

## Extracts of *Potentilla discolor* bunge protect islet $\beta$ -cells against streptozotocin induced injury.

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### Abstract

**Aim:** To investigate whether *Potentilla discolor* Bunge (PDB) extracts could protect islet  $\beta$  cells and promote cell proliferation and insulin secretion.

**Methods:**  $\beta$ -cell injury model was induced by Streptozotocin. Cell viability, insulin secretion and gene expression were measured to investigate the protective effects of PDB extract on RIN-m5f  $\beta$  cells damaged by streptozotocin.

**Results:** PDB extracts inhibited cell damage, improved cell viability, promoted insulin secretion, and increased protein level of p-Fox O1 in RIN-m5f  $\beta$  cells in a dose-dependent manner.

**Conclusions:** PDB extracts exhibit protective role in streptozotocin induced  $\beta$  cell injury model. The mechanism may be related to increased phosphorylation of Fox O1, which increases the number and improves the function of  $\beta$  cells.

**Keywords:** EPD, RIN-m5f, Protective effect, Fox O1.

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### Introduction

Diabetes Mellitus (DM) is a metabolic disorder characterized by systemic control of glucose dysfunction and insulin secretion imbalance. The prevalence of diabetes in Chinese adults has risen to 11.6%, accounting for 1/3 of the number of patients worldwide [1]. According to data released by WHO, diabetes has become the third major disease that threatens human health following cancer and cardiovascular diseases. Islet  $\beta$  cells are the only cells that secrete insulin, the maintenance of normal amount and function of insulin is necessary for the body to secrete adequate insulin to maintain the balance of the body. Streptozotocin (STZ) is a compound derived from *Streptomyces achromogenes* that has been used for the treatment of pancreatic  $\beta$  cell carcinoma. Due to its toxicity to islet  $\beta$  cells, STZ has been used to induce cell or animal model of diabetes [2].

Forkhead box O (FoxO) is a transcription factor that plays an important role in the differentiation, proliferation and survival of various cells such as adipocytes, hepatocytes, and islet  $\beta$  cells. FoxO1 regulates the number of and function of  $\beta$  cells, and plays an important role in the development of diabetes [3,4].

*Potentilla discolor* Bunge (PDB) is a Rosaceae plant with various effects such as clearing heat and detoxicating, curing dysentery to arrest hematochezia. In diabetic rat model, PDB not only significantly reduced blood glucose but also promoted the repair of  $\beta$  cells and the release of insulin [5]. This study

aimed to investigate whether PDB extracts could protect islet  $\beta$  cells and promote cell proliferation and insulin secretion. We established STZ induced damage to RIN-m5f cells as the model and examined the effects of PDB extracts.

### Materials and Methods

#### Reagents

*Potentilla discolor* was identified by Shihua Wang, associate chief pharmacist at Department of Pharmacy at Jingzhou Chinese Medicine Hospital. PDB extracts were prepared as described previously [5]. Roswell Park Memorial Institute (RPMI) 1640 medium, penicillin and streptomycin were from Genom Biopharmaceutical Company. Fetal bovine serum was from HyClone Company. Thiazole blue (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazoliumbromide, MTT) was from AMRESCO Company. Streptozotocin (STZ) was from Sigma. Rat insulin ELISA kit was from Mercodia (Sweden). Fox O1 monoclonal antibody, p-Fox O1 polyclonal antibody and  $\beta$ -actin antibody were from Abcam.

#### Cell culture

Rat RIN-m5f  $\beta$  cells were provided by Collection Center at Wuhan University and cultured in RPMI1640 medium supplemented with 10% fetal bovine serum, penicillin (100 U/ml) and streptomycin (100 mg/ml) at 37°C in a humidified atmosphere containing 5% CO<sub>2</sub> and 95% air. The medium was

changed every two days. Cells were used when they were at the logarithmic phase of growth.

### MTT assay

Cells were seeded into 96-well culture plates and cultured in a humidified chamber at 37°C overnight. Next the cells were treated with 0.25, 0.5, or 1 mg/ml PDB extracts for 48 h. Each day for three consecutive days, viable cells were evaluated with MTT assay kit (Sigma, USA) according to the manufacturer's instructions. 20 µL MTT (5 mg/ml) solution was added to each well in 96-well plates and the plates were incubated at 37°C for 4 h, then 150 µL dimethyl sulfoxide (DMSO) was added to each well in 96-well plates and the plates were incubated at room temperature for 10 min. The absorption value of every well (A) was read at 492 nm using a microplate reader (ELX800, Bio-Tek, USA).

### ELISA

Cells were treated with 0.25, 0.5, or 1 mg/ml PDB extracts for 48 h. The culture medium was collected and insulin content was determined by using ELISA kit according to the manufacturer's instructions.

### Western blot analysis

Cells were treated with 0.25, 0.5, or 1 mg/ml PDB extracts. The cells were collected and total protein was isolated from the cells and quantitated by BSA method. 30 µg protein was loaded onto a 10% Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) and transferred to Polyvinylidene Difluoride (PVDF) membrane. Next, the membrane was incubated with specific antibody for FoxO1, p-FoxO1 or β-actin at 4°C overnight, followed by incubation with secondary antibody for 1 h at room temperature. The membrane was developed using ECL kit (Pierce, Rockford, IL, USA) and exposed to X-ray film. Bands on the films were analysed by Image.plus5.1.

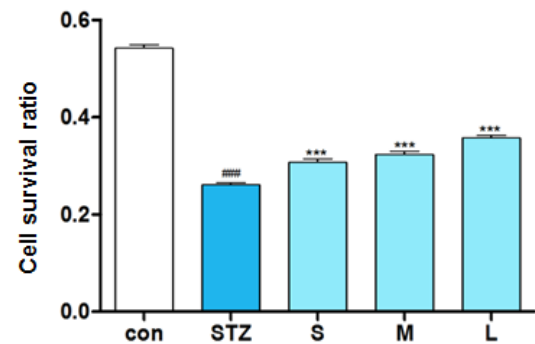
### Statistical analysis

The data were expressed as  $\bar{x} \pm s$  and analysed by SPSS 17.0 software. ANOVA was used to compare the differences among the groups.  $P < 0.05$  indicated that the difference was statistically significant.

## Results

### PDB extracts promotes the survival of RIN-m5f cells after STZ injury

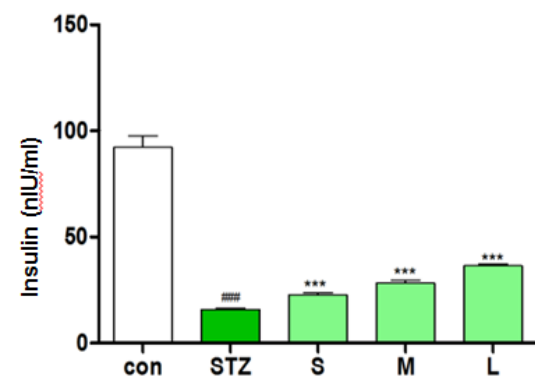
As shown in Figure 1, the number of cells in STZ injury group was significantly lower than that in the control group ( $P < 0.01$ ). Compared with the injury group, 0.25, 0.5 and 1 mg/ml PDB extracts could increase the number of cells in a dose-dependent manner and the differences were significant ( $P < 0.01$ ). These results suggest that PDB extracts effectively repair STZ induced β-cell injury.



**Figure 1.** PDB extracts increase RIN-m5f cell viability. RIN-m5f cells were untreated (con), or treated with STZ (STZ), or treated with STZ plus PDB extracts at 0.25 mg/ml (S), 0.5 mg/ml (M) or 1 mg/ml (L) for 48 h. Cell viability was detected by MTT assay. Data were shown as  $\bar{x} \pm s$  ( $n=5$ ) and analysed by ANOVA. Compared to control group, ### $P < 0.01$ ; compared to STZ group, \*\*\* $P < 0.01$ .

### PDB extracts promotes the secretion of insulin by RIN-m5f cells

Insulin secretion of RIN-m5f cells was determined by ELISA. As shown in Figure 2, after 48 h treatment with PDB extracts, insulin secretion in the injured group significantly decreased compared to control group ( $P < 0.01$ ). Compared to injury group, insulin secretion increased in cells treated by PDB extracts in a dose-dependent manner and the differences were significant ( $P < 0.01$ ). These results suggest that PDB extracts effectively promote STZ injured β-cells to secrete insulin.

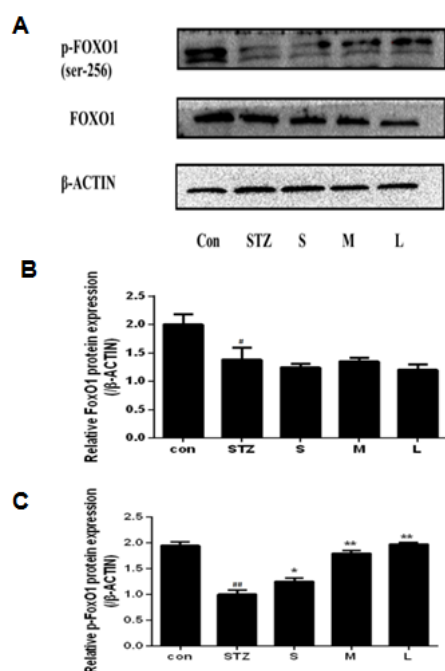


**Figure 2.** PDB extracts increase insulin secretion by RIN-m5f cells. RIN-m5f cells were untreated (con), or treated with STZ (STZ), or treated with STZ plus PDB extracts at 0.25 mg/ml (S), 0.5 mg/ml (M) or 1 mg/ml (L) for 48 h. Insulin secretion was detected by ELISA. Data were shown as  $\bar{x} \pm s$  ( $n=5$ ) and analysed by ANOVA. Compared to control group, ### $P < 0.01$ ; compared to STZ group, \*\*\* $P < 0.01$ .

### PDB extracts promotes the phosphorylation of FoxO1 in RIN-m5f cells

Compared to control group, FoxO1 and p-FoxO1 protein level in injured group decreased ( $P < 0.05$ ). Compared to injury group, FoxO1 protein level did not change significantly but p-FoxO1 protein level increased significantly in cells treated by

PDB extracts in a dose-dependent manner and the differences were significant ( $P < 0.01$ ) (Figure 3). These results suggest that PDB extracts effectively promote FoxO1 phosphorylation in STZ injured  $\beta$ -cells.



**Figure 3.** PDB extracts increase FoxO1 phosphorylation in RIN-m5f cells. RIN-m5f cells were untreated (con), or treated with STZ (STZ), or treated with STZ plus PDB extracts at 0.25 mg/ml (S), 0.5 mg/ml (M) or 1 mg/ml (L) for 48 h. A. Representative blots showing the levels of FoxO1 and p-FoxO1.  $\beta$ -actin was loading control. B. Densitometry analysis of FoxO1 level. Data were shown as  $\bar{x} \pm s$  ( $n=3$ ) and analysed by ANOVA. Compared to control group, # $P < 0.05$ . C. Densitometry analysis of p-FoxO1 level. Data were shown as  $\bar{x} \pm s$  ( $n=3$ ) and analysed by ANOVA. Compared to control group, ### $P < 0.01$ ; compared to STZ group, \* $P < 0.05$ , \*\* $P < 0.01$ .

## Discussion

The damage model of islet cells is mostly induced by high glucose stimulation or STZ treatment [6-9]. The action time of STZ is shorter than high glucose stimulation, and the damage is obvious. Therefore, this study used STZ (4 mmol/L) to induce RIN-m5f  $\beta$ -cell injury model. Maintaining the number of  $\beta$  cells and protecting  $\beta$  cell function is the key to prevent the occurrence and deterioration of type 2 diabetes [10,11]. In this study, we found that PDB extracts could effectively prevent the damage of STZ to the survival and insulin secretion of RIN-m5f  $\beta$ -cells in a dose-dependent manner.

PI3K/AKT signaling pathway is involved in the regulation of the number and function of  $\beta$  cells [12,13]. PI3K/AKT signaling promotes Fox O1 phosphorylation, and phosphorylated Fox O1 could induce  $\beta$ -cell differentiation and insulin secretion, and protected the number and function of  $\beta$  cells [14]. In this study, we found that the expression of FoxO1 and p-FoxO1 in STZ injured  $\beta$  cells decreased, but PDB extracts increased p-FoxO1 level in  $\beta$ -cells in a dose-dependent manner. Therefore, PDB extracts may promote  $\beta$  cell

differentiation and insulin secretion by affecting the phosphorylation of Fox O1.

In conclusion, this study demonstrated that PDB extracts exhibit protective effect on STZ damaged RIN-m5f cells, they increase the number of  $\beta$  cells and promote the release of insulin and the phosphorylation of Fox O1. However, the potential efficacy and possible side effects of PDB in diabetes patients remain unclear. Further clinical trials are needed to confirm the utilization of PDB extracts for the prevention and treatment of diabetes.

## Acknowledgement

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## Conflict of Interest

No

## References

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