



Hypoglycemic activity and mechanism of the sulfated rhamnose polysaccharides chromium(III) complex in type 2 diabetic mice

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ABSTRACT

The sulfated rhamnose polysaccharides found in *Enteromorpha prolifera* belong to a class of unique polyanionic polysaccharides with high chelation capacity. In this study, a complex of sulfated rhamnose polysaccharides with chromium(III) (SRPC) was synthesized, and its effect on type 2 diabetes mellitus (T2DM) in mice fed a high-fat, high-sucrose diet was investigated. The molecular weight of SRPC is 4.57 kDa, and its chromium content is 28 µg/mg. Results indicated that mice treated by oral administration of SRPC (10 mg/kg and 30 mg/kg body mass per day) for 11 weeks showed significantly improved oral glucose tolerance, decreased body mass gain, reduced serum insulin levels, and increased tissue glycogen content relative to T2DM mice ($p < 0.01$). SRPC treatment improved glucose metabolism via activation of the IR/IRS-2/PI3K/PKB/GSK-3 β signaling pathway (which is related to glycogen synthesis) and enhanced glucose transport through insulin signaling cascade-induced GLUT4 translocation. Because of its effectiveness and stability, SRPC could be used as a therapeutic agent for blood glucose control and a promising nutraceutical for T2DM treatment.

1. Introduction

Type 2 diabetes mellitus (T2DM)—also referred to as non-insulin-dependent diabetes mellitus or adult-onset diabetes—is characterized by insulin secretory abnormalities, hepatic glucose overproduction, and insulin resistance in peripheral tissues such as the liver and skeletal muscle [1]. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels [2]. The risk factors of T2DM are relatively well known and include age, obesity, the lifestyle, dietary patterns, and gene–environment interactions [3]. Most of the anti-hyperglycemic agents available for the treatment of T2DM have insulin-dependent mechanisms of action, that is, they stimulate insulin production (e.g., sulfonylureas, glinides, incretin mimetics, and dipeptidyl peptidase-4 inhibitors), improve insulin sensitivity (e.g., thiazolidinediones and biguanides), or directly raise endogenous insulin levels (e.g., basal and prandial types of insulin) [4]. Nonetheless,

the current drug therapies for T2DM patients often have many adverse effects, including weight gain, hypoglycemia, and cardiovascular diseases [3,5]. Therefore, it is necessary to explore novel drugs and therapies to improve treatment efficacy and reduce complications of T2DM.

Trivalent chromium, which is directly related to glucose tolerance factor activity, can enhance the sensitivity of pancreatic islets and accelerate the utilization of glucose in vivo [6,7]. Recently, various chemical forms of chromium(III) compounds, such as chromium picolinate [8] and chromium citrate [9], were tested as nutritional supplements to decrease blood glucose levels and increase insulin levels in T2DM patients. Chromium picolinate is the most well-studied anti-diabetic and anti-obesity chromium complex. Accordingly, its molecular mechanism of action and biological activities have been researched extensively. Accumulating evidence suggests that ligands can significantly improve the bioactivity of chromium and increase its ability to control diabetes. Moreover, novel complexes of chromium(III) with various other ligands

Abbreviations: Man, mannose; Rha, rhamnose; GlcA, glucuronic acid; GalA, galacturonic acid; Lac, lactose; Glc, glucose; Xyl, xylose; Gal, galactose; Ara, arabinose; Fuc, fucoidan; FER, food efficiency ratio (FER (%) = [total body weight gain (g)/total food intake (g)] × 100%); IR, insulin receptor; IRS-1, insulin receptor substrate-1; IRS-2, insulin receptor substrate-2; PI3K, phosphoinositide 3-kinase; PKB, protein kinase B; GLUT4, glucose transporter 4; GSK-3 β , glycogen synthase kinase 3 β ; qRT-PCR, quantitative real time-polymerase chain reaction

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Table 1
Composition analysis and determination of the molecular mass of SRP and SRPC.

Samples	Total sugar content (%)	Sulfate content (%)	Protein content (%)	MW (kDa)	Mole ratio Rhamnose:Glucose:Xylose:Glucuronic acid
SRP	71.19	24.88	1.12	3.78	3.51:1.28:1:1.33
SRPC	70.31	23.22	0.93	4.57	3.65:1.67:1:1.48

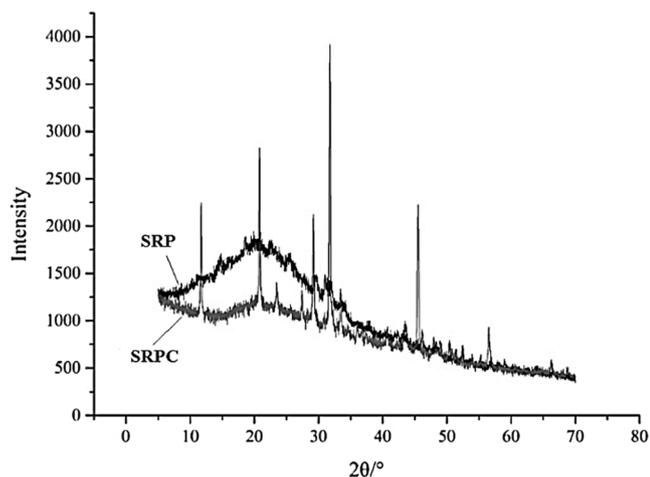


Fig. 1. X-ray diffraction patterns of SRP and SRPC.

have been consistently prepared and evaluated, indicating that the ligand can strongly improve the performance of this type of chromium complex [7,10,11].

Many polysaccharides from marine sources have shown good anti-diabetic activities and are promising candidates for biocompatible ligands of metal ions [5]. *Enteromorpha prolifera* (*E. prolifera*), a green alga found on seashores worldwide, is widely used in the fields of nutrition and medicine [12]. Polysaccharides from *E. prolifera* are a group of sulfated heteropolysaccharides that mainly consist of D-GlcUA p- α -1, 4-3-sulfate-L-Rha p- β -1, 4-D-Xyl p- β -1, and 4-3-sulfate-L-Rha p units. [13]. Recent studies revealed that *E. prolifera* polysaccharides possess excellent chelating properties [12,14,15]. Chi et al. have assessed different extraction techniques to prepare complexes of iron(III) with *E. prolifera* polysaccharides, having an iron content of 208.5 $\mu\text{g}/\text{mg}$ [15].

In this study, a low-molecular-weight sulfated rhamnose polysaccharide (SRP) was prepared and used to synthesize a complex of sulfated rhamnose polysaccharides with chromium(III) (SRPC). Physicochemical properties of SRPC were characterized, and the anti-diabetic effects in T2DM mice fed a high-fat high-sucrose diet (HFSD) were evaluated. This study also investigated the potential mechanism of SRPC action on the PI3K/PKB signaling pathway.

2. Materials and methods

2.1. Materials and chemicals

Chromium(III) picolinate salt was purchased from Energy Chemical (Shanghai, China). Monosaccharide standards (Man, Rha, GlcA, GalA, Lac, Glc, Xyl, Gal, Ara and Fuc) and dextran standards (5, 25, 150, 410, and 670 kDa) were purchased from Sigma-Aldrich Co., Ltd., (St Louis, MO, USA). All other chemicals and reagents were of analytical grade.

2.2. Preparation of SRP and synthesis of SRPC

Crude SRP was extracted following an improved method described by Cui et al. [12]. Then, an SRP with a molecular weight of 3.38 kDa was chosen for this study. After preparation of an aqueous solution of SRP (10 mg/mL), a CrCl_3 solution (1 mol/L) was added to the SRP

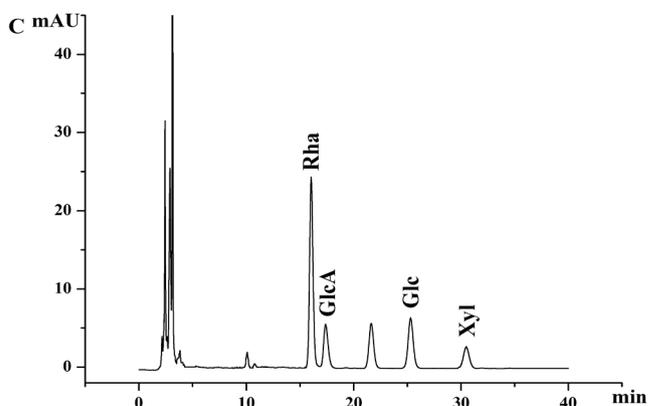
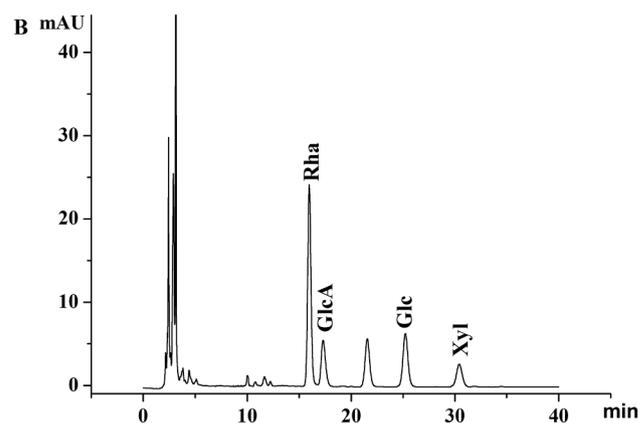
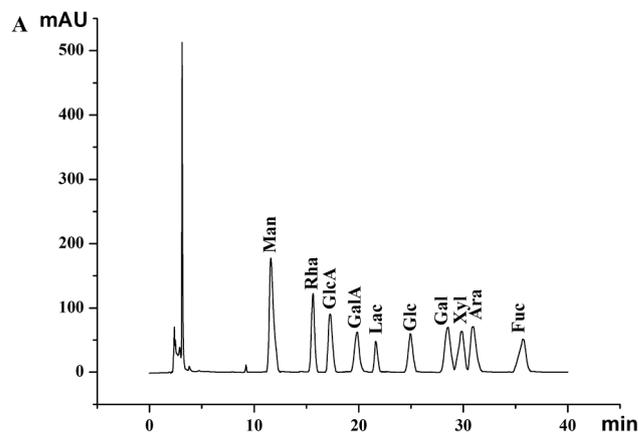


Fig. 2. HPLC analysis of monosaccharide standards, SRP and SRPC. (A) Monosaccharide composition analysis of standards. (B) Monosaccharide composition analysis of SRP. (C) Monosaccharide composition analysis of SRPC.

solution, and pH was adjusted to 7. The reaction mixture was incubated at 60 °C for 3 h and then centrifuged at 3000g for 10 min. The supernatant was concentrated and dialyzed against distilled water to remove unbound Cr^{3+} . After that, the dialysate was concentrated and lyophilized. The obtained powder is hereafter referred to as SRPC.

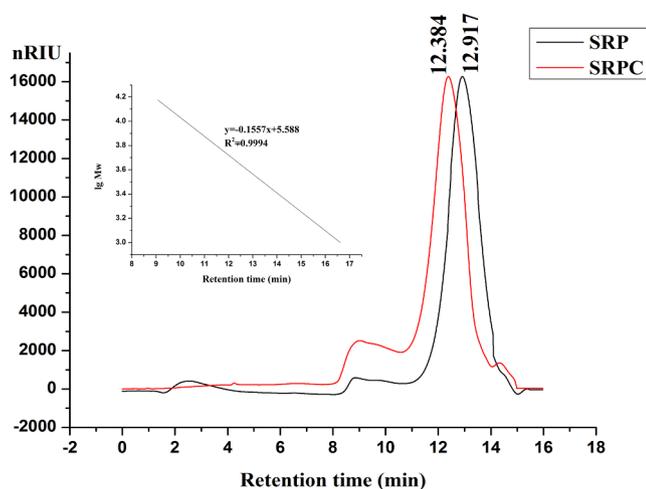


Fig. 3. HPGPC chromatogram of SRP and SRPC.

Table 2

Effects of SRPC on food intake and FER in T2DM mice (n = 9).

Groups	Food intake (g/week)	FER
NC	30.12 ± 1.28	25.43 ± 1.23
DC	23.51 ± 0.84 [#]	52.23 ± 2.09 ^{##}
PC	22.98 ± 1.26 [#]	38.77 ± 2.11 ^{**}
CT	23.44 ± 1.12 [#]	45.35 ± 1.34 [*]
Low-SRP	22.32 ± 1.11 [#]	49.33 ± 1.89
High-SRP	23.29 ± 1.25 [#]	48.69 ± 3.02
Low-SRPC	23.03 ± 0.98 [#]	41.90 ± 2.87 ^{**}
High-SRPC	23.13 ± 0.65 [#]	40.12 ± 2.65 ^{**}

^{##} p < 0.01 relative to the control group.

^{**} p < 0.01 relative to the model group.

[#] p < 0.05 relative to the model group.

2.3. Characterization of SRPC

2.3.1. Components analysis

Total sugar content of the polysaccharide was determined by the phenol-sulfuric acid method [16]. Protein content was measured by the Bradford assay [17]. Sulfate content was determined after hydrolysis with trifluoroacetic acid [18]. The molecular weights of SRP and SRPC were measured by gel permeation chromatography (GPC) on a TSK-gel G4000 PWxl column (30 cm × 7.8 mm, Tosoh, Tokyo, Japan) by means of an Agilent 1260 high-performance liquid chromatography (HPLC) system (Agilent Technologies, Santa Clara, CA, USA). The mono-saccharides of SRP and SRPC were quantified by reversed-phase HPLC after 1-phenyl-3-methyl-5-pyrazolone precolumn derivatization [13].

Table 3

Effects of SRPC on the body mass gain in T2DM mice (n = 9).

Groups	Body mass (g)					
	0 weeks	4 weeks	8 weeks	12 weeks	16 weeks	19 weeks
NC	17.73 ± 1.62	21.33 ± 2.24	23.16 ± 3.38	23.79 ± 4.12	24.63 ± 3.17	25.39 ± 2.68
DC	17.71 ± 1.53	23.34 ± 1.67	25.72 ± 2.34	27.42 ± 2.55 ^{##}	28.74 ± 2.15 ^{##}	29.99 ± 1.76 ^{##}
PC	17.55 ± 1.53	23.4 ± 0.88	25.3 ± 1.41	25.93 ± 2.56 [*]	25.72 ± 2.23 [*]	26.46 ± 1.97 ^{**}
CT	17.83 ± 2.58	24.56 ± 2.33 [†]	27.24 ± 2.09	28.03 ± 1.99	27.92 ± 2.04	28.46 ± 2.37 [†]
Low-SRP	17.07 ± 2.9	23.8 ± 2.34	26.78 ± 3.04	27.34 ± 2.32	27.22 ± 2.26	28.08 ± 3.39 [*]
High-SRP	17.37 ± 2.54	24.27 ± 2.42	27.35 ± 2.23 [†]	28.05 ± 3.33	28.12 ± 1.83	28.71 ± 2.47 [†]
Low-SRPC	17.02 ± 0.85	23.62 ± 1.11	25.88 ± 0.87	26.24 ± 1.01 [†]	26.14 ± 0.84 [†]	26.67 ± 0.61 ^{**}
High-SRPC	17.64 ± 1.04	23.42 ± 1.89	26.21 ± 2.34	26.81 ± 2.48	26.66 ± 2.48	26.92 ± 2.18 ^{**}

^{##} p < 0.01 relative to the control group.

^{**} p < 0.01 relative to the model group.

^{*} p < 0.05 relative to the model group.

Chromium(III) content was determined as described by Stoyanova, with CrCl_3 as a standard [19].

2.3.2. X-ray powder diffraction

The crystal morphology of SRP and SRPC was analyzed on an X-ray diffractometer (D/max 2500; Rigaku, Metropolitan, Japan). The X-ray diffractometer was operated at 40 kV and 30 mA produced by $\text{Cu K}\alpha$ radiation. The X-ray diffraction patterns were recorded over $\theta = 5\text{--}70^\circ$ (where θ is the angle of diffraction) at a rate of $4^\circ/\text{min}$ [15].

2.4. Animals

Male C57BL/6J mice (18 ± 2 g) of specific pathogen-free grade purchased from Jinan Pengyue Experimental Animal Breeding Co., Ltd. (Shandong, China, License ID: SCXK2014-0007) were used for this study. They were housed at $23 \pm 1^\circ\text{C}$ and humidity of 44.5–51.8% on a 12 h light/dark cycle with *ad libitum* access to food and water. The use of animals in this study was approved by the Ethics Committee for Experimental Animal Care at the Ocean University of China (certificate no. SYXK20120014).

2.5. Experimental design

Male C57BL/6J mice were allowed to acclimate for 7 days. For the normal control group (NC), nine mice were selected randomly and fed a low-fat low-sucrose diet (LFSD). The T2DM-model mice were created by means of an HFSD (Research Diets, New Brunswick, NJ, USA; #D12331), which consisted of 20% protein, 25% fat, and 20% carbohydrates, as described by Hu et al. [20] The diet composition and chromium content in the experimental diets are summarized in supplementary Data Table A.1. Blood glucose levels of the mice were measured by collection of a drop of blood from the tip of the tail and testing with a hand-held One Touch Basic Glucose Monitor (Acon Biotech Co., Hangzhou, China). All the mice had normal blood glucose levels (5.33 ± 0.87 mmol/L) at the start of the experiment. After 8 weeks, mice with a blood glucose level greater than 11.1 mmol/L were assumed to be diabetic mice suitable for subsequent experiments.

After establishment of the T2DM model, all the mice were randomly subdivided into eight groups with nine animals each, normal control group (NC), diabetic group (DC), positive control group (PC; 6.8 mg chromium(III) picolinate/kg body mass per day, 840 μg Cr/kg), negative control group (CT; 4.3 mg chromium trichloride hexahydrate/kg body mass per day, 840 μg Cr/kg), low SRP group (10 mg SRP/kg body mass per day), high SRP group (30 mg SRP/kg body mass per day), low SRPC group (10 mg SRPC/kg body mass per day, 280 μg Cr/kg), high SRPC group (30 mg SRPC/kg body mass per day, 840 μg Cr/kg). Therapeutic-agent samples were dissolved in water and administered daily via an orogastric cannula continuously for 11 weeks. In the course of the experiment, the control group was fed the LFSD diet, whereas the

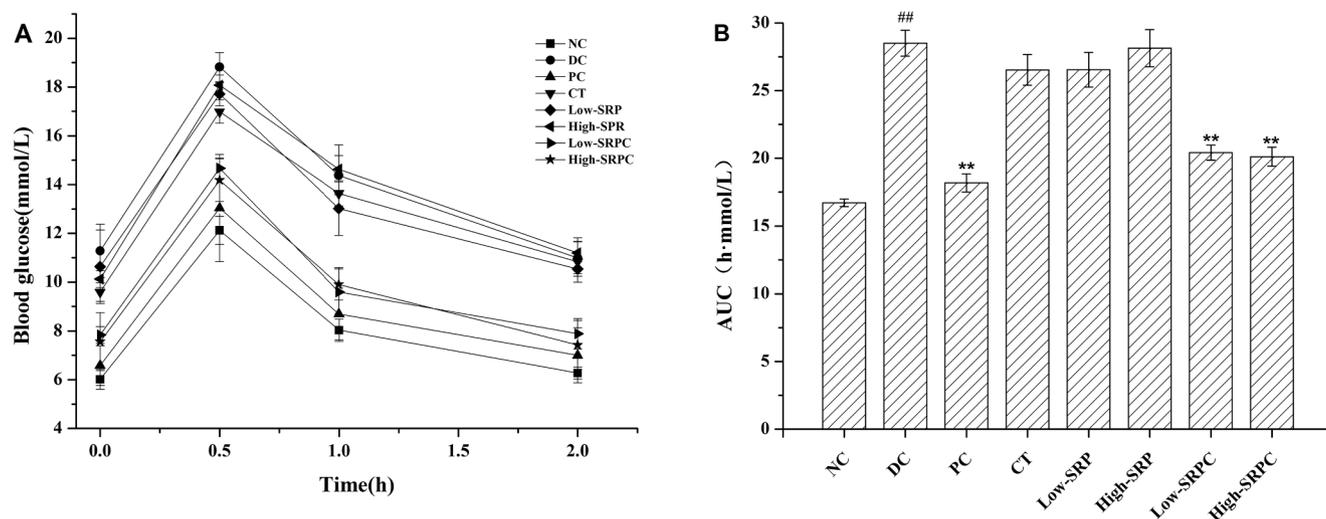


Fig. 4. Effects of SRPC on oral glucose tolerance in T2DM mice ($n = 9$). (A) The impact on blood glucose levels in the oral glucose tolerance test (0–2 h). (B) AUC in the oral glucose tolerance test. $^{##}p < 0.01$ relative to the control group. $^{**}p < 0.01$ relative to the model group.

diabetic mice were fed the HFSD diet and consumed water freely. Both diets were not supplemented with Cr. Food intake and body mass were measured periodically.

2.6. An oral glucose tolerance test

In the last week of the experimental period, this test was performed on mice that had fasted overnight. After oral administration of a glucose aqueous solution (2 g/kg body mass), blood samples were taken from the tail vein of the mice and were analyzed at 0 h (prior to glucose administration) and at 0.5 h, 1 h, and 2 h after glucose administration. The area under the curve (AUC) of each group was calculated, as per formula (1):

$$\text{AUC (h}\cdot\text{mmol}\cdot\text{L}^{-1}) = 0.25 \times A + 0.5 \times B + 0.75 \times C + 0.5 \times D \quad (1)$$

where A, B, C, and D represent blood glucose levels at 0 h, 0.5 h, 1 h, and 2 h, respectively.

2.7. Evaluation of serum insulin level

On the last night of the experiment, all mice were fasted but were given free access to water. Blood was collected from the orbital sinus and immediately centrifuged at 3000g for 10 min at 4 °C, then serum was prepared for determination of insulin levels by an enzyme-linked immunosorbent assay (ELISA; Invitrogen, Carlsbad, CA, USA). HOMA-IR and QUICKI were calculated according to formulas (2) and (3), respectively.

$$\text{HOMIA} - \text{IR} = \text{fasting blood glucose} \times \text{serum insulin} / 22.5 \quad (2)$$

$$\text{QUICKI} = 1 / [\lg(\text{fasting blood glucose}) + \lg(\text{serum insulin})] \quad (3)$$

2.8. Determination of glycogen levels

Glycogen levels of the liver and skeletal muscle were determined by means of relevant kits (Nanjing Jiancheng Bioengineering Institute, China).

2.9. Quantitative reverse-transcription PCR (qRT-PCR)

The mRNA expression levels of glucose metabolism-related genes—IR, IRS-1, IRS-2, PI3K, PKB, GLUT4, and GSK-3 β —were examined by qRT-PCR. For each sample, total RNA was isolated from the liver and muscle (HP Total RNA kit; Tissue RNA Kit; OMEGA, USA), the

first cDNA strand was synthesized by reverse transcription (5X All-In-One MasterMix; abm, Canada), and cDNA was amplified by quantitative PCR with SYBR Green (TOYOBO, Japan) and gene-specific primers (supplementary Data Table A.2). The relative expression of each gene of interest was normalized to β -actin, analyzed by the $2^{-\Delta\Delta C_t}$ method, and expressed as a ratio to the control.

2.10. Statistical analysis

All the data were expressed as a mean \pm standard deviation. Statistical analyses were performed in the SPSS software, version 12.0. 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.

3. Results

3.1. Characterization of SRP and SRPC

SRP was isolated from *E. proliferans* and used to prepare SRPC complexes. As presented in Table 1, the total sugar, sulfate, and protein contents of SRP and SRPC were not significantly different. They contained four monosaccharides: rhamnose, glucose, xylose, and glucuronic acid (Fig. 2). SRPC is homogenous because it yields a single and symmetrically sharp peak in HPLC, with a molecular weight of 4.57 kDa (Fig. 3). After chelation, the chromium content of SRPC was 28 $\mu\text{g}/\text{mg}$.

X-ray diffraction patterns of SRP and SRPC are depicted in Fig. 1. The X-ray diffraction pattern of SRPC is obviously different from that of SRP, which contains additional characteristic peaks at 11.7°, 20.8°, 29.2°, 31.8°, and 45.5°. The broad peaks of SRP disappeared after the coordination reaction, indicating changes in crystal morphology.

3.2. Influence of SRPC on the body mass gain in T2DM mice

Though food intake was higher in LFSD fed mice than that in HFSD fed mice ($p < 0.05$), the FER of the other groups were sharply increased compared with that of the normal control group ($p < 0.01$), as shown in Table 2, indicating that the HFSD used in this study significantly increased the body weight of the rats.

Obesity is a major risk factor of T2DM, and most people with T2DM are overweight or obese at diagnosis; interventions that reduce body mass gain can lower the risk of diabetes [21]. As shown in Table 3, the body mass gain in the diabetic group was remarkably greater than that in the normal control group, indicating that the HFSD diet used in this

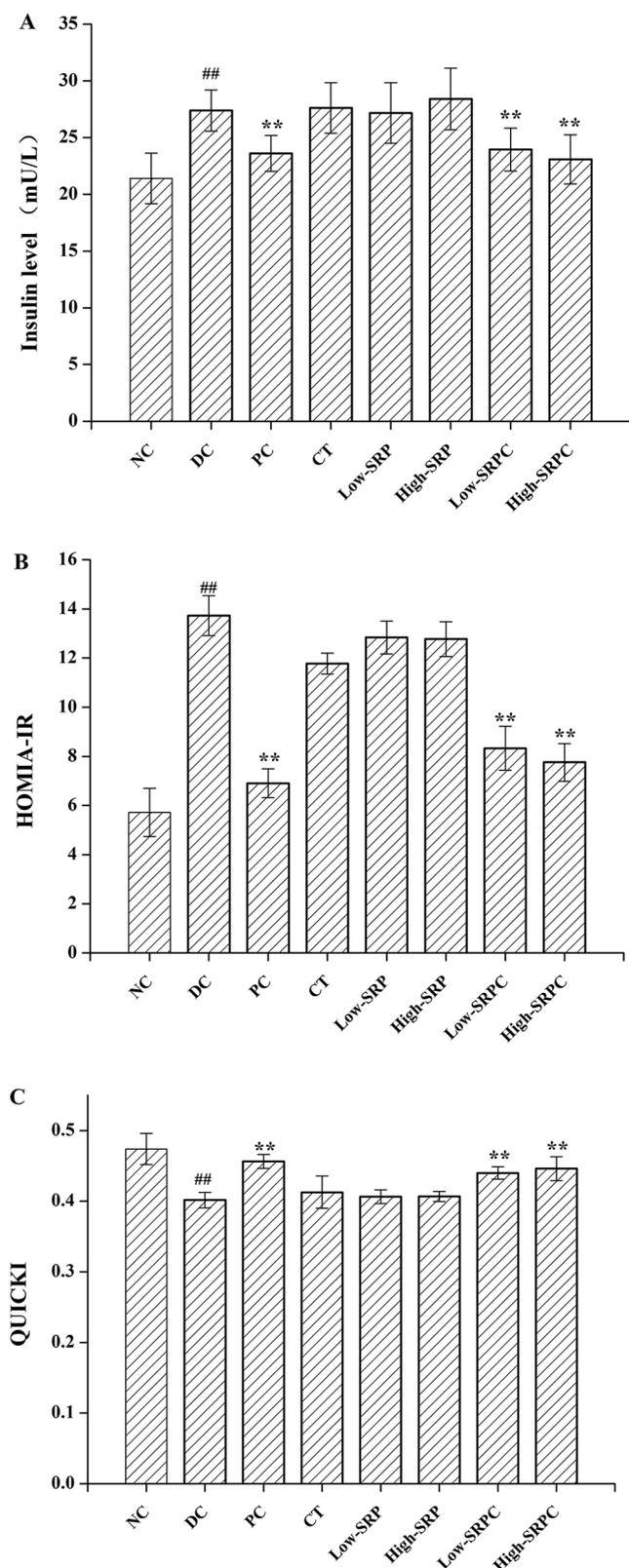


Fig. 5. The influence of SRPC on insulin levels in T2DM mice (n = 9). (A) Serum insulin levels. (B) HOMA-IR. (C) QUICKI. ^{##} p < 0.01 relative to the control group, ^{**} p < 0.01 relative to the model group.

study significantly increased the body mass gain of the mice. Treatment with SRPC observably attenuated the body mass gain relative to the no-treatment group (diabetic group), whereas treatment with SRP did not result in obvious attenuation. The results indicated that SRPC exerted

anti-obesity action and attenuated the body mass gain.

3.3. Effects of SRPC on blood glucose level and oral glucose tolerance

During the last week of the experiment, blood glucose of normal control group was 6.01 ± 0.41 mmol/L. Blood glucose levels of the positive control and SRPC group were reduced to the normal range (7.56 ± 1.19 mmol/L), while other groups still remained at a high level (11.28 ± 1.16 mmol/L).

To investigate blood glucose homeostasis in the diabetic mice, their glucose tolerance was evaluated. After oral administration of glucose, the blood glucose level in all the experimental mice reached its peak within 30 min; in the normal control group, blood glucose gradually returned to baseline after 120 min, but in the diabetic group of mice, blood glucose consistently remained high (Fig. 4A). Administration of SRPC (10 mg/kg and 30 mg/kg) resulted in a significant decrease of the blood glucose level and AUC when compared to the diabetic group (Fig. 4B; p < 0.01). Improvement of the impaired oral glucose tolerance of T2DM mice was also observed after chromium picolinate treatment (p < 0.01). These results indicated that SRPC exerted a hypoglycemic effect on T2DM mice.

3.4. Effects of SRPC on insulin levels

Insulin is the primary hormone for regulation of glucose metabolism. Insulin resistance is typical of T2DM and metabolic syndrome and is often accompanied by hyperinsulinemia and hyperglycemia [4]. As depicted in Fig. 5, the HFSD significantly increased serum insulin levels (p < 0.01), indicating the development of insulin resistance. SRPC treatment remarkably decreased serum insulin levels, in contrast to the diabetic group (p < 0.01). Likewise, chromium picolinate treatment remarkably reduced serum insulin levels in T2DM mice (p < 0.01). The homeostasis model assessment of insulin resistance index (HOMA-IR) and quantitative insulin sensitivity check index (QUICKI) of all groups are shown in Fig. 5B, 5C, respectively. Compared with that in the normal control group, HOMA-IR in the model control group increased significantly (p < 0.01), while QUICKI decreased significantly (p < 0.01). These results show that the HFSD induces severe insulin resistance. The HOMA-IR of low SRPC group decreased significantly (p < 0.01) while QUICKI increased significantly (p < 0.01), when compared to that of model control group. Furthermore, QUICKI of low SRPC group was similar to that of the positive control group. Taken together, these results showed that SRPC ameliorated insulin resistance caused by T2DM and effectively alleviated the main symptoms.

3.5. Effects of SRPC on glycogen levels

Glycogen is the primary intracellular storage form of glucose in hepatic and muscle cells and is thought to be closely related to insulin resistance in T2DM. As illustrated in Fig. 6, glycogen levels in the mice with HFSD-induced diabetes (diabetic group) were significantly lower (p < 0.01). After SRPC treatment for 11 weeks, glycogen levels were significantly higher than those in the diabetic group of mice (p < 0.01) and were similar to those in the positive control group (p < 0.01). Overall, these results suggested that the anti-hyperglycemic activity of SRPC was partially due to glycogen synthesis. This phenomenon may explain insulin sensitization in hepatic and muscle tissues.

3.6. Effects of SRPC on the expression of PI3K/PKB/GSK-3 β signaling pathway

Glycogen synthesis is mainly regulated by the insulin-mediated PI3K/PKB/GSK-3 β signaling pathway. As presented in Fig. 7, IR, IRS2, PI3K, and PKB mRNA expression levels were significantly lower in the diabetic group (p < 0.01), whereas treatment with SRPC or chromium picolinate increased their expression (p < 0.01). Expression of GSK-

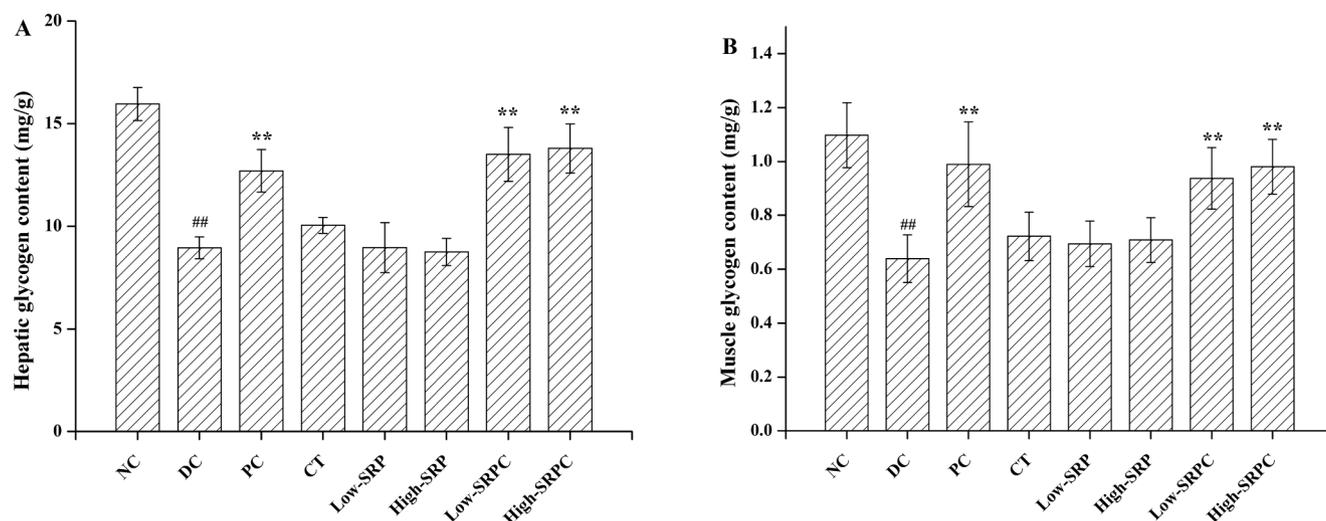


Fig. 6. The impact of SRPC on tissue glycogen content in T2DM mice (n = 9). (A) Hepatic glycogen content. (B) Muscle glycogen content. ^{##}p < 0.01 relative to the control group, ^{**}p < 0.01 relative to the model group.

3 β , a negatively regulated gene in glycogen synthesis, was normalized in the liver of T2DM mice after SRPC or chromium picolinate treatment (p < 0.01). Taken together, these results provided good evidence that SRPC can promote hepatic glycogen synthesis by regulating the insulin-mediated PI3K/PKB/GSK-3 β signaling pathway.

3.7. Effects of SRPC on the expression of PI3K/PKB/GLUT4 signaling pathway

Insulin-dependent glucose transport in skeletal muscle is the main route of glucose disposal. This transport is achieved by translocation of glucose transporter GLUT4 from intracellular sites to the cell surface, a process predominantly mediated by the PKB/GLUT4 signaling pathway, which involves several pivotal genes, such as PKB, PI3K, IRS-1, and IR. As illustrated in Fig. 8, the SRPC-treated group and positive control group showed remarkable increases in mRNA expression as compared to the diabetic group (p < 0.01). These results proved that SRPC can up-regulate the transcription of genes of the insulin-mediated PI3K/PKB/GLUT4 signaling pathway.

4. Discussion

Polysaccharides from *E. proliferifera* are a group of sulfated rhamnose polysaccharides with many beneficial bioactivities, including anticoagulant, antiviral, and anticancer properties [22]. Modern pharmacological studies have revealed that polysaccharides from algae are good candidates for biocompatible ligands of metal ions because of good stability and digestive availability [5,15]. In this study, a low-molecular-weight SRPC was successfully prepared from SRP. After chelation, the chromium content of SRPC reached 28 μ g/mg, indicating that SRP has a good ability to chelate chromium(III). These results are in agreement with other studies indicating that sulfated polysaccharides can chelate metal ions. Cui et al. have treated iron deficiency anemia with sulfated polysaccharide-iron complexes (formed from a 21.25 kDa polysaccharide) that had an iron content of 250 μ g/mg [12].

A high-calorie diet rich in fat and refined sugar is believed to be responsible for the rising prevalence of T2DM [23]. Patients with T2DM can have elevated blood glucose levels, which typically result in even higher insulin values [24]. Impaired glucose tolerance and a gradual loss of β -cell function have also been observed in patients with T2DM [25]. In this study, we found that administration of SRPC (10 mg/kg and 30 mg/kg body mass per day) had a potent hypoglycemic effect on HFSD-induced T2DM in mice. Furthermore, SRPC—but not SRP

alone—improved an oral glucose tolerance metric, relieved insulin resistance (in a blood test), attenuated body mass gain, and increased glycogen content of the liver and muscle. These results suggest that chromium(III) in SRPC has a strong therapeutic effect on T2DM.

Metal ions have been suggested as nutritional interventions in the form of an adjunctive therapy to pharmacological treatment of T2DM [26]. Chromium(III), one of the most common metals on Earth, has gained popularity as a nutritional supplement for diabetic patients [4]. It enhances glucose metabolism by stimulation of insulin action in peripheral tissues. Chromium(III)-supplementation benefits patients with comorbid T2DM and glucose intolerance, it also reverses obesity and insulin resistance caused by a high-fat diet [27]. Chromium is absorbed by passive diffusion and then binds to the iron-transport protein in the blood. Transferrin delivers chromium to the tissues where it binds to a low-molecular-weight organic species that is cleared from the tissues [28].

There are various theories about the molecular basis of the insulin-like effects of chromium: from direct interaction of chromium with insulin to upregulation of insulin receptor amounts by chromium [27]. Studies have shown that the action of chromium involves a direct effect on a number of receptors accessible to insulin, their affinity to the hormone, and modulation of the signal-multiplying proteins by their phosphorylation [10]. Chromium supplementation in animals that are insulin-resistant for either genetic or nutritional reasons indicates that chromium potentiates the actions of insulin, augments the PI3K/PKB insulin signaling pathway, suppresses the negative regulators of insulin signaling, enhances AMPK activity, and attenuates oxidative stress [30].

Typically, the PI3K/PKB signaling pathway in diabetic mice is underactive as compared to normal mice, and its stimulation is accompanied by alleviation of insulin resistance [10]. Chromium has been reported to enhance the kinase activity of IR- β and to increase the activity of downstream effectors of insulin signaling: PI3K and PKB [30]. Glycogen synthesis in the liver is an important mechanism of blood glucose homeostasis and metabolism, regulated via the PI3K/PKB/GSK-3 β pathway [31]. In the present study, SRPC significantly increased the mRNA expression of genes IR, IRS-2, PI3K, PKB, and decreased GSK3 β mRNA expression in the liver of T2DM mice. Glycogen transport regulated by the PI3K/PKB/GLUT pathway is the main route of glucose disposal, and skeletal muscle was analyzed here to investigate the mechanism of action of SRPC on hyperglycemia. SRPC up-regulated GLUT4 at the transcriptional level in skeletal muscle by activating the insulin-dependent IR/IRS-1/PI3K/PKB/GLUT4 signaling cascade. This

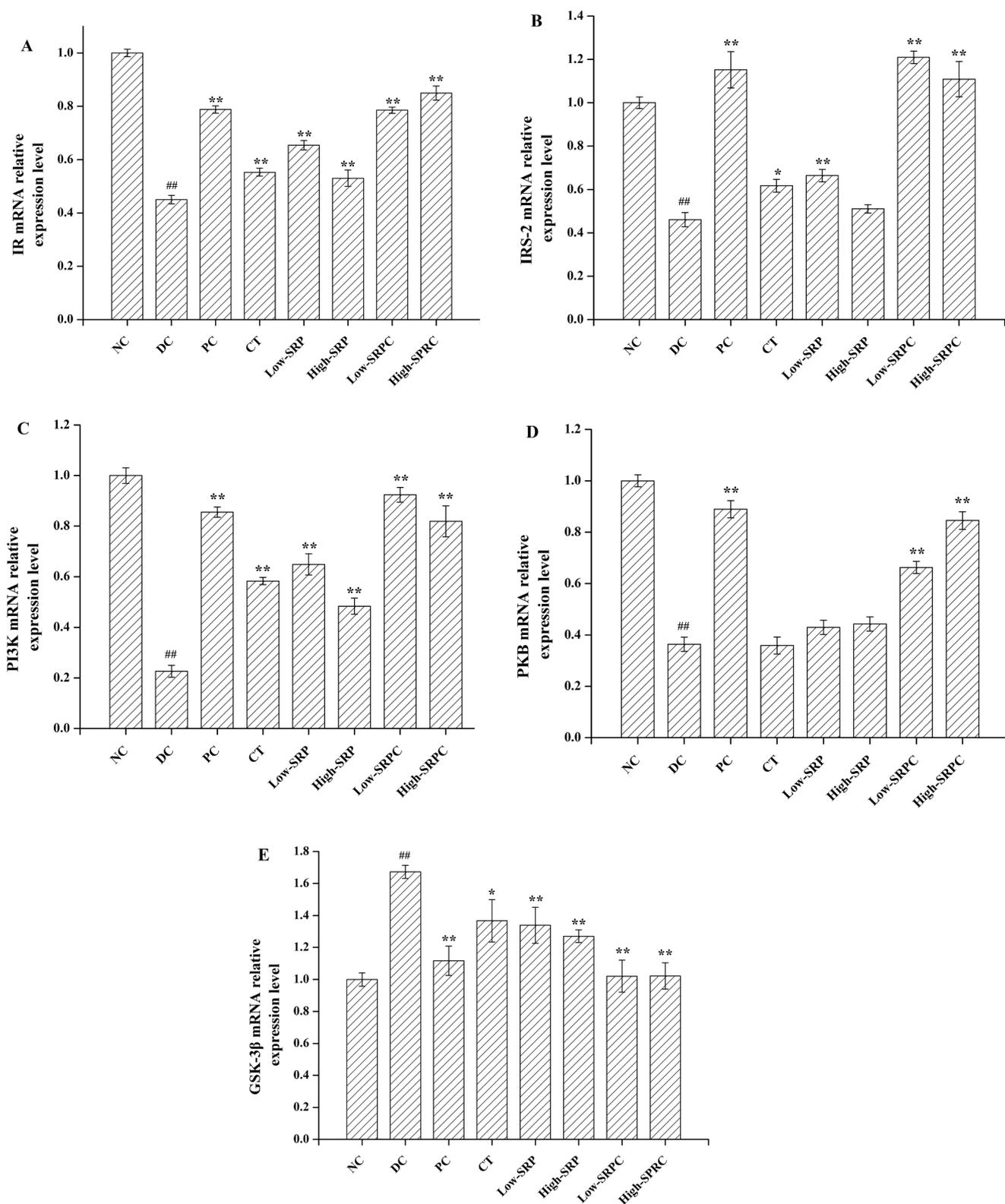


Fig. 7. Effects of SRPC on the mRNA expression levels of IR, IRS-2, PI3K, PKB and GSK-3 β in the liver of T2DM mice (n = 9). β -Actin served as the loading control. (A) IR mRNA relative expression level. (B) IRS-2 mRNA relative expression level. (C) PI3K mRNA relative expression level. (D) PKB mRNA relative expression level. (E) GSK-3 β mRNA relative expression level. ^{##}p < 0.01 relative to the control group, ^{*}p < 0.01 relative to the model group, ^{**}p < 0.05 relative to the model group.

finding revealed that SRPC improved glucose homeostasis by activating the insulin-dependent PI3K/PKB signaling pathway, and this result is consistent with a direct action of chromium on insulin signaling. The beneficial effects of different chromium complexes in animal models of

diabetes and insulin resistance have been reported elsewhere. In a nutritional model of insulin resistance in which mice were fed a high-sucrose diet or a high-fat diet, a triphenylalaninate chromium complex activated the PI3K/PKB signaling pathway and improved hepatic

glucose metabolism [32,33]. In obese insulin-resistant rats, dietary supplementation with chromium picolinate restores insulin resistance by modulating the expression of PI3K, PKB, and GLUT4 [34], in line with our current results. These data confirm a significant role of the PI3K/PKB signaling pathway in the regulation of glucose metabolism in T2DM.

In several animal and human studies, the chromium complex of

picolinic acid, the most popular dietary supplement, has been demonstrated to modulate intracellular pathways of glucose metabolism [29]. This compound is promising therapeutically because it can increase insulin activity and slow the development of T2DM [6]. In the present study, chromium picolinate was employed in the positive control group to demonstrate whether SRPC could achieve the same therapeutic effect on T2DM. Chromium trichloride hexahydrate was applied in the

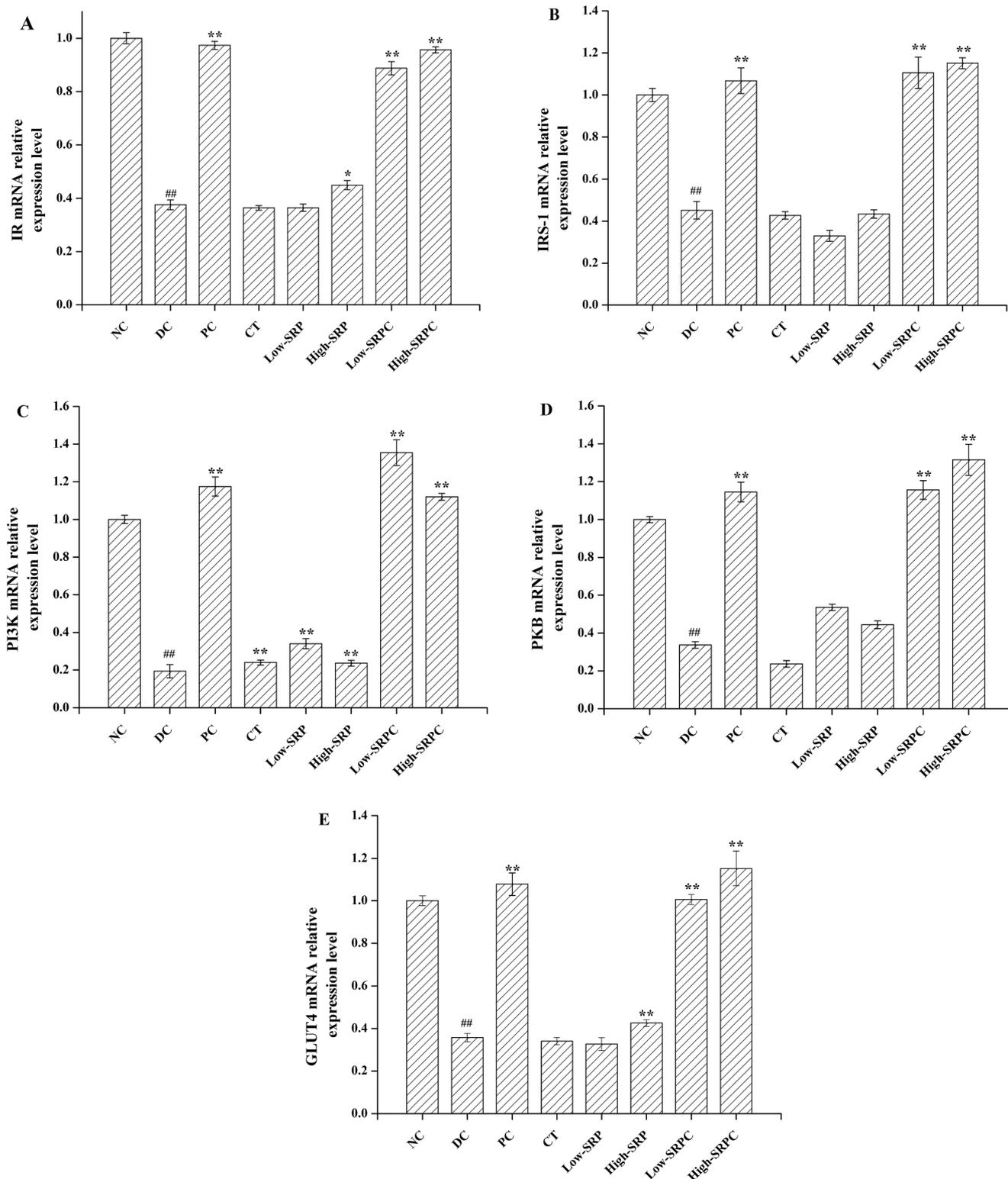


Fig. 8. Effects of SRPC on the mRNA expression levels of IR, IRS-1, PI3K, PKB, and GLUT4 in the muscle of T2DM mice (n = 9). β -Actin served as the loading control. (A) IR mRNA relative expression level. (B) IRS-1 mRNA relative expression level. (C) PI3K mRNA relative expression level. (D) PKB mRNA relative expression level. (E) GLUT4 mRNA relative expression level. ## p < 0.01 relative to the control group, ** p < 0.01 relative to the model group.

negative control group to rule out reagent interference. We confirmed that SRPC at both 280 $\mu\text{g}/\text{d}$ and 840 $\mu\text{g}/\text{d}$ had the same significant therapeutic effect on T2DM mice as did chromium picolinate at 840 $\mu\text{g}/\text{d}$. Martin J. has reported that chromium picolinate can improve glycemia, attenuate body mass gain, and enhance insulin sensitivity when Cr supplementation is at 1000 $\mu\text{g}/\text{d}$ in subjects with T2DM [35]. Ewelina et al. have reported that the chromium propionate complex given orally at doses of 10 or 50 mg/kg body mass per day restored insulin sensitivity and normalized β -cell function in rats with insulin resistance induced by a high-fructose diet [8]. Low doses of SRPC here exerted better anti-diabetic effects, suggesting that SRPC could be a good candidate for a functional food or drug against T2DM.

5. Conclusions

In this study, a low-molecular-weight SRP was obtained from *E. proliferifera* and used for the synthesis of SRPC. The molecular mass of SRPC was found to be 4.57 kDa, and the chromium content was 28 $\mu\text{g}/\text{mg}$. The results in T2DM mice suggest that SRPC (10 mg/kg and 30 mg/kg body mass per day) exerts a significant anti-hyperglycemic effect by improving blood glucose homeostasis in T2DM mice. The insulin resistance of diabetic mice is restored after SRPC treatment, potentially through the PI3K/PKB signaling pathway. In addition, attenuated body mass gains and increased hepatic glycogen levels confirm that SRPC may reverse the problems in glucose metabolism induced by hyperglycemia in the T2DM mice. Thus, SRPC holds promise as a stable and efficient nutraceutical for the treatment of T2DM.

Competing financial interests

The authors declare that they have no competing financial interests.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.bioorg.2019.102942>.

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