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Hypolipidemic Effect of *n*-Butanol Extract from *Asparagus officinalis* L. in Mice fed a High-fat Diet

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During industrial processing of Asparagus (*Asparagus officinalis* L.), around half of each spear is discarded. However, these discarded asparagus (by-products) might be used as food supplements for their potential therapeutic effects. This study evaluated the hypolipidemic effect of *n*-butanol extract (BEA) from asparagus by-products in mice fed a high-fat diet (HFD). Continuous HFD feeding caused hyperlipidemia, oxidative stress and liver damage in mice. Interestingly, while BEA significantly decreased the levels of body weight gain, serum total cholesterol and low density lipoprotein cholesterol, it dramatically increased the high density lipoprotein level when administered at three different doses (40, 80 or 160 mg/kg body weight) for 8 weeks in hyperlipidemic mice. In addition, BEA decreased the levels of alanine transaminase, aspartate transaminase and alkaline phosphatase in serum. Finally, superoxide dismutase activity and the total antioxidation capacity were evidently increased, while the malondialdehyde level and the distribution of lipid droplets were reduced in liver cells of BEA-treated mice. Taken together, the findings of this study suggested that BEA had a strong hypolipidemic function and could be used as a supplement in healthcare foods and drugs or in combination with other hypolipidemic drugs. **Copyright © 2011 John Wiley & Sons, Ltd.**

Keywords: Asparagus officinalis L.; hyperlipidemia; hypolipidemic; hypercholesterolemic.

INTRODUCTION

Hyperlipidemia, including hypercholesterolemia and hypertriglyceridemia, is a major risk factor for the development of cardiovascular diseases (Makni *et al.*, 2008). Elevated levels of plasma total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) and triglyceride (TG) as well as reduced levels of plasma high-density lipoprotein cholesterol (HDL-C) are often associated with an increased risk of coronary heart disease (Smith *et al.*, 2004). In addition, hyperlipidemia can induce oxidative stress in liver (Bolkent *et al.*, 2005). Oxidative stress results from an imbalance between the production of free radicals and their removal by the antioxidant defense system (Lum and Roebuck, 2001). Excessive amounts of oxygen free radicals cause lipid peroxidation and the production of malondialdehyde (MDA), which lead to liver damage (Bolkent *et al.*, 2005). Recently, there has been a special focus on the hypolipidemic and antioxidant activities of dietary plants, some of which have shown promising potential in lowering plasma lipid levels and restoring the antioxidant defense system (Kim *et al.*, 2008; Martinello *et al.*, 2006).

Asparagus (*Asparagus officinalis* L.), a healthy and nutritious vegetable, is commonly consumed in many regions of the world. In addition to its edible value, this plant has been reported to possess various biological activities, such as antimutagenic (juice) (Tang and Gao, 2001), antioxidant (spears) (Sun *et al.*, 2007), antitumor (spears) (Shao *et al.*, 1996), antifungal (roots) (Nwafor and Okwuasab, 2003), hepatoprotective (roots) (Koo *et al.*, 2000), hypoglycemic (roots) (Hannan *et al.*, 2007) and immunoprotective (roots) (Gautam *et al.*, 2004) functions. However, during industrial processing, around half of each spear is discarded, which causes significant waste for producers. In fact, these by-products of asparagus contain many bioactive substances, such as flavonoids (Zhang *et al.*, 2005) steroidal saponins (Fang, 2005) and polysaccharides (Huang *et al.*, 2006), and therefore might be used as food supplements for their therapeutic effects. Nevertheless, only a limited number of studies are available on the pharmacological effects of asparagus by-products so far. Feng *et al.* (2001) reported that asparagus stem bark powder could reduce the serum lipid levels in hyperlipidemic rats when added in the diet. Therefore, it is intriguing to identify the bioactive substances from asparagus by-products and to perform a detailed dose-dependent study for their hypolipidemic effects.

In this study, the hypolipidemic activity of the *n*-butanol extract (BEA) from asparagus by-products was examined in hyperlipidemic mice.

MATERIALS AND METHODS

Plant and preparation of BEA. Prior to selling, freshly harvested asparagus spears were cut to obtain the 15 cm long upper portion (edible part) and the rest (15–18 cm) was considered as by-product. Asparagus by-products were obtained from Shanghai Green Asparagus Co. Ltd (Shanghai China) and identified by Dr Hongqing Li

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(School of Life Science, East China Normal University, Shanghai, China). A voucher specimen was deposited at the herbarium of East China Normal University (ECNU), with registration number zxl001.

Air-dried asparagus by-products (100 g) were first crushed and then extracted twice (3 h each) with 1 L of 70% ethanol at 80 °C under reflux. Then the ethanol extract was concentrated in a rotary evaporator under negative pressure and further freeze-dried to obtain the solid ethanol extract (17.31 g), which was then dissolved in 100 mL 60 °C water and extracted with *n*-butanol three times (1:1). The butanol fractions were dried (3.7 g) and applied to an AB-8 macroporous resin column (Chang Zhou Bao En Chemical plant, Hebei, China), followed by elution with distilled water and 50% (v/v) ethanol sequentially. Finally, the 50% ethanol fractions were concentrated and freeze-dried to produce the solid *n*-butanol extract of asparagus (BEA) (0.45 g). The yield of BEA from the asparagus by-products was approximately 0.50% (w/w). The content of total steroidal saponins and flavonoids in BEA were determined using the spectrophotometric method as described in the Pharmacopoeia of China (2005): the total steroidal saponins content was 52.10% and the total flavonoid content was 2.30%.

Mice and treatment. The hyperlipidemic mouse model was induced according to Huang's procedure (Huang *et al.*, 2004). The license number for using experimental animals was SYXK (SH) 2004-0001, and all experimental procedures were performed according to the regulations described in the Guide for the Care and Use of Laboratory Animals of the U.S. National Institutes of Health (NIH) (National Research Council, 1985). Six-week-old male ICR mice, weighing 20–24 g, were purchased from SIPPR/BK Lab Animal Ltd (Shanghai, China) and were allowed to acclimatize (20±2 °C, 12 h light/12 h dark cycle) for a week. During this time the animals were fed a regular diet (casein 12%, corn starch 60.98%, sucrose 15%, corn oil 7%, mineral mixture 4%, vitamin mixture 1%, cod liver oil 0.02%) *ad libitum*.

After the acclimatization period the mice were randomly divided into two groups: the normal control group (NC) and the experimental group. The former (n=12) was fed a regular diet while the latter (n=60) was fed a high-fat diet (HFD) (15% lard, 2% cholesterol, 0.5% cholic acid added to normal chow) during the entire experimental period of 10 weeks. After feeding the high-fat diet for 2 weeks, the mice were fasted overnight and blood was collected from tail veins. Then plasma total cholesterol (TC) levels were determined by enzymatic colorimetric methods using commercial kits (Shanghai Kexin Biotech Institute, Shanghai, China) according to the manufacturer's instructions. The hypercholesterolemic mice were then subdivided into five groups (n=12 each). HFD group: HFD+distilled water; HFD + SIM group: HFD + 20 mg/kg body weight (BW) simvastatin (Merck, Sharp & Dohme, Hangzhou, China); HFD + BEA40 group: HFD + 40 mg/kg BW *n*-butanol extract of asparagus; HFD + BEA80 group: HFD + 80 mg/kg BW *n*-butanol extract of asparagus; HFD + BEA160 group: HFD + 160 mg/kg BW *n*-butanol extract of asparagus. Vehicle (distilled water), simvastatin and extracts were orally administered to mice by gastric intubation once a day, for 8 weeks as specified.

During the experimental period, the food intake of mice was measured daily. The amount of food ingested was calculated by subtracting the weight of food remaining in the food bin (D_a) from the weight of food placed there 1 day before (D_b). These data were then used to calculate a daily average food intake per animal using the following formula: Average food intake (g)=(D_b−D_a)/12. Body weights were measured weekly and blood was collected for total cholesterol (TC) analysis every other week. At the end of the experiment the animals were fasted overnight and then killed. Blood and liver tissue samples were collected. Tissues were fixed in 2.5% glutaraldehyde for transmission electron microscopy or snap-frozen in liquid nitrogen and stored at −80 °C until needed.

Measurement of serum biochemical values. Serum TC, triglycerides (TG), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) were measured by enzymatic colorimetric methods using commercial kits (Shanghai Kexin Biotech Institute, Shanghai, China) following the manufacturer's instructions. Serum alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP) activities were determined by the method of Reitman and Frankel (1957) using commercial kits (Shanghai Kexin Biotech Institute).

Tissue homogenization. Liver samples (1 g/9 g cold 0.9% saline) were homogenized on ice using a glass homogenizer. The homogenates were centrifuged and the clear supernatants were collected at 4 °C. The superoxide dismutase (SOD) activity, catalase (CAT) activity, malondialdehyde (MDA) level and total anti-oxidation capability (T-AOC) were measured using commercial kits (Nanjing Jianchen Bioengineering Institute, Nanjing, China).

Morphological assays. Small liver pieces were fixed with 2.5% glutaraldehyde, followed by fixing with 1% osmium tetroxide, and then embedded in Epon for transmission electron microscopy. Ultrathin sections were stained with uranyl acetate and lead citrate and examined under a JEM-2100 electron microscope (JEOL, Tokyo, Japan).

Statistical analysis. Results were expressed as mean ± standard deviation. Data were evaluated by one-way analysis of variance using the SPSS program (SPSS Inc., Chicago, USA), and differences in mean values among groups were assessed using Duncan's multiple range test. A value of $p<0.05$ was considered statistically significant.

RESULTS

BEA exerted a protective effect against HFD-induced overweight

Although the initial body weight, the final body weight and the food intake of mice were similar within each group, the body weight was obviously increased in the HFD group compared with the NC group ($p<0.05$) (Table 1). This increase was probably due to the HFD. The results shown in Table 1 also indicated that BEA exerted a protective effect against HFD-induced overweight. Treating hyperlipidemic mice with three different

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Table 1. BEA exerted a protective effect against HFD-induced overweight

Group	Body weight (g)		Weight gain	Average food intake
	Initial	Final	(g)	(g/day)
NC	26.15±2.14	31.44±3.24	5.67±1.49	4.88±0.27
HFD	24.40±2.91	31.69±4.76	7.36±2.15[a]	5.39±0.39
HFD + SIM	24.20±2.77	31.52±3.85	7.31±3.17	5.77±0.97
HFD + BEA40	25.77±4.07	30.84±4.47	5.07±1.83[b]	5.68±0.90
HFD + BEA80	28.07±2.62	32.63±4.93	5.53±1.43[b]	5.34±0.68
HFD + BEA160	27.37±4.03	32.17±4.84	5.17±1.61[b]	5.09±0.73

Values are mean ± SD ($n = 12$). [a]$p < 0.05$ vs the normal control group. [b]$p < 0.05$ vs the high-fat diet group.

doses of BEA (40, 80 or 160 mg/kg BW) resulted in a significant reduction in body weight gain of 31% (p<0.05), 25% (p<0.05) and 30% (p<0.05), respectively, compared with the HFD group.

BEA reduced serum TC and LDL-C levels and increased HDL-C levels in hyperlipidemic mice

The fasting serum TC levels were measured every other week as shown in Fig. 1. After 2 weeks the serum TC levels in the HFD-treated mice were significantly higher, than the NC group (p<0.01), indicating that hypercholesterolemia had been induced. When the hyperlipidemic mice were supplemented with BEA (40, 80 and 160 mg/kg BW) for 6 weeks or longer, a significant hypocholesterolemic effect was observed in these mice. At the end of the 8 week treatment the reduction in BEA40-, BEA80- and BEA160-treated mice was 20% (p<0.01), 20% (p<0.01) and 21% (p<0.01), respectively, compared with the untreated hyperlipidemic mice.

The effects of BEA on serum TG, HDL-C and LDL-C levels were also examined. As shown in Table 2, in the HFD group, the serum HDL-C level was decreased by 18% and the LDL-C level was increased by 19-fold, compared with the NC group. Feeding hyperlipidemic mice with 40 or 160 mg/kg BW BEA significantly increased the HDL-C levels by 42% (p<0.01) and 34% (p<0.01), respectively, compared with the untreated hyperlipidemic controls. The HDL-C levels also tended to increase in the 80 mg/kg BW BEA treated mice, but the pattern of change was statistically insignificant. All three doses of BEA produced a significant reduction in serum LDL-C in comparison with the HFD group (p<0.01), and the percentage reduction was 26%, 22% and 30% in mice receiving 40, 80 and 160 mg/kg BEA, respectively. The TG levels in serum of SIM, BEA40, BEA80, and BEA160 groups were lower than those of the HFD group, though the differences were not statistically significant.

BEA decreased serum AST, ALT and ALP activities in hyperlipidemic mice

Serum AST, ALT and ALP activities in all groups are shown in Table 3. There was an approximate 2-fold, 3-fold and 1.5-fold (p<0.01) increase in serum AST, ALT and ALP activities in the hyperlipidemic mice compared with the NC mice. The BEA at doses of 40, 80 or 160 mg/kg BW produced a significant attenuation in the activity of serum AST by 37%, 30% and 32%, respectively (p<0.01). Also, the three different doses of BEA dramatically decreased the activity of serum ALT by 35%, 38% and 39%, respectively (compared

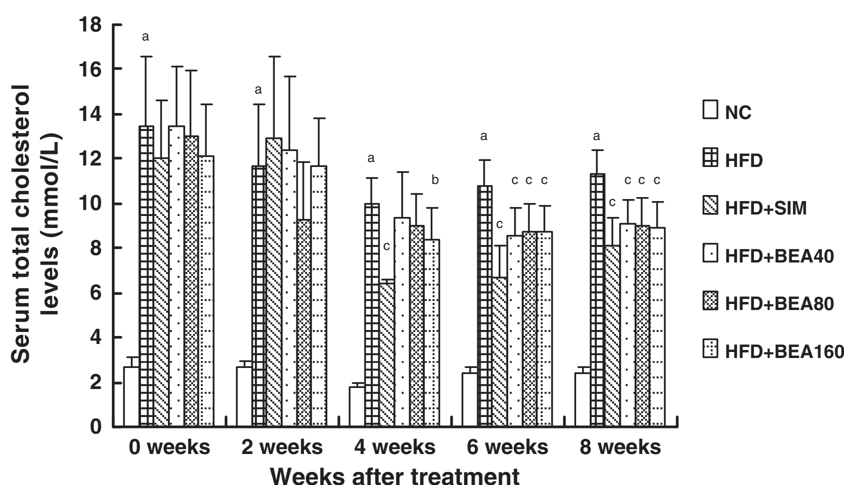

Figure 1. BEA reduced serum TC levels in hyperlipidemic mice: NC, normal control; HFD, high-fat diet + distilled water; HFD + SIM, high-fat diet + 20 mg/kg daily simvastatin; HFD + BEA40, high-fat diet + 40 mg/kg *n*-butanol extract of asparagus; HFD + BEA80, high-fat diet + 80 mg/kg *n*-butanol extract of asparagus; HFD + BEA160, high-fat diet + 160 mg/kg *n*-butanol extract of asparagus. Values are mean ± SD ($n = 12$). [a]$p < 0.01$ vs NC group. [b]$p < 0.05$, [c]$p < 0.01$ vs HFD group.

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Table 2. BEA increased serum HDL-C levels and reduced LDL-C levels in hyperlipidemic mice

Group	TG (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)
NC	0.95±0.19	1.47±0.20	0.54±0.09
HFD	0.76±0.18	1.21±0.26[a]	10.01±1.04[b]
HFD + SIM	0.73±0.11	1.76±0.23[c]	5.80±0.77[c]
HFD + BEA40	0.64±0.075	1.64±0.23[c]	7.40±0.99[c]
HFD + BEA80	0.59±0.09	1.33±0.23	7.82±0.80[c]
HFD + BEA160	0.66±0.10	1.56±0.20[c]	6.92±0.98[c]

Values are mean ± SD (n = 12). [a]$p < 0.05$, [b]$p < 0.01$ vs the normal control group. [c]$p < 0.01$ vs the high-fat diet group.

with the HFD group, $p<0.01$). Moreover, these doses of BEA significantly decreased the serum ALP activity by 17% ($p<0.01$), 21% ($p<0.01$) and 9% ($p<0.05$), respectively, compared with the HFD group.

BEA increased hepatic SOD and T-AOC levels and decreased MDA levels in hyperlipidemic mice

The effects of BEA on hepatic SOD, CAT, MDA and T-AOC levels are shown in Table 4. There was a significant decrease in SOD, CAT activity, T-AOC level and an obvious increase in the MDA level in the hyperlipidemic mice, indicating that the HFD caused an oxidative burden. However, when the hyperlipidemic mice were treated with 40, 80 or 160 mg/kg BW BEA, the SOD activity was markedly increased by 48% ($p<0.01$), 103% ($p<0.01$) and 15% ($p<0.05$), the T-AOC level was elevated by 32% ($p<0.01$), 52% ($p<0.01$) and 40% ($p<0.01$), and the MDA level was decreased by 23% ($p<0.05$), 25% ($p<0.01$) and 18% ($p<0.05$), respectively. However, there was no obvious difference in the CAT level between treated and untreated groups.

BEA decreased lipid deposition and rough endoplasmic reticulum gathering

The effect of BEA was also investigated on the morphology of live cells in the hyperlipidemic mice. As shown in Fig. 2A, rough endoplasmic reticulum (RER), smooth endoplasmic reticulum (SER) and mitochondria (M) were observed in normal hepatocytes. The SER appeared as a network of branching tubules, and sparse RER were localized in the peribiliary regions of the mitochondria.

In the hepatocytes of high-fat-diet mice (Fig. 2B), SER disappeared, while excess lipid droplets congregated and parallel RER emerged. However, in all the BEA treated groups (BEA40-, BEA80- or BEA160-groups), lipid deposition and RER gathering were decreased (Fig. 2D, E, F).

DISCUSSION

This study evaluated the hypolipidemic property of the *n*-butanol extract from asparagus by-products (BEA) in hyperlipidemic mice. After 10 weeks of HFD feeding, mice developed an overweight state, with increased circulating TC, LDL-C and decreased HDL-C levels, indicating that a hyperlipidemic mouse model had been induced (Huang *et al*., 2004).

After treatment with BEA for a period of 8 weeks a decrease in serum TC accompanied by a reduction in LDL-C was observed in the hyperlipidemic mice. The Lipid Research Clinics Coronary Primary Prevention Trial reported that every 1% reduction in plasma total cholesterol leads to a 2% decrease in the risk of coronary heart disease (Sandhya and Rajamohan, 2008). A high LDL-C level is a major risk factor in cardiovascular diseases, thus is the target of many hypocholesterolemic agents (Smith *et al.*, 2004). The BEA showed a

Table 3. BEA decreased serum AST, ALT and ALP activities in hyperlipidemic mice

Group	AST (U/L)	ALT (U/L)	ALP(U/L)
NC	34.05±4.29	34.79±3.96	125.43±8.92
HFD	58.31±4.58[a]	96.97±12.32[a]	173.00±10.70[a]
HFD + SIM	53.30±3.59	90.98±6.93	165.22±10.78[b]
HFD + BEA40	36.78±3.01[c]	63.37±5.38[c]	143.68±5.66[c]
HFD + BEA80	40.89±4.48[c]	60.37±7.61[c]	136.89±4.20[c]
HFD + BEA160	39.72±4.72[c]	58.93±4.78[c]	157.65±6.98[b]

Values are mean ± SD (n = 12). [a]$p < 0.01$ vs the normal control group. [b]$p < 0.05$, [c]$p < 0.01$ vs the high-fat diet group.

Table 4. BEA increased hepatic SOD and T-AOC levels and decreased MDA levels in hyperlipidemic mice

Group	SOD (U/mg prot)	CAT (U/mg prot)	MDA (nmol/mg prot)	T-AOC (U/mg prot)
NC	473.48±31.99	45.35±4.82	3.63±0.42	1.36±0.24
HFD	173.72±15.64[b]	40.46±4.45	6.76±0.66	1.11±0.17[a]
HFD + SIM	231.61±15.62[d]	43.68±5.41	5.16±0.41[d]	2.35±0.34[d]
HFD + BEA40	257.89±18.28[d]	41.15±3.71	5.23±0.45[c]	1.46±0.23[d]
HFD + BEA80	353.12±14.91[d]	42.61±5.58	5.04±0.39[d]	1.69±0.21[d]
HFD + BEA160	199.30±11.52[c]	40.76±4.13	5.53±0.67[c]	1.55±0.20[d]

Values are mean ± SD (n = 12). [a]$p < 0.05$, [b]$p < 0.01$ vs normal control group. [c]$p < 0.05$, [d]$p < 0.01$ vs high-fat diet group.

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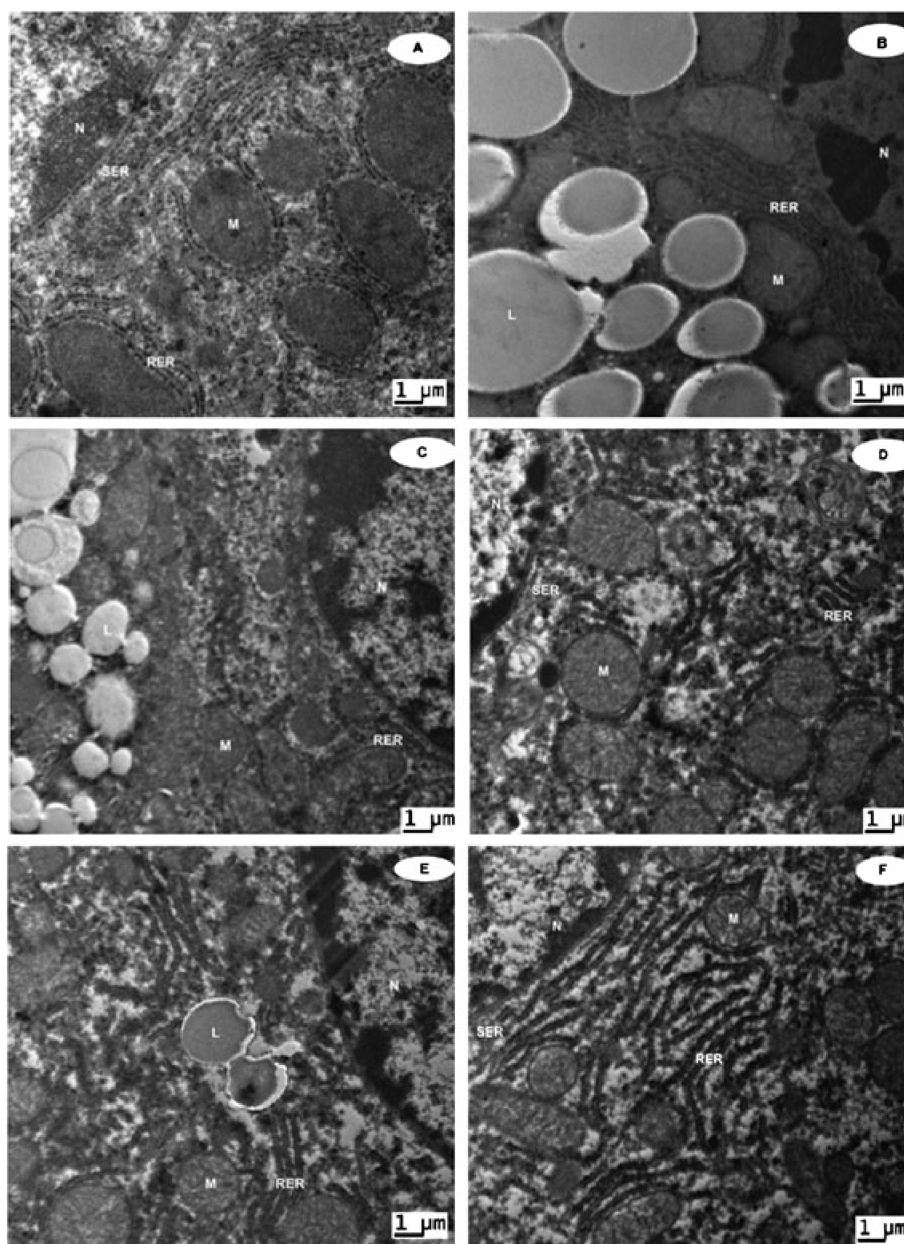

Figure 2. The BEA decreased lipid deposition and rough endoplasmic reticulum gathering in liver cells. (A) Hepatocyte from the normal control group. (B) Hepatocyte from the high-fat diet group. (C) Hepatocyte from the high-fat diet + simvastatin treated group. (D) Hepatocyte from the high-fat diet + BEA40 treated group. (E) Hepatocyte from the high-fat diet + BEA80 treated group. (F) Hepatocyte from the high-fat diet + BEA160 treated group. RER, rough endoplasmic reticulum; SER, smooth endoplasmic reticulum; M, mitochondria; N, nucleus; L, lipid deposition.

hypolipidemic action and induced an increase in HDL-C levels. This lipoprotein, commonly known as 'good cholesterol', facilitates the mobilization of TG and cholesterol from plasma to liver where they are catabolized and eliminated in the form of bile acids (Shah *et al.*, 2001). Therefore BEA would be helpful in decreasing the incidence of cardiovascular diseases by reducing TC and LDL-C levels and increasing HDL-C. The hypolipidemic activity of BEA might be due mainly to the saponin content. Saponin is a type of phytosterol, and can reduce the blood cholesterol level by decreasing the absorption and synthesis of cholesterol (Chen *et al.*, 2008). The significant decrease of serum TC and LDL-C level in treated groups might be due partly to the flavonoid as well, which reduces the blood cholesterol level by inhibiting cholesterol synthesis and increasing the expression of the LDL receptors (Bolkent *et al.*, 2005).

The liver is the primary organ that metabolizes ingested cholesterol (Bolkent *et al.*, 2004). Therefore, the HFD will cause oxidative stress in the liver, which leads to hepatocyte damage involving free radicals and lipid peroxidation (Halliwell and Gutteridge, 1990). It is well known that AST, ALT and ALP levels are the most sensitive indicators for the detection of hepatic cell damage, because they are present in high concentrations in hepatocytes (Kew, 2000). These enzymes will leak into the circulation when hepatocytes or their cell membranes are damaged (Kew, 2000). In our study, the diet with 150 g/kg lard, 20 g/kg cholesterol and 5 g/kg cholic acid not only led to a large increase in MDA level and excessive aggregation of lipid droplets in the liver of

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mice, but also significantly decreased the SOD activity, CAT activity and T-AOC level. These results indicated that the HFD led to oxidative stress in mice and that this oxidative stress resulted in liver damage, causing an increase in ALT, AST and ALP activities. However, treatment with BEA was able significantly to ameliorate the liver damage by increasing hepatic SOD activity and T-AOC level as well as decreasing MDA and lipid levels in hyperlipidemic mice. This amelioration was shown by reduced serum ALT, AST and ALP activities in the BEA treated mice. This implied that BEA had a protective role on hyperlipidemia-caused hepatic damage by decreasing the lipid level and restoring the antioxidant defense system.

In summary, BEA exhibited strong hypolipidemic and hepatoprotective actions caused by high fat feeding. The findings suggest that BEA could be used as a supplement in health-care food and drugs, or in combination with other hypolipidemic drugs. Also, our study could be economically beneficial to producers by suggesting the use of asparagus by-products.

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Conflict of Interest

The authors have declared that there is no conflict of interest.

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