https://doi.org/10.46341/PI2022008 UDC 581.524.1

RESEARCH ARTICLE

Hypoglycemic effect of polysaccharides isolated from shoots of *Actinidia* arguta

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Received: 14.03.2022 | Accepted: 13.07.2022 | Published online: 01.08.2022

Abstract

Diabetes mellitus can result in hyperglycemia caused by insufficient insulin secretion or insulin resistance. As such, plant extracts that exert a hypoglycemic effect with limited side effects are of interest to the medical and healthcare fields. The hypoglycemic effect of polysaccharides extracted from the branches of *Actinidia arguta* was explored in mice in this study. Sixty male Kunming mice were subsequently randomly assigned to one of six groups. The body weight, fasting blood glucose level, serum lipids, and oxidative stress parameters were assessed weekly during the 28-day study period. Pancreatic tissue from sacrificed mice was harvested at the end of the study and dissected for analysis. Polysaccharide AABP3 prevented body weight loss and decreased the fasting blood glucose level in diabetic mice compared with control mice. It also had a beneficial effect on serum dyslipidemia and oxidative stress parameters and was comparable in its protective effect to metformin. Histopathological examination of the pancreas revealed that AABP3 could protect and ameliorate pancreatic damage that may occur in diabetes mellitus in mice. AABP3 may be considered a potential candidate for developing a functional food or natural product for treating diabetes and its complications.

Keywords: Actinidia arguta, polysaccharides, hypoglycemic effect, diabetes mellitus, streptozotocin

Authors' contributions: Qiang Niu and Jian Shen for Co-author wrote the manuscript and performed the experiments. Lili Li, Huiming Zhang and Nadiia Skrypchenko analyzed and interpreted data, created figure. Honglai Sun performed the animal experiments. Dejiang Liu, Lihong Wang and Natalia Zaimenko designed the experiments, revised and proved the manuscript.

Funding: This work was supported by the central government supports the reform and development fund of local colleges and universities for Outstanding Young Talents Project (Grant Nr 2020YQ09).

Competing Interests: The authors have no conflicts of interest to declare.

Statement of ethics: Sixty male Kunming mice (weighing 20 ± 2 g) were purchased from Yanbian University. The license number is SCXK (Ji) 2017-0003.

Introduction

Diabetes mellitus (DM) is a group of metabolic diseases characterized by hyperglycemia

caused by insufficient insulin secretion, insulin resistance, or both (Wang et al., 2017). Chronic hyperglycemia in diabetic patients can result in damage to the eyes, kidneys, heart, blood vessels, and other organs, leading to dysfunction (Ye et al., 2019). According to the different pathogeneses of diabetes, the condition can be divided into four main types: type I diabetes, type II diabetes, gestational diabetes, and secondary diabetes. Among them, type II diabetes accounts for about 90% of diabetes cases (Stumvoll et al., 2005; Kahn et al., 2006). Among patients suffering with type II diabetes, insulin may be secreted but the quantity is relatively insufficient due to insulin resistance, meaning the secreted insulin cannot play an effective role and an increase in blood glucose results. Because patients with this kind of diabetes can produce insulin themselves, they can control their blood sugar through diet or oral hypoglycemic drugs without requiring insulin treatment & Nauck, 2006; Ridderstrale (Drucker & Groop, 2009). At present, insulin and some oral hypoglycemic drugs are mainly used in the clinical treatment of diabetes, including biguanides, thiazolidinediones, and sulfonylureas. However, these drugs have some side effects, such as hypoglycemia and gastrointestinal disorders (Chen et al., 2019). Large number of natural drugs have been studied for the treatment of diabetes and reported in the literature with promised results achieved (Fu et al., 2012; Cao, 2013; Xu et al., 2014; Chen et al., 2016; Wang et al., 2017; Liu et al., 2017). An increasing number of plant polysaccharides have been studied and found to have a good hypoglycemic effect, as well as low toxicity and limited side effects, in the search for effective and safe treatment options for DM. As a result, these polysaccharides have become a hotspot in medicine and healthcare (Zhang et al., 2016).

Actinidia arguta (Siebold & Zucc.) Planch. ex Miq. belongs to the Actinidiaceae Engl. & Gilg family, and is a perennial and deciduous liana (Li et al., 2019). It is called the "king of fruits" because of its high content of amino acids, minerals, antioxidants, vitamin C and dietary fiber (Skrypchenko, 2017; Shin et al., 2019). The hypoglycemic effect of polysaccharides extracted from the branches of A. arguta has not been reported in detail. As such, in this study, we explored the impact of A. arguta polysaccharides on the body weight, fasting blood glucose (FBG) level, blood lipid index, and serum biochemical index of diabetic mice.

Material and methods

Materials and chemicals

Total cholesterol (TC), triglyceride (TG), lowdensity lipoprotein-C (LDL-C), high-density lipoprotein-C (HDL-C), malondialdehyde (MDA) and superoxide dismutase(SOD) were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). All the other reagents and solvents used for the extraction and isolation were of analytical grade and purchased from local firms.

Preparation of polysaccharides

The branches of A. arguta were offered by Agriculture and Forestry Experimental Practice Base of Jiamusi University and were authenticated by senior experimentalist Dejiang Liu in Jiamusi University. The conditions for polysaccharide extraction were as follows: liquid-material ratio, 29 mLg⁻¹; extraction temperature, 80°C; extraction time, 128 min. After centrifugation at 5000 rpm for 10 min, the supernatant was concentrated to a certain volume under reduced pressure using a rotary evaporator, and precipitation was achieved with 80% (v/v) ethanol/water overnight. The resulting precipitates were collected and freeze-dried. The crude polysaccharides were deproteinized via the trichloroacetic acid method and then decolorized by an AB-8 macroporous adsorption resin. The crude polysaccharides were then applied to a diethylaminoethyl (DEAE-52) cellulose anionexchange column and eluted with distilled water followed by gradient solutions (0.1, 0.2, and 0.3 mol L⁻¹ NaCl) at a flow rate of 1 mL min⁻¹. The eluents were collected in 5 mL fractions, and the polysaccharides were determined by the phenol-sulfuric acid method. An elution curve was drawn with the tube number (x-axis) and absorbance (y-axis). The resulting four polysaccharides were named A. arguta branch polysaccharide (AABP): AABP1, AABP2, AABP3, and AABP4. AABP3 was the major polysaccharide and was thus selected to study its hypoglycemic effect in mice.

Experimental animals

Sixty male Kunming mice (weighing 20 ± 2 g) were purchased from Yanbian University. The license number is SCXK (Ji) 2017-0003. All

mice had free access to tap water and were provided a standard laboratory diet prior to the experiment. They were housed at 22 ± 3 °C with a 12 h light-dark cycle.

Experimental design

The mice were randomly divided into six groups (10 mice per group). Group 1: mice were intragastrically administered distilled water every day during the experimental period (normal control group, NC). Group 2: (STZ)-induced streptozotocin diabetic mice were intragastrically administered distilled water (model control group, MC). Group 3: STZ-induced diabetic mice were intragastrically administered 10 mg kg⁻¹ body weight AABP3 (low-dose group, AABP3-L). Group 4: STZ-induced diabetic mice were intragastrically administered 20 mg kg⁻¹ body weight AABP3 (medium-dose group, AABP3-M). Group 5: STZ-induced diabetic mice were intragastrically administered 40 mg kg⁻¹ body weight AABP3 (high-dose group, AABP3-H). Group 6: STZ-induced diabetic mice were intragastrically administered 15 mg kg⁻¹ body weight metformin (positive control group, PC).

The NC mice were fed a normal diet for four weeks after one week of acclimatization prior to the onset of the study. Mice in groups 2 to 6 were fed a high-fat and high-sugar diet for four weeks. After this time, fasted mice were given an intraperitoneal injection of STZ dissolved in cold citrate buffer once at a dose of 80 mg kg⁻¹ body weight. NC mice received an equal volume of cold citrate buffer injection. The blood glucose levels of the mice were measured by the collection of a drop of blood from the tip of the tail, which was subsequently tested with a GA-3 blood glucose meter. Mice with a blood glucose level greater than 11.1 mmol L⁻¹ were assumed to be diabetic three days after intraperitoneal injection. Body weight and FBG levels were monitored weekly throughout the whole experimental period (28 days). After 12 h of fasting following the last intragastric gavage, blood samples were collected from the eve vein by removal of the eyeball. Serum was then harvested by centrifuging the blood samples at 3500 rpm for 10 min and stored at -20°C for further assay. The pancreas of mice were harvested and dissected for analysis.

Determination of serum lipid levels

The serum triglyceride (TC), total cholesterol (TG), low-density lipoprotein-cholesterol (LDL-C) and high-density lipoproteincholesterol (HDL-C) levels were tested following the manufacturers' instructions.

Determination of serum biochemical indexes

The levels of malondialdehyde (MDA) and superoxide dismutase (SOD) in serum supernatant were tested following the manufacturers' instructions.

Histopathological analysis

Pancreatic tissue fixed in a 10% formalin solution was embedded in paraffin using a standard tissue-embedding procedure. Paraffin-embedded sections were stained with hematoxylin and eosin (H&E) for histological examination. All slices were visualized and captured using an inverted optical microscope XDS-18 (Beijing Rongxing Guangheng technology Co., LTD).

Statistical analysis

All the experimental data were expressed as mean \pm standard deviation (SD) in each group. Comparisons between the groups were performed by one-way analysis of variance (ANOVA) and the least significant difference (LSD) test. Data with p < 0.05 were considered statistically significant.

Results

Effect of AABP3 on body weight

Reduction of body weight is a symptom associated with DM; therefore, the body weight of mice in each group was monitored every week during the 28-day study period. The experimental results are shown in Table 1. There was no significant difference in body weight between the mice in all test groups $(20\pm2 \text{ g}; p>0.05)$ before the study. However, all STZ-induced diabetic groups presented a significant loss of body weight compared to the NC group prior to gavage (p<0.01). The AABP3-L, AABP3-M, and AABP3-H groups showed a significant increase in body weight (p<0.01) compared with the MC group. The effect of AABP3-H on the body weight of

Test groups	7 days (g)	14 days (g)	21 days (g)	28 days (g)
NC	44.25 ± 1.27	48.00 ± 1.33	49.90 ± 0.97	52.00 ± 0.88
MC	41.00 ± 1.58 °	37.60 ± 3.27 °	32.75 ± 2.87 °	30.80 ± 2.50 a
AABP3-L	40.85 ± 1.27	42.65 ± 1.53	43.95 ± 1.70	$45.90 \pm 2.22 \ ^{\rm b}$
AABP3-M	41.00 ± 1.63	43.40 ± 1.54	45.05 ± 1.59	47.35±1.53 ^b
ААВРЗ-Н	41.65 ± 1.47	44.50 ± 2.04	46.30 ± 1.70	$49.40 \pm 1.33 \ ^{\rm bc}$
PC	40.80 ± 2.78	45.10 ± 2.28	47.35 ± 1.76	49.90 ± 1.29

Table 1. The effect of AABP3 on the body weight of diabetic mice (values are expressed as mean \pm SD; n = 10 per group).

Note. a – compared with the NC group: p < 0.01; **b** – compared with the MC group: p < 0.01; **c** – compared with the PC group: p > 0.05.

diabetic mice was similar to that observed in the PC group (p>0.05). The data indicate that AABP3 possesses the ability to protect and ameliorate body weight loss in diabetic mice. diabetic mice was similar to that of the PC group (p > 0.05). The data indicate that AABP3 possesses the ability to lower FBG in diabetic mice.

Effect of AABP3 on FBG levels

Hyperglycemia is characteristic of DM and the FBG level in serum is usually monitored to reflect changes in blood glucose. The FBG levels recorded in this study are shown in Table 2. There was no significant difference in the FBG levels of mice in all test groups (p > 0.05) before embarking on the experiment. However, compared with the NC group, all STZ-induced diabetic groups presented a significant increase in FBG prior to gavage (p < 0.01). Compared with the MC group, the AABP3-L, AABP3-M, and AABP3-H groups showed a significant decrease in FBG (p < 0.01). The effect of AABP3-H on the FBG levels of

Effect of AABP3 on blood lipids

Abnormal lipid metabolism leads to an increase in TC, TG and LDL-C, and a decrease in HDL-C in the diabetic condition (Rangika et al., 2015; Akindele et al., 2015; Emordi et al., 2016). The results of the blood lipid analysis performed in this study are shown in Table 3. TC, TG and LDL-C levels were significantly increased in the MC group compared to the NC group (MC vs NC: 7.29 ± 0.09 vs 3.14 ± 0.10 mmol L⁻¹, 2.42 ± 0.12 vs 0.92 ± 0.08 mmol L⁻¹ and 0.84 ± 0.03 vs 0.39 ± 0.03 mmol L⁻¹, respectively; p<0.01), while HDL-C was significantly decreased from 1.13 ± 0.06 mmol L⁻¹ in the NC group to 0.42 ± 0.08 mmol L⁻¹ in the MC group (p<0.01).

Table 2. The effect of AABP3 on the FBG levels of diabetic mice (values are expressed as mean \pm SD; n = 10 per group).

Test groups	7 days (g)	14 days (g)	21 days (g)	28 days (g)
NC	4.56 ± 0.52	5.17 ± 0.46	5.30 ± 0.48	5.43 ± 0.55
MC	25.88 ± 3.79 ^a	27.82±2.55 °	28.58±2.88 ª	29.23±2.33 ª
AABP3-L	23.88 ± 3.95	21.17 ± 3.34	19.95 ± 3.43	18.43 ± 2.55 b
AABP3-M	25.21±3.88	21.58 ± 3.81	19.40 ± 2.78	17.91 ± 3.01 ^b
AABP3-H	22.84 ± 4.12	18.82 ± 4.78	15.56 ± 4.19	10.71 ± 2.99 bc
PC	22.85 ± 2.79	17.48 ± 3.20	14.51±3.83	10.42 ± 2.22

Note. a – compared with the NC group: p < 0.01; **b** – compared with the MC group: p < 0.01; **c** – compared with the PC group: p > 0.05.

Test groups	TC (mmol L ⁻¹)	TG (mmol L⁻¹)	HDL-C (mmol L ⁻¹)	LDL-C (mmol L⁻¹)
NC	3.14 ± 0.10	0.92 ± 0.08	1.13 ± 0.06	0.39 ± 0.03
MC	7.29 ± 0.09 ^a	2.42 ± 0.12 a	0.42 ± 0.08 a	0.84 ± 0.03 °
AABP3-L	6.01 ± 0.19 b	1.75 ± 0.11 $^{\rm b}$	$0.67\pm0.08~^{\rm b}$	0.72 ± 0.05 $^{\rm b}$
AABP3-M	5.27 ± 0.23 b	1.45 ± 0.12 $^{\rm b}$	0.77 ± 0.65 b	0.63 ± 0.05 b
AABP3-H	$4.37 \pm 0.12 \ ^{\rm bc}$	$1.23\pm0.08~{\rm bc}$	$0.98\pm0.06~^{\rm b}$	$0.54 \pm 0.04 \ ^{\rm b}$
PC	3.67 ± 0.15	1.13 ± 0.09	1.00 ± 0.10	0.48 ± 0.04

Table 3. The effect of AABP3 on the blood lipids of diabetic mice (values are expressed as mean ± SD; n = 5 per group).

Note. a – compared with the NC group: p < 0.01; **b** – compared with the MC group: p < 0.01; **c** – compared with the PC group: p > 0.05.

After four weeks of gavage, the TC, TG, and LDL-C levels in mice treated with AABP3-H were significantly decreased to 4.37 ± 0.12 , 1.23 ± 0.08 , and 0.54 ± 0.04 mmol L⁻¹ (p<0.01), respectively, while HDL-C was significantly increased to 0.98 ± 0.06 mmol L⁻¹ compared with the MC group (p<0.01). The effect of AABP3-H on blood lipid changes associated with DM in mice was similar to that of the PC (p>0.05). The data indicate that AABP3 had a beneficial effect on dyslipidemia.

Effect of AABP3 on oxidative stress parameters in the serum

Recently, many studies have demonstrated that DM is typically associated with the increased generation of free radicals or an impaired antioxidant defense mechanism, resulting in diabetes-induced pathological consequences (Liu et al., 2014; Pan et al., 2014; Adefegha et al., 2014). As such, we investigated the changes in MDA and SOD in this study, and the results are shown in Fig. 1. The level of MDA was significantly increased from 6.07 ± 0.25 nmol L⁻¹ in the NC group to 11.90 ± 0.82 mmol L⁻¹ in the MC group (p < 0.01), while the level of SOD was significantly decreased from 22.78±0.26 U mL⁻¹ in the NC group to 20.71±0.16 U mL⁻¹ in the MC group (p < 0.01). The level of MDA in mice administered AABP3-H was significantly decreased to 6.49 ± 0.56 nmol L⁻¹ (p<0.01) after four weeks of gavage, while the level of SOD was increased considerably to 22.43 ± 0.14 U mL⁻¹ compared with the MC group (p < 0.01). The effect of AABP3-H on the oxidative stress changes observed in diabetic mice was better than that of the PC. The data indicate that AABP3 had a beneficial impact on the serum oxidative stress parameters of diabetic mice.

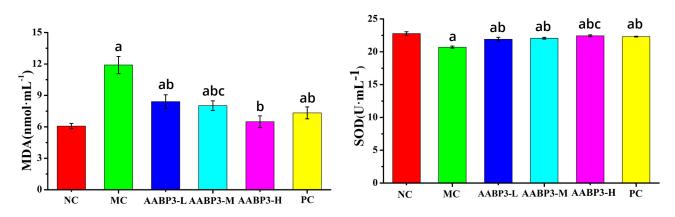


Figure 1. The effect of AABP3 on serum biochemical indexes in diabetic mice after treatment with FPLP for four weeks (values are presented as mean ± SD (n = 5): $\mathbf{a} - p < 0.05$ compared with NC group; $\mathbf{b} - p < 0.05$ compared with MC group; $\mathbf{c} - p > 0.05$ compared with PC group).

Histopathological observation of the pancreas

Pancreas slices observed via histopathology are shown in Fig. 2. The architecture of the pancreatic islets in the NC group was characterized by clear, round or oval boundaries and the islet cells had normal morphology and were arranged closely. However, the pancreatic islets in the MC group exhibited irregular shapes and the islet cells were extensively destroyed. AABP3-H improved the detrimental changes observed in the pancreas tissue of diabetic mice compared with the MC group. The islet cells had normal morphology and the empty areas were reduced in size. This result suggests that AABP3 may protect and ameliorate pancreatic damage in DM.

Discussion

Diabetes is a serious health issue worldwide, producing significant morbidity and mortality, and there is no route to cure diabetes completely. Patients with type II diabetes can control their blood sugar level by oral administration of some hypoglycemic drugs, but currently, some hypoglycemic drugs can cause toxic and other side effects such as hypoglycemia and gastrointestinal diseases. Therefore, developing new drugs with good curative effects and little side effects is of great significance. At present, STZ and alloxan are the main chemical drugs for inducing type II diabetes. After injecting alloxan, blood sugar may recover, while when injecting STZ, mice will die if the dose is too high. Therefore, this experiment can simulate diabetes caused by environmental factors by feeding a high-fat and high-sugar feed combined with low-dose intraperitoneal injection of STZ to induce a type II diabetes mouse model, artificially destroying islet beta cells to damage islets, and the induction method is suitable for establishing a type II diabetes model. In this experiment, after 28 days of gastric administration of AABP3 in mice with type II diabetes, the symptoms of "three more and one less" diabetes in mice in the administration group were recovered, and the fasting blood glucose level was significantly reduced. The contents of TC, TG, LDL-C, and HDL-C in the four indexes of blood lipid of mice in the administration

group were significantly reduced, indicating that the abnormal phenomenon of blood lipid in diabetic mice was improved. The content of MDA in serum decreased obviously, and the content of SOD increased obviously, which indicated that AABP3 could reduce blood sugar by inhibiting peroxidation. It was found that AABP3 high dose group has a certain protective effect on the pancreas STZ-induced diabetic mice through of histological observation. It is concluded that AABP3 regulates the blood sugar level and lipid metabolism of diabetic mice and alleviates insulin resistance, thus playing a certain protective role on the pancreas of mice. AABP3-H had the most significant therapeutic effect in DM and showed similar results to the PC, suggesting AABP3-H acts in a dose-dependent manner. It can thus be concluded that AABP3 may be considered a potential candidate for developing a functional food or natural product for treating DM and its complications. Polysaccharides and other hypoglycemic drugs obtained from natural plants have good effects, low toxic and side effects, and complex mechanisms of action. Subsequent experiments can further explore the hypoglycemic mechanism of this polysaccharide on diabetic mice and explore some toxic and side effects of the polysaccharide. In the pruning process of A. arguta, a large number of branches will be discarded, and the discarded branches will be recycled for utilization. The polysaccharide in the branches will be extracted and purified, and its hypoglycemic mechanism will be explored. The added value of A. arguta can be increased, which can not only avoid waste but also reduce pollution and achieve the purpose of waste utilization.

Conclusions

In summary, according to the present findings, our study demonstrated that AABP3 is the main fraction of polysaccharides extracted from the branches of A. *arguta*. In *in vivo* assays, AABP3 revealed a potent hypoglycemic effect on STZinduced diabetic rats for its ability to reduce glucose levels and ameliorate lipid metabolism and oxidative stress. Further studies in animal models and human volunteers need to be done to substantiate these findings. AABP3

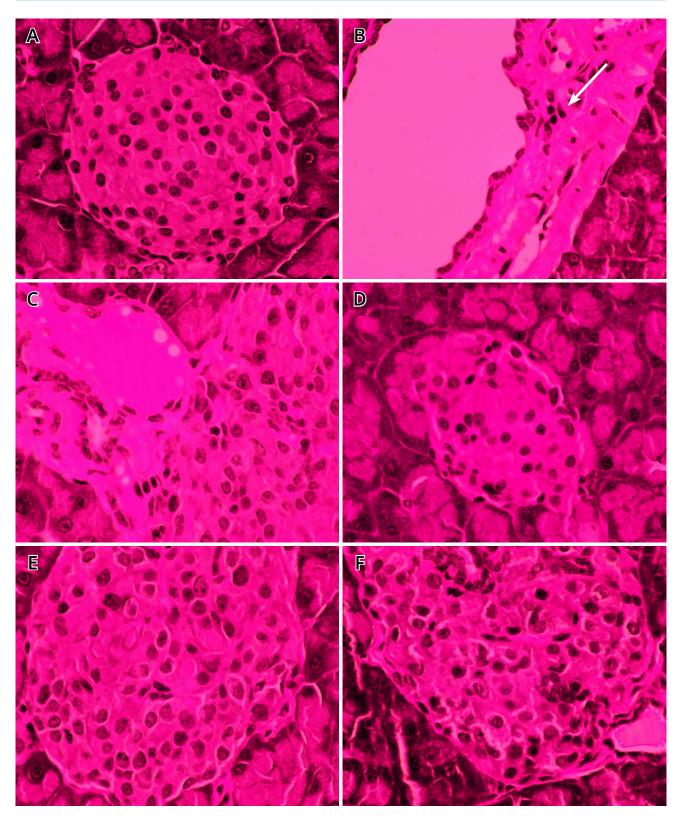


Figure 2. Histopathological study of pancreas tissue from mice (H&E staining, ×400 magnification): **A** – NC group; **B** – MC group; **C** – AABP3-L group; **D** – AABP3-M group; **E** – AABP3-H group; **F** – PC group.

may be considered a potential candidate for developing functional food or natural products for treating diabetes and its complications.

Therefore, an important stage of further research is the study of the qualitative and

quantitative characteristics of polysaccharides extracted from the shoots of A. *arguta*, for the development of methods of quality control of raw materials at the creation of drugs with a hypoglycemic effect.

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Гіпоглікемічний ефект полісахаридів, виділених з пагонів Actinidia arguta

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Цукровий діабет може призвести до гіперглікемії, спричиненої недостатньою секрецією інсуліну або інсулінорезистентністю. Тому рослинні екстракти, які мають гіпоглікемічний ефект з обмеженими побічними ефектами, представляють інтерес для медицини та охорони здоров'я. У цьому дослідженні на мишах досліджували гіпоглікемічний ефект полісахаридів, виділених з пагонів Actinidia arguta. Шістдесят самців мишей Куньмін були випадковим чином розподілені в шість груп по десять особин. Протягом 28-денного періоду дослідження щотижня оцінювали масу тіла, рівень глюкози в крові натщесерце, ліпіди сироватки та параметри окисного стресу. В кінці дослідження були відібрані зразки тканини підшлункової залози дослідних мишей для подальшого аналізу. Встановлено, що полісахарид ААВРЗ запобігав втраті маси тіла мишей та знижував рівень глюкози в крові натщесерце у мишей з діабетом порівняно з контролем. Він також мав сприятливий вплив на сироваткову дисліпідемію та параметри окисного стресу та за своїм захисним ефектом був подібним до метформіну. Гістопатологічне дослідження підшлункової залози показало, що полісахарид ААВРЗ може захищати та зменшувати пошкодження підшлункової залози, які можуть виникати при цукровому діабеті у мишей. ААВРЗ можна розглядати як потенційного кандидата для розробки функціонального харчового або натурального продукту для лікування діабету та його ускладнень.

Ключові слова: Actinidia arguta, полісахариди, гіпоглікемічний ефект, цукровий діабет, стрептозотоцин