Effect of aerobic exercise combined with ginkgo polysaccharide on weight, blood glucose and glycosylated serum protein in diabetic rats

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Abstract: Streptozotocin copied diabetic rats model were studied through aerobic exercise combined with ginkgo polysaccharide. Aimed to offering the updating experimental basis to prevent diabetic through aerobic exercise combined with ginkgo polysaccharide, the effect of aerobic exercise combined with ginkgo polysaccharide were discussed on the weight, blood glucose, glucosylated serum protein and the secretion ability of islet β cells, oral glucose tolerance test, islet cell pathological morophological changes and positive expression of Bax and Bcl-2. At the end of the experiment, the parameters changes were detected, which were body weight, weight of pancreas islet, Fasting blood glucose(FGB), glycosylated serum protein(GSP), FINS, C-peptide, glucose kinase (GK), ultramicro total atpase, ultramicro Na^+K^+-atpase, altramicro $Ca^{2-}M^{2+}$-atpase, β cell function index (HBCI), and oral glucose tolerance test (OGTT). HE dyeing technology was applied on insulin for pathological morphology observation. The positive expression changes of islet β cells Bcl-2 and Bax were detected by immunohistochemical staining and semi-quantitative method of stereology. Experimental results proved that body weight, GK, ultra micro Na^+K^+-atpase, ultra micro $Ca^{2+}M^{2+}$-atpase, C peptide were significantly increased after the intervention of ginkgo polysaccharide ($P<0.05$), correspondingly, insulin levels were dramatic increased ($P<0.01$), while blood glucose and GSP were decreased significantly ($P<0.05$), the ultramicro total atpase decrease, without statistically significance. After the exercise intervention, body weight, ultra micro Na^+K^+-atpase, ultra micro $Ca^{2+}M^{2+}$-atpase, C peptide significantly increased ($P<0.05$), insulin level apparently increased ($P<0.01$), blood glucose and GSP dramatically decreased ($P<0.05$), GK and ultra micro total atpase had an elevated trend, while without statistically significance. After the rational exercise combined with ginkgo polysaccharide intervention, GK, ultra micro total atpase, ultra micro Na^+K^+-atpase, ultra micro $Ca^{2+}M^{2+}$-atpase and insulin level significantly increased ($P<0.05$), body weight and c peptide significantly increased ($P<0.01$), blood glucose and GSP obviously decreased ($P<0.01$). The pathological changes of insulin in diabetic rats were improved. The increased ratio of Bcl-2/Bax enabled the pancreatic β cell developed in the direction of repair and regeneration. The combination of aerobic exercise and ginkgo polysaccharide could help to increase insulin secretion in diabetic rats, and increase insulin reserve and secretion capacity. Then it can control the weight boss of diabetic rats, along with the blood glucose. So it could lead to the development of the pancreas islets of diabetic rats in the direction of repair and regeneration.

Keywords: Exercise, PGBL, diabetes, islet β cells, function.

INTRODUCTION

With the development of society, the improvement of people's living standards, the transformation of lifestyles, and the deterioration of the living environment, the human spectrum of diseases and the death spectrum have also changed. Among them, diabetes has become a frequently-occurring and common disease that seriously affects human health, and is known as the "third killer" that causes death after tumors and cardiovascular diseases (Newsholme *et al.*, 2007). According to the report of the World Health Organization (WHO) in 1997, the number of diabetic patients worldwide has reached 125 million and it is expected to increase to 299 million by 2025.

Diabetes mellitus is a disease characterized by the absolute or relative deficiency of insulin and the increase in blood sugar and urine glucose and it also leads to disorders of metabolism of sugar, fat, and protein. At present, the cause of diabetes is not yet fully elucidated, but there is a more accepted theory that diabetes is a single disease caused by non-single factors, but a complex disease syndrome, and genetic, autoimmune and environmental factors (Bedford *et al.,* 1979).

For a long time, the treatment of diabetes was mainly based on Western medicines and there were great side effects along with the curative effect. With the development of sports human science and the etiology of diabetes, people have a better understanding of the prevention and treatment of diabetes. There has been extensive research on the combination of exercise, medicine and diet in the prevention and treatment of diabetes and considerable progress has been made. Appropriate exercise, medical nutrition therapy, blood glucose monitoring, drug therapy, and diabetes education are collectively referred to as 5 wagons for the modern treatment of diabetes (Lin *et al.*, 2014). Among them, scholars agree that a period of aerobic exercise can improve the sensitivity of insulin receptors in diabetic

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patients, and increase the use of glucose in peripheral organs, and improve glucose tolerance and blood lipid levels. In the area of drugs, especially Chinese medicine, Chinese medicine experts have also explored many drugs that are effective in preventing and treating diabetes, such as *aloe vera*, corn silk, puerarin, oysters, purslane, astragalus, ephedra, panax pseudoginseng, raw land, and mulberry leaves. *Ginkgo biloba* polysaccharide has the effects of activating blood and relieving pain, relieving phlegm and meridians, converging lung and relieving asthma and indicating blood stasis syndrome. A large number of experimental studies and clinical research data show that *Ginkgo biloba* extract can improve heart and brain blood circulation, expand cardiovascular and cerebrovascular, improve blood rheology, eliminate free radicals, and have good curative effect on cardiovascular and neurological diseases (Wang and Zhong, 2014). In addition, *Ginkgo biloba* extract also protects digestive, respiratory, urogenital and regulatory material metabolism, anti-radiation, anti-organ transplant rejection and other effects (Cao and Xiao, 2013). At the present stage, the clinical application is becoming more and more extensive, and it shows certain therapeutic value for some refractory diseases. However, its application to the anti-oxidation research of diabetes is rare both at home and abroad, and the research on the prevention and treatment of diabetes by exercise combining *Ginkgo biloba* polysaccharides and exercise is rarely reported.

In this experiment, diabetic rats were subjected to aerobic exercise and *Ginkgo biloba* polysaccharide intervention, and the effects of aerobic exercise combined with *Ginkgo biloba* polysaccharide on the structure and function of pancreatic islet β cells in diabetic rats were observed and the mechanism was explored to provide a theoretical basis and experimental basis for the exercise combined with *Ginkgo biloba* polysaccharides for the treatment of diabetes.

MATERIALS AND METHODS

Experimental material

The subjects were normal control rats and diabetic rats. The rats were provided by the experimental animal of Gansu Medical College, and fed following the national rodent feed standard on a free diet, room temperature 23~25^{o}C, relative humidity 40~60%, nature lighting, feed room and utensils ultraviolet disinfection once a week.

Experimental group

Eight rats were randomly selected from 50 healthy 8-month-old male Wister rats (SPF type) as control group (N group), the rest 42 rats were enrolled into the diabetic model group. Streptozotocin (STZ) were injected into abdominal cavity of diabetic model group as 60mg/kg. 0.1% citric acid salt of buffer volume were injected into abdominal cavity of the rats in control group. After models were successfully established, diabetic silent group (D group), diabetic aerobic exercise group (DS group), diabetic Ginkgo polysaccharide group (DM group) and diabetic aerobic exercise medicine group (DMS group) were randomly selected from the 42 rats, eight rats in each group. The rats in DS group and DMS group were performed six weeks formal training (six days of training per week, rest at Sunday, intensity 20m/min, and 30 minutes). Each rat in DM group and DMS group toke ginkgo polysaccharide 200mg/kg daily. Rats in D group toke the same volume of distilled water for six weeks.

Medicine

The rats in DM group and DMS group were given ginkgo polysaccharide 200mg per calculated weight kilogram daily. The rats in other group were taken the same volume of distilled water. The medicine or distilled water were taken at 9:00am-11:00am every day and continued 5 weeks.

Treadmill training method

The Bedford treadmill training method was modified. In normal control group, the rats had normal cage life without formal exercise; In D group and DM group, the rats were caged without formal exercise; In DS group and DMS group, rats were trained one week to adept to the formal training, from 15m/min for 15 minutes, to 20m/min for 30 minutes. The formal 6-week training was 20m/min for 30 minutes everyday from 4:00 PM to 6:00PM, and treadmill was without slop. During the training, body weight was measured once a week and diet changes and mental state were observed, the rats were free to drink and eat.

Experimental specimen preparation

Experimental specimen enthanasia and blood specimen preparation

In order to minimize the effects of furious exercise, rats were fasted 12~16 hours at the end of the last training. Then body weight was calculated, and 2% pentobarbital sodium 50mg per kilogram were intraperitoneal injected for anesthesia. The rats were beheaded for blood. 6ml blood were kept in the test tube, the rest blood samples were kept in 4^{o}C and centrifuged 10 minutes in 3000r/min, and the serum was separated and kept in -20^{o}C. this procedure shall be finalized within 2 hours. The serum insulin will be determined later.

Preparation of islet specimen

The pancreas were quickly dissected on the ice tray and washed in the precooled 0.9% physiological saline, Filter paper was used to absorb the water on the pancreas. Electronic balance scale was used to calculate the pancreas weight. Half of the head of pancreas was longitudinal cut for 2mm square tissue block and put into 10% formalin for fixing the specimen. The rest pancreas

tissue was covered by tinfoil and placed in liquid nitrogen tank cooling for hours, then preserved at -20°C. The frozen pancreatic tissue samples were taken out before the test, and placed on the ice tray and melted. According to the proportion of weight (g) and saline solution (g) was 1:9. The ice water was added for automatic homogenate in order to gain 10% pancreatic tissue slurry, then centrifuged 3000r/min for 15 minutes at 4°C. The centrifugal precipitation was removed, the supernatant fluid was kept at -20°C.

Paraffin embedded pancreas tissue

The conventional paraffin was buried after two days of 10% formalin sample fixation. The procedure was below: tap water rinse for 20 minutes, distilled water risen for 10 minutes, 50% ethanol risen 30 minutes, 70% ethanol risen for 60 minutes, 85% ethanol risen for 60 minutes, 95% ethanol risen for 60 minutes, 100% ethanol risen for 20 minutes, 100% ethanol risen for 20 minutes, 1/2 xylene combined with 1/2 ethanol for 30 minutes, xylene I 20 minutes, xylene II 20 minutes, 1/2 xylene combined with 1/2 ethanol for 30 minutes, paraffin wax I for 60 minutes, paraffin II for 60 minutes, and embedding.

Test index and methods

Test of weekly weight changes of rats

On the seventh day of every week, rats' body weight was measured one by one and the weekly increased weight was the previous week calculated weight minus the week calculated weight.

Pancreas weight

The pancreas was removed and washed in pre-cooled 0.9% saline water. The surface water was absorbed. The weight was calculated.

OGTT method

After 10~15 hours fasting, rats were performed OGTT test at 7:00AM-9:00AM. The dose was 2.2g of 0.4g/ml glucose per calculated kilogram. Tail-cut method was applied to withdraw the tail vein blood to detect the blood glucose at the 30th minutes, the 1st hour, the 2nd hour and the 3rd hour (Yang *et al.*, 2006). Orally taken a certain amount of glucose, then the blood glucose concentration was tested and indirectly understand the islet βcells reserve function to speculate the islet secretion (Liu *et al.*, 2005).

Reagent drugs and instructions

Medicine and reagent

Medicine and Reagent	Company Name
glycated serum protein (GSP) kit	Nanjing Jiancheng Biotechnology Co., Ltd.
ultramicro total atpase kit	Nanjing Jiancheng Biotechnology Co., Ltd.
ultramicro Na^+K^+-atpase kit	Nanjing Jiancheng Biotechnology Co., Ltd.
Ultramicro $Ca^{2+}Mg^{2+}$-atpase kit	Nanjing Jiancheng Biotechnology Co., Ltd.
FINS kit	Shanghai Yanhui Biotechnology Co., Ltd.
c-peptide kit	Shanghai Yanhui Biotechnology Co., Ltd.
glucokinase (GK) kit	Shanghai Yanhui Biotechnology Co., Ltd.
Immunohis to chemistry kit	Wuhan Boster Biological Technology., Ltd.
Anti-rabbit Bax kit	Wuhan Boster Biological Technology., Ltd.
Anti-rabbit Bcl-2 kit	Wuhan Boster Biological Technology., Ltd.
DAB chromogenic kit	Wuhan Boster Biological Technology., Ltd.

Instrument

1) BCPT-96 rats treadmill (Hangzhou Qianjiang Technology and Trade Co. Ltd., China)
2) D-78532 high-speed freezing centrifuge (Beijing Grinde International Technology Co. Ltd., China)
3) BT224S analytical balance (Beijing Seidolis Instrument System Co. Ltd., China)
4) 203-3 dry-wet thermometer (Shaanxi Medical Instrument Factory, China)
5) UVmini-1240 UV visible spectrophotometer (Shimuzu Production Institute, Japan)
6) Liquid Nitrogen Tank (Chengdu Jinfeng Liquid Nitrogen Container Co. Ltd., China)
7) YQ-3 Electric homogenizer (Jintan Medical Instrument Factory, Jiangsu Province, China)
8) BCD-216F Refrigerator (Qingdao Haier Co. Ltd., China)
9) Tissue Slicing Machine (Leica Instrument Co. Ltd., Germany)
10) Dyeing VAT (Tangshan Kaiping Tongsheng Medical Procelain Factory, Hebei Province, China)
11) Olympus Microscope (Beijing Pressey Instrument Co. Ltd., China)

Data and results processing

All the experimental data were based on the WINDOWS XP operation platform and were expressed as the x ± SD and processed by SPSS 13.0 statistical software. The comparison of components was conducted with t test, the difference was marked as $P<0.05$, the significant difference was marked as $P<0.01$.

RESULTS

Rats weight changes

Compared with they are not injected STZ, the weight of model rats were significantly risen ($p<0.01$); Compared

Table 1: The first week of treadmill training in rats adapted training program

Week	Day	Intensity(m/min)	Duration(min)
	The 1st day	15	15
The 1st week	The 2nd day	18	15
	The 3rd day	20	15
	The 4th day	20	20
	The 5th day	20	25
	The 6th day	20	30

Table 2: Formal training in rats with treadmill training program

Week	Day	Intensity(m/min)	Duration(min)
The 2nd week	6	20	30
The 3rd week	6	20	30
The 4th week	6	20	30
The 5th week	6	20	30
The 6th week	6	20	30

Table 2: The changes of body weight before and after injected STZ and experimental end in rats ($\overline{X} \pm SD$)

Group	Before injected STZ(g)	After injected STZ(g)	Experimental end(g)
N(n=8)		235±13.14	325±12.39**
D(n=8)			247±12.59
DM(n=8)	230±12.68	211±10.17*	274±13.11#
DS(n=8)			270±12.14&
DMS(n=8)			290±10.59##

Table 3: The changes of body weight before and after injected STZ and experimental end in rats ($\overline{X} \pm SD$)

Group	Before injected STZ(mmol/l)	After injected STZ (mmol/l)	Experimental end (mmol/l)
N(n=8)		4.91±0.19	4.88±0.15**
D(n=8)			19.21±0.74
DM(n=8)	4.98±0.17	21.58±0.92*	13.65±0.72#
DS(n=8)			15.48±0.56&
DMS(n=8)			7.14±0.59##

Note:*compared with before injected STZ, the blood glucose of model rats were significantly risen ($p<0.01$); #compared with D group, the blood glucose of DM group rats were significantly decreased($p<0.05$); &compared with D group, the blood glucose of DS group rats were significantly decreased ($p<0.05$); ##compared with D group, the blood glucose of DMS group rats were very significantly decreased ($p<0.01$); **compared with D group, the blood glucose of N group rats are very significantly increased ($p<0.01$).

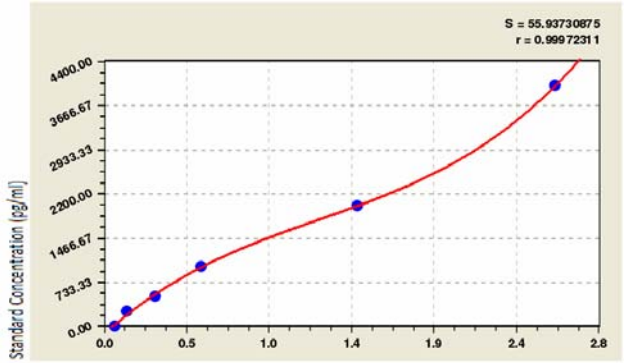
with D group, the weight of DM group rats, DS group rats and DMS group rats were significantly increased($p<0.05$). And compared with D group, the weight of DMS group rats were very significantly increased ($p<0.01$). Moreover, the weight of DM group rats were increased most. So aerobic exercise and ginkgo polysaccharide assist diabetic rats to gain weight, the aerobic exercise combined with ginkgo polysaccharide were better.

Rats blood glucose changes

Compared with they are not injected STZ, the blood glucose of model rats were significantly risen ($p<0.01$); Compared with D group, the blood glucose of DM group and DS group rats were significantly decreased ($p<0.05$). And compared with D group, the blood glucose of DMS group rats were significantly increased ($p<0.01$). Moreover, the blood glucose of DMS group rats were decreased most. Soboth the aerobic exercise and ginkgo polysaccharide helps steadily decrease the blood glucose in diabetic rats, and the effect of aerobic exercise combined with ginkgo polysaccharide was better.

Pancreas islets weight and GSP level at the end of experiment

Compared to N group, the pancreas islets weight of D group obviously decreased ($P<0.01$), while GSP level increased significantly ($P<0.01$); the pancreas islets weight of MD group, DS group and DMS group increased, compared to D group.



Coefficient Data: a=-131.27561, b=2598.7986, c=-1276.0097, d=344.36161

Fig. 1: C-peptide(C-P) standard curve 3rd degree polynomial y=a+bx+cx^2+dx^3…

DISCUSSION

Effect of aerobic exercise combined with ginkgo polysaccharide on the weight of diabetic rats

Weight loss was one of the most obvious sign of diabetes. When diabetes occurred, body could not make full use of glucose. In order to meet the needs of the energy, body strengthened the consumption of fat and protein. Too much fat consumption and protein's negative nitrogen balance enabled patients to be thinner physically and the weight loss. This experiment results showed that the weight of the rats in DM group, DS group and DMS group increased obviously at the end of experiment, compared to D group, 274±13.11g/w, 270±12.14g/w, 290±10.59g/w in perspective. The rats weight in DMS group was most significant (P<0.01). It indicated that rational aerobic exercise and ginkgo polysaccharide could effectively regulate the weight loss of diabetic rats, and rational aerobic exercise combined with ginkgo polysaccharide had a better effect. The treatment of ginkgo polysaccharide could increase rats weights, and was more effective than the treatment of rational aerobic exercise. The statistical difference of this two treatment was not significant.

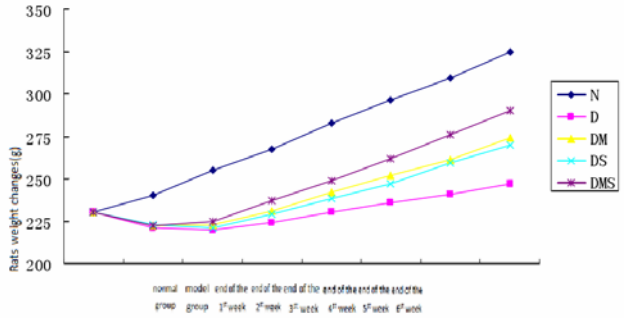


Fig. 3: The change of body weight weekly

The effects of aerobic exercise combined with ginkgo polysaccharide on blood glucose and GSP

Sugar was an important energy source for the body. According to the dietary structure of China, approximately 70% energy that human body required were provided by sugar in food. After digestion and absorpiton, sugar in food entered blood circulation in the form of glucose and could be used directly by body cells and could be stored in liver and muscle in the form of glycogen to be liver glycogen and muscle glycogen. The quantity of stored liver glycogen was not huge, its man function was to maintain the blood glucose level to be relatively stable, i. e. the consumption of blood glucose could be supplied by liver glycogen in time, and excessive blood glucose could be preserved in liver when it was synthesized to be glycogen. Blood glucose was maintained in a state of dynamic balance (Wang, 2007). The occurrence of diabetes was due to the insufficient secretion or relative lack of insulin or insulin resistance. The body would be in a state of sustained high blood glucose, which caused changes in body inner stability. GSP was synthesized by non enzymatic action of synthesized protein and sugar. Its synthesis process was slow and its synthesis rate was positive correlated to the surrounding glucose environment. Therefore, the determination of GSP could reflect the average blood glucose level of previous one two month before the test.

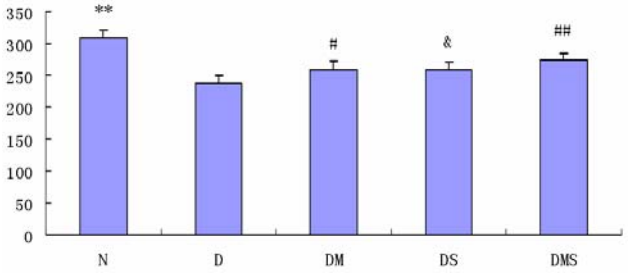


Fig. 6-2: changes in body weight in each group at the end of experiment

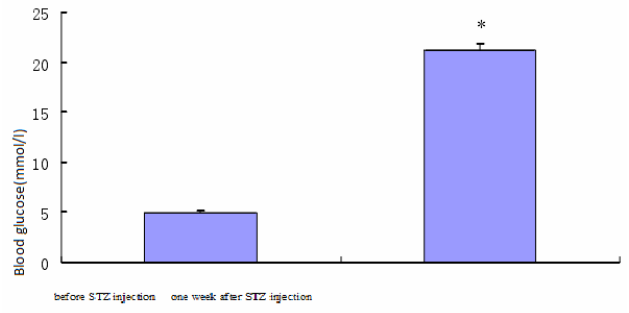


Fig. 4: The changes of body weight before and after injected STZ

A large number of theories and practices proved that rational and regular aerobic exercise could effectively control blood sugar, which increased the energy consumption and increased the blood glucose utilization rate, and increased the secretion of insulin and improved the sensitivity of peripheral tissues to insulin.

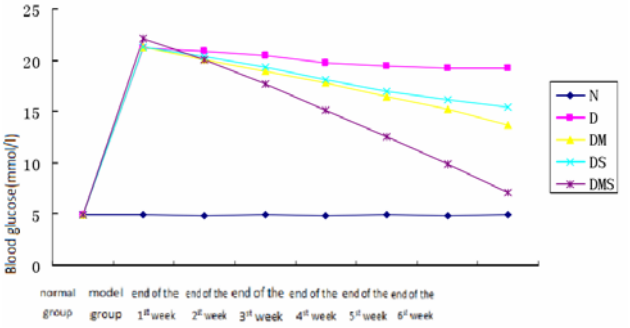


Fig. 5: The rats weekly blood glucose changes during the experiment

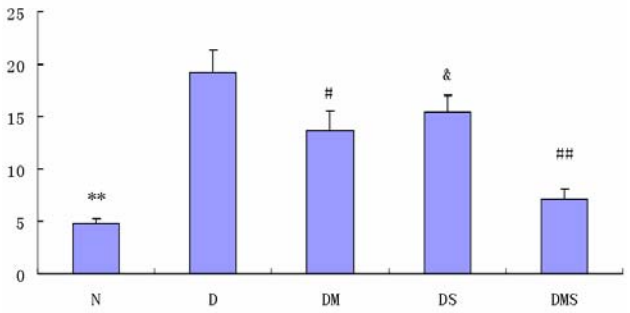


Fig. 6: Rats blood glucose levels at the end of experiment

Some studies put that ginkgo polysaccharide could activate blood, relieve pain, remove stasis, invigorate blood circulation, assist weight loss and relieve asthma. It was widely applied in clinical field, and provided evidence on the treatment of nephrotic sydrome, diabetic nephropathy. This experiment results indicated that the blood glucose level in DM group and DS group decreased significantly compared to D group ($P<0.05$), among which the blood glucose level in DMS group dropped more obviously ($P<0.01$). The results of GSP and blood glucose was same. The experimental results showed that rational aerobic exercise and ginkgo polysaccharide could help the blood glucose control and the elimination of GSP, the combination of two could a achieve a better effect.

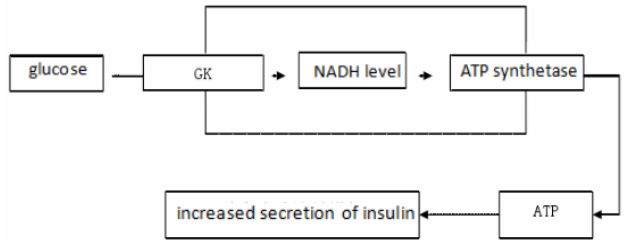


Fig. 7: two-door model of joint control of GK and ATP synthesis enzyme glucose-responsive insulin regulation

CONCLUSIONS

Diabetic rats model could successfully established through STZ 50mg per calculated body weight. Ginkgo polysaccharide was successfully extracted through petroleum. Aerobic exercise, ginkgo polysaccharide and the combination of the two could help to control the weight boss of diabetic rats, along with the blood glucose. Aerobic exercise, ginkgo polysaccharide and the combination of the two treatment could increase insulin secretion in diabetic rats, and increase insulin reserve and secretion capacity. HE stained pathological slices showed that aerobic exercise, ginkgo polysaccharide and the combination of the two could improve the pathological changes of pancreatic islet tissue in diabetic rats. Bax, bcl-2 positive expression showed that aerobic exercise, ginkgo polysaccharide and the combination of the two could lead to the development of the pancreas islets of diabetic rats in the direction of repair and regeneration. So aerobic exercise, ginkgo polysaccharide and the combination of the two could help control the weight loss of diabetic rats, and reduce the blood glucose level and gradually regulate to normal level.

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