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Anti-adipogenic Effects of *Dongchimi* Nano Juice in Mouse 3T3-L1 Adipocytes

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Abstract

The anti-adipogenic effect of *dongchimi* nano juice prepared using a nano-filtering process was investigated by measuring leptin and glycerol levels and the expression of a peroxisome proliferator-activated receptor-γ (PPARγ) gene as indicators of lipid accumulation or lipolysis. Red pepper powder, seeds of red pepper, garlic, and ginger were added in the preparation of *dongchimi*. *Dongchimi* was fermented to reach the optimal fermentation period, followed by nano-filtration in the range of 0.0005~0.1 μm. The lactic acid bacteria of *dongchimi* nano juice were removed completely by a nano-filtering process. Treatment of *dongchimi* nano juice induced glycerol release in the 3T3-L1 adipocytes and decreased the mRNA expression level of PPARγ. These results suggested that *dongchimi* nano juice may enhance lipolysis and modulate adipogenesis in 3T3-L1 cells.

Key words: *dongchimi*, nano-filtering process, 3T3-L1 adipocytes, PPARγ, anti-adipogenic effect

INTRODUCTION

Obesity is a heavy accumulation of fat in the body's fat cells to such a serious degree that it greatly increases the risk of obesity-associated diseases such as heart disease, hypertension, stroke, cancer, diabetes, and osteoarthritis (1-4). For these reasons, many studies have been conducted to develop functional foods. Korean traditional fermented foods could be a major candidate related to antiobesity effect.

Preadipocyte cell lines such as 3T3-L1 cells and the expression of adipocyte-specific related gene facilitate the investigation of regulatory mechanisms of lipid metabolism related to adipocyte differentiation (5,6). For adipogenesis, peroxisome proliferator-activated receptor-γ (PPARγ), CCAAT/enhancer-binding proteins, and adipocyte determination and differentiation-dependent factor 1/sterol regulatory element-binding protein (SREBP)-1 are known to be critical activators (7,8). One of the earliest recognized functions of PPAR was its central role in adipocyte differentiation. Misexpression of PPARγ in fibroblasts is enough to drive these otherwise non-adipogenic cells into adipogenesis (9).

Dongchimi, a water-based kimchi, is a favorite kimchi during winter in Korea (10-12). The origin of *dongchimi* is very different from that of other kimchi. Ingredients such as garlic, ginger, red pepper powder, green onion and salted anchovy juice are used with whole radish in the preparing of *dongchimi*. Additions of some items like leaf mustard, Korean pear, young scallion, and fermented chilies can create a better taste for this of water-based kimchi. Its juice has a particular taste and makes anyone feel refreshed. Recently, many approaches have been conducted to find a new type of traditional food. We are also interested in *dongchimi* juice as a health alternative to soft drinks.

In this study, in order to develop an alternative health soft drink made of *dongchimi* juice as a new type of traditional food, we adopted the nano-filtering process in the preparing of *dongchimi* juice in stead of sterilizing by heat or pasteurization. For manufacturing of nano-filtered *dongchimi* juice (nano juice), *dongchimi* was fermented to reach the optimal fermentation period, followed by nano-filtration in the range of 0.0005~0.1 μm. The anti-adipogenic effects of *dongchimi* nano juice prepared using the nano-filtering process were investigated by measuring leptin and glycerol levels and the expression of PPARγ gene as indicators of lipid accumulation or lipolysis.

MATERIALS AND METHODS

Ingredients and preparations of *dongchimi* juice

Dongchimi was provided by Seojung Cooking Co. (Seoul, Korea). Red pepper powder, seeds of red pepper, garlic, and ginger were added as ingredients for *dongchimi*.

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Dongchimi was fermented for 72 hrs at 25°C to reach the optimal fermentation period (12), followed by nano-filtration in the range of 0.0005～0.1 μm. In order to establish the manufacturing process of *dongchimi* nano juice accompanied with nano-filtration, the *dongchimi* raw juice accompanied without nano-filtration was used as a control.

Cell culture and adipocytes differentiation

3T3-L1 mouse cells were purchased from the American Type Culture Collection (ATCC, USA). Dulbecco's Modified Eagle Medium (DMEM) and fetal bovine serum (FBS) were purchased from Gibco Service Co. (USA). Methylisobutylxanthine (IBMX), dexamethasone (DEX), and insulin (INS) were purchased from Sigma Chemical Co. (USA). The mouse 3T3-L1 pre-adipocytes were grown to confluence in DMEM with 10% FBS at 37°C in a humidified atmosphere of 5 % CO_2. At 1 day post-confluence (designated "day 0"), cell differentiation was induced with a mixture of IBMX (0.5 mM), DEX (0.25 μM), and INS (5 μg/mL) in DMEM containing 10% FBS. On day 2 and day 4, the medium was replaced with DMEM containing 10% FBS and INS (5 μg/mL) only. On day 6 and thereafter the medium consisted of only DMEM plus 10% FBS, which was subsequently replaced every 2 days. At day 8 after inducing differentiation, *dongchimi* raw/nano juices before/after the nano-filtering process were treated into the adipocytes at concentrations of 20 μL/mL and 40 μL/mL, respectively. After 24 hrs, the medium was removed for analysis of leptin, glycerol, and triglyceride levels.

Measurement of leptin, glycerol levels

Measurement of the leptin level was performed with a sandwich enzyme-linked immunosorbent assay (ELISA). Anti-mouse leptin, recombinant mouse leptin, and biotinylated anti-mouse leptin antibodies were purchased from R&D Systems (MN, USA).

The glycerol level was determined using an enzymatic reagent, a free glycerol reagent (Sigma, USA), directed by the protocol of GPO-TRINDER (Sigma, USA).

Reverse transcriptase-PCR analysis of PPARγ mRNA

Total RNA was isolated from 3T3-L1 adipocytes using a Trizol reagent (Invitrogen Co., CA, USA) following the manufacture's recommendations. One μg of total RNA was used for first-strand cDNA synthesis using Superscript II reverse transcriptase (BD Bioscience, Palo Alto, CA). Reverse transcription was performed at 30°C for 10 min, 42°C for 30 min, and 99°C for 5 min to inactivate the avian myeloblastosis virus RTXL. Primers to specifically amplify the genes interested were as follows: for PPARγ gene, forward 5'-GAG ATG CCA TTC TGG CCC ACC AAC TTC GG-3' and reverse 5'-TAT CAT AAA TAA GCT TCA ATC GGA TGG TTC-3'; for β-actin gene, forward 5'-AGC CAT GTA CGT AGC CAT CC-3' and reverse 5'-TCC CTC TCA GCT GTG GTG GTG AA-3'. Amplification was performed in a master-cycler (Eppendorf, Hamburg, Germany) with denaturing at 94°C for 1 min, annealing at 54°C for 1 min, extension at 72°C for 30 sec for 25 cycles and finally 72°C for 7 min. The amplified PCR products were run in 1.0% agarose gels and stained with ethidium bromide, and visualized under UV light.

Statistical analysis

Data were expressed as mean±standard error values. Means with different letters are significantly different ($p<0.05$) by Duncan's multiple range tests. Each experiment was replicated at least 3 times.

RESULTS AND DISCUSSION

Properties of *dongchimi* nano juice

Prepared *dongchimi* was fermented for 72 hrs at 25°C to reach the optimal fermentation period, followed by the nano-filtration process. *Dongchimi* juice prepared using the nano-filtering process was determined as *dongchimi* nano juice. *Dongchimi* raw juice accompanied without nano-filtration was used as a control juice. The physicochemical properties of the *dongchimi* raw and nano juices were compared (Table 1). *Dongchimi* raw and nano juices showed pH 3.69 and pH 3.70 with 0.68% and 0.63% acidity, and 1.32% and 1.37% saltiness, respectively. *Leuconostoc* sp. and *Lactobacillus* sp. numbers of *dongchimi* raw juices were 6.6×10^5 CFU/mL and 1.5×10^5 CFU/mL, respectively. However, the lactic acid bacteria were not found in *dongchimi* nano juices. The lactic acid bacteria were removed completely by using the nano-filtering process. In the preparation of *dongchimi*, heating treatments such as sterilizing by heat or pasteurization and microwave can have negative effects on physicochemical properties. These heating treatments decreased pH, increased total acidity and softerned

Table 1. Physicochemical properties of *dongchimi* juices in nano-filtering process

	pH	Acidity (%)	Saltiness (%)	*Leuconostoc* sp.	*Lactobacillus* sp.
Raw juice	3.69	0.68	1.32±0.01	$6.6\pm0.8\times10^6$	$1.5\pm0.3\times10^5$
Nano juice	3.70	0.63	1.37±0.01	0	0

texture. Therefore, nano-filtering was the recommendable process in the aspect of quality for *dongchimi* nano juice.

Effects of *dongchimi* nano juice on leptin secretion

Leptin secretions caused by the *dongchimi* raw/nano juices prepared by usig the nano-filtering process were examined (Fig. 1). In a fed state, circulating leptin levels are correlated with the extent of obesity (13,14). To investigate the effect the of the nano-filtering process on a lipid accumulation in the 3T3-L1 adipocytes, the leptin secretions were measured. Although treatment of *dongchimi* raw and nano juices reduced the leptin secretions, there was no significant difference in leptin secretion among the groups treated with different concentrations and the filtering process.

Effects of *dongchimi* nano juice on lipolysis

Lipolytic activities of *dongchimi* nano juice were investigated in order to examine the effect of *dongchimi* nano juice on lipid accumulation associated with lipolysis. The lipolytic responses of differentiated adipocytes were determined by measuring the amount of glycerol released in the medium (Fig. 2). *Dongchimi* nano juice considerably raised glycerol levels in the medium, compared to *dongchimi* raw juice. *Dongchimi* nano juice treated with 20 μL/mL and 40 μL/mL increased glycerol levels in ratios of 15% and 53% to the control, respectively. These results demonstrate that the nano-filtering process for manufacturing of *dongchimi* nano juice induced lipolysis by the increased lipolytic activity of adipocytes.

mRNA expression of PPAR γ

To determine whether *dongchimi* nano juice affects the expression of PPAR γ, RT-PCR analysis of the adipogenic transcription factor was conducted (Fig. 3). Treatment of *dongchimi* nano juice reduced mRNA expression of PPAR γ in cultured 3T3-L1 adipocytes, compared to the groups treated with *dongchimi* raw juice or the control cell. PPAR γ is a nuclear receptor superfamily of transcription factors and is predominantly expressed in adipose tissue. These transcription factors appear to function as dominant activators of adipocyte differentiation (15). PPAR γ is a major coordinator of adipocyte gene expression and differentiation (16). PPAR γ is induced prior to the transcriptional activation of most adipocyte-specific genes, and the expression of PPAR γ is sufficient to induce growth arrest and to initiate adipogenesis in exponentially growing fibroblast cell lines (17).

The anti-adipogenic effect of *dongchimi* nano juice could be revealed by lipolysis and the decreased expression of PPAR γ mRNA as indicators of lipid accumulation. That is, *dongchimi* nano juice prepared by using a nano-filtering process blocked the lipid accumulation and induced lipolytic activity in 3T3-L1 adipocytes, which was accompanied with inhibition of adipogenesis through down-regulated expression of PPAR γ.

From these results, the anti-adipogenic effect of *dongchimi* nano juice could be induced by lipolysis. The possible active components responsible for the effect could be radish, garlic, red pepper powder and some products created during the fermentation of *dongchimi*. Several studies have reported the on antiobesity effects of radish, garlic and red pepper powder, which are related to de-

Fig. 1. Effects of *dongchimi* nano juices prepared by using a nano-filtration process on leptin secretions.
Adipocytes were treated for 24 hours at "day 8" after inducing differentiation with vehicle alone (control) or 20, 40 μL/mL of *dongchimi* raw/nano juices. Data are expressed as mean ±standard error values.

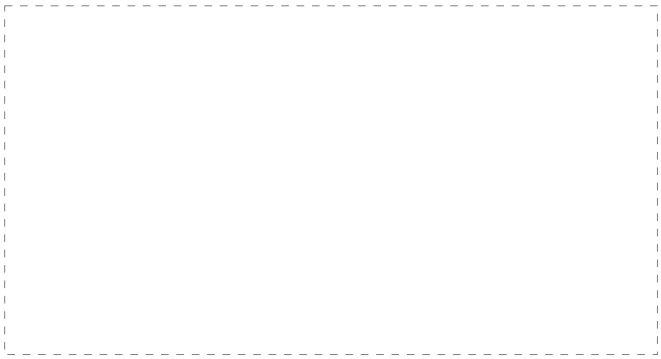

Fig. 2. Effects of *dongchimi* nano juices on glycerol secretions. Data are expressed as mean±standard error values. Means with different letters are significantly different ($p<0.05$) by Duncan's multiple range tests.

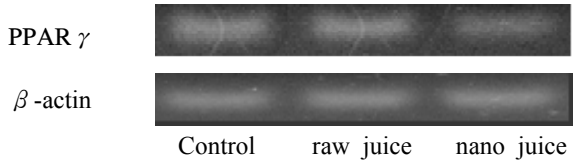

Fig. 3. Effects of *dongchimi* raw and nano juices on mRNA expression of PPAR γ.
Starting at day 8 after inducing differentiation, adipocytes were cultured with or without 40 μL/mL of *dongchimi* nano juice for 24 hours.

creases in body weight, serum lipids, adipose tissues in rats, and body fat gain (18-21). Also, these Korean major spices have an interaction among themselves during fermentation. The fermentation products can affect the anti-adipogenic activity of *dongchimi* nano juice. Nanoparticle size products can work actively due to the filtering of any interruption products. More studies on the active compounds produced during fermentation will be conducted in the future.

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