IJIE Int. J. Indust. Entomol. 35(2) 123-128 (2017)
ISSN 1598-3579, http://dx.doi.org/10.7852/ijie.2017.35.2.123

Mature silkworm powder reduces blood alcohol concentration and liver injury in ethanol-treated rats

Da-Young Lee[1], Kyung-Sook Hong[1], Sun-Mi Yun[1], Moon-Young Song[1], Sang-Deok Ji[2], Jong-Gon Son[2], and Eun-Hee Kim[1]*

[1]*College of Pharmacy and Institute of Pharmaceutical Sciences, CHA University, Seongnam, 13488,*
[2]*Department of Agricultural Biology, National Institute of Agricultural Science, Rural Development Administration, Wanju, 55365, Republic of Korea*

Abstract

Hangover due to alcohol consumption causes social and physical problems. There is a growing interest in edible insects worldwide. We have previously published a new technology to make hard mature silkworm, *Bombyx mori*, into edible form, steamed and freeze-dried mature silkworm larval powder (SMSP). In this study, AIN-76 or SMSP (0.1 and 1 g/kg rat body weight) containing diets in SD rats were pretreated for 2 weeks, and ethanol (3 g/kg rat body weight) was administered as an oral gavage and sacrificed after 3 hours. As a result, blood alcohol and aldehyde levels were significantly decreased in SMSP fed rats. In addition, liver injury markers, alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were significantly decreased in SMSP group compared to ethanol group. TNF-α, an inflammatory cytokine, and malondialdehyde (MDA), an oxidative stress marker, also showed a dose-dependent decrease in the group receiving SMSP. Conclusively, consumption of SMSP not only reduced hangover induced by ethanol, but also decreased liver damage, oxidative stress, and inflammatory response.

Int. J. Indust. Entomol. 35(2), 123-128 (2017)

Received : 14 Dec 2017
Revised : 15 Dec 2017
Accepted : 18 Dec 2017

Keywords:
Steamed and freeze-dried mature silkworm larval powder,
Ethanol,
Blood alcohol concentration,
Blood aldehyde concentration,
liver damage,
TNF-α

Introduction

Worldwide, people consumed 6.2 liters of alcohol in average in 2010 (WHO, 2014). Alcohol consumption is highest in Europe and the Americas, as well as in other regions such as Asia, Australasia, and Africa (Shield *et al*., 2013). Drinking alcohol is associated with a number of health problems, including diabetes, psychosis, cardiovascular disease, cirrhosis, and cancer (Scoccianti *et al*., 2014). Alcohol is a small molecule that spreads easily through the cell membrane, and many factors affect absorption and metabolism (Louvet and Mathurin, 2015). Alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) are known as the major enzymes in the alcohol metabolism pathway (Cequera and Garcia de Leon Mendez, 2014). ADH converts alcohol to acetaldehyde, and ALDH alters acetaldehyde to acetate (Park *et al*., 2012). Acetaldehyde, a metabolite of alcohol metabolism, can cause hangover symptoms. Acute alcohol consumption can cause hangover symptoms due to acetaldehyde, which is a major metabolite of alcohol metabolism (Jung *et al*., 2016). Therefore, the development of new functional food that

***Corresponding author.**
Eun-Hee Kim
College of Pharmacy and Institute of Pharmaceutical Sciences, CHA University, Seongnam, 13488, Republic of Korea
Tel: +82-31-881-7179
E-mail: ehkim@cha.ac.kr

can smoothly and effectively control alcohol metabolism can control diseases caused by alcohol consumption (Sung *et al*., 2012).

Silkworm, *Bombyx mori*, a major species in the sericulture, has long been used. Silkworms have traditionally been used only to produce fabrics using silk (Cho *et al*., 2016). The silkworms were difficult to eat due to the large silk gland, but it was easier to eat because of the technique of processing the mature silkworm larvae recently discovered (Ji *et al*., 2015). In addition, steamed and freeze-dried mature silkworm larval powder (SMSP) contains high amounts of amino acids, vitamins and essential minerals (Ji *et al*., 2016a). Recently silkworms have been used to produce foods with various health improving effects (Ji *et al*., 2016b). The silkworm excrement powder has been reported to inhibit alcoholic hepatotoxicity in rats (Kim *et al*., 2008). Silkworm pupae powder ingestion has reported that fat metabolism was increased in rats (Ryu, 2014). Moreover, the silkworm has been studied to have the effect of decreasing hyperlipidemia and hyperglycemia in rats (Kim *et al*., 2008). In the present study, we investigated the protective effect of SMSP in ethanol-treated rats.

Materials and Methods

Steamed and freeze-dried mature silkworm larval powder (SMSP) Production

Silkworm (also known as Baekokjam (Lee *et al*., 1984)) was used in the present study. SMSP was made as previously published (Ji *et al*., 2015). Briefly, the live larvae of the *Bombyx mori* fed with mulberry leaves were freeze-dried for 24 hours after being steamed using a cooking machine (KumSeong Ltd., Boocheon, Korea) without electric pressure. Larvae were then grinded by hammer mill (HM001, Korean Pulverizing Machinery Co. LTD., Incheon, Korea) and a disk mill (Disk Mill01, Korean Pulverizing Machinery Co. LTD).

Animal and Diets

The Sprague-Dawley (SD) rats in the experiment were purchased from Orient bio (Seoul, Korea). SD rats were housed in individual cages on a 12 h light/dark cycle in a temperature controlled (24 °C) environment during a week of acclimatization, with *ad libitum* access to water and a rodent chow diet (Haran 2018s). After acclimatization, the SD rats were randomized into four groups (n = 10). Each group was fed an experimental diet: the AIN-76A (Normal, Alcohol group) or diets containing SMSP (0.1 and 1 g/kg). The experimental diets were administered for 2 weeks in the form of pellets. Then, all groups except the normal group were orally administered alcohol and after 3 hours all groups were sacrificed. All animal experiments protocols were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of animal center of the CHA University (reference number: IACUC 150021).

Blood and Tissue Samples

After 3 hours of alcohol treatment, blood was drawn from the cardiac puncture under carbon dioxide anesthesia to sacrifice all rats. Blood samples were treated with heparin, and plasma samples obtained by centrifugation at 3,000 rpm for 15 min at 4 °C. The liver was removed, rinsed with phosphate-buffered saline, frozen in liquid nitrogen and stored at -80 °C until used.

Biochemical Assays

Blood alcohol level was assayed using commercial kit (Cell Biolabs, INC. Cat No. STA-620). Place 10 μL of the diluted standards and blood samples in a 96-well plate. Add 90 μL of prepared reaction mixture to each well. Cover the plate wells to protect the reaction from light. Incubate at 37 °C for 30 minutes. Read absorbance in the 540 nm on a microplate reader. The aldehyde assay was also used in the commercial kit (Abcam, Cat No. ab112113). Place the aldehyde containing blood samples in to 96-well plate. Add 50 μL 2 X yellow reaction mixture into each well. Incubate the reaction mixture at room temperature for 60 minutes, protected from light. Measure the plate at 405 nm on an absorbance plate reader.

Serum biochemical parameters of hepatic injury

Hepatic injury was evaluated biochemically by measuring the activities of alanine aminotransferase (ALT/GPT), aspartate aminotransferase (AST/GOT) and alkaline phosphatase (ALP) in serum using Hitachi automatic analyzer 7600-210 (Hitachi high-technologies corporation, Tokyo, Japan)

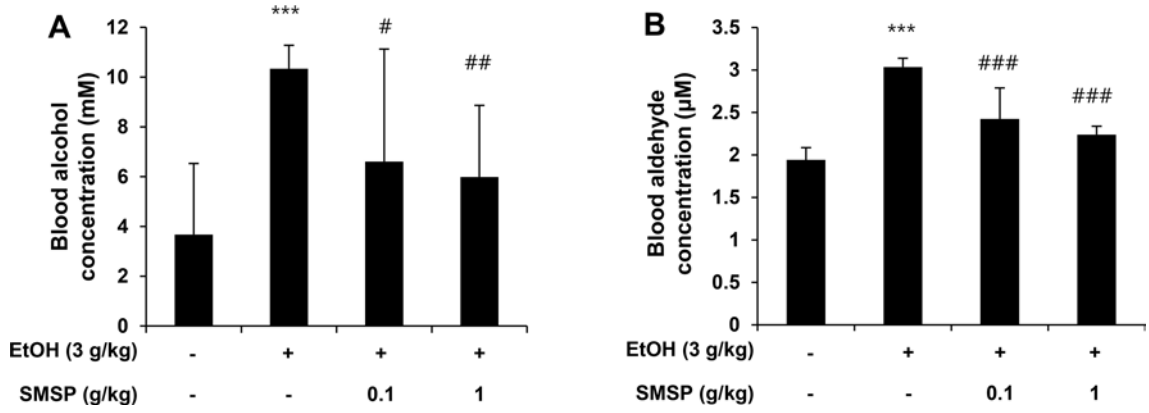

Fig. 1. SMSP alleviated ethanol-induced alcoholic hangover in rats. The concentrations of (A) blood alcohol and (B) blood aldehyde were significantly increased by ethanol administration, whereas the pretreatment with SMSP decreased the amounts of blood alcohol and blood aldehyde in ethanol-treated rats. Data shown as mean ± SD (n = 10). Statistical significance with the controls was analyzed by one-way ANOVA. ***, P <0.001 (*vs* Normal group); #, P < 0.05, ##, P < 0.01, ###, P < 0.001 (*vs* Ethanol group).

ELISA

For the measurement of TNF-α in serum and malondialdehyde (MDA) in tissues, we used the commercial Quantikine ELISA kit (R&D Systmes, MN) and specific spectrophotometric assay for MDA (Oxis International Inc, CA) as following manufacturer's instruction, respectively.

Statistical Analysis

Results are expressed as the mean ± standard deviation. The statistical significance was analyzed by one-way analysis of variance (ANOVA). Statistical significance was accepted at P < 0.05.

Results and Discussion

SMSP decreases blood concentrations of alcohol and aldehyde in ethanol-treated rats

Alcohol is a small molecule, it can pass through biological membranes and spread to all tissue of the body (Cederbaum, 2012; Louvet and Mathurin, 2015). About 10% of the absorbed alcohol is removed by kidneys, lungs and sweat, but the absorption and metabolism of the remaining alcohol is influenced by personal factors such as age, body weight, race, gender, and health status (Louvet and Mathurin, 2015). Most of the ethanol is metabolized by the ADH enzyme to produce acetaldehyde, and acetaldehyde is metabolized to acetate by ALDH (Guo and Ren, 2010). In this study, the rats fed the SMSP diet for 2 weeks were treated with ethanol to induce hangover, and the levels of blood alcohol and aldehyde was measured as well as liver injury markers, inflammatory cytokine TNF-α and oxidative stress marker MDA.

To investigate the protective effect of SMSP, we used ethanol-treated rat model. Single dose of 3 g/kg ethanol was intragastrically injected into 6-week old SD rat after pretreatment with diet containing SMSP for two weeks. Blood alcohol concentration in 1 g/kg SMSP group (5.99 ± 2.88 mM, P < 0.01) was significantly lower compared to the ethanol-treated group (10.34 ± 0.94 mM) (Fig. 1A). Blood aldehyde concentration in 1 g/kg SMSP group (2.24 ± 0.09 μM, P < 0.001) was also significantly lower compared to the ethanol-treated group (3.03 ± 0.11 μM) (Fig. 1B). These results suggest that SMSP can prevent alcohol-induced hangover by lowering blood concentrations of alcohol and aldehyde.

Alcohol consists of three forms: methyl alcohol (methanol), isopropyl alcohol, and ethyl alcohol (ethanol). Methyl alcohol and isopropyl alcohol are prohibited to ingest because of their toxicity. However, ethanol, alcohol as commonly called, is known as a component of beer, wine, and liquor (Guo and Ren, 2010). Alcohol has become an addictive drug worldwide (Park *et al*., 2013). Although proper drinking is not harmful (Elkind *et al*., 2006), abuse of alcohol causes serious medical, social, and economic problems (Guo and Ren, 2010). Hangover is characterized by physical and mental discomfort caused by alcohol. Physically, symptoms such as headache, fatigue,

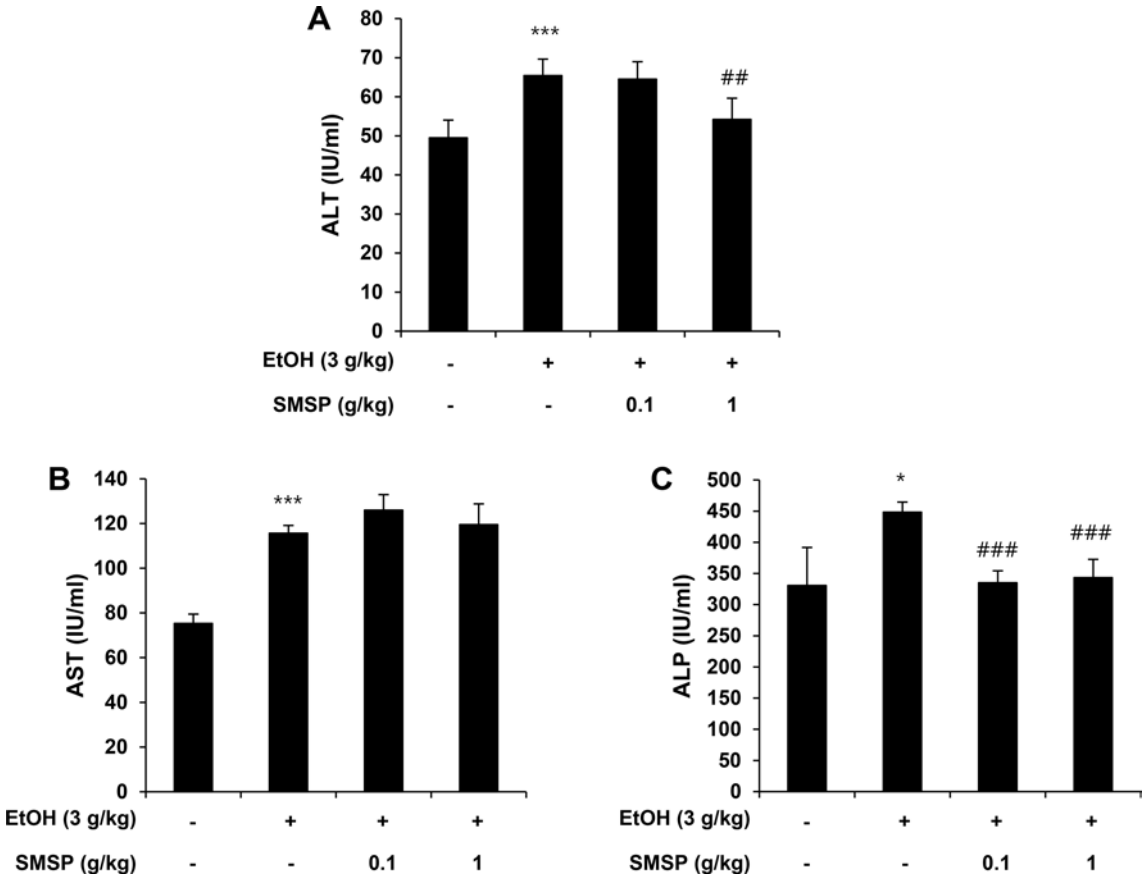

Fig. 2. SMSP decreased ethanol-induced liver injury in rats. The serum levels of ALT (A), AST (B) and ALP (C) were significantly increased by ethanol administration, whereas the pretreatment with SMSP decreased the enzyme activities of ALT and ALP in rats with ethanol-treated rats. Data shown as mean ± SD (n = 10). Statistical significance with the controls was analyzed by one-way ANOVA. *, $P < 0.05$, ***, $P < 0.001$ (*vs* Normal group); ##, $P < 0.01$, ###, $P < 0.001$ (*vs* Ethanol group).

flushing, muscle aches and thirst appear, and mental symptoms such as mood disorder, depression, anxiety, and dizziness appear. A hangover usually starts several hours after drinking and can last for hours (Swift and Davidson, 1998). Acetaldehyde, the major metabolite of alcohol, is known to be the main cause of hangover and is a key generator of free radicals and a known carcinogen, and causes alcoholism. Moreover, acetaldehyde causes mitochondrial dysfunction, damages acetaldehyde metabolism, accumulates acetaldehyde and causes a vicious cycle (Goedde *et al*., 1989; Swift and Davidson, 1998; Guo and Ren, 2010). Therefore, it is very important to reduce the concentration of acetaldehyde in the blood (Sung *et al*., 2012). In this study, the plasma concentrations of alcohol and acetaldehyde were measured. SMSP significantly reduced plasma concentration of alcohol and acetaldehyde in the alcohol-induced hangover model, suggesting that SMSP is the mediator of hangover relief.

SMSP protects liver damage, inflammation and oxidative stress in ethanol-treated rats

To determine the ethanol-induced liver damage, the levels of indicators in the serum, ALT, AST and ALP were measured by the colorimetric method. The activity of ALT (65.43 ± 4.20 IU/L, $P < 0.001$) was significantly induced in ethanol-treated rats, which was significantly suppressed by pretreatment with 1 g/kg SMSP-fed group (54.20 ± 5.40 IU/L, $P < 0.01$) (Fig. 2A). In addition, the activity of ALP (448.25 ± 16.22 IU/L, $P < 0.01$) was also significantly induced in ethanol-treated rats, which was significantly suppressed by pretreatment with 1 g/kg SMSP-fed group (343.25 ± 29.33 IU/L, $P < 0.001$) (Fig. 2C). However, the activity of AST showed no significant difference in SMSP-treated group (Fig. 2B). To investigate whether SMSP protects against ethanol-induced injury, the representative inflammatory cytokine TNF-α and MDA, the marker for oxidative stress were

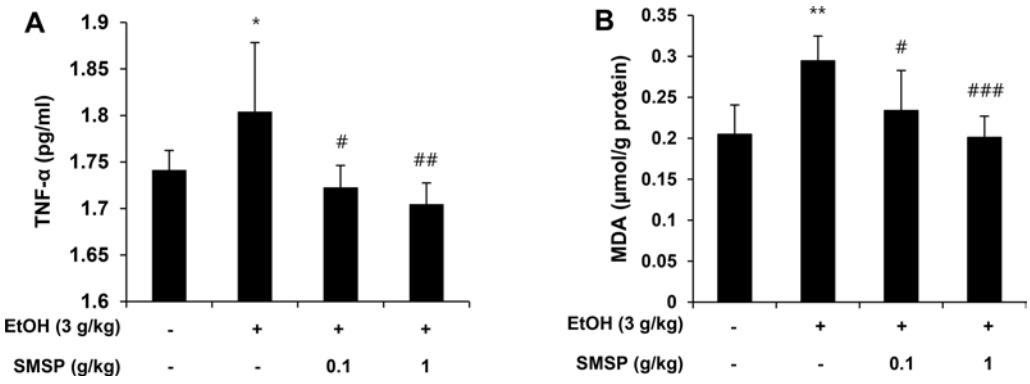

Fig. 3. SMSP pretreatment prevent ethanol-induced inflammation and oxidative stress in rats. The levels of TNF-α (A) and MDA (B) were significantly increased by ethanol administration, whereas the pretreatment with SMSP decreased the levels of TNF-α and MDA in ethanol-treated rats. The Data shown as mean ± SD (n = 10). Statistical significance with the controls was analyzed by one-way ANOVA. *, $P < 0.05$, **, $P < 0.01$ (*vs* Normal group); #, $P < 0.05$, ##, $P < 0.01$, ###, $P < 0.001$ (*vs* Ethanol group).

analyzed. As expected, the levels of TNF-α and MDA induced by ethanol treatment were significantly reduced in a dose-dependent manner in SMSP group (Fig. 3).

Inflammation is a physiological reaction caused by infection or tissue damage (Medzhitov, 2008). Innate immune cells such as macrophages, stimulate neutrophils to accumulate at the injury site, as well as release inflammatory cytokines and chemokines. Neutrophils induce reactive oxygen species and antimicrobial peptides to be released, and they work to eliminate foreign substances (Baumann and Gauldie, 1994; Gao *et al*., 2008; Wang *et al*., 2010). In macrophages, alcohol causes direct oxidative stress and induces the production of inflammatory cytokines. In particular, TNF-α increases the incidence of alcoholic liver disease and induces apoptosis (Mandrekar and Szabo, 2009). The TNF-α induces not only in chronic alcoholics but also in alcohol consumption models (McClain and Cohen, 1989; Khoruts *et al*., 1991). In this experiment, we analyzed inflammatory cytokine and liver damage markers by acute administration of alcohol at 3 g/kg to rats pretreated with SMSP for 2 weeks. TNF-α, an inflammatory cytokine, was significantly increased in the alcohol-treated rat group compared to the normal group, and decreased in the SMSP pretreated group in a dose-dependent manner. In the liver injury marker AST, there was no effect of SMSP, but ALT and ALP showed a significant decrease in the pretreated group of SMSP compared to the alcohol group. These results suggest that SMSP may not only prevent inflammation but also protect liver damage.

Insects are rich in fats, proteins, vitamins, minerals, and fiber, so they have been used for poultry, aquaculture feed, and human foods (van Huis, 2013). The mulberry silkworm, *Bombyx mori*, also used as a food source in many countries (Pereira *et al*., 2003). The SMSP contains a large amount of crude proteins, and includes essential minerals such as calcium, potassium, magnesium, phosphorus, and sulfur. It also contains a large amount of unsaturated fatty acids as well as fatty acids such as palmitic acid, oleic acid, and linolenic acid. In addition, SMSP contains amino acids that are helpful for health, especially aspartic acids, serine, glycine, alanine, and tyrosine (Ji *et al*., 2015; Ji *et al*., 2016a). In this experiment, the hangover resolution effect of SMSP is presumed to have affected the metabolism of proteins, amino acids, minerals, fatty acids, and unsaturated fatty acids contained in SMSP. In summary, the present study suggests that SMSP effectively improves alcohol-induced hangover as well as inflammation and liver damage induced by ethanol administration.

Conflict of Interests

All authors have nothing to disclose and have no commercial or financial interest in the products described in this paper.

Acknowledgements

This work was supported by Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, Forestry and Fisheries (IPET) through Agri-Bio industry Technology Development Program, funded by Ministry of Agriculture, Food and Rural Affairs (MAFRA) (317004-4)

References

Baumann H, Gauldie J (1994) The acute phase response. Immunol Today 15, 74-80.

Cederbaum AI (2012) Alcohol metabolism. Clin Liver Dis 16, 667-685.

Cequera A, Garcia de Leon Mendez MC (2014) Biomarkers for liver fibrosis: advances, advantages and disadvantages. Rev Gastroenterol Mex 79, 187-199.

Cho JM, Kim KY, Ji SD, Kim EH (2016) Protective Effect of Boiled and Freeze-dried Mature Silkworm Larval Powder Against Diethylnitrosamine-induced Hepatotoxicity in Mice. J Cancer Prev 21, 173-181.

Elkind MS, Sciacca R, Boden-Albala B, Rundek T, Paik MC, Sacco RL (2006) Moderate alcohol consumption reduces risk of ischemic stroke: the Northern Manhattan Study. Stroke 37, 13-19.

Gao B, Jeong WI, Tian Z (2008) Liver: An organ with predominant innate immunity. Hepatology 47, 729-736.

Goedde HW, Singh S, Agarwal DP, Fritze G, Stapel K, Paik YK (1989) Genotyping of mitochondrial aldehyde dehydrogenase in blood samples using allele-specific oligonucleotides: comparison with phenotyping in hair roots. Hum Genet 81, 305-307.

Guo R, Ren J (2010) Alcohol and acetaldehyde in public health: from marvel to menace. Int J Environ Res Public Health 7, 1285-1301.

Ji SD, Kim NS, Lee JY, Kim MJ, Kweon HY, Sung GB, *et al*. (2015) Development of processing technology for edible mature silkworm. J Seri Entomol Sci 53, 38-43.

Ji SD, Kim NS, Kweon H, Choi BH, Kim KY, Koh YH (2016a) Nutrition composition differences among steamed and freeze-dried mature silkworm larval powders made from 3 *Bombyx mori* varieties weaving different colored cocoons. Int J Indust Entomol 33, 6-14.

Ji SD, Kim NS, Kweon H, Choi BH, Yoon SM, Kim KY, *et al*. (2016b) Nutrient compositions of *Bombyx mori* mature silkworm larval powders suggest their possible health improvement effects in humans. J Asia-Pac Entomol 19, 1027-1033.

Jung S, Lee SH, Song YS, Lee SY, Kim SY, Ko KS (2016) Effect of Beverage Containing Fermented Akebia quinata Extracts on Alcoholic Hangover. Prev Nutr Food Sci 21, 9-13.

Khoruts A, Stahnke L, McClain CJ, Logan G, Allen JI (1991) Circulating tumor necrosis factor, interleukin-1 and interleukin-6 concentrations in chronic alcoholic patients. Hepatology 13, 267-276.

Kim YS, Kim KY, Kang PD, Cha JY, Heo JS, Park BK, *et al*. (2008) Effect of Silkworm (*Bombyx mori*) Excrement Powder on the Alcoholic Hepatotoxicit in Rats. J Life Sci 18, 1342-1347.

Lee S, Hong K, Sohn K, Mah Y, Kim K (1984) Breeding of new silkworm variety "BaegOkJam". Res Rept RDA 26, 58-64.

Louvet A, Mathurin *P* (2015) Alcoholic liver disease: mechanisms of injury and targeted treatment. Nat Rev Gastroenterol Hepatol 12, 231-242.

Mandrekar P, Szabo G (2009) Signalling pathways in alcohol-induced liver inflammation. J Hepatol 50, 1258-1266.

McClain CJ, Cohen DA (1989) Increased tumor necrosis factor production by monocytes in alcoholic hepatitis. Hepatology 9, 349-351.

Medzhitov R (2008) Origin and physiological roles of inflammation. Nature 454, 428-435.

Park JH, Kim SJ, Hwang I, Bae KC, Bae JH, Song DK (2013) Green tea extract co-administered with a polymer effectively prevents alcoholic liver damage by prolonged inhibition of alcohol absorption in mice. Alcohol Alcohol 48, 59-67.

Park JH, Kim Y, Kim SH (2012) Green tea extract (Camellia sinensis) fermented by Lactobacillus fermentum attenuates alcohol-induced liver damage. Biosci Biotechnol Biochem 76, 2294-2300.

Pereira NR, Ferrarese-Filho O, Matsushita M, de Souza NE (2003) Proximate composition and fatty acid profile of *Bombyx mori* L. chrysalis toast. J Food Compos Anal 16, 451-457.

Ryu SP (2014) Silkworm pupae powder ingestion increases fat metabolism in swim-trained rats. J Exerc Nutrition Biochem 18, 141-149.

Scoccianti C, Lauby-Secretan B, Bello PY, Chajes V, Romieu I (2014) Female breast cancer and alcohol consumption: a review of the literature. Am J Prev Med 46, S16-25.

Shield KD, Rylett M, Gmel G, Kehoe-Chan TA, Rehm J (2013) Global alcohol exposure estimates by country, territory and region for 2005--a contribution to the Comparative Risk Assessment for the 2010 Global Burden of Disease Study. Addiction 108, 912-922.

Sung CK, Kim SM, Oh CJ, Yang SA, Han BH, Mo EK (2012) Taraxerone enhances alcohol oxidation via increases of alcohol dehyderogenase (ADH) and acetaldehyde dehydrogenase (ALDH) activities and gene expressions. Food Chem Toxicol 50, 2508-2514.

Swift R, Davidson D (1998) Alcohol hangover: mechanisms and mediators. Alcohol Health Res World 22, 54-60.

van Huis A (2013) Potential of insects as food and feed in assuring food security. Annu Rev Entomol 58, 563-583.

Wang HJ, Zakhari S, Jung MK (2010) Alcohol, inflammation, and gut-liver-brain interactions in tissue damage and disease development. World J Gastroenterol 16, 1304-1313.

WHO (2014) Global status report on alcohol and health. 1-85.