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## Effect of dianthus superbis total flavonoids on hyperglycemia in an alloxan mouse model

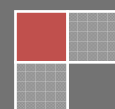
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### ABSTRACT

Objective: Research on the effect of *Dianthus superbis* total flavonoids on induced hyperglycemia in an alloxan mouse model. Methods: The positive control group was given metformin solution; the *Dianthus superbis* total flavonoids high, medium and low dose groups were given different concentrations of total flavonoids *Dianthus superbis* solution; both the blank and model groups were given the same volume of saline. After 10 days, all groups except the blank one were tail vein injected with alloxan to make the hyperglycemic mouse model. The blood glucose and liver glycogen levels in mice from each group were detected and pancreas biopsy was also performed. Results: High, medium and low doses of *Dianthus superbis* total flavonoids significantly reduced the blood glucose levels, increased glycogen and improved the pathological changes in the alloxan-induced hyperglycemic mouse model. Conclusion: The hypoglycemic effect of *Dianthus superbis* total flavonoids is related to promoted glycogen synthesis, and can enhance and improve the function of islet cells.

### KEYWORDS

*Dianthus superbis* total flavonoids; Alloxan; Hyperglycemic.



## INTRODUCTION

Diabetes is a metabolic disease characterized by sustained hyperglycemia, which is due to either lacking of insulin production or the cells of the body not responding rightly to insulin<sup>[1]</sup>. It is a common and worldwide disease. According to the World Health Organization, there are up to 300 million people contracting this disease and the prevalence rate is about 20%-40%. Currently, most of hypoglycemic drugs applied clinically are chemicals, which are not suitable for long-term use due to toxic side effects and the accompanying symptoms. Diabetes is also known as "Xiao Ke" in traditional Chinese medicine. It has been recorded in the "Yellow Emperor", which provides long-term medical practice experience on behalf of physicians. The treatment of diabetes has gradually deepened, and current medicine aims to cure the root cause. Hence, developing Chinese medicine to treat diabetes has greater advantages. This article to study the effect of *Dianthus superbis* on diabetic mice, promote the development of diabetes to *Dianthus superbis* treatments.

## EXPERIMENTAL

### Material and Methods

Experimental reagents and drugs Metformin Hydrochloride Tablets was from Shanghai Pharmaceutical Group Co., Ltd. Xinyi Pharmaceutical Factory (production batch number 080508). *Dianthus superbis* total flavonoids (content 55 %) were from Tianyuan Bio-preparation plant in Xi'an (production batch number 20080526). Sodium Chloride Injection solution was from Zhengzhou yonghe pharmaceutical co., LTD. (production batch number 20080718). Formaldehyde was purchased from Shandong LaiYangShi both chemical co., LTD. (production batch number 20051108). Glucose kits (production batch number 20081107) and liver glycogen checkerboard/GSP determination kits (production batch number 20081229) were all from Nanjing Institute of Biological Engineering.

The experimental instrument Electronic balance, Shanghai jing day electronic instrument co., LTD; 723 visible spectrophotometer, Shanghai precision scientific instrument co., LTD; the thermostatic water bath pot, a constant scientific instrument co., LTD., Shanghai; TDL - 40 b centrifuge, Shanghai anting scientific instrument factory.

Animals KM male mice, whose weight were 18 ~ 21g, were supplied by the Experimental Animal Center of Hebei Province (Animal permit number: 812077)

60 KM male mice, weighing 18~20g, were randomly divided into the blank group (BG), model group (MG), positive control group and the *Dianthus superbis* total flavonoids high, medium and low dose groups, which can also be called as the *Dianthus superbis* alkaloids in HD, MD and LD, respectively. After feeding normally for 3 days, the *Dianthus superbis* total flavonoids high, medium and low dose mice were given 200mg/kg, 100mg/kg and 50mg/kg of *Dianthus superbis* total flavonoids solution, respectively. The concentration of the solution for each group was 10mg/ml, 5mg/ml and 2.5mg/ml (ig volume 0.2ml/10g). The positive control group was given metformin hydrochloride solution (200mg/kg, concentrations 10mg/ml, ig volume 0.2ml/10g). The MG and BG mice were given the same volume of saline. All mice were administered once daily for 10 consecutive days. After fasted 8h and perfusion for 90min, each mouse, except the blank group, was tail vein injected with freshly prepared alloxan solution (60mg/kg; 12mg/ml; 0.05ml/10g). 72h after injection, blood, serum was separated, and OD value of each tube was measured. Based on the OD values, the values of blood glucose were calculated. The mice were then killed and 75mg of liver was weighed. OD values were measured and subsequently used to calculate the values of glycogen. Pancreas was fixed in 10% formalin solution for biopsy.

### Detection Method

Data were analyzed using windows statistical software SPSS 13.0. The differences of measurement data between groups were analyzed using ANOVA.

## RESULT AND DISCUSS

### Impact on blood sugar and liver glycogen in the alloxan-induced hyperglycemic mouse model

**TABLE 1: Effect of *Dianthus superbis* total flavonoids on blood sugar and liver glycogen in the alloxan-induced hyperglycemic mouse model**

Group	N	Dose (g/kg)	Blood sugar (mmol/l)	Liver glycogen (mg/g liver wet weight)
BG	10		5.279±1.037 **	27.252±5.503 **
MG	10		20.532±4.403	11.652±5.503
Positive control group	10	0.2	13.757± 3.612 **	14.924±6.563
<i>Dianthus superbis</i> alkaloids in HD	10	0.2	16.544±5.121 *	18.990± 10.079 *
<i>Dianthus superbis</i> alkaloids in MD	10	0.1	16.656±6.507	15.801± 9.156
<i>Dianthus superbis</i> alkaloids in LD	10	0.05	18.336±4.378	16.631±8.567

**Note:** compared with the model control group, \*\*  $P < 0.01$ , \*  $P < 0.05$

From TABLE 1 shows that compared with the blank group, the blood glucose levels of model group mice were significantly elevated, while the glycogen levels were significantly lower ( $P < 0.01$ ). It suggests that the model copied successfully. Compared with the model group, the levels of blood sugar in the alloxan-induced hyperglycemia mouse model were reduced in mice treated with the positive control and high, medium and low doses of *Dianthus superbis* total flavonoids. *Dianthus superbis* total flavonoids high dose was significantly more effective ( $P < 0.05$ ), and *Dianthus superbis* total flavonoids medium and low doses also had an effect. Compared with the model group, glycogen synthesis was promoted in the positive control group and *Dianthus superbis* total flavonoids high, medium and low dose groups. *Dianthus superbis* total flavonoids high dose was significantly more effective ( $P < 0.05$ ), and *Dianthus superbis* total flavonoids high, medium and low dose groups showed superior effect to positive control group. The results indicate that the hypoglycemic effect of *Dianthus superbis* total flavonoids is related to promoted glycogen synthesis.

**Impact on pancreatic tissue in the alloxan-induced hyperglycemic mouse model**

**TABLE 2 : Effect of *Dianthus superbis* total flavonoids on pancreatic tissue in the alloxan-induce hyperglycemic mouse model**

group	n	-	+	++	+++
BG	10	10	0	0	0
MG	10	0	2	1	7
Positive control group	10	0	4	6	0
<i>Dianthus superbis</i> alkaloids in HD	10	2	6	2	0
<i>Dianthus superbis</i> alkaloids in MD	10	0	5	5	0
<i>Dianthus superbis</i> alkaloids in LD	10	0	3	7	0

Note:“-”Bulky islet cells, abundant cytoplasm and normally sparse nucleus, “+” Atrophic islet cells, decreased cytoplasm and sparse nucleus, “++”Partially atrophic islet cells, reduced cytoplasm and some of dense nuclei, “+++”Markedly atrophic islet cells, decreased cytoplasm and intensive nucleus.

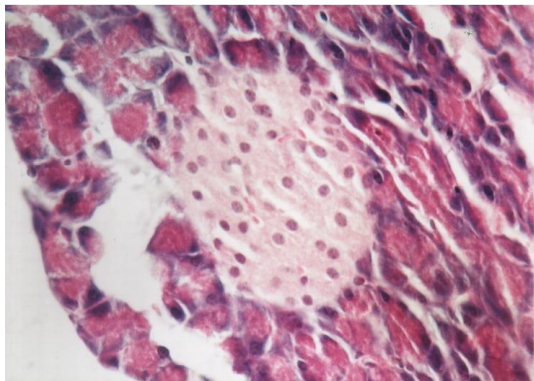


Photo 1 Pancreatic tissue from the Blank group

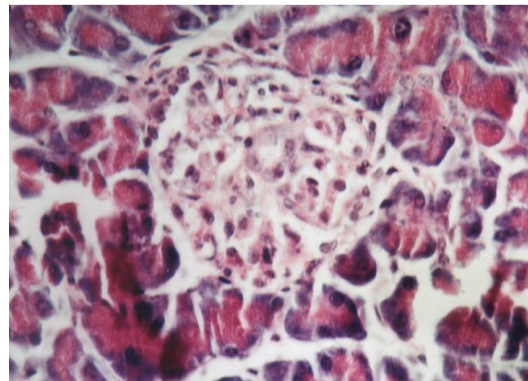


Photo 2 Pancreatic tissue from the from the model group

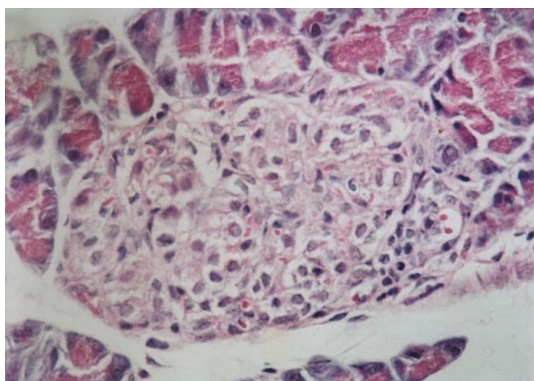


Photo 3 Pancreatic tissue from the positive control group

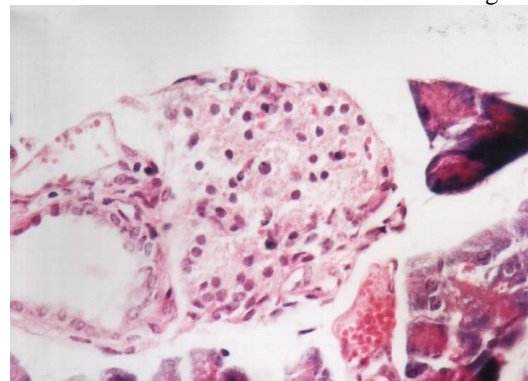


Photo 4 Pancreatic tissue from the *Dianthus superbis* alkaloids in HD group

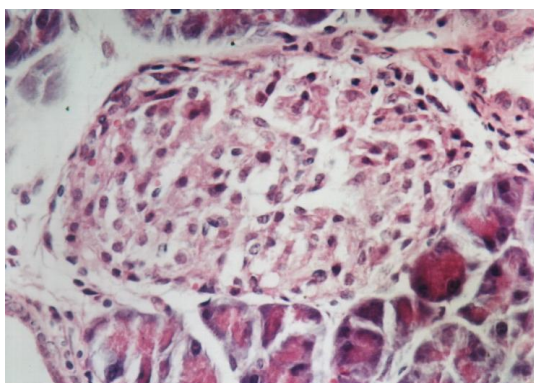


Photo 5 Pancreatic tissue from the *Dianthus superbus* alkaloids in MD group

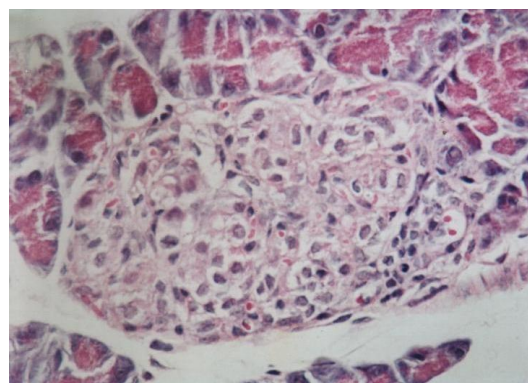


Photo 6 Pancreatic tissue from the *Dianthus superbus* alkaloids in LD group

**Figure 1 : The pathological photos of pancreatic tissue in the alloxan-induced hyperglycemic mouse model**

TABLE 2 and Figure 1 show that the mice in the blank group had bulky islets, abundant cytoplasm, sparse nucleus and normal intercalated duct cells and heart cells. Compared with the blank group, the model group had significantly shrink pancreatic islet cells, reduced cytoplasm and intensive nucleus, while intercalated duct cells and heart cells were normal. Compared with the model group, the positive control mice had volume-increased pancreatic islet cells, and the cytoplasm was rich. However, the nuclei were still dense. In addition, intercalated duct cells and heart cells were also normal. *Dianthus superbus* total flavonoids high dose significantly increased the volume of islet cells, and the mice had abundant cytoplasm, sparse nucleus and normal intercalated duct cells and heart cells. The *Dianthus superbus* total flavonoids medium dose group showed significantly increased volume, abundant cytoplasm and sparse nucleus, while intercalated duct cells and heart cells were normal. The mice treated with low dose of *Dianthus superbus* total flavonoids had small volume of islet cells of mice. The cytoplasm was not rich and the nuclei were dense. However, intercalated duct cells and heart cells were normal. Overall, the main pathological change in experimental models was markedly atrophic islet cells, while pancreatic intercalated duct cells and heart cells were normal. Each group with medicine had restored and improved the effect on islet cell, and the effect was dose-dependent. The best effect is from high dosed of *Dianthus superbus* total flavonoids, followed by medium and low doses of *Dianthus superbus* total flavonoids.

## CONCLUSIONS

Alloxan is a  $\beta$  cytotoxic agent. In the body, it can be selectively absorbed by pancreatic  $\beta$  cells, activating the free radical chain reaction and excessive free radicals, which directly undermine the structure of  $\beta$  cells. It leads to the damage and necrosis of islet  $\beta$  cells, reducing the insulin level and giving the symptoms of diabetes, such as high blood sugar<sup>[2-3]</sup>. Alloxan is safe and reliable. It is one of the commonly used drugs in constructing animal models of diabetes at domestic<sup>[4]</sup>.

*Dianthus superbus* is the dry aboveground plant of Caryophyllaceae *Dianthus superbus* and *Dianthus*. It is distributed in a lot of places in China, and mainly produced in Hebei, Henan, Liaoning, Jiangsu, etc. It has diuretic, Poxue-pass-through and other effects. Clinically, it is mainly used to treat cream card, amenorrhea and irregular menstruation embolism. It has been reported that it is an ancient and modern treatment for diabetes.

Liver is one of the most important organs for glucose metabolism. Decreased liver glycogen synthesis and increased gluconeogenesis are the main reason for the elevated of blood sugar. The results show that high, medium and low doses of *Dianthus superbus* total flavonoids could reduce the level of blood sugar and promote glycogen synthesis in the alloxan-induced hyperglycemia mouse model. High dose of *Dianthus superbus* total flavonoids was significantly more effective ( $P < 0.05$ ), implicating that the hypoglycemic effect of *Dianthus superbus* total flavonoids is associated with promoted glycogen synthesis. Additionally, the hypoglycemic effect of Chinese herbal medicine is multifaceted, which can not only improve the general symptoms, such as thirst, fatigue, weakness, etc, but also promote the body's metabolism and microcirculation and enhance the resistance to insulin. The results show that each group treated with medicine had restored and improved effect on islet cells, and the effect was dose-dependent. The best effect was showed in the *Dianthus superbus* total flavonoids high dose group.

Diabetes has a high prevalence rate in modern life, seriously affecting the patients' quality of life. Currently, scientists working on either western medicine or traditional Chinese medicine are actively looking for a reliable method to better treat diabetes. This study clarifies the treatment of diabetes *Dianthus superbus* total flavonoids mechanism of action to treat diabetes, and it also provides the reliable basis for clinical application of *Dianthus superbus* on diabetes treatment, promoting the clinical application of diabetes drugs as well as expanding the scope of traditional Chinese medicine treating modern diseases.

### ACKNOWLEDGEMENT

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### REFERENCES

- [1] S.Y.Fang, M.H.Xu, H.X.Gu et al.; Effects of radix pueraria flavonoids in da bie shan zone on blood glucose and lipid level of diabetic mice induced by alloxan, *Journal of West an hui University*, **28**, 7-9 (2012).
- [2] R.H.Guo, H.L.Wang, Y.M.Zhai et al.; Effects of soybean trypsin inhibitor on glucose tolerance and hepatic glycogen in alloxandiabetic mice, *Journal of Clinical Rehabilitative Tissue Engineering Research*, **15**, 4477-4480 (2011).
- [3] Y.L.Cai, X.Z.Jin, L.H.Piao et al.; The effect of hazel flower on glycogen content in alloxan diabetic mice, *Yanbian Daxue Xuebao.*, **30**,100-102 (2007).
- [4] Z.Z.Lin, T.Chen; The study of gypenosides hypoglycemic effect in experimental diabetic mice, *Longyan University*, **29**, 51-53 (2011).