Original Article

Antihyperglycemic activity of *Prunella vulgaris* L. in streptozotocin-induced diabetic mice

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Prunella vulgaris L. (Labiatae) has been reported to have a wide range of health benefits in oriental medicine. This study for the first time is to examine the antihyperglycemic effects of *P. vulgaris* in streptozotocin (STZ) - induced diabetic ICR mice (STZ diabetic mice). The effects of *P. vulgaris* L. aqueous-ethanol extract (PVE) on blood glucose, exogenous insulin sensitivity and plasma insulin levels were investigated. In four doses of extracts from the spikes of *P. vulgaris*, extract at dose of 100 mg/kg significantly suppressed the rise in blood glucose after 30 min in the acute glucose tolerance test. Furthermore, this dose was applied in the fellow experiments. A significant decrease in blood glucose levels was observed after treatment of PVE. A combination of PVE and glibenclamide produced a greater effect in blood glucose level than using glibenclamide or PVE alone. PVE enhanced and prolonged the antihyperglycemic effects of exogenous insulin on STZ diabetic mice. Plasma insulin levels were increased with glibenclamide treatment in STZ diabetic mice, whereas such effect was not observed with PVE. These results indicated that *P. vulgaris* enhances the antihyperglycemic effects of exogenous insulin without stimulating insulin secretion, indicating that insulin sensitivity is increased in STZ diabetic mice.
Abbreviations: STZ = Streptozotocin; PVE = *Prunella vulgaris* L. aqueous-ethanol extract; NEFA = Non esterified fatty acids; b.w. = Body weight

Key Words: *P. vulgaris*, glucose tolerance, insulin sensitivity, streptozotocin

Introduction

Diabetes mellitus (DM) is a chronic disease caused by inherited and/or acquired deficiency in production of insulin by the pancreas, or by the ineffectiveness of the insulin produced. Such a deficiency results in increased concentrations of glucose in the blood, which in turn damage many of the body's systems, in particular the blood vessels and nerves.[1] Chronic hyperglycemia during diabetes causes glycation of body proteins that in turn leads to secondary complications affecting eyes, kidneys, nerves and arteries.[2] The therapeutic measurements include use of insulin and other agents like amylin analogues, alpha glycosidase inhibitors like acarbose, miglitol and voglibiose, sulphonylureas, biguanides for the treatment of hyperglycemia. These drugs also have certain adverse effects like causing hypoglycemia at higher doses, liver problems, lactic acidosis and diarrheam.[3-4]

Apart from currently available therapeutic options, many herbal medicines have been recommended for the treatment of diabetes. Herbal drugs are prescribed widely because of their effectiveness, less side effects and relatively low cost.[5] China has a rich history of using various potent herbs and herbal components for treating diabetes. Extracts from the spikes of *P. vulgaris* has been reported to have a wide range of health benefits, such as prevents oxidative stress, lipid peroxidation, obesity, hypercholesterolemia and hyperlipidemia.[6-7] In addition, *P. vulgaris* is traditionally used as folk medicine in the treatment of diabetes mellitus in south of China, which is prepared as an infusion and taken orally 1-2 time per day. It has been reported a very good effectiveness in the control of blood glucose as a folk medicine.[8] However, studies on the use of *P. vulgaris* focused on other effects, and there has been no research for the antidiabetic effect of *P. vulgaris*. STZ is widely used to induce diabetes in experimental animals by causing the selective destruction of pancreatic β-cells that secret insulin.[9] The present investigation was undertaken to study the antihyperglycemic effects of aqueous-ethanol extracts of *P. vulgaris* on STZ diabetic mice.

Materials and methods

Preparation of extract

The spikes of *P. vulgaris* was obtained from Tongrentang Enterprise Co. Ltd. (Beijing, China), and authenticated by Dr. B. Zhao, Department of Plant Sciences, China Agricultural University. The freshly obtained fruiting spikes of *P. vulgaris* were air-dried at 40 ºC in the dark. The materials were stored in airtight glass jars at 4 ºC refrigerator prior to use. Five hundred grams of dried *P. vulgaris* spikes were extracted for 1h with aqueous ethanol (30/70 v/v) under reflux at 80 ºC for three times. The combined solution was vacuum-filtered through a filter paper and concentrated in a rotating vacuum evaporator, at 40-45 ºC.

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The viscous residue was freeze-dried to obtain a dry solid mass with a yield of 45.36g weights (yield 9%, w/w). A stock solution with a concentration of 10 mg/ml was prepared by dissolving the aqueous-ethanol extract of *P. vulgaris* in distilled water and stored at -20ºC prior to use.

Chemicals
STZ, insulin and glibenclamide were all purchased from Sigma Chemical Co. (St. Louis, MO, USA). Insulin radioimmunoassay kit was purchased from China Institute of Atomic Energy (Beijing). Glibenclamide, also known as glyburide, was dissolved initially in a small amount of ethanol and diluted with sterile water to a dose volume of 0.1 ml/10 g body weight (b.w.) for oral administration.

Experimental animals
Male ICR mice (at the age of 6 weeks) were obtained from the Experiment Animal Center of Beijing, and the research was conducted in accordance with the internationally accepted principles for laboratory animal use and care as found in the European Community guidelines. Feed (containing 64% carbohydrate, 20.6% protein, 4.16% fat and 4.92% fiber) and water were supplied ad libitum. Their housing was maintained at a temperature of 20–24 ºC, relative humidity of 50–70%, and a 12 h light/dark cycle. Mice had been housed in groups of ten in the same cage for 1 week before treatment.

Diabetes was induced in overnight fasted mice by intravenous injection via tail vein of 60 mg/kg b.w. of STZ freshly prepared in ice-cold 0.1M sodium citrate buffer, pH 4.5.[10] Normal control mice received an equivalent amount of buffer intravenously. Six days after STZ injection, mice were anesthetized with light ether and tail vein blood glucose concentration were measured in all mice and STZ-injected animals having a fasting blood glucose level lower than 250 mg/dL were excluded from the subsequent experiments. Measurement of blood glucose was carried out by use glucose check strips (Johnson & Johnson medical (China) Ltd.)

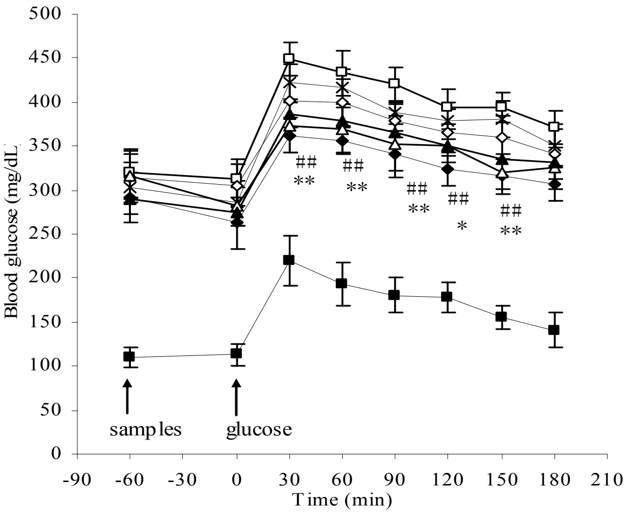

Figure 1. Dose-response effect of PVE on glucose tolerance test in fasting diabetic mice (n=10). (■) normal control; (□) diabetic control; (×) 50 mg/kg PVE; (◇) 75 mg/kg PVE; (△) 100 mg/kg PVE; (▲) 125 mg/kg PVE; (◆) 5 mg/kg glibenclamide. Statistics are shown for 100 and 125 mg/kg PVE * $p<0.05$, ** $p<0.01$, and 5 mg/kg glibenclamide ## $p<0.01$ significantly different relative to diabetic control mice.

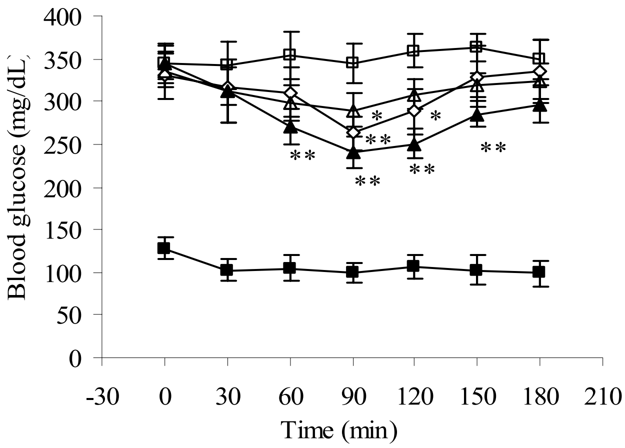

Figure 2. Effects of single and combined administration of PVE and glibenclamide on blood glucose level in diabetic mice (n=10). (■) normal control; (□) diabetic control; (◇) 100 mg/kg PVE; (△) 5 mg/kg glibenclamide; (▲) combination of 100 mg/kg PVE + 5 mg/kg glibenclamide. Statistics are shown for 100 mg/kg PVE * $p<0.05$, ** $p<0.01$, combination of 100 mg/kg PVE and 5 mg/kg glibenclamide ** $p<0.01$, and 5 mg/kg glibenclamide * $p<0.01$ significantly different relative to diabetic control mice.

Oral glucose tolerance test
After the diabetic state was confirmed, the normal and STZ diabetic mice were randomly divided into seven groups (n=10 in each group) as shown in Fig 1. Twenty-four hours after confirmation of the diabetic state, after overnight fast, normal and STZ diabetic mice were oral administered with distilled water, PVE or glibenclamide by gavage, 60 min prior to the challenge with 2 g/kg b.w. glucose intraperitoneally.[11] Mice were anesthetized with light ether and blood samples were taken by distal venesection of the tail vein, just before oral administration of the PVE or water and the glucose, and were then taken subsequently at 30 min intervals for a period of 180 min.

Compare with the dosage of *P. vulgaris* used as folk medicine, four doses (50, 75, 100, 125 mg/kg b.w.) of PVE were used in oral glucose tolerance test. The mice of normal group and diabetic control group were given the same volume of distilled water instead. Glibenclamide-treated STZ diabetic mice were given at dose of 5 mg/kg b.w.[11]

Blood glucose test
After oral glucose tolerance test, these mice would be feed for 1 week before to use. The normal and STZ diabetic mice were checked the blood glucose again and randomly divided into five groups (n=10 in each group) as shown in Fig 2. PVE (100 mg/kg b.w.) and glibenclamide (5 mg/kg b.w.) were given orally. In order to examine the effect of different samples on blood glucose in 180 min, samples were given with the single oral administration and blood glucose levels were monitored in fasted mice, at 0, 30, 60, 90, 120 and 180 min after a single oral administration.

Insulin sensitivity
These mice had been feed for three days after blood glucose test. The normal and STZ diabetic mice were checked the blood glucose again and randomly divided into four groups as shown in Fig 3. The mice of normal group and diabetic control group were given orally the same volume

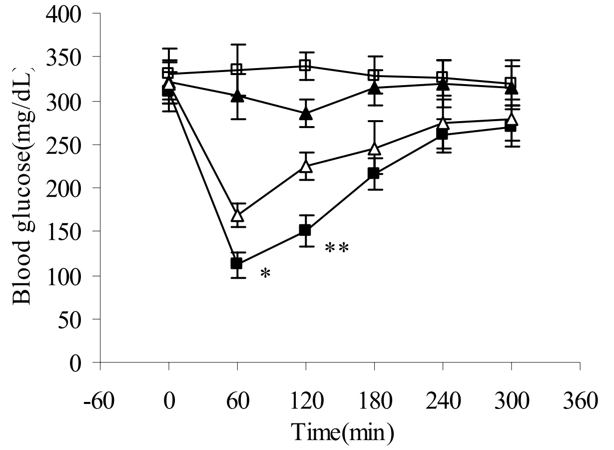

Figure.3. Effects of PVE on comparative insulin sensitivity in diabetic mice (n=10). (□) diabetic control group; (■) combination of 100 mg/kg PVE + 2.5 IU/kg insulin; (▲) 100 mg/kg PVE; (△) 2.5 IU/kg insulin. Statistics are shown for combination of 100 mg/kg PVE and 2.5 IU/kg insulin * p<0.05, ** p<0.01 significantly different relative to insulin-treated diabetic mice.

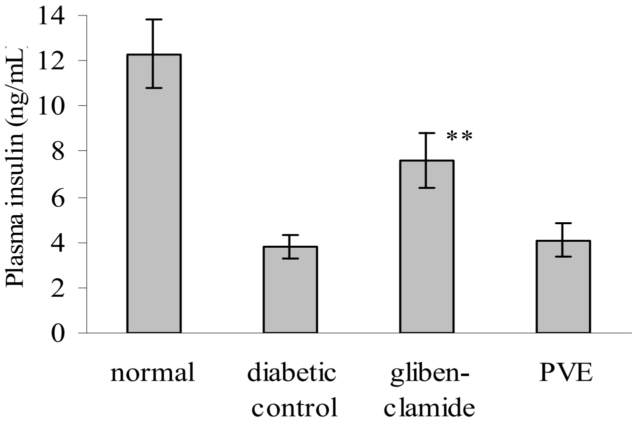

Figure.4. Effects of 100 mg/kg PVE on plasma insulin levels in diabetic mice. Statistics are shown for 5 mg/kg glibenclamide ** p<0.01 significantly different relative to diabetic control mice.

of distilled water instead. Insulin was injected at a dose of 2.5 IU/kg b.w. intraperitoneally[12]. The PVE (100 mg/kg b.w.) and distilled water were administered orally. Blood samples for glucose analysis were taken prior to the administration of PVE, distilled water or the insulin, and subsequently at 60 min intervals for a period of 300 min.[11]

Plasma insulin level

These mice had been feed for three days after insulin sensitivity test. The PVE (100 mg/kg b.w.) and glibenclamide (5 mg/kg b.w.) were administered orally. The mice of normal group and diabetic control group were given orally distilled water (same volume to PVE) instead. Blood samples (150ul) were taken by distal venesection of the tail vein 20 min after treated with different samples. Blood samples were collected into centrifuge tubes containing enough K_3EDTA to achieve a final concentration of 1.7 mg/ml and centrifuged at 3,000 × g for 15 minutes at 4 ± 2 ºC immediately after collection. Plasma was separated and stored at –20 ºC before further assay. Plasma insulin was measured with insulin radioimmunoassay kit.

Statistical analyses

Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Duncan's multiple range tests. P-values of less than 0.05 were considered to be statistical significance. The blood glucose levels were expressed as mean ± SD for ten animals in each group.

Results

Blood glucose levels of PVE-treated groups at different doses after oral glucose challenge are shown in Fig. 1. The maximum rise in blood glucose occurred 30 min after the oral glucose challenge and all doses of PVE and glibenclamide suppressed the rise in glucose levels, to different degrees (Fig 1). Glibenclamide and PVE (100 and 125 mg/kg) significantly (p<0.01) suppressed the rise in blood glucose. In the four doses of PVE, the response of 100 mg/kg was maximum; at the higher dose (125 mg/kg), the response was not higher that at 100 mg/kg. Thus, the dose of 100 mg/kg was chosen as the test dose for subsequent studies.

PVE produced a significant (p<0.01) decrease in blood glucose at 90 min after administrated the dose of 100 mg/kg in diabetic mice, which was then sustained for a further 30 min (Fig 2). In order to investigate whether PVE would further increase the antihyperglycemic action of glibenclamide, PVE and glibenclamide were administered at same time and the blood glucose level monitored for 180 min thereafter. This combined administration of PVE and glibenclamide produced a greater (p<0.01) and sustained fall in blood glucose compared to glibenclamide or PVE alone (Fig 2).

Exogenous insulin treatment alone produced an antihyperglycemic effect in STZ diabetic mice (Fig 3). In the group treated with combination of PVE and exogenous insulin, a significant (p<0.05) decrease in blood glucose was observed 60 min. This enhancement by PVE of the antihyperglycemic effects of exogenous insulin was sustained significantly (p<0.01) during a further period of 60 min.

Plasma insulin of normal and diabetic mice was measured during this study (Fig 4). Plasma insulin levels did not change significantly in the PVE treated group compare to diabetic control group. In addition, glibenclamide treatment increased insulin secretion in STZ diabetic mice (Fig 4).

Discussion

Plants have played a major role in the introduction of new therapeutic agents. A medicinal plant, *Galega officinalis*, led to the discovery and synthesis of metformin [13] but it is still an extensive demand for new oral antidiabetic drugs without side effect. STZ diabetic mice are one of the animal models of human insulin-dependent diabetes mellitus[14]; characterized by high fasting blood glucose levels and drastic reduction in plasma insulin concentration.[15]

This study was undertaken to evaluate the antihyperglycemic activity of the aqueous-ethanol extract of *P. vulgaris* in STZ diabetic mice. The antihyperglycemic effect of different doses of PVE was studied in oral glucose tolerance test. Furthermore, the dose of the maximum response was used in blood glucose test, comparative insulin sensitivity and plasma insulin levels to study the antihyperglycemic effect of PVE, the combination of PVE and glibenclamide, and the combination of PVE and insulin.

The results from the dose response to *P. vulgaris* in STZ diabetic mice indicated that four doses of PVE all suppressed the hyperglycemic response to an acute glucose challenge and produced a fall in the blood glucose level. Two doses of 100 and 125 mg/kg shown significant difference and the response of 100 mg/kg were maximal; thus, we chose the dose of 100 mg/kg as the test dose for subsequent studies.

The combined administration of *P. vulgaris* and glibenclamide significantly decreased blood glucose level in STZ diabetic mice, and the antihyperglycemic activity was more pronounced between 60 and 150 min. The same dose of *P. vulgaris* also enhanced and prolonged the antihyperglycemic effects of exogenous insulin. However, antihyperglycemic activity was not due to a change in endogenous plasma insulin secretion. In present study, similar antihyperglycemic effects of *P. vulgaris* were found in the STZ diabetic mice for the first time. The results from the oral glucose tolerance tests, blood glucose tests, and comparative insulin sensitivity tests in STZ diabetic mice suggest that *P. vulgaris* has the potential to lower blood glucose levels.

Glibenclamide, a sulphonylurea, is antihyperglycemic drug.[4] It is known to stimulate insulin secretion from the pancreas, Whereas, *P. vulgaris* did not affect insulin levels after repeated oral administration. It suggests that *P. vulgaris* influences the blood glucose by other mechanisms than conventional sulphonylurea antihyperglycemic drugs. The improvement of glucose tolerance produced by *P. vulgaris*, in the absence of any change in circulating insulin levels, is similar to the action of metformin,[4] which acts by increasing the insulin sensitivity of target tissues.

The present study with the comparative insulin sensitive tests indicates that the antihyperglycemic effect of insulin is enhanced and prolonged, which could result from increased tissue metabolism or from suppressed levels of Non-esterified Fatty Acids (NEFA). Excess plasma NEFA can inhibit insulin-stimulated glucose utilization in muscle[16] and promote hepatic production of glucose.[17] Whereas, reduction of plasma NEFA concentration improves glucose utilization[18], enhances the suppression of hepatic glucose production by insulin.[19] On the other way, insulin sensitizer like thiazolidinediones is known to act through the nuclear receptor, PPARγ and their antidiabetic activity correlates with the order of potency for PPARγ transactivation.[20-21] It is necessary to elucidate whether there is significant activation of PPARγ in a transactivation assay after PVE administration.

On the other hand, no significant change in plasma insulin levels was noted in STZ diabetic mice after *P. vulgaris* treatment. It seems then that *P. vulgaris* extract reduced blood glucose levels without stimulating insulin secretion. The mechanism involved in this pharmacological effect, therefore is extra-pancreatic. *P. vulgaris* may exert its antihyperglycemic action by other mechanisms such as stimulation of glucose uptake by peripheral tissues[22], inhibition of endogenous glucose production.[23]

Some constituents in the *P. vulgaris* have been identified, such as, phenolic acids (rosmarinic, caffeic *et al.*), triterpenoids (methyl oleanolate, methyl ursolate, methyl maslinate *et al.*)[24-26], flavonoids (quercetin, campherol, rutin *et al.*)[27], tannins and polysaccharide.[28-29] The antihyperglycemic activity of the *P. vulgaris* may be due to any one or more of the constituents in the extract, for example, triterpenoids and flavonoids.

The antihyperglycemic mechanisms of *P. vulgaris* extract remain unclear and further studies are required to elucidate site(s), cellular and molecular mechanisms of *P. vulgaris* extract. The measurement of lipogenesis, lipolysis, NEFA, and glucose uptake could help to elucidate the possible physiological mechanisms for the antihyperglycemic effect of *P. vulgaris*.

In conclusion, the results from this study indicated that *P. vulgaris* could induce antihyperglycemic effects without stimulating insulin secretion. The use of this plant for diabetes treatment is promising but the precise active substance(s), site(s) and mechanism(s) of its pharmacological effect are still to be determined.

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