Selenium-enriched *Bifidobacterium longum* protected alcohol and high fat diet induced hepatic injury in mice

**YI Hong-Wei**, **ZHU Xiao-Xiao**, **HUANG Xiao-Li**, **LAI Yu-Zhu**, **TANG Yue**

Department of Pharmacology, School of Medicine, Southeast University, Nanjing 210009, China

Available online 20 Mar., 2020

**[ABSTRACT]** The objective of this study was to verify the protective effect of *Bifidobacterium longum* (BL) and the synergistical effect of Selenium and BL on alcohol plus high fat diet (HFD) induced hepatic injury in mice. We also want to explore the mechanism of Selenium-enriched *Bifidobacterium longum* (SeBL). C57BL/6 mice were treated with alcohol plus HFD with or without different dosage of BL or SeBL for 4 weeks. Serum levels of ALT, AST, TC, TG, LDL-C, HDL-C, FFAs, TNF-α, IL-6 and IL-1β, hepatic MDA level, SOD activity, the mRNA levels of AMPK, PPAR-α and SREBP1 were invested. SeBL inhibited lipid accumulation in hepatocytes; reduced serum AST and ALT levels; improved dyslipidemia; decreased serum FFAs, TC, TG and LDL-C levels. SeBL also inhibited alcohol plus HFD-induced hepatocyte oxidative stress through decrease in hepatic MDA levels and increase in SOD activity. SeBL also regulated lipid metabolism related genes such as AMPK, PPAR-α and SREBP1. Although BL had similar effect as SeBL, SeBL is more effective than BL. SeBL protected mice from alcohol plus HFD-induced hepatic injury in mice because of its inhibitory effect on hepatocellular oxidative stress, lipogenesis and inflammation. Selenium enhanced the protective effect of BL.

**[KEY WORDS]** Selenium-enriched *Bifidobacterium longum*; Alcoholic liver disease; Non-alcoholic fatty liver disease; Oxidative stress; Inflammation

**[CLC Number]** R965 **[Document code]** A **[Article ID]** 2095-6975(2020)03-0169-09

**Introduction**

Increasing clinical evidence supports some of the proposed health benefits related to the use of probiotics, particularly in managing digestive system diseases. Probiotics are defined as monocultures or mixed cultures of microorganisms that can be administrated to potentially improve the properties of the gut microbiota. Intake of *L. salivarius* and *S. faecium* reduced gut-derived microbial lipopolysaccharide and *E. coli* of mild (not severe) alcoholic hepatitis patients [1]. Probiotics (containing *B. bifidum* and *L. plantarum*) therapy led to a decrease in serum aspartate aminotransferase (AST) with standard therapy [2]. A potential mechanism is that the probiotics transforms the composition of intestinal microbiota, which leads to reductions in alcohol-induced dysbiosis, intestinal permeability, bacterial translocation, endotoxemia, and consequently, the development of alcoholic liver disease (ALD), a major cause of morbidity and mortality worldwide.

Although probiotics had been used in treatment of ALD [3] or non-alcoholic fatty liver disease (NAFLD) [4]. The protective effect of Selenium-enriched *Bifidobacterium longum* (SeBL) was not reported before. We hypothesized that Selenium enhanced protective of *Bifidobacterium longum* (BL) on hepatic injury. In present study, we compared the effect of SeBL and BL on alcohol and high fat diet (HFD) induced hepatic injury in mice.

Consistently high levels of alcohol intake leads to ALD. The spectrum of ALD encompasses fatty liver, hepatic inflammation and necrosis, progressive fibrosis and hepatocellular carcinoma. Heavy alcohol drinking is also associated with disorders unrelated to the liver, such as infections, malignancies, cardiovascular events and diseases of the nervous system, pancreas and kidneys [5]. Globally, alcohol use was the seventh leading risk factor for both deaths and disability-adjusted life-years (DALYs) in 2016, accounting for 2.2% of age-standardised female deaths and 6.8% of age-standardised male deaths. Among the population aged 15–49 years, alcohol use was the leading risk factor globally in 2016, with 3.8% of female deaths and 12.2% of male deaths attributable to alcohol use [6].

The cornerstone of management of patients with ALD is to stop them from drinking alcohol, as continued alcohol intake is the single most important risk factor for progression of ALD. Malnutrition is present in 20%–60% in outpatient alco-
surgical cirrhosis patients and almost 100% in hospitalized alcoholic hepatitis patients. Providing adequate nutrition is important in the management of patients with cirrhosis. Corticosteroids, Pentoxifylline (a phosphodiesterase inhibitor that blocks transcription of tumor necrosis factor α (TNF-α) to decrease serum levels of the gene product), disulfiram, naltrexone, acamprosate, baclofen and antioxidant drugs (such as vitamin E and silibinin) can help some patients achieve sustained abstinence; but drug treatment is not successful in all patients. Despite intensive research in the last two decades, there is currently no Food and Drug Administration-approved therapy for treating ALD [7].

NAFLD is defined as the presence of 5% of hepatic steatosis, in the absence of competing liver disease etiologies, such as chronic viral hepatitis, use of medications that induce steatosis such as amiodarone or tamoxifen, and other chronic liver diseases, such as autoimmune hepatitis, hemochromatosis, Wilson’s disease, or significant alcohol consumption. Excess caloric consumption leading to obesity and related comorbidities is a leading risk factor for NAFLD [8]. Clinically, NAFLD patients tend to be obese, with insulin resistance and/or type 2 diabetes, dyslipidemia, hypertriglyceridemia, and hypertension, which are all risk factors for cardiovascular diseases [9-10]. Although NAFLD is well recognized as the most common cause of liver disease, there are currently no approved pharmacotherapies.

It has been recognized for many years that being overweight or obese and consuming excess amounts of alcohol synergistically promote the development and progression of liver steatosis, hepatitis, cirrhosis, and hepatocellular carcinoma in patients [11]. Alcohol administration for four weeks caused significant hepatic damage as evidenced by increases in hepatic lipid droplets, plasma alanine aminotransferase (ALT) level and inflammatory cytokine level, which were aggravated with the combination of HFD [12]. In present study, we used chronic consumption of alcohol with an HFD to produce an appropriate model for hepatic damage in mice and investigated the protective effect of probiotics.

Antioxidants, both natural and synthetic, have been proposed and utilized as therapeutic agents in hepatic damage. Selenium is of fundamental importance to human health. It is an essential component of several major metabolic pathways, including thyroid hormone metabolism, antioxidant defense systems, and immune function. Serum Selenium levels were significantly lower in both ALD and NAFLD compared to healthy controls and non-malignant disease controls; moreover, hepatic Selenium levels were also reduced in both ALD and NAFLD compared to controls [13]. In this study, we found that Selenium could enhance the protective effect of BL on alcohol plus HFD-induced hepatic damage in mice. Therefore, SeBL will be a potential nutritional supplement.

Materials and Methods

Animals

Male C57BL/6 mice were purchased from Experimental Animal Center of Nanjing Medical University (Nanjing, China). Mice were housed under 12 h light/12 h dark cycle and fed standard chow and tap water ad libitum for a week before treatment. BL and SeBL were gifts of Jiangsu Dexi BioScience Co. Ltd. The bacterial line was DD98. The number of alive bacterial was 10^7/mg. Mice were divided into eight groups: normal diet (ND) group, 10% alcohol plus HFD group and 10% alcohol plus HFD with 3 dosage of BL groups and 3 dosage of SeBL groups respectively. The HFD contains 10% lard and 2% cholesterol, 0.2% cholate, 5% whole milk powder, 5% custard powder and 77.8% ordinary forage. All studies were approved by the Animal Ethics Committee of Southeast University (Nanjing, China) and done in accordance with the Guide for the Care and Use of Laboratory Animals.

Biochemical Measurements

Serum TC, TG, LDL-C, HDL-C, ALT, AST, GGT, LDH and FFAs, hepatic MDA and SOD activity were measured using colorimetric detection kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

Histological Evaluation

Formalin-fixed/paraffin-embedded liver sections were stained with H&E. Liver pathology was evaluated in a blind manner by a certified veterinary pathologist and scored as follows. For steatosis: grade 0, absent; grade 1, < 33% of the parenchyma; grade 2, 34%–66% of the parenchyma; grade 3, > 67% of the parenchyma. For inflammation: grade 0, absent of foci of inflammation; grade 1, fewer than one foci per two 20 × fields; grade 2, one foci per two 20 × fields; grade 3, one to two foci per one 20 × field; or grade 4, more than two foci per one 20 × field.

Enzyme linked immunosorbent assay (ELISA)

Serum IL-1β, IL-6 and TNF-α were detected according to the manual of ELISA kit (R&D systems, Inc, Minneapolis, MN).

RNA Isolation and Gene Expression Analysis

Total RNA was extracted and isolated using TRIzol reagent (LifeTechnologies, Grand Island, NY) according to the manufacturer’s instructions. The first strand of cDNA was reverse transcribed using the ThermoScript reverse transcription-polymerase chain reaction (RT-PCR) system kit (Life Technologies, Grand Island, NY) at 50 °C for 50 min. The mRNA levels of targeted genes were determined by real-time PCR and normalized using β-Actin. The following primers were used for β-Actin: forward 5′-GGCGTATTCCCCCTCCATCG-3′, reverse 5′-CCAGTTGGTAACATGCAAT-GT-3′; SREBP1: forward 5′-TGACCCGGCTATTCCGTGGA-3′, reverse 5′-CTGGGCTGAGCAATACAGTTTC-3′; PPAR-α: forward 5′-AGAGCCCCATCTGTCTCTCTG-3′, reverse 5′-TCTGGTAGTCTGAAAACCAA-3′; AMPK: forward 5′-GTCAAAAGCCCCAGATAGTTTAATGGGTT-3′.

Statistical Analysis

Values were expressed as the mean ± SEM. Significance between groups was evaluated by one-way analysis of vari-
ance (ANOVA) followed by a Student-Newman–Keuls post hoc test. *P < 0.05 was considered statistically significant.

**Results**

SeBL ameliorated alcohol plus HFD-induced mice hepatic steatosis

Prolonged alcohol plus HFD intake resulted in mice hepatic steatosis and hepatocytes damage. The body weight decreased in the first week after alcohol plus HFD intake, and then increased slowly. There were no significant difference between the body weight of these mice fed with normal diet (ND) and alcohol plus HFD (Fig. 1A). The liver index of these mice fed with alcohol and HFD for 4 weeks increased, however, no significant difference was found among different groups (Fig. 1B). Consistently high fat and high cholesterol diet lead to metabolic disease such as diabetes. Therefore, we detected the serum glucose levels after 12 h fast. Serum glucose levels increased significantly. BL and SeBL partially decreased the serum glucose levels; however, there were no significant difference between these groups (Fig. 1C). The serum glucose levels were below 5 mmol·L\(^{-1}\) because the duration of alcohol plus HFD intake was not long enough. Alcohol plus HFD also induced hepatic lipid deposition. Hematoxylin/eosin (H&E) staining showed steatosis formation and inflammatory cells infiltration in mice liver. Large droplet fat appeared as a single or several areas of clearing within affected hepatocytes. The involved hepatocytic nucleus was displaced eccentrically by the fat droplet. BL and SeBL inhibited lipid accumulation in hepatocytes and ameliorated hepatic steatosis and hepatocytes damage (Fig. 1D). Steatosis score and inflammation score decreased after the administration of BL or SeBL, but only 100 mg·kg\(^{-1}\)SeBL had significant protective effect on ameliorating steatosis and inflammation (Fig. 1E–F).

**SeBL inhibited alcohol plus HFD-induced hepatic injury in mice**

The serum ALT and AST levels increased significantly after 4-weeks alcohol plus HFD intake, at the same time the serum γ-glutamyltransferase (GGT) and lactate dehydrogenase (LDH) increased too (Fig. 2). Both BL and SeBL de-

---

**Fig. 1** SeBL inhibited alcohol plus HFD-induced liver damage. C57BL/6 mice were fed with alcohol plus HFD for 4 weeks, at the same time these mice were administrated with several dosage of BL and SeBL respectively. Mice body weight (A), liver index (B) and serum glucose (C) were measured. Representative images of H&E staining of the liver tissues from each treatment group were shown (D) a) control; b) alcohol plus HFD; c-e) alcohol plus HFD and 25, 50, 100 mg·kg\(^{-1}\) BL respective treatment; f-h) alcohol plus HFD and 25, 50, 100 mg·kg\(^{-1}\) SeBL respective treatment. Liver steatosis score (E) and inflammation score (F) were measured as described in material and methods. The value was presented with mean ± SEM (n = 6), *P < 0.05, **P < 0.01, ***P < 0.001
increased ALT levels, but only 100 mg·kg⁻¹ SeBL decreased AST level (Fig. 2A). Although BL decrease serum GGT and LDH levels, there were no significant difference between model group and BL treated groups. 100 mg·kg⁻¹ SeBL significantly decreased mice serum GGT (Fig. 2B) and LDH levels (Fig. 2C). Although both BL and SeBL inhibited alcohol plus HFD-induced hepatic injury, SeBL had more potential protective effect.

**SeBL improved alcohol plus HFD-induced dyslipidemia**

Alcohol plus HFD resulted in rat dyslipidemia, such as serum levels of TG, TC, FFAs and LDL-C increased, while HDL-C levels decreased [14]. In the present study, we also found that alcohol and HFD had synergetic effect on promoting mice dyslipidemia. The mice serum levels of triglyceride (TG), total cholesterol (TC) and low density lipoprotein-cholesterol (LDL-C) increased significantly, but serum high density lipoprotein-cholesterol (HDL-C) levels decreased after 4-weeks alcohol plus HFD intake (Fig. 3A). At the same time, the serum free fatty acids (FFAs) levels also increased significantly (Fig. 3B). 100 mg·kg⁻¹ BL inhibited the increase in mice TG, TC and LDL-C levels induced by alcohol plus HFD intake. But 25 mg BL almost had no significant effect on dyslipidemia (Fig. 3A). 50 and 100 mg·kg⁻¹ SeBL significantly decreased TG and TC levels, meanwhile, 100 mg·kg⁻¹ SeBL decreased LDL-C levels significantly (Fig. 3A). Neither BL nor SeBL had effect on HDL-C levels. 100 mg·kg⁻¹ BL decreased FFAs levels, whereas, 50 and 100 mg·kg⁻¹ SeBL significantly inhibited FFAs levels in a dose dependent manner (Fig. 3B). To further evaluate the effect of BL and SeBL on hepatic steatosis in mice, Oil Red O staining was performed. As shown in Fig. 3C, HFD and alcohol induced lipid deposition in hepatocyte, moreover, BL and SeBL decreased lipid accumulation. Compared with the BL group, SeBL significantly ameliorated hepatic steatosis. Therefore, we drew a conclusion that SeBL performed a more powerful rescue from dyslipidemia induced by alcohol plus HFD intake.

**SeBL inhibited alcohol plus HFD-induced oxidative stress in hepatocytes**

Overmuch calorie especially lipid intake resulted in lipid accumulation, increase in intra-hepatocellular β-oxidation and production of reactive oxygen species (ROS). Intracellu lar oxidative stress increase production of malondialdehyde (MDA) and decrease the activity of superoxide dismutase (SOD). In the present study, we found that 100 mg·kg⁻¹ BL inhibited the increase in MDA and the decrease in SOD activity induced by alcohol plus HFD intake. SeBL decreased MDA and increased SOD activity in a dose dependent manner (Fig. 4). Therefore, Selenium was conducive to inhibition of alcohol plus HFD-induced hepatocellular oxidative stress. Selenium also enhanced the anti-oxidative effect of BL.

**SeBL recovered the balance of hepatocellular lipid metabolism**

Alcohol plus HFD intake induced intra-hepatocellular lipid accumulation. The major mechanism was overmuch FFAs intake-induced increase in lipogenesis and decrease in lipolysis. We detected the genes that regulate lipogenesis and lipolysis. Alcohol plus HFD intake decreased the mRNA levels of adenosine monophosphate-activated protein kinase (AMPK) and peroxisome proliferator-activating receptor α (PPAR-α) (Fig. 5A, B), at the same time the mRNA levels of sterol regulatory element binding transcription factor 1 (SREBP1) increased (Fig. 5C). AMPK and PPAR-α involve in lipolysis while SREBP1 involves in lipogenesis. Only
SeBL significantly increased AMPK and PPAR-α mRNA levels and decreased SREBP1 mRNA level (Fig. 5). BL had no significant effect on mRNA levels of these genes. SeBL with high dosage such as 100 mg·kg\(^{-1}\) regulated these genes associated with the balance of intracellular lipid metabolism. Improvement of the lipid metabolism involved in the inhibitory effect of SeBL on alcohol plus HFD-induced hepatocytes steatosis.

**SeBL inhibited alcohol plus HFD-induced pro-inflammatory factors release**

Alcohol plus HFD caused the damage of hepatocytes, infiltration of inflammatory cells and release of pro-inflammatory factors such as interleukin 6 (IL-6), TNF-α and interleukin 1β (IL-1β). We found that BL inhibited alcohol plus HFD-induced TNF-α release. SeBL inhibited IL-6, TNF-α and IL-1β release (Fig. 6). SeBL inhibited alcohol plus HFD-induced inflammation in a dose dependent manner. SeBL had more potential inhibitory effect on alcohol plus HFD-induced inflammatory factors release.

**Discussion**

In present study, we confirmed the protective effect of BL and SeBL on alcohol plus HFD-induced hepatic injury in mice. SeBL had more potential protective effect than BL. Furthermore, we have explored the molecular mechanism which might be mediated through anti-oxidation, inflammation inhibition and restored the balance of lipid metabolism in mice liver.

A major unmet need in the study of ALD is the lack of a reliable animal model that mimics the entire spectrum of this disease in humans. An ideal animal model of alcoholic hepatitis should at least include inflammation, steatosis, cell damage, fibrosis and the activation of regeneration. Indeed, owing to marked differences in alcohol metabolism between rodents and humans, most animal models with simple ethanol feeding lead to steatosis, mild liver inflammation and no fibrosis, with major differences between mice strains in terms of hepatic injury [15]. Limuro et al. reported that female rodents were more susceptible to chronic alcohol-induced hep-
Fig. 4  SeBL inhibited alcohol plus HFD-induced hepatic oxidative stress. After 4-weeks alcohol plus HFD intake with BL or SeBL administration, livers of C57BL/6 mice were collected. SOD activity (A) and MDA (B) were measured as described in material and methods. The value was presented with mean ± SEM (n = 6), *P < 0.05, **P < 0.01, ***P < 0.001

Excessive calorie and alcohol intake accelerated liver damage in patients and animal [11, 14, 18]. The public health impact of NAFLD, which is strongly associated with overweight or obesity and the metabolic syndrome, is significant given the worldwide disease burden and the associated morbidity and mortality. ALD caused by long-term consumption of excessive amounts of alcohol, is a major cause of morbidity and mortality globally and represents one of the most prevalent common diseases. In present study, we found that HFD synergistically exacerbated alcohol induced mice hepatic steatosis (Figs. 1D, 3C). BL and SeBL ameliorated alcohol plus HFD-induced hepatic damage.

The diagnosis of ALD is often difficult to make due to reliance on accurate history, suspicion and lack of accurate biochemical testing and physical exam findings to determine severity of liver disease. The leakage of hepatocellular AST and ALT is a prominent sign of hepatic injury. We found that ALT was more specific for hepatic damage than AST and appeared to be compatible with the histologic findings in the liver (Fig 2A). This result is consistent with the previously published data in that alcoholic hepatitis altered serum ALT more than AST after exposure to alcohol [19]. Among the various biomarkers of chronic alcohol abuse, GGT has a good sensitivity for detecting excessive ethanol consumption [20].
Fig. 6  SeBL inhibited pro-inflammatory factor release induced by alcohol plus HFD intake. After 4-weeks alcohol plus HFD intake, C57BL/6 mice serum were collected. IL-6 (A), TNF-α (B) and IL-1β (C) were measured as the manufacturer’s instructions. The value was presented with mean ± SEM (n = 6). *P < 0.05, **P < 0.01, ***P < 0.001

BL or SeBL was sufficient to protect against changes in hepatic steatosis and serum ALT, AST and GGT in mice receiving both alcohol plus HFD (Fig. 2).

Germ-free mice develop more hepatic injury after alcohol feeding and more fibrosis after administration of a liver toxin [21–22]. In present study, BL and SeBL were used as healthy microbiota to restore to eubiosis. *Bifidobacterium* is saccharolytic bacteria that can ferment carbohydrates to lactic acid. Lactic acid is known to be effective in inhibiting the growth of pathogenic bacteria. Probiotic bacteria also enhance intestinal barrier function by promoting intestinal epithelial cell survival and growth, at the same time, the immune system may be modulated to suppress the release of pro-inflammatory cytokines such as TNF-α and to induce the release of protective cytokines such as interleukin 10 and transforming growth factor β [3]. We found that BL and SeBL also inhibited pro-inflammatory cytokines such as IL-1β, IL-6 and TNF-α release (Fig. 6). Recently, probiotics have been shown to be effective in reducing or preventing the progression of hepatic damage. BL supplementation can be effective for improvement of some anthropometric, inflammatory and oxidative indices in patients with NAFLD [23]. Oral supplementation with BL attenuates hepatic fat accumulation [24]. Lactobacillus but not BL were used to reduce alcohol-induced hepatic inflammation by attenuation of TNF-α production [25]. Although BL had been used to ameliorate hepatic injury, the protective effect of SeBL were not reported. We found that SeBL had more potential protective effect on alcohol plus HFD-induced hepatic injury in mice.

Every gram SeBL used in present study contained about 0.352 mg Selenium. Selenium is incorporated into selenoproteins that have a wide range of pleiotropic effects, ranging from antioxidant and anti-inflammatory effects to the production of active thyroid hormone. Low selenium status has been associated with increased risk of mortality, poor immune function, and cognitive decline. Higher selenium status or selenium supplementation has antiviral effects, is essential for successful male and female reproduction, and reduces the risk of autoimmune thyroid disease [26].

Alcohol metabolism leads to accumulation of ROS, mainly hydrogen peroxide (H₂O₂) and superoxide anion O₂⁻ [27]. As alcohol dehydrogenase, CYP2E1 and aldehyde dehydrogenase are mainly expressed in hepatocytes, most of the direct cellular toxicity of ethanol affects these cells. Chronic exposure to ethanol induces glutathione depletion, which makes hepatocytes more sensitive to oxidative stress [28], as reduced glutathione protects cells against ROS. Selenium is a cofactor of plasma Glutathione peroxidases (GSH-Px) which is synthesized in the kidney. GSH-Px play an important role in ROS metabolism. The biochemical function of glutathione peroxidase is to reduce lipid hydroperoxides to their corresponding alcohols and to reduce free hydrogen peroxide to water. In present study, we found that SeBL reduced hepatic MDA level and increased SOD activity (Fig. 4). SeBL also had more potential protective effect on alcohol plus HFD-induced hepatic injury.

In present study, H&E staining showed hepatocytal macrosteatosis that represented aberrations of lipid metabolism such as excess delivery, altered intracellular metabolism, possibly increased synthesis, and abnormal export mechanisms. The increase in serum TG, TC and LDL-C levels indicated dyslipidemia after alcohol plus HFD intake for 4 weeks, which indicated disbalance of hepatic lipid metabolism (Fig. 3). SeBL increased AMPK and PPAR-α mRNA levels,
decreased SREBP1c mRNA level (Fig. 5). SREBP1c exerts its deleterious role by increasing fatty acid biosynthesis through fatty acid synthase and enzymes responsible for fatty acid desaturation such as stearoyl-CoA desaturase [39]. Conversely, PPAR-α prevents ethanol-induced steatosis [40]. Chronic alcohol consumption promotes steatosis by disrupting hepatic lipid metabolism via SREBP and PPAR-α, which are directly influenced by AMPK. AMPK regulates the relative concentrations of intracellular malonyl Coenzyme A and long-chain acyl-Coenzyme A — the key metabolites responsible for the balance between fat synthesis and fat degradation pathways — is also implicated in hepatic fat metabolism [37]. AMPK downregulation also causes a decrease in fatty acid oxidation mediated by acetyl-CoA carboxylase and carnitine palmitoyltransferase 1. Therefore, the inhibitive effect of SeBL on lipid deposition and hepatic steatosis mediated through lipid metabolism regulation (Fig. 1). Nevertheless, the detailed mechanism was still not clear.

Intake of alcohol or a diet rich in saturated fatty acids, cholesterol, and fructose impairs the intestinal barrier by weakening the mucus-associated defense, impairing tight junction function and causing intestinal inflammation with subsequent translocation of bacterial pathogens into the bloodstream and to the liver. After entering the portal vein blood, endotoxins reach the liver and mediate hepatic injury by activating Kupffer cells through Toll-like receptors (TLRs) and other pathogen recognition receptors. Ligand binding causes Kupffer cells to release the proinflammatory cytokines TNF-α, IL-1β, IL-6, IL-12, and IL-18 and ROS [39]. SeBL inhibited proinflammatory cytokines such as IL-6, TNF-α and IL-1β levels (Fig. 6). Metabolism of alcohol generates a number of metabolites, including acetate, ROS, acetaldehyde, and epigenetic changes, that can induce inflammatory responses. Acute and chronic inflammatory conditions have been closely linked to the pathogenesis of ALD and NAFLD. A high level of circulating TNF-α was found in patients with alcoholic hepatitis without liver diseases, furthermore, elevated IL-6 is found in chronically alcohol-fed animals, even those without apparent liver disease [39]. Reduction of proinflammatory factors greatly facilitated amelioration of alcohol plus HFD-induced hepatic injury in mice.

**Conclusion**

Our results indicate that SeBL and BL prevented alcohol plus HFD-induced hepatic steatosis and damage via inhibiting oxidative stress, proinflammatory factors and restore the balance of lipid metabolism. Although BL and SeBL had similar protective effect, SeBL had more profound effects than BL because SeBL contains Selenium that reduced hepatic cellular ROS. SeBL represents a promising nutritional supplement for alcohol or HFD-induced hepatic injury.

**References**


