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Research Paper

Evaluation Of Antidiabetic and Antihyperlipidemic Activity of Catharanthus Pusillus on Alloxan-Induced Diabetic Albino Wistar Rats

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ABSTRACT

Background: Diabetes mellitus and secondary hyperlipidemia present severe global clinical challenges. This study evaluated the antidiabetic, antihyperlipidemic, and pancreatic enzyme-modulating activity of the 70% ethanolic leaf extract of *Catharanthus pusillus* in alloxan-induced diabetic Albino Wistar rats. **Methods:** Diabetes was induced using alloxan monohydrate (150" mg/kg" , i.p.). Experimental groups (n=6) received vehicle, standard treatment (Metformin 50" mg/kg" + Atorvastatin 10" mg/kg"), or *C. pusillus* extract (250" mg/kg" and 500" mg/kg" p.o.) for 28 days. Fasting blood glucose (FBG), serum lipid profiles (TG, TC, LDL-C, HDL-C), and serum α -amylase activity were systematically quantified. **Results:** Extraction yield was 17.38% w/w" . Phytochemical analysis confirmed the presence of alkaloids, carbohydrates, proteins, flavonoids, cardiac glycosides, phenolics, and steroids. Treatment with *C. pusillus* (500" mg/kg") significantly reduced blood glucose from 397.33 $\pm$ 6.83" mg/dL" (Day 0) to 77.83 $\pm$ 6.49" mg/dL" (Day 28; P<0.001), normalized TG (142.5 $\pm$ 1.61" mg/dL"), TC (203.0 $\pm$ 6.04" mg/dL"), and LDL-C (133.3 $\pm$ 5.55" mg/dL"), while significantly increasing HDL-C (41.17 $\pm$ 1.53" mg/dL" ; P<0.01). Serum α -amylase levels were restored to 357.2 $\pm$ 6.16" U/L" (P<0.001). **Conclusion:** *Catharanthus pusillus* possesses potent antidiabetic and antihyperlipidemic efficacy, supporting its therapeutic potential in metabolic disorder management.

INTRODUCTION

Diabetes mellitus is a progressive metabolic disorder characterized by chronic hyperglycemia resulting from impaired insulin secretion, defective insulin action, or both. Extended hyperglycemia accelerates macrovascular and

microvascular complications, frequently accompanied by secondary hyperlipidemia—a major driver of cardiovascular morbidity.

While conventional oral hypoglycemic agents (e.g., sulfonylureas, biguanides) and lipid-lowering drugs (e.g., statins) are widely used, their administration is often limited by adverse

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reactions, secondary failure, and cost constraints. Natural remedies offer a valuable source of alternative therapeutics. *Catharanthus pusillus* (Family: Apocynaceae) is a recognized medicinal plant with established antimicrobial and antioxidant properties, but its combined antidiabetic and antihyperlipidemic effects require formal preclinical evaluation.

2. MATERIALS AND METHODS

2.1 Plant Collection, Authentication, and Extraction

Fresh leaves of *Catharanthus pusillus* were collected from the region surrounding Jaipur, Rajasthan, India, and authenticated by a botanist. The leaves were shade-dried and coarsely pulverized. Powdered material (250 g) was extracted with 70% ethanol in a Soxhlet apparatus at 60--70°C for 35 hours. The filtrate was concentrated under reduced pressure at 40°C to yield a dark green crystalline residue.

Percentage Yield (%)

$$= \left(\frac{\text{Weight of Dried Extract}}{\text{Weight of Raw Powdered Material}} \right) \times 100$$

$$\begin{aligned} \text{Percentage Yield} &= \left(\frac{43.45 \text{ g}}{250 \text{ g}} \right) \times 100 \\ &= 17.38\% \text{ w/w} \end{aligned}$$

2.2 Qualitative Phytochemical Screening

Standard qualitative biochemical assays were conducted to screen for primary and secondary plant metabolites.

2.3 Experimental Animals and Ethical Maintenance

Healthy Albino Wistar rats of either sex (200 ± 50 g) were housed under standard environmental conditions ($25 \pm 2^\circ\text{C}$, relative humidity 50–60%, 12-hour light/dark cycle) with free access to

standard pellet diet and water *ad libitum*, adhering to CPCSEA guidelines.

2.4 Induction of Diabetes and Secondary Hyperlipidemia

After an overnight fast, diabetes was induced via a single intraperitoneal injection of freshly prepared alloxan monohydrate (150 mg/kg body weight in 0.9% normal saline). Animals were monitored for 24–48 hours for stable hyperglycemia. Rats exhibiting fasting blood glucose (FBG) levels > 200 mg/dL were included in the study. To establish hyperlipidemia, diabetic animals were concurrently maintained on a high-fat diet (HFD).

2.5 Experimental Animal Grouping

Animals were randomly assigned to five groups ($n = 6$ per group):

- **Group I (Normal Control):** Healthy rats receiving vehicle (0.5 mL distilled water/rat/day p.o.).
- **Group II (Diabetic Control):** Diabetic/HFD rats receiving vehicle (0.5 mL distilled water/rat/day p.o.).
- **Group III (Standard):** Diabetic rats receiving Metformin (50 mg/kg p.o.) + Atorvastatin (10 mg/kg p.o.).
- **Group IV (Extract Low Dose):** Diabetic rats receiving *C. pusillus* extract (250 mg/kg p.o.).
- **Group V (Extract High Dose):** Diabetic rats receiving *C. pusillus* extract (500 mg/kg p.o.).

Daily oral treatment was administered for 28 consecutive days.

2.6 Biochemical Determinations

- **Fasting Blood Glucose:** Blood samples were collected from the retro-orbital plexus at Day 0, Day 7, Day 14, and Day 28. FBG was quantified using the Glucose Oxidase-Peroxidase (GOD-POD) enzymatic method.



- **Lipid Profile Parameters:** On Day 28, serum Total Cholesterol (TC), Triglycerides (TG), and High-Density Lipoprotein Cholesterol (HDL-C) were determined using commercial enzymatic assay kits (CHOD-PAP and GPO-Trinder methods) on a semi-automated biochemical analyzer. Low-Density Lipoprotein Cholesterol (LDL-C) was calculated using Friedewald's formula:

$$\text{LDL-C} = \text{Total Cholesterol} - \text{HDL-C} - \left(\frac{\text{Triglycerides}}{5} \right)$$

- **Serum α -Amylase Activity:** Measured at the conclusion of the 28-day treatment period using kinetic enzymatic methods.

2.7 Statistical Analysis

Data are expressed as Mean $\pm$ Standard Error of the Mean (SEM) ($n = 6$).

Statistical significance was evaluated using one-way Analysis of Variance (ANOVA) followed by Bonferroni's post-hoc test. $P < 0.05$ was considered statistically significant.

3. RESULTS

3.1 Extract Yield and Solubility Profile

The 70% ethanolic leaf extract yielded 17.38% w/w. The extract was soluble in water, methanol, and DMSO, partially soluble in chloroform, petroleum ether, and ethyl acetate, and insoluble in acetone.

3.2 Phytochemical Composition

Qualitative phytochemical screening confirmed the presence of alkaloids, carbohydrates, proteins/amino acids, flavonoids, cardiac glycosides, phenolics, tannins, and steroids.

Phytochemical Class	Test Conducted	Inference / Result
Alkaloids	Mayer's, Wagner's, Hager's Tests	Present
Carbohydrates	Molisch's Test	Present
	Barfoed's Test	Absent
Proteins & Amino Acids	Biuret Test	Present
Flavonoids	Alkaline Reagent Test, Lead Acetate Test	Present
Glycosides	Borntrager's Test	Absent
	Legal's Test	Absent
	Keller-Killiani Test (Cardiac)	Present
Tannins & Phenolics	Ferric Chloride Test, Lead Acetate Test	Present
	Gelatin Test	Absent
Saponins	Froth / Foam Test	Absent
Steroids & Triterpenoids	Salkowski's Test	Absent
	Liebermann-Burchard Test	Present

3.3 Effect on Fasting Blood Glucose Levels

Alloxan administration induced severe hyperglycemia in Groups II–V (> 390 mg/dL).

Daily oral administration of *C. pusillus* extract produced a time- and dose-dependent reduction in FBG over the 28-day period.

Group / Treatment	Day 0 (mg/dL)	Day 7 (mg/dL)	Day 14 (mg/dL)	Day 28 (mg/dL)
Group I (Normal Control)	77.67 $\pm$ 6.56	75.83 $\pm$ 4.62	77.67 $\pm$ 8.66	78.17 $\pm$ 3.31
Group II (Diabetic Control)	405.50 $\pm$ 9.09	403.83 $\pm$ 10.34 ^{###}	403.17 $\pm$ 11.72 ^{###}	401.17 $\pm$ 10.38 ^{###}
Group III (Standard)	397.00 $\pm$ 7.07	237.50 $\pm$ 9.81 ^{***}	129.17 $\pm$ 10.70 ^{***}	74.33 $\pm$ 4.76 ^{***}



Group IV (250 mg/kg Extract)	398.67 ± 8.19	282.67 ± 9.89***	133.67 ± 9.31***	79.67 ± 6.44***
Group V (500 mg/kg Extract)	397.33 ± 6.83	246.50 ± 8.12***	130.67 ± 4.27***	77.83 ± 6.49***

Values are Mean ± SEM ($n = 6$). ### P

< 0.001 vs Normal Control; *** P

< 0.001 vs Diabetic Control.

By Day 28, the high-dose extract (500 mg/kg) restored blood glucose levels (77.83 ± 6.49 mg/dL) to levels comparable to both the standard drug combination (74.33 ± 4.76 mg/dL) and normal control rats (78.17 ± 3.31 mg/dL).

3.4 Effect on Serum Lipid Profile

Diabetic control rats exhibited severe dyslipidemia, marked by significant elevations in TG, TC, and LDL-C alongside a reduction in HDL-C ($P < 0.001$). Administration of *C. pusillus* extract for 28 days improved lipid parameters in a dose-dependent manner.

Experimental Group (n=6)	TG (mg/dL)	TC (mg/dL)	LDL-C (mg/dL)	HDL-C (mg/dL)
Group I (Normal Control)	99.29 ± 2.76	124.40 ± 6.58	44.49 ± 8.14	60.00 ± 3.65
Group II (Diabetic Control)	181.60 ± 3.53###	294.00 ± 12.26###	237.70 ± 10.53###	20.00 ± 1.82###
Group III (Standard)	106.70 ± 2.06***	137.00 ± 5.86***	62.32 ± 3.73***	53.33 ± 5.27***
Group IV (250 mg/kg Extract)	162.60 ± 1.11***	255.70 ± 4.78*	197.40 ± 5.11**	25.83 ± 2.38 ^{ns}
Group V (500 mg/kg Extract)	142.50 ± 1.61***	203.00 ± 6.04***	133.30 ± 5.55***	41.17 ± 1.53**

Values are Mean ± SEM ($n = 6$). ### P

< 0.001 vs Normal Control; * $P < 0.05$, ** P

< 0.01, *** P

< 0.001, ns = not significant vs Diabetic Control.

3.5 Effect on Serum α -Amylase Activity

Alloxan-induced pancreatic islet injury led to a sharp drop in serum α -amylase levels in the diabetic control group (176.2 ± 24.70 U/L) compared to normal controls (589.0 ± 16.45 U/L; $P < 0.001$). Treatment with *C. pusillus* extract for 28 days produced a dose-dependent recovery in serum α -amylase activity.

Experimental Group (n=6)	Serum α -Amylase Activity (U/L)	Statistical Significance
Group I (Normal Control)	589.0 ± 16.45	Baseline
Group II (Diabetic Control)	176.2 ± 24.70	$P < 0.001$ ### vs Normal Control
Group III (Standard)	492.2 ± 22.30	$P < 0.001$ *** vs Diabetic Control
Group IV (250 mg/kg Extract)	291.3 ± 7.99	$P < 0.001$ *** vs Diabetic Control
Group V (500 mg/kg Extract)	357.2 ± 6.16	$P < 0.001$ *** vs Diabetic Control

DISCUSSION

Alloxan monohydrate induces diabetes through selective toxic destruction of pancreatic β -cells via redox cycling and generation of reactive oxygen species (ROS). The resulting insulin deficiency

triggers catabolic states, persistent hyperglycemia, hyperphagia, polydipsia, and secondary dyslipidemia.

In this study, oral administration of 70% ethanolic extract of *Catharanthus pusillus* for 28 days effectively attenuated alloxan-induced



hyperglycemia. The observed reduction in fasting blood glucose (397.33 to 77.83 mg/dL at 500 mg/kg) suggests enhanced peripheral glucose uptake and potential regeneration or protection of functional β -cell architecture. These findings align with earlier reports on related Apocynaceae species and polyphenol-rich botanical extracts that stimulate GLUT-4 translocation via IRS-1/PI3K/Akt signaling cascades.

The extract also improved the secondary lipid abnormalities associated with diabetic states. Insulin deficiency typically enhances hormone-sensitive lipase activity, elevating circulating free fatty acids and hepatic VLDL/LDL synthesis. *C. pusillus* treatment at 500 mg/kg significantly reduced serum TG, TC, and LDL-C, while increasing protective HDL-C levels. This lipid-modulating effect may stem from the activation of Lecithin-Cholesterol Acyltransferase (LCAT) and tissue Lipoprotein Lipase (LPL), facilitating cholesterol esterification and triglyceride clearance.

Furthermore, the recovery of serum α -amylase activity (176.2 to 357.2 U/L at 500 mg/kg) indicates partial restoration of exocrine and endocrine pancreatic physiological balance following extract administration. Overall, the presence of synergistic bioactive phytoconstituents—including alkaloids, flavonoids, and phenolics—underpins the dual antidiabetic and antihyperlipidemic activities of *Catharanthus pusillus*.

CONCLUSION

The 70% ethanolic leaf extract of *Catharanthus pusillus* demonstrates potent antidiabetic and antihyperlipidemic activity in alloxan-induced diabetic Wistar rats. Treatment for 28 days normalized fasting blood glucose levels, improved atherogenic lipid parameters, and restored pancreatic enzyme activity in a dose-dependent

manner. These results validate the traditional medicinal utility of *C. pusillus* and highlight its potential as a candidate for developing novel phytotherapeutic agents targeting metabolic disorders.

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