

Improving Effects of *Brassica oleracea* L. var. *italica* Sprout Extract on Alcoholic Liver Dysfunction

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Abstract - Alcoholic fatty liver disorder has become a frequent health concern worldwide. To investigate the effects of *Brassica oleracea* (*B. oleracea*) sprout extract (BOE), the present study was designed with alcoholic fatty liver in the rat. Initially, the effects of BOE on liver parameters were examined. Male rats were divided into five groups. The normal control group was fed the normal diet, and the BOE group was fed the high fat diet and ethanol with/without BOE for 4 weeks. After 4 weeks feeding period, rats were sacrificed and their livers and blood were used for fatty liver-related biomarkers analyses. As a result, BOE ameliorated fatty liver-related enzymes profiles in liver tissues and also reduced blood alcohol concentration in rat model. We demonstrated that BOE protected the high fat diet and alcohol-induced fatty liver in rat model. Furthermore, BOE increased detoxificative abilities against alcohol.

Key words – Alcoholic liver dysfunction, *Brassica oleracea* sprout extract, Fatty liver, High fat diet

Introduction

Major effective compound of *B. oleracea*, sulforaphane (4-methylsulfinylbutyl isothiocyanate) has been well identified as the most potent naturally occurring inducer of phase II enzymes (Fahey *et al.*, 1997; Yoshida *et al.*, 2015). Especially, dietary sulforaphane is known to ameliorate various injuries such as carbon tetrachloride-induced intestinal ischemia reperfusion etc (Baek *et al.*, 2008). In these reports, the ability of orally ingested sulforaphane-riched BOE to induce ameliorative effect was suggested as the basis for protection from these injuries.

Sulforaphane is a metabolite of glucoraphanin via hydrolyzation to sulforaphane by myrosinase (Bones and Rossiter, 1996; Zhang and Talalay, 1998). *B. oleracea* sprouts have

been reported to induce detoxification enzymes in vitro and in vivo (Zhang *et al.*, 2006). In other hands, *B. oleracea* synthesized naphthoquinone derivatives such as vitamin K families such as phyloquinone (Blanco and Blanco, 2017) by various plants including cabbage, cauliflower, tomatoes, spinach, and soybean, olive, and sunflower oils. Indeed, many phytochemicals were reported many medicinal effects such as cancer (Mokhtari *et al.*, 2018; Ravichandiran *et al.*, 2019), metabolic disorder (Teng *et al.*, 2019), muscle inflammation (Sun *et al.*, 2015), atherosclerosis (Shehatou and Suddek, 2016) and temporal/sustained neurodegeneration (Klomprens and Ding, 2019; Oh *et al.*, 2013). Fortunately, few reports have evaluated already the effects of ingestion of *B. oleracea* sprouts on liver function via various mechanisms such as regulation of ER stress (Lei *et al.*, 2018) and activation of nuclear erythroid 2-related factor 2 (Nrf2) (Xu *et al.*, 2019), protection of inflammation in hepatic tumorigenesis (Chen *et al.*, 2016). However, no has been reported on the improve-

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ment of lipidemic liver disorder with alcohol metabolism in the blood. Therefore, we investigate the effects of BOE on HFD and alcohol-induced liver with *in vivo* model. These reports show that alcoholic liver injuries are reduced by intake of BOE in *in vitro* and *in vivo*;

Materials and Methods

Preparation of BOE

To obtain BOE, *B. oleracea* were germinated from guaranteed seed and sprouts (3 days after budding) (KOREGON, Ansong, Korea) (20 kg) extracted with ten-fold volume of 70% ethanol at 40–60°C for 12 h. The extract was filtered by 0.45 μm pore size filter (EMD Millipore, MA, USA) and concentrated with rotary evaporator (N-1300E, EYELA, Tokyo, Japan). Finally, the concentrated extract was sterilized by filtration through MF-Millipore filters (EMD Millipore, MA, USA), freeze dried (Bontero, IIShinBioBase Co Ltd, Korea), packed in plastic tubes (Nunc, CA, USA), and stored at -80°C (Thermo-Fisher, CA, USA) until use (Anydoctor HealthCare Co. Ltd. Seoul, Korea).

Reagents and kits

All guaranteed reagents were purchased from Sigma-Aldrich Co (MO, USA). Venous whole blood was taken from inferior vena cava, and then serum samples were sent to the Green Cross Laboratories (GC lab, GiHeung, Korea) on the same day. Parameters including hepatic enzymes such as alanine aminotransferase (ALT), alkaline phosphatase (ALP), and gamma-glutamyl transferase (GGT) as well as the lipid profile including, total cholesterol (T-CHO), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), and blood ethanol concentration were examined.

Animal model

We performed alcohol-induced fatty liver models (approval No WKU-17-67, Ethics Committee of the Wonkwang University). For the experiment using the ethanol-induced fatty liver model, male Sprague-Dawley rats aged 6 weeks were purchased from Orient Bio (Sungnam, Korea). After the acclimatization period, rats were divided into Normal control (n = 10), fatty liver model (n = 10), fatty liver model (high fat

diet for 4 weeks) + 30 mg/kg BOE (n = 10), fatty liver model + 100 mg/kg BOE, and fatty liver model + 300 mg/kg BOE (n = 10) groups. Control and fatty liver groups were fed the normal diet, and the BOE treat groups were fed the BOE diet for 4 weeks. Fatty liver was induced in the fatty liver model (high fat diet + ethanol) group and fatty liver + BOE groups by oral administration of 6 mL/kg ethanol (15 mL/kg as 40% ethanol, v/v). The normal group was orally administered same solvent.

Histologic experiments

Histological experiments were conducted following common methods. Briefly, the dissected liver tissue was fixed in 10% formalin solution and embedded with paraffin. The paraffin sections (4–6 μm thickness) and stained with hematoxylin and eosin (H&E) following routine methods. Images were taken in randomly chosen microscopic views from individual slide of each animals using a microscope (TE-2000, Nikon, Tokyo, Japan)(Xie *et al.*, 2018). Slides were graded for steatosis according to the Kleiner's methods (Kleiner *et al.*, 2005). A score of steatosis grade was assigned to describe the extent of lipid accumulated spaces in the hepatocytes (grade 0: <5%; grade 1: 5–33%; grade 2: 33–66%; grade 3: >66%) (Wang *et al.*, 2015).

Statistical analysis:

All data are expressed as the Mean \pm S.E.M. A one-way analysis of variance (ANOVA) test employed followed by Tukey's multiple range tests to compare each group. Student's t-test was used for comparisons of two groups. Statistical analyses conducted using SPSS for Windows software (Ver 10.0, Chicago, IL, USA) and data with different superscript letters are significantly different when p value is less than 0.05.

Results and Discussion

Single treatment of BOE to hepatocytes cell line had low cytotoxic effect on the concentration of dietary intake in rats (data not shown). The levels of body weight gain food intake, and water intake after HFD (control group) and HFD + EtOH administration (ethanol group), shown no significantly changed

in the various dose of BOE group compared with each group

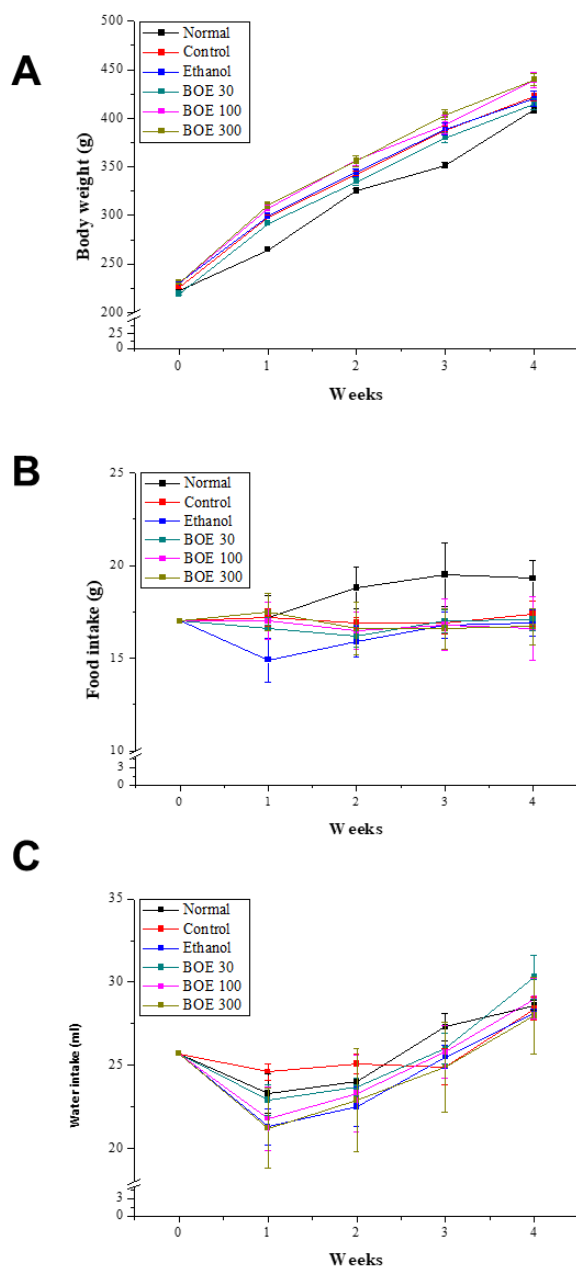


Fig. 1. Changes of general profiles of BOE-administrated rats. After 4 weeks with normal diet (normal group) or high fat diet (control and ethanol group), BOEs were treated indicated dosages. (A) Body weight (B) food intake, and (C) water intake profiles were monitored and drawn as the line-scatter graphs. ^{a,b,c} Values in the row with different superscript letters are significantly different by Tukey's multiple range tests at $P < 0.05$. Data are shown as mean \pm SEM.

(Fig. 1). Indeed, administration of different dose of BOE also shown no significant changes between each group. Whereas, the levels of ALP, ALT as a blood enzyme marker of functional integrity on liver were markedly reduced by administration of BOE in a dose dependent manner (Fig. 2). In other study, *B. oleracea* sprout extract-containing diet induced low increases of body weight than normal diets (Kikuchi *et al.*, 2015; Lai *et al.*, 2008). Similarly, we also show no significant changes of weight gain in the animals fed with direct administration of BOE (Fig. 1A). Therefore, these results suggested that BOE has ameliorative effects on HFD- and EtOH-induced liver

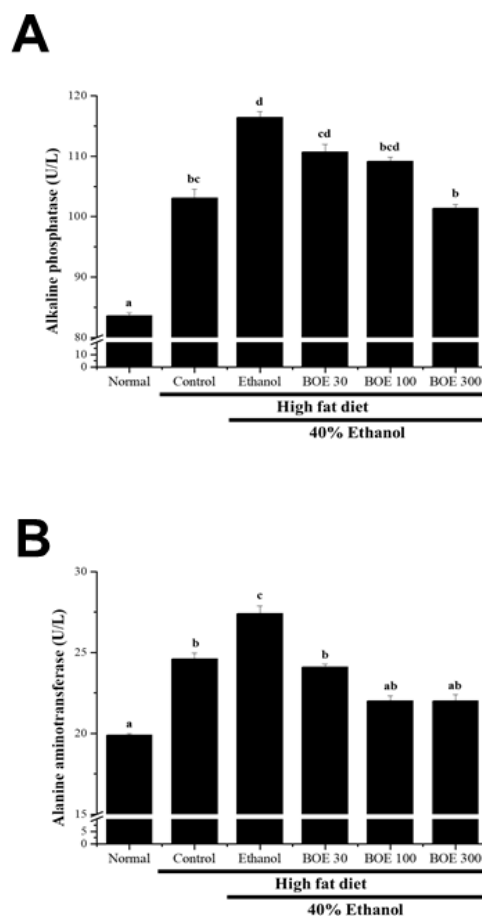


Fig. 2. Changes of liver-related enzymes profiles in serum on HFD/alcohol-induced fatty liver model by BOE-administration. Normal diet (normal group) or high fat diet (control and ethanol group), BOEs were treated indicated dosages for 4 weeks and then measured (A) alkaline phosphatase (ALP) and (B) alanine aminotransferase (ALT). ^{a,b,c} Values in the row with different superscript letters are significantly different, $P < 0.05$. Data are shown as mean \pm SEM.

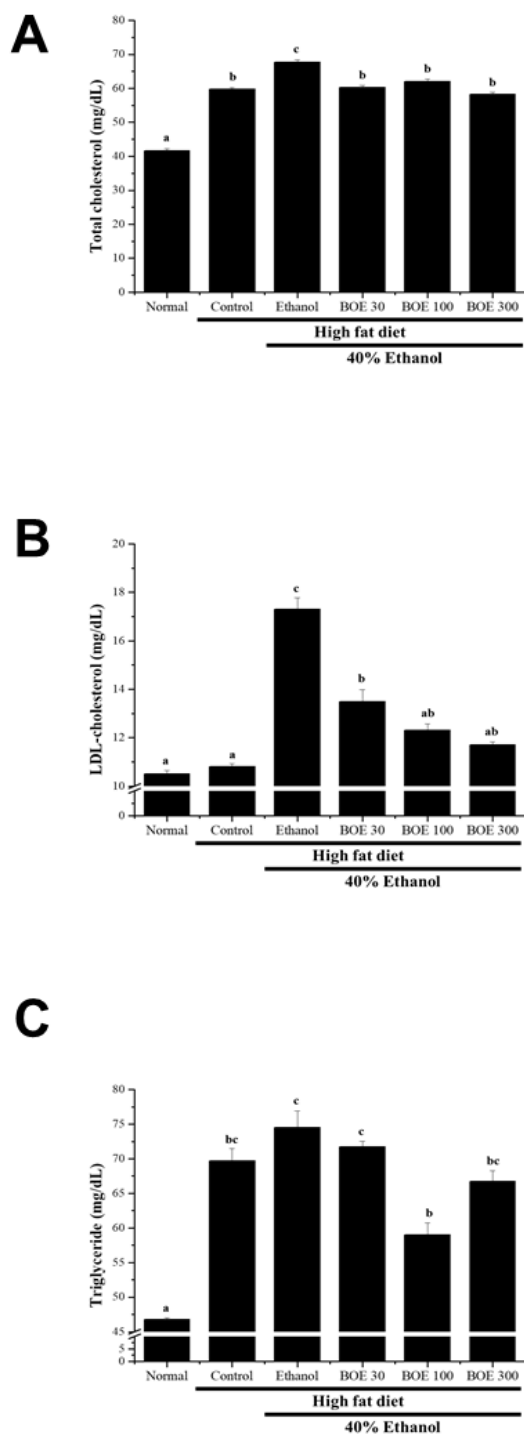


Fig. 3. Changes of blood lipid profiles on HFD/alcohol-induced fatty liver model by BOE-administration. (A) Total cholesterol (T-CHO), (B) low-density lipoprotein cholesterol (LDL-C), and (C) triglyceride (TG) levels in serum. ^{a,b,c} Values in the row with different superscript letters are significantly different by Tukey's multiple range tests at $P < 0.05$. Data are shown as mean \pm SEM.

disorder and BOE had effects to more different mechanisms to other organs, but may require long-term administration of BOE for observe of chronic effects, if necessary.

Taken together, we measured blood levels of T-CHO, LDL-C, and TG (Fig. 3). In control and EtOH groups were significantly increased these lipid markers in blood, whereas administration of BOE was recovered compared with the control and/or EtOH group. Although, LDL-C was reduced by administration BOE in a dose dependent manner (Fig. 3B), however T-CHO and TG were shown lower dose dependency (Fig. 3A and C). Indeed, the level of high-density lipoprotein cholesterol (HDL-C) shows no significant changes in any groups (data now shown). Therefore, we need further examines specific mechanisms of BOE on lipid metabolism on same model in future.

Taken together, we investigated effects of BOE administration on alcohol degradation in blood. Levels of blood alcohol were highly elevated at 1hr after EtOH administration. Interestingly, chronic administration of BOE were significantly reduced blood alcohol levels at same time point in a dose dependent manner and decreased area under curve (AUC) (Fig. 4). These results suggested that improving effects of BOE in animal liver function were also related against to

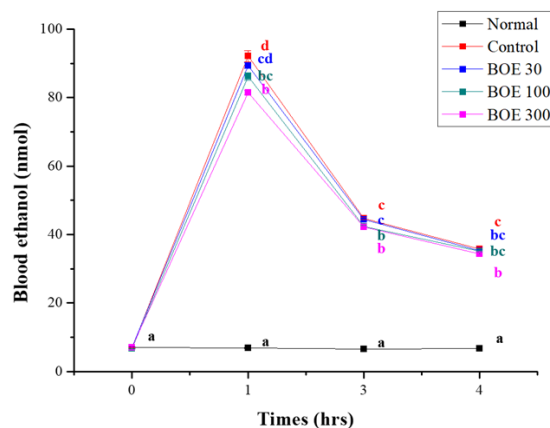


Fig. 4. Effects of BOE on reduction of blood alcohol concentration on the HFD/alcohol-induced fatty liver. Ethanol was single administrated to both group and measured blood alcohol described in Materials and Methods. ^{a,b,c} Values in the row with different superscript letters are significantly different by Tukey's multiple range tests at $P < 0.05$. Data are shown as mean \pm SEM.

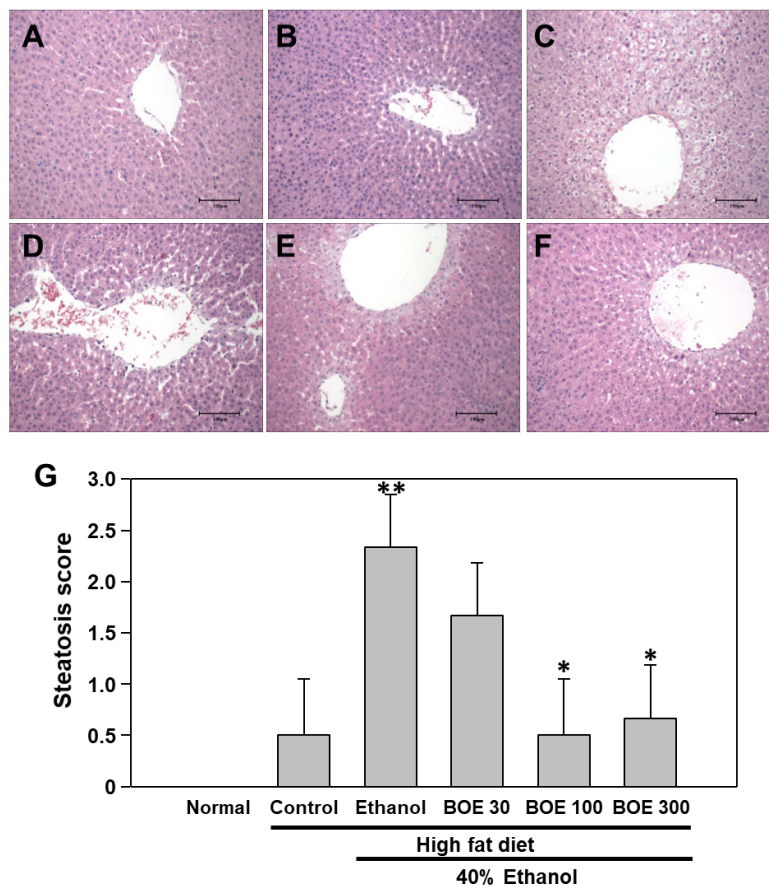


Fig. 5. Ameliorative effects of BOE on high fat diet- and HFD/alcohol-induced fatty liver model. Tissue sections slide of (A) normal, (B) HFD, (C) HFD + EtOH, (D) HFD + EtOH + BOE 50 mg/kg, (E) HFD + EtOH + BOE 100 mg/kg, and (F) HFD + EtOH + BOE 300 mg/kg were stained with H&E methods and presented (G) steatosis scores. Scale bar = 100 μ m. Magnification: $\times 20$. ** indicates significantly difference between normal and EtOH group by Student's T-test at $P < 0.01$. * indicates significantly difference with HFD+EtOH group by Student's T-test at $P < 0.05$. Data are shown as mean \pm SEM.

alcohol metabolism.

Based on these data, we performed histological experiments for evaluate that improving effects against HFD- and alcohol-induced fatty liver and its complication. Liver steatosis, abnormal retention of lipids within cells, is primary lipid metabolic problem. As a result, high fat diet and ethanol administration were markedly increased lipid and architecture malformation in liver tissue. In contrast, BOE administration were significantly reduced lipid droplet area in a dose dependent manner (Fig. 5) and relatively higher degree of steatosis accompanied by the presence of various size fat vacuoles (Fig 5B). These results suggest that administration BOE have ameliorative effects alcohol- and HFD-induced hepatic steatosis via reduction of lipid accumulation in

hepatocytes.

In summary, we showed that BOE ameliorated liver functions and lipid metabolism in normal and fatty liver with EtOH administration in rat model. Moreover, BOE improved ability alcohol metabolism in rats model also. Our data suggest that protection from various liver injuries resulted from induction of anti-oxidation such as GSH synthesis and GST activity. Indeed, some other mechanisms may contribute to suppression of HFD and/or alcohol-induced liver injury with other liver problem (Xie *et al.*, 2017). Therefore, we conclude that BOE had defensive and protected function to the hepatic-disorders of various types of excess lipid accumulation and alcohol consumption through acceleration of metabolism and antioxidation in the liver.

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Conflict of Interest

The authors declares that there is no conflict of interest regarding the publication of this paper.

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