Research Article

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Hypolipidaemic and hepatoprotective effects of ethanolic and aqueous extracts from *Asparagus officinalis* L. by-products in mice fed a high-fat diet

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Abstract

BACKGROUND: Asparagus (*Asparagus officinalis* L.) by-products, i.e. the parts of the spears discarded during industrial processing, might have potential use as food supplements for their therapeutic effects. In this study the hypolipidaemic and hepatoprotective effects of ethanolic (EEA) and aqueous (AEA) extracts from asparagus by-products were evaluated in mice fed a high-fat diet (HFD).

RESULTS: Continuous HFD feeding caused obvious hyperlipidaemia and liver damage in mice. However, both EEA and AEA significantly decreased the levels of body weight gain, serum total cholesterol and serum low-density lipoprotein cholesterol in hyperlipidaemic mice when administered at a daily dose of 200 mg kg^{-1} for 8 weeks. Also, serum high-density lipoprotein cholesterol levels were evidently increased in the AEA-treated group. Moreover, both EEA and AEA dramatically decreased the activities of alanine and aspartate transaminases in serum. Finally, superoxide dismutase activity and total antioxidant capacity were increased and malondialdehyde level and the distribution of lipid droplets decreased in liver cells of both EEA- and AEA-treated mice.

CONCLUSION: The findings of this study suggest that both EEA and AEA have strong hypolipidaemic and hepatoprotective properties and could be used as supplements in healthcare foods and drugs or in combination with other hypolipidaemic drugs.

Keywords: *Asparagus officinalis* L.; hypolipidaemic; hyperlipidaemia; hepatoprotective

INTRODUCTION

Hyperlipidaemia, including hypercholesterolaemia and hypertriglyceridaemia, is a major risk factor in the development of cardiovascular diseases.[1] Indeed, the Lipid Research Clinics Coronary Primary Prevention Trial has concluded that every 1% reduction in plasma total cholesterol (TC) leads to a 2% decrease in the risk of coronary heart disease.[2] Elevated levels of plasma 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[3] and fatty liver disease.[4] Nowadays, a logical strategy to prevent or treat cardiovascular disease is to target hyperlipidaemia with drugs and/or dietary intervention.[5] Recently, there has been a special focus on the hypolipidaemic effects of dietary plants, some of which have shown promising potential in lowering cholesterol levels in plasma.[6]

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 demonstrated to possess various biological activities such as antimutagenic (juice),[7] hypolipidaemic (juice),[8] antioxidant (edible parts of spears),[9] antitumour (edible parts of spears),[10] antifungal (roots),[11] hepatoprotective (roots),[12] hypoglycaemic (roots)[13] and immunoprotective (roots)[14] functions. However, during industrial processing, around half of the total length 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,[15] steroidal saponins[16] and polysaccharides[17] and might have potential use 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. Therefore the active fractions of asparagus by-products and a detailed dose-dependent study of their physiological effects remain to be validated.

In this study the hypolipidaemic and hepatoprotective properties of ethanolic (EEA) and aqueous (AEA) extracts from asparagus by-products were examined in hyperlipidaemic mice.

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MATERIALS AND METHODS

Plant material and preparation of AEA and EEA

Prior to selling, freshly harvested asparagus spears were cut to obtain the 15 cm length upper portion (edible part), and the rest of the spear (15–18 cm) was considered as a by-product. Asparagus by-products were obtained from Shanghai Green Asparagus Co. Ltd (Shanghai, China) and identified by Dr Hongqing Li (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 700 g L^{-1} ethanol at 80 °C under reflux. The extracted solution was then concentrated in a rotatory evaporator under reduced pressure and further freeze-dried to produce the solid ethanolic extract (15.1 g). At the same time the residue of asparagus by-products was air dried in a ventilated cabinet and then boiled twice (2 h each) in 1 L of water. Finally, the water-extracted solution was concentrated and further freeze-dried to produce the solid aqueous extract (6.1 g).

The major active ingredients in EEA and AEA were determined: the content of soluble dietary fibre was analysed as described previously;[18] total flavonoids and steroidal saponins were quantified using a spectrophotometric method.[19]

Animals and treatment

A hyperlipidaemic mouse model was induced by the procedure of Huang *et al.*[20] The licence number for using experimental animals was SYXK(SH) 2004-0001, and all experimental procedures conformed to the regulations described in the *Guide for the Care and Use of Laboratory Animals* of the US National Institutes of Health (NIH).[21] Six-week-old male ICR mice weighing 20–24 g were purchased from SIPPR/BK Lab Animal Ltd (Shanghai, China) and allowed to acclimatise (20 ± 2 °C, 12 h light/12 h dark cycle) for 1 week. During this time the animals were raised on a regular diet (120 g casein, 609.8 g corn starch, 150 g sucrose, 70 g corn oil, 40 g mineral mixture, 10 g vitamin mixture and 0.2 g cod liver oil kg^{-1}) *ad libitum*.

After the acclimatisation period the mice were randomly divided into two groups: a normal control (NC) group and an experimental group. The former ($n = 12$) was fed a regular diet while the latter ($n = 48$) was fed a high-fat diet (150 g lard, 20 g cholesterol, 5 g cholic acid and 825 g normal chow kg^{-1}) during the entire experimental period of 10 weeks. After feeding mice the high-fat diet for 2 weeks, hypercholesterolaemia was successfully induced. The hypercholesterolaemic mice were then subdivided into four groups ($n = 12$ each): HFD group, high-fat diet + distilled water; HFD + SIM group, high-fat diet + 20 mg kg^{-1} body weight (BW) simvastatin (Merck, Sharp & Dohme, Hangzhou, China); HFD + EEA group, high-fat diet + 200 mg kg^{-1} BW ethanolic extract of asparagus; HFD + AEA group, high-fat diet + 200 mg kg^{-1} BW aqueous 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 according to the following formula: average food intake (g) = $(D_b - D_a)/12$. Body weights were measured weekly and blood was collected for TC analysis every other week. At the end of the experiment the animals were fasted overnight and then sacrificed. Blood and liver tissue samples were collected. Tissue samples were fixed in 25 g L^{-1} glutaraldehyde for transmission electron microscopy or snap-frozen in liquid nitrogen and stored at −80 °C until needed.

Measurement of serum biochemical values

Serum TG, TC, HDL-C and LDL-C levels were measured by enzymatic colorimetric methods using commercial kits (Shanghai Kexin Biotech Institute, Shanghai, China). Serum alanine transaminase (ALT) and aspartate transaminase (AST) activities were determined by the method of Reitman and Frankel[22] using commercial kits (Shanghai Kexin Biotech Institute).

Tissue homogenisation

Liver samples were homogenised to 100 g L^{-1} with cold 9 g L^{-1} saline using glass equipment on ice. The homogenates were centrifuged and the clear supernatants were collected at 4 °C. Superoxide dismutase (SOD) activity, catalase (CAT) activity, malondialdehyde (MDA) level and total antioxidant capacity (T-AOC) were measured using commercial kits (Nanjing Jianchen Bioengineering Institute, Nanjing, China).

Morphological assays

Small liver pieces were prefixed with 25 g L^{-1} glutaraldehyde and postfixed with 10 g L^{-1} osmium tetroxide and 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 ($n = 12$). 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. $P < 0.05$ was considered statistically different.

RESULTS

Effects of EEA and AEA on body weight and weight gain in hyperlipidaemic mice

Although the initial body weight, final body weight and food intake of mice were similar in all groups, there was an obvious increase in body weight gain in the HFD group compared with the NC group ($P < 0.05$). This increase was probably due to the HFD. The results shown in Table 1 also indicate that EEA and AEA exerted a protective effect against HFD-induced overweight. Treatment of hyperlipidaemic mice with EEA and AEA resulted in a significant reduction in body weight gain of 28% ($P < 0.05$) and 38% ($P < 0.01$) respectively compared with the HFD group.

Effects of EEA and AEA on serum lipid levels in different groups

As shown in Table 2, fasting serum TC levels were measured every other week. After 2 weeks a significant increase in serum TC levels was observed in HFD-treated mice compared with the NC group ($P < 0.01$), indicating that hypercholesterolaemia had been induced. When hyperlipidaemic mice were supplemented with EEA or AEA for 6 weeks or longer, a significant hypocholesterolaemic effect was observed. At the end of the 8 week treatment the reduction in EEA- and AEA-treated mice was 16 and 20% respectively compared with untreated hyperlipidaemic mice ($P < 0.01$).

Table 1. Body weight and weight gain in different groups of mice

Group	Body weight (g)		Weight gain (g)	Average food intake (g day^{-1})
	Initial	Final		
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*	5.39 ± 0.39
HFD + SIM	24.20 ± 2.77	31.52 ± 3.85	7.31 ± 3.17	5.77 ± 0.97
HFD + EEA	27.13 ± 3.66	32.64 ± 5.33	5.32 ± 2.01†	5.06 ± 0.54
HFD + AEA	27.95 ± 2.67	32.65 ± 3.76	4.57 ± 1.68††	4.66 ± 0.70

NC, normal control; HFD, high-fat diet + distilled water; HFD + SIM, high-fat diet + 20 mg kg^{-1} daily simvastatin; HFD + EEA, high-fat diet + 200 mg kg^{-1} ethanolic extract of asparagus; HFD + AEA, high-fat diet + 200 mg kg^{-1} aqueous extract of asparagus. Values are expressed as mean ± standard deviation ($n = 12$).
* $P < 0.05$ *vs* NC; † $P < 0.05$,
†† $P < 0.01$ *vs* HFD.

Table 2. Serum total cholesterol levels in different groups of mice

Group	Total cholesterol (mmol L^{-1})				
	0 weeks	2 weeks	4 weeks	6 weeks	8 weeks
NC	2.70 ± 0.39	2.68 ± 0.30	1.81 ± 0.19	2.37 ± 0.27	2.42 ± 0.22
HFD	13.46 ± 3.14**	11.67 ± 2.76**	10.01 ± 1.17**	10.74 ± 1.18**	11.32 ± 1.08**
HFD + SIM	12.02 ± 2.59	12.90 ± 3.64	6.38 ± 0.21††	6.64 ± 1.50††	8.10 ± 1.26††
HFD + EEA	13.73 ± 2.86	11.45 ± 2.00	9.64 ± 1.81	8.04 ± 1.10††	9.48 ± 1.09††
HFD + AEA	13.16 ± 2.17	10.03 ± 2.67	7.85 ± 1.85††	7.57 ± 1.27††	9.02 ± 1.30††

NC, normal control; HFD, high-fat diet + distilled water; HFD + SIM, high-fat diet + 20 mg kg^{-1} daily simvastatin; HFD + EEA, high-fat diet + 200 mg kg^{-1} ethanolic extract of asparagus; HFD + AEA, high-fat diet + 200 mg kg^{-1} aqueous extract of asparagus. Values are expressed as mean ± standard deviation ($n = 12$).
** $P < 0.01$ *vs* NC;
†† $P < 0.01$ *vs* HFD.

Table 3. Serum triglyceride (TG), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) levels (mmol L^{-1}) in different groups of mice

Group	TG	HDL-C	LDL-C
NC	0.95 ± 0.19	1.47 ± 0.20	0.54 ± 0.09
HFD	0.76 ± 0.18	1.21 ± 0.26*	10.01 ± 1.04**
HFD + SIM	0.73 ± 0.11	1.76 ± 0.23††	5.89 ± 0.77††
HFD + EEA	0.65 ± 0.08	1.33 ± 0.19	8.14 ± 0.93††
HFD + AEA	0.67 ± 0.10	1.48 ± 0.26†	8.10 ± 0.90††

NC, normal control; HFD, high-fat diet + distilled water; HFD + SIM, high-fat diet + 20 mg kg^{-1} daily simvastatin; HFD + EEA, high-fat diet + 200 mg kg^{-1} ethanolic extract of asparagus; HFD + AEA, high-fat diet + 200 mg kg^{-1} aqueous extract of asparagus. Values are expressed as mean ± standard deviation ($n = 12$).
* $P < 0.05$, ** $P < 0.01$ *vs* NC;
† $P < 0.05$, †† $P < 0.01$ *vs* HFD.

The effects of EEA and AEA on serum TG, HDL-C and LDL-C levels were also examined. As shown in Table 3, in the HFD group, serum HDL-C was decreased by 18% while serum LDL-C was increased 19-fold. Treatment of hyperlipidaemic mice with AEA evidently increased HDL-C levels by 22% compared with untreated hyperlipidaemic mice ($P < 0.01$). The levels of this particular cholesterol fraction also tended to increase in EEA-treated mice, but the pattern of change remained statistically insignificant. Both EEA and AEA decreased the high serum LDL-C levels in hyperlipidaemic mice by 19% in comparison with the HFD group ($P < 0.01$). However, there was no obvious difference in TG levels between treated and untreated groups.

Effects of EEA and AEA on serum AST and ALT activities in different groups

Serum AST and ALT activities in all groups are shown in Fig. 1. There was a significant increase ($P < 0.01$) in serum AST and ALT activities in hyperlipidaemic mice compared with NC mice. EEA and AEA produced a significant increase in the activity of serum AST by 13 and 22% respectively compared with the HFD group ($P < 0.01$). Also, EEA and AEA dramatically decreased the activity of serum ALT by 31 and 40% respectively compared with the HFD group ($P < 0.01$).

Effects of EEA and AEA on hepatic SOD, CAT, MDA and T-AOC levels in different groups

The effects of EEA and AEA on hepatic SOD, CAT, MDA and T-AOC levels are shown in Table 4. There was a significant decrease in SOD activity, CAT activity and T-AOC level and an obvious increase in MDA level in hyperlipidaemic mice after induction, indicating that the HFD caused an oxidative burden. However, when hyperlipidaemic mice were treated with EEA and AEA, the SOD activity was markedly increased by 12% ($P < 0.05$) and 36% ($P < 0.01$), the T-AOC level was elevated by 14% ($P < 0.05$) and 32% ($P < 0.01$) and the MDA level was decreased by 19% ($P < 0.05$) and 32% ($P < 0.01$) respectively.

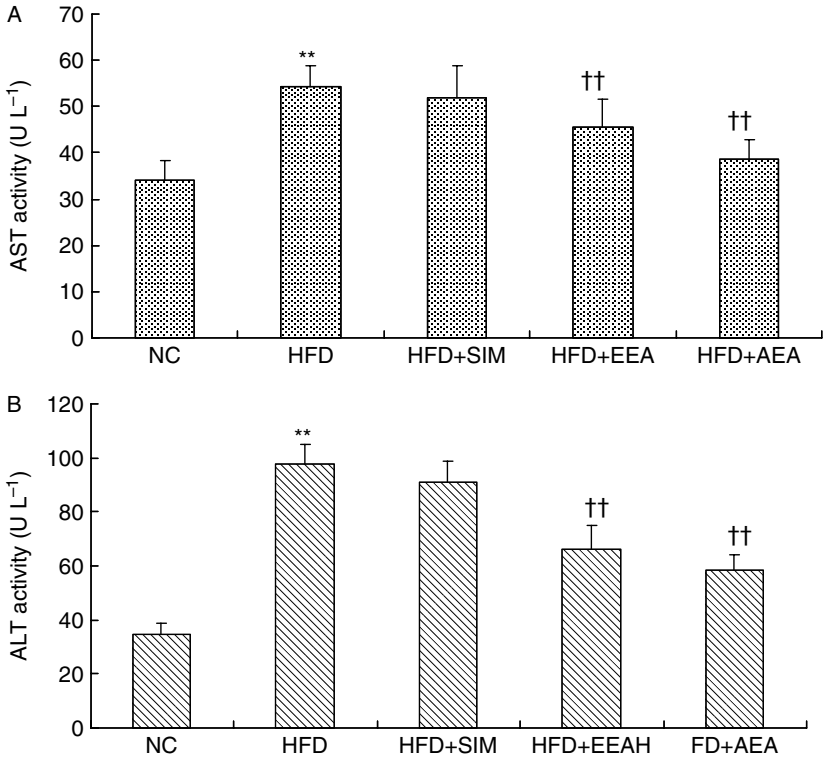

Figure 1. Serum (A) aspartate transaminase (AST) and (B) alanine transaminase (ALT) activity levels in different groups of mice: NC, normal control; HFD, high-fat diet + distilled water; HFD + SIM, high-fat diet + 20 mg kg^{-1} daily simvastatin; HFD + EEA, high-fat diet + 200 mg kg^{-1} ethanolic extract of asparagus; HFD + AEA, high-fat diet + 200 mg kg^{-1} aqueous extract of asparagus. Values are expressed as mean ± SD ($n = 12$). $^{**}P < 0.01$ *vs* NC; $^{††}P < 0.01$ *vs* HFD.

Table 4. Hepatic superoxide dismutase (SOD), catalase (CAT), malondialdehyde (MDA) and total antioxidant capacity (T-AOC) levels (U mg^{-1} protein) in different groups of mice

Group	SOD	CAT	MDA	T-AOC
NC	373.48 ± 22.01	57.45 ± 5.82	2.93 ± 0.22	1.66 ± 0.24
HFD	173.72 ± 15.64**	49.45 ± 5.45*	4.47 ± 0.36**	1.11 ± 0.16**
HFD + SIM	231.61 ± 15.62††	53.87 ± 5.41	3.18 ± 0.34††	2.35 ± 0.34††
HFD + EEA	194.30 ± 8.88††	52.61 ± 3.71	3.63 ± 0.57†	1.27 ± 0.33†
HFD + AEA	236.14 ± 10.38††	50.15 ± 5.58	3.04 ± 0.35††	1.47 ± 0.24††

NC, normal control; HFD, high-fat diet + distilled water; HFD + SIM, high-fat diet + 20 mg kg^{-1} daily simvastatin; HFD + EEA, high-fat diet + 200 mg kg^{-1} ethanolic extract of asparagus; HFD + AEA, high-fat diet + 200 mg kg^{-1} aqueous extract of asparagus. Values are expressed as mean ± standard deviation ($n = 12$).
* $P < 0.05$, ** $P < 0.01$ *vs* NC;
† $P < 0.05$, †† $P < 0.01$ *vs* HFD.

Hepatocytes viewed by transmission electron microscope

We also investigated the morphological effects of EEA and AEA on the liver of hyperlipidaemic mice. As shown in Fig. 2A, rough endoplasmic reticulum (RER), smooth endoplasmic reticulum (SER) and mitochondria were observed in normal hepatocytes. SER appeared as a network of branching tubules, while sparse RER was localised in the peribiliary regions of mitochondria.

In the hepatocytes of HFD-treated mice (Fig. 2B), SER disappeared, while excess lipid droplets congregated and parallel RER emerged. However, in all experimental treated groups, specific decreases in lipid deposition and in the gathering of RER were observed (Figs 2D and 2E). These results suggest that EEA and AEA might have hepatoprotective effects.

Major active ingredients in EEA and AEA

The major active ingredients in EEA and AEA are presented in Table 5. The three main active ingredients were soluble dietary fibre, steroidal saponins and total flavonoids. Soluble dietary fibre was present at high levels in both EEA and AEA, though its content in AEA was three times that in EEA. The content of steroidal saponins was almost 80 g kg^{-1} in EEA compared with <5 g kg^{-1} in AEA. The content of total flavonoids in EEA was twice that in AEA.

DISCUSSION

The purpose of this study was to evaluate the hypolipidaemic and hepatoprotective properties of ethanolic and aqueous

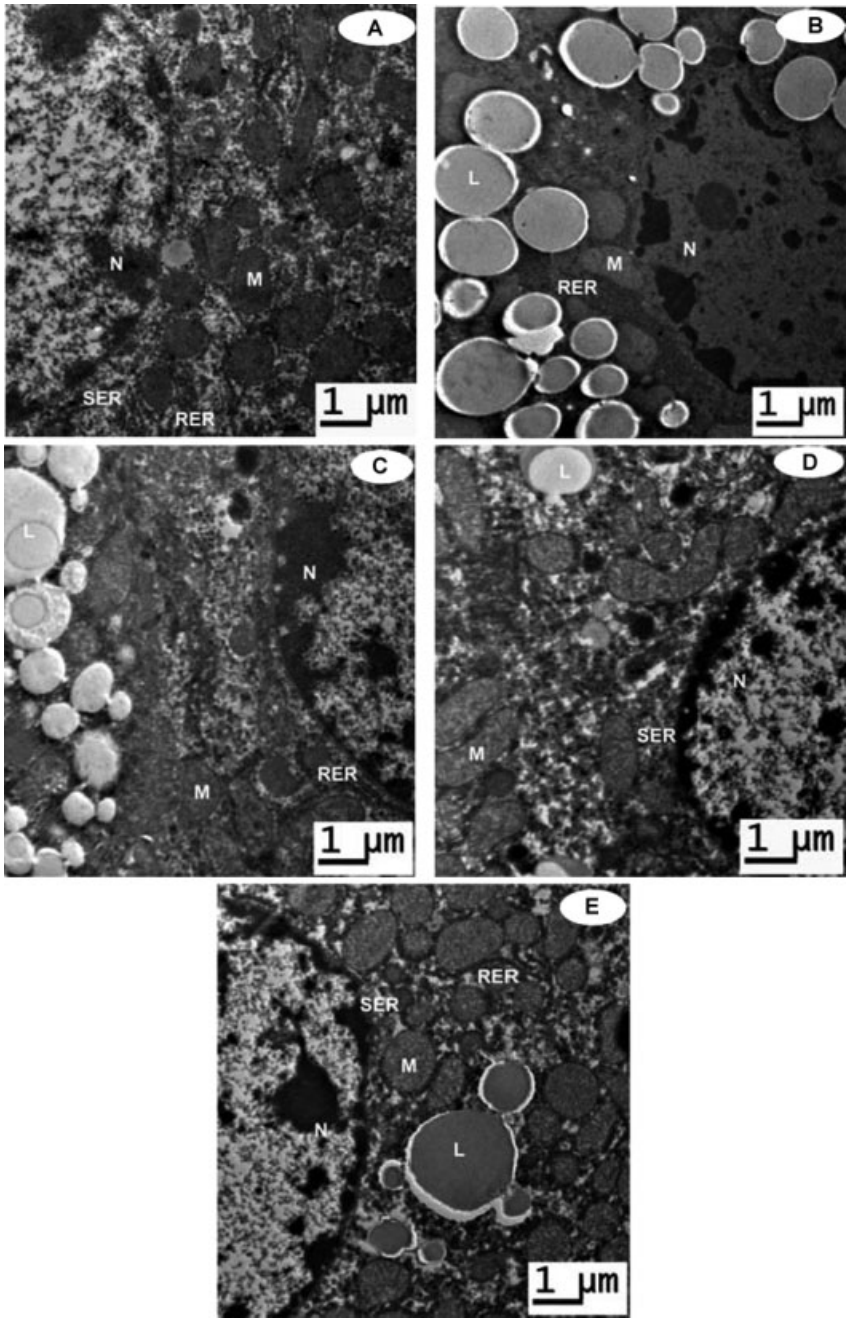

Figure 2. Transmission electron micrographs of hepatocytes from (A) normal control, (B) high-fat diet, (C) simvastatin-treated, (D) ethanolic extract-treated and (E) aqueous extract-treated groups of mice. RER, rough endoplasmic reticulum; SER, smooth endoplasmic reticulum; M, mitochondria; N, nucleus; L, lipid deposition.

Table 5. Major active ingredients (g kg^{-1}) in ethanolic (EEA) and aqueous (AEA) extracts of asparagus

Active ingredient	EEA	AEA
Soluble dietary fibre	153.4 ± 8.1	434.6 ± 14.5
Total flavonoids	73.2 ± 5.8	35.0 ± 3.1
Steroidal saponins	79.4 ± 2.6	2.5 ± 0.12

extracts from asparagus by-products in hyperlipidaemic mice. After 10 weeks of HFD feeding, HFD control mice developed an overweight state, with increased circulating TC and LDL-C and decreased HDL-C levels, indicating that a hyperlipidaemic mouse model had been induced.[20]

After treatment with EEA and AEA for a period of 8 weeks a decrease in serum TC accompanied by a reduction in its LDL fraction was observed in hyperlipidaemic mice. LDL-C is a major risk factor in cardiovascular diseases and is also the target of many hypocholesterolaemic therapies. AEA also showed hypolipidaemic action, causing an increase in HDL-C level. This lipoprotein, commonly known as 'good cholesterol', facilitates the mobilisation of TG and cholesterol from plasma to the liver, where they are catabolised and eliminated in the form of bile acids.[23] These

results suggest that EEA and AEA would be helpful in decreasing the incidence of cardiovascular diseases through a reduction in TC and LDL-C and an increase in HDL-C. The conspicuous hypolipidaemic activity of EEA and AEA might be mainly due to their soluble dietary fibre content, which reduced cholesterol and bile acid absorption in the intestinal lumen and decreased insulin secretion. Firstly, greater faecal excretion of bile acids leads to their decreased enterohepatic circulation, followed by an increase in the conversion of cholesterol to bile acids in the liver and an increase in cholesterol uptake from the circulation. Secondly, most soluble fibres decrease the rate of glucose absorption and attenuate the rise of plasma glucose and insulin levels, leading to a reduced level of cholesterol synthesis in the liver, because insulin promotes hepatic biosynthesis of cholesterol.[24] The significant decrease in serum TC and LDL-C levels in treated groups might be partly due to flavonoids and saponins as well. Saponins are a type of phytosterols and can reduce blood cholesterol level by decreasing the absorption and synthesis of cholesterol.[24] Flavonoids are known to reduce blood cholesterol level by inhibiting cholesterol synthesis and increasing the expression of LDL receptors.[25]

It has been reported that HFD treatment can cause liver damage.[26] When mice are fed an HFD, their liver, the primary organ that metabolises cholesterol ingested in excess, is affected by oxidative stress induced by the HFD. This oxidative stress leads to cell damage involving free radicals and lipid peroxidation.[27] In order to rule out possible damage to the liver by the HFD and understand the hepaprotective effect of EEA and AEA, we examined the activities of ALT and AST in serum and the morphology of liver cells in mice. It is well known that AST and ALT levels are the most useful and powerful indicators for the detection of hepatic cell damage, because both are present in high concentrations in hepatocytes.[28] These enzymes leak into the circulation when hepatocytes or their cell membranes are damaged.[28] In our study the diet with 150 g kg^{-1} lard, 20 g kg^{-1} cholesterol and 5 g kg^{-1} cholic acid not only led to a large increase in MDA level and excessive aggregation of lipid droplets in the liver of mice but also significantly decreased SOD activity, CAT activity and T-AOC level. These results indicate that the HFD led to oxidative stress in these mice and that this oxidative stress resulted in liver damage, eventually causing an increase in ALT and AST activities. However, treatment with EEA and AEA was able to significantly ameliorate the liver damage by increasing hepatic SOD activity and T-AOC level as well as decreasing MDA and lipid levels in hyperlipidaemic mice. This observation implies that both EEA and AEA have a hepatoprotective effect by decreasing the lipid level and restoring the antioxidant defence system.

CONCLUSION

Both ethanolic and aqueous extracts from asparagus by-products exhibited strong hypolipidaemic and hepatoprotective action when administered at a daily dose of 200 mg kg^{-1} for 8 weeks in hyperlipidaemic mice. Our findings suggest that these extracts could be used as supplements in healthcare foods and drugs or in combination with other hypolipidaemic drugs. Thus utilisation of asparagus-processing waste in this way could be economically beneficial to producers.

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