Original Article
Food Science and Technology
ISSN 0101-2061 (Print)
ISSN 1678-457X (Online)
DOI: https://doi.org/10.1590/fst.77422

The antioxidant activity of Chuju polysaccharide and its effects on the viscera of diabetic mice

Jianting YANG[1*], Jinpeng HU[1], Guoliang ZHOU[2], Min WEI[1], Yan LIU[1]

Abstract

Chuju polysaccharide, which is extracted from a traditional Chinese flower, contains diabetes benefits. In this study, the free radical scavenging ability and reducing force of the Chuju polysaccharides were determined. The Chuju polysaccharides were given intragastrically to the diabetic mice. Results have shown that three Chuju polysaccharides had certain scavenging effects on DPPH radical, hydroxyl radical and superoxide anion radical, which are stronger than VE, but weaker than VC. The scavenging effects of three Chuju polysaccharides on hydroxyl radical were as follows: D2 > D1 > D3. The reducing force of the three components was also shown as the same, indicating that Chuju polysaccharide had strong antioxidant activity. According to the histological anatomy and pathological section analysis of mice, the three components of Chuju polysaccharide could have a certain protective effect on liver, kidney and pancreas. The components with stronger antioxidant activity contain a more significant protective effect. Together, these findings indicated that Chuju polysaccharides possess great potential as a diet supplement or medication for diabetes.

Keywords: Chuju polysaccharide; diabetes; mouse model; antioxidant; viscera.

Practical Application: The results indicate that Chuju polysaccharide has strong antioxidant activity. Chuju polysaccharides has great potential as a diet supplement or medication for diabetes.

1 Introduction

Chuju, *Dendranthema morifolinm (Ralllal)* Tzvel. cv. is a kind of chrysanthemum, which is a genuine medicinal material produced in Chuzhou, Anhui Province, China (Hu et al., 2017). In traditional Chinese medicine, it is believed that Chuju has lots of health benefits, such as brightening eyes, calm liver, and so on (Han et al., 2015; Zhou et al., 2018). Chuju has been used to treat headache, dizziness, and pain in China and many other countries (Tao et al., 2019).

According to previous researches, Chuju has many functions such as bacteriostasis, antioxidant, hypoglycemic and so on (Li et al., 2022). Studies have shown that total flavonoids of Chuju can significantly prolong the coagulation time and increase the content of NO in the serum of diabetic rats (Wang et al., 2021). Aqueous extracts of Chuju can effectively prevent hyperglycemia induced by a high-fat diet, which may be related to its antioxidant activity (Uppin et al., 2020; Wang et al., 2017).

Researchers have found that aqueous extracts of Chuju have different scavenging abilities for DPPH free radical and ABTS free radical when Chuju is dried in different ways (Xie et al., 2018). Reportedly, the drying methods of Chuju polysaccharide can affect the scavenging rate, which has shown that Chuju polysaccharide has good antioxidant activity (Wang et al., 2017). Chuju polysaccharides can significantly prolong the swimming time of weight-bearing mice, and reduce the contents of BLA and BUN in fatigue mice. Moreover, it also can increase the content of HG and improve the respiratory times of decapitated mice. Furthermore, after administrating Chuju polysaccharide, respiratory maintenance time and hypoxia tolerance survival time of mice can be prolonged under atmospheric pressure (Yue et al., 2018).

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by high blood glucose due to insufficient insulin secretion or insulin secretion disorder (Olt, 2015). The number of people (20-79 years) with diabetes is 537 million in 2021 on the globe. This number is predicted to rise to 643 million in 2030 and 783 million in 2045 (International Diabetes Federation, 2021). Various factors contribute to increased the risk of DM, but the main factors are lifestyle, dietary, genetic, obesity and age. Hyperglycemia-induced oxidative stress and apoptosis in pancreatic β-cells have been found to play major role in the development of DM (Lim et al., 2018). Therefore, reducing the oxidative stress and apoptosis in pancreatic β-cells may contribute to the prevention and treatment of DM (Qiao et al., 2022).

In our previous work, Chuju polysaccharides can reduce the blood glucose level of diabetic mice induced by alloxan, and the reduction is positively correlated with the dose (Yang et al., 2022b). In this work, the antioxidant activities of Chuju polysaccharides and their effects on diabetic mice were studied, to provide a basis for revealing the hypoglycemic mechanism of Chuju polysaccharides.

Received 16 June, 2022
Accepted 14 Aug., 2022
[1] *School of Food Engineering, Anhui Science and Technology University, Chuzhou, People's Republic of China*
[2] *School of Life and Health Sciences, Anhui Science and Technology University, Chuzhou, People's Republic of China*
*Corresponding author: jiantingyang@163.com

2 Materials and methodsa

2.1 Materials and reagents

Chuju polysaccharide was prepared by the enzymatic method according to previous reports (Yang et al., 2022b). Three Chuju polysaccharide components D1, D2 and D3 were purified by DEAE-cellose-52, which were collected and freeze-dried, respectively. Chuju polysaccharide solution (100 mg/mL) was prepared with deionized water for further experiments.

2.2 Free-radical scavenging of DPPH

The scavenging activities of digestion samples against DPPH were determined according to the previous study with slight modifications (Sipahli et al., 2022). Briefly, samples with different concentrations were prepared with polysaccharide stock solution. 2 mL sample and 2 mL DPPH (0.1 mM) were mixed and kept in the dark for 30 min at 25 °C. The absorbance at 517 nm was measured (SpectraMax 190, Molecular Devices, USA). 2 mL DPPH in anhydrous ethanol (0.1 mM) and 2 mL anhydrous ethanol were taken for control experiments. VC and VE were selected as positive controls. The DPPH free-radical scavenging rate of the sample was calculated as the following Equation 1:

$$\text{Free-radical scavenging }(\%) = \left[A0 - \left(Ai - Aj\right)\right] / A0 \times 100 \quad (1)$$

Where A0: absorbance control, Aj: absorbance of test sample, Ai: absorbance of sample.

2.3 Hydroxyl radical scavenging activity

The activity was carried out according to the method described previously with slight modifications (Wang et al., 2022). 2 mL sample was mixed with 1 mL of 9 mM $FeSO_4$, 2 mL of 9 mM salicylic acid-ethanol solution and 2 mL of 8.8 mM H_2O_2. The reactions were carried out at room temperature for 1 h and the absorbance was read at 510 nm. VC and VE were selected as positive controls. The capability of scavenging hydroxyl radicals was calculated using the above equation.

2.4 Superoxide radical scavenging activity

Superoxide radical scavenging activity was measured by the modified method of Byun et al. (2021). After extract with pyrogallol (7 mM) for 4 min, 1 mL HCl (10 M) was added to the mixed solution. Then, the absorbance was calculated at 420 nm. VC and VE were selected as positive controls. The capability of scavenging hydroxyl radicals was calculated using the above equation.

2.5 Determination of reducing force

The reducing force of methanolic extracts was determined according to the method of Babbar et al. (2011). 2 mL sample was mixed with 2.5 mL phosphate buffer (0.2 M, pH 6.6) and 2.5 mL, 1% potassium ferricyanide ($K_3Fe(CN)_6$). The mixture was incubated at 50 °C for 20 min and centrifuged at 3500 rpm after the addition of 2.5 mL of 10% trichloroacetic acid. 2.5 mL supernatant was collected and mixed with 0.5 mL, 0.1% FeCl3. The absorbance was measured at 700 nm. VC and VE were selected as positive controls. An increase in absorbance was directly correlated to an increase in reducing force.

2.6 Animal model

Male mice were fed with the high-fat diet, which contained 2% lard, 6% soybean, 1% egg white, 1% fish meal and 3% egg yolk powder, for 2 weeks. After fasting for 12 h, mice were intraperitoneally injected with alloxan (120 mg/kg), which was prepared with saline. After an interval of 6 h, mice were intraperitoneally injected with 20% glucose solution, and fed with 5% glucose solution within 24 h to prevent death caused by hypoglycemia. After 7 d, the fasting blood glucose of mice was higher than 7.8 mmol/L, and the blood glucose was higher than ≥ 11.1 mmol/L (GA-6, Sinocare, China). Further, mice were identified as II diabetes mellitus models (insulin resistant type).

2.7 Animal treatment

According to body weight and blood glucose, mice were divided into 5 groups randomly with 10 mice in each group. Mice were treated according to Table 1, meanwhile a high-fat diet was given for 4 weeks. Blood was taken from the tail every week, and the changes in blood glucose were measured after 12 h of fasting. After fasting for 12 h before the last administration, the blood glucose of mice in each group was measured. Then, 20% urethane was injected intraperitoneally (1.0 g/kg) for anesthesia. Moreover, blood was collected from the abdominal aorta and serum was separated. The liver, kidney and pancreas of each mouse were collected and fixed with 4% paraformaldehyde.

2.8 Histopathological analysis

Organ tissues, including liver, kidney and pancreas, were washed and fixed in 4% buffered paraformaldehyde. Furthermore, they were dehydrated with an alcohol series of different grades, embedded in paraffin, and cut into 4 cm sections (LEICARM2245, Leica, Germany). Sections were stained with hematoxylin and eosin, detected under a microscope (IX73, Olympus, Japan).

2.9 Statistical analysis

Results are expressed as the mean ± standard deviation for all analyzed indices. One-way analysis of variance was conducted, and the level of significance was set to $P < 0.05$. Statistical analyses were performed by using SPSS version 19.0 (SPSS Inc., Chicago IL, USA).

Table 1. Experimental group and dosage.

Experiment groups	Treatment methods
Control group	Saline, i.g. 5 mL
Model group	Saline, i.g. 5 mL
Positive control group	Metformin, 150 mg/kg, i.g. 5 mL
D1 group	Chuju polysaccharide D1, 500 mg/kg, i.g. 5 mL
D2 group	Chuju polysaccharide D2, 500 mg/kg, i.g. 5 mL
D3 group	Chuju polysaccharide D3, 500 mg/kg, i.g. 5 mL

3 Results

3.1 DPPH radical scavenging activity of Chuju polysaccharide

As can be seen in Figure 1, Chuju polysaccharide has a certain scavenging activity on DPPH free radical and stronger than that of VE, but its scavenging activity is weaker than VC. Furthermore, Chuju polysaccharide D2 contains the highest scavenging activity on DPPH.

3.2 Hydroxyl radical scavenging activity of Chuju polysaccharide

The hydroxyl radical scavenging activity of Chuju polysaccharide is shown in Figure 2. Similarly, hydroxyl radical scavenging activity of Chuju polysaccharide is weaker than VC, but stronger than VE. Moreover, the hydroxyl radical scavenging activity of three Chuju polysaccharides is D2 > D1 > D3.

3.3 Scavenging activity of Chuju polysaccharide on superoxide anion free radical

As is shown in Figure 3, the superoxide anion radical scavenging activity of Chuju polysaccharide is following concentration-dependent changes. Same as before, the superoxide anion radical activity of Chuju polysaccharide is weaker than VC and stronger than VE. The Chuju polysaccharides D3 contains the lowest scavenging activity on superoxide anion radical.

3.4 Result of reducing force of Chuju polysaccharides

The reducing force of Chuju polysaccharide is shown in Figure 4. It can be seen that the reducing force of D2 is the strongest in Chuju polysaccharides, but still weaker than VC. Furthermore, the reducing force of Chuju polysaccharide D3, which contains the lowest power, is stronger than VE.

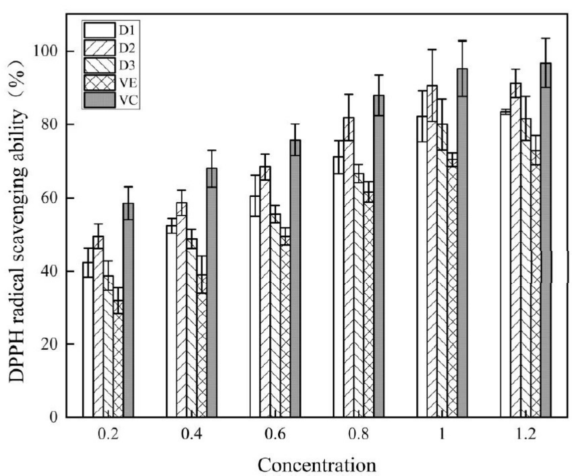

Figure 1. Scavenging DPPH free radical rate of different Chuju polysaccharides. Data are presented as means ± SD (standard deviation).

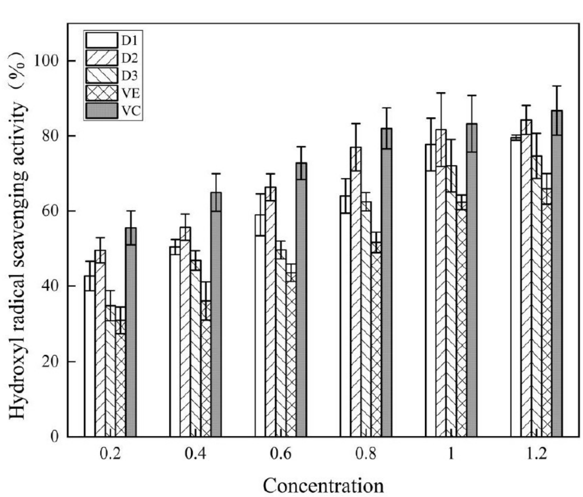

Figure 2. Scavenging hydroxyl radical rate of different Chuju polysaccharides. Data are presented as means ± SD (standard deviation).

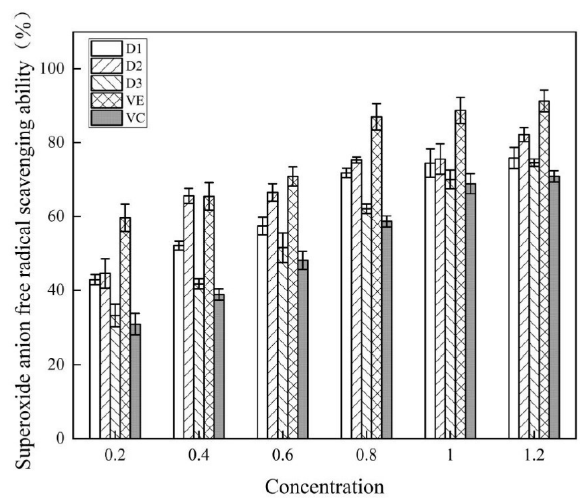

Figure 3. Scavenging radical rate of different Chuju polysaccharides. Data are presented as means ± SD (standard deviation).

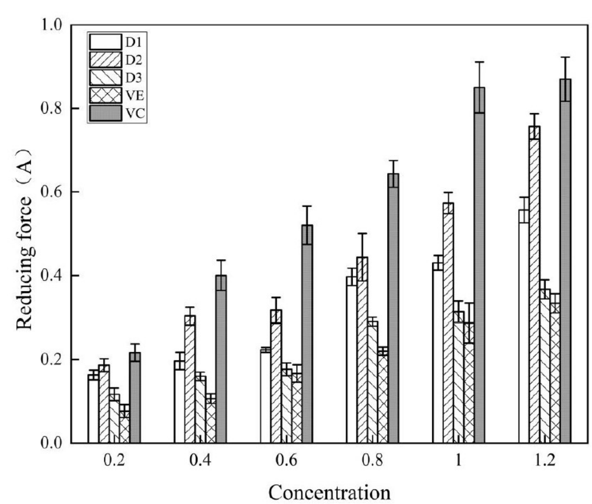

Figure 4. Reducing force of different Chuju polysaccharides. Data are presented as means ± SD (standard deviation).

3.5 Anatomical results of liver and kidney of mice with different treatments

From Figure 5, it is found that the liver and kidney of mice in the blank group are complete and smooth, which is easy to peel off with good hardness. The liver of the positive control group was incomplete and difficult to peel off. Furthermore, they also had strong adhesion with other organs, which contains a great peculiar smell. The liver and kidney of Chuju polysaccharide groups are relatively complete, with good surface gloss and certain hardness, indicating that the three Chuju polysaccharides have a certain protective effect.

3.6 Pathological changes in tissues

The liver plays an important role in drug metabolism, so it can best reflect the significant impact of glucose metabolism and lipid metabolism (Saidov & Isroilov, 2002). Moreover, the degree of liver injury has the most significant impact on metabolism. The results of pathological changes in mouse liver after different treatments are shown in Figure 6.

In the blank group, the liver cells of normal mice are evenly distributed with an obvious nucleus, and the distribution of sinusoidal space is normal. Compared with the blank group, the distribution of liver cells in the positive control group was uneven, and the distribution of sinusoidal space was significantly increased. After treatment with Chuju polysaccharide, the liver injury can be significantly improved. Moreover, evenly distributed cells and fewer cracks in hepatocytes, indicating that Chuju polysaccharides have certain protective effects on the liver. Furthermore, the effects of D2 and D3 are more obvious than those of D1.

The kidney is an important urinary organ, which can produce urine and remove metabolites, wastes and toxins in the body (Herder & Roden, 2022; Liu et al., 2022). Kidney disease is the most obvious complication of diabetes, which can seriously affect the structure of the glomerulus and gradually harden it, resulting in increased glomerular filtration rate and high filtration, resulting in proteinuria. Therefore, the renal structure of diabetic mice can be used to analyze the damage degree of diabetic mice to kidney organs. The results of pathological sections are shown in Figure 7.

In the blank group, the kidney structure was complete, which contains the normal size of glomerulus, and the edge of the structure was clear with no thickening. The structure of renal tubules is complete and continuous, and the stroma is evenly distributed. There is no obvious expansion and thickening. In the positive control group, it can be seen that the glomerulus has certain atrophy and the structural edge is thickened, but the glomerular structure is complete and the interstitial distribution is not obvious. After polysaccharides treatments, the kidney structure of diabetic mice was relatively complete. Moreover, the

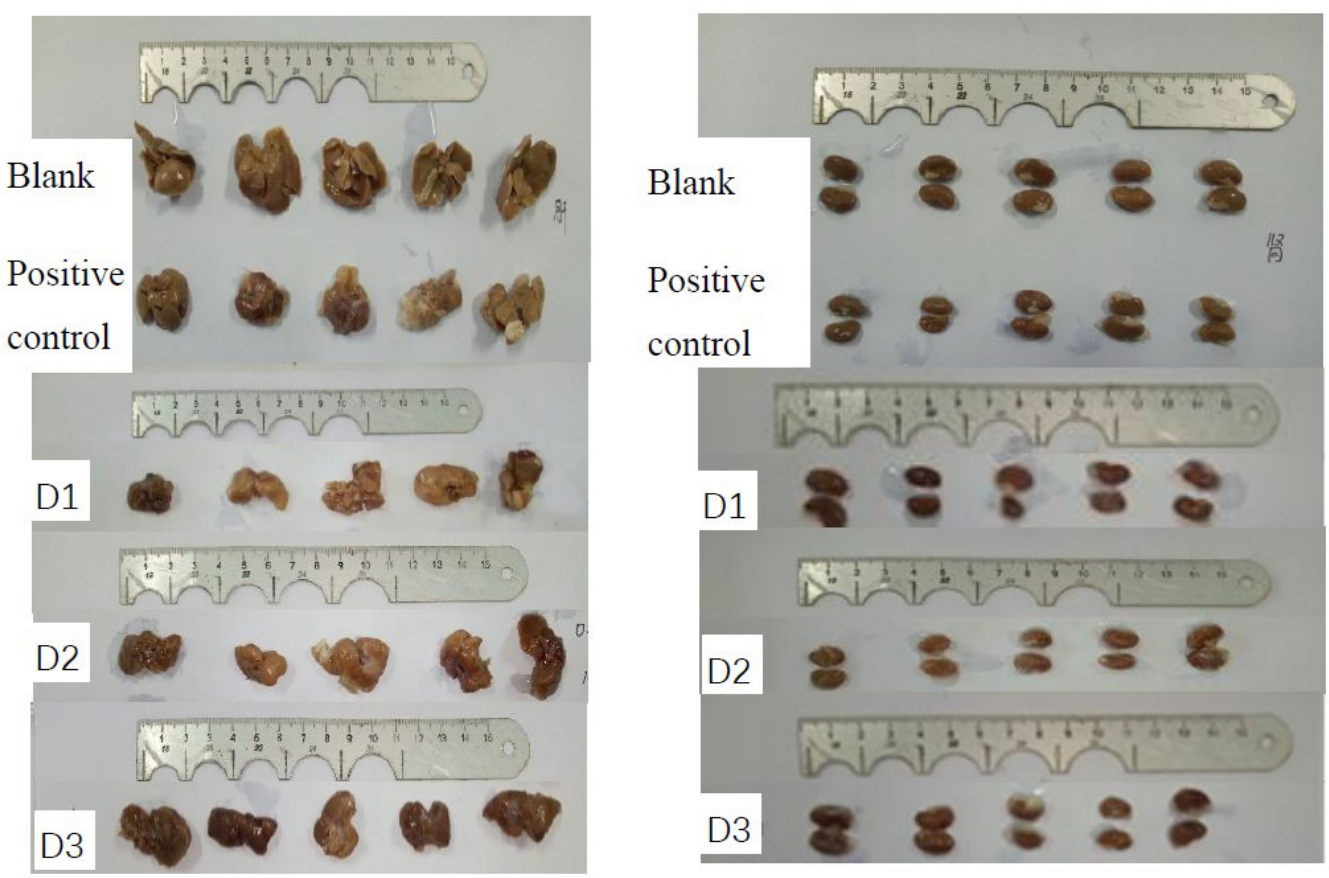

Figure 5. The liver and kidney anatomical map of the mice from different group. Data are presented as means ± SD (standard deviation) (n = 10).

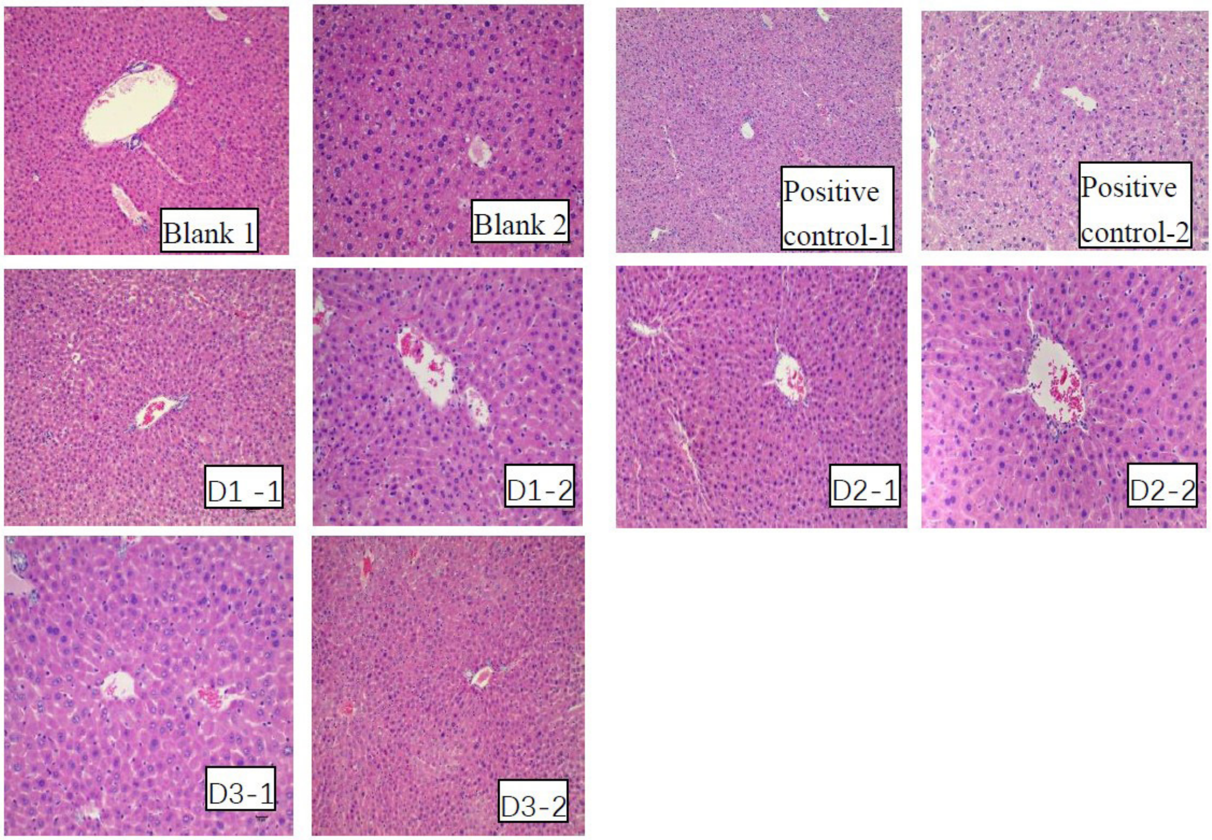

Figure 6. The liver pathological section of mice in D1, D2 and D3 Chuju polysaccharide (1, × 100; 2, × 400) (n = 10).

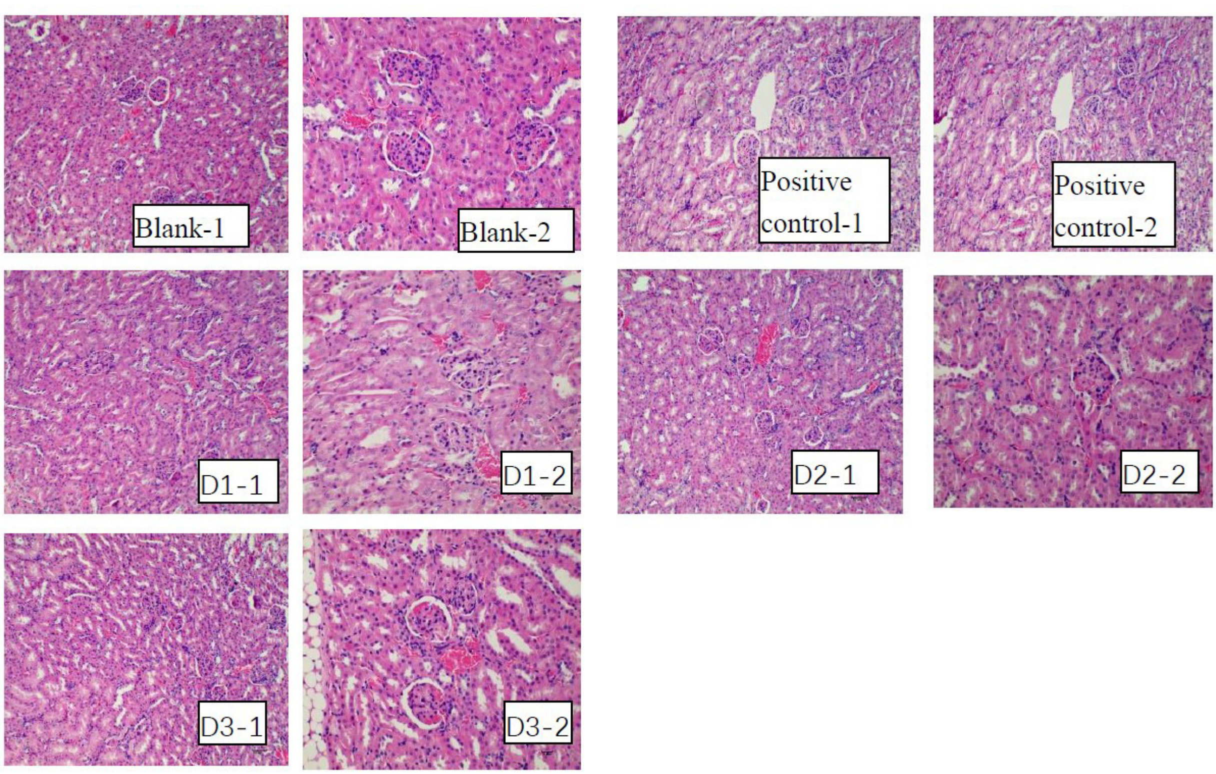

Figure 7. The kindey pathological section of mice in D1, D2 and D3 Chuju polysaccharide. Data are presented as means ± SD (standard deviation) (n = 10).

thickening of glomerular margin became small. Furthermore, the interstitium was significantly improved, indicating that Chuju polysaccharides had certain restorative effects on renal structure and function.

The pancreas is one of the important digestive organs. It is mainly located at the bend of duodenum and has a soft texture, which mainly secretes digestive enzymes and insulin (Kaimala et al., 2022). In the blank group, the pancreatic structure was complete, which contained evenly distributed cells (Figure 8).

The structures of glandular lobules and connective tissue were clearly discernible. Moreover, the islet structure is complete, alongside a dense nucleus and uniform cytoplasm. In the positive control group, there was a thickening of adipose tissue between cells. Moreover, in most cytoplasmic cells, vacuoles appeared. And, nuclear of cells has become unclear. In polysaccharides treatment groups, the proliferation of adipose tissue was smaller than that of medium and low dose groups. Moreover, the vacuoles of cytoplasm cells were significantly improved. Meanwhile, cytoplasmic cells have been more abundant. Also, the structures of glandular lobules and connective tissues were more clear, indicating that Chuju polysaccharides had a certain recovery function on the pancreas of diabetic mice.

4 Discussion and conclusion

In clinical practice, DM mainly reflects hyperglycemia and hyperlipidemia, which is accompanied by "eat more, drink more, urinate more, but lose weight" symptoms (Chang et al., 2021). Type II diabetes is mainly manifested in insufficient insulin secretion or poor insulin effect. The insulin effect is closely related to the liver, pancreas and kidney of animals. These organs are attacked by oxidation, and their cell distribution, nucleus and fissure distribution will change significantly (Wilson & Castle, 2020). In this experiment, four mice were treated with alloxan. The fasting and the changes of blood glucose after the meal were used to screen the diabetic mice.

Diabetic complications are caused by metabolic changes, for the chronic hyperglycemia and diabetes. They often exhibit islet resistance, hypertension and hyperlipidemia. Physiological characteristics are glucose intolerance, obesity, hypertension and premature atherosclerosis (Muche et al., 2020). Damage to other organs is manifested as acute pancreatitis, diabetic nephropathy, diabetic peripheral neuropathy, such as insulin resistance, hypertension and dyslipidemia (Yang et al., 2022a). These changes are also associated with oxidative (Krycer et al., 2020). Therefore, antioxidant methods can be used to deal with

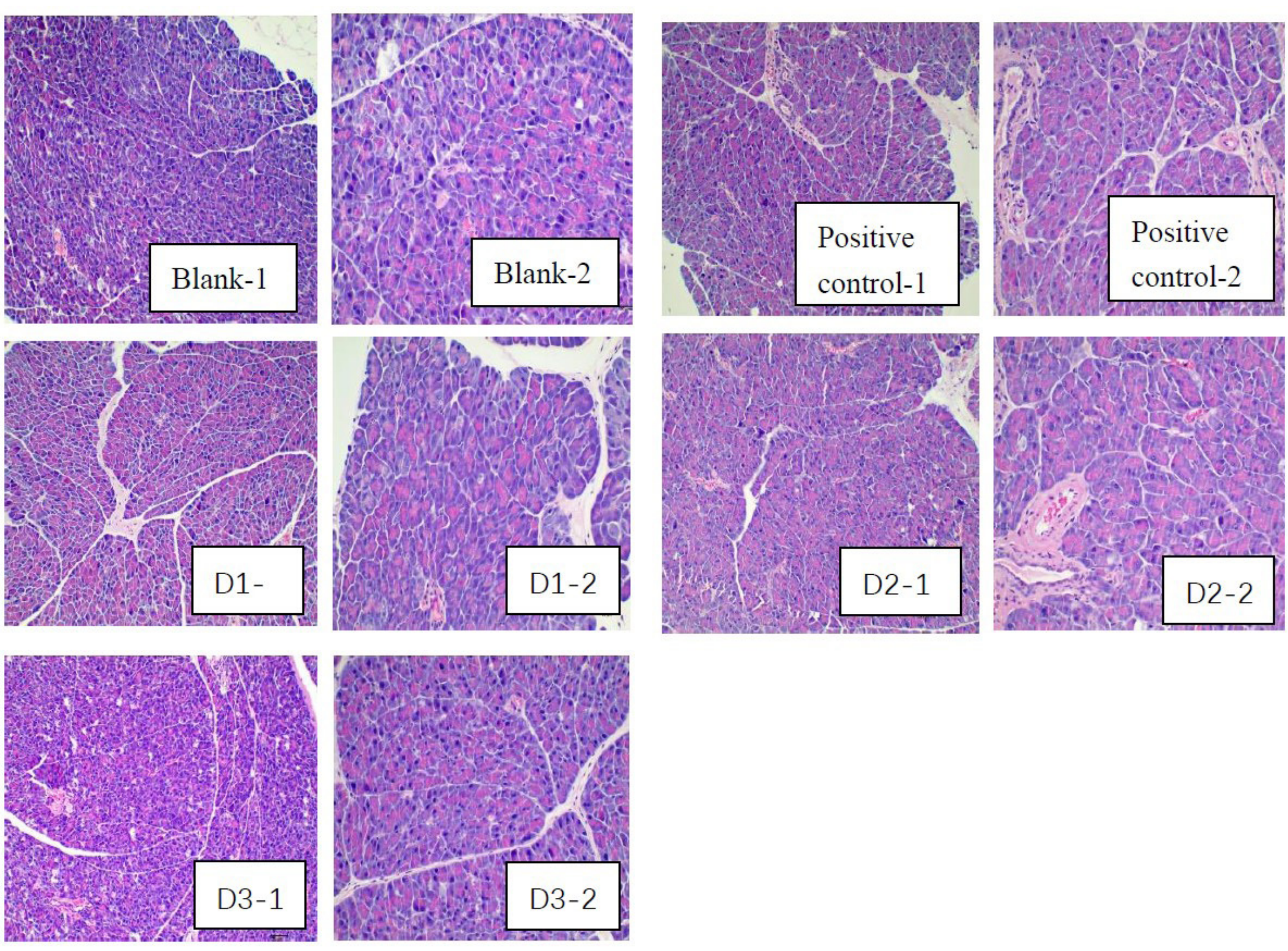

Figure 8. The pancreas pathological section of mice in D1, D2 and D3 Chuju polysaccharide. Data are presented as means ± SD (standard deviation) (n = 10).

oxidative damage. In this experiment, three kinds of polysaccharides separated from Chuju were used to treat diabetic mice. The study has laid the foundation for further research.

The methods of treating diabetes are main in the following aspects: improvement β-Cell dysfunction, enhanced insulin secretion function, improve glucose metabolism and so on (Rossello et al., 2019). Polysaccharides are long polymerized multi molecular substances linked by glycosidic bonds in monosaccharides, which are complex in structure and play a key role in the treatment of diabetes by altering the mechanism of the metabolic process through structural metabolism (Huang et al., 2012; Zhang et al., 2019). In this study, we carried out a pharmacological analysis of polysaccharides from Chuju by detecting their influence on diabetic mice.

In the results, three Chuju polysaccharides (D1, D2 and D3) all have certain scavenging effects on DPPH free radical, hydroxyl and superoxide anion free radical. Furthermore, their clean effects are stronger than VE, but weaker than VC. The scavenging effects of three Chuju polysaccharides on hydroxyl free radical is D2 > D1 > D3. Moreover, the reducing force also shows similar results, indicating that Chuju polysaccharides have strong antioxidant activity. The histological structure of mice is dissected and analyzed combined with pathological sections. It can be seen that the three components of Chuju polysaccharide have a certain protective effect on the liver, which has a good protective effect on the kidney and pancreas. The stronger the antioxidant activity was, the more obvious the protective effect was.

Conflict of interest

The authors have declared that no competing interest exists.

Acknowledgements

This work was supported by Natural Science Research Subject from Education Department of Anhui Province (No. KJ2017A516).

References

Babbar, N., Oberoi, H. S., Uppal, D. S., & Patil, R. T. (2011). Total phenolic content and antioxidant capacity of extracts obtained from six important fruit residues. *Food Research International*, 44(1), 391-396. http://dx.doi.org/10.1016/j.foodres.2010.10.001.

Byun, N. Y., Heo, M. R., & Yim, S. H. (2021). Correlation of anti-wrinkling and free radical antioxidant activities of Areca nut with phenolic and flavonoid contents. *Food Science and Technology*, 41(4), 1041-1049. http://dx.doi.org/10.1590/fst.35520.

Chang, P. Y., Chiou, S. T., Lo, W. Y., Huang, N., & Chien, L.-Y. (2021). Stressors and level of stress among different nursing positions and the associations with hyperlipidemia, hyperglycemia, and hypertension: a national questionnaire survey. *BMC Nursing*, 20(1), 250. http://dx.doi.org/10.1186/s12912-021-00777-y. PMid:34903232.

Han, Y., Zhou, M., Wang, L., Ying, X., Peng, J., Jiang, M., Bai, G., & Luo, G. (2015). Comparative evaluation of different cultivars of Flos Chrysanthemi by an anti-inflammatory-based NF-kappa B reporter gene assay coupled to UPLC-Q/TOF MS with PCA and ANN. *Journal of Ethnopharmacology*, 174, 387-395. http://dx.doi.org/10.1016/j.jep.2015.08.044. PMid:26320691.

Herder, C., & Roden, M. (2022). A novel diabetes typology: towards precision diabetology from pathogenesis to treatment. *Diabetologia*. In press. http://dx.doi.org/10.1007/s00125-021-05625-x. PMid:34981134.

Hu, J., Ma, W., Li, N., & Wang, K. J. (2017). Antioxidant and anti-inflammatory flavonoids from the flowers of Chuju, a medical cultivar of Chrysanthemum Morifolim Ramat. *Journal of the Mexican Chemical Society*, 61(4), 282-289.

Huang, H. Y., Korivi, M., Chaing, Y. Y., Chien, T. Y., & Tsai, Y. C. (2012). Pleurotus tuber-regium polysaccharides attenuate hyperglycemia and oxidative stress in experimental diabetic rats. *Evidence-Based Complementary and Alternative Medicine*, 2012, 856381. http://dx.doi.org/10.1155/2012/856381. PMid:22973406.

International Diabetes Federation – IDF. (2021). *IDF diabetes atlas* (10th ed.). Brussels. Retrieved from https://www.diabetesatlas.org

Kaimala, S., Kumar, C. A., Allouh, M. Z., Ansari, S. A., & Emerald, B. S. (2022). Epigenetic modifications in pancreas development, diabetes, and therapeutics. *Medicinal Research Reviews*, 42(3), 1343-1371. http://dx.doi.org/10.1002/med.21878. PMid:34984701.

Krycer, J. R., Elkington, S. D., Diaz-Vegas, A., Cooke, K. C., Burchfield, J. G., Fisher-Wellman, K. H., Cooney, G. J., Fazakerley, D. J., & James, D. E. (2020). Mitochondrial oxidants, but not respiration, are sensitive to glucose in adipocytes. *The Journal of Biological Chemistry*, 295(1), 99-110. http://dx.doi.org/10.1074/jbc.RA119.011695. PMid:31744882.

Li, R., Xue, Z., Li, S., Zhou, J., Liu, J., Zhang, M., Panichayupakaranant, P., & Chen, H. (2022). Mulberry leaf polysaccharides ameliorate obesity through activation of brown adipose tissue and modulation of the gut microbiota in high-fat diet fed mice. *Food & Function*, 13(2), 561-573. http://dx.doi.org/10.1039/D1FO02324A. PMid:34951619.

Lim, Y. J., Kim, J. H., Pan, J. H., Kim, J. K., Park, T. S., Kim, Y. J., Lee, J. H., & Kim, J. H. (2018). Naringin protects pancreatic beta-cells against oxidative stress-induced apoptosis by inhibiting both intrinsic and extrinsic pathways in insulin-deficient diabetic mice. *Molecular Nutrition & Food Research*, 62(5), 1700810. http://dx.doi.org/10.1002/mnfr.201700810. PMid:29314619.

Liu, H., Sridhar, V. S., Boulet, J., Dharia, A., Khan, A., Lawler, P. R., & Cherney, D. Z. I. (2022). Cardiorenal protection with SGLT2 inhibitors in patients with diabetes mellitus: from biomarkers to clinical outcomes in heart failure and diabetic kidney disease. *Metabolism: Clinical and Experimental*, 126, 154918. http://dx.doi.org/10.1016/j.metabol.2021.154918. PMid:34699838.

Muche, A. A., Olayemi, O. O., & Gete, Y. K. (2020). Predictors of postpartum glucose intolerance in women with gestational diabetes mellitus: a prospective cohort study in Ethiopia based on the updated diagnostic criteria. *BMJ Open*, 10(8), e036882. http://dx.doi.org/10.1136/bmjopen-2020-036882. PMid:32868358.

Olt, S. (2015). Relationship between vitamin D and glycemic control in patients with type 2 diabetes mellitus. *International Journal of Clinical and Experimental Medicine*, 8(10), 19180-19183. PMid:26770553.

Qiao, Y., Zhang, L., Hou, C., & Li, F. (2022). Platycodin D protects pancreatic β-cells from STZ-induced oxidative stress and apoptosis. *Food Science and Technology*, 42, e63521. http://dx.doi.org/10.1590/fst.63521.

Rossello, X., Ferreira, J. P., McMurray, J. J., Aguilar, D., Pfeffer, M. A., Pitt, B., Dickstein, K., Girerd, N., Rossignol, P., & Zannad, F. (2019). Impact of insulin-treated diabetes on cardiovascular outcomes following high-risk myocardial infarction. *European Heart Journal. Acute Cardiovascular Care*, 8(3), 231-241. http://dx.doi.org/10.1177/2048872618803701. PMid:30259764.

Saidov, A. B., & Isroilov, R. I. (2002). Cardiomyocyte ultrastructure in rats with different metabolizing capacities of the liver during acute myocardial infarction. *Bulletin of Experimental Biology and Medicine*, 133(6), 556-558. http://dx.doi.org/10.1023/A:1020221522900. PMid:12447463.

Sipahli, S., Dwarka, D., Amonsou, E., & Mellem, J. (2022). In vitro antioxidant and apoptotic activity of *Lablab purpureus* (L.) sweet isolate and hydrolysates. *Food Science and Technology*, 42, e55220. http://dx.doi.org/10.1590/fst.55220.

Tao, M., Sun, Y.-H., Tao, Y.-G., Miao, W.-J., & Ge, F. (2019). Comparison of physicochemical characteristics of submicron powder and common powder of Chrysanthemum Morifolium Chuju. *Current Topics in Nutraceutical Research*, 17(2), 172-179.

Uppin, V., Acharya, P., Bheemanakere, K. B., & Talahalli, R. R. (2020). Hyperlipidemia downregulate brain antioxidant defense enzymes and neurotrophins in rats: assessment of the modulatory potential of EPA plus DHA and zerumbone. *Molecular Nutrition & Food Research*, 64(20), 2000381. http://dx.doi.org/10.1002/mnfr.202000381. PMid:32918393.

Wang, C., Chen, H., Jiang, H. H., Mao, B. B., & Yu, H. (2021). Total flavonoids of Chuju decrease oxidative stress and cell apoptosis in ischemic stroke rats: network and experimental analyses. *Frontiers in Neuroscience*, 15, 772401. http://dx.doi.org/10.3389/fnins.2021.772401. PMid:34955724.

Wang, J., Li, X., Xing, S., Ma, Z., Hu, S., & Tu, C. (2017). Bio-organic fertilizer promotes plant growth and yield and improves soil microbial community in continuous monoculture system of Chrysanthemum morifolium cv. Chuju. *International Journal of Agriculture and Biology*, 19(3), 563-568. http://dx.doi.org/10.17957/IJAB/15.0339.

Wang, K. W., Sheng, X. Y., Chen, X. J., Zhu, X. Y., & Yang, Y. (2022). Characterization and antioxidant activities of polysaccharide extracted from Benincasa hispida var. chieh-qua How. *Food Science and Technology*, 42, e88421. http://dx.doi.org/10.1590/fst.88421.

Wilson, L. M., & Castle, J. R. (2020). Recent advances in insulin therapy. *Diabetes Technology & Therapeutics*, 22(12), 929-936. http://dx.doi.org/10.1089/dia.2020.0065. PMid:32310681.

Xie, Y., Zhang, Z., Li, F., Fan, X., Xiao, X., & Wang, J. (2018). Rapid analysis of salicylic acid and vanillin in Chuju continuous cropping soil using near infrared spectroscopy. *Fresenius Environmental Bulletin*, 27(6), 4518-4523.

Yang, F. M., Zhang, Y. L., Yang, N., Yu, X., Gao, X., & Zhao, M. Y. (2022a). Parthenolide regulates DNMT1-mediated methylation of VDR promoter to relieve podocyte damage in mice with diabetic nephropathy. *Food Science and Technology*, 42, e51221. http://dx.doi.org/10.1590/fst.51221.

Yang, J. T., Zhou, G. L., Wei, M., & Liu, Y. (2022b). Effects of Chuju polysaccharides on glucose metabolism in diabetic mice. *Current Topics in Nutraceutical Research*, 20(1), 90-96.

Yue, J., Zhu, C., Zhou, Y., Niu, X., Miao, M., Tang, X., Chen, F., Zhao, W., & Liu, Y. (2018). Transcriptome analysis of differentially expressed unigenes involved in flavonoid biosynthesis during flower development of *Chrysanthemum morifolium* 'Chuju'. *Scientific Reports*, 8(1), 13414. http://dx.doi.org/10.1038/s41598-018-31831-6. PMid:30194355.

Zhang, Z., Zhang, L., & Xu, H. (2019). Effect of Astragalus polysaccharide in treatment of diabetes mellitus: a narrative review. *Journal of Traditional Chinese Medicine*, 39(1), 133-138. PMid:32186034.

Zhou, Y., Huang, G., Li, X., Chen, F., Liu, H., Yang, Y., & Yang, J. (2018). Determination of the Aglycon moieties of glycosidically bound compounds in Flos Chrysanthemi by GC x GC-TOFMS. *Acta Chromatographica*, 30(3), 195-199.