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Original Article

Lactic acid bacteria-fermented product of green tea and *Houttuynia cordata* leaves exerts anti-adipogenic and anti-obesity effects

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ABSTRACT

Obesity is associated with higher risks of developing diabetes and cardiovascular disease. Green tea, rich in polyphenolic compounds such as epigallocatechin gallate (EGCG) and epigallocatechin (EGC), has been shown to display anti-obesity effects. *Houttuynia cordata* leaves have also been shown to exhibit anti-obesity effects due to their chlorogenic acid content. Lactic acid bacteria are able to increase the production of polyphenolic compounds. This study aims to develop a novel anti-obesity fermentation product by combining *H. cordata* leaf tea with green tea, using *Lactobacillus paracasei* subsp. *paracasei* NTU 101 (NTU 101) for fermentation due to the advantages of bioconverting the polyphenolic compounds. The regulation of adipogenesis factors and the anti-obesity effect of the NTU 101-fermented tea were evaluated in an *in vitro* 3T3-L1 pre-adipocyte model and an *in vivo* obese rat model, respectively. The results show that the NTU 101-fermented tea, which contained higher EGCG, EGC, and chlorogenic acid levels than unfermented tea, was able to inhibit the lipogenesis of mature 3T3-L1 adipocytes by the stimulation of lipolysis. Furthermore, the body weight gain, body fat pad, and feeding efficiency of obese rats, induced with a high fat diet, were decreased by the oral administration of NTU 101-fermented tea. The significant anti-obesity effect was probably due to lipolysis. However, NTU 101 bacteria cells and EGCG may also act as functional ingredients to contribute to the anti-obesity effects of NTU 101-fermented products.

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1. Introduction

The main cause of obesity is a greater intake than expenditure of energy, but there are various other causes including environmental factors (such as diet, lifestyle, lack of exercise, and medication), genetic factors, and psychological stresses. However, diet and lifestyle are considered the main causes of modern-day obesity. Obesity is believed to result from an increase in the number or size of adipocytes. The proliferation and differentiation of preadipocytes into mature adipocytes is a key stage in the development of obesity [1].

In *in vitro* studies, 3T3-L1 preadipocytes are mainly used to explore adipocyte differentiation and the associated molecular mechanisms. 3T3-L1 preadipocytes are a representative cell type for typical obesity and are the most widely used fat cells in studies on the differentiation of preadipocytes into adipocytes [2]. This differentiation results in triglyceride (TG) accumulation. During preadipocyte differentiation, substantial increases in gene expression of CCAAT/enhancer-binding protein α (*C/EBP*α) and peroxisome proliferator-activated receptor γ (*PPAR*γ) occur [3]. The mitogen-activated protein kinase (MAPK) pathway regulates the mRNA expression of *C/EBP*α and *PPAR*γ, which in turn inhibits the differentiation of 3T3-L1 preadipocytes into adipocytes [4]. When entering the differentiation stage, the characteristics of mature adipocytes become increasingly more apparent: externally, the cells change from fibrous to circular and intracellular lipid droplets accumulate [2,5].

Many natural products such as herbs and teas perform multiple health functions due to their functional compounds. However, these compounds in herbs and teas cannot be easily absorbed or used by the human body. These substances can undergo bioconversion mediated by microorganisms in which they are broken down into smaller molecules via enzymatic reactions to facilitate their utilization and health benefits [6–8]. For this reason, probiotics have been added to foods and Chinese herbal medicines for fermentation, to increase the nutrient content in foods and enhance the activity of the original ingredients, improve the nutritional value of the food products, and add new value [9]. It has been suggested that the addition of microorganisms into Chinese herbal medicine for fermentation results in the breakdown of the original ingredients, increasing the efficacy of the medicine [10], the alteration of the environmental factors for the original ingredients, increasing their bioavailability [9], or the synthesis of new active substances via microbial metabolism.

Lactobacillus paracasei subsp. *paracasei* NTU 101 (NTU 101), a strain isolated from infant feces, is resistant to stomach acid and bile in natural conditions. This study used NTU 101 to ferment herbal extracts (green tea and *Houttuynia cordata* leaves) and used bioconversion mechanisms to improve the levels of active ingredients in the fermented products. Green tea is rich in epigallocatechin gallate (EGCG), which was reported in a previous study to promote the expression of genes related to lipid oxidation in skeletal muscle [11]. *H. cordata* leaves are rich in chlorogenic acid, which has been shown to confer beneficial effects on lipid and glucose metabolism. It was suggested that the administration of chlorogenic acid to rats fed a high-fat diet will lead to the reduction of serum TG and cholesterol levels [12]. We used NTU 101 to ferment green tea and *H. cordata* leaf tea and aimed to increase EGCG and chlorogenic acid levels through NTU 101 bioconversion during the fermentation process. We thereby aimed to produce a fermented product, rich in NTU 101, EGCG, and chlorogenic acid. We further investigated the effects of the fermented product on body fat reduction, with the expectation that the results of this study may help to develop a new type of health food that contains lactic acid bacteria and can reduce body fat.

2. Materials and methods

2.1. Chemicals

LC grade acetonitrile, chloroform, methanol, and dimethyl sulfoxide were purchased from Merck Co. (Darmstadt, Germany). Tryptone, yeast extract, peptone, malt extract, potato dextrose agar, and Bacto-agar were purchased from Difco Co. (Detroit, MI, USA). Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum were purchased from Invitrogen Life Technologies (Carlsbad, CA, USA). Dexamethasone, isobutylmethylxanthine, insulin, oil-red O, heparin, and *p*-nitrophenyl butyrate were purchased from Sigma Chemical Co. (St Louis, MO, USA). Trypan blue stain was purchased from Gibco BRL Life Technologies Inc. (Gaithersburg, MD, USA).

2.2. Microorganism and seed cultures

L. paracasei subsp. *paracasei* NTU 101 (NTU 101; DSMZ 28047), originally isolated from an infant [13], was maintained on MRS agar at 37 °C for 48 h. Seed cultures were prepared by transferring a loopful of colony from MRS agar into a 500-mL glass bottle containing 500 mL MRS medium. The cultures were incubated at 37 °C for 48 h. After that, inoculum sizes of 5% were transferred to a submerged cultured substrate.

2.3. Bioconversion fermentation of herbal tea L. paracasei subsp. paracasei NTU 101

H. cordata leaf (36 g) and green tea (36 g) were mixed and extracted with RO water (2 L) at 95 °C for 1 h for the preparation of the herbal tea. This tea was defined as the unfermented herbal tea and a portion was set aside and used as a sample in the animal test after freeze-drying. After cooling, the herbal tea (2 L) was used as the bioconversion fermentation medium for NTU 101. The cultures were incubated at 37 °C for 10 h. After submerging the culture, the NTU 101-fermented tea was dried by freeze dryer. The dried product was analyzed for viable cell number, EGCG, epicatechin gallate (ECG), and chlorogenic acid respectively. It was also used as samples in the cell test and animal test for the evaluation of its anti-adipogenesis and anti-obesity effects.

2.4. Determination of EGCG, ECG, and chlorogenic acid

The NTU 101-fermented tea and the unfermented herbal tea (10%, w/v) were filtered with a 0.45 μm pore sized filter and analyzed by high-performance liquid chromatography (HPLC, Model L-2130, Hitachi Co., Tokyo, Japan) on a C_{18} column

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