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Original Research Article

Pharmacognosy for efficient natural therapy

Screening of Anti-Diabetic Activity of *Physalis Minima Fruits*

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Abstract: The plants are a vast resource of phytoconstituents which could treat different diseases. The methanolic extract of *Physalis minima* fruits was screened for anti-diabetic activity. The literature survey revealed that these studies were not reported earlier. So, the present research study was aimed to reveal the anti-diabetic activity of *Physalis minima* fruits. The results concluded that the extract possess anti-diabetic activity. The study showed good significance in repair of damaged β-cells in diabetic *Physalis minima* fed rats, when compared to diabetic control animals. Ithas been concluded that *Physalis minima* extract showed hypoglycaemic activity on prolonged treatment and is also regenerating β- cells and so it could be used in the treatment of diabetes mellitus.

KeyWords: *Anti-diabetic, Methanolic extract, Physalis minima, hypoglycaemic activity, Diabetes mellitus.*

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1. INTRODUCTION

Diabetes mellitus is a congregation of metabolic disorders signalized by high blood sugar, hyperlipidaemia, and protein metabolism due to lack of insulin secretion and/or insulin action. It is related with diminished life standard and aggravated risk aspects for mortality and morbidity. If it is not treated, high blood sugar from diabetes can affect nerves, eyes, kidneys, and other organs [1].The diabetes mellitus can be classified into different categories as follows:

- Type 1 diabetes (an autoimmune disorder): The immune system strikes and damages cells in the pancreas organ from which insulin is secreting. The reason for such strike is not clear. About 10 percent of people during 2014, worldwidewere suffering from diabetes possess Type 1.[2]
- Type 2 diabetes: This category of diabetes exists due to resistance to insulin or pancreas is unable to produce enough insulin, resulting in higher sugar levels in the blood.
- Pre-diabetes: It is a diabetic condition in which blood sugar is higher than normal, but not yet high enough to be type 2 diabetes.
- Gestational diabetes: It is diabetic condition in which blood sugar level increases during pregnancy. Insulin-blocking hormones secreted by the placenta are the cause for gestational diabetes.[3]

The main goal of diabetes treatment and management is to keep up optimum concentration of blood glucose (normal blood glucose levels are less than 100mg/dL (fasting for at least eight hours) and less than 140mg/dL (two hours after eating)). The therapy of diabetes includes oral hypoglycemic agents and insulin. The four classes of oral hypoglycemic agents have been considerably used *viz.* insulin secretagogues, biguanides, thiazolidinediones and alpha-glucosidase inhibitors. Each category of drugs acts through various mechanisms which induce secretion of insulin curtails hepatic gluconeogenesis, promotes insulin receptor sensitivity and prolonged digestion and absorption of carbohydrates. The usage of oral drugs is restricted by the adverse side effects which include blood, skin and gastrointestinal reactions, hypoglycemic coma and dysfunction of liver and kidney[4]. These are contraindicated in pregnancy. Recently, there is increased attention to plant drugs as they cause lesser side effects as compared to oral hypoglycemic agents The past studies have proved terpenoids, coumarins, flavonoids, and other secondary plant metabolites, including glutamic acid and arginine exhibit anti-diabetic properties[5, 6]. *Physalis minima, also* known as "wild cape gooseberry", belongs to family Solanaceae. It is equatorial perennial herb with cream to yellowish flowers. It bears edible yellowish fruits covered with papery layer that turns straw brown on ripening [7, 8]. The results of the preliminary phytochemical analysis in chloroform, diethyl ether, ethanol, ethyl acetate and methanol extracts of stem, leaves and fruits have shown presence of alkaloids, flavonoids, cardiac glycosides, phenols, saponins, steroids, tannins and terpenes[9]. The past studies reported that the plant possesses diuretic, anti-inflammatory, analgesic, antipyretic, antibacterial, anti-diabetic activities [10-13] however, the fruits of the plant were not included in those studies. The present study was aimed to study the anti-diabetic activity of methanolic extract of fruits of *Physalis minima*.

2. MATERIALS AND METHODS

2.1 Animals

The present work on animals was carried out at St. John College of Pharmacy, Hasanparthy, Warangal, Telangana state, India, as per the guidelines of OECD 423.The protocol of the experiment on animals was approved by Institutional Animal Ethics Committee (IAEC) with Regd. No.067/IEAC/StJCOP/2017, St. John College of Pharmacy, Hasanparthy, Warangal, Telangana state, India.The rats were divided into six groups containing six animals in each group (n=6) and were orally fed with increasing doses (50, 100 mg/kg body weight) of methanolic extract suspended in Gum acacia in water. After administration of extract, the animals were monitored during the first 30 min for their gross behavioural changes, locomotion, convulsions and mortality.

2.2 Collection of Plant Material

The fruits of *Physalis minima* were gathered from the fields of Jammikunta, Karimnagar, Telangana, India. The plant parts were authenticated and deposited at the herbarium of University College of Pharmaceutical Sciences, Satavahana University, Karimnagar, Telangana, India.

2.3 Preparation of the Extract

The fruits of *Physalis minima* (2.0kg) were kept for maceration with methanol for seven days. The extracts were concentrated in desiccators[14].

2.4 Chemicals

All the chemicals used for the investigation were of analytical grade.

2.5 Drugs

Streptozotocin was bought from Merck Millipore, Mumbai, India.

2.6 Induction of Diabetes[15]

The animals (male Wistar rats) were made diabetic by intraperitoneal administration of a freshly made solution of Streptozotocin in citrate buffer saline (pH-4.5) with a dose of 50 mg/kg body weight. The rats became diabetic within a week of Streptozotocin treatment as the observed blood sugar levels were 300-350mg/kg.

2.7 Drug administration

The methanolic extract of fruits of *Physalis minima* was suspended in 0.3% carboxymethyl cellulose (CMC) and given through oral gavage at doses of 200 and 400 mg/kg of body weight per day.

2.8 Method of Evaluation

Six groups of adult male Wistar rats weighing 250-300g animals were taken for the study each containing 6 rats. The grouping of the animals was done as tabulated (Table 1).On the 40th day, the animals were anesthetized with diethyl ether which was fast overnight 12 hrs. Blood was collected by pricking the tail to freshly prepared EDTA tubes. Then the

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tubes were centrifuged at 3,000 rpm for 20 minutes in micro centrifuge at 4°C. The plasma was separated to test the blood glucose level, lipid profile and serum insulin. The liver and muscle pieces were drained and dried on tissue paper, weighed and stored at -80^{0}C for determining glycogen contentBy using the Grover method.

2.9 Biochemical Analysis

For the estimation of blood parameters like blood glucose, serum total cholesterol, liver glycogen, glycosylated haemoglobin and serum insulin biochemical analysis was performed. Blood glucose was determined using glucometer, glycosylated haemoglobin by using the diagnostic kit from Biosystems. Liver glycogen was evaluated using the Grover method. Tissue glycogen was determined in fed rats by method described by Morales *et al*[16]. Serum total cholesterol was estimated by the Cholesterol oxidase/peroxidase method and triglycerides by the phosphate oxidase/ peroxidase method. Serum HDL- cholesterol and LDL-cholesterol were determined by diagnostic kit. Serum insulin was assayed by radioimmunoassay kit.

3. STATISTICAL METHOD

The statistical evaluation was done by ANOVA (One way). Student's t-test and probability level at $p<0.05$ values are selected as significant. The values denoted are mean ± SD using SPSS 14.0 Version.

4. RESULTS AND DISCUSSION

4.1 Determination of fasting blood glucose level

The fasting blood glucose level in untreated animals was within normal range (Table-2) before the start of experiment. 24hrs after the treatment with STZ the blood glucose level significantly increased to the range of 300-350mg/dl. Treatment with extract of *Physalis minima* resulted in significantly reduced blood glucose levels ($P<0.005$). The animals treated with *Physalis minima* started showing decreased fasting blood glucose from 10th day of treatment. On the 40th day of treatment the fasting blood glucose levels were 185.52 ± 20.06 and 112.85±13.68 mg/dl at the 200 and 400mg/kg dose levels of *Physalis minima* respectively. Thus, *Physalis minima* fruit extract at 400mg/kg dose level proved to have significant hypoglycaemic activity in diabetic rats.

4.2 Evaluation of Glycosylated hemoglobin and Body weight

In diabetes, the RBC's are highly liable to oxidative stress and thus show increased levels of glycosylated haemoglobin. The present investigation proved to reduce HbA1c level to control group of animals which were treated with *Physalis minima* extract and even pick up in body weight was noticed. The results are shown in Table 3.

4.3 Evaluation of Glycogen, and insulin level in tissue

Insulin has a vital role in reducing blood glucose level by rise in glycogenesis in liver and muscles. In diabetes, Glycogen amount of skeletal muscles and liver specifically reduces. In diabetic animals, the serum insulin reduces[17].On the 40th day of the experiment, animals treated with *Physalis minima* extract (200 and 400 mg/kg), liver glycogen level (20.79±0.43 and 25.84±1.52) was significantly increased with respect to diabetic control group (12.31±0.63)(Table 4) but this was not restored to the normal group glycogen level (35.20±0.89) (Table3). In the present investigation, we confirm that daily administration of Physalis extract (400mg/kg) resulted to be effective, in satisfactory restore back to near normal capacity of liver to synthesize glycogen [18, 19]. The insulin level was restored to (16.85±2.18^b). When compared to diabetic rats (5.52±1.56^a) the value is almost in comparison with control rats (17.85±1.26). This indicated an overall blood glucose control due to the improvement in insulin secretion to a considerable level in PM extract treated rats.

4.4 Determination of lipid profile level

In about 40% of diabetic patients, the frequent occurrences found are irregularities in lipid profile[20]. It is linked with hypertriglyceridemia and hypercholesterolemia. Inadequate acute insulin at first elevates movement of free fatty acid from adipose tissue, which causes in raised production of cholesterol-LDL particles. Hypertriglyceridemia is a condition which results in hypoinsulinemia, intolerance of glucose and insulin resistance. Notable level of decrease in total cholesterol and increase in HDL cholesterol is a very preferable biochemical state to avoid chances of atherosclerosis and ischemic conditions. *Physalis minima* extract was more significant in reducing the serum lipids. This hypolipidemic effect may be because of a rise in insulin production that eventually led to a reduction in the production of cholesterol and fatty acid. These extracts enhanced the biochemical parameters. On 40th day of experiment, cholesterol, triglycerides in the *Physalis minima* extract given groups were decreased as compared to diabetic induced control groups and normal rats which were untreated There was a notable betterment by treatment of *Physalis minima* extract. In standard PM treated group, HDL was significantly improved than normal & LDL is regained as normal when compared to STZ treated group (Table 5).

Table 1.Grouping of test animals

Group	Animal
I	Normal rats + saline
II	Normal rats +(400mg/kg) PMM
III	Diabetic rats (STZ (50mg/Kg)
IV	Diabetic rat +(200mg/kg) PMM
V	Diabetic rat +(400mg/kg) PMM
VI	Diabetic rat + insulin (3IU/kg)

Table 2. Effect of *Physalis minima* extract on blood glucose levels in untreated and untreated rats

Groups	Blood Glucose level in mg/dl			
	Day 0	Day 10	Day 20	Day 40
I	80.2 ± 5.6	83.3 ± 5.1	79.2 ± 6.3	82.5 ± 6.6
II	82.4 ± 5.9	85.32 ± 4.5	88.6 ± 5.5	89.9 ± 6.3
III	380.25 ± 16.8	394.06 ± 21.5[a]	423.72 ± 15.6[a]	440.56 ± 16.6[a]
IV	360.45 ± 18.2	300.42 ± 15.5[a]	222.51 ± 10.7[a,b]	185.20 ± 11.5[a,b]
V	385.35 ± 16.5	280.22 ± 11.4[a]	200.65 ± 13.5[a,b]	102.25 ±10.8[b]
VI	365.61 ± 12.9	99.25 ± 10.4[b]	84.65 ± 8.6[b]	87.54 ± 10.2[b]

p< 0.05 by comparison with Streptozotocin diabetic rats.
[a] p < 0.05 by comparison with normal rats.
[b] p < 0.05 by comparison with Streptozotocin diabetic rats.

Table 3. Effect of *Physalisminima*extract on body weight and Glycosylated haemoglobin in normal and diabetic rats after 40 days of treatment

Groups	Body weight (g)			
	Before Induction	After induction	After treatment	Glycosylated hemoglobin
Normal rats + saline	210.4 ±6.5	203.3 ± 6.6	209 ± 5.7	0.53 ± 0.04
Normal rats + (400mg/kg)PMM	206.5 ± 3.8	206.0 ± 3.6	216 ± 3.5[b]	0.43 ± 0.07[b]
Diabetic rats(STZ 50mg/Kg)	203.2 ± 4.1	196.0 ± 3.9	147.5 ± 4.2[b]	0.89 ± 0.05[a]
Diabetic rat + (200mg/kg)PMM	200.1 ± 4.9	192.1 ± 4.8	154.5 ± 5.1[b]	0.50 ± 0.08[b]
Diabetic rat +(400mg/kg)PMM	195.0 ± 3.5	191.1 ± 3.3	201.5 ± 6.0[b]	0.45 ± 0.07[b]
Diabetic rat + insulin (3IU/kg)	198.0 ± 4.9	194.3 ± 5.4	207.3 ± 8.1	0.43 ± 0.06[b]

p< 0.05 by comparison with streptozotocin diabetic rats

Table 4. Effect of *Physalis minima* extract on Muscle glycogen liver glycogen and plasma insulin in normal and diabetic rats after 40 days of treatment

Groups	Muscle glycogen (mg/g tissue)	Liver glycogen (mg/g tissue)	Plasma insulin (µU/ml)
I	8.11 ± 0.97	43. 52 ± 4.6	16.52 ± 1.44
II	8.3 ± 0. 89[b]	45.22 ± 4.4[b]	14.90 ± 1.63[b]
III	1.84 ± 0.54[a]	16.40 ± 4.1[a]	4.23 ± 1.74[a]
IV	7.43 ± 0.87[b]	40.25 ± 4.24[b]	11.66 ± 1.81[b]
V	8.54 ± 0.52[b]	44.23 ± 4.64[b]	15.87 ± 2.07[b]
VI	8.62 ± 0.58[b]	46.68 ± 4.35[b]	4.29 ± 2.3[a]

[a]p< 0.05 by comparison with normal rats.
[b]p< 0.05 by comparison with streptozotocin diabetic rats.

Table 5. Effect of *Physalis minima* extract on Total cholesterol, Triglyceride Serum HDL and Serum LDL in normal and diabetic rats after 40 days of treatment

Groups	Total cholesterol (mg/dL)	Triglyceride (mg/dL)	HDL (mg/dL)	LDL (mg/dL)
I	11 ± 4.18	16.17 ± 1.81	54.98 ± 4.1	80.12 ± 5.25
II	95.13 ± 3.78[b]	14.45 ± 1.26[b]	52.72 ± 4.36[b]	79.26 ± 3.14 [b]
III	241.96 ± 4.52[a]	38.39 ± 2.31[a]	33.67 ± 3.85[a]	146.4 ± 3.8[a]
IV	161.25 ± 6.71[b]	26.32 ± 1.45[b]	46.32 ± 3.8[b]	82.66 ± 4.26[b]
V	112.25 ± 7.65[b]	16.52 ± 1.15[b]	64.78 ± 3.96[b]	87.88 ± 4.32[b]
VI	98.42 ± 5.54[b]	15.56 ± 0.86[b]	55.61 ± 6.15[b]	88.42 ± 1.2[b]

[a]p< 0.05 by comparison with normal rats.
[b]p< 0.05 by comparison with streptozotocin diabetic rats.

5. CONCLUSION

These results indicate that *Physalis minima* fruit extract possesses significant hypoglycemic and hypolipidemic activity in long-term therapy and also have the ability to regenerate β-cells. So, it could be used as a drug in the treatment of Diabetes mellitus.As it has regenerating capability, the drug might be used to treat the patients with pre-diabetes or patients at early stages of diabetes.To minimise the adverse effects of synthetic drugs, i would like to contribute my work with herbal drugs to cure diabetes.

6. AUTHORS CONTRIBUTION STATEMENT

Mrs. K. Sunitha and Mr. A. Srinivas Nayak have gathered the data, collected the plant and was authenticated by Dr. E. Narasimha Murthy, Taxonomist. We altogether discussed and worked to evaluate the activity of drug and designed the manuscript. Altogether contributed to the final manuscript.

7. CONFLICT OF INTEREST

Conflict of interest declared none.

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