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The study of ethanol extract of *Epilobium angustifolium* L. on blood sugar level in type II diabetic rats

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Abstract: *Epilobium angustifolium* (EA) is well known as a traditional medicinal plant in many countries with multiple health effects. However, the chemical composition and anti-diabetic effect of EA has not been reported. In our study, the composition and anti-diabetic effects of ethanol extracts from EA *in vivo* and in streptozotocin (STZ)-induced type II diabetic rats were investigated. EA ethanol extracts exhibited protection effect on H_2O_2 induced oxidative stress damage INS-1 cells, reduce the body weight loss, blood glucose level and increase insulin level when compared with those of diabetic rats. Following 21 days of EA treatment at 9.2 and 18.4mg/kg, BW increased by 15.85% and 15.53%, respectively, which were extremely higher than diabetic group (9.50%). The fasting blood glucose level of EA 9.2mg/kg group rats significantly decreased by 60.43% and insulin level increased by 2.78 times, respectively. Corresponding to that, the fasting blood glucose level of EA 18.4mg/kg group rats decreased by 52.61% and insulin level increased by 2 times, respectively. Collectively our data suggest that ethanol extract of EA has remarkably hypoglycemic effect in type 2 diabetes and EA might be a promising functional food or medicine for T2DM treatment.

Keywords: *Epilobium angustifolium*, hypoglycemic, ethanol extract.

INTRODUCTION

Epilobium angustifolium (EA), also known as *Chamerion angustifolium*, is a perennial herbaceous plant in the willow herb family Onagraceae (Fleming, 1998) (fig. 1). It is native throughout the temperate Northern Hemisphere and widely distributed in Europe, Northern Asia and North America, such as Italy, Belgium, Britain, Austria, China, Russia, Canada, United States and so on (Fleming, 1998; Vitalone *et al*., 2001; Blaschek *et al*., 2007; Vitalone *et al*., 2003). EA is well known as a traditional medicinal plant in folk medicine for treating benign prostate hyperplasia (Wichtl, 2004), acute and general prostatitis (Vitalone *et al*., 2003), kidney problems and urinary tract diseases (Vogl *et al*., 2013), skin burns (Piterskaya, 2012), mouth ulcers and persistent coughs (Fleming, 1998; Granica *et al*., 2014). Moreover, EA is also commonly referred to as tea and used for the treatment of sleeping disorders, gastritis and stomach ulceration in Russia and treatment of disorders of the prostate, kidneys and urinary tract in Austria (Vitalone *et al*., 2003). While American Indians have used it for skin infections and rectal bleeding (Lebeda *et al*., 2004), in China, willow herb has been documented as relief of multiple diseases such as menstrual disorders, diarrhea, gastric fever, liver-gallbladder disease, joint sprain, cold damp and hot in the traditional medicine books or local medicinal plants records (Da-shan, 1997; Du, 2006).

Aqueous extracts from EA have been ascribed a variety of beneficial properties, including analgesic (Battinelli *et al*., 2001), anti-inflammatory (Juan *et al*., 1988; Vogl *et al*., 2013), anti-fungal (Jones *et al*., 2000), anti-microbial (Battinelli *et al*., 2001; Kremer *et al*., 2013), anti-proliferative (Kiss *et al*., 2006; Stolarczyk *et al*., 2013; Vitalone *et al*., 2001; Vitalone *et al*., 2003), anti-exudative, anti-oxidative (Shikov *et al*., 2006; Stajner *et al*., 2007; Onar, 2012) and anti-androgenic activities (Hiermann *et al*., 1986). It has been confirmed to be rich in the flavonoid quercetin-3-O-D-glucuronide and myricetin 3-O-glucuronide which were strongly activity against in the paw edema of rat (Hiermann *et al*., 1998), and the effect of non-steroidal analgesics was similar to that of lysine acetylsalicylate (Slacanin *et al*., 1991). Trolox and ascorbic acid with high radical scavenging activity are highly abundant in the extracts of EA in comparison to most of well-known antioxidants (Toth *et al*., 2009). Especially during the massive blooming stage, EA has been proved highest amount of flavonoids and radical scavenging activity with optimal extraction rate of methanol and water (75/25 v/v, %)(Maruška *et al*., 2014).

Recent investigations on the cytotoxic and genotoxic effects of EA extract have revealed that high concentration of EA extracts has strong inhibition on the cell viability and DNA damage of hepatocellular carcinoma (Oleshchuk *et al*., 2017). For example, Oenothein B, a polyphenol isolated from EA shown stimulation on bovine and human T cells and endopeptidase in prostate cancer cells, inhibition on the proliferation of prostate cells and hyaluronidase and lipoxygenase (Kiss *et al*., 2011; Ramstead *et al*., 2015; Ramstead *et al*., 2012). Furthermore, previous studies

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have demonstrated that EA extracts can activate phagocyte functional responses and inhibit the activity of specific metallopeptidases due to oenothein B (Schepetkin *et al*., 2009; Kiss *et al*., 2004).

Fig. 1: The *Epilobium angustifolium* plant.

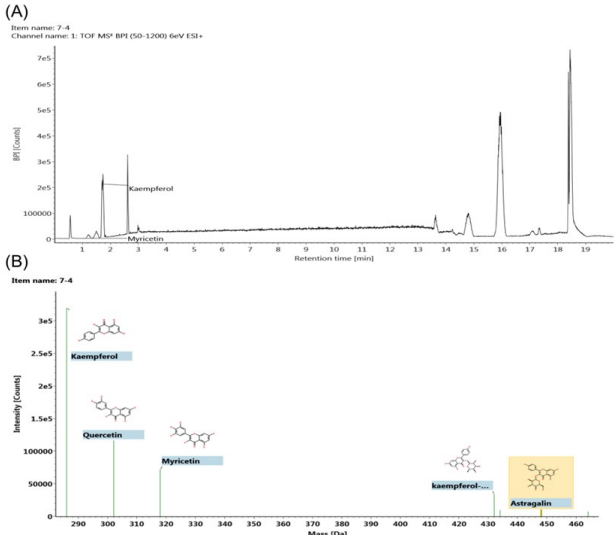

Fig. 2: UPLC-QTof-MS BPI chromatogram of ethanol phase.

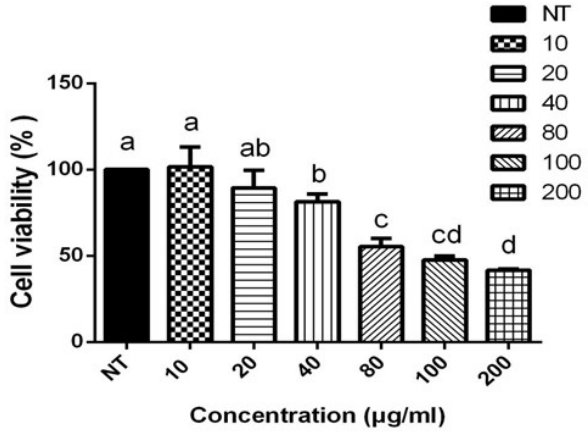

Fig. 3: Cell viability of INS-1 cells treated by 70% ethanol extract of *Epilobium angustifolium* in different concentration. All data are expressed as means ± SD. Different letters indicate significant differences ($p<0.05$). All measurements were run in triplicate (n=3).

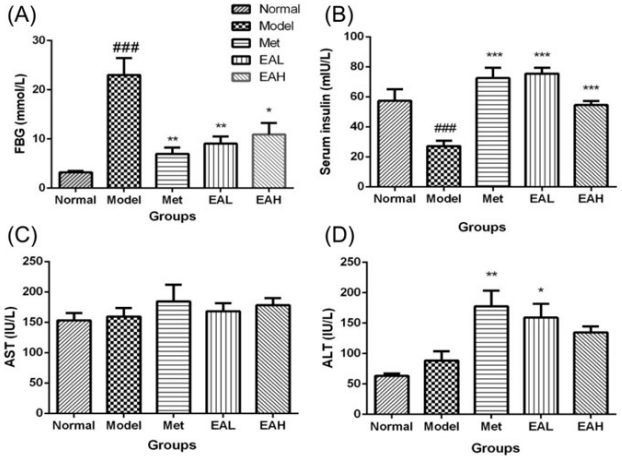

Fig. 4: Effects of 70% ethanol extract of *Epilobium angustifolium* on blood parameters in comparison to STZ induced diabetic rat. (a) FBG, fasting blood glucose; (b) Serum insulin; (c) AST, aspartate aminotransferase; (d) ALT, alanine aminotransferase. All group rats were sacrificed after 4 weeks of treatment. Model: STZ-treated; Met: Metformin; EAL: *Epilobium angustifolium* low concentration (9.2 mg/kg body weight); EAH: *Epilobium angustifolium* high concentration (18.4 mg/kg body weight); The results were expressed as Mean±SD, n=10. Statistically significant difference between groups: $^{###}P<0.001$ vs Normal control; $*P<0.05$, $**P<0.01$, $***P<0.001$ vs model group.

Although EA was used in traditional antidiabetic formulations of Russia, Belarus and Ukraine (Sharafetdinov *et al*., 2017), the composition of the formulation and its chemical constituents and bioactive substances have not been reported. The main purpose of this paper was to analyze the chemical components of the EA ethanol extract and its hypoglycemic effect. To achieve this purpose, UPLC-QTOF-MS was used to analyze the chemical components and streptozotocin (STZ)-induced type II diabetic rats model was used to investigate the hypoglycemic effect. Our study revealed that the ethanol extract of EA exhibited protection effect on H_2O_2 induced oxidative stress damage INS-1 cells, reduce the body weight loss, blood glucose level and increase insulin level when compared with those of diabetic rats. Our data suggest that ethanol extract of EA has remarkably hypoglycemic effect in type 2 diabetes and EA might be a promising functional food or medicine for the treatment of T2DM.

MATERIALS AND METHODS

Plant materials, chemicals and equipments

The whole plants of *Epilobium angustifolium* (EA) was harvested from Xilingol grassland of China. It was

identified by botanists of Inner Mongolia University. Streptozotocin (STZ) was purchased from Sigma-Aldrich, USA. Metformin hydrochloride (tablets) was purchased from Jiangsu Suzhong Pharmaceutical Co., Ltd. (Jiangsu, China). UPLC/QTof (Waters, USA) used in this study to analyze the chemical composition of EA water extract. HPLC grade acetonitrile, leucine enkephalin and formic acid solution was purchased from Fisher Scientific, Waters and Sigma-Aldrich, USA, respectively. The organic solvents, such as anhydrous ethanol, petroleum, *n*-butanol and ethyl acetate, were purchased from the chemical reagent company (Tianjin, China). Water for HPLC using was produced by Milli-Q water instrument (Massachusetts, United States).

EA ethanol extract preparation

EA plants dried to constant weight were pulverized in a multifunction grider (GX-25, Zhejiang, China) and stored at 4°C condition. Accurately weigh the 0.1g EA powder sample and put it into a 1.5 mL centrifuge tube, 1.0mL of 70% ethanol was added, room temperature sonicated (40 kHz,150W) for 30min, centrifugation was performed at 2000×g for 10 min, then the solvent was removed in a nitrogen stream and finally the sample was put into a 4°C condition for later use.

UPLC-QTof-MS analysis

Test conditions as previously reported by our laboratory (Saren *et al*., 2018), in short, the condition of chromatographic analysis were as follows: A Vanguard HSS T3 guard column was connected by the column (ACQUITY BEH Shield RP18, 2.1×100mm Column, 1.7 μm) at the temperature of 40°C. Water with 0.1% formic acid was used as mobile phase A, and acetonitrile with 0.1% formic acid was used as mobile phase B. The time and concentration of gradient elution of mobile phase were as follows: 75%→80% B, 0→1.5 min; 80% B, 1.5→11 min; 80%→100% B, 11→13 min; 100% B, 13→17 min; 100%→80% B, 17→17.1 min; 80%→75% B, 17.1→20 min; and the flow rate of mobile phase was 0.4mL/min. The conditions of mass spectrometry was ESI positive ion mode, and the mass detection range was from 100 to 1200 Da. The capillary voltage was 3Kv, the sample cone was 40V and the extraction cone was 4V. The temperature of source was 100°C and that of desolvation was 400°C. The desolvation gas, lock mass, and accuracy error threshold were 800L/h, 556.2766 (positive ion mode) and 5mDa, respectively. MassLynx 4.2 was using for data collection. According to the chemical composition report of EA and the collected chemical composition information, "Chemdraw" software was used to draw the chemical composition structure, and A database of theoretical relative molecular mass and the molecular formula were established.

Animals

Male Wistar rats in adult weighing about 210-235g were purchased from the Experimental Animal Center (Hohhot, China). Animals were domesticated in laboratory conditions for seven days before the experiment. The rats were fed at standard temperature (22±1°C) and humidity (50±5%) alternating 12-hour light/dark cycles. The animals got standard pellet of food and tap water for free. All animal experiments involved in this research were carried out according to the guidelines of the Animal Care and Use Committee of Inner Mongolia University (NO. SYXK2014-0002). We try our best to reduce the number of animals used in experiments and to relieve the pain of animals in the process of experiments.

Rat model of diabetes mellitus

Citrate buffer with concentration of 0.1M and pH of 4.5 was used to prepare fresh STZ for animal injection. Prior to the use of STZ injections, animals fasted for 12h and were provided with only free drinking water. STZ with dose of 45mg/kg (body weight) was intraperitoneally injected into Wistar rats and the control group was replaced with citrate buffer. The level of fasting blood glucose (FBG) in rats which was higher than 11.1 mmol/L after 72 h of injection were identified as Type II diabetes mellitus (T2DM) for follow-up studies.

Experimental design

EA sample was extracted twice with 20 times volume (A 30-g sample of the EA was extracted twice with 600mL) 70% ethanol (at 50°C) for duration of 4h each time. The solid residue was removed by centrifugation and the extract was kept at 4°C after vacuum freeze drying. Fifty animals were divided into 5 groups, 10 rats in each group, and the grouping method was random. The first two groups were treated as normal control (PBS only as vector) and diabetic model group (STZ treatment) respectively. Animals in the third group were given metformin orally (26mg/100g body weight) and animals in the other groups were EAL (EA low concentration, 9.2 mg/kg body weight) and EAH (EA high concentration, 18.4mg/kg body weight) which was given EA for 21 days after STZ administration. All groups were on the regular pellet diet throughout the study. Body weight was monitored daily during the experiment.

Sample collection and H&E staining

On day 22 (after diabetes), the animals were sacrificed by an aesthesia (chloroform) after a night fasting. Cardiac puncture was used for blood samples collection. And the blood was coagulated at room temperature for 30 min, then centrifuged at 3000×g for 10min. Finally, the supernatant was taken to obtain the serum. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels in rat serum were then measured by a special biological kit (Biosino Bio-Technology and

Table 1: Compounds identified in ethanol extract of *Epilobium angustifolium* by UPLC-QTof-MS.

Component name	Formula	Neutral mass (Da)	Observed neutral mass (Da)	Observed m/z	Mass error (mDa)	Observed RT (min)	Adducts
Astragalin	$C_{21}H_{20}O_{11}$	448.1006	448.0997	471.0889	-0.9	1.53	+Na, +K
Guajavarin	$C_{20}H_{18}O_{11}$	434.0849	434.0843	457.0735	-0.6	1.46	+Na, +H, +K
Isoquercetin	$C_{21}H_{20}O_{12}$	464.0955	464.0954	487.0846	-0.1	1.24	+Na, +H, +K
Kaempferol	$C_{15}H_{10}O_6$	286.0477	286.0479	287.0551	0.1	1.72	+H, +Na
Kaempferol-3-O-rhamnoside	$C_{21}H_{20}O_{10}$	432.1056	432.1050	455.0942	-0.6	1.73	+Na, +H, +K
Myricetin	$C_{15}H_{10}O_8$	318.0376	318.0381	319.0454	0.6	1.01	+H
Quercetin	$C_{15}H_{10}O_7$	302.0427	302.0431	303.0503	0.4	1.50	+H

Table 2: Characterization of flavonoids of *Epilobium angustifolium.*

No.	Component name	Chemical formula	Reference
1	1,2,3,4,6-O-galloyl-glucoside	$C_{41}H_{32}O_{26}$	(Hofmann *et al*., 2016)
2	3-O-methylquercetin O-arabinoside	$C_{21}H_{20}O_{11}$	(Howard and Mabry, 1970)
3	Annulatin O-arabinoside	$C_{21}H_{20}O_{12}$	(Howard and Mabry, 1970)
4	Annulatin O-glucoside	$C_{22}H_{21}O_{13}$	(Howard and Mabry, 1970)
5	Annulatin O-rhamnoside	$C_{22}H_{22}O_{12}$	(Howard and Mabry, 1970)
6	Astragalin	$C_{21}H_{20}O_{11}$	ND
7	Digalloylrhamnoside	$C_{20}H_{20}O_{15}$	(Hofmann *et al*., 2016)
8	Guajavarin	$C_{20}H_{18}O_{11}$	ND
9	Isoquercetin	$C_{21}H_{20}O_{12}$	ND
10	Kaempferol	$C_{15}H_{10}O_6$	ND
11	Kaempferol 3-O-arabinoside	$C_{20}H_{18}O_{10}$	(Kachlicki *et al*., 2008)
12	Kaempferol 3-O-glucoside	$C_{21}H_{20}O_{11}$	(Kachlicki *et al*., 2008)
13	Kaempferol 7-O-glucuronide	$C_{21}H_{18}O_{12}$	(Nakanishi *et al*., 2007)
14	Kaempferol 7-O-p-coumaroylglucoside	$C_{30}H_{26}O_{13}$	(Piasecka *et al*., 2015)
15	Kaempferol 7-O-rhamnoside	$C_{21}H_{20}O_{10}$	(Kachlicki *et al*., 2008)
16	Kaempferol-3-O-rhamnoside	$C_{21}H_{20}O_{10}$	ND
17	Myricetin	$C_{15}H_{10}O_8$	ND
18	Myricetin 3-O-rhamnoside	$C_{20}H_{16}O_{13}$	(Kachlicki *et al*., 2008)
19	Myricetin 7-O-glucoside	$C_{21}H_{20}O_{13}$	(Kachlicki *et al*., 2008)
20	Myricetin-3-O-arabinoside	$C_{20}H_{18}O_{12}$	(Kachlicki *et al*., 2008)
21	Myricetin-3-O-caffeoyl-glucoside	$C_{30}H_{26}O_{16}$	(Piasecka *et al*., 2015)
22	Quercetin	$C_{15}H_{10}O_7$	ND
23	Quercetin 3-O-arabinoside	$C_{20}H_{18}O_{11}$	(Kachlicki *et al*., 2008)
24	Quercetin 7-O-glucoside	$C_{21}H_{20}O_{12}$	(Kachlicki *et al*., 2008)
25	Quercetin 7-O-rhamnoside	$C_{21}H_{20}O_{11}$	(Kachlicki *et al*., 2008)
26	Quercetin glucuronide	$C_{21}H_{18}O_{13}$	(Nakanishi *et al*., 2007)
27	Quercetin-3-O-feruloylglucoside	$C_{31}H_{28}O_{15}$	(Piasecka *et al*., 2015)
28	Quercetin-7-O-[galloyl]-glucoside	$C_{28}H_{24}O_{16}$	(Kachlicki *et al*., 2008)
29	Quercetin-7-O-caffeoylglucoside	$C_{30}H_{26}O_{15}$	(Piasecka *et al*., 2015)
30	Quercetin-7-O-p-coumaroylglucoside	$C_{30}H_{26}O_{14}$	(Piasecka *et al*., 2015)
31	Quercetin-O-[digalloyl]-glucoside	$C_{35}H_{28}O_{20}$	(Piasecka *et al*., 2015)

Table 3: Effects of 70% ethanol extract of *Epilobium angustifolium* on body weight in STZ induced diabetic rat.

Days	Normal	Model	Met	EAL	EAH
0*	255.17 ± 2.10^{Ad}	217.50 ± 4.30^{Cb}	239.83 ± 5.71^{Bc}	237.67 ± 2.86^{Bb}	235.00 ± 1.05^{Bb}
7	273.67 ± 2.46^{Ac}	225.50 ± 2.69^{Cab}	260.83 ± 5.29^{ABb}	245.00 ± 3.57^{BCb}	241.50 ± 1.07^{BCb}
14	292.50 ± 1.35^{Ab}	234.67 ± 3.18^{Cab}	272.67 ± 5.40^{ABa}	260.00 ± 6.35^{BCab}	256.33 ± 3.56^{BCab}
21	307.50 ± 1.97^{Aa}	238.17 ± 3.62^{Ca}	281.00 ± 6.29^{Ba}	275.33 ± 7.59^{Ba}	271.50 ± 4.53^{Ba}
WG%	20.51 ± 0.63^{A}	9.50 ± 0.61^{C}	17.16 ± 0.97^{B}	15.85 ± 1.92^{B}	15.53 ± 1.37^{B}

WG%: Weight gain rate (%). Normal: Normal control; Model: STZ-treated; Met: Metformin; EAL: *Epilobium angustifolium* low concentration (9.2mg/kg body weight); EAH: *Epilobium angustifolium* high concentration (18.4mg/kg body weight). Values indicate mean ± stand error of the mean (SD) of more than 8 rat per group. *0: The body weights of rat in the 0 row referred to the beginning body weights of rat after the T2DM development and did not refer to the initial body weight. Different lowercase letters in the same column indicate significant differences among the four weeks treatment for single group ($p<0.05$). Different capital letters in the same row indicate significant differences between five groups ($p<0.05$).

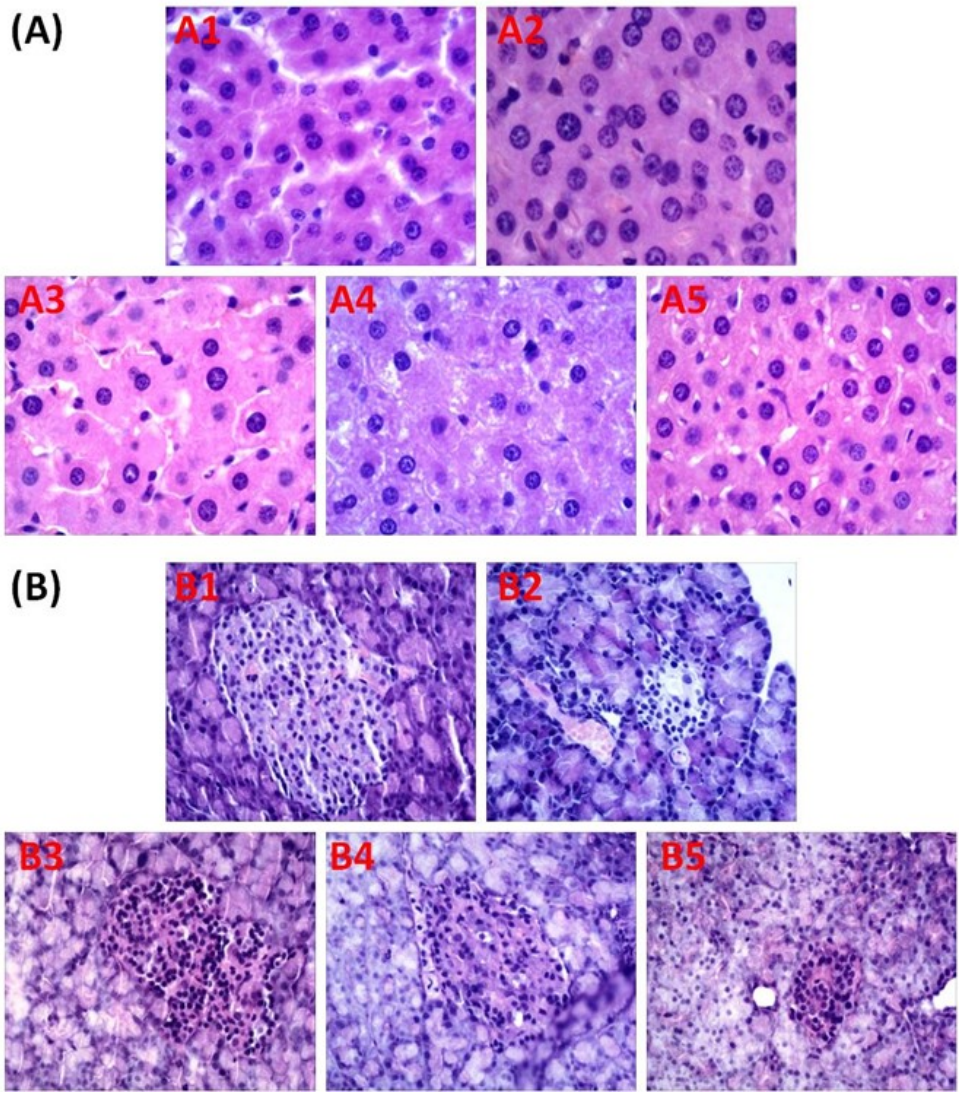

Fig. 5: Effects of 70% ethanol extract of *Epilobium angustifolium* on liver and pancreas in comparison to STZ induced diabetic rat. (A) Liver (H&E stain, 400×): A1 (normal control); A2 (Model, STZ-treated); A3 (Metformin control); A4 (*Epilobium angustifolium* low concentration 9.2mg/kg); A5 (*Epilobium angustifolium* high concentration 18.4mg/kg). (B) Pancreas (H&E stain, 200×): B1 (normal control); B2 (Model, STZ-treated); B3 (Metformin control); B4 (*Epilobium angustifolium* low concentration 9.2mg/kg); B5 (*Epilobium angustifolium* high concentration 18.4 mg/kg).The red arrow indicates the location of the islets.

Science Inc., Beijing, China). An ACCU-CHECK instrument (Roche, China) was used to measure blood glucose level. Rat insulin concentration was determined according to the method described in the ELISA kit which was provided by Nanjing Jiancheng Bioengineering Research Institute (Nanjing, China).

In histopathological analysis, the liver and pancreas were removed and fixed in 4% paraformaldehyde (PFA) fixative, followed by 5μm thick paraffin sections and staining for microscopic examination. Tissue samples and rat serum were preserved at -80°C for subsequent analysis.

Cell culture and cell viability measurement

The INS-1 cells required for this experiment were provided by a biological company of Shanghai (Zhong Qiao Xin Zhou Biotechnology Co., Ltd, Shanghai, China). The INS-1 cells were cultured in RPMI-1640 at 37°C and containing 5% CO_2. In addition, the cell culture environment also included the following components: 100 U/mL penicillin, 50μmol/L β-mercaptoethanol (Beijing Biotopped Science & Technology Co., Ltd, Beijing, China), 100μg/mL streptomycin (Life Technology, Carlsbad, CA, USA) and 10% fetal bovine serum (Hyclone). INS-1 cells were inoculated in a 96-well plate at a density of 2.0×10^4 cells per well. After incubation for

24 h, 70% ethanol extract of EA was finally added to cell concentrations of 10, 20, 40, 80, 100 and 200μg/mL. Another 24 h later, MTT assay kit was used to determine the cell viability.

Data processing and analysis

The unpaired t-test or the single factor analysis of variance (ANOVA) and Dunnett multiple comparison test were used to determine the significant difference. Statistical significance was considered at the 95% confidence level ($P<0.05$) and 99% confidence level ($P<0.01$). All results were represented by the mean ± SD. GraphPad Prism 8.0.2 software was used for data processing and statistical analysis.

RESULTS

Chemical compounds and characterization

The whole plant of EA was dried at 70°C to constant weight, and then crushed and extracted twice with ethanol. Analysis by UPLC-QTof-MS revealed multiple components in the extract, which were established via contrasting the molecular formula and fragmentation patterns with documented data in the literature and 7 compounds were identified (fig. 2, table 1), including quercetin, kaempferol, kaempferol-3-O-rhamnoside, guajavarin, myricetin. In this study, the chemical component of EA ethanol extract was identified as flavonoids and the identified results were compared with the flavonoids of EA extract reported in the literature as shown in table 2. Astragalin, kaempferol, myricetin and quercetin were first report in our study.

Cytotoxicity test

To assess possible cytotoxicity of ethanol extract of EA, INS-1 cells (pancreatic -cell line) were treated with enhanced EA extracts and their viability was measured by MTT assay. The results showed that when the concentration of EA was 10, 20 and 40μg/ml, the cell viability was higher than 80% (fig. 3), indicating that the concentration of EA treatment ranged from 10 to 40 μg/ml without obvious cytotoxicity to INS-1 cell during the period of incubation. However, when EA concentration was higher than 80% μg/ml, the cell viability was less than 60% and the cell viability was less than 50% when treated with EA at 100 and 200μg/ml (fig. 3), indicating that high concentration (≥80μg/ml) of EA could induce cytotoxicity in INS-1 cells.

Evaluation of hypoglycemic effect

The body weight (BW) gain rate in model group after 4 weeks was only 9.50%, which was extremely less than that of normal control group (20.51%) ($p<0.05$) (table 3). Treatment with metformin resulted in a 17.16% increase in BW. Following 21 days of EA treatment at 9.2 and 18.4mg/kg, BW was increased by 15.85% and 15.53 %, respectively (table 3). Compared with the model group, BW increased significantly after 4 weeks in EA group, but there was no obvious difference in the gain of BW between EAL (9.2mg/kg), EAH (18.4mg/kg) and the positive control drug metformin group (15.85%, 15.53% vs. 17.16%) (table 3). In this research, the fasting blood glucose value (FBG) test showed that the value of model group was dramatically higher than that of normal group after STZ injection ($p<0.01$, fig. 4a). Compared with the model group, the FBG decreased significantly after EA intake for 4 weeks (EAL decreased by 60.43%, EAH decreased by 52.61%, $p<0.01$), and especially EAL with 9.2mg/kg EA extracts could be as effective as positive control metformin (decreased by 69.56%). EAL (dose: 9.2mg/kg) group exhibited a lower FBG value than EAH at dose 18.4mg/kg group. Serum insulin levels were also quantified in different treatment groups, the results showed that serum insulin level in model group was markedly less than that in normal group ($p<0.01$), and EA group showed an extremely significant increase in insulin level (EAL increased by 2.78 times, EAH increased by 2 times, respectively, $p<0.01$) compared with the model group (fig. 4b). As can be seen from fig. 4c, there was no significant difference in AST levels in serum among normal group, model group, metformin group, EAL group and EAH group (fig. 4c). However, compared with the model group, the serum ALT level in EAL and metformin treatment groups were abnormally higher ($p<0.05$) (fig. 4d). In addition, research on the pathological changes of the liver and pancreas, macrovesicular cell increase and hepatocellular degeneration were obviously found in model group rats liver (fig. 5A2), but no significant change in metformin treatment group (fig. 5 A3) and EA treatment group (fig. 5 A4, A5), especially the number of macrovesicular cell in EAL and metformin group even lower than the normal group. It was shown in fig. 5B, the pancreas of rats in normal group showed normal islets (fig. 5B1). Islets destruction and reduction in the number was obvious in model group and EAH group (Model group fig. 5B2, EAH fig. 5B5) but no significant change in metformin (fig. 5B3) and EAL group (fig. 5B4).

DISCUSSION

Chemical composition

These compounds in Table 1 may be the bioactive molecules of EA, because previous studies have demonstrated that they have significant anti-oxidant activities in reducing, ABTS radical scavenging, DPPH radical scavenging, and high-lipid peroxidation inhibitory activity (Annabella and Olta, 2018; Granica *et al*., 2014; Kaškonienė *et al*., 2015; Kiss *et al*., 2004; Maruska *et al*., 2014). These components of ethanol extracts could improve the prooxidant/antioxidant balance, elevate

antioxidant levels, lower the levels of free radicals and then suppress insulin resistance which were damaged by hyperglycemia (Aragno *et al*., 2004). Oxidative stress is considered to play a crucial part in the occurrence and development of diabetes mellitus (Phamhuy *et al*., 2008; Saha and Mazumder, 2013), and various risk factors of diabetes mellitus can induce oxidative stress reaction which further leads to pancreatic cell damage and insulin secretion dysfunction (Zheng *et al*., 2015). The glycosides, such as annulatin O-glucoside, kaempferol 3-O-glucoside, myricetin 7-O-glucoside, and quercetin 7-O-glucoside in Table 2 were reported in the literature (Howard and Mabry, 1970; Kachlicki *et al*., 2008). Although the Kaempferol-3-o-rhamnoside was our first discovery, the kaempferol-7-o-rhamnoside which was very similar has been detected by Kachlicki (Kachlicki *et al*., 2008). Guajavarin and isoquercetin were the first to be found in EA extract.

EA treatment improves symptoms in diabetic mice

BW loss is one of the typical features of diabetes induced by STZ and high-fat diet(Chen *et al*., 2018; Sezik *et al*., 2005) and the regulation of BW has a large influence on the improvement of insulin sensitivity. As shown in Table 3, EA treatment could alleviate the body weight loss of diabetic rats as well as metformin, and thereby might improve the quality of life. Hyperglycemia is considered to be another important characteristic of diabetic rats and FBG is an important parameter of diabetes alleviation (Man *et al*., 2016). As shown in Fig 4a, FBG value in model group was significantly higher than that in normal group, which might be caused by impaired insulin secretion induced by STZ (Li *et al*., 2015; Wang *et al*., 2016). EA treatment can significantly reduce FBG value, but the bioavailability of high dose EA was not linearly higher than those of low dose. Insulin resistance is another important feature of diabetes except for hyperglycemia, which is closely related to insulin secretion disorder and hyperglycemia caused by STZ injection (Wang *et al*., 2017). In our study, the results of serum insulin level of model group showed that it might be directly related to pancreatic damage. However, EA significantly blocked the trend of insulin level decline, and it might be caused by the recovery of the pancreatic function (fig. 4b). In particular, the serum insulin level of EAL group was even higher than that of normal control group, further indicating that low-concentration EA enhanced the insulin secretion function of the pancreas. Furthermore, AST and ALT levels of serum are usually used as biomarkers to indicate liver health. AST and ALT results in this study suggest that EA might be slightly toxic to the liver, which was consistent with the results of cell viability assay (fig. 3), but much less toxic than metformin (fig. 4d). It indicated that low-concentration EA could prevent islet destruction in rats with diabetes and play a protective role on islets. According to the results of previous literature studies, the protective function of liver and pancreas may be closely related to the rich manganese in EA (FIREWEED, 2018; Kazi *et al*., 2008). The protective effect of EA in the liver may be due to the fact that manganese normalize the secretion and synthesis of insulin, thereby regulating the unpredictable drop of blood glucose, and finally restoring the normal life of diabetic rats.

Sustainability analysis

In this paper, the chemical composition of the ethanol extract of EA were analyzed by UPLC-QTOF-MS and 7 flavonoid compounds were determined by comparing molecular formulas and fragment patterns with those in the literature, but the chemical structures of these components were not identified by NMR. At the same time, the bioactive components of each component and their contribution to the reduction of blood glucose will be the main content of our next research.

CONCLUSION

In this paper, the hypoglycemic effects of ethanol extract from *Epilobium angustifolium* (EA) *in vitro* and in STZ induced diabetic rats were investigated for the first time. The study demonstrated that the main chemical constituents of EA ethanol extracts were sterols, flavonoids and fatty acids. Cell activity test results showed that EA had a protective effect on the oxidative stress induced by H_2O_2 in INS-1 cells, and its toxicity was far lower than that of metformin. The results of hypoglycemic effect test showed that EA ethanol extracts could significantly ameliorate the body weight loss and blood glucose level of diabetic rats, thus significantly improves the hyperglycemia status. EA ethanol extracts significantly reduced insulin resistance in diabetic rats, and the AST and ALT levels were extremely increased in liver tissue. In addition, histological evaluation displayed that EA ethanol extracts could protect liver and pancreas from damage and dysfunction, and could have hypoglycemic effect on the prevention and treatment of diabetes. In summary, EA can be used as a potential candidate drug for T2DM treatment or as a functional food for T2DM prevention.

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