Physalis peruviana Linn. fruit extract improves insulin sensitivity and ameliorates hyperglycemia in high-fat diet low dose STZ-induced type 2 diabetic rats

M. Sathyadevi, E.R. Suchithra and S. Subramanian*
Department of Biochemistry, University of Madras, Guindy Campus, Chennai 600 025, India

Received on: 26-03-2014; Revised on: 20-04-2014; Accepted on: 21-04-2014

ABSTRACT

Physalis peruviana Linnaeus, belonging to the family Solanaceae and genus Physalis, possess a wide array of pharmacological properties. In the absence of systemic studies in the literature, the present study was aimed to explore the antidiabetic potential of the Physalis peruviana fruits in high fat diet fed-low dose STZ induced experimental type 2 diabetes in rats. Phytochemical screening of the fruit extract was performed. Diabetic rats were treated with Physalis peruviana fruit extract at a dosage of 200 mg/kg b.w daily for 30 days. Metformin (200 mg/kg, b.w) was used as a standard drug. The physiological criterions such as food and fluid intake were recorded. The levels of fasting blood glucose, plasma insulin and HbA1c were also estimated. Intraportaline insulin tolerance test was performed. The level of glycogen content in liver tissue was estimated. The activities of serum aspartate transaminase, alanine transaminase and alkaline phosphatase were assayed. The fruit extract supplementation attenuated the elevated levels of glucose, glycosylated hemoglobin, AST, ALT and ALP. The insulin level in liver tissue was estimated. The activities of serum aspartate transaminase, alanine transaminase and alkaline phosphatase were assayed. The results showed that Physalis peruviana fruit extracts are non toxic in nature and possess significant antidiabetic properties which might be attributed to the presence of biologically active ingredients present in the fruit extract.

Keywords: Physalis peruviana; Type 2 diabetes; high fat diet; Streptozotocin; antidiabetic

INTRODUCTION

Type 2 diabetes mellitus is a heterogeneous metabolic disorder characterized by persistent hyperglycemia with a concomitant decline in insulin action (insulin resistance), in the context of insulin resistance and relative lack of insulin. The global increase in the prevalence of diabetes is due to population growth, aging, urbanization and an increase of obesity and physical inactivity. Roughly 80% of people with diabetes are in developing countries, of which India and China share the larger contribution. The use of plants for medicinal purposes is as old as our civilization. The World Health Organization (WHO) estimates that 80 percent of the world’s population relies on herbal medicine for some aspect of primary health care. In the developing world, herbal medicine is used in industrialized nations by alternative medicine practitioners such as naturopaths. However, most of the medicinal plants used in herbal medicine have not received scientific validation.

One such traditionally used medicinal plant that lacks scientific scrutiny is Physalis peruviana. Cape gooseberry (Physalis peruviana) is a fruit that belongs to the Solanaceae family has been grown in Egypt, South Africa, India, New Zealand, Australia and Great Britain. It is also known as Uchuva in Colombia and goldenberry or wild tomato in English speaking countries. P. peruviana L. is an herbaceous, semi-shrub, upright, and perennial in subtropical zones plant, it can grow until reach 0.6 to 0.9 m and in some cases can grow up to 1.8 m. A single plant may yield 300 fruits each containing around 200 seeds.

Colombia is the largest producer of P. peruviana fruits followed by South Africa. Colombia produces 11,500 ton/year of P. peruviana L.fruit. The plant does not tolerate clay soils because it has superficial roots. Each fruit is protected by the calyx. The shelf life of the fruit with calyx is one month while without calyx is 4 to 5 days or so. The fruit juice of P. peruviana L can be used as preservative for jams and jellies. The fruits are sweet when ripe, with a characteristic mildly tart flavor. So far, there are no studies that indicate the possible adverse effects of fruits.

Different parts of the plant show antioxidant, anti-inflammatory and antiproliferative effects on hepatoma cells. In addition, the fruit has excellent potential as a foodbased strategy for anti-diabetic and anti-hypertensive products. P. peruviana, possess anticancer, antibacterial, antipyretic, immunomodulatory, and have been used for the treatment of malaria, asthma, hepatitis, dermatitis, and rheumatism.
Physalis peruviana contains biologically active components e.g. physalins, withanolides, phytosterols and polyunsaturated fatty acids e.g. linoleic acid and oleic acid. Among its major components are high amounts of vitamins A, B and C as well as the presence of essential minerals, magnesium, calcium, potassium, sodium and phosphorus which are classified as macronutrients, while the iron and zinc are considered as micronutrients. Furthermore, it has been added that Cape gooseberry pulp, seed and pomace pulp might serve as excellent dietary sources for vitamin K1, α-linoleic acid, essential fatty acids, tocopherols and carotenoids.

In absence of systemic studies in the literature, the present study was aimed to evaluate the antidiabetic effects of Physalis peruviana fruits in high fat fed- low dose STZ induced experimental diabetes in rats.

Materials and Methods

Plant Material
Physalis peruviana fruits were collected from Theni, Tamilnadu. The plants were identified and authenticated by a qualified taxonomist and a voucher specimen was deposited at CAS in Botany, University of Madras, Chennai.

Preparation of plant extract
Mature Physalis peruviana fruits were dehusked, washed, crushed in an air oven at 50°C then powdered in an electrical grinder, which was then stored in an airtight brown container at 5°C until further use. The powdered fruits were delipidated with petroleum ether (60-80°C) for overnight. It was then filtered and soxhalation was performed with 95% Ethanol. Ethanol was evaporated in a rotary evaporator at 40–50°C under reduced pressure. The 100gm of dried powder of Physalis peruviana L yields 17g.

Preliminary phytochemical screening
The ethanolic extract of Physalis peruviana fruits were subjected to qualitative phytochemical screening for the presence of major phytochemicals.

Experimental animals
Male albino rats of Wistar strain weighing (160-180g) were procured from Tamilnadu Veterinary and Animal Sciences University (TANUVAS), Chennai. The rats were housed in spacious polypropylene cages lined with husk. The experimental rats were maintained in a controlled environment (12:12 ± 1h light/dark cycle) and temperature (30°C ± 2°C). Animals were acclimatized to standard husbandry conditions for one week to eliminate the effect of stress prior to initiation of the experiments. The rats were fed with commercial pellet fed rats chow (Hindustan Lever Ltd., Bangalore, India), and had free access to water ad libitum. The experiments were designed and conducted in strict accordance with the current ethical norms approved by Ministry of Social justices & Environment, Government of India and Institutional Animal Ethical Committee guidelines (IAEC NO. 09/03/2012).

High fat diet fed streptozotocin induced diabetes
The rats were divided into two dietary regimens by feeding either normal or high fat diet (HFD) for the initial period of two weeks. After two weeks of dietary manipulation, the groups of rats fed with HFD was injected intraperitoneally with a low dose of STZ (35 mg/kg b.w) dissolved in 0.1M cold citrate buffer, pH 4.5). One week after STZ injection, the rats were screened for blood glucose level. Rats having fasting blood glucose (FBG) >250mg/dl that exhibited random hyperglycaemia and glycosuria were selected for the experiment. The rats were allowed to continue to feed on their respective diets until the end of the experiments.

Toxicity and dosage fixation studies
The acute toxicity of Physalis peruviana fruits was studied in the control rats according to OECD guideline 423. Graded doses of Physalis peruviana fruits dissolved in water were given orally and the animals were observed continuously for the first 2 hours followed by every hour up to 6 hours and daily thereafter for fourteen days for any signs of morbidity, mortality and behavioural toxicity. Physalis peruviana fruits were found to be non-toxic up to 2 g/kg b.w.

Graded doses of Physalis peruviana fruits (100, 200, 300, 400 mg/kg b.w) was administered to HFD + STZ induced diabetic rats for various periods of treatment. From the data obtained, the optimum dosage was fixed as 200 mg/kg b.w for 30 days. The animals were divided into four groups, comprising a minimum of six animals in each group as follows:

Group 1 – Control rats.
Group 2 – HFD + STZ (i.p. 35mg/kg b.w) induced rats.
Group 3 – Physalis peruviana fruit extract (200 mg/kg b.w.) treated diabetic rats
Group 4 – Diabetic rats treated with metformin (200 mg/ kg b.w/ day) in aqueous solution orally for 30 days.

At the end of the treatment period, the rats were fasted overnight, anesthetized and sacrificed by cervical decapitation. The blood was collected with and without anticoagulants for plasma and serum separation, respectively.

Intraperitoneal insulin tolerance test
At the end of the experimental period, fasting blood samples were withdrawn through retro-orbital bleeding from the control and experimental groups of rats. Four more blood samples were collected at 30, 60, 90 and 120 min intervals after the intraperitoneal administration of a bolus of insulin (2 unit/kg b.w). All the blood samples were collected with EDTA for the determination of glucose by using glucose oxidase peroxidase/diagnostic enzyme kit (Span Diagnostic Chemicals, Surat, India) and the analysis was performed according to the manufacturer’s instructions.
Biochemical parameters

Fasting blood glucose level was estimated according to the method of Sasaki et al., 1972. Plasma insulin was assayed using the Ultra-sensitive ELISA kit for rat insulin (Linco Research, St Charles, MO, USA). Glycosylated hemoglobin (HbA1c) levels were estimated according to the method of Nayak and Pattabiraman. Urine sugar was detected using commercially available diastix urine strips. The activities of AST, ALT and ALP were assayed. The liver and muscle tissues were dissected out and washed with ice-cold saline for determination of glycogen content.

Statistical analysis

The results were expressed as mean ± S.E.M of six rats per group and statistical significance was evaluated by one-way analysis of variance (ANOVA) using SPSS (version 16) program followed by LSD. Values were considered statistically significant when p < 0.05.

RESULTS

The levels of food and fluid intake of the control and experimental groups of rats are shown in Figure 1 and 2 respectively. HFD-STZ induced rats showed significant (p<0.05) increase in food and fluid intake when compared with control group of rats. Subsequent to the oral administration of fruit extract as well as metformin to diabetic group of rats, the levels were found to be similar to that of control group of rats.

Liver and muscle glycogen content of HFD/STZ diabetic control rats showed a highly significant decrease as compared to normal control (Figure 4). However, treatment with the fruit extract and metformin improved the levels of glycogen content in liver and muscle tissues. Table 1 shows the phytochemical screening of the fruit extract. The fruit extract was found to contain flavonoids, alkaloids, glycosides, saponins and phenols.

The effect of fruit extract as well as metformin treatment on the level of blood glucose in the experimental groups of rats receiving an intraperitoneal insulin challenge is shown in Figure 3. The blood glucose level is significantly (p<0.05) reduced in diabetic rats treated with fruit extract as well as metformin than that of the diabetic group of rats.

Liver and muscle glycogen content of HFD/STZ diabetic control rats showed a highly significant decrease as compared to normal control (Figure 4). However, treatment with the fruit extract and metformin improved the levels of glycogen content in liver and muscle tissues. Table 1 shows the phytochemical screening of the fruit extract. The fruit extract was found to contain flavonoids, alkaloids, glycosides, saponins and phenols.

The effect of fruit extract on the levels of blood glucose, fasting plasma insulin and glycosylated hemoglobin (HbA1c%) in HFD/STZ diabetic rats is shown in Table 2. The levels of blood glucose and HbA1c% was found to be significantly elevated in diabetic rats as compared with normal control. Oral administration of fruit extract to diabetic rats significantly improved the altered level. The levels of plasma insulin were moderately decreased in HFD-STZ induced diabetic rats. Diabetic rats treated with fruit extract as well as metformin showed improved insulin level.

Figure 1. Effect of Physalis perruviana fruit extract on food intake of experimental groups of rats.

Unit: g/rat/day for food intake.
Values are given as mean ± S.E.M [n=6] for groups of six rats in each. One-way ANOVA followed by post hoc test LSD.
Statistical significance was compared within the groups as follows: a control rats; b diabetic control rats. Values are statistically significant at *p<0.05
Figure 2. Effect of *Physalis perruviana* fruit extract on fluid intake of experimental groups of rats.

Unit: g/rat/day for water consumption.

Values are given as mean ± S.E.M [n=6] for groups of six rats in each. One-way ANOVA followed by post hoc test LSD. Statistical significance was compared within the groups as follows: a control rats; b diabetic control rats. Values are statistically significant at *p < 0.05.

Figure 3. Effect of *Physalis perruviana* extract on intraperitoneal insulin tolerance test in experimental groups of rats.

Values are given as mean ± S.E.M for groups of six rats in each. One-way ANOVA followed by post hoc test LSD. Statistical significance was compared within the groups as follows: a control rats; b diabetic control rats. Values are statistically significant at *p < 0.05.
Figure 4. Effect of *Physalis perruviana* extract on the levels of glycogen content in liver and muscle tissues in experimental groups of rats.

Values are given as mean ± S.E.M (n=6). One-way ANOVA followed by post hoc test LSD. *p<0.05 when compared with *control rats; *diabetic control rats.

Table 1: Phytochemical analysis of *P. peruviana* fruit extract

<table>
<thead>
<tr>
<th>Phytoconstituents</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
</tr>
<tr>
<td>Phytosterols</td>
<td>+</td>
</tr>
<tr>
<td>Diterpenes</td>
<td>-</td>
</tr>
<tr>
<td>Triterpenoids</td>
<td>-</td>
</tr>
<tr>
<td>Glycosides</td>
<td>+</td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>-</td>
</tr>
<tr>
<td>Phenols</td>
<td>+</td>
</tr>
</tbody>
</table>

Table 2. The levels of blood glucose, glycosylated hemoglobin (HbA1c), plasma insulin and urine sugar in control and experimental groups of rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Blood glucose</th>
<th>HbA1c</th>
<th>Insulin</th>
<th>Urine sugar</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>87.66 ± 4.74</td>
<td>5.17 ± 0.30</td>
<td>15.67 ± 0.19</td>
<td>Nil</td>
</tr>
<tr>
<td>Diabetic</td>
<td>298.09 ± 9.05</td>
<td>12.60 ± 0.46</td>
<td>10.29 ± 0.32</td>
<td>+++</td>
</tr>
<tr>
<td>Diabetic + <em>P. peruviana</em></td>
<td>134.18 ± 5.94</td>
<td>7.22 ± 0.29</td>
<td>12.45 ± 0.49</td>
<td>Nil</td>
</tr>
<tr>
<td>Diabetic + metformin</td>
<td>120.27 ± 6.85</td>
<td>6.97 ± 0.19</td>
<td>14.50 ± 0.46</td>
<td>Nil</td>
</tr>
</tbody>
</table>

*Units: mg/dl for blood glucose, % hemoglobin for HbA1c, µU/ml for plasma insulin, +++ indicates more than 2% sugar. Results are expressed as mean ± S.E.M [n=6]. One-way ANOVA followed by post hoc test LSD. Values are statistically significant at P<0.05. The results were compared with *control rats, *diabetic rats.*
Enzyme activities are expressed as:

**DISCUSSION**

Extracts of medicinal plants are known to contain different chemopreventive or chemotherapeutic compounds, which possess more than one mechanism of action. The bioactive components present in the fruit of *P. peruviana* L. make this to be considered as a natural functional food, because the physiological properties associated with its nutritional composition. The phytochemical analysis of the fruit extract revealed the presence of biologically active ingredients such as flavonoids, alkaloids, glycosides, saponins and phenols. It has been reported that *Physalis peruviana* fruits contain high levels of polyphenols and rich in vitamins A and C content which readily accounts for its antioxidant nature. The main active components of vitamin A in fruits are α-carotene, β-carotene and β-cryptoxanthin. The most common carotenoids is β-carotene, because none of the other carotenoids is present in provitamin A which has half the activity of β-carotene, also is less extensive in nature. Carotenoids are responsible for the orange colour in the fruit of *P. peruviana* L. The benefits associated of the fruit of *P. peruviana* L., are mainly due to their nutritional composition because, besides having good nutritional characteristics contains biologically active components that provide health benefits and reduce risk for certain diseases.

High fat diet initiates the insulin resistance which is one of the important features of type 2 diabetes. Streptozotocin (STZ) is widely used to reproducibly induce both insulin-dependent and noninsulin-dependent diabetes mellitus possibly by inducing β cell death through alkylation of DNA. Low-dose STZ has been known to induce a mild impairment of insulin secretion which is similar to the feature of the later stage of type 2 diabetes. Hence in the present study, HFD-STZ induced animal model was used to assess the antidiabetic efficacy of *Physalis Peruviana* fruit extract.

Diabetes mellitus is characterized by classical symptoms such as polyphagia and polydipsia which were exhibited in HFD-STZ diabetic rats and this may be attributed to the impaired glucose homeostasis as result of insulin inefficiency. In the present study, the HFD-STZ induced diabetic rats showed increased food and fluid consumptions. However, oral treatment with the fruit extract to diabetic group of rats decreased food and fluid consumptions which could be due to improved glycemic status.

HFD-STZ induced diabetic rats displayed frank hyperglycemia indicating the state of insulin resistance in HFD-STZ induced rats. The fruit extract decreased the levels of blood glucose and glycosylated hemoglobin indicating the beneficial effects of the fruits in maintaining glucose homeostasis. Treatment with the fruit extract to diabetic rats facilitates insulin stimulated glucose uptake into the peripheral tissues which is evident from intraperitoneal insulin tolerance test. The levels of plasma insulin were moderately decreased in HFD-STZ induced diabetic rats. Though the level was not decreased significantly, the insulin level in HFD-STZ diabetic rats could not facilitate glucose uptake due to insulin resistance. There was improved insulin sensitivity and a mild increase in insulin level in diabetic rats treated with the fruit extract indicating that the fruit extract exhibit significant insulin sensitization activity as well as improvement in the glucose homeostasis probably due to improved pancreatic β-cell function which is evident from improved plasma insulin level.

Liver and skeletal muscle is the primary site of glucose disposal in insulin-stimulated state. Elevated endogenous glucose production is a common abnormality associated with diabetes that, in concurrence with deprived pancreatic function and reduced glucose clearance, contributes to the hyperglycemia characteristic of diabetes. Insulin regulates the metabolism by modulating the uptake and utilization of glucose in target organs such as liver, skeletal muscle and adipose tissue by controlling the activities of numerous metabolic enzymes. In the present study, the glycogen levels were significantly increased in liver tissues upon treatment with the fruit extract indicating the improved glucose utilization and storage.

Increased activities of AST, ALT and ALP levels are associated with hepatic damage or the changes in the permeability of hepatocyte membrane. Because AST is widely distributed in the body including primarily the liver and muscles and redblood cells, ALT is considered as a more specific enzyme for liver function. In addition, higher level of liver-function enzyme e.g., ALT and AST in serum are not only used for the identification of liver damage but also for the hepatic IR, metabolic syndrome as well as T2D. A strong correlation exists between serum ALT level and insulin resistance but not for the AST, and it has been indicated as a predictor of T2D in human subjects. Oral administration of the fruit extracts to HFD-STZ induced diabetic rats decreased the activity of these enzymes to their basal levels, indicating its non toxic nature.

### Table 3. Effect of *P. peruviana* fruit extract on the activities of serum transaminases and alkaline phosphatase in the control and experimental groups of rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>AST (µmol of pyruvate liberated/h/mg of protein)</th>
<th>ALT (µmol of phenol liberated/min/mg of protein)</th>
<th>ALP (µmol of inorganic pyrophosphate liberated/h/mg of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>80.81 ± 2.64</td>
<td>20.77 ± 0.44</td>
<td>73.65 ± 0.94</td>
</tr>
<tr>
<td>Diabetic</td>
<td>131.30 ± 2.78</td>
<td>44.99 ± 1.65</td>
<td>143.50 ± 6.94</td>
</tr>
<tr>
<td>Diabetic + <em>P. peruviana</em></td>
<td>71.48 ± 1.44</td>
<td>25.49 ± 0.83</td>
<td>94.17 ± 1.73</td>
</tr>
<tr>
<td>Diabetic + metformin</td>
<td>84.45 ± 1.94</td>
<td>19.90 ± 0.84</td>
<td>71.19 ± 1.05</td>
</tr>
</tbody>
</table>

Enzyme activities are expressed as: AST and ALT - µmol of pyruvate liberated/h/mg of protein, ALP - µmol of phenol liberated/min/mg of protein. Results are expressed as mean ± S.E.M [n=6]. One-way ANOVA followed by post hoc test LSD. The results were compared with “Control rats,” “Diabetic rats. Values are statistically significant at *P<0.05.”
CONCLUSION
The results of the present study indicate that *Physalis peruviana* fruits possess significant antidiabetic activity in type 2 diabetic rats. The fruit extract improves insulin sensitivity in HFD fed-STZ-induced diabetic rats which is evident from the results of intraperitoneal insulin tolerance test and plasma insulin levels. Also, the nutritional composition and the presence of biologically active ingredients in the fruits make it considerable for the treatment of diabetes mellitus. However, further studies are in progress to understand the plausible mechanism by which the fruit extract attenuates hyperglycemia, and improves insulin sensitivity in HFD-STZ induced T2D in experimental rats.

ACKNOWLEDGEMENT
The research fellowship (UGC-BSR) of the University Grants Commission (UGC), New Delhi, India, to Mrs. M. Sathyadevi is gratefully acknowledged.

REFERENCES
29. King J: The hydrolases-acid and alkaline phosphatases. In:


**Source of support:** University Grants Commission (UGC), New Delhi, India; **Conflict of interest:** None