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In vitro and in vivo protective effect of *Ganoderma lucidum* polysaccharides on alloxan-induced pancreatic islets damage

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Abstract

This study was undertaken to investigate the protective effect against alloxan-induced pancreatic islets damage by *Ganoderma lucidum* Polysaccharides (*Gl*-PS) isolated from the fruiting body of *Ganoderma lucidum* (Leyss. ex Fr.) Karst. In vitro, alloxan caused dose-dependent toxicity on the isolated pancreatic islets. Pre-treatment of islets with *Gl*-PS for 12 h and 24 h significantly reversed alloxan-induced islets viability loss. *Gl*-PS was also found to inhibit the free radicals production induced by alloxan in the isolated pancreatic islets using confocal microscopy. *Gl*-PS dose-dependently increased serum insulin and reduced serum glucose levels when pretreated intragastrically for 10 days in alloxan-induced diabetic mice. It was found that the pancreas homogenates had higher lipid peroxidation products in alloxan-treated mice than in the *Gl*-PS-treated animals. Aldehyde fuchsin staining revealed that alloxan caused nearly all the β cells disappearing from the pancreatic islets, while *Gl*-PS partly protected the β cells from necrosis. Alloxan (60 mg/kg) induced NF-κB activation in the pancreas at 30 min after injection, pretreatment with *Gl*-PS inhibited alloxan-induced activation of NF-κB. These results suggest that *Gl*-PS was useful in protecting against alloxan-induced pancreatic islets damage in vitro and in vivo; one of the mechanisms is through its scavenging ability to protect the pancreatic islets from free radicals-damage induced by alloxan.

Keywords: *Ganoderma lucidum* Polysaccharides; Alloxan; Pancreatic islets; Free radicals; NF-κB

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Introduction

Diabetes mellitus (DM) is a group of metabolic disorders with different underlying etiologies, characterized by hyperglycemia due to underutilization of glucose. Now, it affects 6% of the population in the world. Insulin and oral hypoglycemic agent including biguanides, sulfonylureas and thiazolidinediones are the main way to treat DM and are effective in controlling hyperglycemia, but these kinds of drugs also have prominent side effects. There is an urgent need to look for the efficacious agents with lesser side effects (Rang and Dale, 1991).

Plants have always been utilizable sources of drugs and many of the currently available drugs have been directly or indirectly from plants (ADA, 1997). In accordance to the recommendations of the WHO expert committee on diabetes mellitus, it seems important to investigate the hypoglycemic agents from the plant origin, which were used in traditional medicine (Alarcon-Aguilara et al., 1998).

Ganoderma lucidum (Leyss. ex Fr.) Karst is a medicinal mushroom, which belongs to the polyporacceae of aphyllophorales. Its fruiting body is called "Lingzhi" in China and "Reishi" in Japan. For hundreds of years, this mushroom has been regarded as a traditional Chinese medicine or a folk medicine used for the prevention and treatment of various human diseases, such as chronic bronchitis, hepatitis, hypertension, hypercholesterolemia, tumorigenic disease and immunological disorders in China and other Asian countries (Lin, 2001).

Ganoderma lucidum is reported to contain some biologically active components, such as polysaccharides and triterpenes. It has been shown that *Ganoderma lucidum* Polysaccharides (*Gl*-PS) have antitumor (Maruyama et al., 1989; Miyazaki and Nishjima, 1981; Zhang and Lin, 1999) and immunomodulatory (Xia and Lin, 1989; Lei and Lin, 1992, 1993) activities. *Gl*-PS is also reported to have hypoglycemic activity by increasing plasma insulin and by affecting the hepatic key enzymes in the carbohydrate metabolism in the normal and alloxan-induced diabetic mice (Hikino et al., 1985, 1989; Luo and Yang, 2000). Alloxan is a prompt and potent inducer of diabetes in experimental animals because of its damaging effect on insulin-producing β cells (Lenzen and Panten, 1988; Oberley, 1988). It has been generally accepted that alloxan-induced hyperglycemia is mainly due to its ability to induce oxygen free radicals, which damage the pancreas (Heikkila et al., 1974, 1976; Winterbourn and Munday, 1989). Recently, it has been reported that *Gl*-PS has the ability to scavenge the free radicals (Gui et al., 1996; You and Lin, 2002; Shi et al., 2002; Lee et al., 1999; Kim and Kim, 1999; Lin et al., 1995). In the present study, we investigate the effects of *Gl*-PS on the alloxan-induced damage in vivo and in vitro.

Methods

Materials

Collagenase (Type V), 2′7′-dichlorodihydrofluorescein diacetate(DCHF-DA), Alloxan (Alloxan monohydrate), Thiobarbituric acid(TBA), 3-[4,5-dimethylthiazol-2-yl]-2,5-dephenyl tetrazolium bromide(MTT) were purchased from Sigma Chemical Co.; Iodoacetic acid was purchased from Merck; Cell culture materials were purchased from GIBCO-BRL. T_4 polynucleotide kinase and oligonucleotids were purchased from Promega, Poly[dI-dC] was purchased from Pharmacia and [r-^{32}P] ATP was purchased

from Amersham. Radioimmunoassay(RIA) kits for insulin test were purchased from China Institute of Atomic Energy, Beijing, China. All the other chemicals used were of the highest grade available commercially.

Preparation of Gl-PS

Ganoderma lucidum (Leyss. ex Fr.) Karst was collected in Fujian province, China. The fruiting body of *Ganoderma lucidum* (Leyss. ex Fr.) Karst was authenticated by Professor MAO Xiaolan, Institute of Microbiology of Chinese Academy of Science. *Ganoderma lucidum* Polysaccharides (*Gl*-PS) were extracted by hot water from the fruiting body of *Ganoderma lucidum* (Leyss. ex Fr.) Karst, followed by ethanol precipitation, reserve dialysis and protein depletion. The yield of *Gl*-PS was 0.82% (w/w) in terms of the fruiting body of *Ganoderma lucidum*. It is a polysaccharide peptide with a molecular weight of 584,900 and has 17 amino acids. The ratio of polysaccharides to peptides is 93.51%: 6.49%. The polysaccharides consist of rhamnose, xylose, fructose, galactose, mannose and glucose with molar ratios of 0.793:0.964:2.944:0.167:0.384:7.94 and are linked by β-glycosidic linkages. It is a hazel-colored and water-soluble powder (Lin et al., 2002).

Isolation and pre-incubation of pancreatic islets

Male Wistar rats weighting 60 ~ 70 g purchased from the Institute of Animal, Chinese Academy of Medical Science, Beijing, China were used in the in vitro studies. Pancreatic islets were isolated from the rats by collagenase digestion (Rabinovitch, 1985). In brief, the pancreas was removed and digested with in Hank's buffer (PH 7. 4) at 37 °C in shaking waterbath for 10 min. Digestion was stopped by adding cold Hank's buffer. The undigested pancreas is allowed to remain in the vial and add collagenase solution again for another 5 min. After washing three times with the same buffer, islets were filtrated through 105 μm-nylon filter. Islets through the filter were cultured in RPMI-1640 medium containing 20 mM HEPES, 300 mg/L L-glutamine, 100,000 IU/L penicillin, 100 mg/L streptomycin, and 7% fetus serum under 95% O_2 and 5% CO_2 atmosphere at 37 °C. Twenty four-hours later, the cell suspensions were decanted, centrifuged and diluted in the same culture medium for 3-day incubation. After aspirating and removing the culture medium and the unattached cells, fresh standard culture medium containing 2.5 μg/ml iodoacetic acid were added for 3 ~ 5 h, then remove the medium and replace the same culture medium for another 3 ~ 5 days incubation for the experimental use.

Cytotoxity of alloxan

Cytotoxicity of alloxan on the pancreatic islets was determined using a MTT reduction assay. Pancreatic islets were seeded onto 96 well plates at a cell concentration of 2×10^5 cells/well in 160 μl of RPMI-1640 medium containing 5% FCS and were preincubated overnight. After preincubation, the islets were incubated at 37 °C for 30 min without or with different concentration of alloxan 20 μl (dissolved in cold normal saline and the final concentration is 0.2 mM ~ 6.4 mM). Then 20 μl MTT solution (5 mg/ml in PBS) were added to each well and the plate were further incubated for another 4 h at 37 °C. Supernatant were then discarded and 200 μl of DMSO was added to the incubation mixtures and mixed to thoroughly dissolve the dark blue crystal formazan. The absorbance at 570 nm (formation of formazan) and 655 nm(reference) were recorded with a spectrophotometer.

Effect of Gl-PS on the cytotoxicity of alloxan

Pancreatic islets were seeded onto 96 well plates at a cell concentration of 2×10^5 cells/well in 140 μl of RPMI-1640 medium containing 5% FCS and were preincubated overnight. After preincubation, the islets were incubated at 37 °C for 8 h or 20 h without or with different concentration of *Gl*-PS(dissolved in RPMI-1640 and the final concentration is 1.5625 μg/ml ~ 100 μg/ml). Then 20 μl alloxan solution (dissolve in cold normal saline and the final concentration is 3.2 mM) was added to the 96 well plates for 30 min and later the cell cytotoxicity was measured by MTT method, which mentioned above.

Time series scanning of cell fluorescence of free radicals

After isolation and pre-incubation in the 35 mm-petri dishes as mentioned above, islets were then incubated for 24 h without or with *Gl*-PS(dissolved in RPMI-1640 and the final concentration is 100μg/ml). Then the islets were washed with PBS and loaded with the ROS fluorescent probe 2′,7′-dichlorodihydrofluorescein diacetate at a final concentration of 10 μM in PBS for 30 min. The islets were washed with PBS for three times and incubated in the PBS for the confocal microscope investigation. Alloxan (the final concentration is 3.2 mM) was added to the medium and record the image for 40 min under confocal microscope (German TCS-SP2 confocal microscope) with an argon-krypton laser was used. Islets without *Gl*-PS and alloxan were as control group. The changes of free radicals over time and the fluorescent images were recorded every 20 s. Cells were imaged via the epifluorescence mode with a 20× immersion lens at Exl 488 nm and Eml 530 nm. The cell images were stored on computer and transformed to the cell fluorescence intensity using Leica confocal software.

Drug administration and induction of diabetes in mice

The experimental animals were male albino Swiss mice weighing 18 ~ 22 g, which were purchased from the Department of Experimental Animals, Peking University Health Science Center, Beijing China. All procedures were carried out in accordance with the Institute's ethical committee's requirements for experimental use of animals. Mice were divided into five groups. Groups 1 and group 2 were normal and diabetic control animals given saline intragastrically; group 3,4,5 animals were respectively given intragastrically *Gl*-PS 50, 100 and 200 mg/kg body weight. These pretreatments were administrated for ten consecutive days. On the seventh day of the experiment, 8 hours after *Gl*-PS administration, diabetes was induced by a single intravenous injection of alloxan 60 mg/kg to the 24-hour fasted mice, control mice received only saline. On the tenth day, 3 hours after the last intragastric administration, the overnight-fasted mice were decapitated under mild ether anesthesia.

Determination of serum glucose and insulin

Blood samples were collected from the retroorbital venous plexus and the serum was separated for measurement of the levels of glucose by the glucose-oxidase method and insulin by using a commercially available radioimmunoassay kit with porcine insulin as the standard.

Determination of pancreatic lipid peroxides products

The duodenal regions of pancreas were removed and homogenized in the cold PBS buffer for the determination of the contents of lipid peroxides products using the method of thiobarbituric acid and were expressed in terms of malonaldehyde (MDA) formed (Ohkawa et al., 1979). Protein content of tissue homogenates was measured by Bradford method (Bradford, 1976) using bovine serum albumin as the standard protein.

Aldehyde fuchsin staining of the pancreas

The splenic regions of pancreas were fixed in Bouin's fixative, 5 μm thick sections were treated with Halmi modified Gomori's aldehyde fuchsin reagent (Halmi, 1952; Gomori, 1950). The β cells, with amaranth granules, sharply contrasted with the α cells which were stained saffron yellow. The β cells were counted in 10 islets/slide under a light microscope at 50-fold magnification, in order to determine the mean value of β cells/islet.

Determination of NFκB activation in vivo

Normal and diabetic control animals were given saline, and *Gl*-PS-treated mice were pretreated intragastrically with *Gl*-PS (50 and 100 mg/kg) for consecutive seven days. 8 hours after last *Gl*-PS administration, diabetes was induced by a single intravenous injection of alloxan 60 mg/kg to the 24-hour fasted mice, normal control mice received only saline. Mice were killed at 30 min after alloxan injection, pancreas were immediately removed. Crude nuclear extracts were prepared from the pancreas as described previously (Dereckere and Gannon, 1994) and the effect of *Gl*-PS on the NFκB activation were determined using an electrophoretic mobility shift assay(EMSA). Double-stranded synthetic oligonucleotides probes for NFκB (5′-AGTGAGGGGACTTTCCCAGGC-3′) were end-labeled using [r-^{32}P] and T_4 polynucleotide kinase. Binding reactions containing equal amounts of protein (~ 10 μg) and labeled oligonucleotide probes were performed for 30 min at 37 °C in binding buffer(4% glycerol, 1 mM $MgCl_2$, 0.5 mM ethylenediaminetetraacetate, 0.5 mM dithiothretol, 50 mM NaCl, 10 mM Tris, pH 8.0, 50 μg/ml poly[dI-dC]). Specific binding was confirmed using 100 fold excess unlabelled NFκB

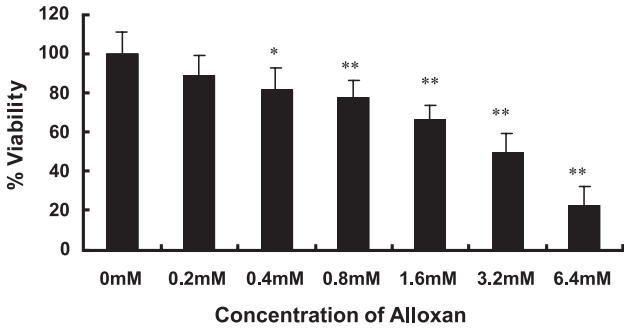

Fig. 1. Effect of different concentration of alloxan on the pancreatic islets viability. Pancreatic islets were incubated with different concentration for 30 min, and then the pancreatic islets viability was detected by MTT assay. n = 6. *P < 0.05 **P < 0.01 vs. 0 mM control group.

oligonucleotides as a specific competitor. Protein-DNA complexes were separated using 8% non-denaturing polyacrylamide gel electrophoresis followed by radiomography to detect the level of retardation produced by binding to NFκB probe.

Statistical analysis

Results obtained are expressed as mean ± S.D. Statistical analysis was performed by one-way ANOVA followed by least-significant difference (LSD). *P* values below 0.05 were considered significant.

Results

Effect of cytotoxicity of alloxan on the pancreatic islets

Alloxan administration to the isolated pancreatic islets for 4.5 h caused dose-dependent toxicity. At 3.2 mM alloxan, nearly 50% of the islets were necrotic and at the concentration of 6.4 mM, most of the islets (80%) were necrotic (Fig. 1).

Effect of Gl-PS on the cytotoxicity of alloxan

Preincubation of *Gl*-PS for 12 h partly protected the islets toxicity induced by 3.2 mM alloxan at the concentration of 100 μg/ml. Prolong the incubation time to 24 h, *Gl*-PS protected the islets from being destroyed by alloxan in a concentration-dependent manner. At the concentrations of 25 μg/ml and 100 μg/ml, *Gl*-PS increased the cell viability from 47.87% in cell treated with alloxan only to 70.3% and 80.15% respectively (Figs. 2 and 3).

Effect of Gl-PS on the time series scanning of free radicals induced by alloxan in pancreatic islets

Fluorescent intensity remained unchanged in the normal control islets. In the alloxan control group, at 10 min after alloxan addition, the fluorescence began to increase and the fluorescence increase

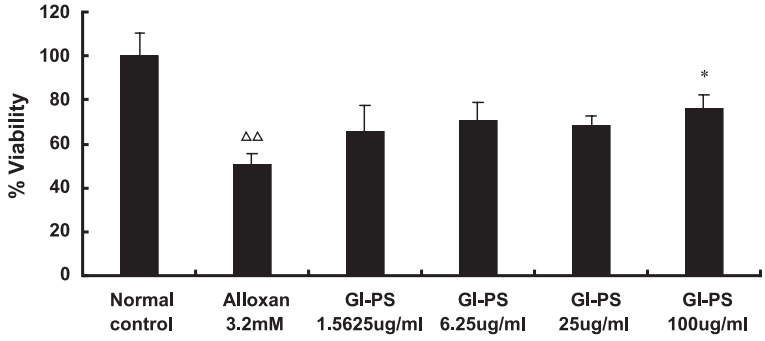

Fig. 2. Effect of *Gl*-PS for 12 h on the pancreatic islet viability induced by alloxan. n = 6. Pancreatic islets were preincubated with different concentration of *Gl*-PS for 8 h and then alloxan 3.2 mM were added to the culture medium for 30 min except the control group. The pancreatic islets viability was detected by MTT assay. $^{\triangle\triangle}P < 0.01$ vs. control group; $^{*}P < 0.05$ vs. alloxan 3.2 mM group.

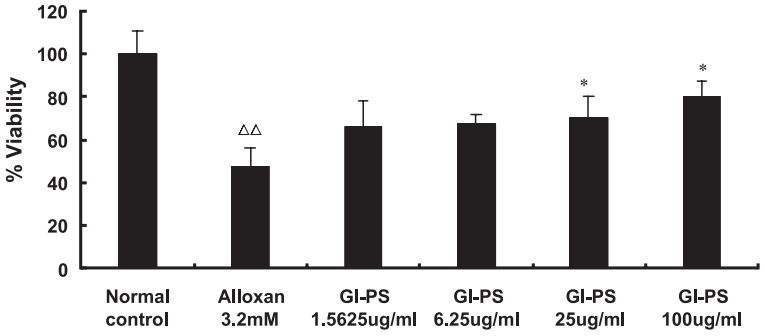

Fig. 3. Effect of *Gl*-PS for 24 h on the pancreatic islet viability induced by alloxan. n = 6. Pancreatic islets were preincubated with different concentration of *Gl*-PS for 20 h and then alloxan 3.2 mM were added to the culture medium for 30 min except the control group. The pancreatic islets viability was detected by MTT assay. $^{\triangle\triangle}P < 0.01$ vs. control group; $^{*}P < 0.05$ vs. alloxan group.

significantly 20 min later as compared to that of 0 min, it reached maximum at 30 min and maintained at this level for 10 min at least, the fluorescence increase 4.5-time fold. Addition of *Gl*-PS100 μg/ml and alloxan nearly at the same time, the fluorescent intensity also increased significantly. Fluorescence of free radicals in the islets preincubated with *Gl*-PS100 μg/ml for 24 h were inhibited completely (Fig. 4).

Effect of Gl-PS on serum glucose and insulin levels in alloxan-induced hyperglycemic mice

As shown in Table 1, Alloxan 60 mg/kg administration to mice caused the expected rise in serum glucose and reduction in serum insulin 72 h later ($p < 0.01$ compared to untreated controls).

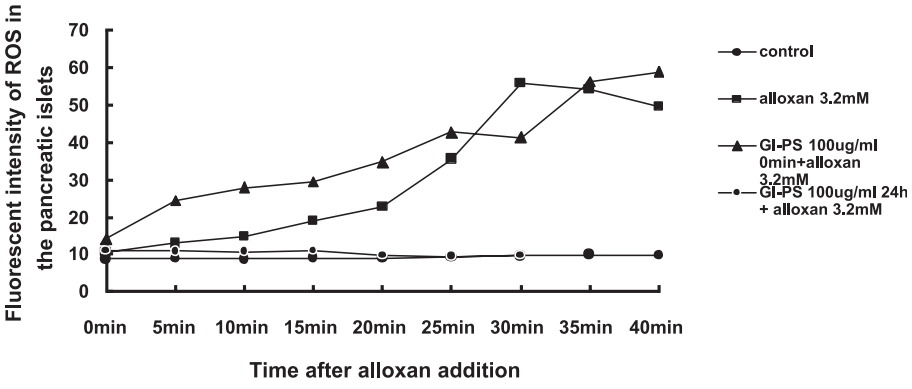

Fig. 4. Effect of *Gl*-PS 100 μg/ml on the time series scanning of free radicals induced by alloxan (3.2 mM) in pancreatic islets. Islet were incubated incubated at 37 °C for 24 h without or with 100 μg/ml *Gl*-PS (dissolved in RPMI-1640). Then the islets were loaded with the free radicals fluorescent probe (DCHF-DA) at a final concentration of 10 μM in PBS for 30 min and the cells were washed with PBS for three times. Alloxan (the final concentration is 3.2 mM) was added to the medium and record the image for 40 min under confocal microscope. Islets without *Gl*-PS and alloxan were as control group. The cell images were stored on computer and transformed to the cell fluorescence intensity. Each point represents mean islet fluorescent intensity of free radicals. n = 15 ~ 20.

Table 1
Effect of *Gl*-PS on serum glucose and insulin levels in alloxan-induced diabetic mice

Group	n	Serum glucose (mmol/L)	Serum insulin (mIU/L)
Normal control	10	5.57 ± 1.35	14.36 ± 5.21
Diabetic control	13	26.36 ± 5.03[a]	8.49 ± 1.49[b]
Gl-PS 50 mg/kg i.g	13	23.45 ± 5.05	9.31 ± 2.06
Gl-PS 100 mg/kg i.g	13	22.39 ± 5.40	9.29 ± 1.76
Gl-PS 200 mg/kg i.g	13	15.09 ± 5.71*	11.83 ± 2.62*

Diabetic mice were pretreated with *Gl*-PS for 10 consecutive days and diabetes was induced by a single intravenous injection of alloxan 60 mg/kg. Values are given as mean ± S.D.
[a] $P < 0.01$ vs. the normal control group.
[b] $P < 0.05$ vs. the normal control group.
*$P < 0.05$ vs. the diabetic control group.

Pretreatment with *Gl*-PS decreased serum glucose levels and increased insulin levels, especially with *Gl*-PS 200 mg/kg, which showed statistical significance ($p < 0.05$) as compared to the group receiving alloxan alone.

Effect of Gl-PS on the MDA content in the pancreas of alloxan-induced hyperglycemic mice

The pancreas MDA levels were significantly higher in the diabetic control mice ($p < 0.05$) as compared to the normal control group. Pretreatment with *Gl*-PS 200 mg/kg decreased the pancreas MDA significantly as compared to the diabetic control ($p < 0.05$) (Table 2).

Effect of Gl-PS on the aldehyde fuchsin staining of the pancreas in alloxan-induced hyperglycemic mice

In the normal pancreas, the β cells occupied about 80% of each islet; the average number of β cells per islet was 34. Alloxan caused nearly all the β cells to be absent in the Langerhan's islets, 0 ~ 3 β cells commonly appeared in one islet, which led to the reduction in the number and size of the islets.

Table 2
Effect of *Gl*-PS on the MDA content in the pancreas of alloxan-induced diabetic mice

Group	n	Pancreas MDA (nmol/mgprot)
Normal control	10	0.66 ± 0.10
Diabetic control	13	0.91 ± 0.22[a]
Gl-PS 50 mg/kg i.g	13	0.81 ± 0.14
Gl-PS 100 mg/kg i.g	13	0.79 ± 0.19
Gl-PS 200 mg/kg i.g	13	0.72 ± 0.10*

Diabetic mice were pretreated with *Gl*-PS for 10 consecutive days and diabetes was induced by a single intravenous injection of alloxan 60 mg/kg. Values are given as mean ± S.D.
[a] $P < 0.05$ vs. the normal control group.
*$P < 0.05$ vs. the diabetic control group.

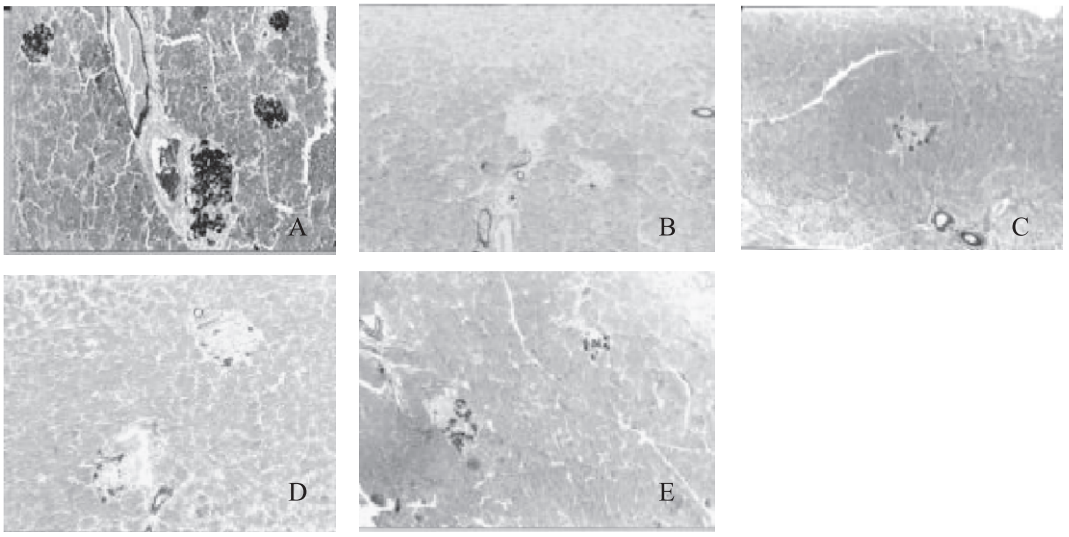

Fig. 5. Mouse pancreas stained with Gomori's aldehyde fuchsin reagent at 50-fold magnification. The splenic regions of pancreas were removed, and fixed in Bouin's fixative and stained with Gomori's aldehyde fuchsin reagent The β cells, with amaranth granules, sharply contrasted with the α cells which were stained saffron yellow. Diabetic mice were pretreated with *Gl*-PS for 10 days. A: normal control; B: diabetic control; C: *Gl*-PS 50 mg/kg; D: *Gl*-PS 100 mg/kg; E: *Gl*-PS 200 mg/kg.

Pretreatment with *Gl*-PS (50 mg/kg, 100 mg/kg and 200 mg/kg) partly protected the β cells from necrosis, the number of β cells were increased, the average number of β cells per islet was 5, 10 and 19 respectively, especially with the dose of 200 mg/kg (Figs. 5 and 6).

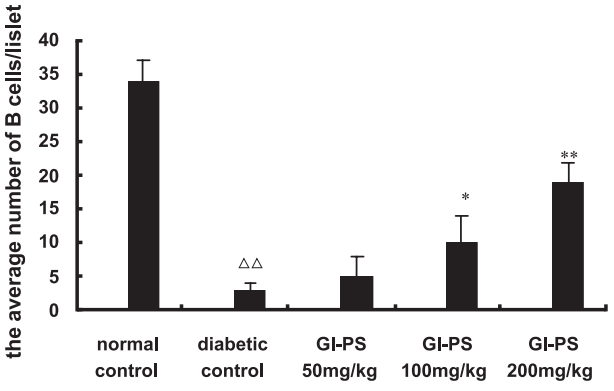

Fig. 6. Protective effect of *Gl*-PS on the average number of β cells/islet in alloxan-induced diabetic mice. n = 6. The splenic regions of pancreas were removed, and fixed in Bouin's fixative and stained with Halmi modified Gomori's aldehyde fuchsin method. The pancreas were counted in 10 islets/slide under light microscope at 50-fold magnification to estimate the mean value of β cells numbers/islet. Values are given as mean ± S.D. Diabetic mice were pretreated with *Gl*-PS for 10 days. $^{\triangle\triangle}P < 0.01$ vs. the normal control group; $^{*}P < 0.05$, $^{**}P < 0.01$ vs. the diabetic control group.

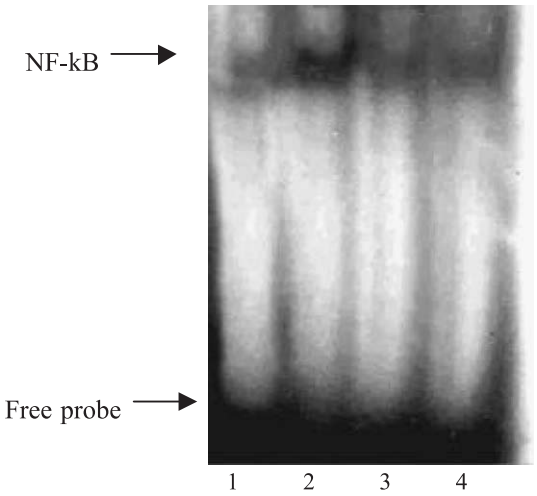

Fig. 7. Effect of *Gl*-PS on the NF-κB activation in pancreas induced by alloxan. *Gl*-PS-treated mice were pretreated with *Gl*-PS (100 and 200 mg/kg) for consecutive seven days. 8 hours after last *Gl*-PS administration, diabetes was induced by a single intravenous injection of alloxan 60 mg/kg to the 24-hour fasted mice, control mice received only saline. Mice were killed at 30 min after alloxan injection, pancreas were immediately removed. Crude nuclear extracts were prepared from the pancreas and the effect of *Gl*-PS on the NFκB activation were determined using an electrophoretic mobility shift assay (EMSA). Lane 1: control; Lane 2: alloxan-treated group; Lane 3: *Gl*-PS 100 mg/kg group; Lane 4: *Gl*-PS 200 mg/kg group. Results are the representative of two separate experiments.

Effect of Gl-PS on the alloxan-induced NFκB activation

Similar to previous studies, significant NFκB activation in the pancreas was observed 30 min after alloxan injection as compared to the normal control. Pretreatment with *Gl*-PS (100 and 200 mg/kg) partly inhibited the activation of NFκB (Fig. 7).

Discussion

Alloxan is a derivative of pyrimidine, which was reduced to dialuric acid by biological reducing agent; Dialuric acid readily autoxidize, establishing a redox cycle for generation of superoxide anions, hydrogen peroxide and hydroxyl radicals, thus lead to tissue injury (Deamer and Heillila, 1971). Pancreas is especially susceptible to alloxan-induced-free radical damage probably due to its low levels of key enzymes that scavenge oxygen free radicals (Malaisse, 1982). It is suggested that the free radical species responsible for the alloxan toxicity is hydroxyl radicals (Heikklia et al., 1976). Hydroxyl radicals can induce lipid peroxidation in membranes as well as attack DNA, RNA and proteins as well as other cell components. This reactivity can explain why alloxan rapidly affects the function of β cells (Jain and Logothetopoulos, 1976). As in the whole animal, the toxic effects of alloxan are confined to β cells and other endocrine cells and the exocrine parenchymal cells are not damaged (Anne et al., 1999). We found that isolated pancreatic islets incubated with alloxan for 4.5 h suffer concentration-dependent decrease in cell viability, this result was consistent with previous

studies of the alloxan toxicity on the isolated islets (Jorns et al., 1997). In the present study, we used DCHF-DA as the free radicals fluorescent probe and found that alloxan could act as an exogenous free radicals generator in isolated pancreatic islets. In vivo study, 72 h after alloxan injection to the fasting mice, the lipid peroxidation product MDA content of pancreas increased, which result in the damage of pancreas β cells, and led to the reduction of serum insulin contents and elevation of glucose levels ultimately.

The scavenging effect of *Gl*-PS has been reported by many researchers (Gui et al., 1996; You and Lin, 2002; Shi et al., 2002; Lee et al., 1999; Kim and Kim, 1999; Lin et al., 1995). In this study, preincubation with *Gl*-PS for 12 h and 24 h protected isolated pancreatic islets from being destroyed by alloxan in a time and concentration-dependent manner. The results of preincubation of *Gl*-PS for 24 h on the time series scanning of cell fluorescence of free radicals also suggest that *Gl*-PS has the ability to scavenge the free radical induced by alloxan completely, while alloxan addition soon after *Gl*-PS can't inhibit the free radical formation. These results indicate that *Gl*-PS exerted its protective effect intracellularly, rather than extracellularly reacting with alloxan in the culture medium. The findings that pretreatment with *Gl*-PS can reduce the lipid peroxidation product MDA content of pancreas, decrease serum glucose levels and increase serum insulin significantly in alloxan-induced hyperglycemia agree with the idea that it is able to alleviate alloxan toxicity. The results show that *Gl*-PS can protect the animals from being damaged by free radicals directly; this in turn may contribute to the regulation of blood glucose.

Some researchers have predicated that critical determination in β cell death leading to IDDM may be the activation of NFκB. NFκB is a transcription factor which can be activated by oxidants and other stresses (Siebenlist et al., 1994; Baldwin, 1996). It has been reported that alloxan induce maximal NFκB activation at 30 min after injection in vivo (Ho et al., 2000). Once activated, NFκB translocates to the nucleus and binds to DNA and upregulates the expression of several genes such as iNOS and TNFα. These target proteins initiate and propagate the immune/inflammatory response resulting in the invasion immune cells into the pancreas that produce and amplify the free radical production and destroy theβcell ultimately (Ho et al., 2000). Some antioxidants such as PBN and N-acetylcysteine inhibit the NFκB activation by influencing the redox cycle state of the cell and reduce the severity of alloxan-induced diabetes (Ho et al., 1999, 2000). Our results indicate that *Gl*-PS pretreatment inhibit the alloxan-induced NFκB activation and subsequent β cell death in pancreas, thus also reduce the severity of diabetes as we found in the animal experiments.

Although it is generally believed that alloxan-induced hyperglycemia is mainly due to its ability to induce oxygen free radicals, there were also other mechanisms. It has been suggested that alloxan destroys β-cell function by inhibiting the enzyme activity of glucokinase and hexokinase through oxidation of two thiol groups which are in the glucose-binding site of the enzyme, this reaction is not caused by alloxan-generated free radicals (Lenzen and Munday, 1991). It was also found that alloxan significantly reduced the ex vivo mRNA and protein expression of glucose transporter 2 (GLUT2) (Walde et al., 2002). This may be the reason why *Gl*-PS inhibited the islets free radical formation induced by alloxan completely, while it could not protect the pancreatic islets from being destroyed by alloxan completely.

In summary, pretreatment with *Gl*-PS was found to produce an antihyperglycemic effect in alloxan-induced hyperglycemic mice. One mechanism may be through its scavenging ability to protect the pancreatic islets from free radicals-damage induced by alloxan in vivo and in vitro.

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