNA Synthesis Kit (Vazyme Biotech Co., Ltd, China) was used to reverse-transcribe RNA into cDNA. SYBR Green Master Mix Kit (Vazyme Biotech Co., Ltd, China) was chosen for amplification on a CFX System (Bio-Rad). The gene-specific primers as shown in Table 1 were referred

Table 1
Primers sequences for RT- PCR.

Gene	Direction	Sequence
Glyceraldehyde phosphate dehydrogenase (GAPDH)	Forward	5′-CAAGGTCATCCATGACAACTTTG-3′
	Reverse	5′-GTCCACCACCCTGTTGCTGTAG-3′
GLUT9	Forward	5′-GAGATGCTCATTGTGGGACG-3′
	Reverse	5′-GTGCTACTTCGTCCTCGG-3′
URAT1	Forward	5′-GCTACCAGAATCGGCACGCT-3′
	Reverse	5′-CACCGGGAAGTCCACAATCC-3′