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PHYTOCHEMICAL SCREENING AND *IN VIVO* ANTIDIABETIC ACTIVITY OF HYDROALCOHOLIC EXTRACT LEAVES OF *LILIUM CANDIDUM*

Abhay Rawat, Arpit Shrivastava, Prateek Kumar Jain, Harshita Jain*
Adina Institute of Pharmaceutical Science, Sagar (M.P.)

***Correspondence Info:**
Harshita Jain
Adina Institute of Pharmaceutical Science, Sagar (M.P.)
Email:
harshi.jain1987@gmail.com

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ABSTRACT

The present study aimed to evaluate the phytochemical composition and antidiabetic potential of the hydroalcoholic leaf extract of *Lilium candidum* in streptozotocin-induced diabetic rats. Preliminary phytochemical screening confirmed the presence of bioactive compounds including alkaloids, phenols, saponins, and proteins. Quantitative estimations revealed substantial levels of total alkaloids and phenolic content. *In vivo* experiments demonstrated that oral administration of the extract at doses of 100 and 200 mg/kg for 30 days significantly reduced blood glucose levels, improved serum insulin, and decreased glycosylated hemoglobin levels in diabetic rats. Additionally, the extract positively modulated lipid profiles by reducing total cholesterol and triglyceride levels. The results were comparable to those observed with the standard drug glibenclamide. These findings support the traditional use of *Lilium candidum* as an antidiabetic agent and encourage further studies for its development as a natural therapeutic option for diabetes management.

Keywords: *Lilium candidum*, hydroalcoholic extract, phytochemical screening, antidiabetic activity, blood glucose, streptozotocin, lipid profile, insulin, glycosylated hemoglobin.

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia resulting from defects in insulin secretion, insulin action, or both. It is one of the leading causes of morbidity and mortality worldwide, affecting an estimated 537 million adults in 2021, with projections reaching 643 million by 2030 (International Diabetes Federation, 2021). Chronic hyperglycemia in diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels (American Diabetes Association, 2022). Current pharmacological treatments for diabetes, including insulin and oral hypoglycemic agents, although effective, are often associated with adverse effects, limited availability in rural areas, and poor patient compliance (Patel *et al.*, 2012; Kumar *et al.*, 2013).

In this context, the search for safer and more effective antidiabetic agents from natural sources has gained momentum. Medicinal plants have been extensively explored for their therapeutic benefits in the management of diabetes owing to their minimal side effects, affordability, and the presence of diverse bioactive compounds such as alkaloids, flavonoids, saponins, glycosides, and tannins known for their antihyperglycemic activity (Grover *et al.*, 2002; Modak *et al.*, 2007). Among these, *Lilium candidum* (family: Liliaceae), commonly known as Madonna lily, is a

traditional medicinal plant reputed for its wide range of pharmacological activities including anti-inflammatory, antimicrobial, and antioxidant effects (Nawrot *et al.,* 2021).

Lilium candidum has been traditionally used in herbal medicine for treating respiratory infections, skin diseases, and inflammatory conditions. Recent phytochemical investigations have revealed the presence of bioactive constituents such as alkaloids, saponins, phenolics, and proteins in various parts of the plant, suggesting potential therapeutic benefits (Bulgakov *et al.,* 2020). However, limited scientific data is available on its antidiabetic efficacy, particularly concerning the hydroalcoholic extract of its leaves.

The present study was therefore undertaken to investigate the phytochemical constituents and evaluate the in vivo antidiabetic activity of hydroalcoholic leaf extract of *Lilium candidum* in streptozotocin-induced diabetic rats. The study aims to provide scientific validation to the traditional use of the plant and explore its potential as a natural antidiabetic agent.

MATERIALS AND METHODS

Extraction procedure

Following procedure was adopted for the preparation of extract from the shade dried and powdered herbs:

Defatting of plant materials

Leaves of *Lilium candidum* were shade dried at room temperature. 50 gram of dried leaves of *Lilium candidum* was coarsely powdered and subjected to extraction with petroleum ether by maceration. The extraction was continued till the defatting of the material had taken place.

Extraction by maceration process

Defatted leaves of *Lilium candidum* were extracted with hydroalcoholic solvent (ethanol: water: 70:30) using maceration process (24hrs). The extract was evaporated above their boiling points. Finally the percentage yields were calculated of the dried extract (Harborne, 1998).

Determination of percentage yield

The percentage yields of extract were calculated by using following formula:

$$\text{Percentage yield} = \frac{\text{Weight of Extract}}{\text{Weight of powder drug Taken}} x100$$

Qualitative evaluation

Phytochemical tests are conducted to identify and determine the quantity of specific phytochemical compounds present in a plant extract or plant material. These tests employ various chemical, chromatographic, and spectroscopic techniques to isolate, separate, and characterize the phytochemicals. The choice of tests depends on the nature of the phytochemical of interest and the available resources. Phytochemical tests were done as per the standard method (Kokate; 1997).

Quantitative studies of bioactive constituents

Estimation of total alkaloids content

The plant extracts (1mg) was dissolved in methanol, added 1ml of 2 N HCl and filtered. This solution was transferred to a separating funnel, 5 ml of bromocresol green solution and 5 ml of phosphate buffer were added. The mixture was shaken with 1, 2, 3 and 4 ml chloroform by vigorous shaking and collected in a 10-ml volumetric flask and diluted to the volume with chloroform. A set of reference standard solutions of atropine (40, 60, 80, 100 and 120 µg/ml) were prepared in the same

manner as described earlier. The absorbance for test and standard solutions were determined against the reagent blank at 470 nm with an UV/Visible spectrophotometer. The total alkaloid content was expressed as mg of AE/100mg of extract (Hossain *et al.*, 2011).

Estimation of total phenol content

The total phenol content of the extract was determined by the modified folin-ciocalteu method (Hossain *et al.*, 2011). 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 10- 50µg/ml was prepared in methanol. 10 mg of dried extract was dissolved in 10 ml methanol and filter. Two ml (1mg/ml) of this extract was for the estimation of phenol. 2 ml of extract and each standard was mixed with 1 ml of folin-ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 10min for colour development. The absorbance was measured at 765 nm using a spectrophotometer.

In vivo* antidiabetic activity of hydroalcoholic of *Lilium candidum

Animals

Healthy adult male albino Wistar rats (150-200 g) were used for the study. Rats were housed in polypropylene cages lined with husk in standard environmental conditions. (temperature 25±2° C; relative humidity 55±10%; and 12:12 light:dark cycle,) The rats were fed on a standard pellet diet *ad libitum* and had free access to water. The experiments were performed after approval of the protocol by the Institutional Animal Ethical Committee (IAEC) and were carried out in accordance with the current guidelines for the care of laboratory animals.

Acute toxicity

An acute toxicity study was carried out for the hydroalcoholic extract of *Lilium candidum*, OECD guidelines no 420. Male albino mice were weighed (25-30 g, 10 weeks old) and grouped into A, B, C, D, E, and F. Group A animals served as control and received distilled water, while groups B, C, D, E and F were orally administered upto 2000 mg/kg, respectively. The animals were observed at 2, 6, 24 and 48 hr after extract administration to detect changes in autonomic or behavioral responses. Mortality was observed for 24 hrs (Khandelwal, 2008).

Induction of Experimental Diabetes in Rats

Induction of diabetes: Diabetes was induced in rats by a single intraperitoneal (i.p.) injection of streptozotocin (STZ, Sigma Chemical Co. USA) at a dose of 60 *mg/kg* b.w. freshly dissolved in 0.1 M cold citrate buffer of pH 4.5; 48 *hr* later, blood samples were collected, and blood glucose levels were determined to confirm the development of diabetes. Those animals which showed hyperglycemia (blood glucose levels >240 *mg/dl*) were used in the experiment (Pandhare *et al.*, 2011).

Experimental Protocol

Animals were divided into five groups of 6 rats each.

The rats were divided into five groups of 6 animals (n = 6) each as below:

Group I- Normal control (received distilled water 10 ml/kg b.w., p.o.)

Group II- Diabetic control untreated (received distilled water 10 ml/kg b.w., p.o.)

Group III- Diabetic treated with standard drug glibenclamide (0.25 mg/kg/day, p.o.)

Group IV- Diabetic treated with hydroalcoholic extract of *Lilium candidum* (100 mg/kg/day, p.o.)

Group V-Diabetic treated with hydroalcoholic extract of *Lilium candidum* (200 mg/kg/day, p.o.)

For 30 days, blood glucose levels were measured on the study's 1st, 15th and 30th day. Finally, on day 30, blood was collected to estimate various parameters.

Estimation of lipid profile

The TG and TC levels were estimated using standard kits obtained from Span Diagnostics, India (Szkudelski, 2001).

Estimation of serum insulin

Serum insulin concentration was determined by radioimmunoassay kit done spectrophotometrically using standard kits (Lenzen, 2008).

Estimation of glycated hemoglobin

After 30 days experimental period, blood was withdrawn by retro orbital puncture under light ether anesthesia and the glycated hemoglobin was estimated.

Statistical analysis

The results were expressed as mean± S.E.M. Statistical difference was tested using one-way variance analysis (ANOVA) followed by Tukey's Post hoc tests. A difference in the mean p value <0.05 was considered as statistically significant.

RESULTS AND DISCUSSION

The present investigation was conducted to evaluate the antidiabetic potential of hydroalcoholic extract of *Lilium candidum* leaves in streptozotocin-induced diabetic rats. The phytochemical analysis (Table 1 & 2) revealed that the extract contains several bioactive compounds, including alkaloids, saponins, carbohydrates, phenols, and proteins, which are known for their therapeutic properties in metabolic disorders. The percentage yield of the extract was found to be 10.5%, with a solid, dark green, and characteristic odor. Quantitative estimation (Table 3) showed the presence of total alkaloids (0.869 mg/100 mg) and total phenols (0.756 mg/100 mg), confirming the presence of compounds with potential antioxidant and antidiabetic activity.

In the *in vivo* studies, significant changes were observed in blood glucose levels over the 30-day treatment period (Table 4). Diabetic control rats showed a progressive rise in glucose levels (395.41 ± 4.75 mg/dL on day 30), indicating successful induction of diabetes. Treatment with the standard drug glibenclamide significantly reduced glucose levels to 124.45 ± 1.85 mg/dL ($p<0.001$). The groups treated with *Lilium candidum* extract at doses of 100 and 200 mg/kg also showed substantial reductions in glucose levels to 145.65 ± 1.45 and 135.60 ± 1.65 mg/dL, respectively, with statistical significance ($p<0.01$ and $p<0.001$), demonstrating the extract's potent hypoglycemic effect.

Biochemical parameters (Table 5) further validated the antidiabetic effect. Diabetic rats showed elevated levels of total cholesterol and triglycerides (95.65 ± 0.30 and 115.25 ± 1.50 mg/dL, respectively), which are typical markers of diabetic dyslipidemia. Treatment with the hydroalcoholic extract resulted in a significant reduction in lipid levels, comparable to glibenclamide, indicating improved lipid metabolism likely due to the phytoconstituents present in the extract.

Moreover, serum insulin and glycosylated hemoglobin (HbA1c) levels (Table 6) were significantly altered in diabetic rats. Diabetic

control animals exhibited a sharp decline in insulin levels (7.00 ± 0.15 µU/mL) and a marked increase in HbA1c (0.65 ± 0.010 mg/g Hb), reflecting impaired glucose regulation. Treatment with *Lilium candidum* extract significantly improved insulin secretion (14.8–15.6 µU/mL) and reduced HbA1c levels (0.26–0.35 mg/g Hb), indicating enhanced glycemic control and beta-cell preservation.

These results suggest that the hydroalcoholic extract of *Lilium candidum* exhibits significant antidiabetic activity, possibly due to the synergistic effects of its bioactive constituents such as alkaloids and phenolic compounds. The improvement in glycemic, lipid, and insulin profiles supports its potential role as a complementary therapy in diabetes management. Further studies on the mechanism of action and identification of specific active components are warranted to substantiate these findings.

Table 1: Physical characteristics of extract

Extract	Consistency	Colour	Odour	% Yield
Hydroalcoholic	Solid	Dark green	Characteristic	10.5

Table 2: Qualitative chemical tests of extract of *Lilium candidum*

S. No.	Bioactive constituents	Test	Hydroalcoholic extract
1	Alkaloids	Hager's Test	+ve
2	Carbohydrates	Fehling's Test	+ve
3	Glycosides	Legal's Test	-ve
4	Saponins	Froth Test	+ve
5	Phenols	Ferric Chloride Test	+ve
6	Flavonoids	Lead acetate Test	-ve
7	Proteins	Xanthoproteic Test	+ve
8	Diterpenes	Copper acetate Test	-ve

+ ve = Present, - ve =Absent

Table 3: Estimation of total alkaloids and phenol content in *Lilium candidum*

S. No.	Total alkaloids content	Total phenol content
	mg/100mg	
1.	0.869	0.756

Table 4: Effect of hydroalcoholic extract of *L. candidum* treatment on blood glucose in rats

Group	Treatment	Blood glucose (mg/dL)		
		Day 1	Day 15	Days 30
I	Normal	94.00±4.12	95.00±3.45	93.00±3.45
II	Diabetic Control	285.00±2.25	296.58±1.25	395.41±4.75
III	Standard drug glibenclamide (0.25 mg/kg/day, p.o.)	258.65±1.85	248.74±3.15***	124.45±1.85 ***
IV	Diabetic + extract of *Lilium candidum* (100 mg/kg/day, p.o.)	267.74±0.85	275.65±1.10 **	145.65±1.45**
V	Diabetic + extract of *Lilium candidum* (200 mg/kg/day, p.o.)	265.50±1.58	258.74±1.20 ***	135.60±1.65 ***

Table 5: Effect of hydroalcoholic extract of *L. candidum* treatment on biochemical parameters in rats

Group	Treatment	Total Cholesterol (TC) (mg/dL)	Triglycerides (TG) (mg/dL)
I	Normal	71.25±0.90	72.85±0.20
II	Diabetic Control	95.65±0.30 #	115.25±1.50 #
III	Standard drug glibenclamide (0.25 mg/kg/day p.o.)	76.12±0.35 ***	82.45±0.65 ***
IV	Diabetic + extract of *Lilium candidum* (100 mg/kg/day p.o.)	85.45±0.45 **	83.12±1.25 *
V	Diabetic + extract of *Lilium candidum* (200 mg/kg/day p.o.)	78.95±0.25 **	81.74±0.85 **

Table 6: Effect of hydroalcoholic extract of *L. candidum* treatment on serum parameters in rats

Group	Treatment	Serum Insulin (µU/mL)	Glycosylated Hemoglobin (mg/g Hb)
I	Normal	19.5±0.50	0.20±0.008
II	Diabetic Control	7.00±0.15#	0.65±0.010#
III	Standard drug glibenclamide (0.25 mg/kg/day p.o.)	16.5±0.40***	0.25±0.008***
IV	Diabetic + extract of *Lilium candidum* (100 mg/kg/day p.o.)	14.8±0.40**	0.26±0.007*
V	Diabetic + extract of *Lilium candidum* (200 mg/kg/day p.o.)	15.6±0.40**	0.35±0.005**

Values are expressed as mean±S.E.M (n = 6). Values are statistically significant at $^{\#}$ p<0.001 vs. normal group; *P < 0.001, **P < 0.01vs. diabetic control group (One-way ANOVA followed by Tukey's post hoc test).

CONCLUSION

The present study successfully demonstrated that the hydroalcoholic extract of *Lilium candidum* leaves possesses significant antidiabetic potential in streptozotocin-induced diabetic rats. Phytochemical screening confirmed the presence of therapeutically active constituents such as alkaloids, phenols, saponins, and proteins. The extract exhibited a notable hypoglycemic effect, improved lipid profiles, enhanced insulin levels, and reduced glycosylated hemoglobin, with results comparable to the standard antidiabetic drug glibenclamide. These findings scientifically validate the traditional use of *Lilium candidum* in managing diabetes and support its further exploration as a natural, plant-based therapeutic agent for diabetes mellitus.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

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