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Full Length Research Paper

Preparation and antidiabetic activity of polysaccharide from *Portulaca oleracea L.*

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Extraction parameters of polysaccharide from *Portulaca oleracea* L. (POP) and antidiabetic activity of POP on alloxan induced diabetic mice were studied. Better extraction parameters of POP were obtained by the single factor test, as follows: extraction temperature 95°C, extraction time 5 h, and ratio of solvent to raw material 40. POP treatment (200, 400 mg/ kg body weight) for 28 days resulted in a significant decrease in the concentration of fasting blood glucose (FBG), total cholesterol (TC) and triglyceride (TG) in diabetes mellitus mice. Furthermore, POP significantly increased the concentration of high-density lipoprotein cholesterol (HDLc) and serum insulin level in diabetes mellitus mice. Our data demonstrated POP at the dose of 400 mg/kg body weight (bw) exhibited optimal effect. The above results suggest that polysaccharide extracted from *P. oleracea* L. can control blood glucose and modulate the metabolism of glucose and blood lipid in diabetes mellitus mice.

Key words: Extract, *Portulaca oleracea* L., polysaccharide, antidiabetic.

INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia, abnormal lipid and protein metabolism along with specific long-term complications affecting the retina, kidney and nervous system (David et al., 1997). For a long time, diabetics have been treated with several medicinal plants or their extracts based on the folklore medicine (Akhtar and Ali, 1984). Synthetic hypoglycemic agents can produce serious side effects and in addition, they are not suitable for use during pregnancy. Therefore, the search for more effective and safer hypoglycemic agents has continued to be an important area of active research.

Portulaca oleracea L. grows widely in different areas of the world including north of China. The plant is known in folk medicine in some parts of China as hypotensive and antidiabetic (Meng and Wu, 2008). *P. oleracea* L. contains many compounds, including free oxalic acids, alkaloids, omega-3 fatty acids, coumarins, flavonoids, polysaccharide, cardiac glycosides, and anthraquinone glycosides (Mohammad et al., 2004). Polysaccharide from *P. oleracea* L. (POP) has been recently studied for their physiological and pharmaceutical activities. The purpose of this study was to investigate better extraction parameters of POP and antidiabetic activity of POP on alloxan induced diabetic mice for the use of this plant in the treatment of diabetes.

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MATERIALS AND METHODS

Plant materials

P. oleracea L. was collected in Hebei province in July and the material was identified by Mr. Wang Guang Yao, a botanist of Jilin Agriculture Science and Technology College. A voucher specimen has been deposited in herbarium of Jilin Agriculture Science and Technology College. Fresh and intact *P. oleracea* L. was picked to shade dried as experimental material.

Drugs and reagents

Alloxan was purchased from Sigma Co. (USA). Glucose Analyzer and strips were purchased from Roche Diagnostic Co. (USA). Reagents for total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-c) were obtained from Beijing Chengxinde Biochemistry Reagent Company (Beijing, China). Reagents for serum insulin were purchased from Adlitteram Diagnostic Laboratories Co. (USA).

Extraction of POP

The shade dried *P. oleracea* L. were ground in a high speed disintegrator (Model HDV, Dongying Hongjiu Traditional Medicine Machine Company, Shandong, China) to obtain a fine powder; then they were extracted in a Soxhlet apparatus with a mixture of chloroform-methanol (2:1, 75℃), and pretreated with 80% ether twice to remove some coloured materials, oligosaccharides, and some small molecule materials. The organic solvent was volatilized and pretreated dry powder was obtained, as described previously (Zhao and Liu, 2008). The pretreated dry powder (20.0 g) was extracted with deionized water(water– powder (ml/g) ranging from 20:1 to 50:1) at pH 6.5 - 7.5 (adjusting the suspension pH by 0.1 mol/l NaOH or HCl), while the temperature of the water bath ranged from 70 to 100℃ and was kept steady (within ±1.0℃). The water–*P. oleracea* L. slurry in a 2.0 L stainless steel boiler in the water bath was stirred with an electric mixing paddle for a given time (extraction time ranging from 1 to 6 h) during the entire extraction process. The mixture was centrifuged (2000 g, 20 min), then the supernatant was separated from insoluble residue with nylon cloth (pore diameter: 38 um). The extracts were then defatted by the method of Sevag (Sevag et al., 1938), precipitated by the addition of ethanol to a final concentration of 80% (v/v), and the precipitates were collected by centrifugation (2000 g, 20 min). It was then solubilized in deionized water and lyophilized to get POP. The contents of polysaccharides were determined by phenol-sulfuric acid method and by reference to glucose, and wavelenth in spectrophotometer (Model V-5100, Chenxiyongchuang Science and Technology Company, Beijing, China) was set at 490 nm (Li and Wang, 2005).

Experimental animals

Male mice of original Kun-ming strain, weighing 18 - 22 g, were used for the study. Housed individually in polypropylene cages, maintained under standard conditions (12 h light and 12 h dark cycle, 25 ± 30℃, 35 - 60% humidity), the animals were fed with standard diet and water *ad libitum*. The approval of this experiment was obtained from the Institutional Animal Ethics Committee of Yanshan University (Qinhuangdao, China). After 1 week of acclimation, the fasted mice were induced with a single injection of 4% alloxan prepared freshly at a dose of 200 mg/kg bw (Chen et al, 2005; Yang et al, 2006). Diabetes was confirmed by the determination of tail vein blood glucose levels on the third day after administration of alloxan. Mice having blood glucose levels greater than 11.10 mmol/L were considered diabetic and were used for the study.

Antidiabetic activity of POP

POP and Glibenclamide (Glib) were dissolved in distilled water and were fed by gavage to mice once a day. Forty male mice were randomly divided into five equal groups as follows:

i.) Normal control group (NC): normal control mice administered water daily for 28 days.
ii.) Diabetic control group (DC): diabetic control mice administered water daily for 28 days.
iii.) Diabetic + POP (200 mg/kg) group (DLP): diabetic mice administered *P. oleracea* L. polysaccharide (200 mg/kg) daily for 28 days.
iv.) Diabetic + POP (400 mg/kg) group (DHP): diabetic mice administered *P. oleracea* L. polysaccharide (400 mg/kg) daily for 28 days.
v.) Diabetic + Glibenclamide (4 mg/kg) group (DG): diabetic mice administered reference drug Glib (4 mg/kg) daily for 28 days.

During POP and Glib supplement for 28 days, fasting blood glucose level was measured for once every week. Blood was collected from tip of the tail vein and fasting blood glucose level was measured by using a glucose analyzer. On 28th day of experiment, the mice were sacrificed by decapitation under light ether anesthesia and blood was collected from dorsal aorta and serum was separated by centrifugation for 5 min and was kept at -20℃ for the biochemical assay of total cholesterol (TC), high-density lipoprotein cholesterol (HDLc), triglyceride (TG) and for serum insulin assay. TC, TG, and HDL-c were determined by enzyme methods. Serum insulin level was estimated by insulin-ELISA kit according to the manufacturer's instruction. All results were expressed as mean ± SD and were analyzed by SPSS for Windows, version 13.0(SPSS Inc, Chicago). The Duncan test and one way analysis of variance were used for comparisons (Jung et al., 2005; Achyut et al., 2007). The values were considered significant when $P < 0.05$.

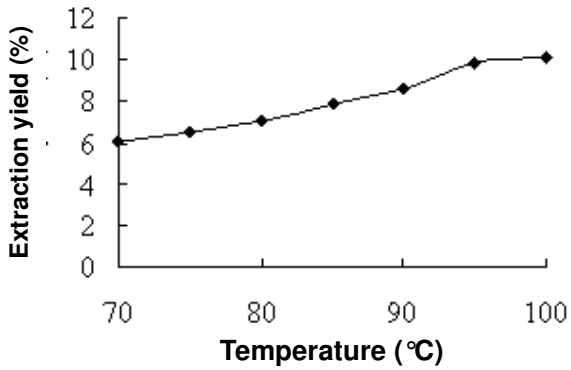

Figure 1. Effect of extraction temperature on extraction yield of POP.

RESULTS

Effect of temperature on extraction yield of POP

As far as the extraction temperature is concerned, the higher the temperature, the higher the extraction yields of polysaccharides. As shown in Figure 1, extraction yield increased from 6.04 to 9.81% with the increasing temperature. However, on the other hand, a relatively high extraction temperature (at 100℃) was detrimental to the extraction yield. Only a bit of the extraction yield was increased as the temperature was higher than 95℃ because of destruction of enzyme activity at high temperature in this reaction system (Shogren et al., 2006).

Therefore, the suitable temperature for higher yield of the polysaccharides was considered to be 95℃.

Effect of extraction time on extraction yield of POP

Extraction time is another factor that would influence the extraction efficiency (Ray et al., 2006). With the increase of the extraction time from 1 to 5 h in the extraction system, the extraction yield quickly increased from 4.52 to 5.97% (Figure 2). When the extraction time continued to lengthen, the extraction yield increased little. Because longer extraction time could delay and lengthen production cycle, 5 h of extraction time was adopted in the present work.

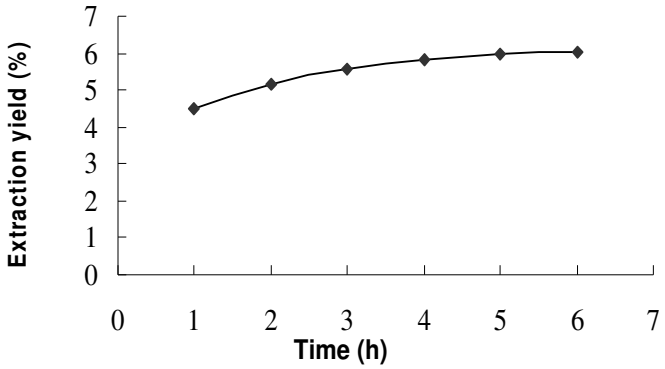

Figure 2. Effect of extraction time on extraction yield of POP.

Effect of ratio of solvent to raw material on extraction yield of POP

Ratio of solvent to raw material was another factor affecting extraction yield of polysaccharides (Sun and Tomkinson, 2002). The ratio of solvent to raw material was set at 20, 25, 30, 35, 40, 45 and 50. With the increase of the ratio of solvent to raw material from 20 to 40 in the extraction system, the extraction yield quickly increased from 7.32 to 10.72% (Figure 3). It can be seen from Figure 3 that extraction yield of polysaccharides in the extraction system gives higher value when the ratio of solvent to raw material was 40. In addition, the extraction yield of polysaccharides increased little when the ratio of solvent to raw material surpassed 50. Therefore, the suitable ratio of solvent to raw material for higher total yield of the polysaccharides was considered to be 40.

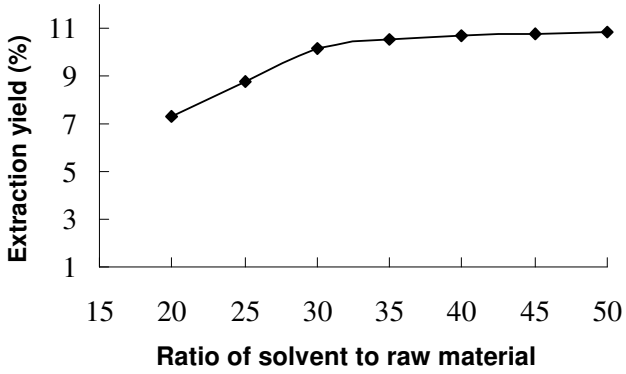

Figure 3. Effect of ratio of solvent to raw material on extraction yield of POP.

Effect of POP on fasting blood glucose levels in mice

The alloxan-induced diabetic mice exhibited hyperglycemia. In the diabetic groups, a significant ($P < 0.05$) increase in FBG was detected as compared to the normal control group. But these abnormal increases in blood glucose levels significantly ($P < 0.05$) and dose-dependently decreased in the POP-administered groups as compared to the diabetic control group from 7 days after administration. In the DG group, decrease was also significant ($P < 0.05$) from 7 days after administration. The NC and DC groups did not show any significant variation on the blood glucose level throughout the experimental period ($p > 0.05$). The results are shown in Table 1.

Effect of POP on blood lipids levels in mice

Diabetes mellitus is usually complicated with hyperlipoproteinemia. The present results showed that the TC and TG levels were significantly elevated in the diabetic control group as compared to the normal control group ($P < 0.05$), and serum HDL-c level, a friendly lipoprotein, was decreased in diabetic control group as compared to the normal control group ($P < 0.05$). After supplementation with POP and glibenclamide, the alteration in lipid metabolism was partially attenuated as evidenced by decreased serum TG and TC levels and by increased HDL-c concentration in diabetic mice. The response was better in the DHP group compared to the DLP group which is comparable to that of the DG group. The results are shown in Table 2.

Effect of POP on blood serum insulin levels in mice

Serum insulin level of the normal control group was higher than that of the diabetic control group, which indicated that alloxan would damage the pancreas islet cells. After 28 days of the POP supplementation to the mice, there was a significant elevation in serum insulin level as compared to the diabetic control group ($p < 0.05$), which implied that treatment with POP improved the insulin secretion on diabetic mice. In the DHP group, the insulin level was higher than that of the DLP group. The results implied that POP improved the function of

Table 1. Effect of POP on blood glucose level (mmol/L) in mice.

Group	Number of animals	Days after dosing (day)				
		0	7	14	21	28
NC	8	5.15±0. 0.17	5.14±0.17	5.10±0.13	5.04±0.08	5.16±0.10
DC	8	15.36±0.39	15.33±0.26	15.33±0.35	15.15±0.15	15.23±0.29
DLP	8	15.25±0.30	12.67±0.24	11.29±0.28	10.56±0.32	9.76±0.29
DHP	8	15.39±0.28	11.92±0.26	10.48±0.30	9.47±0.29	8.33±0.17
DG	8	15.292±0.29	9.06±0.14	7.24±0.21	6.47±0.24	5.68±0.33

n = 8; (mean ± S.D., g); P < 0.05 as compared with normal control group; P < 0.05 as compared with diabetic control group. NC = Normal control; DC = diabetic control; DLP = diabetic + POP (200 mg/kg); DHP = diabetic + POP (400 mg/kg); and DG = diabetic + glibenclamide (4 mg/kg).

Table 2. Effect of POP on blood lipids (mmol/L) in mice.

Group	Number of animals	TG	TC	HDL-c
NC	8	1.64±0.02	2.70±0.02	1.64±0.01
DC	8	2.05±0.04℃	3.34±0.04℃	0.77±0.02℃
DLP	8	1.86±0.02℃	3.16±0.02℃	0.84±0.02℃
DHP	8	1.77±0.02℃	3.12±0.02℃	1.11±0.03℃
DG	8	1.72±0.01℃	3.03±0.05℃	1.39±0.02℃

n = 8; (mean ± S.D., g); P<0.05 as compared with normal control group; P < 0.05 as compared with diabetic control Group. NC = Normal control; DC = diabetic control; DLP = diabetic + POP (200 mg/kg); DHP = diabetic + POP (400 mg/kg); and DG = diabetic + glibenclamide (4 mg/kg).

islet cells and stimulated the insulin secretion. The results are shown in Figure 4.

DISCUSSION

The methods for the isolation of polysaccharides from the plant are various in different studies. Different extraction methods and fractions could affect not only the ingredients but also the physiological activity of the extract (Chao et al., 2006). On the basis of the single factor test in this study, it can be concluded that better extraction parameters of POP are as followings: extraction temperature 95℃, extraction time 5 h, and ratio of solvent to raw material 40, while extraction yield of FIBL was 10.91 %.

Effective control of the blood glucose level is a key step in preventing or reversing diabetic complications and improving the quality of life in both type 1 and type 2 diabetic patients (Abraira et al., 1995; Ohkubo et al., 1995). Antihyperglycemic potency of the POP in diabetic mice has been indicated here by the study of FBG level as it is an important basal parameter for monitoring of diabetes. The present study showed that alloxan-induced diabetic mice presented obvious hyperglycemic symptoms, but POP produces a significant antihyperglycemic effect when oral administration to alloxan-diabetic mice. The dosage of 400 mg/kg is more effective than that of 200 mg/kg.

Diabetes is also associated with hyperlipidemia. The

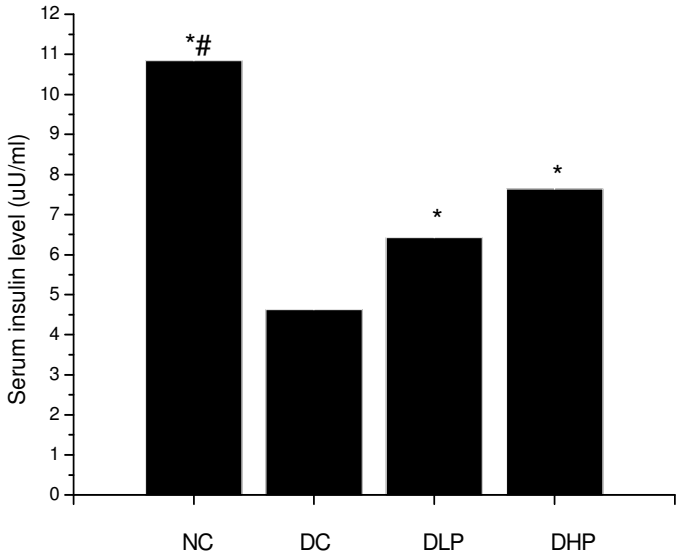

Figure 4. Effect of POP on serum insulin level in mice. n = 8; (mean ± S.D., g); P < 0.05 as compared with diabetic control group; P < 0.01 as compared with diabetic control group. NC = Normal control; DC = diabetic control; DLP = diabetic + POP (200 mg/kg); and DHP = diabetic + POP (400 mg/kg).

serum TC and TG decreased significantly in diabetic mice after POP supplementation. These effects may be due to low activity of cholesterol biosynthesis enzymes or

low level of lipolysis which are under the control of insulin (Sharma et al., 2003). This POP supplementation also results in the significant attenuation in the level of HDL-c in serum toward the control level which again strengthens the hypolipidemic effect of the POP.

Alloxan could damage pancreatic β cell, resulting in a decrease in endogenous insulin secretion, which decreased utilization of glucose by the tissues consequently. In this study, we have observed that POP increased the concentration of serum insulin in alloxan-induced diabetic mice. The possible mechanism of action of POP 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.

Our research has indicated that POP possesses antidiabetic activities and the dose of 400 mg/kg bw represents the optimal level for effecting a positive diabetic response in mice. Therefore, POP should be considered as a candidate for future studies on diabetes. The further studies are in progress to elucidate the molecular and cellular mechanism of POP.

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