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Full Length Research Paper

Hypoglycemic effects and mechanisms of *Portulaca oleracea* L. in alloxan-induced diabetic rats

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Portulaca oleracea* L. (POL) has been used as one of the traditional oriental medicines to treat bacteria, virus, antherasis, caducity, diabetes and for enhancing immunity. This study aims at revealing effects of POL on alloxan-induced diabetic rats and its mechanisms. According to thin layer chromatography assay, the main compounds of POL included organic acid, flavonoids, alkaloids, terpene ansteroid, hydroxybenzene, saponin and polysaccharide. The rats were divided into four groups: normal control (NC), diabetes control (DC), diabetes + POL high dose (400 mg/kg), diabetes + POL low dose (200 mg/kg). The diabetic rats were administrated with POL or dH_2O daily for 28 days. The POL treatment resulted in significant decreases of fasting blood glucose, total cholesterol and triglycerides. POL also showed a tendency of improvement body weight gain on diabetic rats. Furthermore, the DC group had low serum insulin level comparing with that of NC group, at the same time, the insulin levels were dose-dependently raised in the POL-treated groups than that of DC group. According to single cell gel electrophoresis and LD_{50} analysis, POL was proved to be nontoxic to the animals. The results indicate that POL would alleviate the blood glucose and lipid rising associated with diabetes, and improve the abnormal glucose metabolism and increase insulin secretion by restoring the impaired pancrease β cells in alloxan-induced diabetic rats, which suggest that POL has the hypoglycemic potential and could be useful on the diabetes therapy.

Key words: *Portulaca oleracea* L., diabetes, hypoglycemia, hypolipidemia, insulin.

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder characterized by hyperglycemia and alterations in carbohydrate, fat and protein metabolism, associated with absolute or relative deficiencies in insulin secretion and/or insulin action. The symptoms of diabetes are polyuria, polydipsia, polyphagia, pruritus and unexpected weight loss, and so on (Velasco Plaza et al., 1993; Wong and Tzeng et al., 1993). About 100 million people around the world have been diagnosed with diabetes by the year 2010, it is projected that 215 million people will have the disease (Zimmet, 1999). Recently, appropriate hypoglycemic agents have been focused on plants used in traditional medicine, because some natural products in traditional medicine may be better treatments than currently used drugs (Rates, 2001). However, the compounds and precise antidiabetic mechanisms of most herbs remain to be indistinct.

Portulaca oleracea L. (POL) (Chinese name Ma-Chi-Xian) is widely used in China not only as an edible plant, but also as a traditional Chinese herbal medicine for alleviating pain and swelling. It also has the abilities of defending bacteria, virus, antherasis, caducity, diabetes, and for enhancing immunity (Jian et al., 1986), but the mechanism of action and its compounds have not been clarified. In this study, we analyzed the compounds of POL extract and investigated its hypoglycemic effects. Activities of the extract were determined by comparing the changes in blood glucose, body weight, serum triglyceride (TG) and total cholesterol (TC) levels in alloxan-induced diabetic rats. Furthermore, the serum insulin levels in four groups were determined by Elisa,

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and the toxicity of POL was assayed in single-cell gel electrophoresis and LD_{50} experiments.

MATERIALS AND METHODS

Reagents and herb

A glucose analyzer (GT-1640) and strip were purchased from Arkray Inc. (Japan). Alloxan was obtained from Sigma Co. (USA). GF254-TLC plates were bought from Qingdao Haiyang Chemical Co. Other chemicals were analytic grade. Dried POL were purchased from a local drug store and authenticated by the specialists.

Preparation of *P. oleracea* L. extracts and characterization

The air-dried *P. oleracea* L. (500 g) were powdered, and the powder was extracted with dH_2O (1 g/10 ml) with constant stirring for 4 h and then filtered. Residue was extracted again as above method one time. The filtrates were combined and dried in a rotary evaporator under reduced pressure at 55℃, and then freeze-dried in a lyophilizer. The yield of the extract was approximately 20%. The extract was examined by thin layer chromatography (TLC) analysis to identify the main compounds. The POL solution was dotted on the TLC plates, and n-butanol:acetic acid: H_2O (4:3:1) was used as the solvent system (Gao, et al., 2007) , then the indicators were sprayed on the plates, respectively. The plates were heated at 105℃ for 10 min. in an oven. Nine kinds of indicator system were used to identify the compounds of POL, which included iodine and potassic iodide determining alkaloide, ferric trichloride determining hydroxybenzene, phenol and sulfuric acid determining polysaccharide, and so on (Lu, et al., 2005).

Preparation of experimental animals

Wistar rats were bred in the Animal Department of Beijing Institute of Traditional Medical and Pharmaceutical Sciences. Male rats (180 - 200 g) were maintained in standard environmental conditions of temperature (22-25℃), relative humidity (60 - 70%), dark/light cycle, and fed a standard diet and water *ad libitum*. All animal procedures were performed according to the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health, as well as guideline of the Animal Welfare Act. After 1 week of acclimatization, the rats were subjected to a 16 h fast. Diabetes was induced with a single injection of alloxan 150 mg/kg body weight i.p. The alloxan was freshly dissolved in ice-cold 0.9% sodium chloride solution. After five days, the rats with marked hyperglycemia (fasting blood glucose >11.1 mmol/L) were selected and used for the study. Thirty-two rats (8 normal rats, 24 alloxan-induced diabetic rats) were chosen and divided into four groups: normal control group (NC), diabetes control group (DC), diabetes + POL low dose group (DM + POL LD) and diabetes + POL high dose group (DM + POL HD).

Acute term effect of *P. oleracea* L. extract on blood glucose level

On day 1, the acute blood glucose test was performed to assess the hypoglycemic effect of POL extract. After overnight fasting with free access to water, the rats were orally administrated using gavage with POL extract suspension (200, 400 mg/kg b.w.), which was dissolved in dH_2O with dose 200 mg/kg b.w. for POL LD group, and 400 mg/kg b.w. for POL HD group, or administrated with same volume dH_2O only for normal and diabetic control groups. Tail blood sample was taken at 0, 0.5, 1, 1.5, 2.0 and 4 h after the POL administration. Blood glucose level of blood sample at various time points was measured by glucose oxidase method.

Long term effect of *P. oleracea* L. extract on blood glucose level

The POL treated rats each was given with POL extract, which were dissolved in dH_2O, on 200 mg/kg b.w. (for low dose group) and 400 mg/kg b.w. (for high dose group) daily by gavage for 28 days. On contrast, the control rats (NC and DC) were given with same volume of dH_2O only. At day 0, 7, 14, 21 and 28, blood samples were collected from tail veins following overnight fasting and measured. At the same time, the body weight of each rat was recorded.

Effect of *P. oleracea* L. on serum triglyceride, total cholesterol and insulin levels

On day 29, the rats were fasted overnight; the blood samples were collected in a sterile tube by sinoocular puncture under ether anesthesia, and left to stand at room temperature for 2 h, then centrifuged at 1500 × g for 15 min at 4℃. The supernatant was immediately separated from the pellet to prepare serum and determine the levels of TG and TC using an automated chemistry analyzer (OLYMPUS, Japan), following the manufacturer's instructions (Center of Medical Science and Technology, Capital Medical University, Beijing, China). Serum insulin level was then determined by insulin-ELISA kit (Insulin ELISA kit, Adlitteram Diagnostic Laboratories Co., USA) according to the manufacturer's instruction.

LD_{50} experiment

To determine acute toxicity of a single oral administration of POL, different doses of the POL (0.8, 1.2, 1.6, 2.0 g/kg) were administrated to different rats (6 rats were used for each group, control rats received dH_2O). Mortality and general behavior of the animals were observed periodically for 48 h. The animals were observed continuously for the initial 4 h and intermittently for the next 6 h and then again at 24 and 48 h following drug administration.

Single cell gel electrophoresis

Single cell gel electrophoresis (SCGE) was performed according to the method of Singh et al. with some modification (Singh et al., 1988). The blood was collected from 10 rats and lymphocytes were separated from whole blood using a Ficoll Paque lumphocytes separation medium and then suspended in PBS (Collins and Dusinska et al., 2002). Cells were incubated in RPMI 1640 (10% fetal bovine serum) medium and exposed to the test compounds, which included dH_2O control (same volume), POL (50, 100 µg/ml, final concentration) or H_2O_2 (5 µmol/L), cultured at 37℃ in a 5% CO_2, 95% air incubator for 1 h.

Then the cells were centrifuged at 4℃ and suspended in a small volume of PBS. The cells were mixed with 0.5% low melting-temperature agarose at 37℃, and then placed on precleaned microscope slides covered with thin layer of 0.5% normal melting-point agarose. The slides were covered with a third layer of low melting-point agarose. The slides were immersed in a lysing solution for 1 h to lyse the cells and permit DNA unfolding. Electrophoresis was conducted at 25 V for 20 min. After

Table 1. Compounds of POL extracts by TLC analysis.

Indicator	Examined components	Ratio of flow (Rf)	Color
Iodine / potassic iodide	Alkaloide	0.2543	Brown
Ferric trichloride / water	Hydroxybenzene	0.2231	Purple
Acetic anhydride / sulfuric acid	Terpene ansteroid	0.2562	Prunosus
Phosphomolybdic acid / ethanol	Saponin	0.2447	Dark blue
Phenol / sulfuric acid	Polysaccharide	0.2783	Brown
10% KOH	Anthraquinone	-	-
10% NaOH	Flavone	0.2418	Yellow
Sulfuric acid / ethanol	Lignin	-	-
Bromophenol blue / ethanol	Organic acid	0.2198	Dark yellow

"-"means without any spot on the plates.

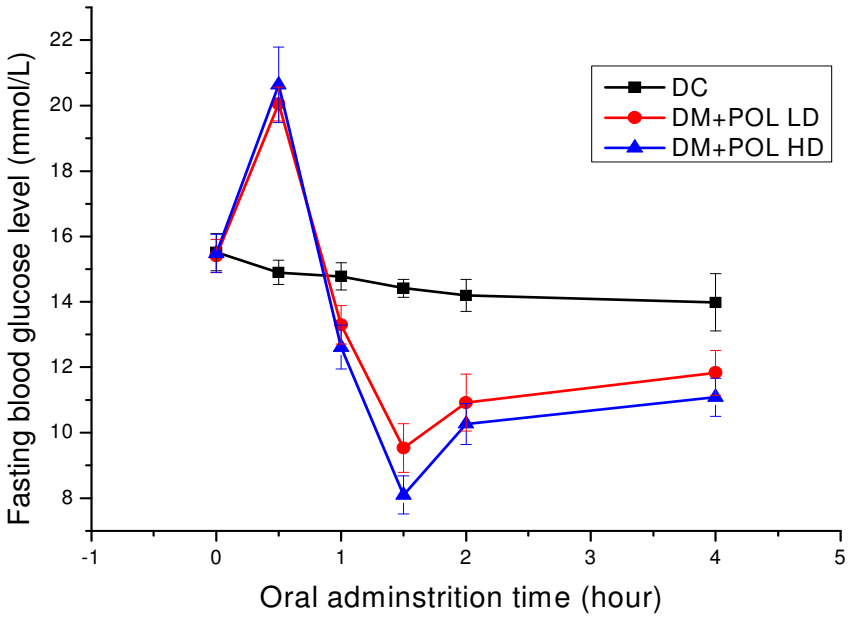

Figure 1. Effect of POL on blood glucose in the acute blood glucose test.

electrophoresis, the slides were washed gently to remove alkali and detergents with Tris Buffer, rinsed with dH_2O, and then stained with ethidium bromide (20 µg/ml). The slides were evaluated under a fluorescence microscope (Nikon). Four different cultures were analyzed, the tail lengths of 100 cells per culture evaluated and categorized. The assay was repeated three times to avoid selection bias.

Statistical analysis

Statistical analyses were performed using the SPSS 13.0 statistical software package. Data are expressed as mean ± S.E. The effects of POL on acute term and long term blood glucose levels were determined using two-way ANOVA repeated measures, followed by the Turkey test.

Differences in body weight, blood lipid and serum insulin levels among normal/diabetic control rats and POL-treated low/high dose rats were analyzed using one-way ANOVA, followed by Scheffe test, and the differences between the NC and DC groups was compared using Student's T-test. Results were considered significantly different at the level of $p < 0.05$.

RESULTS

Components of *P. oleracea* L. in the thin layer chromatography assay

The results of POL TLC assay were shown in Table 1. There were seven main compounds in POL, including organic acid, flavonoids, alkaloids, hydroxybenzene, terpene ansteroid, saponin and polysaccharide.

Effect of *P. oleracea* L. on acute blood glucose test

There was no significant difference at 0 min among the DC and POL treated groups (Figure 1). However, the supplementation of POL decreased the blood glucose levels of the diabetic rats. The hypoglycaemic effect of POL became significant following oral administration 1 h, reached the peak at 1.5 h ($p < 0.01$), and was still

Table 2. Effect of POL treatment on blood glucose in the long term blood glucose test.

Groups	Blood glucose levels (mmol / L)				
	Day 0	Day 7	Day 14	Day 21	Day 28
NC	4.83 ± 0.38	4.68 ± 0.43	4.93 ± 0.34	4.77 ± 0.53	4.8 ± 0.46
DC	15.52 ± 0.56	15.62 ± 0.92	15.18 ± 0.65	15.88 ± 1.15	16.08 ± 0.39
DM+POL LD	15.4 ± 0.51	12.61 ± 0.61	11.22 ± 0.65*	10.31 ± 0.41*	9.76 ± 0.92*
DM+POL HD	15.48 ± 0.58	12.2 ± 0.70	10.98 ± 0.80*	9.68 ± 0.86*	8.33 ± 0.45*

Each value represents mean ± S.E. of six rats per group, * Represents statistical significance vs. diabetes control ($p < 0.01$).

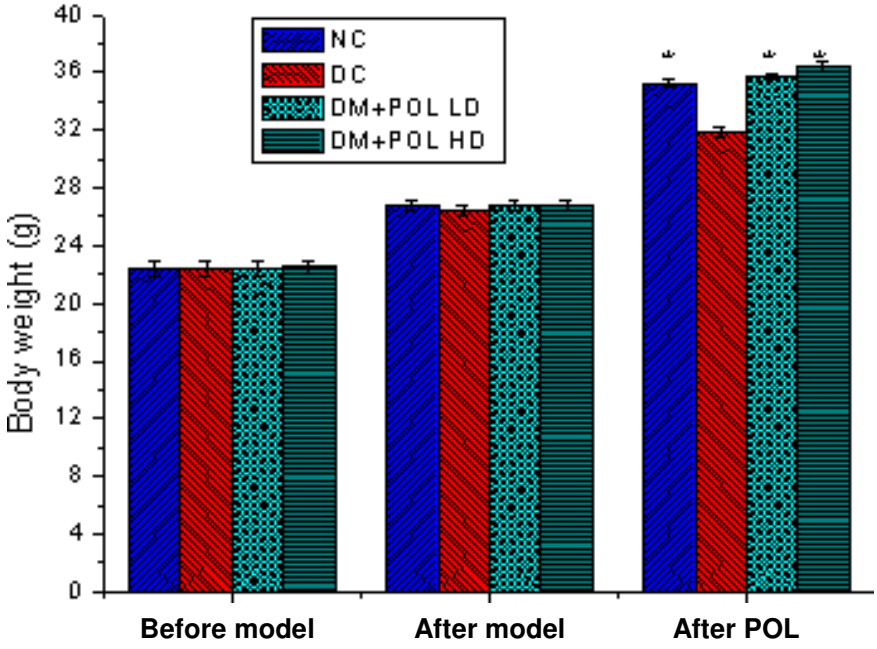

Figure 2. Effect of POL treatment on body weight of the rats, * Represents statistical significance vs. diabetic control group ($p < 0.05$).

significant at 4 h, which was shown in the dosage dependent manner. There was no significant change for blood glucose levels in DC group rats during the acute glucose test ($p > 0.05$). The decrease rates of blood glucose (at low and high dose POL treated groups) were 23.16 and 28.44%, respectively. The results indicated that POL has acute hypoglycaemic potential.

Effect of *P. oleracea* L. on long term blood glucose test

During POL treatment for 4 weeks, the level of blood glucose was measured for once every week. The results were summarized in Table 2. Before inducement of diabetic phenotype, there was no significant difference on blood glucose levels among the groups ($p > 0.05$). The blood glucose levels in the POL treated groups showed no significant difference at the end of the first week of drug administration ($p > 0.05$), but those in the two POL treated groups were all lower than that in the DC group ($p < 0.01$) after 14 days treatment. On the day 28, blood glucose levels were decreased in the POL LD and POL HD groups by 36.58 and 46.17%, respectively. The NC and DC rats did not show any significant variation on the blood glucose level throughout the experimental period ($p > 0.05$). These results indicated POL decreases hyperglycemia in diabetic rats.

Effect of *P. oleracea* L. on body weight of rats

Changes of body weights in control and experimental groups were listed in Figure 2. There was no significant difference on initial body weights (before model) among the four groups ($p > 0.05$). POL treatment improved the

Table 3. Effect of POL treatment on TC and TG levels.

Groups	TG (mmol/L)	TC (mmol/L)
NC	1.651 ± 0.014	2.709 ± 0.031
DC	2.069 ± 0.047	3.335 ± 0.063
DM+POL LD	1.853 ± 0.025*	3.159 ± 0.030*
DM+POL HD	1.766 ± 0.016**	3.136 ± 0.051**

Each value represents mean ± S.E. of six rats per group, * Represents statistical significance vs. diabetes control ($p < 0.05$). ** Represents statistical significance vs. diabetes control ($p < 0.01$).

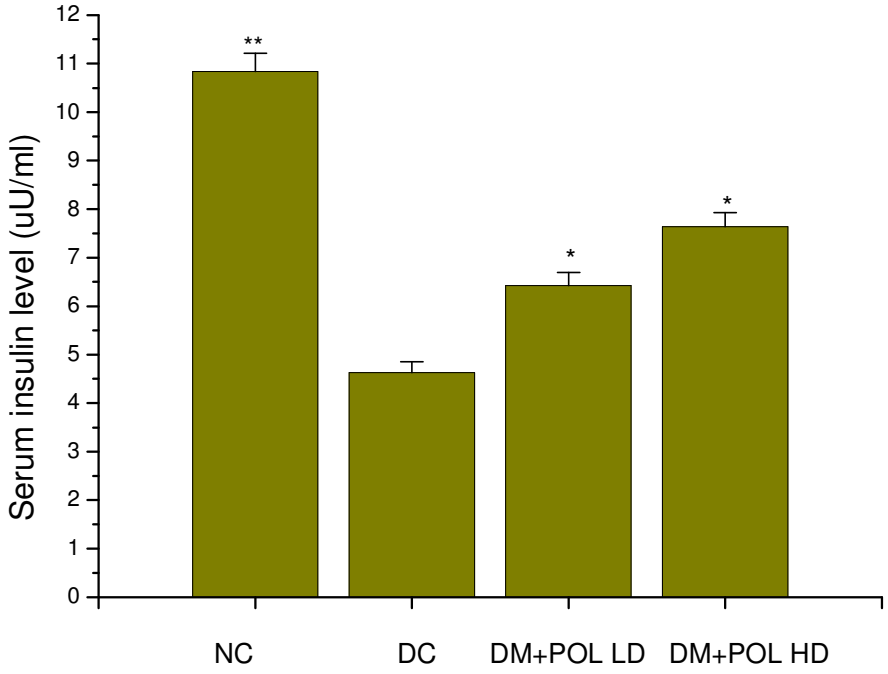

Figure 3. Effect of POL treatment on serum insulin level, *Represents statistical significance vs. diabetic control group ($p < 0.05$), ** Represents normal group vs. diabetic control group ($p < 0.01$).

weight gain comparing to diabetic control rats. By the end of the experiment, the body weight of the normal control group was significantly increased. In contrast, the rats of the diabetic control group slightly increase body weight during the experiment period ($p > 0.05$). Following POL treatment for 4 weeks, the body weight of rats in both POL treated groups was significantly increased than that of DC group ($p < 0.01$).

Effect of *P. oleracea* L. on serum total cholesterol and triglycerides levels

Serum TG and TC levels were determined on the day 29, and the results were summarized in Table 3. The serum TG and TC levels were significantly higher in the DC group than that of the NC group. TG levels in DM + POL LD and HD groups were lower than that of DC group ($p < 0.01$), meanwhile TC levels in DM+ POL LD and HD groups were decreased than that of DC group ($p < 0.05$, 0.01). The hypolipidemic effect of POL was not shown dose-dependently, although the levels of TG and TC in DM + POL high dose group were lower than those of DM + POL low dose group ($p > 0.05$).

Effect of *P. oleracea* L. on serum insulin levels

The serum insulin levels of four groups were determined on the day 29, and the results were summarized in Figure 3. The serum insulin level of NC group was higher than that of DC group, which indicated that alloxan would

Table 4. Percentage DNA in the tail of lymphocytes in SCGE assay.

Scores	Cells 0	Cells 1	Cells 2	Cells 3	Cells 4	Percentage DNA in the tail
dH_2O culture	281	19	0	0	0	3.84
POL (50 µg/ml)	276	24	0	0	0	3.93
POL (100 µg/ml)	279	21	0	0	0	2.9
H_2O_2 (5 µmol/L)	0	6	137	146	17	45.55

Cells 0 represent the number of undamaged cells, and Cells 4 represents the number of the most heavily damaged cells.

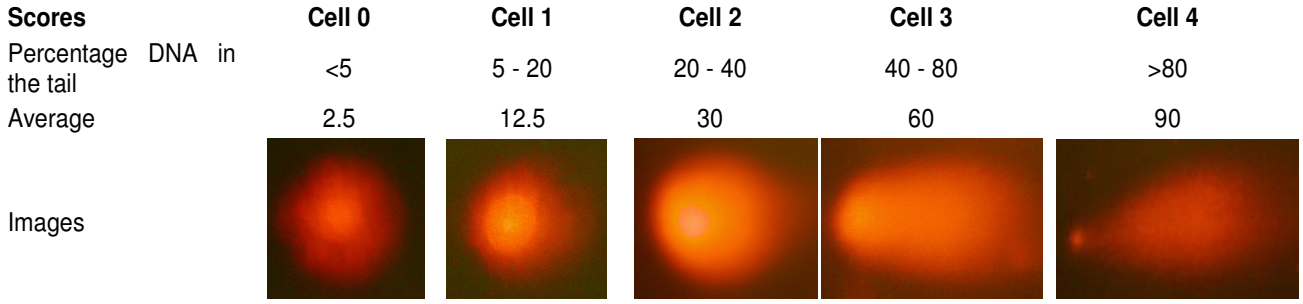

Figure 4. Single cell gel electrophoresis images of different damaged lymphocytes,Visual classification of DNA damage, according to the relative proportion of DNA in the tail (cells 0-4), obtained in SCGE. Cell 0 represents undamaged cells, and cell 4 represents the most heavily damaged cell.

damage the pancreas islet cells. With 28 days of POL treatment, serum insulin level in POL treated groups was significantly higher than that of diabetic control group ($p < 0.05$), which implied that POL treatment improved the insulin secretion on diabetic rats. In the POL-treated HD group, the insulin level was higher than that of the LD group, which implied that POL improved the function of islet cells and stimulated the insulin secretion.

LD_{50} experiment

The behavior of the treated rats appeared normal during the experiment. No toxic effect was found up to 10 times of effective dose of the water extract and there was no death occurred in any of these groups. Only the consumption of food was increased in 8 and 10 time high doses but remaining normal after 4 h.

Single cell gel electrophoresis

Four different disposed methods of the cultured lymphocytes were assayed by single cell gel electrophoresis. The results were shown in Figure 4 and Table 4. The percentage DNA in the tail [(2.5 × cells0 + 12.5 × cells 1 + 30 × cells 2 + 60 × cells 3 + 90 × cells 4)/∑cells] was calculated to express the amount of DNA damage (Collins and Dusinska, et al., 2002). The result showed that POL-treated lymphocytes were not damaged, and the images were similar with dH_2O-treated cultures, but H_2O_2-treated cells were heavily damaged.

DISCUSSION

P. oleracea L. has been used as a traditional medicine for many years in China, owing to its therapeutical properties of anti-bacteria, anti-virus, anti-antherasis, anti-caducity, and enhancing immunity. We used TLC assay to identify the main compounds of POL, which included organic acid, flavonoids, alkaloids, terpene ansteroid, hydroxybenzene, saponin and polysaccharide. According to documents, polysaccharide and flavone are usual antidiabetic compounds in herbs (Lu et al., 2005; Zhao et al., 2006; Singab et al., 2005). Further work on isolation and purification of each compound from POL will be done to identify the hypoglycemic effective compounds.

Alloxan is cytotoxic to the pancreatic β cells, so it is an effective diabetes-induced agent. It has been widely used to induce diabetes mellitus in experimental animal models allowing investigation of hypoglycemic agents in the treatment of diabetes (Wu et al., 2006; Kar et al., 2003). Alloxan injection consistently produced symptoms of diabetes mellitus including hyperglycemia, decreased insulin levels, polyuria and weight loss (Kar et al., 2003).

In the acute blood glucose test, the blood glucose level of DC rats remained high during the experiment. When diabetic rats were treated 0.5 - 1 h with POL extract, the glucose level rise slightly, and after 1.5 h, the blood

glucose level was decreased significantly, and the hypoglycemic effect could be maintained for 4 h. The result indicated that the some components of POL may increase the glucose level initially. Whereas, the main effect of POL is hypoglycemic activity, which may be a new aspect to consider with respect to post meal glucose reduction requested. Furthermore, when the diabetic rats were treated for 28 days with low and high doses of POL extract, the blood glucose levels were decreased, and the effect was shown in dose dependent manner, which suggested that POL had long term hypoglycemic ability. For a comprehensive understanding of these influences further investigations would be necessary.

The body weight is usually decreased in diabetes status (Jayakar et al., 2004; Junod et al., 1969; Craft et al., 1983). In our study, a significant decrease in body weight was observed during 28 days after alloxan-induced compared with that of NC group. When the diabetic rats were treated with POL for 28 days, the decrease of body weight was improved.

DM is a metabolic disorder that usually affects carbohydrate, fat and protein metabolisms, followed with multi-organs regression in the later period and hyperlipidemia is associated with hyperglycemia (Failla and Kiser, 1981; Okon et al., 2007). In the DC group, the levels of TC and TG were raised significantly compared with normal rats. In the POL treated groups, TC and TG levels were significantly decreased. The results indicated that POL extract not only possessed significant hypoglycemic ability, but also had hypolipidemic effect in alloxan-induced diabetic rats.

Alloxan could damage pancreatic β cell, resulting in a decrease in endogenous insulin secretion, which decreased utilization of glucose by the tissues consequently (Xie et al., 2005). In this study, we have observed that POL decreased the level of blood glucose and increased the concentration of serum insulin in alloxan-induced diabetic rats. The possible mechanism of POL extract could be correlated with promoting insulin secretion by closure of K+-ATP channels, membrane depolarization and stimulation of Ca^{2+} influx, an initial key step in insulin secretion (Ryle et al., 1984). A further study should be designed to address this hypothesis. Under normal conditions, insulin increased the receptor-mediated removal of low density lipoprotein cholesterol and hypercholestrolemia were reported to occur in diabetic rats (Iwasaki et al., 2007). Accumulation of TGs is one of the risk factors in Coronary Heart Disease (CHD). The significant increase of the level of triglycerides in plasma of diabetic control rats might be due to the lack of insulin. Since under normal condition, insulin activated the enzyme of lipoprotein lipase and hydrolysis triglycerides (Morikawa et al., 2007). POL reduced the level of triglycerides by the increase of insulin level in plasma in alloxan-induced diabetic rats and might prevent the progression of CHD. The SCGE assay is a rapid, simple, visual and sensitive technique for measuring DNA breakage in individual mammalian cells (Collins and Dusinska, 2002; Singh,et al.,1988). Cells embedded in agarose on microscope slides are subjected to lysis, unwinding of DNA and electrophoresis at high pH. After staining with a fluorescent dye, cells with DNA damage display increased migration of genetic material from the cell nucleus. The damage is quantified by measuring the displacement between the genetic material of the nucleus ('comet head') and the resulting 'tail'. The torsional moment of the tail has been suggested to be an appropriate index of induced DNA damage in considering both the migration of the genetic material as well as the relative amount of DNA in the tail. We did the single cell gel assay for POL treated cell. SCGE and LD_{50} results showed that POL had not any toxic effect to the animals.

It could be concluded that the extract of POL had hypoglycemic potential by stimulating insulin secretion and prevented atherosclerosis. Thus POL could be a candidate for therapeutic pharmaceutical against diabetes mellitus. Further investigation should be done on purification and identification of the anti-diabetic ingredients of POL.

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