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Anti-obesity and anti-hepatosteatosis effects of dietary scopoletin in high-fat diet fed mice

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ABSTRACT

The effects of scopoletin on non-alcoholic fatty liver in obese mice were investigated. Mice were fed high-fat diet (HF) with or without two doses of scopoletin (0.01 and 0.05%, w/w) for 16 weeks. Both doses of scopoletin led to similar reductions in body weight, visceral fat, serum levels of leptin, lipid, TNFα, IL-6, IFNγ and MCP-1, insulin resistance and hepatic lipid accumulation, whereas they increased serum adiponectin and faecal lipid levels. Ingenuity pathway analysis revealed that hepatic gene networks related to lipid concentrations, inflammation of organs, quantity of adipose tissue, proliferation of cell and necrosis were down-regulated in the scopoletin group. The top up- or down-regulated genes were *Cidea*, *Apoa4*, *Cyp7a1*, *Errfi1*, *Col1a1*, *Mmp13*, *Cdkn1a*, *Gdf15* and *Saa1*, which emerged as associated genes related to hepatic steatosis and inflammation. These results indicate that scopoletin may ameliorate HF-induced hepatic dysfunction via regulation of lipid metabolic and inflammatory genes.

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Abbreviations: ALT, alanine aminotransferase; *Apoa4*, apolipoprotein A-IV; AST, aspartate aminotransferase; β-oxidation, fatty acid β-oxidation; *Cdkn1a*, cyclin-dependent kinase inhibitor 1A; *Cidea*, cell death-inducing DFFA-like effector A; *Col1a1*, collagen, type 1, alpha 1; CPT, carnitine palmitoyltransferase; *Cyp7a1*, cholesterol 7 alpha-hydroxylase; *Errfi1*, ERBB receptor feedback inhibitor 1; FAS, fatty acid synthase; FFA, free fatty acid; G6P, glucose-6-phosphate; G6Pase, glucose-6-phosphatase; *Gapdh*, glyceraldehyde-3-phosphate dehydrogenase; *Gdf15*, growth differentiation factor 15; GK, glucokinase; HDL-C, HDL-cholesterol; H&E, haematoxylin and eosin; HF, high-fat diet; HF-LS, high-fat diet with 0.01% scopoletin; HF-HS, high-fat diet with 0.05% scopoletin; HOMA-IR, homeostatic index of insulin resistance; IL-1β, interleukin-1 beta; IL-6, interleukin-6; IFNγ, interferon gamma; IPA, ingenuity pathway analysis; IPGTT, intraperitoneal glucose tolerance test; IR, insulin resistance; MCP-1, monocyte chemoattractant protein-1; *Mmp13*, matrix metallopeptidase 13; NAD^+, nicotinamide adenine dinucleotide$^+$; NADH, nicotinamide adenine dinucleotide; NADPH, nicotinamide adenine dinucleotide phosphate; NC, normal diet; NAFLD, non-alcoholic fatty liver disease; PAP, phosphatidate phosphohydrolase; PEPCK, phosphoenolpyruvate carboxykinase; *Saa1*, serum amyloid A1; TC, total cholesterol; TG, triacylglycerol; Trichrome, Masson's trichrome; TNFα, tumour necrosis factor alpha

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1. Introduction

Non-alcoholic fatty liver disease (NAFLD) is a common cause of chronic liver disease that increases in prevalence with increasing incidence of obesity and type 2 diabetes (Vernon, Baranova, & Younossi, 2011). NAFLD is generally characterized by fat accumulation exceeding 5% of hepatic tissue, which encloses a spectrum ranging from steatosis to steatohepatitis, fibrosis and cirrhosis (Ganji, Kashyap, & Kamanna, 2015; Machado & Cortez-Pinto, 2005). Obese patients with hepatosteatosis and steatohepatitis have higher levels of inflammatory biomarkers relative to age-, sex- and obesity-matched controls (Ganji et al., 2015; Zhang, Yang, & Yu, 2015). Previous studies have indicated that systemic inflammation is strongly associated with hepatic steatosis and cardiometabolic disorders in obese individuals (Acharyya et al., 2007). Hepatic proinflammatory cytokines such as TNFα, IL-6 and IL-1β are overproduced in fatty liver (Shoelson, Lee, & Goldfine, 2006) and crucial effectors of insulin resistance (Raso et al., 2013). Although weight loss is essential to ensuring successful outcomes to the treatment of obesity-induced liver disease, numerous natural compounds have also been investigated for their treatment (Duarte et al., 2015).

Scopoletin (6-methoxy-7-hydroxycoumarin) is a naturally occurring coumarin, which is found in many edible plants and fruits, such as oat (*Avena sativa*), elephant garlic (*Allium ampeloprasum*), celery (*Apium graveolens*), red pepper (*Capsicum annuum*), chili pepper (*Capsicum frutescens*), carrot (*Daucus carota*), chicory (*Cichorium intybus*), lemon (*Citrus limon*), grapefruit (*Citrus paradisi*) and sweet potato (*Ipomoea batata*) (Carpinella, Ferrayoli, & Palacios, 2005; Matsumoto, Mizutani, Sakata, & Shimizu, 2012). Scopoletin has been reported to possess antioxidant (Shaw, Chen, Hsu, Chen, & Tsai, 2003), antitumoural (Chang et al., 2012), antihypertension (Ojewole & Adesina, 1983) and anti-hyperglycaemic activity (Chang et al., 2015). Moreover, it has been shown to ameliorate synovial inflammation and destruction of cartilage and bone in adjuvant arthritis (Netzer et al., 2015). Mandukhail, Aziz, and Gilani (2010) suggested that scopoletin reduced the risk of hypercholesterolaemia, hypertriglyceridaemia and hyperglycaemia associated with high-fat diet.

Gnonlonfin, Gbaguidi, Gbenou, Sanni, and Brimer (2011) reported cassava (*Manihot esculenta* Crantz), which is one of the most important food crops in tropical regions, contains scopoletin at between 4.1 and 11.1 mg/kg dry weight. Dietary intake of berries is known to have beneficial effects on human diseases, such as cardiovascular disease, obesity and some cancers (Firuzi, Miri, Tavakkoli, & Saso, 2011). Recently, goji berries (the fruit of *Lycium barbarum*) became a popular food supplement in China and southeastern Asia, and scopoletin has been reported to be one of the main phenolic components in the ethyl acetate extract of goji berries (present at 8 mg/kg on extract) (Forino, Tartaglione, Dell'Aversano, & Ciminiello, 2016). Scopoletin has been recommended as a marker constituent for the quality control of noni products (Issell, Franke, & Fielding, 2008). Noni (*Morinda citrifolia* Linn), which is also known as the Indian mulberry, hog apple and cheese fruit, has antitumour, hypotensive and immune enhancing effects (Nayak & Shettigar, 2010). Pandy, Narasingam, Kunasegaran, Murugan, and Mohamed (2014) reported a scopoletin concentration in the methanol extract of noni fruit was 18.95 μg/mg. Furthermore, in a previous study, we reported that the ethyl acetate fraction of *Artemisia iwayomogi* exhibited hepatoprotective effects in alcohol and high-fat fed mice (Lee, Seo, Yun, Kim, & Lee, 2011) and that its main component was scopoletin (204 mg/g) (Seo, Jeong, & Yun, 2010). However, it is still unclear whether scopoletin can regulate NAFLD through metabolic and transcriptomic mechanisms. Therefore, this study was designed to elucidate the role of scopoletin in obesity-associated hepatosteatosis and inflammation via whole genome expression analysis.

2. Materials and methods

2.1. Animals

Four-week-old male C57BL/6J mice were purchased from Jackson Laboratory Center (Bar Harbor, ME, USA) and individually housed under a controlled temperature (22 ± 2 °C) and a 12 h light-dark cycle. After a one week adaptation period, mice were randomly divided into four groups of nine and fed a normal diet (NC), high-fat diet (HF, 20% fat and 1% cholesterol) or HF with 0.01 or 0.05% scopoletin diet (HF-LS or HF-HS; TCI Co., Ltd, Tokyo, Japan) for 16 weeks. The compositions of the experimental diets were based on the AIN-76 semisynthetic diet (American institute of nutrition, 1977). Body weight and food intake were measured once a week and daily, respectively, during the feeding period.

At the end of the experimental period, mice were fasted for 12 h and then anaesthetized with ether. Blood samples were subsequently taken from the inferior vena cava for serum biomarker analysis, after which the liver and adipose tissues were removed, rinsed with a physiological saline solution and stored immediately at –70 °C until analysis. The present study was approved by the Sunchon National University Institutional Animal Care and Use Committee (SCNU IACUC-2013-11).

2.2. Serum adipokines, cytokines, chemokine and liver damage markers

Serum adiponectin and leptin levels were determined using a quantitative sandwich enzyme immunoassay kit (R&D System, Minneapolis, MN, USA). Tumour necrosis factor alpha (TNFα), interleukin-6 (IL-6), interferon gamma (IFNγ), and monocyte chemoattractant protein-1 (MCP-1) levels were determined using a multi-detection kit (BioRad, Hercules, CA, USA) and Luminex 200 Labmap system (Luminex, Austin, TX, USA). The serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were measured using an automated chemistry analyser (Fuji-Dri-Chem 3500; Fujifilm, Tokyo, Japan).

2.3. Insulin resistance biomarkers

At week 8, 12 and 16, the 6 h fasting serum glucose concentrations were measured using a glucometer (GlucoDr supersensor, Allmedicus, Anyang, Korea). Serum insulin level