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Protective effects of sodium selenite on insulin secretion and diabetic retinopathy in rats with type 1 diabetes mellitus

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Abstract: The aim of the present study was to determine the effects of sodium selenite on pancreatic β cells and diabetic retinopathy in type 1 diabetes mellitus (T1DM) rats. Diabetes was induced by administration of streptozotocin (STZ) and both diabetic and control animals were treated with sodium selenite to measure body weight, food and water intake as well as blood glucose level. Additionally, immunohistochemistry was performed to detect the levels of insulin secretion in pancreatic β cells. Apoptosis level of pancreatic cells in rats was determined by apoptosis kit. Retinal tissues were stained with hematoxylin-eosin and the area of retinal capillary was measured by Image-Pro Plus 6.0 software. Food and water intake coupled with blood glucose level were increased while body weight of rats was decreased in STZ group. After treatment with sodium selenite, High-Sel group and Low-Sel group showed decreased food intake coupled with blood glucose level and concomitantly increased body weight (vs. STZ group). Of note, the insulin secretion in pancreatic β cells as well as serum insulin levels were strikingly heightened while apoptosis level of pancreatic tissues was lowered in the High-Sel group (vs. STZ and Low-Sel groups). Additionally, both High-Sel and Low-Sel groups showed a small area of retinal capillary (vs. STZ group). Sodium selenite could promote the levels of insulin secretion in pancreatic β cells of T1DM rats, and concomitantly ameliorate diabetic retinopathy.

Keywords: Diabetes, sodium selenite, insulin, pancreatic β cells.

INTRODUCTION

Diabetes mellitus (DM), mainly including type 1 diabetes and type 2 diabetes, is a chronic complicated metabolic disease (B, 2012; Jangir & Jain, 2014). Importantly, type 1 DM (T1DM) is an autoimmune disease characterized by the progressive destruction of β-pancreatic cells and the insufficiency of insulin production (Groele *et al.*, 2017; Zare Javid *et al.*, 2020). Besides, T1DM, known as insulin-dependent DM, mostly occurs in early childhood or adolescence (Kondrashova & Hyoty, 2014; Endesfelder *et al.*, 2016). Patients with T1DM have traditionally been treated with long-term insulin injections to maintain normal blood glucose levels, however, insulin injection therapy can only temporarily lower blood glucose levels, and cannot alleviate DM complications such as nephropathy, neuropathy, and retinopathy (Hsiao *et al.*, 2020). Therefore, it is urgent to find new therapeutic targets to improve the treatment of T1DM patients.

Selenium (Se) is an essential trace element for humans and animals, which is needed for a broad variety of physiological functions including thyroid hormone metabolism, protection against oxidative stress, and immunity associated functions (Kedzierska *et al.*, 2017). Diabetic retinopathy is a common complication of DM that is triggered by multiple factors including reactive oxygen species (Cecilia *et al.*, 2019). Previous study have shown that Se may have potential therapeutic use in treating diabetes due to its anti-oxidative stress activity and the regulation of glucose transport and glycometabolism (Lu *et al.*, 2016). Sodium selenite is a common dietary form of Se, which is recognized as essential to animal and human nutrition (Shilo *et al.*, 2003). The amelioration of serum, liver and kidney antioxidant enzymes activities by sodium selenite in diabetic rats has been reported (Ahmadvand *et al.*, 2014). However, the effects of sodium selenite on diabetic retinopathy have not been elucidated. Therefore, the present study was designed to investigate the effect of sodium selenite on diabetic retinopathy in streptozotocin (STZ)-induced rats.

MATERIALS AND METHODS

Establishment of T1DM model

Male Sprague-Dawley (SD) rats (200~250g) were purchased from Jinan Pengyue Experimental Animal Breeding Co., Ltd. (Shandong, China). The animals were acclimated and maintained in an environmentally controlled breeding room (temperature, 25°C; humidity, 55%; light/dark cycle, 12 h). They were group-housed in cages and had free access to water and food. This study was approved by the ethics committee of Shandong First Medical University & Shandong Academy of Medical Sciences (#2019022). All efforts have been made to minimize the suffering of rats.

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All rats fasted for 10 h after 1 week feeding. Then, STZ (Solarbio Science and Technology Corporation, Beijing, China) was dissolved in a 0.1 M saline-citrate buffer (pH 4.5) and rats were subjected to intraperitoneal injection of 55 mg/kg STZ. Control rats were injected with comparable volumes of vehicle (saline-citrate buffer).

One week after STZ-induced model establishment, rats were randomly divided into 3 groups: STZ group, Low-Sel group (1 mg/kg/day) and High-Sel group (2 mg/kg/day). Briefly, sodium selenite (Sigma-Aldrich, Merck KGaA, Darmstadt, Germany) was dissolved in distilled water and then injected into rats at 9 am after they fasted overnight and were weighed. Rats in Low-Sel group and High-Sel group separately received sodium selenite (1mg/kg/day) and sodium selenite (2mg/kg/day). Rats in Control group and STZ control group received distilled water.

Measurement of body weight, food and water intake, blood glucose and serum insulin levels in rats

After fasting for 10 h, blood glucose levels of rats were tested with glucometer (ACCU-CHEK), and then the changes in body weight and fasting blood glucose levels were measured every 6 days. Food and water consumptions per cage were record every day throughout the experiment (3 ~ 4 rats/cage), and the average value was calculated every 6 days. The diet during fasting was not measured. After the injection of sodium selenite for 30 days and fasting for 12 h, rats were anesthetized with ether and blood samples were collected. After centrifugation, 2 μL of serum from each rat was collected and sent to Taian City Central Hospital (Shandong Province, China) for insulin level detection. Thereafter, eyeball and pancreas tissues were collected and fixed in 4% paraformaldehyde for 24h. After dehydration, samples were embedded in paraffin and sliced into sections of 5~6μm in thickness.

Immunohistochemistry (IHC)

The sections of rat pancreatic tissues were dewaxed and rehydrated, followed by incubation overnight with insulin antibody (ab181547, abcam, Cambridge, UK) at 4°C. After being washed with PBS three times, sections were incubated with the secondary antibody at room temperature for 1 h. The reaction was terminated after DAB staining for 1~3 min after PBS washing (three times). Nuclei were stained with hematoxylin for 3 min. After dehydration, transparentization and mounting processes, sections were observed and photographed under an optical microscope. Three fields were taken from each section of which the optical density and area were measured by IPP6.0 software. Mean optical density (MOD) values were calculated as follows: MOD =integrated optical density/area. The data were normalized as a basis for evaluating insulin secretion in pancreatic β cells.

Following dewaxing and rehydration, sections were stained with apoptosis kit (Roche, 11684817910) and observed under an optical microscope. Each section was photographed in 3 fields at random and IPP6.0 software was used to detect the optical density and area. MOD = integrated optical density/area. The data were normalized to evaluate the apoptosis level of pancreatic tissues.

H&E staining

Rat retinal tissues were digested with proteinase K, and then mounted onto glass slides for H&E staining. After that, tissues were observed and photographed under an optical microscope. Finally, the area of retinal capillary was determined by IPP 6.0 software.

STATISTICAL ANALYSIS

Statistical analysis was conducted using Graph Pad Prism 7 software. Statistical difference was determined by *T*-test between two sets of data, while differences among multiple groups were determined by one-way analysis of variance and verified by Tukey's post hoc test. Statistical significance was defined as $P<0.05$.

RESULTS

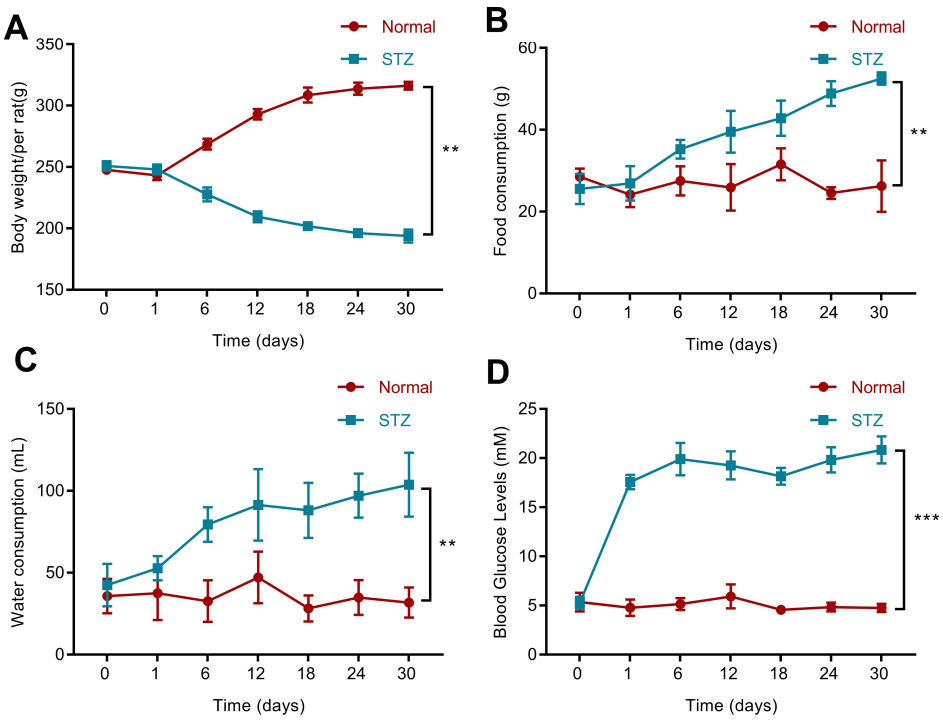
Establishment of STZ-induced T1DM in rats

T1DM rats were induced by administration of STZ. Body weight, food consumption, water intake and blood glucose level of diabetic rats were recorded and analyzed at different time points. As shown in fig. 1A, there was a reduction in body weight for rats in the STZ group as compared to the Normal group by day 6 ($P<0.05$). Moreover, the total food and water intake of the rats were measured. Results showed an increase in food and water intake in STZ group compared to the Normal group from the 6th day (fig. 1B-C, $P<0.05$). Additionally, analysis of blood glucose level in rats showed that STZ group had increased blood glucose level from day 1 as compared to Normal group (fig. 1D, $P<0.05$). Above data indicated a successful establishment of T1DM rat model.

Sodium selenite ameliorates T1DM in rats

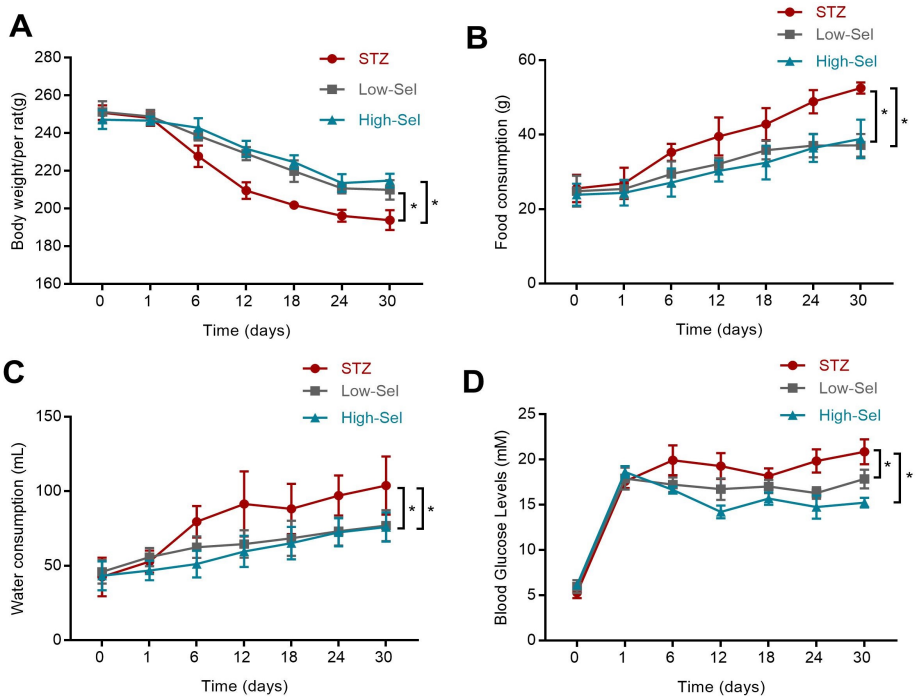
Different doses of sodium selenite were intragastrically administrated on T1DM rats. As depicted in fig. 2A, the body weight in the STZ group was clearly lower than that of Low-Sel group and High-Sel group from day 6 ($P<0.05$). Comparison between Low-Sel group and High-Sel group disclosed that rats in High-Sel group had slightly elevated body weight than Low-Sel group, but no significant difference was observed ($P>0.05$).

The measurements on food consumption of rats are shown in fig. 2B. After 6 days, STZ group had higher food consumption when compared to Low-Sel and High-Sel groups ($P<0.05$), while food consumption of rats in the High-Sel was clearly lower than in Low-Sel group ($P<0.05$). After 18 days, there was a reduction in food consumption in the Low-Sel group ($P>0.05$) and until on day 24, rats in High-Sel group had similar food consumption to that in Low-Sel group.



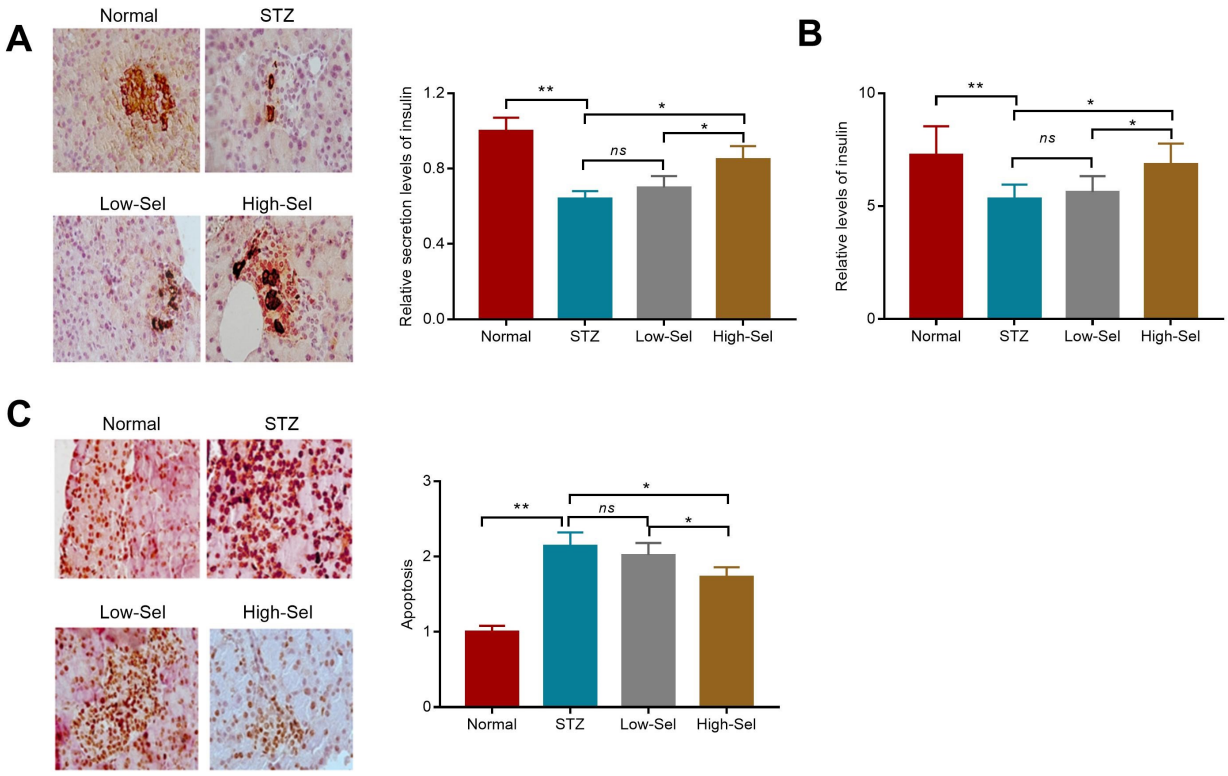
Note: After injection of STZ, body weight (A), food consumption (B), water intake (C) and blood glucose level (D) of rats were measured. Data were presented as mean ± standard deviation, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$; STZ, streptozotocin; T1DM, type 1 diabetes mellitus.

Fig. 1: STZ induces T1DM in rats



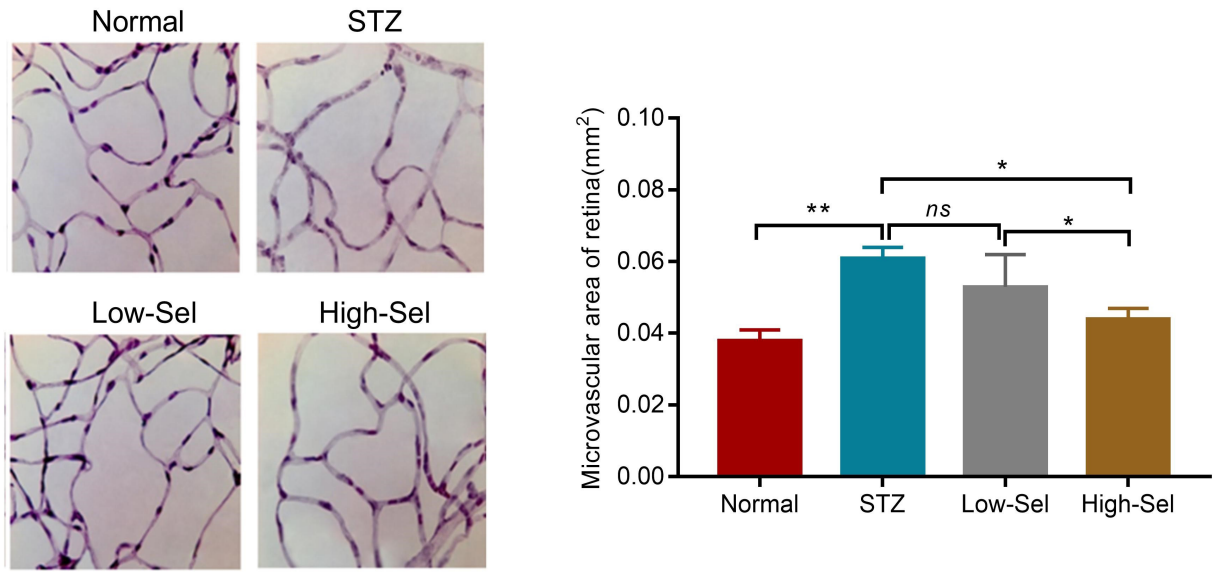
Note: Sodium selenite was intragastrically administrated to T1DM rats to determine the changes in body weight (A), food consumption (B), water intake (C) and blood glucose level (D) of rats. Data were presented as mean ± standard deviation, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$; T1DM, type 1 diabetes mellitus.

Fig. 2: Sodium selenite attenuates T1DM in rats



Note: After T1DM rats were treated with sodium selenite, IHC was performed to detect relative secretion levels of insulin (A). The serum insulin levels in rats were measured (B). Cell apoptosis level of pancreatic tissues in rats was monitored by apoptosis kit (C). Data were presented as mean ± standard deviation, $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$; T1DM, type 1 diabetes mellitus.

Fig. 3: Sodium selenite stimulates insulin secretion from pancreatic β cells



Note: Following intragastrically administration to T1DM rats by sodium selenite, the area of retinal capillaries in rats was measured. Data were presented as mean ± standard deviation, $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$; T1DM, type 1 diabetes mellitus.

Fig. 4: Sodium selenite hinders diabetic retinopathy in rat models

Fig. 2C shows water intake of rats in each group. The results manifested that STZ group had increased water intake level from day 1, which was elevated significantly from day 6 (vs. High-Sel group and Low-Sel group) ($P<$ 0.05). Additionally, comparison between High-Sel group and Low-Sel group showed that High-Sel group exhibited decreased water intake as compared to Low-Sel group from day 1 ($P<0.05$), while there was no obvious differences of water intake level between High-Sel group and Low-Sel group from day 12. Similar water intake was found between High-Sel group and Low-Sel group on day 24 ($P > 0.05$).

Detection on blood glucose level in rats after 12 days revealed that blood glucose level of rats in STZ group was obviously heightened than both High-Sel and Low-Sel groups and meanwhile High-Sel group showed reduced blood glucose level (vs. Low-Sel group) (fig. 2D, $P<0.05$). The aforementioned results indicated that sodium selenite treatment could clearly elevate the body weight and reduce food and water intake of T1DM rats. In addition, sodium selenite could also decrease the blood glucose level in T1DM rats.

Sodium selenite augments insulin secretion from pancreatic β cells

To further explore the role of sodium selenite in T1DM, different doses of sodium selenite was applied to treat T1DM rats for 30 days. As shown in fig. 3A, IHC displayed that insulin was mainly located in the cytoplasm of pancreatic β cells. Besides, relative secretion levels of insulin in the High-Sel group were significantly higher than in both STZ and Low-Sel groups ($P<0.05$), while no obvious difference was confirmed between Low-Sel group and STZ group ($P>0.05$).

Additionally, the serum insulin levels in rats were detected (fig. 3B). Results revealed that High-Sel group had distinctly elevated relative levels of serum insulin as compared to STZ group and Low-Sel group ($P<0.05$), while there was no notable difference of relative levels of insulin between Low-Sel group and STZ group ($P>0.05$). Further, we measured apoptosis level of pancreatic tissues in rats (fig. 3C). The detection revealed that the apoptosis level of pancreatic tissues in STZ group was strikingly higher than that in High-Sel group ($P<0.05$), and no significant difference was found between the Low-Sel group and STZ group ($P>0.05$). Collective data presented that high dose of sodium selenite potentiates insulin secretion in pancreatic β cells significantly as compared to low dose of sodium selenite.

Sodium selenite suppresses diabetic retinopathy in rat models

Retinopathy was noted as the most common diabetic complication in diabetic patients, which is featured by increased proliferation of retinal capillaries. In this regards, the area of retinal capillary in rats was determined to assess proliferation of retinal angiogenesis, as shown in fig. 4. High-Sel group revealed a small area of retinal capillary when compared with STZ group ($P<0.05$), whilst area of retinal capillary between STZ group and Low-Sel group showed no significant difference ($P>0.05$), suggesting sodium selenite could block diabetic retinopathy in rats.

DISCUSSION

At present, pancreas and islet cell transplantation are options for patients with T1DM. However, a shortage of donors and an annual decline in the incidence of insulin-dependent diabetes are the Achille's heel of these therapeutic approaches. To date, therapies for T1DM are limited in efficiency and the mechanism triggering T1DM is not clear. The aim of this study was to investigate and determine the therapeutic effects of sodium selenite in T1DM rats.

The longer-term outcome of adolescent diabetes in terms of weight, eating disorder, and glycemic control is uncertain (Bryden *et al.*, 1999). Initially, in the present study, T1DM rats were induced by administration of STZ, and the results showed that STZ-induced T1DM resulted in markedly suppressed body weight and concomitantly increased total food and water intake coupled with blood glucose level of rats. Previous research has shown that Se altered mechanical and electrical cardiac activities of diabetic rats (Ayaz *et al.*, 2004). To decipher the functional role of sodium selenite in T1DM rats, different doses of sodium selenite was intragastrically administrated on T1DM rats. Intriguingly, our results revealed that both Low-Sel and High-Sel groups had clearly higher body weight than STZ group. Besides, total food and water intake coupled with blood glucose level of rats in the Low-Sel group and High-Sel group were significantly suppressed. Therefore, we can come to the conclusion that treatment with sodium selenite could clearly ameliorate T1DM in rats.

T1MD induced by STZ in animal models is characterized by a loss of β-cells of islets of Langerhans in the pancreas, leading to insulin deficiency (Yin *et al.*, 2010). Besides, a previous study has shown that combination of cerium oxide nanoparticles/sodium selenite could improve rat pancreatic islets by reducing oxidative stress (Pourkhalili *et al.*, 2012). The mechanism by which sodium selenite ameliorates T1DM rats has then been explored. Serum insulin, insulin secretion and apoptosis level of pancreatic tissues were examined after sodium selenite treatment. The detection manifested that High-Sel group had distinctly elevated relative secretion levels of insulin as well as serum insulin levels in rats as compared to both STZ and Low-Sel groups. In addition to measuring insulin secretion as well as serum insulin, we also measured the apoptosis level of pancreatic tissues, which showed STZ group had strikingly higher apoptosis level than High-Sel group. Herein, our study confirmed that treatment with high dose

of sodium selenite could promote insulin secretion in pancreatic β cells obviously.

Despite the recognized potential of intensive insulin therapy for controlling glycemic levels and delaying the onset of diabetes-associated complications, patients with T1DM do not meet or maintain glycemic targets (Gomez-Peralta *et al.*, 2020). Diabetic retinopathy is one of the leading causes of visual impairment and blindness in patients with T1DM. Thus, to find a novel therapy to target diabetic retinopathy is essential for reducing the morbidity and mortality associated with diabetes (Cheung *et al.*, 2010). Herein, the functional effects of sodium selenite on diabetic retinophathy in our study were then elaborated. Retinopathy, one of the most common diabetic complications, is featured by increased proliferation of retinal capillaries. Supplementation with Se exerted protective effects against diabetic retinophathy and protected the retinal capillaries from damage (Thornber & Eckhert, 1984; Gonzalez de Vega *et al.*, 2018). In this regards, the area of retinal capillary in rats was determined to assess proliferation of retinal angiogenesis after sodium selenite treatment. Results showed that High-Sel group and Low-Sel group revealed a small area of retinal capillary when compared with STZ group whilst area of retinal capillary between High-Sel and Low-Sel groups showed no significant difference, suggesting sodium selenite could block diabetic retinopathy in rats.

On the basis of experiment results, we can conclude that the antidiabetic effect of sodium selenite may be partially explained by the suppression of blood glucose levels. Sodium selenite also reduces diabetic retinophathy of diabetic rats, but the underlying mechanism for action is still unclear. Our experimental study exhibited the protective effects of sodium selenite in experimental diabetes possibly caused by increasing the level of insulin secretion in pancreatic β cells. It is expected that the results of these experiments will provide a better basis for the future treatment of diabetes.

CONCLUSION

Our findings revealed that sodium selenite promoted the proliferation of pancreatic β cells and insulin secretion. Taken together, the present study indicates that sodium selenite has a key function in T1DM and may serve as a novel therapeutic target of T1DM.

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