

The aqueous extract of *Asparagus officinalis* L. by-product exerts hypoglycaemic activity in streptozotocin-induced diabetic rats

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Abstract

BACKGROUND: The inedible bottom part of asparagus (*Asparagus officinalis* L.) spears, around one-third to one-half of the total length, is always discarded as by-product. Since it still contains various bioactive substances, this by-product might have potential usage in food supplements for its therapeutic effects. In this study the hypoglycaemic effect of the aqueous extract of asparagus by-product (AEA) was evaluated in a streptozotocin (STZ)-induced diabetic rat model.

RESULTS: Continuous administration of AEA for 21 days significantly decreased fasting serum glucose and triglyceride levels but markedly increased body weight and hepatic glycogen level in diabetic rats. In an oral glucose tolerance test, both the blood glucose level measured at 30, 60 and 120 min after glucose loading and the area under the glucose curve showed a significant decrease after AEA treatment.

CONCLUSION: The results of this study demonstrate that AEA has hypoglycaemic and hypotriglyceridaemic functions, suggesting that it might be useful in preventing diabetic complications associated with hyperglycaemia and hyperlipidaemia.

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Keywords: *Asparagus officinalis* L.; streptozotocin; diabetic rats; hypoglycaemic effect

INTRODUCTION

Asparagus (*Asparagus officinalis* L.) is a healthy and nutritious vegetable that is rich in many bioactive phytochemicals such as carotenoids,¹ steroidal saponins,^{2–4} flavonoids,^{4,5} phenolics,^{6,7} dietary fibre⁸ and oligosaccharides.⁹ Besides culinary quality, asparagus and its extract have been reported to possess antioxidant,^{5,7,10,11} antitumour,^{12,13} hypolipidaemic,¹⁴ hypoglycaemic¹⁴ and antifungal¹⁵ properties.

The edible shoot of asparagus is approximately 25–35 cm in length, which is about one-half to two-thirds of the full length of the stem. The remaining woody part (inedible bottom part) is always discarded as by-product. However, this by-product of asparagus still contains many bioactive substances such as flavonoids, steroidal saponins and dietary fibre,^{16,17} which suggests its potential usage in food supplements for its therapeutic effects. Since the by-product has a similar bioactive composition to the edible part of asparagus, we hypothesise that this by-product still possesses biological functions. It was previously reported that the high-dietary-fibre powder obtained from asparagus by-product exhibited antioxidant activity,¹⁶ and results from our laboratory showed that oral administration of the aqueous extract of asparagus by-product (AEA), which contained saponins, flavonoids and polysaccharides, had a potent hypolipidaemic effect in mice fed a high-fat diet.¹⁷ However, whether AEA shows a hypoglycaemic effect is still unknown. Therefore in this study we evaluated the possible hypoglycaemic effect of AEA in streptozotocin (STZ)-induced diabetic rats.

MATERIALS AND METHODS

Plant material

The asparagus by-product used in this study was obtained from Shanghai Green Asparagus Co. Ltd (Shanghai, China). It comprised the bottom part of asparagus spears (10–25 cm), the top part (the edible part, 30–35 cm) having been taken as food. The corresponding asparagus spears were collected from Chongming in March 2009 and authenticated by Dr Hongqing Li (School of Life Science, East China Normal University, Shanghai, China). A voucher specimen (No. zx1001) was deposited in the herbarium of East China Normal University.

Preparation of aqueous extract

A total amount of 100 g of dried and powdered asparagus by-product was suspended in 20 volumes of distilled water at room temperature and stirred intermittently for 2 h. The mixture was then boiled for 30 min and filtered. The resulting filtrate was sequentially concentrated in a vacuum rotary evaporator and further freeze-dried to produce the solid aqueous extract (AEA, 12.01 g). Phytochemical screening of AEA revealed the

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presence of proteins, carbohydrates, saponins, phenols and flavonoids. The contents of total and reducing sugars were determined by the phenol-sulfuric acid and dinitrosalicylic acid methods^{18,19} as 434.58 ± 14.51 and 70.10 ± 4.91 mg g⁻¹ ($n = 5$) respectively. The content of steroidal saponins was determined as 25.03 ± 1.18 mg g⁻¹ ($n = 5$) as described previously.²⁰ The content of total flavonoids was determined as 35.01 ± 3.06 mg g⁻¹ ($n = 5$) by spectrophotometry as described previously.²¹

Animals and diet

Male Sprague-Dawley rats weighing 240–280 g were purchased from Super-B&K, Ltd (Shanghai, China) and housed in an air-conditioned room with controlled temperature (23–25 °C) and automatic lighting (12/12 h light/dark cycle). The animals were fed a standard chow diet obtained from Shanghai SLAC Laboratory Animal Co. Ltd (Shanghai, China) and water *ad libitum*. The standard chow diet contained 92 g water, 221 g crude protein, 52.8 g crude fat, 52 g crude ash, 41.2 g crude fibre, 12.4 g calcium, 9.2 g phosphorus, 7.2 g DL-methionine/leucine mixture, 13.4 g lysine and 520 g nitrogen-free extract kg⁻¹.

The experimental animal protocol used in the study was approved by the Science and Technology Commission of Shanghai Municipality (licence number SYXX (SH) 2004-0001). All protocols of animal maintenance and handling were in accordance with NIH standards established in the Guidelines for the Care and Use of Experimental Animals.²²

Induction of diabetes

After a 10 h fast the test rats were randomly divided into two groups: a normal control group and an STZ-induced diabetic group. Diabetes was induced in rats by a single intraperitoneal injection of 47 mg kg⁻¹ body weight (BW) of STZ (Sigma, St Louis, MO, USA) freshly dissolved in 0.1 mol L⁻¹ citrate buffer (pH 4.5). Normal control rats were injected with citrate buffer only. In some cases, STZ injection may trigger massive insulin release and result in fatal hypoglycaemia. To prevent this, rats were fed 30 mg mL⁻¹ glucose solution for 24 h. Five days after injection, rats with fasting serum glucose levels between 10 and 26 mmol L⁻¹ were used as diabetic rats for further study.

Experimental design

In this experiment a total of 60 rats (ten normal rats, 50 STZ-induced diabetic rats) were used and divided into six groups of ten rats each. The normal control rats were treated orally with distilled water daily for 3 weeks. The STZ-induced diabetic rats were randomly assigned to five groups. One served as diabetic control group and was treated with distilled water daily for 3 weeks. The others served as drug groups and were treated through gastrogavage either with glibenclamide at a dose of 10 mg kg⁻¹ BW day⁻¹ or with AEA at 100, 200 or 400 mg kg⁻¹ BW day⁻¹ for 3 weeks. During the experiment, all animals were carefully monitored every day.

Serum glucose concentrations were estimated on days 0, 14 and 21. At the end of the experiment, all rats were anaesthetised (chloral hydrate, 450 mg kg⁻¹) after an 8 h fast. Blood samples were taken from the abdominal aorta, and sera were obtained for biochemical analysis. All samples were stored at -70 °C until use.

Oral glucose tolerance test

After 1 week of administration an oral glucose tolerance test (OGTT) was performed on the animals after a 16 h fast. Distilled

Table 1. Effect of AEA on fasting serum glucose level in STZ-induced diabetic rats

Group	Serum glucose (mmol L ⁻¹)		
	0 days	14 days	21 days
Normal control	5.37 ± 0.69 ^{bb}	6.98 ± 1.27 ^{bb}	6.20 ± 1.55 ^{bb}
Diabetic control	17.04 ± 5.67 ^{aa}	26.64 ± 4.12 ^{aa}	26.80 ± 2.76 ^{aa}
Diabetic + AEA 100	16.55 ± 4.81 ^{aa}	21.83 ± 6.02 ^{aa}	21.19 ± 5.91 ^{aa}
Diabetic + AEA 200	16.57 ± 4.92 ^{aa}	16.48 ± 6.91 ^{aa,bb}	16.01 ± 7.99 ^{aa,bb}
Diabetic + AEA 400	16.57 ± 4.81 ^{aa}	20.27 ± 6.45 ^{aa,b}	19.69 ± 5.04 ^{aa,b}
Diabetic + glibenclamide	16.67 ± 5.01 ^{aa}	19.58 ± 7.66 ^{aa,b}	21.88 ± 7.85 ^{aa}

Each value represents mean ± SD of ten rats in each group. Statistical significance: ^{aa} $P < 0.01$ compared with normal control group; ^b $P < 0.05$ and ^{bb} $P < 0.01$ compared with diabetic control group.

water (normal control and diabetic control), AEA at doses of 100, 200 and 400 mg kg⁻¹ BW and glibenclamide at a dose of 10 mg kg⁻¹ BW were administered orally to normal and diabetic rats. After 1 h, glucose (2 g kg⁻¹ BW) was administered intragastrically to animals as a 300 mg mL⁻¹ solution. Blood glucose levels from the tail vein were analysed using blood glucose sensors (MediSense Optium Xceed) and electrodes (Optium, Abbott Diabetes Care Inc., Alameda, CA, USA) at 0 (just before the oral administration of glucose), 30, 60 and 120 min after glucose loading. Calculation of the area under the blood glucose curve (AUC) was made according to the formula²³

$$\begin{aligned} \text{AUC} = & (\text{basal glycaemia} + \text{glycaemia } 0.5 \text{ h}) \times 0.25 \\ & + (\text{glycaemia } 0.5 \text{ h} + \text{glycaemia } 1 \text{ h}) \times 0.25 \\ & + (\text{glycaemia } 1 \text{ h} + \text{glycaemia } 2 \text{ h}) \times 0.5 \end{aligned}$$

Biochemical analysis

Serum glucose, triglyceride, total cholesterol and high-density lipoprotein cholesterol (HDL-C) levels were determined using commercial enzyme-linked kits purchased from Kexin Biotechnology Institute (Shanghai, China). Hepatic glycogen was quantified using a kit from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Serum insulin was measured by a double-antibody radioimmunoassay (RIA) method using a rat insulin RIA kit (Millipore, Billerica, MA, USA).

Statistical analysis

All data are presented as mean ± standard deviation (SD). Data were evaluated by one-way analysis of variance using SPSS Version 14.0 (SPSS Inc., Chicago, IL, USA), and differences between means were assessed by Duncan's multiple range test. The level of significance was set at $P < 0.05$ for all statistical tests.

RESULTS

Changes in serum glucose level

The serum glucose levels measured after an 8 h fast are shown in Table 1. In sera from STZ-induced diabetic rats, glucose levels were increased about three- to four fold over those in sera from normal

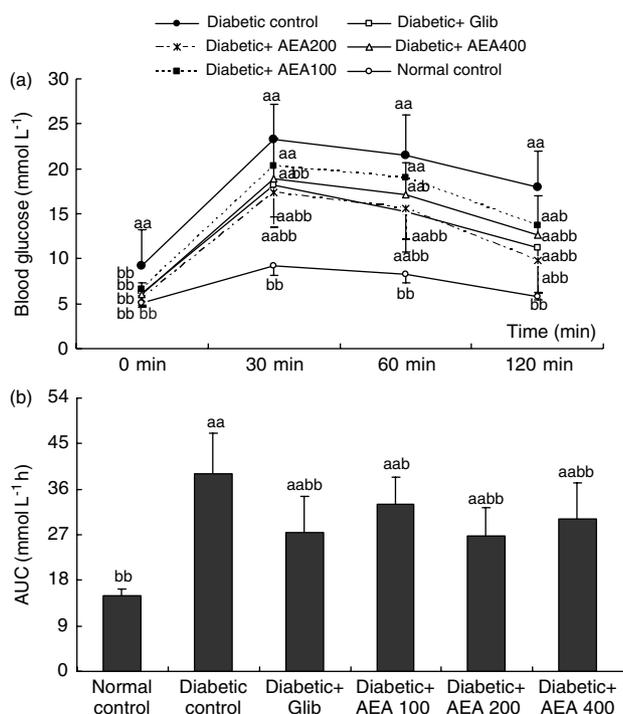


Figure 1. (a) Blood glucose level and (b) AUC of OGTT (2 g glucose kg⁻¹ BW) in normal and diabetic rats. Statistical significance: ^a*P* < 0.05 and ^{aa}*P* < 0.01 compared with normal control group; ^b*P* < 0.05 and ^{bb}*P* < 0.01 compared with diabetic control group. Glib, glibenclamide.

control rats. Administration of 200 and 400 mg AEA kg⁻¹ BW for 14 or 21 days produced a significant decrease in serum glucose in comparison with the diabetic control group (*P* < 0.05), with reductions of 38.14 and 17.96% on day 14 and 40.26 and 29.91% on day 21 respectively.

Oral glucose tolerance test

In the OGTT the blood glucose level of diabetic control rats was dramatically elevated at 30, 60 and 120 min after glucose loading, whereas the blood glucose elevation was significantly attenuated in glibenclamide- and AEA-treated diabetic rats (Fig. 1(a)). Glibenclamide and 200 and 400 mg kg⁻¹ AEA caused a significant hypoglycaemic effect at 30, 60 and 120 min (*P* < 0.01 or *P* < 0.05), whereas 100 mg kg⁻¹ AEA only decreased the blood glucose level at 120 min (*P* < 0.05).

The AUC for the glucose response during the OGTT revealed a significant increase in the diabetic control group (38.96 ± 8.03 mmol L⁻¹ h) compared with the normal control group (15.03 ± 1.24 mmol L⁻¹ h) (Fig. 1(b)). Compared with the diabetic control group, the 100, 200 and 400 mg kg⁻¹ AEA-treated groups showed a reduction in AUC by 15.30, 31.47 and 22.72% respectively (*P* < 0.05 or *P* < 0.01), and glibenclamide treatment also reduced the AUC by 29.30% (*P* < 0.01).

Serum insulin and hepatic glycogen levels

During the experiment, serum insulin and hepatic glycogen levels were measured in all rats. Both serum insulin (Fig. 2) and hepatic glycogen (Fig. 3) levels were markedly lower (*P* < 0.05) in diabetic rats than in normal control rats. After the 21 day treatment period, diabetic rats treated with AEA showed no significant differences in serum insulin (*P* > 0.05), whereas the hepatic glycogen level

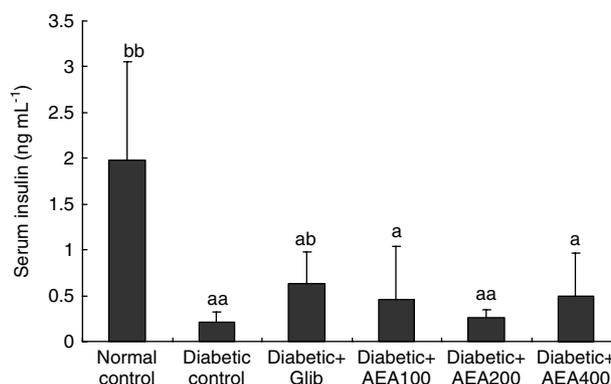


Figure 2. Serum insulin level in normal and diabetic rats. Statistical significance: ^a*P* < 0.05 and ^{aa}*P* < 0.01 compared with normal control group; ^b*P* < 0.05 and ^{bb}*P* < 0.01 compared with diabetic control group. Glib, glibenclamide.

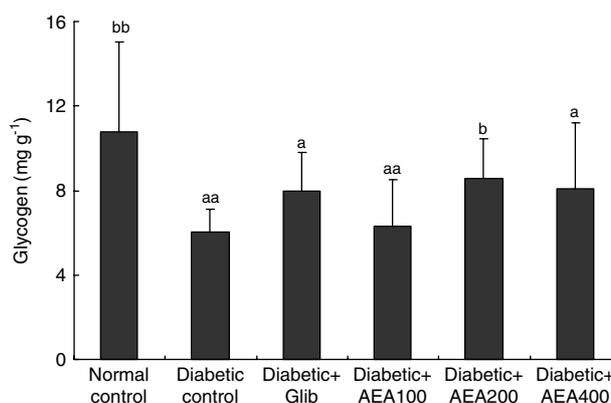


Figure 3. Hepatic glycogen level in normal and diabetic rats. Statistical significance: ^a*P* < 0.05 and ^{aa}*P* < 0.01 compared with normal control group; ^b*P* < 0.05 and ^{bb}*P* < 0.01 compared with diabetic control group. Glib, glibenclamide.

of the 200 mg kg⁻¹ AEA-treated group was found to increase significantly by 41.86% compared with the diabetic control group (*P* < 0.05).

Serum lipid profile and body weight

Table 2 shows the changes in serum lipid parameters in the different experimental groups. Compared with normal control rats, serum total cholesterol levels of diabetic rats were markedly increased whereas body weights were significantly decreased (*P* < 0.01) (Fig. 4).

Daily administration of AEA at 100 and 400 mg kg⁻¹ to diabetic rats for 21 days significantly reduced serum triglyceride levels by 37.10 and 38.71% respectively (*P* < 0.05) compared with diabetic control rats. Meanwhile, glibenclamide supplementation caused a significant reduction of 16.86% (*P* < 0.05) in total cholesterol levels of diabetic rats. As can be seen in Fig. 4, diabetic rats clearly gained body weight after being supplied with glibenclamide and the three different doses of AEA (*P* < 0.05).

DISCUSSION

It is known that treatment of rats with high-dose STZ is an established model for type 1 diabetes, as high-dose STZ severely

Table 2. Effect of AEA on serum triglyceride, total cholesterol and HDL-C levels in STZ-induced diabetic rats

Group	Triglyceride (mmol L ⁻¹)	Total cholesterol (mmol L ⁻¹)	HDL-C (mmol L ⁻¹)
Normal control	1.05 ± 0.45	1.38 ± 0.22 ^b	0.68 ± 0.07
Diabetic control	1.24 ± 0.37	1.72 ± 0.43 ^a	0.85 ± 0.11
Diabetic + AEA 100	0.78 ± 0.29 ^b	1.69 ± 0.25 ^a	0.87 ± 0.14
Diabetic + AEA 200	0.97 ± 0.41	1.78 ± 0.26 ^a	0.85 ± 0.32
Diabetic + AEA 400	0.76 ± 0.33 ^b	1.74 ± 0.25 ^a	0.92 ± 0.13
Diabetic + glibenclamide	1.17 ± 0.43	1.43 ± 0.13 ^b	0.85 ± 0.14

Each value represents mean ± SD of ten rats in each group. Statistical significance: ^a*P* < 0.05 compared with normal control group; ^b*P* < 0.05 compared with diabetic control group.

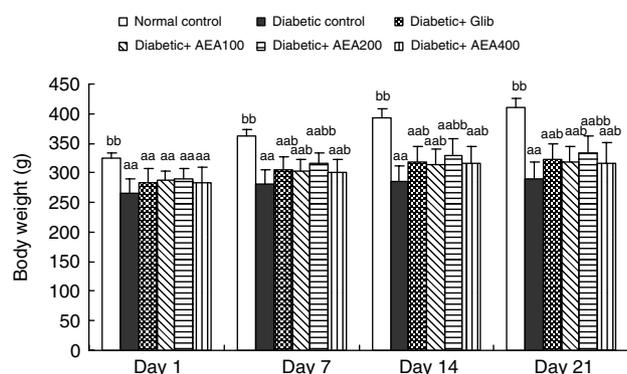


Figure 4. Body weight of normal and diabetic rats. Statistical significance: ^{aa}*P* < 0.01 compared with normal control group; ^b*P* < 0.05 and ^{bb}*P* < 0.01 compared with diabetic control group. Glib, glibenclamide.

impairs insulin secretion, leading to hyperglycaemia in rats. In our investigation the administration of STZ to rats resulted in hyperglycaemia, glucose intolerance, hypoinsulinaemia, increased serum cholesterol and decreased body weight. With this diabetes model in rats the antidiabetic effect of AEA was evaluated. Our normoglycaemic study revealed that AEA at doses of 200 and 400 mg kg⁻¹ showed a hypoglycaemic effect. In the OGTT, both the blood glucose level measured at three time points after glucose loading and the mean glucose concentration calculated from the AUC showed a significant decrease after AEA treatment, which was consistent with the hypoglycaemic activity of AEA in the fasting serum glucose test. These results suggested the AEA could improve the glucose intolerance in diabetic rats after glucose loading and might be effective as a hypoglycaemic agent in response to glucose ingestion.

Our results also showed that AEA lowered the elevated serum glucose levels in diabetic rats without significantly increasing serum insulin levels, which suggested that AEA did not have any insulin-stimulating effect and that the hypoglycaemic effect of AEA observed in our study was not associated with the stimulation of insulin secretion. Hence we suspected that AEA worked through an insulin-independent pathway when it lowered the glucose level in our study.

Diabetes mellitus is known to impair the normal capacity of the liver to synthesise glycogen.^{24,25} Moreover, results of previous investigations showed that the hepatic glycogen levels of STZ-induced diabetic animals were significantly lower than those of

normal rats,^{26–28} and the activities of glycogenolytic enzymes, i.e. phosphorylase (both a and b), phosphorylase kinase and protein kinase (in the presence or absence of cyclic AMP), were significantly decreased.²⁶ In the present study the hepatic glycogen levels of diabetic rats were markedly decreased, which was consistent with the previous observations, whereas this decline was significantly increased by AEA. This finding might be the result of activation of the glycogen synthase system by the extract. From this result we suspected that AEA could probably improve glucose homeostasis by stimulating glucose utilisation in peripheral tissues (liver). However, this speculation needs to be verified in further studies.

Diabetes is a metabolism-associated disease, particularly closely related to lipid metabolism, affecting the plasma lipid and lipoprotein profile.²⁹ Controlling serum lipid levels through dietary or drug therapy is also a major approach to treating diabetes and related complications.³⁰ As shown in our study, AEA had a definite hypotriglyceridaemic effect in STZ-induced diabetic rats. This result suggested that AEA would be helpful in the prevention of diabetic complications by improving dyslipidaemia.

Based on a large number of chemical and pharmacological studies, numerous active hypoglycaemic compounds have been listed, including polysaccharides, flavonoids, terpenoids, sterols, saponins, alkaloids, xanthenes, glycosides, alkyldisulfides, aminobutyric acid derivatives, guanidine and peptides.^{31–33} In the present study, phytochemical screening of AEA revealed the presence of polysaccharides, flavonoids and saponins. Therefore we speculate that the hypoglycaemic activity of AEA might be mainly due to its polysaccharide, flavonoid and saponin contents. However, this hypothesis needs further validation. Currently, work is being carried out in our laboratory to isolate the polysaccharides from AEA and evaluate their possible hypoglycaemic/hypolipidaemic effects. Once we have purified the active ingredients from AEA, the mechanism of their hypoglycaemic and hypolipidaemic effects will be elucidated.

CONCLUSION

This study demonstrated that AEA had hypoglycaemic function and improved the syndromes of glucose intolerance, hyperlipidaemia and weight loss in diabetic rats. The results suggested that treatment with AEA would be helpful in preventing diabetic complications by improving hyperglycaemia and hyperlipidaemia. The hypoglycaemic mechanism of AEA needs further investigation.

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