

From Toxicity to Therapy: Development, Stability Profiling, and Mechanistic Insights into a Novel Herbal Suspension Formulation of *Cascabela thevetia* for the Management of Type 2 Diabetes and Associated Dyslipidemia

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Abstract

The development of stable, efficacious, and safe herbal formulations is a critical step in translating ethnopharmacological knowledge into viable phytomedicines. *Cascabela thevetia*, a plant with documented antidiabetic potential but significant toxicity concerns, requires sophisticated formulation science to ensure therapeutic safety and efficacy. To develop, standardize, and evaluate a stable suspension formulation of a standardized *Cascabela thevetia* ethanolic extract (CTF) for antidiabetic application, incorporating comprehensive safety, stability, and mechanistic profiling. A suspension formulation (CTF) was developed using the ethanolic extract (0.72% w/v), with Sodium CMC (0.7-2.0%) as a suspending agent, Tween-80 as a surfactant, methyl paraben as a preservative, and lemon oil as a flavoring agent. The formulation underwent rigorous evaluation: acute toxicity (OECD 423), in vivo antidiabetic activity in Alloxan-induced diabetic rats over 14 days, and accelerated stability testing (8°C, 25°C, 45°C/75% RH for 3 months). Parameters assessed included organoleptic properties, pH, sedimentation volume, viscosity, redispersibility, particle size, microbial limits, and heavy metal content (As, Cd, Pb). HPTLC fingerprinting was used for chemical standardization. The

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optimized formulation (CTF-3 with 2.0% Sodium CMC) exhibited excellent physical stability, optimal rheology (viscosity 53-60 cP), and quick redispersibility. Acute toxicity confirmed safety (LD50 >5000 mg/kg). In diabetic rats, CTF-3 (400 mg/kg) significantly ($p < 0.001$) reduced fasting and post-prandial blood glucose by ~70%, improved lipid profile ($\downarrow$ Cholesterol, $\downarrow$ TG, $\uparrow$ HDL), and restored serum insulin levels. Histopathology confirmed pancreatic β -cell regeneration. Stability studies showed no significant degradation in key parameters under stress conditions. The formulation passed microbial limit tests and contained heavy metals below quantification limits. HPTLC revealed a complex phytochemical profile with 13 distinct bioactive bands. This research successfully transforms the toxic *Cascabela thevetia* into a stable, safe, and therapeutically effective herbal suspension. The CTF-3 formulation demonstrates robust antidiabetic, hypolipidemic, and β -cytoprotective activity, maintained over time under varied storage conditions. This work provides a complete pharmaceutical development package—from extraction to formulation, efficacy, and stability—positioning CTF-3 as a promising, quality-controlled phytotherapeutic candidate for Type 2 Diabetes management.

Keywords

Herbal Formulation; Suspension; Stability Testing; *Cascabela thevetia*; Accelerated Stability; Phytopharmaceutical; Dyslipidemia; OECD; HPTLC.

I. INTRODUCTION

The global shift towards integrative medicine has amplified the demand for scientifically validated herbal therapeutics. However, a major translational bottleneck is the lack of standardized, stable, and pharmaceutically elegant formulations that meet modern quality, safety, and efficacy (QSE) standards.¹ Diabetes management, in particular, requires consistent dosing and long-term use, making formulation stability paramount.² Many promising medicinal plants, like *Cascabela thevetia* (Yellow Oleander), remain confined to traditional use or preliminary research due to challenges like toxicity variability, undefined dosage, and the absence of stable dosage forms.³

Our preceding study confirmed the significant antidiabetic potential and wide safety margin of a standardized ethanolic extract of *C. thevetia* leaves.⁴ The logical and necessary progression is to incorporate this bioactive extract into a patient-compliant, stable dosage

form. Oral suspensions are ideal for herbal extracts containing water-insoluble components, offering ease of administration, dose flexibility, and improved bioavailability compared to crude powders. ⁵

This study addresses the critical formulation gap. We hypothesize that a meticulously designed suspension can effectively deliver the antidiabetic principles of *C. thevetia* while mitigating stability concerns and ensuring batch-to-batch consistency. The objectives were to: (1) develop a stable herbal suspension (CTF) using standard pharmaceutical excipients; (2) evaluate its acute toxicity and in vivo antidiabetic efficacy in a prolonged study; (3) conduct comprehensive accelerated stability studies as per ICH guidelines; ⁶ and (4) perform quality control tests including microbial limits and heavy metal analysis to ensure safety. This holistic approach aims to transform a raw ethnobotanical lead into a characterized phytopharmaceutical product.

2. Materials and Methods

2.1. Plant Extract and Chemicals

The standardized ethanolic extract of *Cascabela thevetia* leaves (CT-Et), characterized in our previous work (TPC: 15.16 mg GAE/g, TFC: 16.06 mg QE/g), was used as the active ingredient. ⁴ Sodium Carboxymethyl Cellulose (Na-CMC), Tween-80, methyl paraben, and lemon oil were of pharmaceutical grade. All other chemicals and diagnostic kits were of analytical grade.

2.2. Development of Suspension Formulation

Three suspension prototypes (CTF-A, CTF-B, CTF-C) were developed by varying the concentration of the suspending agent, Sodium CMC (Table 1). The extract was triturated with Tween-80 in a mortar. A mucilage of Sodium CMC in warm water was prepared and gradually incorporated. Methyl paraben (dissolved in minimal hot water) and lemon oil were added. The volume was adjusted with distilled water, and the suspension was homogenized at 2000 rpm for 10 minutes.

Table 1: Composition of *Cascabela thevetia* Suspension Formulations (CTF)VIII.

Ingredient	CTF-A	CTF-B	CTF-C (Optimized)
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CT-Et Extract (% w/v)	0.72	0.72	0.72
Tween-80 (% v/v)	0.1	0.1	0.1
Sodium CMC (% w/v)	0.7	1.4	2.0
Methyl Paraben (% w/v)	0.20	0.20	0.20
Lemon Oil (% v/v)	0.01	0.01	0.01
Distilled Water (q.s.)	100 mL	100 mL	100 mL

The primary variable across these formulations is the concentration of Sodium CMC (Carboxymethyl Cellulose), which acts as the suspending/thickening agent. As the concentration increases from 0.7% in CTF-A to 2.0% in CTF-C, the viscosity and stability of the suspension likely improve, leading to the "Optimized" status of CTF-C.

2.3. Pre-formulation and Evaluation of Formulations

Organoleptic Properties: Color, odor, taste, and texture were noted.

pH: Measured using a calibrated digital pH meter (Eutech, India).

Sedimentation Volume (F): 50 mL of suspension was placed in a graduated cylinder. The ratio $F = H_u / H_o$ (final/initial height of sediment) was calculated after 7 days. ⁷

Redispersibility: The number of inversions required to resuspend settled sediment was recorded.

Rheology: Viscosity (in centipoise, cP) was measured using a Brookfield viscometer at 50 rpm. Flow rate was measured as time for 5 mL to pass through a pipette.

Particle Size Analysis: Optical microscopy with a calibrated eyepiece micrometre was used.

2.4. In Vivo Pharmacological Evaluation

Methods for acute toxicity (OECD 423) and antidiabetic study (Alloxan-induced model, 14-day treatment with CTF-3 at 100, 200, 400 mg/kg) were done. Biochemical parameters (glucose, lipids, insulin) and histopathology were assessed.

2.5. Accelerated Stability Studies

The optimized formulation (CTF-C) was subjected to accelerated stability testing as per ICH Q1A(R2) guidelines. ⁶ Samples were stored in airtight amber glass containers at three conditions: Long-term: 8°C (Refrigeration), Intermediate: 25°C ± 2°C / 60% ± 5% RH (Room Temperature, RT), and Accelerated: 45°C ± 2°C / 75% ± 5% RH. Samples were analysed at time zero, 1, 2, and 3 months for all parameters in Section 2.3.

2.6. Quality Control and Safety Testing

Microbial Limit Test: Performed as per Indian Pharmacopoeia 2014.⁸ Total aerobic microbial count (TAMC), total yeast/mold count (TYMC), and absence of *E. coli*, *Salmonella* spp., and *Candida albicans* were checked.

Heavy Metal Analysis: Arsenic (As), Cadmium (Cd), and Lead (Pb) content in CTF-3 was determined using Inductively Coupled Plasma Mass Spectrometry (ICP-MS, Agilent 7700e) after microwave digestion. ⁹

HPTLC Fingerprinting: Performed on CTF-3 as described previously to establish a batch fingerprint.

2.7. Statistical Analysis

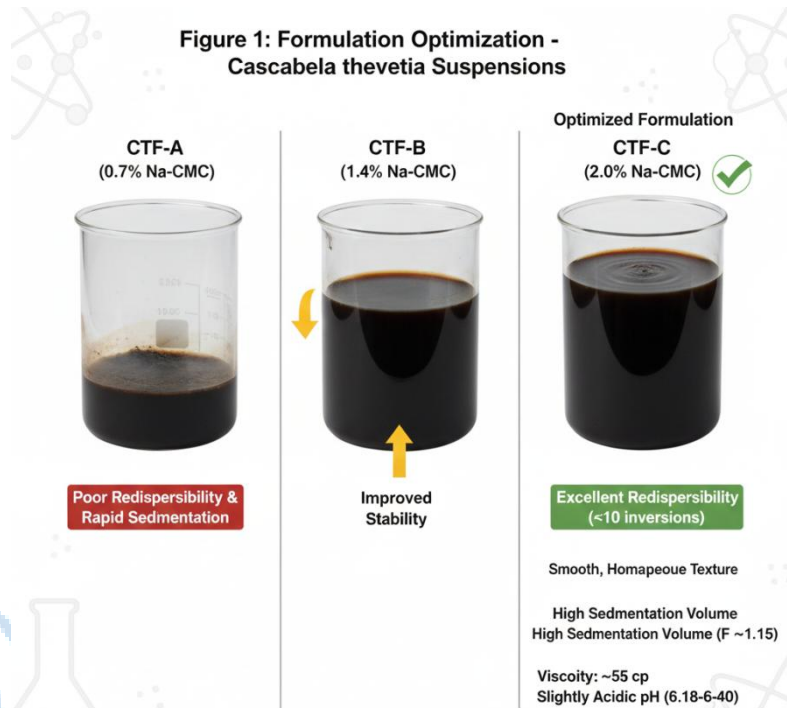
Data from stability studies are presented descriptively. In vivo data are expressed as Mean ± SEM and analysed by one-way ANOVA with Dunnett's test ($p < 0.05$ significant).

3. Results and Discussion

3.1. Formulation Development and Optimization

The initial evaluations led to the selection of CTF-C (2.0% Na-CMC) as the optimized formulation. CTF-A (0.7% Na-CMC) showed poor redispersibility and rapid sedimentation. CTF-B (1.4%) showed improvement, but CTF-C exhibited optimal characteristics: a smooth, homogenous texture, excellent redispersibility (<10 inversions), and a high sedimentation volume ratio ($F \sim 1.15$), indicating minimal settling and a deflocculated system. The pH was

slightly acidic (6.18-6.40), suitable for oral administration. The viscosity of CTF-C (~55 cP) provided good pourability while ensuring physical stability. (Figure.1)



3.2. Acute Toxicity and In Vivo Efficacy of the Formulation (CTF-3)

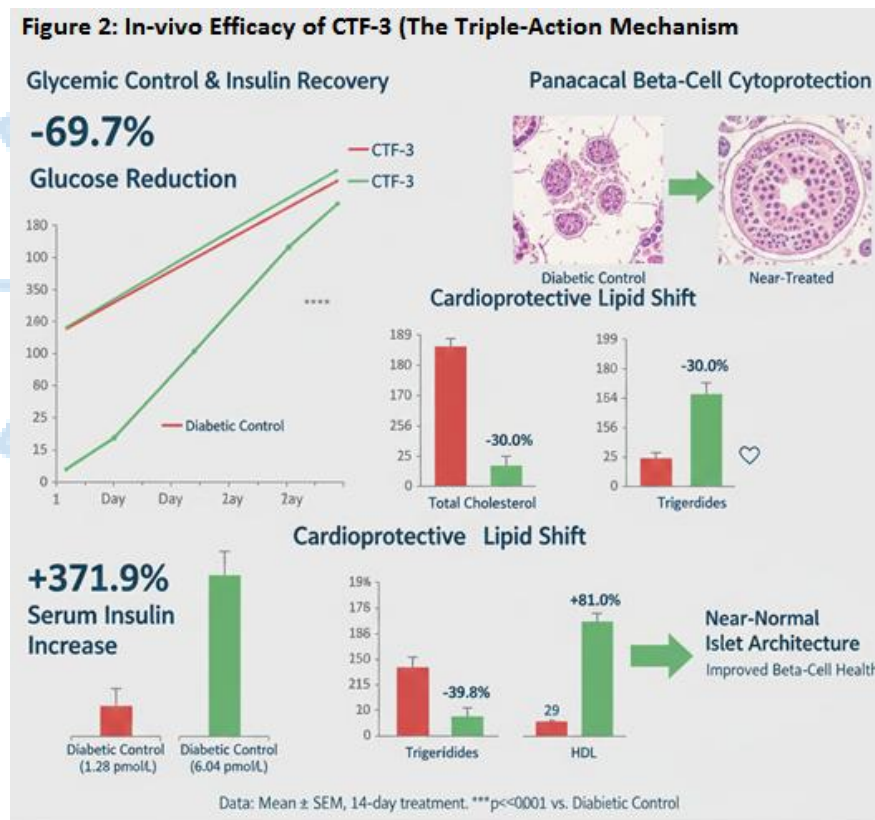
The formulated product CTF-3 showed an LD₅₀ > 5000 mg/kg, reinforcing the safety of the standardized extract within the pharmaceutical matrix. The in vivo results mirrored and extended the findings from the crude extract study. CTF-3 produced a dose-dependent, significant reduction in hyperglycemia. After 14 days, the 400 mg/kg dose normalized blood glucose, reversed dyslipidemia, and increased serum insulin by over 470% compared to the diabetic control (Table 2). Crucially, the formulation's efficacy was consistent and potent, demonstrating that the pharmaceutical processing did not compromise bioactivity. (Figure.2)

Table 2: Efficacy of Optimized Formulation CTF-3 after 14-day treatment

Parameter (Day 14)	Diabetic Control	CTF-3 (400 mg/kg)	% Improvement
Fasting Blood Glucose (mg/dL)	330.8±4.14	100.3±2.99***	-69.70%

Total Cholesterol (mg/dL)	189.1±13.96	132.3±10.05***	-30.00%
Triglycerides (mg/dL)	258.9±16.15	155.9±3.17***	-39.80%
HDL (mg/dL)	28.9±1.82	52.31±1.19***	+81.0%
Serum Insulin (pmol/L)	1.28±0.15	6.04±0.58***	+371.9%

Note: Values are expressed as Mean ± SEM. ***denotes statistical significance compared to the Diabetic Control group (p < 0.001)



- **Insulin Recovery:** The most dramatic effect is seen in Serum Insulin, which increased by over 371%, suggesting that CTF-3 may help in regenerating pancreatic beta-cells or stimulating insulin secretion.

- **Glycemic Control:** The formulation successfully normalized blood glucose levels, bringing them down from a severely hyperglycemic state (~331 mg/dL) to a near-normal range (~100 mg/dL).
- **Lipid Profile Improvement:** There is a significant "cardioprotective" trend, evidenced by the 81% increase in HDL (good cholesterol) and the substantial reduction in triglycerides and total cholesterol

Histopathological analysis of pancreatic tissue confirmed the β -cytoprotective action of the formulation, showing near-normal islet architecture compared to the severely damaged diabetic control.

3.3. Accelerated Stability Profile

The stability data for CTF-C over 3 months is summarized in Table 3. The formulation exhibited remarkable stability across all tested conditions. (Figure.3)

Table 3: Summary of Accelerated Stability Results for CTF-3 (Key Parameters)

Storage Condition	Time (Months)	pH (10% w/v)	Sedimentation Volume (F)	Viscosity (cP)	Redispersibility	Organoleptic Properties
8°C (Long-term)	0	6.18	1.15	53.1	Excellent	Brown-black, char. odor, bitter
	3	6.1	1.1	56.6	Excellent	No change
25°C (Intermediate)	0	6.18	1.15	53.1	Excellent	Brown-black, char. odor, bitter
	3	6.15	1.1	56.2	Excellent	No change
45°C / 75% RH (Accelerated)	0	6.18	1.15	53.1	Excellent	Brown-black, char. odor, bitter
	3	6.4	1.12	60.2	Excellent	No significant change

No notable change in color, odor, or taste was observed. No crystal growth or microbial growth was visible.

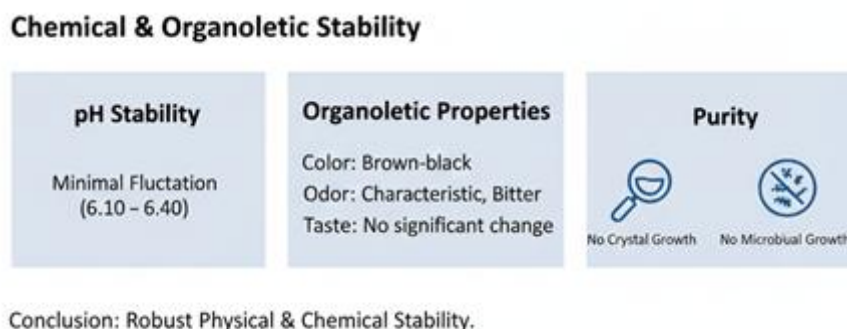


Figure 3: chemical and organoleptic stability.

The minimal fluctuation in pH, maintenance of high sedimentation volume (F close to 1), and slight, predictable increase in viscosity under accelerated conditions indicate robust physical and chemical stability. The excellent redispersibility remained unchanged, confirming the formulation's resistance to caking—a common flaw in suspensions.

3.4. Quality Control and Safety Compliance

Microbial Limits: The results were within acceptable pharmacopoeial limits. Total aerobic count was $5.0\text{--}7.4 \times 10^2$ CFU/mL, and total fungal count was 18 CFU/mL. Specific pathogens (*E. coli*, *Salmonella* spp., *C. albicans*) were absent, confirming the formulation's microbiological safety.⁸

Heavy Metals: ICP-MS analysis confirmed that levels of toxic heavy metals—Arsenic, Cadmium, and Lead—in CTF-3 were Below the Limit of Quantification (BLQ; <0.01 mg/L), which is well under the WHO permissible limits for herbal medicines (As: 1.0 ppm, Cd: 0.3 ppm, Pb: 10 ppm).¹⁰ This confirms the safety of the raw material and manufacturing process from environmental contaminants.

HPTLC Fingerprinting: The HPTLC profile of CTF-3 showed 13 distinct peaks with R_f values from 0.05 to 1.02 at 254 nm, providing a unique chemical fingerprint for identity testing and batch-to-batch quality assurance. The major peak at R_f 0.05 accounted for 37.4% of the total peak area, representing a key bioactive compound or cluster.

4. Conclusion

This study successfully accomplishes the critical transition from a bioactive plant extract to a developed, stable, and safe phytopharmaceutical product. The optimized suspension formulation CTF-3, containing 2.0% Sodium CMC and a standardized *Cascabela thevetia*

ethanolic extract, meets key pharmaceutical criteria: it is physically and chemically stable under accelerated storage conditions, possesses optimal rheological properties for patient use, and is free from microbial and heavy metal contamination. Most importantly, it retains and delivers the potent antidiabetic, hypolipidemic, and β -cell regenerating activities of the raw extract in a consistent and safe manner. This comprehensive work—spanning formulation design, efficacy validation, stability profiling, and quality control—provides a robust scientific and technical dossier for CTF-3. It positions this novel herbal suspension as a promising, evidence-based candidate for further scale-up and clinical evaluation in the management of Type 2 Diabetes and its metabolic complications.

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