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# Barley sprout extracts reduce hepatic lipid accumulation in ethanol-fed mice by activating hepatic AMP-activated protein kinase

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ABSTRACT

Chronic alcohol consumption leads to hepatic lipid accumulation and alcoholic fatty liver disease. Previously, we demonstrated that barley sprout extract, which contains saponarin as an active compound, reduces hepatic steatosis. In this study, we investigated the effect of barley sprout extracts (BSE) on hepatic lipid accumulation in a mouse model of alcoholic fatty liver disease. Seven-week-old C57BL/6 mice were fed an alcohol-containing diet (5% ethanol) and a low or high dose of BSE (100 or 200 mg/kg body weight, respectively) for 10 days. The high dose of BSE significantly decreased hepatic lipid accumulation compared with the ethanol-only control group. In the second animal study, mice were fed an alcohol-containing diet for 10 days, followed by a 45% high-fat diet with oral administration of BSE (100 or 200 mg/day/kg body weight) for 4 weeks. Mice in both BSE-fed groups showed reduced hepatic steatosis. In the livers of mice fed BSE, phosphorylation of AMP-activated protein kinase (AMPK) was increased, and expression of hepatic autophagy markers was elevated. In cultured hepatocytes, BSE (200 μg/mL) increased the rate of fatty acid oxidation and reduced that of fatty acid synthesis. Taken together, these findings suggest that BSE promotes degradation of lipid droplets and subsequent activation of fat oxidation by activating AMPK in the liver, thus protecting against development of hepatic steatosis in alcohol-fed mice. Saponarin, a major flavonoid in BSE and an activator of AMPK, increased the activity of microsomal triglyceride transfer protein, which suggests that the reduction in hepatic triglyceride levels was mediated by this component of BSE. In conclusion, BSE ameliorated hepatic steatosis in a mouse model of ethanol-induced fatty liver by activating AMPK, an effect possibly mediated by the saponarin component.

## 1. Introduction

Alcoholic fatty liver disease (AFLD) is a major chronic liver disease worldwide (Arteel, Marsano, Mendez, Bentley, & McClain, 2003). Excessive alcohol consumption results in lipid accumulation in the liver, which leads to fatty liver, alcoholic hepatitis, and alcoholic cirrhosis (Diehl, 2002; Livero & Acco, 2016). Hepatic steatosis is the most common early symptom of AFLD and is characterized by the accumulation of lipid droplets within hepatocytes without prominent infiltration of inflammatory macrophages (Yeh & Brunt, 2014). No drug has been approved for AFLD (Gao & Bataller, 2011); therefore, prevention of hepatic steatosis due to alcohol consumption is an important issue (Louvet & Mathurin, 2015). Prevention of hepatic steatosis is particularly important in patients with AFLD because of the possibility of progression to more severe forms of AFLD (You & Crabb, 2004). Hepatic lipid metabolism is highly complex and regulated by multiple pathways, including that centered around AMP-activated protein kinase (AMPK) (Viollet et al., 2006). AMPK is a crucial regulatory protein for cellular energy homeostasis, and its activation stimulates oxidation of fuel molecules, including stored fats, and suppresses anabolic metabolism, such as lipid and protein syntheses (Hardie, 2008; Mihaylova & Shaw, 2011). Thus, activation of AMPK could be an effective strategy for reducing hepatic steatosis.

Several natural substances induce AMPK activation (Ajmo, Liang, Rogers, Pennock, & You, 2008; Liu et al., 2014; Noh et al., 2011). The sprouts from natural grains such as barley have potent antioxidant activity and high levels of flavonoids (Park, Seo, & Kang, 2015). In a previous study, we showed that barley sprouts contain high levels of

*Abbreviations:* AFLD, alcoholic fatty liver disease; AMPK, AMP-activated protein kinase; BSE, barley sprout extract; GC–MS, gas chromatography mass spectrometry; H & E, hematoxylin and eosin; PPAR, peroxisome proliferator-activated receptor; FOXO3, forkhead box O3; HFD, high fat diet; C, control; E, EtOH; L, LBSE; H, HBSE; HC, HFD-control; HE, HFD-EtOH; HL, HFD-LBSE; HH, HFD-HBSE; WAT, white adipose tissue

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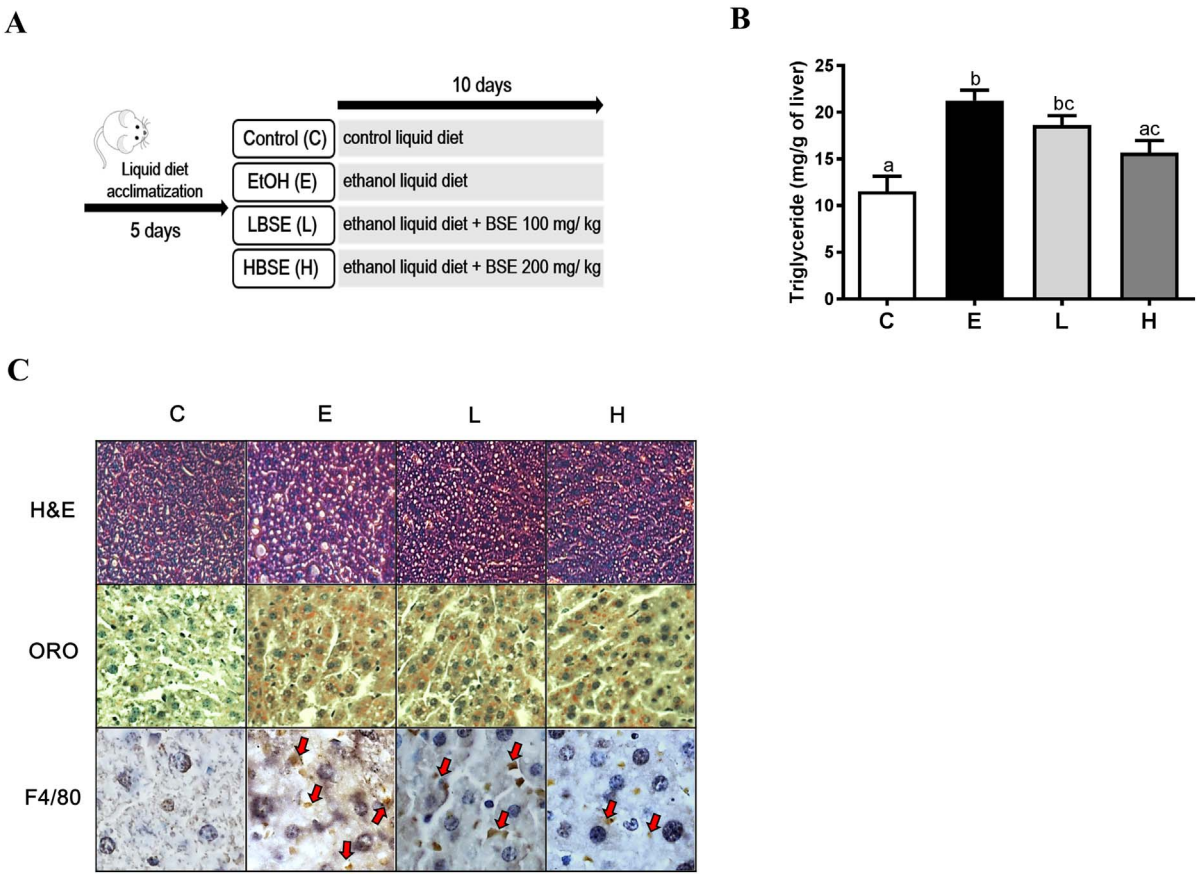


**Fig. 1.** BSE ameliorated hepatic lipid accumulation in EtOH-fed mice. BSE reduced hepatic triglyceride concentrations, lipid accumulation, and macrophage infiltration into liver tissue. After daily oral administration of BSE for 10 days, EtOH-fed mice were euthanized, and organs were collected. (A) Experimental design. (B) Hepatic triglyceride concentrations. (C) H & E staining, Oil red O staining, and F4/80 staining of mouse livers. Images were obtained and analyzed as described in the Materials and methods section (magnification × 200). Data are means ± SEM, and the significance of differences was determined by one-way ANOVA. Different letters indicate significant differences among the groups. BSE, barley sprout extract; C, control; E, EtOH; L, LBSE; H, HBSE.

polyphenols and flavonoids; the major flavonoid identified in barley sprouts was saponarin (Lee et al., 2015; Seo, Lee, Jia, Wu, & Lee, 2015). In addition, we reported that barley sprout ethanol (EtOH) extract (80% v/v; BSE) exerted hypocholesterolemic and hypoglycemic effects by stimulating AMPK activity and its signaling pathways in cultured hepatocytes and in high-fat diet (HFD)-fed mice (Lee et al., 2015). Thus, in this study, we investigated the effects of BSE on lipid accumulation and steatosis in the liver during or after ingestion of alcohol using a mouse model of AFLD. The results suggest that BSE can alleviate AFLD by activating the AMPK signaling pathway and inducing expression of autophagy-related proteins. Moreover, saponarin was at least in part responsible for these effects.

## 2. Materials and methods

### 2.1. Sample preparation and composition analysis

Barley sprouts (*Hordeum vulgare* L.) were harvested at the Department of Functional Crops, National Institute of Crop Science, Rural Development Administration (Jeonju, Republic of Korea) as described previously (Seo et al., 2013). Briefly, barley seeds (*Kunalbori 1*) were imbibed in water for 1 day prior to germination and kept in the dark for 2 days at 22–25 °C. The germinated barley was grown in a growth chamber (DSGC768, Dongseo Science, Anyang, Republic of Korea) under 60% relative humidity. Thirty days after germination, barley leaves were harvested, freeze-dried and extracted using EtOH (80%, v/v) at room temperature for 24 h. The extracts were filtered and evaporated under a vacuum and subsequently freeze-dried to yield dry powders. BSE composition was analyzed as described previously (Seo et al., 2013). Total polyphenols were determined by the Folin–Ciocalteu method, as described previously (Singleton, Orthofer, & Lamuela-Raventos, 1999). To quantify policosanols, the lipophilic fraction was isolated by adding hexane, and gas chromatography mass spectrometry (GC–MS) analysis was performed using the Agilent Technologies 7890A series GC system coupled to the 5975C single quadrupole MS (Agilent Technologies, Palo Alto, CA, USA) with the HP-5MS (5% diphenyl–95% dimethylsiloxane co-polymer) capillary GC column (30 m × 0.25 μm × 0.25 μm film thickness; Agilent Technologies). The oven temperature was programmed to increase from 150 to 325 °C at a rate of 4 °C/min and then maintained at 320 °C for 5 min. Helium was used as the carrier gas at a flow rate of 1.8 mL/min. The sample (1 μL) was injected into the GC by an autosampler (Agilent Technologies). The split ratio was 1:5. For MS detection, the electron impact ion source and transfer line temperatures were set to 200 and 280 °C, respectively, and the ionization energy was set to 70 eV. Data collection and analysis were conducted using the GC–MSD Chemstation (Agilent Technologies) (Seo et al., 2013). The results showed that BSE contained 167.4 mg/g total polyphenols, 58.0 mg/g flavonoids, and 19.4 mg/g saponarin (Supplemental Table 1).

### 2.2. Animal experiments

Mice were fed EtOH to induce AFLD (Bertola, Mathews, Ki, Wang, & Gao, 2013). C57BL/6 male mice (6 weeks old, 20–23 g) were purchased from Samtako (Kyunggido, Korea). Two animal experiments were performed. In the first experiment, mice were fed a commercial