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Evaluation of antidiabetic activity of medicinal plant extracts used by tribal communities in rural areas of Warangal district, Andhra Pradesh, India

Esampally Sucharitha, Mamidala Estari*
Infectious Diseases and Metabolic Disorders Research Lab, Department of Zoology, Kakatiya University, Warangal, Andhra Pradesh, India.

***Correspondence:** estari08@gmail.com

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Abstract
The prevalence of diabetes mellitus (DM) is increasing with ageing of the population and life style changes associated with rapid urbanization and westernization. *Physalis minima* is widely used in Indian medicine by the tribal communities to treat various diseases including diabetes. The present study was aimed at assessing the hypoglycemic effects of extracts from *P. minima* in alloxan-induced diabetic rats. The powdered plant parts were successfully extracted with boiling water using soxhlet extractor. The Wister strains of male albino rats were used for the present study. The antihyperglycemic activity of the crude aqueous extracts of *P. minima* different parts were studied in alloxan-induced diabetic rats. The toxicity study results showed that the medium lethal dose (LD_{50}) of the extracts is higher than 1 g/kg body weight and hence, in a single dose administration, the plant extracts had no adverse effect. There is no significant level of reduction in fasting blood glucose level was noticed for the aqueous extracts of root and stem of *P. minima*. On chronic administration, the effect of *P. minima* flower and leaf causes a fall in fasting blood sugar of rats. These findings clearly established that the antidiabetic efficacy of the flower and leaf extract of *P. minima* are almost equal and both exhibited more potent antidiabetic activity by reducing the blood glucose level significantly than all other root and stem extracts.

Keywords: Diabetes mellitus; *Physalis minima*; hypoglycemic; Warangal; India.

Introduction

Diabetes mellitus (DM) is a serious health problem being the third greatest cause of death all over the world, and if not treated, it is responsible for many complications affecting various organs in the body (El-Hilaly *et al*., 2007). The chronic hyperglycemia of diabetes is associated with long term damage, dysfunction, and failure of various organs (Lyra *et al*., 2006). In diabetic rats, the utilization of impaired carbohydrate leads to accelerate lipolysis, resulted in hyperlipidemia (Morel and Chisolm, 1989). Despite the presence of known antidiabetic medicine in the pharmaceutical market, diabetes and the related complications continued to be a major medical problem. Recently, some medicinal plants have been reported to be useful in diabetes worldwide and have been used empirically as antidiabetic and antihyperlipidemic remedies (Mitra *et al*., 1996). More than 400 plant species having hypoglycemic activity have been available in literature (Oliver-Bever, 1986; Roy *et al.*, 2005), however, searching for new antidiabetic drugs from natural plants is still attractive because they contain substances which take alternative and safe effect on DM.

Physalis minima is an annual herb indigenous to many parts of the tropics, including the Amazon. It can be found on most continents in the tropics, including Africa, Asia, and The Americas. It grows up to 1 m high, bears small, cream-colored flowers, and produces small, light yellowish-orange, edible fruit sometimes referred to as cape gooseberry. The fruit is about the size of a cherry tomato and like tomatoes, it contains many tiny edible seeds inside. *P. minima* propagates easily from the many seeds the fruit contains, spontaneous clumps of plants can be found along river banks and just about anywhere the soil is disturbed and the canopy is broken (allowing enough sunlight to promote its rapid growth) (Parmar and Kaushal, 1982). Plant derivatives with hypoglycemic properties have been used in folk medicine and traditional healing systems around the world from very ancient time. As per the

ethnobotanical literature on traditional phytotherapy of Indian medicinal plants, the species *P. minima* is consistently used by the tribal communities for the treatment of diabetes as well as in modern medicine. There is no any scientific evidence is available of this plant to treat diabetes. Therefore this work has been taken with the aim of producing an inventory of this plant used by traditional healers in Adilabad district of Andhra Pradesh to treat diabetes.

Materials and Methods

Preparation of plant extracts

The stem, leafs, root, and flowers of *P. minima* was collected from the rural areas (Mangapeta, Eturnagaram, and Venkatapur) of Warangal district, Andhra Pradesh, India, identified and authenticated from botanist Dr. A.V.S.S. Raju, Department of Botany, Kakatiya University, Warangal and the voucher specimens of *P. minima* are preserved in the Department of Zoology. The collected plant parts of stem, leafs, root, and flowers were cleaned and washed well with water. Then 50 g of selected plant parts were dried under shade at 25°C for 5 days in the absence of sunlight and grounded well to fine powder. The powdered plant parts (nearly 30 g) were successfully extracted with boiling water using soxhlet extractor are then cooled and filtered using Whatman No. 1 filter paper. The filtrate was centrifuged at 10,000 rpm at room temperature (25°C) and the sediment was discarded. The supernatant was concentrated upto 100 mL on rotavapor under reduced pressure. The concentrated crude extract was lyophilized into powder (5 g) and used for the study.

Experimental animals

The Wister strains of male albino rats weighing between 100 and 150 g were obtained for the present study, from National Institution of Nutrition, Hyderabad. The animals were housed in larger spacious cages and they were fed with commercial pelleted rat chow marketed by Hindustan Lever Ltd., Bangalore, India, under the trade name Gold Mohur Rat Feed and had free access to water *ad labium*. The animals were well acclimatized to standard environmental conditions of temperature (22 ± 5°C) and humidity (55 ± 5%) and 12 h light dark cycles throughout the experimental period. The animals used in the present study were approved by the Institutional Animal Ethical Committee (No.77/Pharm-Zoo/KU/IAEC/2012).

Acute toxicity test

Seven main groups of male Wister albino rats were selected to study the acute toxicity of all plant extracts under investigation. All groups received one oral dose of 100, 250, 500, 650, 800, 950, and 1100 mg of plant extract/kg body weight. Animals were kept under close observation for 24 h after administering the extract, and then they were observed daily for 3 days for any change in general behavior and/or other physical activities. After 24 h, there were no died animals representing the safety action of all extracts.

Experimental design

The rats were segregated into 7 groups with minimum of 8 rats in each group.

Group I: Normal control rats
Group II: Diabetic control rats
Group III: Diabetic rats treated with stem extract of *P. minima*
Group IV: Diabetic rats treated with leaf extract of *P. minima*
Group V: Diabetic rats treated with root extract of *P. minima*
Group VI: Diabetic rats treated with flower extract of *P. minima*
Group VII: Diabetic rats administered with tolbutamide (150 mg/kg b.w.) in aqueous solution orally for 28 days.

Treatment protocol

Test extracts (250 mg/kg b.w.), standard drug Tolbutamide (150 mg/kg b.w.), and control (2 mL saline) were administered orally, every 24 h for a period of 28 days. The experimental rats were carefully monitored everyday, no sign of toxicity was noticed on the behaviors and general health of the rats when exposed to the plant extract. Animals described as festered were deprived of food for at least 12 h but allowed free access to drinking water. The blood samples were obtained through the tail vein puncturing with hypodermic needy under light ether anesthesia. 0.2 mL of blood was withdrawn at interval of initial, 1, 3, and 5 h of administration of single dose (for acute study) and at the end of 7, 14, 21, and 28 days (prolonged study). At the end of the experimental period, the fasted rats were then sacrificed by cervical decapitation. Blood was collected with Ethylenediamine tetraacetic acid (EDTA) as anticoagulant and centrifuged at 3000 rpm for 15 min to separate plasma. The blood without EDTA was centrifuged at 6000 rpm for 5 min for serum separation.

Oral glucose tolerance test

Fasted rats were divided into six groups of 6 rats in each. Group I served as normal control and received distilled water, Group II served as diabetic control, Groups III–VI received the different extracts at a dose of 250 mg/kg body weight, and Group VII received standard drug tolbutamide (150 mg/kg b.w.). After 30 min of extract administration, the rats of all groups were orally treated with 2 g/kg of glucose. Blood samples were collected from the rat tail vein just prior to glucose administration and at 30, 60, and 90 min after glucose loading. Blood glucose levels were measured immediately by using Glucometer.

Alloxan-induced diabetic model

Alloxan monohydrate was first weighed individually for each animal according to its weight and then solubilized with 0.2 mL saline just prior to injection. Diabetes was induced by injecting it at a dose of 150 mg/kg body weight, intraperitonially (Manoharan and Punitha, 2006). After 1 h of alloxan administration, the animals were given feed *ad libitum* and 5% dextrose solution was also given in feeding bottle for a day to overcome the early hypoglycemic phase. The animals were kept under observation, after 48 h, blood glucose was measured by Glucometer. One group served as a control which received vehicle alone. The diabetic rats (glucose level $>$ 300 mg/dl) were separated and divided into six different groups for experimental study, with each group containing 8 animals. Group II were left untreated and served as diabetic controls.

Estimation of blood glucose

The blood glucose was measured in all the groups by using glucose enzyme reagent system manufactured by Span Diagnostic Private Ltd., Surat, India. The system uses glucose oxidase method for estimating glucose in blood to evaluate the hypoglycemic activity.

Statistical analysis

Data were analyzed by comparing values for different treatment groups with the values for individual controls. Results are expressed as mean ± S.D. The significant differences among values were analyzed using analysis of variance (one-way ANOVA) in latest computer software programme.

Results and Discussion

Acute toxicity test

A preliminary toxicity study was designed to demonstrate the appropriate safe dose range that could be used for subsequent experiments rather than to provide complete toxicity data on the extract. Acute toxicity studies conducted revealed that the administration of graded doses of stem, leaf, root, and flower crude extracts (upto a dose of 1100 mg/kg) of *P. minima* did not produce significant changes in behaviors such as

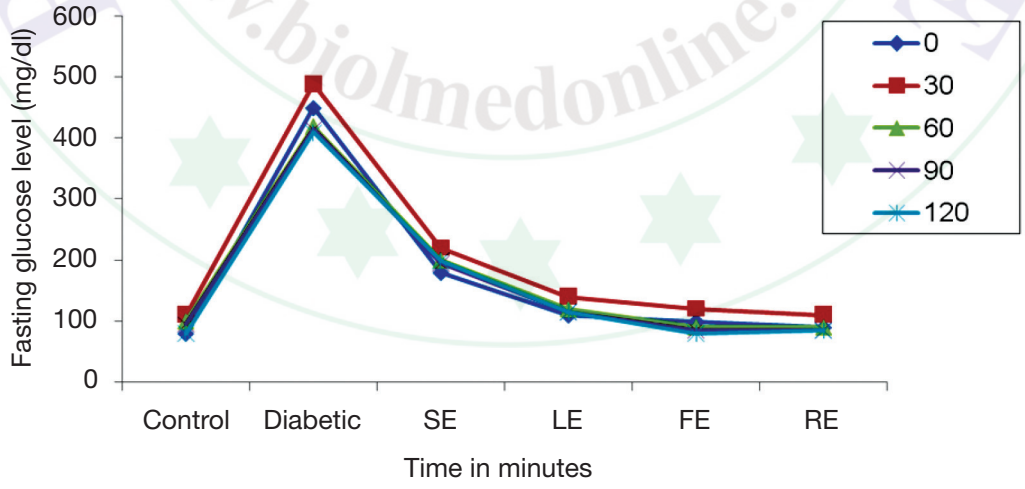

Figure 1: Effect of *P. minima* extracts on blood glucose level in OGTT in normal rats.
The data are indicated as mean ± S.E.M. (n = 6). SE = Stem extract treated rats, LE = Leaf extract treated rats, FE = Flower extract treated rats, and RE = Root extract treated rats.

alertness, motor activity, breathing, restlessness, diarrhea, convulsions, coma, and appearance of the animals. No death was observed upto the dose of 1 g/kg body weight. The mice were physically active. These effects were observed during the experimental period (72 h). The result showed that in single dose; the plant extracts had no adverse effect, indicating that the LD_{50} could be greater than 1 g/kg body weight in mice.

Oral glucose tolerance test

The experiment showed that Oral Glucose Tolerance Test (OGTT) measures the body ability to use glucose, the body's main source of energy (Grover *et al*., 2002). This test can be used to diagnose prediabetes and diabetes. We made an attempt for the first time to study the effect of *P. minima* plant extracts in hyperglycemic rats. *P. minima* stem, leaf, flower, and root aqueous extracts show good oral glucose tolerance. The effect of different extracts on glucose tolerance test in normal rats is shown in Figure 1. At 30 min after glucose administration, the peak of blood glucose level increased rapidly from the fasting value and then subsequently decreased. The other extracts of *P. minima* exhibited remarkable blood glucose lowering effect at 90 min.

Effect of P. minima on blood glucose level

The mean fasting glucose levels for Group I–VII are indicated in Table 1 (for acute study) and Table 2 (for prolonged study). Results in Table 1 show that there was an elevation in blood glucose levels in alloxan treated diabetic rats when compared with normal rats. The intraperitoneal injection of alloxan in Wister rats produced hyperglycemial impaired glucose tolerance and insulin resistance. Among the administration of the extracts of various plant parts of *P. minima*, only the flower and leaf extracts and tolbutamide tends to bring the fasting blood glucose level towards the normal in the acute study. There is no significant level of reduction in fasting blood glucose level was noticed for the aqueous extracts of root and stem of *P. minima*.

On chronic administration (Table 1) the effect of *P. minima* flower (133.77 ± 3.16) and leaf (134.33 ± 2.28) causes a fall in fasting blood sugar of rats. The fall is evident even in the 1st week and goes on progressively increasing till at the end of 4 weeks and the fall in the fasting blood sugar was nearly equal to that of reference drug tolbutamide (126.60 ± 2.55). The antidiabetic activity of root and stem is not significant in prolonged study also. These findings clearly established that the antidiabetic efficacy of the flower and leaf extract of *P. minima* are almost equal and both exhibited more potent antidiabetic activity by reducing the blood glucose level significantly than all other root and stem extracts.

The extract may have the properties to stimulate or regenerate the β-cells of islets of langerhans for the secretion of insulin and are most effective for controlling diabetes by various mechanisms which may finally lead to improvement of carbohydrate metabolizing enzymes towards the re-establishment of normal blood glucose level. In our study, it is found that extract have also hypoglycemic effect in glucose induced hyperglycemic rats. The extracts of plant enhanced glucose utilization. So the blood glucose level was significantly reduced in the

Table 1: Effect of *P. minima* on blood glucose level of alloxan-induced diabetic rats after acute treatment.

Group (*n*)	Dose	Initial	1 h	3 h	5 h
Normal control	2 mL saline	97.62 ± 0.29	98.33 ± 0.30	98.74 ± 0.27	98.95 ± 0.48
Diabetic control	2 mL saline	179 ± 2.74*[a]	186 ± 3.10*[a]	189 ± 3.49*[a]	193 ± 2.941*[a]
Diabetic + *P. minima* stem	250 mg/kg b.w.	177 ± 4.22[b]	176 ± 3.41[b]	175.7 ± 4.22[b]	173.7 ± 4.32[b]
Diabetic + *P. minima* leaf	250 mg/kg b.w.	178.3 ± 4.15	171.2 ± 3.69*[b]	166.6 ± 3.22*[b]	154.8 ± 3.81*[b]
Diabetic + *P. minima* root	250 mg/kg b.w.	175.9 ± 3.78[b]	175.2 ± 3.12[b]	174.9 ± 3.15[b]	173.4 ± 3.40[b]
Diabetic + *P. minima* flower	250 mg/kg b.w.	178.5 ± 3.04	171.2 ± 3.57*[b]	169.1 ± 3.40*[b]	151.8 ± 3.76*[b]
Tolbutamide	100 mg/kg b.w.	168.2 ± 3.82	161.5 ± 4.35*[c]	156.0 ± 3.90*[c]	149.31 ± 2.53*[c]

Values are given as mean ± S.D. (*$p < 0.05$ significant vs. control).

n = Number of animals in each group (8 rats per group), values are statistically significant at $p < 0.05$.

Statistical significance was compared within the groups as follows:

[a]Diabetic control rats were compared with normal control rats.

[b]*P. minima* extract treated diabetic rats were compared with diabetic control rats.

[c]Tolbutamide treated diabetic rats were compared with diabetic control rats.

Table 2: Effect of *P. minima* on blood glucose level of alloxan-induced diabetic rats after prolonged treatment.

Group (*n*)	Initial	7th day	14th day	21st day	28th day
Normal control	97.62 ± 0.29	88.56 ± 0.854	85.38 ± 1.27	84.37 ± 1.79	85.03 ± 0.20
Diabetic control	179 ± 2.74	187.6 ± 3.98*a	209.00 ± 2.28*a	231.76 ± 1.91*a	244.63 ± 2.91*a
Diabetic + *P. minima* stem	177 ± 4.22	196.8 ± 4.03	189.09 ± 1.40	184.90 ± 0.83	181 ± 3.40
Diabetic + *P. minima* leaf	178.3 ± 4.15	151.7 ± 5.03*b	134.33 ± 2.28*b	127.31 ± 3.16*b	108.00 ± 1.70*b
Diabetic + *P. minima* root	175.9 ± 3.78b	172.9 ± 4.02b	170.33 ± 3.34b	168.20 ± 2.20b	168.97 ± 3.06b
Diabetic + *P. minima* flower	178.5 ± 3.04	149.8 ± 4.26*b	133.77 ± 3.16*b	126.02 ± 1.92*b	102 ± 3.95*b
Tolbutamide	168.2 ± 3.82*c	132.4 ± 3.82*c	126.60 ± 2.55*c	106.74 ± 2.74*c	99.21 ± 1.98*c

Values are given as mean ± S.D. (*$p < 0.05$ significant vs. control).
n = Number of animals in each group (8 rats per group), values are statistically significant at $p < 0.05$.
Statistical significance was compared within the groups as follows:
[a]Diabetic control rats were compared with normal control rats.
[b]*P. minima* extract treated diabetic rats were compared with diabetic control rats.
[c]Tolbutamide treated diabetic rats were compared with diabetic control rats.

glucose loaded rats (Tables 1 and 2). The present investigation established that the leafs and flowers of the plant *P. minima* have antidiabetic activities. However, further studies were warranted, to observe their effects on diabetic model and to find out the exact mechanism of such action and to isolate bioactive compounds responsible for observed activities.

Conclusion

Alloxan, a β-cytotoxin, causes a massive destruction of β-cells of the islets of langherhans, resulting in reduced synthesis and release of insulin. The function of the insulin system is suppressed, which leads to high level of hyperglycemic and eventually to death, but the different extracts of *P. minima* showed portent antidiabetic effect in alloxan-induced diabetic rats and reduced the mortality rate significantly. The present investigation has also opened avenues for further research, especially with reference to the different dose studies and development of potent formulation for DM from *P. minima*. Activity guided fractionation, formulation, and its evaluation will be a need for the future study.

Ethical Approval

The study was approved by the animal ethics committee of Department of Zoology, Kakatiya University, Warangal, Andhra Pradesh.

Conflict of Interests

None declared.

Authors' Contributions

Both authors contributed equally to this work.

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