Research Article

Hypoglycemic potential of *Elaeagnus angustifolia* fruit polysaccharide on streptozotocin–induced type 2 diabetic mice

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Abstract

In this study, the hyperglycemic potential of *Elaeagnus angustifolia* fruit polysaccharide in both normal healthy and streptozotocin-induced diabetic mice was investigated. Results showed no significant effect of *E. angustifolia* fruit polysaccharide on blood glucose level in normal control group, while *E. angustifolia* significantly suppressed the rise in blood glucose of diabetic mice. In addition, in the first two weeks of administration, the body weight was decreased both in negative control group and *E. angustifolia* groups, however, *E. angustifolia* (800 mg/kg) was recovered to the begining weight in the fourth week. *E. angustifolia* (800 mg/kg) could markedly reduce the levels of total cholesterol, triglyceride and improve the level of high density lipoprotein-cholesterol. The results suggest that *E. angustifolia* could be considered as an ingredient of functional foods for diabetes.

Introduction

Diabetes is a metabolic disease which has the characteristic of hyperglycemia. With the development of the society and the improvement of people's living standard, the incidence of diabetes also rises sharply (Li et al., 2007; Kim et al., 2010). It is necessary to find an effective and low-toxicity hypoglycemic drug.

For the past few years, numerous extracts have been isolated from plant and used as a promising source of therapeutic agents for diabetes mellitus such as *Coptis chinensis* (Jiang et al., 2013), *Rhizophora mangle* cortex (Andrade-Cetto et al., 2017), *Lilium lancifolium* (Zhang et al., 2014), pumpkin (Wang et al., 2017a), peach gum-derived polysaccharide (Wang et al., 2017b), *Momordica charantia* (Xu et al., 2015), *Talinum triangulare* (Xu et al., 2015), *Ocimum gratissimum* (Livia et al., 2014), *Citrus macroptera* Montr (Uddin et al., 2014), *Eugenia jambolana* (Kishalay et al., 2015), *Prunus cerasus* L. (Fatima et al., 2017), *Gaultheria trichophylla* (Fiaz et al., 2017), *Rhizo-*

phora mangle (Adolfo et al., 2017) and so on.

Elaeagnus angustifolia commonly refer to as" sliver berry", "Russian olive", "oleaster", "wild olive" and "qi li xiang" in mandarin belongs to *Elaeagnus* genus. The fruit from *E. angustifolia* contains higher content of polysaccharide which can reduce blood lipid and lower hypertension (Bendaikha et al., 2014). Furthermore, several researchers have reported that *E. angustifolia* polysaccharide have significant anti-oxidant capacity, anti-inflammatory capacity, anti-mutagenic capacity (Du et al., 2016; Hamidpour et al., 2017). Some experts researched the effect of *E. angustifoli* polysaccharides on immune functions of immunosuppressive mice and they found that *E. angustifolia* polysaccharides markedly increased the immunity function by increasing the spleen and thymus index of mice (Lian et al., 2009). However, hypoglycemic potential of *E. angus*so is far. Therefore, our research was undertaken to investigate the hypolipidemic effects of *E. angustifolia* polysaccharide on type 2 diabetic mice.

Materials and Methods

Chemicals and reagents

Streptozotocin was purchased from the Sigma Chemical Company (USA). Disodium hydrogen phosphate and sodium dihydrogen phosphate were obtained from Beijing chemical company (China), and acarbose was gained from Bayer Health Care Company (China).

Collection of plant material

The ripe fruits of *E. angustifolia* were obtained from Gu lang Town, Gansu Province in China. They were crushed to 0.38 mm before use.

Preparation of polysaccharide from E. angustifolia

The powdered *E. angustifolia* (0.5 kg) was soaked in 95% ethanol in a tightly closed round bottom flask at 85°C for 2 hours in order to get rid of fat. The residue was filtered through Buchner funnel and then soaked in 2 L of sterile double-distilled water in (ammonium sulfate/ polyethylene glycol) and further heated at 95°C in water bath for 2 hours to ensure maximum yield of water soluble compounds. The liquid extract was then cooled and concentrated by using a rotary evaporator at 80°C. The ethanol (80%) was poured into the extract and put in the 4°C for 24 hours and then concentrated at 4,000 rpm for 15 min (Chen et al., 2015). The extraction solution was deproteinized by the Sevag method (Pan, 2015) repeated three times. The activated carbon powder was used for decolorization and the operation was repeated several times until no color remains (Yu et al., 2017). The dialysis bags were used to remove small molecule impurities (including monosaccharides, oligosaccharides and some salt ions, etc). As listed above, 83.7% purified *E. angustifolia* polysaccharide was obtained.

Experiment animals

Adult male mice (22-27 g) were obtained from the animal facility of the biomedicine unit, Lanzhou University. All the animals were housed in standard conditions with 12/12 hours light-dark cycle at a temperature of 25 ± 5°C and a humidity of 35-60%. All of them could get tap water and rodent chow *ad libitum*.

Effect on blood glucose of normal mice

The male mice (n=40) were chosen and adapted to the experimental environment after a week. Then they were divided into 4 groups and each group had 10 mice. The normal control group were given an equal amount of citrate buffer whereas other three groups received *E. angustifolia* at three different concentrations (400, 600 and 800 mg/kg) for 1 week. After fasted for 3 hours in the last administration, the blood was taken from the tail and blood glucose meter was used to determinate blood glucose level.

Induction of diabetes

A total of 80 healthy male mice were selected to adapt to the experimental environment. A week later, 70 were randomly selected and injected with freshly prepared streptozotocin 150 mg/kg (streptozotocin dissolved with citrate buffer with pH 4.3). The remaining 10 healthy mice used as the normal control group, were injected with equal amount of saline. After 72 hours, blood glucose levels were measured. Mice with blood glucose levels ≥16.7 mmol/L were considered diabetic and were used for the study (Junod, 1967).

Experiment design

The mice were divided into six groups with 10 mice in each group. Group I, mice were administered intragastrically with 0.9% physiological saline for 28 days (normal control); Group II, the diabetic mice were administered intragastrically with 0.9% saline for 28 days (negative control); Group III, the control mice were administered intragastrically with acarbose for 28 days (positive control); Group IV, the diabetic mice were administered intragastrically with *E. angustifolia* at 400 mg/kg for 28 days; Group V, diabetic mice were administered intragastrically with *E. angustifolia* at 600 mg/kg for 28 days; Group VI, diabetic mice were administered intragastrically with *E. angustifolia* at 800 mg/kg for 28 days.

Effect on body weight, blood glucose and lipid levels

During the experimental process, the weight of the experimental mouse was measured once a week. After four weeks, the blood sampling was taken from the eyeball and then measured the blood glucose level, blood lipids including high density lipoprotein cholesterol (HDL-C), total cholesterol as well as triglyceride were measured (Xiong et al., 2013).

Statistical analysis

Results were expressed as mean ± standard deviation (SD). Data were analyzed by one-way analysis of variance (ANOVA) followed by Duncan's multiple-range test using the SPSS 17.0 statistical software program. Differences were considered significant at $p < 0.05$.

Results

Effect on blood glucose level in diabetic mice

Compared to the normal control group, the diabetic mice showed an obvious increase of blood glucose level over the completed period ($p < 0.05$; Table I). Results showed no significant difference among negative control group, positive control group and *E. angustifolia* treated groups in the first day. However, the blood glucose level of the positive control group and *E.*

Table I

Effect of *E. angustifolia* on blood glucose level of diabetic mice

Group	Blood glucose level (mmol/L)		
	0 week	2 weeks	4 weeks
Normal control	6.4 ± 0.7[b]	6.1 ± 1.0[d]	6.3 ± 0.1[e]
Negative control	20.7 ± 1.2[a]	22.6 ± 2.1[a]	24.3 ± 1.2[a]
Positive control	20.6 ± 0.9[a]	18.6 ± 1.5[c]	15.0 ± 1.1[d]
Extract (400 mg/kg)	21.2 ± 2.2[a]	20.5 ± 1.4[b]	18.1 ± 1.6[b]
Extract (600 mg/kg)	21.0 ± 1.8[a]	20.5 ± 1.5[b]	16.5 ± 1.6[c]
Extract (800 mg/kg)	21.8 ± 1.7[a]	19.9 ± 1.9[b]	15.8 ± 1.5[cd]

Values represent the mean ± standard error (n = 10). Means followed by different superscripts in the same column are significantly different at (p<0.05)

angustifolia treated groups were lower than that in the negative control group after two weeks of administration. The blood glucose level of the mice in the experimental group (except negative control group) continued to decrease, while negative control group showed an increasing trend. In the fourth week, the blood glucose level in *E. angustifolia* (800 mg/kg) group was 15.8 mmol/L, which is 34.8% lower than that of negative control group. Above all, blood glucose level of *E. angustifolia* (800 mg/kg) group showed no significant difference (p>0.05) with that of positive control in the fourth week. It can be seen that *E. angustifolia* (800 mg/kg) group had a better effect on inhibiting blood glucose level.

Effect on body weight in diabetic mice

As shown in Table II, the body weight of diabetic mice in negative control group, positive control group and *E. angustifolia* groups decreased in the first two weeks. In addition, the body weight in diabetic mice was lower than that of in normal control. After two weeks of administration, the body weight of the positive control group and *E. angustifolia* groups were increased gradually, instead, it showed a continuous increase in the negative control group. In the fourth week, the body weight of the negative control group continually

decreased which was the symptom of diabetes, however, the body weight of *E. angustifolia* (800 mg/kg) group was recovered to the began weight. These results indicated that *E. angustifolia* can alleviate the weight loss of diabetic mice.

Effects on blood lipid in diabetic mice

Due to abnormal metabolism of lipids, the accumulation of total cholesterol and triglyceride in diabetic patients increased, while HDL decreased
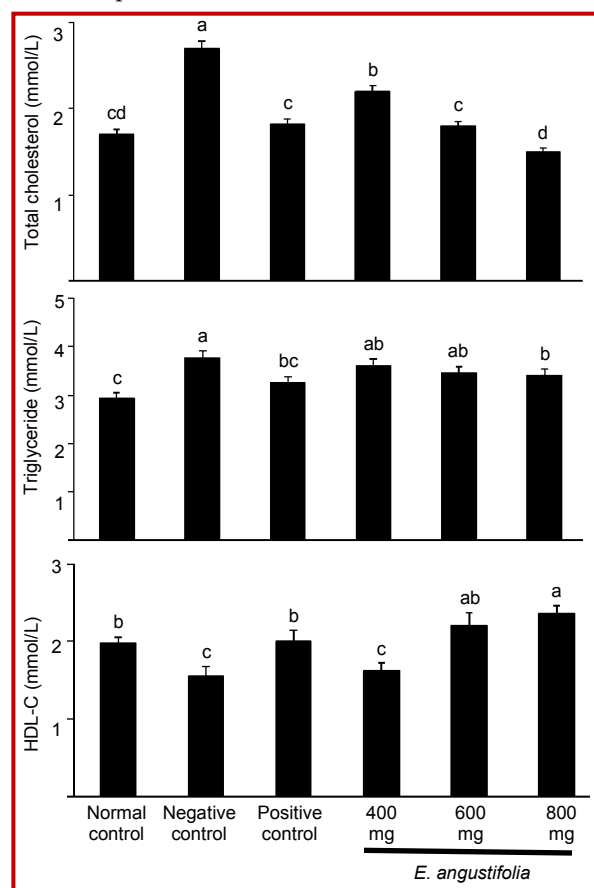

Figure 1: Effect of *E. angustifolia* on total cholesterol, triglyceride and HDL-C levels in diabetes mice. Different lowercase letters indicate significant differences between means (p<0.05)

Table II

Effect of *E. angustifolia* on body weight of diabetic mice

Group	Body weight (g)				
	0 week	1 week	2 weeks	3 weeks	4 weeks
Normal control	24.9 ± 1.5[ab]	28.4 ± 2.0[a]	32.2 ± 2.0[a]	36.2 ± 0.9[a]	39.4 ± 1.3[a]
Negative control	24.5 ± 1.8[ab]	21.6 ± 1.8[d]	19.9 ± 0.8[d]	17.1 ± 1.5[d]	16.9 ± 0.8[e]
Positive control	24.1 ± 1.5[a]	23.7 ± 1.8[bc]	24.0 ± 0.2[b]	25.3 ± 0.7[b]	26.5 ± 0.0[b]
Extract (400 mg/kg)	23.9 ± 1.1[b]	22.5 ± 2.1[cd]	21.2 ± 0.8[c]	23.7 ± 1.7[c]	23.0 ± 0.8[d]
Extract (600 mg/kg)	24.8 ± 1.7[ab]	23.4 ± 2.1[bc]	22.2 ± 1.2[c]	23.5 ± 0.5[c]	23.3 ± 0.8[cd]
Extract (800 mg/kg)	24.7 ± 1.8[ab]	23.8 ± 2.4[b]	23.2 ± 2.4[b]	23.6 ± 1.3[c]	24.1 ± 0.9[c]

Values represent the mean ± standard error (n = 10). Means followed by different superscripts in the same column are significantly different at (p<0.05)

(Wang et al., 2017a). The results in Figure 1 showed the effect of different doses of *E. angustifolia* on blood lipid levels of normal and diabetic male mice. The levels of total cholesterol and triglyceride negative control group were higher than those in other groups, and had the lower level of HDL-C which was a serious complication of diabetes mellitus. Compared with negative control group, the levels of total cholesterol and triglyceride in *E. angustifolia* (400, 600 and 800 mg/kg) groups were significantly lower. It is worth noting that total cholesterol in *E. angustifolia* (800 mg/kg) group was 1.5 mmol/L which was lower than 1.8 mmol/L in positive control group. The HDL-C was higher in *E. angustifolia* (800 mg/kg) group among all the treated groups. It is obvious that diabetes mellitus symptoms are effectively alleviated by *E. angustifolia* treatments.

Discussion

In the present study, polysaccharide from *E. angustifolia* fruit was purified. The diabetic mice showed an obvious increase of blood glucose compared with normal control mice. Similar results were reported with the polysaccharide from *Lilium lancifolium* (Zhang et al., 2014). This result was similar to Guo et al. (2017) who found that fasting blood glucose of PPSC group was significantly lower compared with negative control. In addition, 15.8 mmol/L glucose level in *E. angustifolia* (800 mg/kg) group is lower than that reported by Wang et al. (2017b) who noted the blood glucose in PSD -H group was 17.0 mmol/L. As known, streptozotocin can directly damage the pancreatic islet β-cells in mice, making it unable to normally secrete insulin thus cause the rise of blood glucose level. Results showed that *E. angustifolia*. fruit polysaccharide can significantly (p<0.05) reduce blood glucose in diabetic mice, it's presumably due to reducing the damage of streptozotocin to pancreatic β-cells and enhancing the secretion of islets.

The initial loss of body weight in streptozotocin-induced diabetic mice has explained by Jiang et al. (2013) who pointed out that the loss of body weight was consistent with the clinical symptom of patients with diabetes mellitus. The body weight in streptozotocin-induced diabetic mice was lower than that of normal control, it could be due to protein dehydration and fat metabolism (Guo et al., 2017). Our results showed that body weight in *E. angustifolia* groups was increased gradually which was in line with Pan et al. (2014) who reported that the weight gain was increased in DHP, DOP, DNP and high dosages DNP groups of alloxan-induced diabetic mice and Guo et al. (2017) who noted that the PPSC group showed a higher increase in body weight of alloxanised mice than negative control. The body weight in *E. angustifolia* (800 mg/kg) group was higher than that of in negative control group in the fourth week, it probably due to the improvement of

glucose uptake and utilization in cells thus reducing the consumption of tissue protein and fat (Guo et al., 2017).

Our results showed lower levels of total cholesterol and triglyceride as well as higher HDL level in *E. angustifolia* groups compared with negative control group. Similarly, Fu et al. (2012) reported that ASP group could raise the ratio of HDL/total cholesterol and reduce the total cholesterol, triglyceride levels, especially at the dose of 200 mg/mL. However, Wu et al. (2015) found that total cholesterol of *Talinum triangulare* polysaccharides-treated streptozotocin-induced diabetic mice showed no significant difference compared with negative control (p>0.05) and higher than positive control. It is worth mentioning that total cholesterol in *E. angustifolia* (800 mg/kg) group was lower than that of in positive control group. This found was in line with Pan et al. (2017) who noted that 800 mg/kg corn silk poly-saccharides showed lower total cholesterol than that of in positive control group.

Conclusion

E. angustifolia fruit polysaccharide has hypolipidemic effect presented in a significant reduction in total cholesterol, triglyceride and an improvement in HDL-C of diabetic mice.

Ethical Issue

All experimental process of animals were approved by the Ethics Committee for Animal Experimentation of the University, and carried out in accordance with the Regulations of Experimental Animal Administration issued by the State Committee of Science and Technology of People's Republic of China.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Acknowledgement

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