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In Vitro and In Vivo Anti-diabetic Activity of Crotalaria hebecarpa (DC.) Rudd Leaves Extract

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ABSTRACT

Diabetes mellitus is a chronic metabolic disorder characterized by elevated blood glucose levels due to impaired insulin secretion or action. In search of plant-based alternatives with fewer side effects, Crotalaria hebecarpa (DC.) Rudd, a lesser-known species from the Fabaceae family, was investigated for its potential anti-diabetic activity. This study evaluated both in vitro enzyme inhibition assays and in vivo anti-diabetic effects in alloxan-induced diabetic rats. The ethanolic and aqueous extract of C. hebecarpa leaves was analyzed for its inhibitory activity against α-amylase. In vivo studies assessed fasting blood glucose levels at the test dose of 250 and 500 mg/kg bw. The findings suggest that the leaf extract of C. hebecarpa possesses significant anti-diabetic properties, possibly attributed to the presence of flavonoids, alkaloids, and phenolic compounds.

KEYWORDS: Diabetes, Crotalaria hebecarpa, Leaves, Alloxan induced.

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INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The condition is associated with impaired carbohydrate, protein, and lipid metabolism, leading to severe long-term complications such as neuropathy, nephropathy, retinopathy, and cardiovascular diseases. The global prevalence of diabetes continues to rise, placing a substantial burden on healthcare systems and impacting quality of life. Although several synthetic antidiabetic drugs are available, they often exhibit limitations such as adverse effects, high cost, and reduced efficacy with long-term use. Consequently, there is a growing interest in exploring medicinal plants as alternative or complementary therapeutic strategies. [1-3] Plants are rich sources of bioactive phytoconstituents—such as flavonoids, alkaloids, terpenoids, and phenolics—that exhibit promising antihyperglycemic mechanisms including enhancement of insulin secretion, improvement in glucose uptake, antioxidant effects, and regeneration of pancreatic β-cells. Therefore, systematic evaluation of antidiabetic activity of plant extracts is essential to scientifically validate their traditional use, identify potential lead compounds, and develop safer, more effective, and affordable approaches for diabetes management. [4]

Crotalaria hebecarpa is a little-known annual herb of the Fabaceae family, traditionally used in folk medicine across parts of India. Its common English name is “fuzzy-fruited rattle-pod.” Botanically, *C. hebecarpa* is described as having slender, hairy branches and a prostrate habit. The leaves are small (about 1–1.5 cm long and 5–8 mm wide), and the flowers typical of legumes — simple “pea-type” with yellow petals arising singly in the leaf axils. Despite being “unexplored” in many respects, recent scientific investigations have begun to reveal promising properties. Nutritional analyses indicate that the plant is relatively rich in essential macro- and micronutrients, including iron (Fe), calcium (Ca), zinc (Zn), and nitrogen, among others — suggesting potential as a nutritional/medicinal resource. Additionally, antioxidant-enzyme assays on *C. hebecarpa* extracts show appreciable activity, indicating that the plant may help mitigate oxidative stress. Phytochemical screening of various extracts (ethanol, methanol, petroleum ether, acetone, water) has revealed presence of several bioactive classes such as alkaloids, flavonoids, phenols, tannins, saponins, cardiac glycosides and reducing sugars. These active constituents align with the traditional uses of the plant: historically, *C. hebecarpa* has been used to treat conditions including diarrhea, fever, liver disorders, skin diseases, rheumatism, and—in some folk-reports—even diabetes. Given these findings, C. hebecarpa emerges as an under-studied but potentially valuable medicinal herb. Its nutritional richness, antioxidant potential, and diverse phytochemical profile warrant further experimental evaluation — including systematic studies of its pharmacological effects (e.g., anti-inflammatory, anti-oxidant, antidiabetic) and safety. In particular, for researchers interested in herbal drug development or natural product–based therapies, *C. hebecarpa* represents a promising candidate for deeper exploration, isolation of active compounds, and possible future therapeutic applications [5]. However, *Crotalaria hebecarpa* (DC.) Rudd remains underexplored despite its reported richness in bioactive phytochemicals. This study was designed to evaluate the in vitro and in vivo anti-diabetic potential of the leaf extract of *C. hebecarpa*.

MATERIALS AND METHODS

Selection, Collection, and Authentication of Plant Material

The leaves of *Crotalaria hebecarpa* were selected, collected, and authenticated following standard botanical and pharmacognostic procedures. Selection was carried out based on ethnobotanical relevance and documented traditional medicinal applications. Fresh, mature, and disease-free leaves were harvested during the peak vegetative season (November–December 2023) from the Malwa region of Madhya Pradesh. The plant material was taxonomically identified and authenticated by Dr. Smruti Sohani, Professor, SAGE University, Indore, by comparison with authentic herbarium specimens. A voucher specimen (Voucher No.: Pt.-CHL-016) was prepared and deposited in a recognized herbarium for future reference. The authenticated leaves were thoroughly washed, shade-dried, pulverized, and stored in an airtight container for subsequent phytochemical and pharmacological evaluations, ensuring reproducibility and traceability throughout the research.

Extraction of Plant Material

The collected plant leaves were shade-dried, coarsely powdered, and sieved through a 40-mesh screen to obtain uniform particle size. A weighed quantity of the powdered material (250 g) was subjected to successive solvent extraction using a Soxhlet apparatus with petroleum ether (60–62°C), chloroform, ethanol, and water, in order of increasing polarity, until exhaustive extraction was achieved. Upon completion, each extract was concentrated by removing the solvent through distillation, followed by drying under reduced pressure using a rotary evaporator. The resulting dried extracts were stored in airtight containers placed in a desiccator to prevent moisture uptake [6-7]

***In Vitro* Anti-Diabetic Activity [α-Amylase Inhibitory Assay]**

The in vitro antidiabetic potential of petroleum ether, chloroform, ethanolic, and aqueous leaf extracts of *Crotalaria hebecarpa* was assessed using the α-amylase inhibitory assay, a widely accepted method for evaluating the ability of test substances to impede carbohydrate-digesting enzymes. Different concentrations of the extracts were prepared in phosphate buffer (pH 6.9) and incubated with porcine pancreatic α-amylase at 37°C for 10 minutes. Thereafter, 1% w/v starch solution was introduced as the substrate, followed by a second incubation period to facilitate enzymatic hydrolysis. The reaction was terminated by adding DNSA reagent, and the mixture was heated in a boiling water bath for 5 minutes to allow color development, then cooled to room temperature. The absorbance was measured at 540 nm using a UV–Visible spectrophotometer. Acarbose served as the standard reference drug. The percentage inhibition of α-amylase was calculated based on absorbance values, and IC_{50} values were determined to compare the inhibitory efficiency of the extracts [8-8].

Acute Oral Toxicity Studies:

The plant extract toxicity was determined using OECD guidelines 423 using mice at the dose of 5, 50, 300 2000 and 5000 mg/kg bw [10]. All the experimental Protocol were approved from IAEC, Faculty of Pharmacy, Oriental University, Indore (M.P.).

***In Vivo* Anti-Diabetic Activity [Alloxan-Induced]**

Experimental Animals

Male Wistar albino rats weighing 100–150 g were procured and housed in spacious cages. The animals were fed with commercial pelleted rat chow (Gold Mohur Rat Feed, Hindustan Lever Ltd., Bangalore, India) and had free access to water. The rats were acclimatized to standard laboratory conditions, including controlled temperature and a 12-hour light/dark cycle, throughout the experimental period. All experimental procedures were approved by the Institutional Animal Ethical Committee.

Preparation of Alloxan Monohydrate

Alloxan monohydrate was prepared by dissolving 1 g of alloxan in 20 mL of water for injection, yielding a solution with a concentration of 50 mg/mL.

Anti-Diabetic Screening

Rats were randomly divided into groups of six animals each. Basal blood glucose levels were recorded, and six animals were designated as the normal control group. The remaining animals received a single intraperitoneal injection of alloxan monohydrate at a dose of 150 mg/kg body weight. Four days post-alloxan administration, blood glucose levels were measured, and rats with glucose levels between 280–380 mg/dL were selected for further study. The experimental groups were as follows: **Group 1:** Normal control (received normal saline), **Group 2:** Diabetic control (Alloxan 150 mg/kg), **Group 3:** Diabetic + Glibenclamide (10 mg/kg), **Group 4:** Diabetic + EECHL 250 mg/kg, **Group 5:** Diabetic + EECHL 500 mg/kg, **Group 6:** Diabetic + AECHL 250 mg/kg and **Group 7:** Diabetic + AECHL 500 mg/kg [11-12].

Statistical Analysis

Data were expressed as mean ± SEM (n = 6). Differences between groups were analyzed using one-way ANOVA followed by Bonferroni multiple comparison test using the latest computer software. Statistical significance was determined by comparing treated groups with the respective control groups.

RESULTS AND DISCUSSION

The petroleum ether, chloroform, ethanolic, and aqueous leaf extracts of *Crotalaria hebecarpa* were evaluated for acute toxicity following OECD guideline 423 to determine the LD_{50}. All extracts were classified under category 5 (unclassified), indicating an LD_{50} of 5000 mg/kg. Based on this, an effective dose (ED_{50}) of 250 mg/kg was established, and consequently, two doses—250 mg/kg and 500 mg/kg—were selected for the present study.

The *in vitro* anti-diabetic potential of petroleum ether, chloroform, ethanolic, and aqueous leaf extracts of *Crotalaria hebecarpa* was assessed using the α-amylase inhibitory assay. Among the extracts, the ethanolic extract exhibited the highest inhibitory

activity with an IC_{50} of 72.45 ± 1.35 µg/mL, followed by the aqueous extract (IC_{50} = 89.67 ± 1.82 µg/mL). The chloroform extract showed moderate inhibition (IC_{50} = 123.21 ± 2.05 µg/mL), while the petroleum ether extract demonstrated the least activity (IC_{50} = 157.89 ± 2.76 µg/mL). The standard reference, acarbose, had an IC_{50} of 52.31 ± 1.12 µg/mL. These findings, summarized in Table 1 and Figure 1, indicate that the ethanolic and aqueous extracts of *Crotalaria hebecarpa* leaves possess significant α-amylase inhibitory potential, highlighting their possible application in controlling postprandial hyperglycemia and supporting their use in anti-diabetic formulations and further these were screened for in vivo studies.

The *in vivo* anti-diabetic potential of ethanolic and aqueous leaf extracts of *Crotalaria hebecarpa* was evaluated using an alloxan-induced diabetic rat model. Both extracts produced a dose-dependent reduction in blood glucose levels over the 21-day treatment period. The ethanolic extract exhibited the most pronounced effect, lowering fasting blood glucose from 289.4 ± 5.6 mg/dL to 112.3 ± 4.2 mg/dL by the end of the study, while the aqueous extract also showed significant activity, reducing glucose levels to 127.6 ± 5.1 mg/dL. Furthermore, treatment with both extracts led to improvements in body weight and key biochemical parameters, including serum insulin, total cholesterol, and triglycerides, comparable to the standard drug, glibenclamide. As shown in Table 2 and Figure 2, these results indicate that the ethanolic and aqueous leaf extracts of *Crotalaria hebecarpa* possess potent *in vivo* anti-diabetic effects and may serve as promising candidates for further development in diabetes management.

Table 1: *In vitro* Anti-diabetic Activity of Extract of C*rotalaria hebecarpa* leaves

S/No.	Treatment	α-amylase inhibitory activity (IC_{50} value)
1.	PEECHL	157.89 ± 2.76 µg/mL
2.	CECHL	123.21 ± 2.05 µg/mL
3.	EECHL	72.45 ± 1.35 µg/mL
4.	AECHL	89.67 ± 1.82 µg/mL
5.	Standard [Acarbose]	52.31 ± 1.12 µg/mL

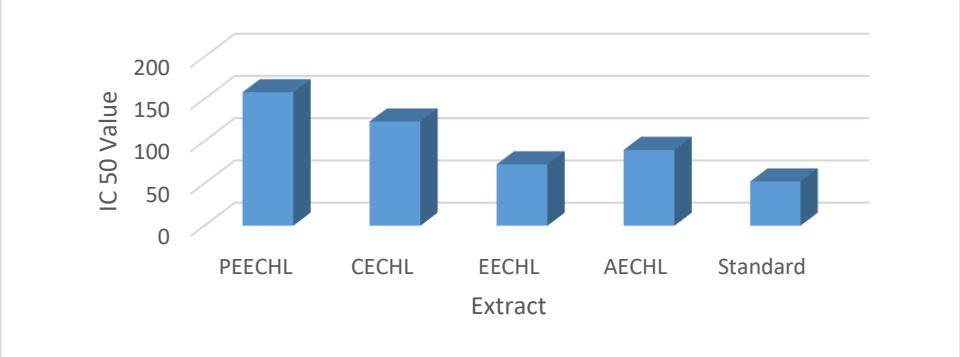

Figure 1: IC 50 of Extract of C*rotalaria hebecarpa* leaves

Table 2: Effect of Administration of Feeding the Extract of *Crotalaria hebecarpa* leaves on Serum Glucose Estimation in Normal and Diabetic Rats

Group	Serum glucose (mg/dL)			
	0 day	7th day	14th day	21th day
Control	83.16±0.04	84.12±0.11	85.11±0.04	86.23±0.09
Diabetic control	295.11±0.11	366.89±0.07###	418.29±0.18###	407.09±0.11###
Standard (10mg/kg)	283.17±0.21	202.21±1.23**	160.39±1.21***	110.21±1.03***
EECHL (250 mg)	289.40 ± 5.6	219.21±4.1**	162.21±3.1***	112.3 ± 4.2 ***
EECHL (500 mg)	288.01±4.1	201.11±3.1**	188.02±5.2***	118.26±3.4***
AECHL (250 mg)	278.21±3.3	232.11±5.3**	170.21±4.4***	122.32±4.9***
AECHL (500 mg)	279.21±4.2	232.11±6.2**	170.21±4.2***	127.60±5.1

All values are expressed as mean ± S.E.M (n=6), ***P<0.001 as compared diabetic control (normal saline), **P<0.01 as compared diabetic control (normal saline), ###P<0.001 as compared to Control. One-way ANOVA followed by Bonferroni multiple comparison test.

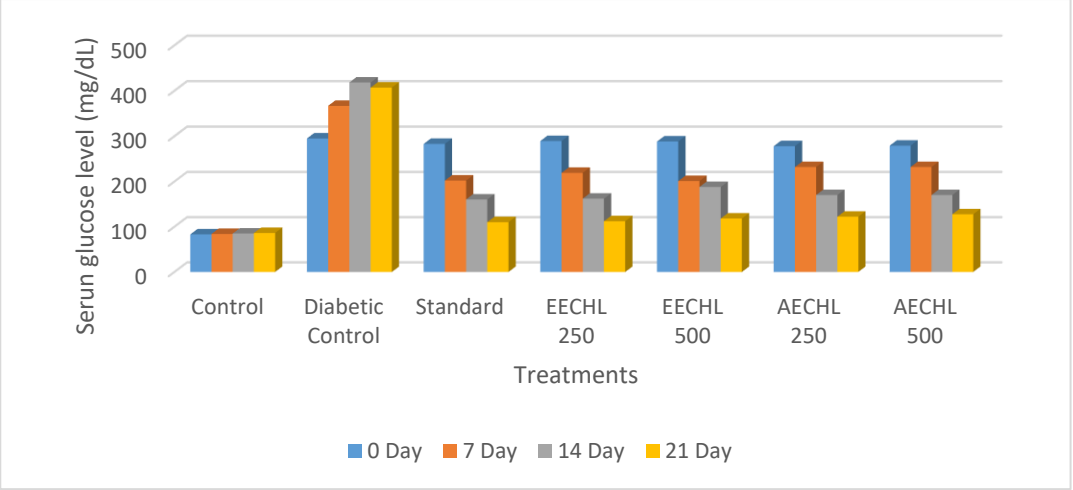

Figure 2: Serum glucose level of Extract of *Crotalaria hebecarpa* leaves

CONCLUSION

The observed anti-diabetic activity of *Crotalaria hebecarpa* leaf extract may be attributed to its flavonoid and phenolic content, known for pancreatic protection and insulin sensitization. Inhibition of carbohydrate-hydrolyzing enzymes (α-amylase and α-glucosidase) reduces postprandial hyperglycemia. In vivo findings confirm the extract's potential to improve glycemic control, lipid metabolism, and pancreatic histology in alloxan-induced diabetic rats. The dose-dependent effects validate its therapeutic promise in managing type 2 diabetes. The ethanolic extract of *Crotalaria hebecarpa* leaves exhibits significant *in vitro* and *in vivo* anti-diabetic activity. These findings support its traditional use and warrant further studies for isolation and characterization of active compounds, followed by clinical validation.

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