CJIM 2003;9(4):289-292

Effects of Portulaca Oleracea on Insulin Resistance in Rats with Type 2 Diabetes Mellitus *

SHEN Lan (沈 岚), and LU Fuer (陆付耳)

ABSTRACT **Objective:** To study the effects of Portulaca oleracea, a Chinese medicinal herb, on insulin resistance in rats with type 2 diabetes mellitus (T2DM). **Methods:** Experimental model of T2DM was established by injection of streptozotocin (25mg/kg) and feeding with high calorie forage. The effects of Portulaca oleracea on oral glucose tolerance, serum levels of insulin, triglyceride, total cholesterol, highdensity lipoproteinscholesterol and free fatty acids, and insulin sensitivity index were all observed. **Results:** Portulaca oleracea could reduce the body weight, improve the impaired glucose tolerance and lipid metabolism, decrease serum free fatty acids, attenuate hyperinsulinemia and elevate insulin sensitivity. **Conclusion:** Portulaca oleracea could improve insulin resistance in rats with T2DM, and the mechanism might be related to its actions in improving lipid metabolism and decreasing free fatty acids.

KEY WORDS diabetes mellitus type 2, Portulaca oleracea, insulin resistance, lipid metabolism, free fatty acid

Insulin resistance (IR) is the common pathophysiological basis for a group of metabolic abnormalities called insulin resistance syndrome, which includes type 2 diabetes mellitus (T2DM), obesity, lipid metabolic disturbance, primary hypertension and atherosclerosis. It was once reported that a Chinese medicinal herb, Portulaca oleracea, used alone could effectively treat diabetes mellitus in humans[1] as it has the action of ameliorating hyperlipidemia, and preventing and curing atherosclerosis[2,3]. But experimental study of Portulaca oleracea on insulin resistance in human or diabetic animals has not been reported so far. Therefore, the present study was carried out on the streptozotocin (STZ) and high calorie forage induced animal model to observe the effects of Portulaca oleracea on insulin resistance in T2DM rats.

METHODS

Reagents

STZ, product of Sigma Company, was made into solution of 1.25% STZ in 0.1 mol/L sodium citratecitric acid buffer solution (pH4.4). Tween 80 was product of Shanghai Chemical Reagent Company, Chinese Medical Corporation, chemical purity grade. Glucose assay kits were purchased from Beijing BHKT Clinical Reagent Co. Ltd., insulin radioimmunoassay kit was from Beijing Northern Biological Technique Institute, free fatty acids (FFA) test kit was from Nanjing Jiancheng Bioengineering Institute and kits for determining serum triglycerides (TG), total cholesterol (TC) and high density lipoproteinscholesterol (HDLC) were all from Zhejiang Dong Ou Biological Engineering Co. Ltd.

Drugs

Portulaca oleracea was obtained from Hubei Medicinal Materials Company, decocted with water, condensed to a concentration containing 1.9 g/ml crude herbs and preserved at -4 ℃. Ethyl polyenoate soft capsules (each capsule weighing 450 mg, containing 315 mg ω3 unsaturated fatty acid) were obtained from Tianjing Zhongyang Pharmaceutical Co. Ltd., the content in which was mixed with tween 80 in 60 ℃ water bath homogeneously before using, and diluted with distilled water to a concentration of ω3 unsaturated fatty acid 14.96 mg/ml with 5% tween 80.

Model Establishment, Grouping and Treatment of the Animals

Female Wistar rats, clean grade, weighing 210-230 g, provided by Hubei Sanitary and Anti-Epidemic Station, were housed individually, one rat per cage, under the 12/12hour light/dark cycle and controlled temperature (22-28 ℃) and humidity (50

*Financially supported by Education Ministry of China (Grant No. 1998-679)

Institute of Integrated Traditional & Western Medicine, Tongji Hospital, Tongji Medical College, Huazhong University of Science & Technology, Wuhan (430030)
Correspondence to: Dr. LU Fuer;
Tel: 027-83662577; Fax: 027-83646605
Email: felu@tjh.tjmu.edu.cn

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- 60 %), and had free access to forage and water.

The high calorie forage was made of basic forage (provided by Experimental Animal Center of Tongji Medical College, Huazhong University of Science and Technology), milk powder, sugar, refined lard and eggs in the proportion of 63.4:30:2:0.2.

All the rats, except those for normal control, were made into model of T2DM in the way described in literature[4] as the following: After 12 hrs of fasting, rats were anesthetized with ethyl ether, and injected with 25 mg/kg of STZ through caudal vein. After 2 weeks of feeding with normal forage, oral glucose tolerance test (OGTT) was conducted on all the rats and those with reduced glucose tolerance were selected and randomly divided into five groups: the model group (without intervention), the Portulaca oleracea (PO) low, moderate and high dose groups (POl, POm, POh) and the ethyl polyenoate (EP) group. They were all fed with the abovementioned high calorie forage. The POl, POm and POh groups were treated with decoction containing 5, 10, 20 g/kg PO per day respectively, equivalent to 2.5, 5, 10 times the dosage used clinically on human adults (2 g/kg·d) and the rats in the EP group were treated with EP 157.5 mg/kg per day, equivalent to 5 times that used on human adults (31.5 mg/kg·d), through gastrogavage every morning for 9 consecutive weeks, while the rats in both the normal control and the model groups were given water of the same volume (about 1ml/100g body weight) instead. OGTT was reexamined 1 week before ending the experiment. At the end of the experiment, a 2.5 ml of blood sample was drawn from the portal vein of each rat through laparotomy after the rats had fasted for 12 hrs and been anesthetized with sodium pentobarbital 50 mg/kg. Then the serum was separated from blood sample immediately within 30 minutes and preserved in -20 ℃ refrigerator for determining fasting blood glucose (FBG), insulin (Ins), TC, TG, HDLC and FFA. Tests were performed within 2 weeks.

Methods for Indexes Detection

OGTT test: After 12 hrs fasting, the rats were given 2 g/kg of 20 % glucose by gastric perfusion. Then blood samples were taken from caudal vein by tailscission immediately after glucose perfusion (T0), 30 (T1), 60 (T2) and 120 minutes (T3) after glucose perfusion for serum glucose determination.

FBG was tested by oxidase method, Ins was measured by radioimmunoassay, and serum levels of TG, TC, HDLC and FFA were measured in strict accordance with the manual in the corresponding kits. Insulin sensitivity index (ISI) was calculated according to the method proposed by LI GW, et al[5], which is the reciprocal of arithmetic product of FBG and Ins, and its natural logarithm was taken for analysis for abnormal distribution.

Statistical Analysis

All data were expressed as mean ± standard deviation ($\bar{x} \pm s$). Variance analysis was used to compare the results among multiple groups and q test to compare data between each one and each another group. A P value of 0.05 or less was considered statistically significant.

RESULTS

Comparison of Body Weight and OGTT among Groups

As shown in Table 1, the increase of body weight in the model group was significantly greater than that in the normal group ($P < 0.05$). All the three groups treated by PO got lowered body weight, with the data in the POm and the POh

Table 1. Comparison of Body Weight and OGTT among Groups ($\bar{x} \pm s$)

Group	n	Body Weight (g)	Blood Glucose (mmol/L) in Different Time Points			
			T0	T1	T2	T3
Normal	10	250.60 ± 8.26	4.16 ± 0.27	6.64 ± 0.69	6.55 ± 0.61	5.83 ± 0.63
Model	9	263.44 ± 9.08 *	5.39 ± 0.70 **	9.48 ± 1.57 **	9.57 ± 2.05 **	8.38 ± 0.90 **
POl	10	253.70 ± 16.06	5.05 ± 0.55	8.92 ± 0.83	8.75 ± 1.57	6.86 ± 0.75 △△
POm	10	248.60 ± 14.81 △	5.33 ± 0.84	8.40 ± 1.51 △	8.02 ± 1.36 △	7.35 ± 1.36 △
POh	11	245.91 ± 14.79 △△	5.23 ± 0.43	8.20 ± 1.18 △	7.96 ± 1.42 △	7.65 ± 0.77
EP	10	265.30 ± 15.48	4.90 ± 0.47	8.67 ± 1.11	8.17 ± 0.88	7.16 ± 0.57 △△

Notes: * $P \leq 0.05$, ** $P < 0.01$, compared with the normal group; △ $P < 0.05$ and △△ $P < 0.01$, compared with the control group

groups showing significant difference ($P < 0.01$, $P < 0.05$) when compared with that in the model group, but only slightly lower than that in the normal group. Body weight in the EP group was somewhat heavier than that in the normal group, but without statistical significance ($P > 0.05$).

The results of OGTT showed that the serum glucose level in the model group at various time points was significantly higher than that in the normal group ($P < 0.01$). There was no significant difference in comparison between all the treated groups and the model group at T0 ($P > 0.05$), but it did show significant difference in the comparison between the model group with the POl group at T3, with the POm group at T1, T2 and T3 ($P = 0.050$, 0.018, and 0.012, respectively), and with the POh group at T1 and T2 (both $P < 0.05$), as well as that between the model group and the EP group at T3 ($P < 0.01$). Above results showed that PO could lower the body weight, and improve the abnormity of glucose tolerance of T2DM rats. EP showed no obvious effect on the body weight, but it did improve the abnormity of glucose tolerance to some extent.

Comparison of FBG, Ins and ISI among Groups

See Table 2. Similar to the data obtained in OGTT 1 week before ending the experiment, FBG was significantly elevated in the model group as compared with that in the normal group ($P < 0.01$), but it showed insignificant difference ($P > 0.05$) as compared with the treated groups. Ins level in the model group was significantly higher ($P < 0.05$), and ISI was significantly lower ($P < 0.05$) than those in the normal group respectively. In the POl, POm and POh groups, Ins was all significantly lower than that in the model group ($P < 0.01$ or 0.05), very close to the normal level, and correspondingly, their ISI was significantly higher than that in model group ($P < 0.01$ or $P < 0.05$). In EP group, Ins and ISI proved to be somewhat different from those in the model group, but without statistical significance ($P > 0.05$). The above results showed that PO could correct the hyperinsulinemia in T2DM rats, improve the sensitivity of insulin, with its effect better than EP.

Table 2. Comparison of FBG, Ins and ISI among Groups ($\bar{x} \pm s$)

Group	n	FBG (mmol/L)	Ins (IU/ml)	ISI
Normal	10	4.58 ±0.54	28.71 ±5.91	-4.85 ±0.31
Model	9	5.22 ±0.51**	36.64 ±7.64*	-5.23 ±0.26*
POl	10	4.80 ±0.50	25.86 ±7.91△△	-4.78 ±0.37△△
POm	10	4.90 ±0.49	27.57 ±8.36△	-4.86 ±0.34△
POh	11	5.03 ±0.44	28.01 ±8.44△	-4.91 ±0.33△
EP	10	5.09 ±0.54	33.34 ±8.97	-5.09 ±0.27

Notes: * $P < 0.05$, ** $P < 0.01$, compared with the normal group; △ $P < 0.05$, △△ $P < 0.01$, compared with the control group

Comparison of Serum TG, TC, HDLC and FFA among Groups

See Table 3. Serum TG, TC and FFA levels were all significantly higher and HDLC levels significantly lower in the model group than those in the normal group ($P < 0.01$), while the former three indexes in all PO treated groups were significantly lower as compared with those in the untreated model group ($P < 0.05$ or 0.01), somewhat close to the normal level ($P > 0.05$), and serum HDLC concentrations in the POl and POm groups were significantly higher than that in the model group ($P < 0.01$, $P < 0.05$), close to normal level ($P > 0.05$). Serum FFA concentrations in all PO treated groups were significantly lower than that in the model group, but still higher than that in the normal group. In EP group, serum HDLC concentration showed no significant difference from that in the model group ($P > 0.05$), while TG, TC, FFA concentrations were similar to those in PO treated groups ($P > 0.05$). The above results showed that PO could significantly improve the hyperlipide

Table 3. Comparison of Serum TG, TC, HDLC and FFA among Groups ($\bar{x} \pm s$)

Group	n	TG (mmol/L)	TC (mmol/L)	HDLC (mmol/L)	FFA (μmol/L)
normal	10	1.93 ±0.60	1.24 ±0.41	1.10 ±0.22	885.51 ±151.39
Model	9	2.84 ±0.74*	1.94 ±0.43*	0.84 ±0.15*	1726.37 ±423.60*
POl	10	2.19 ±0.63△	1.38 ±0.34△△	1.03 ±0.16△△	1280.75 ±279.25△△
POm	10	1.70 ±0.28△△	1.10 ±0.21△△	0.97 ±0.11△	1261.82 ±302.49△△
POh	11	1.87 ±0.54△△	1.25 ±0.23△△	0.86 ±0.09	1175.90 ±236.54△△
EP	10	1.97 ±0.37△△	1.14 ±0.28△△	0.77 ±0.11	1237.88 ±378.19△△

Notes: * $P < 0.01$, compared with the normal group; △ $P < 0.05$, △△ $P < 0.01$, compared with the control group

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mia and lower the serum FFA level in T2DM rats, its effect equivalent to that of EP. However, it showed superiority to EP in raising HDL-C level.

DISCUSSION

In this experiment, a model of T2DM rats was established by injection of a small amount of STZ and high calorie forage feeding. The results showed that in addition to hyperglycemia, insulin resistance, characterized by obesity, abnormal glucose tolerance, hyperinsulinemia and lipid metabolic disorder, was developed in the model animals. It was demonstrated that PO treatment could reduce the body weight, improve the impaired glucose tolerance and lipid metabolism, attenuate hyperinsulinemia and elevate insulin sensitivity in T2DM model rats. Simopoulos, an American scientist, indicated that PO is rich in ω3 unsaturated fatty acid[6], and vitamin E, vitamin C, carotene, glutathione etc., and ω3 unsaturated fatty acid was reported to have effect in alleviating IR[7] and hyperlipidemia, and preventing and curing cardiovascular diseases[8]. EP, which mainly consists of ω3 unsaturated fatty acid, is a wellknown anti-hyperlipidemia agent.

Results of the present study showed that PO has the effect similar to that of EP in decreasing the hyperlipidemia, and is further more superior to EP in improving impaired glucose tolerance and hyperinsulinemia, and increasing serum HDL-C level.

High FFA concentration plays an important role in the development of IR in patients with obesity[9], whose abnormal lipid metabolism could induce IR through multiple links. For instance, the increased lipid depots could lead to the preferential use of FFA in the glucose-FFA cycle, which results in a glucose utilization decrease in the body. Lipids accumulation could activate lipodieresis, decrease the lipoprotein lipase (LPL) activity in adipose tissues, and increase hormonesensitive lipase (HSL) activity, thus resulting in a large amount of FFA release into blood circulation to form FFA hyperlipidemia; FFA activated protein kinase C would affect the insulin signaling cascade[10], which impairs the uptake and disposal of insulin mediated glucose, suppress glycogen synthesis, promote gluconeogenesis, thus leading to hyperinsulinemia and the development of IR. In conclusion, the mechanism of PO in improving insulin resistance in the experimental T2DM rats might be related to its action of improving lipid metabolism and decreasing free fatty acids.

PO is one of the plants that possesses the richest source of ω3 unsaturated fatty acid[6]. With its extensive distribution on land, strong resistance against diseases, and usefulness in both medicinal and edible purposes, it truly deserves our further investigation and exploitation. But, whether its effective component in improving IR is limited to ω3 unsaturated fatty acid or not, and what are its target points and special mechanisms, remain to be further studied.

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(Received November 4, 2002)