Comparison with various mulberry leaves' and fruit's extract in lipid accumulation inhibitory effect at adipocyte model

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Abstract

In relation to the global increase trend of obesity population, there is a demand for the development of foods having high functional activity by mass-extracting anti-obesity active substances using mulberry product such as leaf and fruit. Therefore, we evaluated the anti-obesity efficacy according to varieties by using the mulberry leaves and fruit extracts. At the same time, high active varieties were selected. For this purpose, the effects of the extracts of the mulberry leaf and fruit on 3T3-L1 adipocyte differentiation were examined. As a result, in the case of mulberry leaves, the lipid accumulation inhibitory rate of ‘Cheongolppong’ was higher than that of the control at 500 μg/ml treatment. And in the case of the extract of mulberry fruit, ‘Daesim’ showed the highest lipid accumulation inhibitory rate compared with the control at 50 times of diluted extract.

Introduction

Mulberry leaf is known to contain 1-deoxynojirimycin, a hypoglycemic component (Ju et al., 2015), and is mainly used as a tea (Ju et al., 2016). Recently, consumers are interested in the functional ingredients and efficacy of mulberry products such as mulberry leaf, mulberry fruit, root bark, branch and so on. In this regard, papers on functional ingredients such as rutin (Yun and Lee, 1995; Kim et al., 2014a; Kim et al., 2014b), resveratrol (Kim et al., 2011; Kim et al., 2012), and 1-deoxynojirimycin (Kim et al., 2013) and selection of high-content varieties for mulberry leaves have been reported. In addition, various effects such as lowering of cholesterol, antidiabetics, hypertension inhibition, prevention of arteriosclerosis, prevention of stroke are reported (Park et al., 2013).

Now the sericulture industry in Korea is carrying out researches and policies related to the transition of paradigm called ‘Functional sericulture’. In the case of mulberry fruit, since 2002, the National Institute of Agricultural Sciences has been analyzing the functional ingredient content of mulberry fruit according to the mulberry variety or strain and developing food processing technology using it.

As a result, many studies on the functional components of mulberry fruit have been reported that mulberry fruit contained a large amount of various functional ingredients such as cyanidin-3-glucoside (C3G) (Kim and Kim, 2003), rutin (Kim and Kim, 2004), γ-aminobutyric acid (GABA) (Kim et al., 2004), linoleic...
Materials and methods

Experimental material

The mulberry leaves of this study were collected from various mulberry varieties cultured in National Institute of Agricultural Sciences including ‘Buyoungsang’, ‘Yulbon’, ‘Cheongilppong’, and ‘Cheongolppong’ at the opening stage of 5th leaf. Based on the mulberry cultivation method for silkworms, they are managed in a manner of low-cut training (summer pruning) every year. After the harvesting, the fresh leaves were immediately stored at -70°C in a deep freezing cryocooler (Ilshin Lab Co., Ltd, Korea), and lyophilized to prepare powder.

Meanwhile, mulberry fruits (Fig. 1) were obtained at the different regions and different varieties including ‘Kwasang No. 2’ (Buan, Jeonbuk), ‘Daesim’ (Gongju, Chungnam), ‘Cheongilppong’ (Suwon, Kyunggi), and ‘Iksu’ (Sangju, Kyungbuk). They were kept in a deep freezing cryocooler (Ilshin Lab Co., Ltd, Korea) at -70°C.
Extract preparation

- Mulberry leaf extracts: Mulberry leaf powder was prepared by powdering the fresh mulberry leaves after freezing at -70°C and then vacuum lyophilization. 10 L of 70% fermented alcohol was added to 1 kg of mulberry leaf powder and sonicated (at room temperature, 1 hour, 3 times). The extract solution was filtered with Watman No. 2 filter paper. And then concentrated in vacuum evaporator at 40°C. The manufacturing process is shown in Fig. 2.

- Mulberry fruit extracts: As shown in Fig. 3, to 5 kg of frozen mulberry fruit, 0.1% citric acid-70% fermented alcohol solution (10 L) was added and extracted three times by hand-kneading for 10–20 minutes. The extract solution was filtered with Watman No. 2 filter paper. And then concentrated in vacuum evaporator at 40°C.

3T3-L1 adipocyte culture and differentiation

3T3-L1 preadipocytes were purchased from ATCC (American Type Culture Collection, Manassans, VA, USA). Preadipocyte were cultured in DMEM containing 10% BCS and 1% phenicillin-streptomycine at 37°C and 5% CO². For preadipocyte differentiation, 3T3-L1 differentiation and treatment of samples were carried out according to the treatment of previous study (Jun et al., 2014; Ko et al., 2015; Kwon 2016; Wu et al. 2015) evaluating the anti-adipogenesis physiological activity of the sample during the adipogenesis process. The differentiation of 3T3-L1 preadipocytes was maintained for 2 days at 100% confluency after cell culture in each well for cell differentiation. All adipocytes were induced with adipocyte differentiation with 10% FBS DMEM medium containing MDI and incubated for 48 hours with 10% FBS DMEM containing 1 μg/mL insulin after 72 hours. After 48 hours, the cells were replaced with 10% FBS DMEM to induce adipocyte differentiation.

Oil Red O staining test

On day 9, the medium was removed from the wells, washed twice with D-PBS, and fixed with 10% formaldehyde solution in D-PBS for 30 min at room temperature. After 10% formaldehyde solution was removed, the cells were washed twice with D-PBS, treated with Oil Red O staining solution prepared with isopropyl alcohol, and stained at room temperature for 1 hour. After removing the staining solution and washing twice with D-PBS, D-PBS was completely removed from each well, and 100% isopropyl alcohol was added to each well to elute Oil Red O staining solution. Absorbance was measured at 510 nm.
Results and discussion

Identification of anti-obesity effect

In order to evaluate the anti-obesity activity of mulberry leaves and fruits extract, the amount of fat accumulation in 3T3-L1 cells differentiated into adipocytes was calculated by measuring absorbance at 510 nm through Oil Red O staining (Fig. 4). Lipid accumulation (%) of control was calculated using differentiated adipocytes not treated with mulberry leaves or mulberry fruit extract as a control.

At the same time, Orlistat treatments, which are marketed as prescription drugs under the trade name Xenical by Roche, are also compared in most countries as obesity remedies. Its primary function is to prevent the absorption of fat from human diet by working with lipase inhibitors, and to reduce calorie intake.

Comparison of inhibitory effects of mulberry leaf extract on the lipid accumulation in 3T3-L1 adipocyte cell

The extracts were prepared by using mulberry leaves of different varieties such as 'Buyoungsang', 'Yulbon', 'Cheongilppong', and 'Cheongolppong'. And inhibitory effects on 3T3-L1 adipocyte differentiation and lipid accumulation with mulberry leaf extract was compared.

At the concentration of 10, 50, and 100 μg/ml, the inhibitory effect of fat accumulation was less than or similar to that of control. But, when the mulberry leaf extract was treated at a concentration of 500 μg/ml, the lipid accumulation ratio were 'Buyoungsang' 66.68%, 'Yulbon' 66.09%, 'Cheongilppong' 84.85% and 'Cheongolppong' 42.10%, respectively. So 'Cheongolppong' was selected as high active antiobesity variety for mulberry leaves (Table 1).

As a result, it was found that the high concentration of mulberry leaf extract had an anti-obesity effect, and the degree of anti-obesity effect was also different according to variety.

This result is different from the result of Kwon (2016) which showed 60.42%, 38.24%, and 5.97% inhibition of adipocyte differentiation at 10, 100 and 200 μg/ml concentrations of mulberry leaf extract. This is because the polyphenol components such as rutin present in the mulberry leaves were sufficiently

![Fig. 4. Detection of inhibitory effects on 3T3-L1 adipocyte differentiation and lipid accumulation with mulberry fruit extract by 0:1 Red O staining.](image)

**Table 1.** Inhibitory effect of 70% ethanol mulberry leaf extracts from varieties on the lipid accumulation in 3T3-L1 adipocyte cell.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Concentration (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10</td>
</tr>
<tr>
<td>Control</td>
<td>100.00±1.223</td>
</tr>
<tr>
<td>Varieties</td>
<td></td>
</tr>
<tr>
<td>Buyoungsang</td>
<td>104.18±0.44</td>
</tr>
<tr>
<td>Yulbon</td>
<td>101.43±3.11</td>
</tr>
<tr>
<td>Cheongilppong</td>
<td>111.59±2.61</td>
</tr>
<tr>
<td>Cheongolppong</td>
<td>100.43±2.57</td>
</tr>
</tbody>
</table>

The cells were incubated during differentiation of extracts from various varieties of mulberry leaves and lipid accumulation levels were determined by Oil Red O stain. Extract were incubated with concentration 10, 50, 100 and 500 μg/ml for 8 days. The values were calculated as percentage of Control. Each value is expressed as the mean±SD of three independent experiments.
extracted by hot water extraction (120°C), whereas the extract of this experiment was not obtained by ultrasonic extraction at room temperature.

Therefore, in the future, extraction method should be thoroughly reviewed, and if the search for anti-obesity active substances and the easy processing technology of food materials are developed, the consumption of mulberry leaves will help to improve income of the production farmers.

Assessment of anti-obesity activity of mulberry fruit extract and selection of high-activity variety

The extracts were prepared by using mulberry fruits of different varieties such as ‘Kwasang No. 2’ (Buan, Jeonbuk), ‘Daesim’ (Gongju, Chungnam), ‘Cheongilppong’ (Suwon, Kyunggi), and ‘Iksu’ (Sangju, Kyungbuk). Inhibitory effects on 3T3-L1 adipocyte differentiation and lipid accumulation with mulberry fruit extract was compared.

As a result, when the mulberry fruit extract was diluted at 50 fold concentration, the lipid accumulation ratio were as ‘Kwasang No. 2’ 93.86%, ‘Daesim’ 67.70%, ‘Cheongilppong’ 87.44% and ‘Iksu’ 86.81%, respectively. So, ‘Daesim’ was selected as high active antiobesity variety for mulberry fruits (Table 2).

Especially, ‘Daesim’ is a breeding variety by the National Institute of Agricultural Sciences. It has a very large size and easy to harvest.

As with mulberry leaves, if the anti-obesity active substance of ‘Daesim’ is revealed, it will be consumed as an anti-obesity food and contribute to the public health.

Acknowledgements

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References


Table 2. Inhibitory effect of 0.1% citric acid-70% ethanol mulberry fruit extracts from varieties on the lipid accumulation in 3T3-L1 adipocyte cell.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Control</th>
<th>Varieties</th>
<th>1000</th>
<th>500</th>
<th>100</th>
<th>50</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1000</td>
<td>500</td>
<td>100</td>
<td>50</td>
</tr>
<tr>
<td>Kwasang No. 2</td>
<td>92.92±2.87</td>
<td>92.01±1.62</td>
<td>78.61±0.77</td>
<td>93.86±1.58</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Daesim</td>
<td>86.67±1.67</td>
<td>107.74±1.15</td>
<td>77.82±1.08</td>
<td>67.70±0.34</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cheongilppong</td>
<td>83.44±1.95</td>
<td>110.04±1.67</td>
<td>90.26±0.20</td>
<td>87.44±0.39</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iksu</td>
<td>89.67±0.70</td>
<td>90.66±0.26</td>
<td>88.59±0.61</td>
<td>86.81±0.73</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The cells were incubated during differentiation of mulberry fruit extracts from various varieties and lipid accumulation levels were determined by Oil Red O stain. Extract were incubated with dilution ratio of 50, 100 500 and 1000 fold for 8 days. The values were calculated as percentage of Control. Each value is expressed as the means±SD of three independent experiments.