RESEARCH ARTICLE

Anti-hyperglycemic Activity of the *Piper longum* Dried Fruit Extract on the Experimental Animal Model

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ABSTRACT

Predominately, in the Indian tradition, the species *Piper longum* could play a significant role in the management of several severe disorders such as asthma, tuberculosis, rheumatism, and epilepsy. Mostly, the plant root would be responsible for treating insomnia, rheumatism, and epilepsy, as well as plant leaves for a prevailing stimulant for both the digestive and the respiratory systems. The alcoholic extract of *P. longum* fruits showed antiamoebic, anti-inflammatory, and immunomodulatory potential in experimental animals. In addition to these benefits, the current study concluded that the examination of the ethanolic extract of *P. longum* fruits as an anti-hyperglycemic in streptozotocin (STZ)-induced diabetic animal model (Wistar rats). A diabetic condition could be induced in an animal model by dissolving STZ in a 0.1 M citrate buffer of pH 4.5. It was injected intraperitoneally at a dose of 50 mg/kg body weight in the animal test model. All the test animals were fed with a 5% dextrose solution to control overnight hypoglycemic conditions. Subsequently, 48 hours of hyperglycemic test animals were selected for the study. The observed anti-hyperglycemic effect of ethanolic extract of *P. longum* dried fruits should correspond to that of the reference standard, i.e., glibenclamide. The study reveals that the ethanolic extract of *P. longum* fruits has a promising potential as an anti-hyperglycemic in the STZ-induced diabetic animal test model. So, it is presumed that the ethanolic extract of *P. longum* could be a promising alternative for severe diabetes complications such as hyperglycemia, oxidative stress, etc., in the near future.

Keywords: Anti-hyperglycemic, Dried fruit, *Piper longum*, Streptozotocin.

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INTRODUCTION

Diabetes mellitus (DM) is a long-lasting metabolic condition characterized by high levels of blood glucose due to the impeded insulin release from the pancreas.[1,2] It is well known to harm most of body organs, predominantly eyes, kidneys, blood vessels, heart, and nerves.[3] This elevated blood sugar produces the classical indications of polydipsia, polyuria, and polyphagia.[4] The occurrence of DM has a high risk of major clinical conditions such as heart diseases, stroke, peripheral vascular diseases, retinopathy, neuropathy, renal failure, blindness, amputations, etc.[5] DM marks around 4% globally and would be projected to rise by 5.4% in 2025.[6] Hyperglycemia and hyperlipidemia are two vital characteristics of DM.[7] Type-1 DM is a chronic autoimmune disorder characterized by increased blood glucose level (hyperglycemia), which is due to the insulin deficiency that occurs as the significance of the loss of the pancreatic islet β-cells.[8-11] This category could be classified as idiopathic or immune-mediated. Type-2 diabetes is characterized by insulin resistance, which may be combined with relatively low insulin secretion.[12] On the other hand, gestational diabetes be like type-2 diabetes in some aspects, involving a combination of comparatively insufficient insulin. It occurs in about 2 to 10% of all pregnant women and may improve after the delivery.[13]

In the current investigations, *Piper longum* dried fruits extract has proven promising anti-hyperglycemic in the test animal model. It would be proposed promising alternative for other conventional anti-hyperglycemic medicines in a near future. The pipali or *P. longum* which was mostly used for domestic purposes as a spice and constituent of medicines. Several studies have disclosed that piperine, a chemical constituent isolated from *P. longum,* also act as a central nervous system (CNS) depressant, antipyretic, analgesic, antidiabetic, anti-inflammatory, and hepatoprotective.[14] In addition, piperine could improve the bioavailability of several drugs, such as sulfadiazine, tetracycline, rifampicin,

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streptomycin, pyrazinamide, ethambutol, and phenytoin.[15] The current study has investigated the anti-hyperglycemic activity of the fruits of *P. longum* in STZ-induced diabetic rats.

MATERIALS AND METHODS

Plant Collection and Authentication

The dried fruits of *P. longum* were procured from the grocery shop, in India. The extract of *P. longum* was authenticated by experts in the field.

Experimental Animals

All experiments were conducted as per the protocol of control and management of experimental animals (CPCSEA) guidelines and subsequently following the Institutional Animals Ethical Committee (IAEC) approval. All the test animals (e.g., Wistar rats) weighing 150 to 250 g are obtained from the animal house. All the animals are acclimatized to the animal house before use. They are kept in cages in an animal house with a 12 hours light and dark cycle at a temperature of (25 ± 1°C) with 50 ± 55% of relative humidity (RH). All animals are fed on the pellets and tap water *ad libitum*. All the precautions were taken as per the guidelines of CPCSEA.

Chemicals Required

Streptozotocin (STZ) (50 mg/kg), glibenclamide (20 mg/kg)

Preparation of Extract

Ethanolic extract was prepared by consecutive solvent extraction in a Soxhlet apparatus at a temperature 68 to 70°C. The filtrates were distilled and concentrated under reduced pressure at low temperatures (40–45°C) in Buchi Rotavapor R-200. Furthermore, kept for freeze-drying.[16]

Tnduction of Diabetes in Experimental Animals

A standard drug STZ was dissolved in a 0.1 M citrate buffer of pH 4.5 and injected intraperitoneally at a dose of 50 mg/kg. All the test animals were fed with a 5% dextrose solution to control overnight hypoglycemia. After 48 hours, injected animals with noticeable hyperglycemic condition were selected for the proposed study.

Table 1: Treatment protocol

S No.	*Groups*	*Test animals*	*Treatment and dose*	*Route of administration*
1	I	6	Vehicle - Citrate buffer pH 4.5	Oral
2	II	6	STZ-induced untreated (50 mg/kg of body weight)	Intraperitoneal
3	III	6	Glibenclamide (5 mg/kg of body weight)	Oral
4	IV	6	Test extract of *P. longum* fruit (100 mg/kg of body weight)	Oral
5	V	6	Test extract of *P. longum* fruit (200 mg/kg of body weight)	Oral

Experimental Design

The test animals were alienated into five sets as shown in Table 1. Glibenclamide was used as the reference standard. Animals defined as fasted were deprived of food for at least 12 hours but allowed free access for water. Fasting blood glucose measurement was performed on the 1st, 7th, 14th, and 21st days of the study. A calibrated glucometer monitored an elevated blood glucose levels.

The data were calculated as mean ± SEM and the statistical investigation of data was determined with student t-test and one-way analysis (ANOVA) followed by Duncan's multiple range test (DMRT).

RESULTS

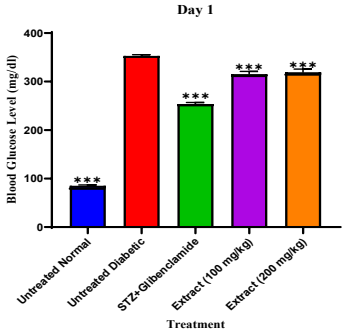
In STZ-treated diabetic rats, blood glucose was elevated to a high level during the study, where body weight decreased. Chronic treatment with ethanolic extract of *P. longum* at 100 and 200 mg/kg significantly ($p < 0.001$) causes a decrease in blood glucose on the 1st, 7th, 14th, and 21st days of dosing are as shown in Table 2.

All values were expressed as a mean ± SEM (n = 6). Statistical comparisons were determined by one-way ANOVA followed by DMRT.

(DMRT) ***$p < 0.001$ when compared with the untreated diabetic rat.

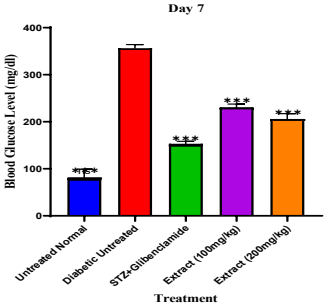
DISCUSSION

In the present study, STZ is used to induce hyperglycemia in test animals. At low doses, STZ (50 mg/kg) partially destroys



***$p < 0.001$ when compared with the untreated diabetic animals.

Figure 1: Effect of *P. longum* on blood glucose level on the 1st day



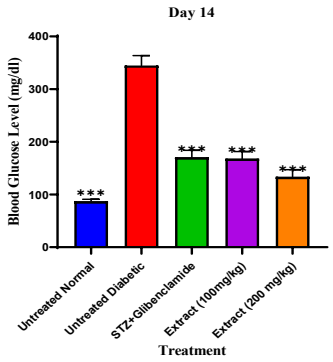
(DMRT) ***$p < 0.001$ when compared with the untreated diabetic rat.

Figure 2: Effect of *P. longum* on blood glucose level on the 7th day

Table 2: Average blood glucose profile

Groups/Treatment	*Average serum glucose mg/dL*			
	Day 1	*Day 7*	*Day 14*	*Day 21*
Untreated Normal	85.17 ± 0.7***	80.67 ± 7.5***	87.17 ± 1.5***	87.67 ± 1.8***
Untreated Diabetic	358.2 ± 0.94	356.3 ± 3.1	344.8 ± 7.6	340.3 ± 8.7
Diabetic + Glibenclamide (2 mg/kg)	253.7 ± 1.2***	153.2 ± 2.1***	170.7 ± 5.3***	99.50 ± 1.4***
Diabetic + Extract (100 mg/kg)	315.3 ± 2.2***	205.8 ± 4.6***	168.0 ± 5.4***	128.5 ± 2.0***
Diabetic + Extract (200 mg/kg)	319.2 ± 2.6***	230.0 ± 3.1***	134.0 ± 5.3***	106.7 ± 2.8***

Values are expressed as mean ± SEM: (n = 6)
*$p < 0.05$, ***$p < 0.001$compared with untreated diabetic rats



(DMRT) ***$p < 0.001$ when compared with the untreated diabetic rat.

Figure 3: Effect of *P. longum* on blood glucose level on the 14th day

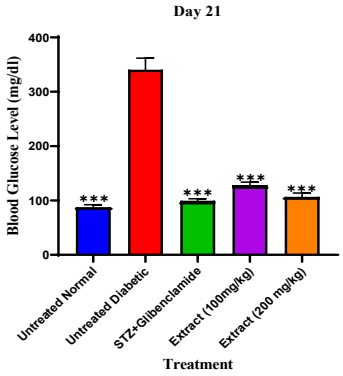


Figure 4: Effect of *P. longum* on blood glucose level on the 21st day

the beta cells, resulting in insufficient insulin secretion. It is a widely recognized animal model and is described to resemble human hyperglycemic non-ketotic DM, which is related with kidney hypertrophy, which may lead to end-stage renal damage, oxidative stress, hepatotoxicity, and hypercholesterolemia. In this study, ethanolic fruit extract of *P. longum* has selected for evaluating anti-hyperglycemic activity in STZ-induced diabetic rat. It was noticed that low dose of STZ (50 mg/kg) destroys only part of beta cells, which secrete an inadequate amount of insulin. Here, continuous treatment with two different doses of ethanolic fruit extract of *P. longum* (100 and 200 mg/kg) for 21 days was given after the induction of DM. Blood glucose determination was done by calibrated glucometer on the 1st (Figure 1), 7th (Figure 2), 14th (Figure 3), and 21st (Figure 4) days. Glucose level decreases gradually during the continuous treatment of the 21 days. After the induction of diabetes, glucose levels of rats were above 400 mg/dL, which get reduced to about 315.3 ± 2.2 and 319.2 ± 2.6 mg/dL after administration of extract at dose 100 and 200 mg/kg, respectively on the 1st day. On the 7th day, glucose level was reduced up to 205.8 ± 4.6, and 230.0 ± 3.1 mg/dL at dose of 100 and 200 mg/kg, respectively. On the 14th day, glucose level decreased to 168.0 ± 5.4 and 134.0 ± 5.3 mg/dL which fell up to normal level on the 21st day, i.e., 128.5 ± 2.0 and 106.7 ± 2.8 mg/dL with a dose of extract at 100 and 200 mg/kg, respectively. The findings were correlated with the previous research findings, in that the blood glucose levels significantly increased in STZ-induced diabetic rats. In the present study, the continuous treatment of test extract of *P. longum* for a period of three weeks caused a significant decrease in blood glucose levels in treated diabetic rats.

CONCLUSION

The current study reveals that the ethanolic extract of *P. longum* dried fruit would be proven a promising anti-hyperglycemic agent. Here, *P. longum* fruit has significantly reduced the TC, TG, LDL-C, and VLDL-C levels with an increase of HDL-C level in treated diabetic animals compared to their counterpart untreated diabetic animals. This could be possible due to the insulin secretagogue activity of the extract.

Ethanolic extract of *P. longum* fruit-treated diabetic rats showed a fall in the atherogenic index and an increase in the proportion of protection against atherogenicity. Subsequently, a decrease in the atherogenic index has due to an increase in HDL-C levels. The existence of a negative correlation between HDL-C and atherosclerosis resulted in an improvement in the protection against atherogenicity in STZ-induced diabetic rats.

In conclusion, it is noted that the *P. longum* fruits show an anti-hyperglycemic effect on diabetes-induced animal models. Therefore, this preliminary study suggested that the ethanolic extract of *P. longum* dried fruits could be a promising alternative for severe diabetes complications such as hyperglycemia, oxidative stress, etc., in the near future. The other comparative studies are currently under evaluation in our laboratory and will be reported at a suitable time.

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ETHICAL COMMITTEE APPROVAL

The IAEC meet held at Vidyabharati College of Pharmacy, Amravati on dated 1 Feb 2022 has been approved 24 Wistar rats for the current study.

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