Phytomedicine 17 (2010) 974–979

Contents lists available at ScienceDirect

Phytomedicine

journal homepage: www.elsevier.de/phymed

Ethanolic extracts of Brazilian red propolis promote adipocyte differentiation through PPARγ activation

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ARTICLE INFO

Keywords:
3T3-L1
Adipocyte differentiation
PPARγ
Red propolis
Adiponectin
TNF-α

ABSTRACT

Aim of the study: The aim of present study was to investigate the effects of ethanolic extracts of red propolis (EERP) on adipogenesis and evaluate the molecular basis for their anti-obesity effects.
Materials and methods: We tested whether EERP alone could induce differentiation of 3T3-L1 cells, regulate the expression of adipocyte-specific genes and reverse inhibitory effects of TNF-α on their differentiation. Next, we performed a luciferase reporter gene assay to test whether EERP could enhance transcriptional activities of PPARγ and adiponectin promoter activities.
Results: EERP strongly induced differentiation of 3T3-L1 preadipocytes into adipocytes, and enhanced the PPARγ transcriptional activity and adiponectin promoter activity. In addition, EERP attenuated the inhibitory effect of TNF-α on adipocyte differentiation and adiponectin production in mature adipocytes.
Conclusion: The present study indicates that EERP enhance differentiation of 3T3-L1 adipocytes in part by its potency of PPARγ activation and are capable of reversing inhibitory effects of TNF-α on adipocyte differentiation and adiponectin expression. These results suggest the value of EERP as a diet supplement for prevention and treatment of obesity and obesity-associated disorders.

Introduction

Propolis, a product collected by honey bees from plants, has been popularly used in folk medicine, and a variety of biological functions have been identified including anti-oxidant (Pascual et al. 1994; Burdock 1998), -microbial (Grange and Davey 1990), -inflammatory (Khayyal et al. 1993), -carcinogenic (Grunberger et al. 1988), and -cariogenic properties (Park et al. 1998; Hayacibara et al. 2005). Reportedly, propolis contains more than 300 compounds (De Castro 2001). The chemical composition of propolis varies depending on the local flora at the site of collection (Marcucci and Bankova 1999). Novel reddish propolis was recently found in northeastern Brazil (Trusheva et al. 2006), and its botanical origin was described as *Dalbergia ecastophyllum (L) Taub.* (Leguminosae) (Daugsch et al. 2008). Fourteen compounds have been so far isolated from the Brazilian red propolis, which include simple phenolics, triterpenoids, isoflavonoids, prenylated benzophenones, and naphthoquinone epoxide (Trusheva et al. 2006). The chemical constituents of red propolis are apparently different from those of Brazilian green propolis.

Obesity is the most common metabolic disorder in the industrialized countries, and is a major risk factor for arteriosclerosis, diabetes and hyperlipidemia. The adipose tissue plays an essential role in the regulation of energy balance (Rosen and Spiegelman 2006). In the obese state, the expression of adipocytokines such as resistin, interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), plasminogen activator inhibitor-1 (PAI-1), monocyte chemotactic protein-1 (MCP-1), and angiotensinogen is increased in enlarged adipocytes, causing insulin resistance and metabolic disorders. On the other hand, the production and secretion of adiponectin from small adipocytes, which increases insulin sensitivity, are reduced (Yamauchi et al. 2001; Guerre-Millo 2004). Peroxisome proliferator-activated receptor γ (PPARγ) that belongs to the superfamily of ligand-activated nuclear hormone receptors is predominantly expressed in the adipose tissue (Rangwala and Lazar 2004). PPARγ controls the expression of genes involved in adipocyte differentiation, and regulates lipid metabolism. PPARγ activation by thiazolidinedione derivatives such as troglitazone, pioglitazone, and rosiglitazone, increases the number of small adipocytes expressing adiponectin, and concomitantly decreases

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doi:10.1016/j.phymed.2010.03.001

that of large adipocytes producing excess amounts of TNF-α and free fatty acid, thereby ameliorating insulin resistance (Okuno et al. 1998). Thus, PPARγ is a therapeutic target for prevention and treatment of insulin resistance and metabolic syndrome. It has been recently reported that oral intake of propolis reduces the accumulation of body fat and dyslipidemia via the change of the expression of proteins involved in adipose depot and lipid metabolism (Ichi et al. 2009). Some other plant-derived constituents also promote adipogenesis and expression of insulin-sensitizing adipocytokines (Shen et al. 2006; Yang et al. 2007; Saito et al. 2007; Hassan et al. 2009; Cho et al. in press).

In the present study, we investigated the effects of ethanolic extracts of red propolis (EERP) on adipogenesis of 3T3-L1 cells, and characterized the underlying molecular mechanisms.

Materials and methods

Reagents

Dulbecco's modified Eagle's medium (DMEM), Cell Counting Kit-8, *p*-hydroxycinnamic acid, and Pikkagene Dual-SeaPancy Luminescence Kit were purchased from Wako (Tokyo, Japan). Charcoal-stripped fetal bovine serum was obtained from Biological Industries (Kibbutz Beit-Ha'Emek, Israel). AdipoInducer Reagent (insulin: INS, dexamethazone: DEX, and isobutylmethylxanthine: IBMX), SYBR Premix Ex Taq, TransIt LT-1, and RNase-free DNase I were from Takara (Otsu, Japan). (±)-Naringenin, daidzein, biochanin A, Oil Red O, TNF-α, and DMSO were purchased from Sigma (St Louis, USA). Oligonucleotide primers were from Rikaken (Nagoya, Japan). TRIzol and Superscript III Reverse Transcription Kit were purchased from Invitrogen (Carlsbad, USA). Quercetin and rutin were obtained from Tokyo Chemical Industry (Tokyo, Japan). Rosiglitazone was purchased from Alexis (Lausen, Switzerland).

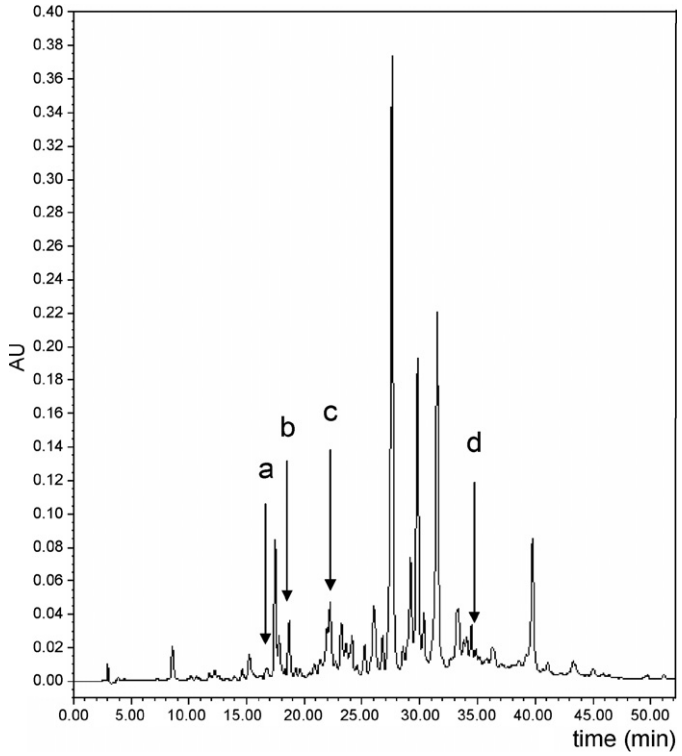

Fig. 1. HPLC chromatogram of EERP. 1, daidzein; 2, quercetin; 3, naringenin; 4, biochanin A.

Preparation of ethanolic extracts of red propolis

Red propolis harvested in the State of Alagoas in Brazil were extracted with 95% (v/v) ethyl alcohol overnight at room temperature under shaking and then filtered through the Advantec No. 9 filter paper to obtain its ethanolic extracts.

HPLC analysis

The main constituents in EERP were analyzed by high performance liquid chromatography (HPLC). The samples were injected into an HPLC system (Waters, Washington, USA) fitted with a CAPCEL PAK C18 column (Φ4.6 mm × 250 mm) (Shiseido, Tokyo, Japan). The mobile phase consisted of 0.1% (v/v) TFA in water and methanol. The constituent was measured at 280 nm with

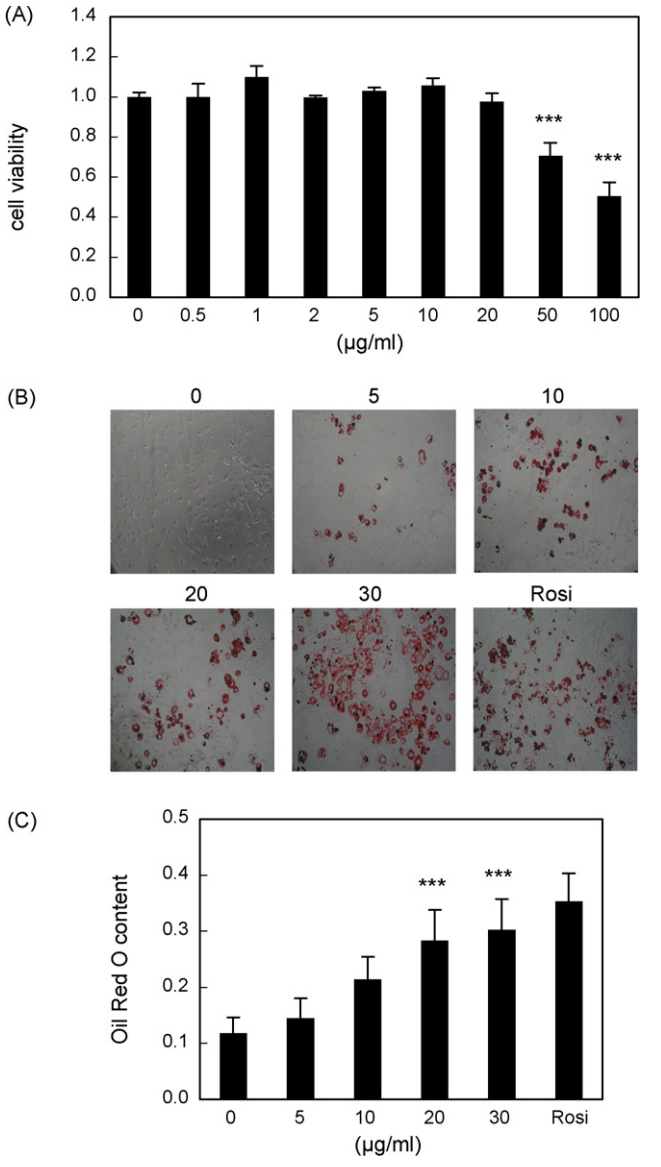

Fig. 2. Effects of EERP on cell viability and adipocyte differentiation of 3T3-L1 preadipocytes. (A) 3T3-L1 preadipocytes were treated with various concentrations of EERP (0–100 μg/ml) for 24 h, and then cell viability was determined. Data are expressed as mean ± SD (n = 3). Asterisks indicate significant differences compared with DMSO-treated control cells (***p < 0.001). (B) Post-confluent 3T3-L1 preadipocytes were treated with EERP (0–30 μg/ml) for 10 days, and stained with Oil Red O. Cells were treated with rosiglitazone (2 μM) as a positive control. (C) The Oil Red O content was measured at a wavelength of 540 nm. Data are expressed as mean ± SD (n = 6) (***p < 0.001).