Saponins extracted from *Asparagus officinalis* L. by products exerts hypoglycemic effect in streptozotocin induced type 2 diabetic rats

Xinglei Zhu¹, Caixia Li², Yudan Zhu³

¹Editorial Department of Journal of Yunnan Normal University, Yunnan Normal University, Kunming 650092, China
²Editorial Department, Kunming Medical University, Kunming 650500, China
³Yunnan Dadi Fenyouan Enviromental Protection Co., Ltd, Kunming 650000, China

*Corresponding author: shidaxuebao@163.com

Abstract. This study aimed to evaluate the hypoglycemic property of saponins from *Asparagus officinalis* L. by-products (SA) in high-fat diet and streptozotocin (STZ)-induced type 2-like diabetic rats. Diabetic rats were administrated with SA at doses of 20, 40 and 60 mg/kg body weight once a day for 45 consecutive days. The diabetic rats showed significant decreased fasting serum glucose and free fatty acid, and significant increased hepatic glycogen and high-density lipoprotein cholesterol after SA treatment. Furthermore, SA administration at dose of 60 mg/kg markedly decreased both the blood glucose level measured after glucose loading and the area under the curve in the oral glucose tolerance test. Overall, the current study suggest that SA could control hyperglycemia and improve glucose tolerance, and may serve as a potential food supplement or natural drug in the management of diabetes.

1. Introduction

Diabetes mellitus is a long-term metabolic disease which characterized by hyperglycemia and impaired glucose tolerance [1]. It is reported that there are nearly 415 million individuals worldwide who have diabetes currently [2]. And about 90% diabetes mellitus patients belong to type-2 diabetes mellitus which begins with insulin resistance and then develops into hyperglycemia and hypoinsulinemia [1, 3]. Although several different kinds of anti-diabetic drugs can be found in the market, these drugs might cause various side-effects such as gastrointestinal reactions and allergic reactions [4, 5]. Therefore, it is very important to develop new anti-diabetic drugs with fewer side-effects. It has been reported that many kinds of plant extract showed hypoglycemic effects and could be used as anti-diabetic agents [1, 2, 5, 6].

Asparagus (*Asparagus officinalis* L.), a healthy and nutritious vegetable, is widely used in salads, vegetable dishes and soups all over the world. The reported bioactive constituents of asparagus include steroidal saponins [7], oligosaccharides [8], flavonoids [9] and phenolics [9]. In addition to its edible value, asparagus and its extracts have been reported to possess antioxidant [3], antitumour [10], antifungal [11], hypolipidaemic [12, 13], hypoglycaemic [14] and immunologic enhancement [15] activities.
Although most of these studies focus on the edible parts and roots of the asparagus, the edible shoot of asparagus is about 50%-70% of the full length of the stem. The remaining woody part (inedible bottom part) is always discarded as by-product during industrial processing, leading to significant resource waste and environmental pollution. In fact, this by-product of asparagus has been reported to be a rich source of steroidal saponins [16], flavonoids [17] and polysaccharides [18, 19] and might have potential use as food supplements or natural drugs. Therefore, the recycling of this by-product of asparagus has become a meaningful task, and it would be profitable in economical and ecological aspects. Our previous studies showed that aqueous, ethanolic [20] and n-butanol [21] extracts from asparagus by-product exhibited strong hypolipidemic property in high-fat-fed mice, and the aqueous extract displayed hypoglycemic activity in streptozotocin-induced diabetic rats [22]. It was reported that juice from asparagus by-product also exhibited hypoglycemic activity in diabetic rats [23]. Saponins is the main bioactive phytochemicals of asparagus by-product and was found to inhibited cancer cell migration and invasion through modulating the Rho GTPase signalling pathway [24-27]. However, whether saponins from *Asparagus officinalis* L. by-products (SA) shows a hypoglycemic effect remains limited. Therefore, the present study was conducted to investigate the possible hypoglycemic effect of SA in streptozotocin (STZ)-induced diabetic rats.

2. Materials and methods

2.1. Plant material
Freshly harvested asparagus spears were cut to obtain the 15-30 cm length top (edible) part for selling as food, and the bottom part of the spear (10-25 cm) was considered as a by-product. Asparagus by-product used in this study was provided by Shanghai Green Asparagus Co. Ltd (Shanghai, China).

2.2. Preparation of saponins from *Asparagus officinalis* L. by-products (SA)
SA was prepared as described previously [24]. Briefly, air-dried asparagus by-product powder was first extracted with ethanol. These ethanol fractions were then extracted with n-butanol to obtain crude saponins. Finally, these crude saponins were applied to an AB-8 macroporous resin column (Changzhou Baoen Chemical plant, Hebei, China) for further purification to produce SA. The steroidal saponins content was quantified as 55% by spectrophotometry as described previously [28].

2.3. Mice and treatment
Male Sprague-Dawley rats (200-220 g) were obtained from Shanghai SIPPR/BK Lab Animal Co., Ltd. (Shanghai, China) and kept in an air-conditioned room with a 12 h light/12 h dark cycle at 23-25 °C. The rats were maintained in clean cages with *ad libitum* access to water and food. All protocols of animal maintenance and handling were in accordance with NIH guideline for care and use of laboratory animals [29], and the animal experimental protocol was approved by the institutional animal ethical committee.

All the rats fed a standard chow diet (92 mL 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 mixture of DL-methionine and DL-leucine, 13.4 g lysine and 520 g nitrogen-free extract kg⁻¹) obtained from Shanghai SLAC Laboratory Animal Co., Ltd (Shanghai, China) 5 days for acclimatization.

2.4. Induction of type 2 diabetes
After 5-day acclimatization, the rats were randomly divided into two groups: fed either the standard chow diet (normal control group, NC, *n*=9) or a high-fat diet (HFD, 180 g lard, 200 g sucrose, 620 g standard chow diet kg⁻¹) for 5 weeks. After 12 h fast, a freshly prepared solution of STZ at 30 mg/kg body weight (BW) in 0.1 mol/L citrate buffer (pH 4.5) was intraperitoneally injected to HFD-treated rats, whereas normal control rats were injected with citrate buffer only. To prevent the fatal hypoglycemia arose induced by STZ injection, the rats were fed 30 mg/mL glucose solution for 24 h
after the injection. One week after injection, rats with fasting serum glucose levels higher than 8 mmol/L were used as type 2 diabetic rats for further study.

2.5. SA treatment
Total 45 diabetic rats were used in subsequent study and divided into 5 groups of 9 animals each. One served as diabetic control group (DC), and was treated with equal volume distilled water. One was treated with 500 mg/kg BW metformin (Met). The other three groups were treated with SA at doses of 20 (SA20), 40 (SA40), 60 (SA60) mg/kg BW. The drugs or vehicles were given through daily oral gavages for 45 days to rats. Animal weights were measured every week throughout the experiment and the dose was adjusted accordingly. Serum glucose levels were analysed on days 0, 23 and 45. After consecutive treatment for 45 days, all rats were fasted 12 h then sacrificed. Blood samples were collected and centrifuged to produce sera for biochemical analysis, and all samples were stored at −80 °C until use.

2.6. Serum biochemical analysis
Fasting serum glucose, total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C) and free fatty acid (FFA) levels were measured by enzymatic colorimetric methods using commercial kits purchased from Shanghai Kexin Biotechnology Institute (Shanghai, China) following the manufacturer’s instruction. Moreover, fasting serum low density lipoprotein-cholesterol (LDL-C) was calculated according to the Friedewald formula [30].

\[
LDL-C = TC - (HDL-C) - \frac{TG}{2}
\]

Fasting serum insulin level was determined by a double-antibody radioimmunoassay (RIA) method using a rat insulin RIA kit (Millipore, Billerica, MA, USA) according to the manufacturer’s protocol.

2.7. Oral glucose tolerance test
Oral glucose tolerance test (OGTT) at a dose of 2 g/kg glucose was performed on the 23-day of SA administration. All the rats were fasted overnight for 16 h. Distilled water, Metformin (500 mg/kg BW) or SA (20, 40, 60 mg/kg BW) was given to the respective group 30 min prior to oral glucose administration. Blood was withdrawn from the tail tip to measure blood glucose (BG) at 0, 30, 60 and 120 min after oral glucose administration using a glucometer (Abbott Diabetes Care, Inc., Alameda, CA). The area under the curve (AUC) was determined according to the following formula [31]:

\[
AUC = \frac{(BG_0 + BG_{30})}{2} \times 0.5 + \frac{(BG_{30} + BG_{60})}{2} \times 0.5 + \frac{(BG_{60} + BG_{120})}{2} \times 1
\]

2.8. Statistical analysis
Statistical analysis were performed using the SPSS 21.0 statistical package (SPSS, Inc.). Data were expressed as mean ± standard deviation (SD). Data were evaluated by one-way analysis of variance followed by Duncan or Games-Howell’s multiple range tests. P-value lower than 0.05 was considered to be statistically significant.

3. Results

3.1. Effect of SA on fasting serum glucose level
After 5-week HFD induction and a single intraperitoneal injection of STZ, a significant increase in fasting serum glucose level was observed in induced rats compared with the NC group (P < 0.01), indicating that diabetes had been induced (Table 1). When diabetic rats were treated with SA at doses of 20 and 60 mg/kg for 23 d, there was a slight, statistically non-significant, decrease in serum glucose level compared with the DC group. However, 40 mg/kg SA treatment significantly reduced the serum glucose level compared with the DC group on day 23 (P < 0.05). When diabetic rats were treated with SA for 45 d, a significant hypoglycemic effect was observed at all doses. The reduction of serum
glucose in 20, 40 and 60 mg/kg SA-treated rats was 46.81% ($P < 0.01$), 48.70 ($P < 0.01$), 35.49% ($P < 0.05$) respectively.

| Group   | n | Fasting Serum glucose (mmol/L) |
|---------|---|--------------------------------|
|         | 0 d | 23 d | 45 d |
| NC      | 9   | 5.55±0.27$^d$ | 5.33±0.35$^d$ | 5.31±0.85$^d$ |
| DC      | 9   | 20.36±1.26$^b$ | 19.53±1.14$^b$ | 23.22±3.09$^b$ |
| Met     | 9   | 19.36±1.22$^b$ | 18.34±2.56$^b$ | 15.15±3.10$^{bc}$ |
| SA20    | 9   | 18.40±3.04$^b$ | 15.24±7.12$^b$ | 12.35±5.05$^{bd}$ |
| SA40    | 9   | 18.87±2.62$^b$ | 13.59±4.40$^{bc}$ | 11.91±2.47$^{bc}$ |
| SA60    | 9   | 18.95±2.04$^b$ | 16.99±3.73$^b$ | 14.98±3.68$^{bc}$ |

Note: Values are represented as mean ± SD. Statistical significance: $^a P < 0.05$ and $^b P < 0.01$ compared with NC group, $^c P < 0.05$ and $^d P < 0.01$ compared with DC group, the same in the figures and tables below.

3.2. Effect of SA on oral glucose tolerance test

As shown in Fig. 1(a), in the OGTT, the blood glucose level of DC rats was dramatically elevated at 30, 60 and 120 min after glucose loading compared with the NC group ($P < 0.01$), suggesting an impaired glucose tolerance state. However, the blood glucose elevation was significantly attenuated in SA60-treated rats at 30 and 60 min ($P < 0.05$). And metformin treatment also prevented the blood glucose levels from rising at 30, 60 and 120 min ($P < 0.01$). The AUC of the OGTT showed a significant increase in DC group compared with the NC group ($P < 0.01$). But this increase was suppressed significantly by SA60 and metformin treatment. SA60 and Met groups showed a reduction in AUC by 12.21% ($P < 0.05$) and 21.55% ($P < 0.01$), respectively.

Figure 1. (a) Blood glucose concentration and (b) AUC of OGTT in normal and STZ-induced diabetic rats

3.3. Effect of SA on fasting serum insulin and hepatic glycogen levels

As shown in Fig. 2 and Fig. 3, both fasting serum insulin and hepatic glycogen levels were significantly decreased in DC rats when compared with the NC group ($P < 0.01$). After 45-day SA treatment this decrease of serum insulin was not changed in diabetic rats ($P > 0.05$), whereas the hepatic glycogen level of SA40 and SA60 group were found to increase significantly by 40.48% and 46.72%, respectively, compared with DC group.
3.4. Effect of SA on Serum Lipid Levels
Serum lipid profiles were measured as shown in Table 2. Compared with NC group, serum TC (P < 0.05) and FFA (P < 0.01) levels of DC rats were markedly increased whereas HDL-C levels were significantly decreased (P < 0.01). The 20 mg/kg SA treatment could significantly reduce serum TC levels by 30% (P < 0.05) compared with DC rats. Meanwhile, administration of SA at 40 and 60 mg/kg produced a marked elevation in serum HDL-C levels by 19% and 23%, respectively, when compared with the DC rats (P < 0.05). Supplementation of SA at 40 and 60 mg/kg also reduced serum FFA levels by 29% and 23%, respectively, compared with DC rats (P < 0.01). Metformin treatment caused a significant reduction in serum TC and FFA levels in diabetic rats by 29% (P < 0.05) and 18% (P < 0.01), respectively. However, there was no obvious difference in TG and LDL-C levels between treated and untreated groups.

Table 2. Effect of SA on fasting serum TC, TG, HDL-C, LDL-C and FFA levels in STZ-induced diabetic rats

| Group | n  | TC (mmol/L) | TG (mmol/L) | HDL-C (mmol/L) | LDL-C (mmol/L) | FFA (mmol/L) |
|-------|----|-------------|-------------|----------------|----------------|--------------|
| NC    | 9  | 1.54±0.19c  | 0.97±0.12   | 0.62±0.07b     | 0.70±0.30d     | 0.30±0.09d   |
| DC    | 9  | 1.87±0.32a  | 0.96±0.19   | 0.48±0.08b     | 0.94±0.26      | 0.56±0.05b   |
| Met   | 9  | 1.32±0.35c  | 0.89±0.24   | 0.50±0.05b     | 0.58±0.49      | 0.46±0.07b   |
| SA20  | 9  | 1.30±0.34c  | 0.83±0.19   | 0.56±0.09      | 0.60±0.34      | 0.61±0.07    |
| SA40  | 9  | 1.40±0.42   | 0.81±0.25   | 0.57±0.10c     | 0.86±0.43      | 0.40±0.11d   |
| SA60  | 9  | 1.64±0.39   | 0.91±0.26   | 0.59±0.10c     | 0.73±0.32      | 0.43±0.08ad  |
4. Discussion

Many related studies focus on diabetes and related treatments [32-36]. The current increase in prevalence of type 2 diabetes is believed to be closely connected with sedentary lifestyle and ingestion of energy-rich food in genetically susceptible individuals [37]. In this study, type 2 diabetic rat models were established with a high-fat diet and intraperitoneal injection of STZ, and these treated rats showed hyperglycemia, glucose intolerance, hypoinsulinemia, decreased hepatic glycogen and increased serum TC and FFA. Then the antidiabetic property of SA was evaluated in this diabetes model. The major finding of the present study is that SA at three different doses significantly lowered the serum glucose concentrations and SA at a high dose of 60 mg/kg markedly decreased both the blood glucose level measured after glucose loading and the area under the curve in the OGTT. These results suggest that SA could control hyperglycemia and improve glucose tolerance, the central feature of the diabetes, and implied its potential therapeutic use in diabetes and hyperglycemia.

In our study, a significant decrease in serum insulin level was observed in diabetic rats, but this reduction could not be improved after a 45-day administration of SA. This result suggested that SA did not lower blood glucose by increasing secretion of insulin from β-cells. Furthermore, it is known that prolonged FFA elevation is capable of causing insulin resistance [38]. Previous reports demonstrated that 4 hours of FFA elevation reduced insulin-mediated glucose uptake by approximately 50% [39]. Nowadays, increased FFA level is prove to be one of the metabolic hallmarks of insulin resistance [38]. The present data showed that FFA was significantly increased in DC group compared with NC group, while SA treatment at dose of 40 and 60 mg/kg were able to effectively reduce the FFA level in diabetic rats. This indicated that SA might be work through increasing the sensitivity of peripheral tissue to insulin, though the further studies are warranted.

Hepatic glycogen is the major storage form of glucose, and its function is to provide a readily available source of glucose for body [40]. The metabolism of hepatic glycogen plays an important role in regulating the blood glucose level, because the liver can absorb and convert circulated glucose into glycogen to lower blood glucose [41-43]. It is well known that diabetes mellitus impair the normal capacity of liver to synthesize glycogen. Previous studies revealed that the content of hepatic glycogen and the activities of glycogen-metabolizing enzymes were obviously decreased in diabetic animals [44, 45]. In this study, the level of hepatic glycogen was markedly decreased in DC group compared with NC group, but this decline was changed by SA administration at doses of 40 and 60 mg/kg. This result indicated that SA may exert its hypoglycemic effect through promoting the absorption and conversion of blood glucose into hepatic glycogen.

Dyslipidemia is a common diabetic complications, and it is the primary risk factor for developing cardiovascular disease in diabetic patients [1, 46, 47]. In this study, the TC levels were markedly reduced in SA20 group and HDL-C levels were significantly elevated in SA40 and SA60 group compared with DC group. These results indicated that SA may be helpful to improve dyslipidemia, and further decrease the incidence of cardiovascular complications in diabetic conditions.

5. Conclusion

SA, which exhibited strong hypoglycemic effect and improved glucose tolerance and dyslipidemia in type-2 diabetic rats, may serve as a food supplement or natural drug in the management of diabetes. Further studies need to be carried out to reveal the precise mechanisms of its hypoglycemic activities. Besides, the current study suggests an economically beneficial way to use asparagus by-products and thereby reduce environmental pollution.

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