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Effect of nano yam polysaccharide on the blood glucose and blood lipid in rats

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Abstract: Chinese yam is the dry rhizome of dioscoreaceae plant. Polysaccharide in yam is one of significant functional components, its pharmacological effects include glucose-lowering, lipid-lowering, anti-tumor, anti-oxidation and enhancing the immune. The effects of nano yam polysaccharide on the metabolism of blood glucose and blood lipid in model rats were systematically investigated in this study. The results showed that the diabetic rat model can been successfully induced by the peritoneal injection of 200mg/kg alloxan. The rats were fed with the high-fat diet for 30d, which could induce a model of hyperlipidemia rat successfully. After the model rats were fed with nano yam polysaccharide of 50mg/ml and 100mg/ml per day for 12d and 30d, respectively. For each nano yam polysaccharide group, the blood glucose level was significantly reduced, the glucose tolerance, glycogen and the content of C-peptide were improved in alloxan rats. Moreover, the symptom of one little and three more in diabetic rats was ameliorated and the contents of TC, TG and LDL-C in the serum for the high fat rats were significantly decreased.

Keywords: Yam polysaccharide, alloxan, C-peptide, liver glycogen, hyperlipidemia.

INTRODUCTION

Diabetes and hyperlipidemia are metabolic disorders that are caused by a variety of causes, they are important risk factors for cardiovascular diseases and have become the major diseases that endanger the human health. Currently, animal models mainly include drug model, genetic model and transgenic model. Drug model has some advantages such simple, practical and easy to repeat, it is widely used in the screening of therapeutic drugs (Olubomehin *et al*., 2013). Alloxan diabetes model is normally used for drug model to study the efficacy of diabetes drugs. Alloxan is a kind of β cytotoxic agents, it can selectively damage β cells from a variety of animals, leading to the decrease of insulin secretion and the experimental diabetes (Ananthi *et al*., 2003 and Bhavapriya *et al*., 2001). Yam is the dried root of dioscoreaceae plant and perennial twining herbaceous plant. Since ancient times, yam is a healthy food that is used for medicine and edible in China (Wang *et al*., 2003). Yam polysaccharide is one of the main active ingredients for yam, has become a research hotspot of yam. Yang *et al.* (2010) established type 2 experimental diabetic rats model by high-calorie diet combined with intraperitoneal injection of streptozotocin, different doses of yam polysaccharide and metformin were used to fill the stomach in order to study the hypoglycemic mechanism of yam polysaccharide on type 2 diabetes rats. The results showed that yam polysaccharide has the obvious lowering-blood sugar function in comparison with the diabetic model group. Chen *et al.* (2006) research results showed that yam can strengthen the metabolism of protein and liver fat for chicken, promote the utilization of amino acids and reduce fat accumulation, thus increasing the nutritional value of chicken. Modern researches have showed that the lipid-lowering and hypoglycemic activities of polysaccharide are increasingly favored by people and nanocrystallization of traditional chinese medicine has become a hot research with the development of nanotechnology (Bilal *et al*., 2011; Chaturvedi *et al*., 2004; Suanarunsawat *et al*., 2010). Based on the nanometer size effect of nano dosage form, drugs have some biological characteristics such as high bioavailability, prolonging' acting time *in vivo*, the slow release and targeting effects. For the pharmacological activity research of yam polysaccharide, the domestic and foreign scholars mostly focused on the extracts of yam polysaccharide (Gao *et al*., 2011; Irudayaraj *et al*., 2013; Wei *et al*., 2002). The systematic and comprehensive studies on hypoglycemic and lipid-lowering activities of nano yam polysaccharide have not been reported. In this paper, the research in this area was carried out to provide a theoretical basis for the clinical application on diabetes and hyperlipidemia of nano yam polysaccharide.

MATERIALS AND METHODS

95% purity of yam polysaccharide was purchased from CiYuan Biotechnology Co. Ltd. in Shanxi (China). 5 capsules were from Strong Pharmaceutical Factory in Chaozhou (China). Acarbose was from YiKa Biological Technology Co. Ltd. in Shanghai (China). α-glycosidase enzyme and alloxan were purchased from Sigma in USA and PNPG was from Aeresco company. Metformin hydrochloride tablets were purchased from Xingyuan

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Pharmaceutical Co. Ltd. in Henan (China). Glucose assay reagent kit, the immune detection reagent kit of C peptide enzyme, glycogen reagent kit, high density lipoprotein cholesterol reagent kit, low density lipoprotein reagent kit, the total cholesterol reagent kit and triglyceride reagent kit were purchased from Jiancheng Biological Engineering Institute in Nanjing (China). All other chemicals were analytical grade.

90 healthy Wistar rats with weight of 180-220 g, 9 weeks of age, half male and half female were purchased from YiSi Experimental Animal Technology Co. Ltd. in Changchun (China). Animal certificate type: SCXK (ji), 2011-0004. Rats were fed with standard laboratory diet and water was given ad libitum. High fat feed with Article No. D12492 was purchased from the Research Diets company in USA.

Preparation and characterization of nano yam polysaccharide

Anhydrous ethanol of 240ml and yam polysaccharide of 10 g were orderly added into the dry agate pot. After the mixture was fully stirred, the agate pot was filled with 200 g agate balls and sealed. The suspension solution was milled for 36h at the ball mill speed of 360r/min and freeze-dried into powder. Finally, the obtained yam polysaccharide nanoparticles were enclosed into the 5 capsules.

Morphology and size distribution of the prepared nano yam polysaccharide particles were respectively investigated by scanning electron microscopy (SEM, JMS-6480A, Japan) and the laser particle size distribution instrument (SALD-2201, Japan).

Hypoglycemic effect of nano yam polysaccharide on alloxan diabetic rats

Establishment of a rat model of alloxan diabetes

After 50 wistar rats were fed with adaptability for 1 week, they were deprived of food for 12h but had free access to water. 40 rats were intraperitoneally injected with 2% alloxan saline solution at a dose of 200mg/kg. After injecting 72 h, they were deprived of food for 12h but had free access to water. Blood was collected from orbital venous plexus and put into the centrifugal tube. Serum was obtained by centrifuging for 15min under 4^{o}C at the speed of 3000 r/min. Other 10 rats were used for blank group and injected with saline solution of equal amounts.

Grouping and dosing drug of alloxan diabetic rat model

The model rats were randomly divided into four groups. Each group consisted of ten animals, that was the positive control group (Positive, metformin hydrochloride tablets group, 70 mg/kg), model group (AD, alloxan diabetic rats), low dose group of nano yam polysaccharide (YP-L, 50 mg/kg), high dose group of nano yam polysaccharide (YP-H, 100mg/kg). Each group of rats was given by gavage at the same time once a day. The blank group was given by empty capsule, the positive control group was given by metformin hydrochloride of 70mg/kg, the model group was given by the excipient capsule without drug, low dose group of yam polysaccharide was given by nano yam polysaccharide of 50mg/kg, the high dose group of yam polysaccharide was given by nano yam polysaccharide of 100mg/kg. After the continuous dosing for 12 days, the body weight, diet and drinking water situation of rats before and after dosing drug were recorded. Fasting and no-ban water on the 13th day, blood was collected from orbital venous plexus and put into the centrifugal tube. Serum was obtained by centrifuging for 15 min under 4^{o}C at the speed of 3000 r/min. The fasting blood glucose, the glucose tolerance and C peptide were tested with reagent kit, respectively.

Determination of blood glucose in diabetic rats

The blood was collected from retinal vein plexus of rat, the serum was obtained by centrifuging for 15min under 4^{o}C at a speed of 3000 r/min. The liquid was added into each tube according to table1 and put in the water bath in 37^{o}C for 15 min. The color could be stable at least 2 h, the absorbance value (A) was tested at 505 nm with the UV spectrophotometer (757CRT, China).

Determination of the glucose tolerance in diabetic rats

Continuous dosing for 12 days, the rats were deprived of food but had free access to water. Glucose of 2.5g/kg was used to fill the stomach. Blood from the orbital venous plexus was collected after filling the stomach for 0, 30 min, 1h and 2h, respectively. Then the blood samples were put into the centrifugal tube, the serum was obtained after centrifuging for 15min uner 4^{o}C at the speed of 3000 r/min. The reagent kits were used to test the blood glucose level to investigate the effect of nano yam polysaccharide on the glucose tolerance in rats. And the calculation equation of blood glucose value was as follow:

AUC mmol/l= 1/2A+B+C+1/2D

(A, B, C and D are the blood glucose level after dosing drug for 0, 0.5 h ,1h and 2h, respectively)

Determination of C peptide in diabetic rats

C peptide reagent kit adopted the sandwich enzyme-linked immunosorbent method of the biotin double antibody. C peptide was added to the monoclonal antibody orifice. Adding C peptide antibodies marked biotin after incubation, the immune complex was formed by bonding mildew avidin-HRP. No combination of enzymes were removed after incubation and washing. There was blue when the substrate A and B were added, the colour eventually turned into yellow under the acid. The depth of color was proportional to the concentration of C peptide. The rats for the model group were deprived of food but had free access to water for 12 h, the blood was collected from retinal vein plexus in rats, and serum was obtained by centrifuging for 15 min under 4^{o}C at a

speed of 3000 r/min. C-peptide in the serum was measured in accordance with the requirements for reagent kit operation.

Determination of liver glycogen in diabetic rats

The fresh livers of wistar rats were taken out and weighed, cooking for 20 min in the boiling water according to the liver weight (mg) and lye volume (μl) ratio of 1:3 and cooling in the cold water. Then, glycogen was prepared into the glycogen test fluid according to the following method: the adding amount of distilled water was 96 × the sample weight when the concentration of hepatic glycogen test liquid was 1%. The preparing test solution mixed with the chromogenic agent according to table 2. After mixture was cooked in the boiling water for 5 min in a test tube, and cooled in the cold water. OD value was determined at 620 nm.

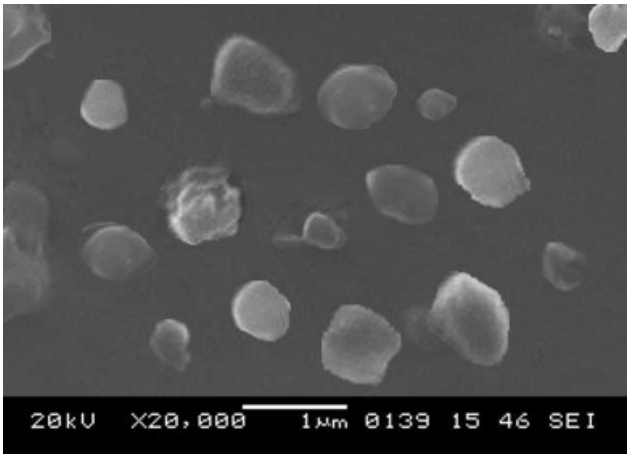

Fig. 1: SEM of nano yam polysaccharide

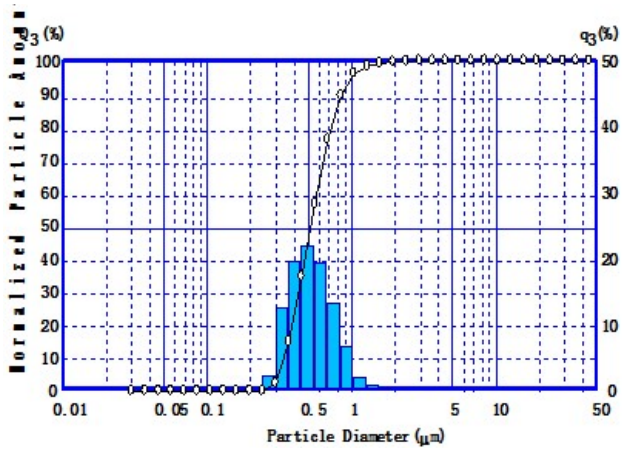

Fig. 2: The average particle size distribution of nano yam polysaccharide

Weight, drinking and diet of diabetic rats

Daily weight, drinking and diet of rats were measured at the same time before and after dosing drugs.

Activity inhibition test of α-glycosidase enzyme

In the cell culture plate of 96-well, PNPG solution of 120 μl was added into each hole with a trace pipetting gun, then adding the yam polysaccharide solution and acarbose solution of 15 μl, the blank hole was filled with the same amount of DMSO. The absorbance at 405 nm was defined A_1. And then the enzyme solution of 15 μl was added into every hole, water bath for 30 min under 37°C and ice bath. The absorbance at 405 nm was measured A_2, $A_{sample} = A_2 - A_1$. The enzyme inhibition rate of every group was calculated according to the following formula.

$$\text{Inhibition rate} = \frac{(A_{DMSO} - A_{sample})}{A_{DMSO}} \times 100\%$$

Lipid-lowering effect of nano yam polysaccharide on the hyperlipidemia rats

Establishment of the hyperlipidemia rat model

After 40 wistar rats were fed with standard laboratory diet for 3 days, rats were randomly divided into 4 groups and each group consisted of ten animals. There were blank control group (normal diet), model group (high fat diet), low dose group of nano yam polysaccharide (50mg/kg), high dose group of nano yam polysaccharide (100mg/kg). Blank control group was fed with normal diet and the other 3 groups were fed with high-fat diet for 30 days.

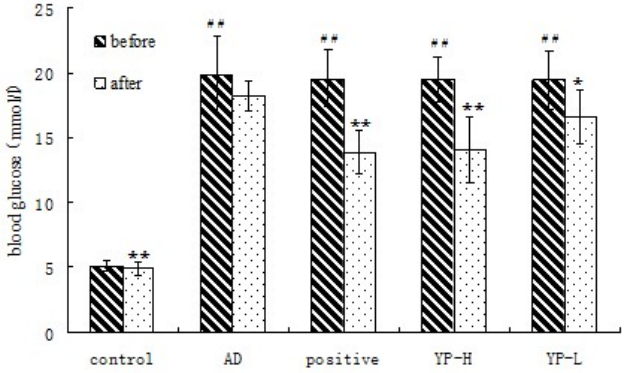

Fig. 3: Concentration of fasting plasma glucose for every group of experimental animals (x±s,n=10) Compared with the model group,**P<0.01, *P<0.05. Compared with the blank group, ## P<0.01, # P<0.05.

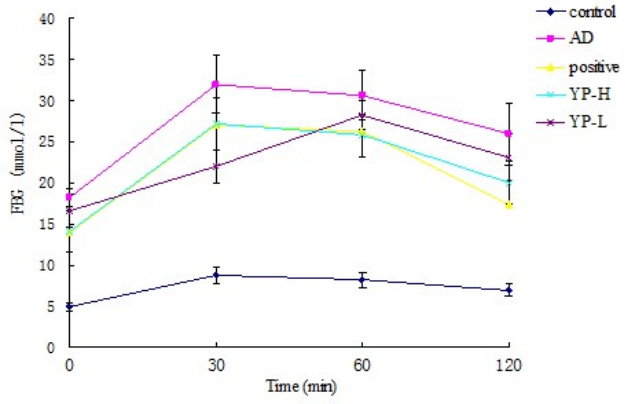

Fig. 4: The relationship between glucose tolerance and time for diabetic rats (x±s, n=10) Compared with the model group,**P<0.01, *P<0.05. Compared with the blank group, ## P<0.01, # P<0.05.

Grouping and dosing drug of hyperlipemia rat model

After establishment of the hyperlipidemia model, the rats were treated by gavage at different doses of nano yam polysaccharide every day. The model group was given with the empty capsules and continuously dosed drug for 30 days. Fasting for 12 h on the 31 days, blood was collected by orbital venous plexus. Blood samples were put into the centrifugal tube, the serum was obtained by centrifuging for 15 min under 4°C at a speed of 3000 r/min. The reagent kits were used to determine the levels of total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C).

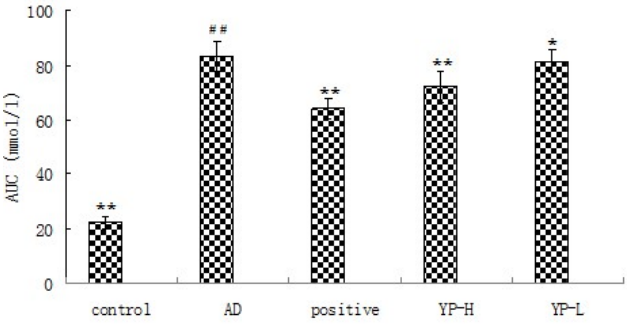

Fig. 5: The area under the blood sugar concentration - time curve for every group of experimental animals (AUC) (x±s, n=10) Compared with the model group,**P<0.01, *P<0.05. Compared with the blank group, ## P<0.01, # P<0.05.

Ethical approval

The manuscript has not been published previously, and it is not under consideration for publication elsewhere. Its publication is approved by all authors and tacitly or explicitly by the responsible authorities where the work was carried out.

STATISTICAL ANALYSIS

All results were showed by using the mean ± standard deviation (X ± SD). The statistical software of SPSS13.0 was used, the comparisons among different groups were statistically processed by one-way ANOVA.

RESULTS

The appearance of the nano yam polysaccharide

Nanopowders of the prepared yam polysaccharide by the ball grinding technology and freeze drying method showed yellow brown. SEM of nano yam polysaccharide is shown in fig. 1. It can be seen from fig. 1 that nano yam polysaccharide was roundness and ball-liked particles, size was uniform.

The size distribution of nano yam polysaccharide particles is shown in fig. 2. From fig. 2, the average particle size of nano yam polysaccharide was 520nm.

Effect of nano yam polysaccharide on fasting blood glucose of alloxan diabetic rats

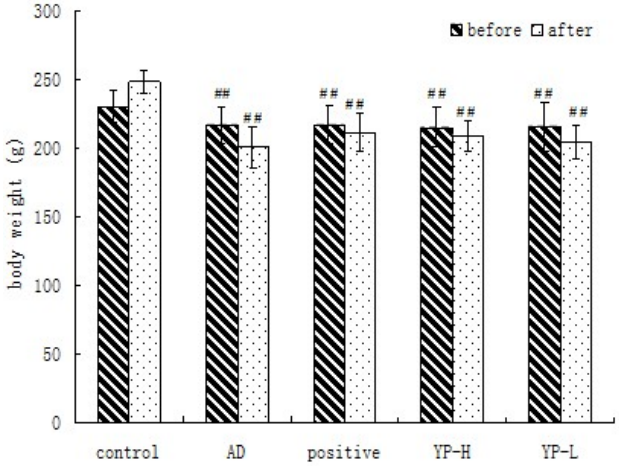

Fig. 6: Body weight of every group of experimental animals (x±s,n=10) Compared with the model group,**P<0.01, *P<0.05. Compared with the blank group, ## P<0.01, # P<0.05.

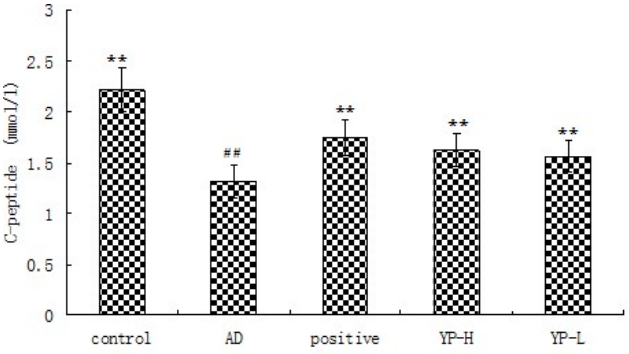

Fig. 7: Concentration of fasting C peptide for every group of experimental animals (x±s,n=10) Compared with the model group,**P<0.01, *P<0.05. Compared with the blank group, ## P<0.01, # P<0.05.

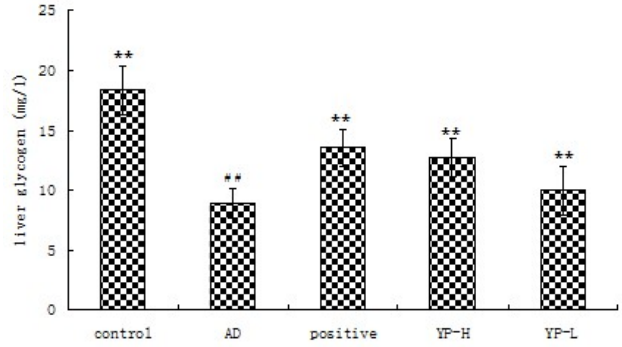

Fig. 8: The content of hepatic glycogen for every group of experimental animals (x±s, n=10) Compared with the model group,**P<0.01, *P<0.05. Compared with the blank group, ## P<0.01, # P<0.05.

The model rats of alloxan diabetic was established. The fasting glucose values of rats before dosing and 12 d after

Table 1: The amount of the added sample in the glucose reagent kit

	Blank tube	Standard tube	Determination tube
Working liquid (μL)	1000	1000	1000
Distilled water (μL)	10		
Standard substance (μL)		10	
Sample (μL)			10

Table 2: The sample amount in liver glycogen reagent kit

Reagent	Blank tube	Standard tube	Testing tube
Distilled water (mL)	1.0		
0.01mg/mL standard (mL)		1.0	
Test fluid of glycogen (mL)			1.0
Chromogenic agent (mL)	2	2	2

Table 3: Drinking and eating amount for every group of experimental animals (x±s, n=3)

group	diet intake (g/d)	water intake (ml/d)
Control group	26	42
Model group	42	141
Positive group	34	94
YP-H	33	78
YP-L	38	92

Table 4: Effect of nano yam polysaccharide on a-glucosidase activity (x±s, n=3)

Group	Concentration (μg/mL)	Absorbance	Inhibition rate (%)
Blank group		0.321±0.045	
Acarbose	0.1	0.259±0.049	19.31%
Low dose group of nano yam polysaccharide	50	0.313±0.036	2.49%
High dose group of nano yam polysaccharide	100	0.221±0.036	31.15%

dosing are shown in fig. 3. Compared with the control group, blood glucose levels increased significantly ($p<0.01$) for every group of diabetic rats before dosing drug, indicating that the alloxan could successfully induce the model of diabetic rats. After intragastric administration with different doses of nano yam polysaccharide and metformin, the concentration of blood glucose significantly decreased for each dose group, and the hypoglycemic effect of yam polysaccharide has a linear relation with the dose. So, nano yam polysaccharide can reduce the blood glucose of diabetic rats.

Effect of nano yam polysaccharide on glucose tolerance of alloxan diabetic rats

After each group of rats were given with 2.5 g/kg glucose, levels of fasting blood glucose at all time points are shown in fig. 4 and fig. 5. Form fig. 4, the blood glucose value of every group increased after intragastric gavage with glucose, while the blood glucose levels dropped after 0.5-2h. The values of blood glucose at different time points for the model group were significantly higher than those for the control group ($p<0.01$). The blood glucose levels for yam polysaccharide dose group and metformin positive control group were significantly lower than those of model group. Form fig. 5 compared with the model group, the area under the glucose curve for each dose group was significantly reduced. In short, the nano yam polysaccharide could significantly improve the glucose tolerance of diabetic rats, and rapidly inhibit a rise of postprandial blood glucose.

Effects of nano yam polysaccharide on weight, drinking, diet in diabetic rats

The weight of rats were measured at the same time before and after dosing drug, the effects of nano yam polysaccharide on weight, drinking and diet in diabetic rats are shown in fig. 6. Compared with normal control group, the weight of diabetic rats before and after dosing drug significantly decreased. After intragastric administration with different doses of nano yam polysaccharide and metformin, each dose group did not significantly inhibit the decrease of body weight of diabetic rats.

Table 3 shows the drinking and eating amount of every group at the same time before and after dosing drug. It could be seen from table 3 that the quantities of eating and drinking for diabetic rats increasedin comparison with the control group. After intragastric administration with different doses of nano yam polysaccharide and

metformin, nano yam polysaccharide could obviously inhibit the amounts of drinking water and diet of diabetic rats.

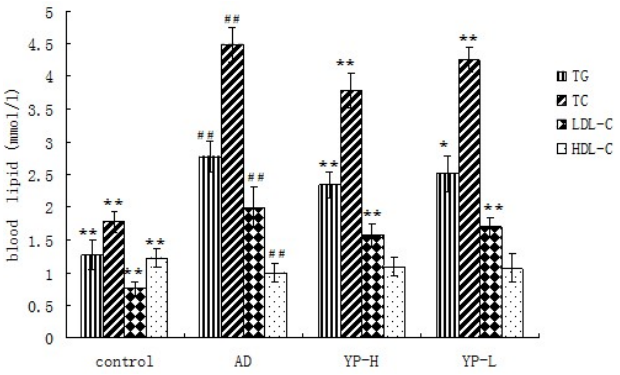

Fig. 9: Concentration of blood lipid for every group of experimental animals (x±s,n=10) Compared with the model group,**P<0.01, *P<0.05. Compared with the blank group, ## P<0.01, # P<0.05.

Effect of nano yam polysaccharide on C-peptide in diabetic rats

Fig. 7 shows the values of fasting C-peptide after dosing drug. From fig. 7, level of C peptide in the serum for the model group significantly decreased in comparison with the control group, indicating that the alloxan could successfully induce the diabetic rat model. Compared with the model group, different doses of nano yam polysaccharide and metformin could significantly increase the content of C peptide in the serum ($p<0.01$), the effect of increasing C peptide for nano yam polysaccharide had a linear relation with the dose.

Effect of nano yam polysaccharide on liver glycogen in diabetic rats

The contents of liver glycogen for each group of rats after dosing drug are shown in fig. 8. It could be seen from Figure 8 that the content of liver glycogen for diabetic rats significantly decreased ($p<0.01$) in comparison with the normal control group. After intragastric administration with different doses of nano yam polysaccharide and metformin, the contents of liver glycogen of diabetic rats significantly increased for the high dose group and metformin group ($p<0.01$). In short, nano yam polysaccharide could decrease the content of liver glycogen, and its effect was linear with the dose.

Inhibition effect of nano yam polysaccharide on α-glycosidase enzyme activity

Inhibition results of nano yam polysaccharide on α-glycosidase enzyme activity are shown table 4. From table 4, inhibition rates of low dose group and high dose group of nano yam polysaccharide on α-glycosidase enzyme were 2.49% and 31.15%, respectively. Moreover, the inhibition rate of high dose group of nano yam polysaccharide on α-glycosidase enzyme was higher than acarbose. In short, nano yam polysaccharide could obviously inhibit the activity of the α-glycosidase enzyme.

Effects of nano yam polysaccharide on blood lipids in diabetic rats

TC, TG, LDL-C and HDL-C levels in the serum for every group of rats are shown in fig. 9. Compared with the control group, TC, TG and LDL-C levels of hyperlipidemia model group significantly increased ($p<0.01$), while HDL-C significantly reduced ($p<0.01$), which suggested that the hyperlipidemia rats model was successfully established. TC, TG and LDL-C levels for different doses of nano yam polysaccharide groups significantly reduced in comparison with the model group, and it was linear with the dose. Difference of HDL-C level was not significant. So, nano yam polysaccharide could inhibit the elevation of blood lipids.

DISCUSSIONS

The value of fasting glucose exceeded 11.1 mmol/L and there was symptoms of more feed, more drink and more urine for rats, namely model was successfully made. Mental condition of normal rats was good, they was living, the fur was thick and glossy, the weight significantly increased. Compared with the normal rats, the diabetic rats showed the dropping spirit, less activity, dark yellowish fur without burnish, weight loss, more drinking, more eating and wet litter.

The liver is a major organ of sugar metabolism, the metabolic process is mainly induced by insulin. The liver decreases the blood glucose by promoting glycogen synthesis and inhibiting glucose dysplasia. Alloxan can result in the decrease of insulin level in diabetes rat, disorder of liver glucose metabolism, reduction of glycogen synthesis and glucose utilization, reinforcement of glucose dysplasia and elevation of blood glucose (Cai *et al*., 2007; Pareek *et al*., 2009). This study suggests the treatment effect of nano yam polysaccharide on alloxan diabetes mice can be caused by several mechanisms. At first, nano yam polysaccharide can obviously inhibit the activity of α-glycosidase enzyme, resulting in reducing the glycogen degradation, promoting the synthesis and storage of glycogen in liver, improving the content of hepatic glycogen, lessening the glucose' release and decreasing the blood sugar. Secondly, nano yam polysaccharide can improve the function of the damaged islet B cell, promote the insulin secretion, increase the content of C peptide in serum and inhibit the sugar dysplasia, thereby lowering the blood glucose level, strengthening the function of glucose tolerance and improving the condition of the sugar metabolic disorder. Meanwhile, nano yam polysaccharide can significantly reduce the contents of TC, TG and LDL- C in the serum of high-fat rat, decrease the accumulation of fat and the swell of fat cells. The number of the insulin receptor on

the cell membrane of fat relatively increases, which causes the relative abundance of insulin and avoides the non-oxidation or transformation into protein or fat of blood glycogen that is caused by the exhaustion of islet β cell and a lack of insulin. Third, LDL-C is one of the important factors that lead to hardening of the arteries. Hyperlipemia has a close relationship with coronary heart disease, especially, the higher TC and TG can induce the great risk of coronary heart disease. Nano yam polysaccharide can reduce TC, TG and LDL-C in the serum of high-fat rats. It plays a crucial role that the disorder of glucose metabolism can be corrected by the metabolic pathway of glucose and lipid, which has the prevention effect for cardiovascular disease.

CONCLUSION

In this study, model of hyperlipidemia rat was successfully induced after rats were fed with high fat diet containing 60% fat for 30 days. Rat enterocoelic was injected with 200mg/kg alloxan, which can successfully induce the diabetic rat model. Nano yam polysaccharide (50mg/kg, 100mg/kg) could significantly reduce the blood glucose level in alloxan diabetic rat, improve the glucose tolerance, liver glycogen and C-peptide levels, inhibit the activity of α-glycosidase enzyme and significantly reduce the contents of TC, TG and LDL-C in the serum of fat rat. Effect of nano yam polysaccharide on HDL-C level in the serum of hyperlipidemia rat was not significant difference.

ACKNOWLEDGEMENTS

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