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Hypoglycemic Effects of Glycosaminoglycan from *Urechis unicinctus* in Diabetic Mice

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ABSTRACT To further utilize glycosaminoglycan from *Urechis unicinctus*, the hypoglycemic effect and possible mechanism of glycosaminoglycan on diabetic mice were evaluated. Diabetes was induced in mice by intraperitoneal injections of streptozotocin for 3 consecutive days and fed with high-sugar and high-lipid fodder. After diabetes was confirmed, the hypoglycemic effect of glycosaminoglycan from *U. unicinctus* was investigated in the diabetic mice. Results demonstrated that glycosaminoglycan could significantly decrease blood glucose concentrations, HOMA-IR, AUG, and liver MDA content in diabetic mice. In addition, it significantly enhanced liver SOD and GSH-Px activity, as well as liver GCK activity and hepatic glycogen levels. Glycosaminoglycan from *U. unicinctus* exhibited efficacy against diabetes, suggesting its potential use as a natural intervention against diabetes.

KEY WORDS: • ***diabetic mice*** • ***glycosaminoglycan*** • ***hypoglycemic effect*** • **Urechis unicinctus**

INTRODUCTION

DIABETES IS A CHRONIC metabolic syndrome, characterized by a relative lack of insulin secretion and/or profound insulin resistance and disturbance of carbohydrate metabolism. It is now the third major disease following cardiovascular diseases and tumors and has become a major threat to human health.[1] Clinical treatment commonly utilizes drugs, including insulin secretion enhancers, insulin sensitizers, and drugs that can reduce carbohydrate absorption.[2] These drugs are associated with varying degrees of side effects, such as hypoglycemia, lactic acidosis, and ketoacidosis.[3] Therefore, extracting hypoglycemic substances from natural biological resources has become the mainstream of the development of new hypoglycemic drugs.

In recent years, pharmacological studies have shown that polysaccharides can reduce high blood sugar levels by clearing excessive free radicals in organisms, improving the antioxidant capacity of organisms, and reducing islet cell damage.[4,5] Some scholars have conducted substantial works on hypoglycemic effects of polysaccharides from plants and obtained remarkable results.[6–15] However, there are fewer studies on the hypoglycemic effects of polysaccharides from animals.[16,17] In the present study, we induced diabetes in mice and treated them with different doses of glycosaminoglycan from *Urechis unicinctus* (a species of marine spoon worm commonly eaten in Asia) to investigate its hypoglycemic effect in diabetic mice and discuss its possible mechanism.

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MATERIALS AND METHODS

Preparation of glycosaminoglycan from U. unicinctus

U. unicinctus was cleaned and made into homogenates with the addition of water. The subtilisin was added into the homogenate for 5 h. The enzymatic hydrolysate was then centrifuged to separate the supernate. The supernate was decolorized with hydrogen peroxide and centrifuged. After adjusting the pH, neutral proteins were removed by centrifugation. The supernate was precipitated with alcohol (the final concentration of ethyl alcohol was 70%) to obtain crude glycosaminoglycan. The purified glycosaminoglycan was obtained by chromatography on a DEAE anion-exchange column (2.6–90 cm). The relative molecular weight of glycosaminoglycan was 450.032 kDa, with 28.37% of sulfate radical content, 24.81% of uronic acid content, and 7.83% of amino sugar content.

Experimental animals

Specific pathogen-free (SPF) Kunming mice weighing 20.12 ± 1.03 g were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd., with the license number of SCXK (Jing) 2012-0001.

Induction of diabetes in mice

After normal feeding for 1 week, mice were administered with intraperitoneal injections of streptozotocin (STZ, 35 mg/kg) for 3 consecutive days and fed with high-sugar and high-lipid fodder. Four weeks later, the blood glucose level after fasting for 8 h was measured, and the mice with blood glucose >15 mM were considered diabetic and selected for the study.

Experimental design

Mice were divided into six groups, including the normal group, the model control, high-, middle-, low-dose groups, and positive control. Each group had 20 mice. High-, middle-, and low-dose groups were treated with intragastric administration of glycosaminoglycan, with the dose of 300, 200, and 100 mg/kg per day, respectively (glycosaminoglycan doses were chosen according to pre-experimental data); the normal group and the model control were treated with intragastric administration of isometric normal saline; and the positive controls were treated with intragastric administration of 200 mg/kg metformin hydrochloride (provided by Tianjin Pacific Chemical & Pharmaceutical Co., Ltd., State Approval Number H12020797). The treatments lasted for 4 weeks.

Effects of glycosaminoglycan on the blood and liver indexes of diabetic mice

On the 29th day, after fasting for 8 h, 10 mice were randomly chosen from each group, and their eyeballs were enucleated to collect blood for blood glucose and serum insulin determination. Mice were sacrificed by cervical dislocation and their livers were instantly removed into precooled normal saline in a ratio of 1:10 w/v, homogenated and centrifuged at 4000 rpm for 15 min. The supernatant was obtained for liver index detection.

Determination of blood glucose and serum insulin

A glucose assay kit (provided by Nanjing Jiancheng Bioengineering Institute) was used to measure the blood glucose levels. The percentage of hypoglycemic rate could be calculated as follows:

Hypoglycemic Rate (HR%) = [(initial blood glucose − final blood glucose)/initial blood glucose] × 100.

The serum insulin was estimated using a radioimmunoassay kit (provided by Beijing Lvyuan Bode Biotechnology Co., Ltd.), and insulin resistance in homeostatic model assessment (HOMA-IR) was calculated according to the following formula.[18]

HOMA-IR = (fasting insulin × fasting glucose)/22.5

Determination of superoxide dismutase activity

The superoxide dismutase (SOD) activity was measured using the SOD kit (provided by Nanjing Jiancheng Bioengineering Institute), and homogenate protein concentration in the liver was measured by the Coomassie brilliant blue (CBB) method. The SOD activity was calculated as follows:

SOD activity (U/mgprot) = [(control tube OD − measuring tube OD)/control tube OD] ÷ 50% × (total volume of reaction liquid/sampling amount) ÷ protein concentration

Determination of malondialdehyde concentration

The malondialdehyde (MDA) concentration was measured using an MDA kit (provided by Nanjing Jiancheng Bioengineering Institute), and the liver homogenate protein was measured by the CBB method. The MDA concentration in the liver was calculated as follows:

MDA concentration (nmol/mgprot) = [(measuring tube OD − control tube OD)/(standard tube OD − blank tube OD)] × standard substance concentration ÷ protein concentration

Determination of glutathione peroxidase activity

The glutathione peroxidase (GSH-Px) activity was measured using the GSH-Px kit (provided by Nanjing Jiancheng Bioengineering Institute), and the liver homogenate protein was measured by the CBB method. The GSH-Px activity unit was defined as follows: per mg protein, after deducting the effect of nonenzymatic reaction per minute, the glutathione concentration in the reaction system was decreased by 1 μM as an enzyme activity unit. The equation was as follows:

GSH-Px activity (U/mgprot) = (nonenzymatic tube OD − enzymatic tube OD)/(standard tube OD − blank tube OD) × standard tube concentration × dilution multiple ÷ reaction time) ÷ (sampling amount × protein concentration)

Determination of glucokinase content

The glucokinase (GCK) content was measured using the double antibody sandwich ELISA method in accordance with the instructions of the GCK ELISA kit (provided by Beijing Lvyuan Bode Biotechnology Co., Ltd.).

Determination of hepatic glycogen content

The hepatic glycogen content was measured using a glycogen assay kit (provided by Nanjing Jiancheng Bioengineering Institute), the equation was as follows:

Hepatic glycogen content (mg/g) = (measuring tube OD/standard tube OD) × standard tube content × dilution multiple × 10 ÷ 1.11

Glucose tolerance test in diabetic mice

The remaining 10 mice in each group were prepared for the glucose tolerance test. Before the last intragastric administration of glycosaminoglycan, mice were fasted for 8 h (not including water), and blood was collected from caudal veins to measure fasting blood glucose (0 h). One hour after intragastric administration, each group was intragastrically administered 2 g/kg glucose solution and the blood glucose was determined after 30, 60, and 120 min. The calculation equation for area under curve (AUC) was as follows:

AUC = 0.5 × 0 h blood glucose + 0.5 h blood glucose + 1.5 × 1 h blood glucose + 2 h blood glucose

Table 1. Effect of Glycosaminoglycan on Blood Glucose in Diabetic Mice (Mean ± SD)

Group	*Dose (mg/kg)*	*Animal number*	*Initial blood glucose (mM)*	*Final blood glucose (mM)*	*Hypoglycemic rate (%)*
Normal	—	10	5.73 ± 0.52	5.77 ± 0.65	—
Model control	—	10	17.97 ± 3.53**	18.65 ± 2.84**	—
Metformin hydrochloride	200	10	17.93 ± 2.77	11.02 ± 2.10^{aa}	38.74 ± 3.46
GAG-1	100	10	17.88 ± 3.32	13.92 ± 2.36^{a}	21.88 ± 6.52^{bb}
GAG-2	200	10	17.72 ± 3.34	12.80 ± 3.36^{aa}	28.42 ± 6.27^{b}
GAG-3	300	10	18.05 ± 3.26	11.90 ± 2.50^{aa}	34.01 ± 8.02

**$P < .01$ as the model control group compared with the normal group.
$^{a}P < .05$, $^{aa}P < .01$ as compared to the model control group.
$^{b}P < 0.05$, $^{bb}P < 0.01$ as compared to the positive control group.

Statistical analysis

All data were expressed as mean ± SD. Statistical analysis was carried out using the F-test (one-way ANOVA) followed by Dunnett's test. Values were considered to be significantly different when the P value was less than .05.

RESULTS

Effects of glycosaminoglycan on blood glucose in diabetic mice

After 28 days of intragastric administration of glycosaminoglycan, the mice were fasted for 8 h and their blood was collected by caudal vein to measure the blood sugar level (Table 1). During the experiment, the fasting blood glucose levels of the normal mice did not change greatly and that of the model control mice were maintained at a relatively high level, which was significantly higher compared with the normal group ($P < .01$); at the end of the experiment, the blood glucose level of the different doses of glycosaminoglycan groups and the positive control was significantly lower compared with the model control ($P < .05$, $P < .01$), showing that glycosaminoglycan from *U. unicinctus* had a remarkable hypoglycemic effect on diabetic mice. Furthermore, the hypoglycemic rate of low and middle glycosaminoglycan dose groups was significantly lower compared with the positive controls ($P < .05$, $P < .01$) and that of the high glycosaminoglycan dose group was obviously lower than the 'positive control, but did not reach a significant level. This indicated that the hypoglycemic effect of glycosaminoglycan was slightly poorer compared with metformin hydrochloride.

Effects of glycosaminoglycan on serum insulin of diabetic mice

Effects of glycosaminoglycan on serum insulin of diabetic mice are shown in Table 2. The insulin level and HOMA-IR in the model control mice were significantly higher compared with the normal mice ($P < .05$, $P < .01$). The insulin levels of glycosaminoglycan dose groups and the positive control were lower compared with the model control, but were not statistically significant. HOMA-IR among the four groups of diabetic mice was significantly lower compared with the model control mice ($P < .01$), indicating that model mice were profoundly insulin resistant, whereas glycosaminoglycan and metformin hydrochloride could relieve the insulin resistance of mice, thus lowering insulin levels.

Effects of glycosaminoglycan on antioxidant index of diabetic mice

The SOD and GSH-Px activity in the model control was significantly lower compared with the normal group ($P < .01$), while the MDA concentration was significantly higher compared with the normal group ($P < .01$); the SOD and GSH-Px activity of the glycosaminoglycan (100 mg/kg)

Table 2. Effect of Glycosaminoglycan on Serum Insulin in Diabetic Mice (Mean ± SD)

Group	*Insulin (mU/L)*	*HOMA-IR*
Normal	6.54 ± 0.97	1.67 ± 0.27
Model control	8.70 ± 1.62*	7.09 ± 0.83**
Metformin hydrochloride	7.41 ± 1.59	3.60 ± 0.88^{aa}
GAG-1	7.34 ± 1.37	4.55 ± 1.13^{aa}
GAG-2	7.19 ± 1.20	4.21 ± 1.84^{aa}
GAG-3	7.03 ± 1.50	3.76 ± 1.29^{aa}

*$P < .05$, **$P < .01$ as the model control group compared with the normal group; $^{aa}P < 0.01$ as compared to the model control group.

Table 3. Effect of Glycosaminoglycan on Liver Antioxidant Activity in Diabetic Mice (Mean ± SD)

Group	*SOD (U/mgprot)*	*GSH-Px (U/mgprot)*	*MDA (nmol/gprot)*
Normal	349.13 ± 49.50	869.60 ± 93.87	13.18 ± 3.67
Model control	196.33 ± 64.64**	437.91 ± 58.64**	25.41 ± 4.61**
Metformin hydrochloride	260.69 ± 67.99	559.66 ± 79.83	18.90 ± 2.41^{a}
GAG-1	243.86 ± 70.95	568.50 ± 81.42^{a}	19.15 ± 4.60^{a}
GAG-2	290.19 ± 55.41^{a}	616.83 ± 86.40^{a}	16.90 ± 3.07^{aa}
GAG-3	317.69 ± 52.97^{aa}	668.27 ± 85.46^{aa}	15.79 ± 2.96^{aa}

**$P < .01$ as the model control group compared with normal group; $^{a}P < 0.05$, $^{aa}P < .01$ as compared to the model control group.
SOD, superoxide dismutase.

Table 4. Effect of Glycosaminoglycan on Liver Glycogen and Glucokinase in Diabetic Mice (Mean ± SD)

Group	*Glucokinase (pg/mL)*	*Liver glycogen (mg/g)*
Normal	860.53 ± 123.39	10.07 ± 1.26
Model control	479.34 ± 68.64**	6.72 ± 0.87**
Metformin hydrochloride	641.32 ± 96.44[a]	9.01 ± 1.30[aa]
GAG-1	574.59 ± 103.05	7.80 ± 1.05
GAG-2	608.76 ± 49.37	8.41 ± 1.20
GAG-3	630.94 ± 104.71[a]	8.87 ± 1.26[a]

**$P < .01$ as the model control group compared with the normal group; [a]$P < .05$, [aa]$P < .01$ as compared to the model control group.

group and metformin hydrochloride (200 mg/kg) group was obviously higher compared with the model control, but not significantly. The GSH-Px activity of the glycosaminoglycan (100 mg/kg) group was significantly higher compared with the model control. The SOD and GSH-Px activity of glycosaminoglycan (200, 300 mg/kg) groups was significantly higher compared with the model control ($P < .05$, $P < .01$). The MDA concentration of glycosaminoglycan (100 mg/kg) group and metformin hydrochloride group was significantly lower compared with the model control. The MDA concentration of glycosaminoglycan (200, 300 mg/kg) groups was significantly lower compared with the model control ($P < .01$), demonstrating that glycosaminoglycan from *U. unicinctus* had better antioxidant ability in diabetic mice than metformin hydrochloride (Table 3).

Effects of glycosaminoglycan on hepatic glycogen and GCK in diabetic mice

The GCK and hepatic glycogen content of the model control was significantly low compared with the normal group ($P < .01$, Table 4). The GCK and hepatic glycogen content of glycosaminoglycan (100, 200 mg/kg) was remarkably higher compared with the model control, but was not significant. The GCK content of glycosaminoglycan (300 mg/kg) group and metformin hydrochloride (200 mg/kg) group was significantly higher compared with the model control. The hepatic glycogen content of high glycosaminoglycan dose group was significantly higher compared with the model control. The hepatic glycogen content of the positive control was significantly higher compared with the model control ($P < .01$).

Effects of glycosaminoglycan on oral glucose tolerance in diabetic mice

As shown in Table 5, blood glucose levels of all experimental groups of mice first increased and then decreased within 2 h of oral administration of glucose. The blood glucose level in the model control was significantly higher compared with the normal group after 0, 30, 60, and 120 min of oral administration of glucose ($P < .01$); the blood glucose level of the positive control, high-, middle-, and low-dose glycosaminoglycan groups was significantly lower compared with the model control before oral administration of glucose ($P < .01$). Thirty minutes after oral administration of glucose, the blood glucose level of low-dose glycosaminoglycan group was significantly lower compared with the model control. Sixty and 120 min after the oral administration of glucose, the blood glucose level of the different doses of glycosaminoglycan groups and the positive control group was significantly lower compared with the model control ($P < .05$, $P < .01$); AUC of the model control was significantly higher compared with the normal group ($P < .01$), AUC of the positive control and the different doses of glycosaminoglycan groups were significantly lower compared with the model control ($P < .05$, $P < .01$). This indicated that glycosaminoglycan and metformin hydrochloride could enhance blood glucose regulation and glucose tolerance in diabetic mice.

DISCUSSION

Normally, experimental animals can be given a small amount of STZ to destroy a part of the islet β-cell function and fed with high-fat fodder so as to make peripheral tissues insensitive to insulin. These treatments may induce the animal models close to the Type 2 diabetes of humans.[19] In the present study, healthy mice were administered with intraperitoneal injections of streptozotocin for 3 consecutive days and fed with high-sugar and high-lipid fodder. In this way, we successfully induced diabetic mice models. We found that the blood glucose concentrations and insulin levels in the model mice were significantly higher compared with the normal group, indicating that profound insulin resistance, which may be attributed to excessive intake of carbohydrates or fats, could cause the body's resistance to insulin and the need for more compensatory insulin secretion in islet β cells.[20] On this basis, effects of glycosaminoglycan from *U. unicinctus* on glucose levels, serum insulin, and oral glucose tolerance of diabetic mice were

Table 5. Effect of Glycosaminoglycan on Oral Glucose Tolerance in Diabetic Mice (Mean ± SD)

Group	*0 min*	*30 min*	*60 min*	*120 min*	*AUG*
Normal	5.82 ± 0.47	7.54 ± 0.73	8.42 ± 0.63	6.85 ± 0.85	14.96 ± 1.26
Model control	19.03 ± 3.49**	25.05 ± 2.35**	31.03 ± 3.30**	22.05 ± 2.49**	51.27 ± 3.90**
Metformin hydrochloride	11.87 ± 2.62[aa]	22.86 ± 3.28	17.95 ± 2.68[aa]	13.36 ± 3.09[aa]	34.54 ± 5.72[aa]
GAG-1	14.10 ± 2.40[aa]	21.08 ± 2.51[a]	26.83 ± 3.67[a]	16.97 ± 3.34[a]	42.67 ± 6.22[a]
GAG-2	12.92 ± 2.54[aa]	24.00 ± 2.90	19.06 ± 2.44[aa]	14.88 ± 2.84[aa]	36.92 ± 4.46[aa]
GAG-3	11.96 ± 2.24[aa]	23.07 ± 2.82	17.90 ± 1.90[aa]	13.93 ± 2.67[aa]	34.91 ± 4.56[aa]

**$P < .01$ as the model control group compared with the normal group; [a]$P < .05$, [aa]$P < .01$ as compared to the model control group.

studied, verifying that glycosaminoglycan could significantly reduce blood sugar levels, HOMA-IR, and AUC in diabetic mice. It obviously improved the glucose tolerance of diabetic mice, eased the symptoms of diabetic mice, and showed good therapeutic effects in diabetic mice.

Insulin resistance leads to decreased blood glucose and blood lipid utilization; the excess carbohydrate and free fatty acid *in vivo* produce lots of free radicals by oxidation itself, cause oxidative stress, and damage the body's cells and tissues.[21,22] Some scholars believed that excessive free radical production may induce diabetes, and a series of complications caused by diabetes are closely related with free radicals.[23] SOD is an important enzyme scavenging oxygen free radical and it directly reflects the antioxidant level of organisms. MDA is one of the main products of lipid peroxidation caused by a free radical and its content can indirectly impair the organisms' antioxidant capacity and scavenging of oxidative products. GSH-Px is an important enzyme of peroxide extensively existing in organisms, which can catalyze glutathione into oxidized glutathione, thus protecting the structure and function of the cell membrane from interference and peroxidative damage. Based on our findings, glycosaminoglycan from *U. unicinctus* can significantly improve SOD and GSH-Px activity in the liver of diabetic mice and significantly decrease MDA concentrations, showing a good antioxidant capacity. It was inferred that the glycosaminoglycan could effectively repair the liver of diabetic mice and therefore obviously improve the GCK and hepatic glycogen content of diabetic mice. Therefore, glycosaminoglycan has a good potential for market development of a natural medicinal product.

In conclusion, glycosaminoglycan from *U. unicinctus* has hypoglycemic effects and the mechanism may be as follows: glycosaminoglycan can improve antioxidant capacity of diabetic mice, repair liver and pancreas tissue, enhance the glucose tolerance and insulin sensitivity index, increase liver glycogen, and decrease blood glucose in diabetic mice.

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AUTHOR DISCLOSURE STATEMENT

No competing financial interests exist.

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