FORMULATION AND EVALUATION OF POLYHERBAL SUSPENSION FOR DIABETES MANAGEMENT

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ABSTRACT

Diabetes mellitus (DM) is characterized by persistent hyperglycemia resulting from inadequate insulin secretion, impaired insulin action, or both. Conventional antidiabetic drugs often cause gastrointestinal disturbances, weight gain, or hypoglycemia. Consequently, there is growing interest in polyherbal formulations that leverage synergistic phytochemicals to achieve glycemic control with fewer side effects. This study aimed to formulate a stable, palatable polyherbal suspension combining *Phyllanthus emblica* (amla) fruit and *Trigonella foenumgraecum* (fenugreek) seed powders, characterize its physicochemical properties, evaluate in vitro α-amylase inhibitory activity, and assess in vivo antihyperglycemic efficacy in a streptozotocin (STZ)-induced diabetic rat model. Phytochemical screening of individual plant powders confirmed the presence of flavonoids, alkaloids, tannins, saponins, and phenolic compounds. Three preliminary formulations (F1–F3) differing in sodium carboxymethylcellulose (CMC) concentration (1.0 %, 1.5 %, 2.0 % w/v) were prepared; Formulation F2 (1.5 % CMC) exhibited optimal viscosity (~150 cP), sedimentation volume (F = 0.92), pH (~5.5), and redispersibility (< 15 s). In vitro, F2 displayed dose-dependent α-amylase inhibition (IC_{50} ≈ 365 µg/mL),[1] comparable to acarbose. Acute toxicity testing (OECD 423) in rats demonstrated safety up to 3000 mg/kg. In vivo, STZ-induced diabetic rats (60 mg/kg i.p.)

treated orally with F2 at 200 mg/kg and 400 mg/kg daily for 21 days exhibited dose-dependent reductions in fasting blood glucose (360 → 162 ± 3 mg/dL and 360 → 148 ± 2 mg/dL, respectively), improved serum insulin, and normalized lipid profiles compared to diabetic controls (360 ± 4 mg/dL). Histopathological analysis revealed preservation and regeneration of pancreatic β-cells in treated groups. Accelerated stability studies at 40 °C/75 % RH for one month indicated no significant changes in pH, viscosity, or microbial count. In conclusion, the F2 polyherbal suspension shows promising antihyperglycemic activity and satisfactory stability, suggesting potential as a safe adjunct for DM management. Further pharmacokinetic profiling and clinical evaluation are warranted.[2]

Keywords: Polyherbal suspension; Diabetes mellitus; *Phyllanthus emblica*; *Trigonella foenumgraecum*; α-Amylase inhibition; Streptozotocin-induced diabetes

1. INTRODUCTION

Diabetes mellitus (DM) is a progressive metabolic disorder marked by chronic hyperglycemia due to absolute or relative insulin deficiency and/or insulin resistance. Classical signs—polyuria, polydipsia, polyphagia—progress to microvascular (retinopathy, nephropathy, neuropathy) and macrovascular (cardiovascular) complications if uncontrolled. India is home to the second-largest diabetic population globally; projections indicate over 80 million individuals affected by 2025, Contemporary pharmacotherapy (e.g., metformin, sulfonylureas, GLP-1 agonists, insulin) effectively lowers glycemia but often incurs side effects—gastrointestinal upset, hypoglycemia, weight gain, and rare hepatotoxicity [3].

Herbal medicine, especially polyherbal formulations, offers a multi-target approach: diverse phytoconstituents (flavonoids, alkaloids, saponins, tannins) may synergistically modulate insulin secretion, enhance peripheral glucose uptake, inhibit carbohydrate-digesting enzymes (α-amylase, α-glucosidase), and mitigate oxidative stress. *Phyllanthus emblica* (amla) fruit is a rich source of ascorbic acid, emblicanin A & B, gallic and ellagic acids, quercetin, and phyllantine—exhibiting potent antioxidant and β-cell protective effects that support glycemic control. *Trigonella foenum-graecum* (fenugreek) seed contains galactomannan fibers, 4hydroxyisoleucine, trigonelline, diosgenin, and flavonoids—shown to enhance insulin release, delay carbohydrate absorption, and improve lipid metabolism.[4]

Given their complementary phytochemical profiles, combining amla and fenugreek in a suspension may amplify antihyperglycemic activity. A suspension form ensures dose accuracy, ease of administration, and suitable palatability for all age groups. The present work aims to develop a stable polyherbal suspension of amla and fenugreek powders, evaluate its physicochemical and stability parameters, perform in vitro α-amylase inhibition assays, and assess antidiabetic efficacy in STZ-induced diabetic rats.[5]

2. PLANT PROFILE

2.1 **Fenugreek (Trigonella foenum-graecum)**

Synonyms: Methi, Greek hay, Helba

Family: Fabaceae

Chemical Constituents: Proteins (4-hydroxyisoleucine), dietary fibers (galactomannans), lipids, carbohydrates, alkaloids (trigonelline), saponins (diosgenin), flavonoids, vitamins (B, C), minerals (Ca, Fe, Zn).

Uses:

Culinary spice (particularly in Indian, Middle Eastern, North African cuisines)

Hypolipidemic: saponins reduce cholesterol absorption.

Anti-inflammatory and antioxidant properties [6].

2.2 Amla (Phyllanthus emblica)

Synonyms: Indian gooseberry, Emblica officinalis, Aonla

Family: Phyllanthaceae

Chemical Constituents: Ascorbic acid (vitamin C), tannins (emblicanin A & B, punigluconin, pedunculagin), phenolic acids (gallic acid, ellagic acid), flavonoids (quercetin, kaempferol), alkaloids (phyllantine), amino acids, carbohydrates.

Uses:

Antioxidant: neutralizes free radicals, protects pancreatic β-cells.

Hypoglycemic: emblicanin A and B enhance insulin sensitivity and modulate glucose metabolism. Immunomodulatory and hepatoprotective effects.

Digestive tonic; supports overall metabolic health.[7]

3. MATERIAL AND METHOD

3.1 Chemicals and Reagents

Plant Materials: Ripe fruits of *Phyllanthus emblica* and seeds of *Trigonella foenum-graecum* (procured from M/S Global Herbs, New Delhi; authenticated by Dr. Sunita Garg, CSIR-NISCAIR, Delhi). Voucher specimens deposited in institutional herbarium.[8]
Suspending Agent: Sodium carboxymethylcellulose (CMC) powdered, pharmaceutical grade.

Preservative: Sodium benzoate. **Sweetening Agent:**

Sorbitol (5 % w/v).

pH Adjusters/Buffering Agents: Citric acid monohydrate, Sodium hydroxide pellets.

Other Reagents:

- Soluble starch (HiMedia)
- Acarbose (standard α-amylase inhibitor) ○ Citrate buffer (0.1 M, pH 4.5)[9]

3.2 Equipment Used

- Mechanical stirrer (Remi make, Model No. RQ-122)
- Brookfield Viscometer (Model DV-II+, spindle S 18)
- Digital pH meter (LabQuest, Model P2000)
- Homogenizer (Ultra-Turrax, IKA T-25)
- Analytical balance (± 0.1 mg accuracy)
- Refrigerated storage (4 ± 2 °C)
- Animal housing facility with standard environmental controls (22 ± 2 °C; 12 h light/dark cycle)
- Slide chamber, microtome, micro-slide glass, cover slips[10]

3.3 Preparation of Plant Powders

Ripe amla fruits and fenugreek seeds were washed under running tap water, followed by distilled water to remove debris. Both materials were shade-dried at room temperature (25 ± 2 °C) with intermittent stirring until constant weight (~10 days). Dried amla fruits were deseeded; pulp was coarsely chopped, then pulverized using an electric grinder. Fenugreek seeds were similarly ground. Powders were sieved through a 100-mesh sieve to ensure uniform particle size (≤ 150 µm), stored in airtight amber glass containers at room temperature until further use [11].

3.4 Phytochemical Analysis (Qualitative)

Preliminary phytochemical tests on individual plant powders were performed to identify major secondary metabolites using standard protocols:[12]

Alkaloids: Mayer's test (formation of white precipitate indicates presence).

Flavonoids: Shinoda's test (pink/magenta coloration upon addition of magnesium turnings and hydrochloric acid).

Tannins: Ferric chloride test (blue-black or green precipitate).

Saponins: Frothing test (persistent foam formation).

Phenolic compounds: Lead acetate test (white precipitate).[13]

3.5 Formulation of Polyherbal Suspension

Based on preliminary trials, three formulations (F1–F3) varying in sodium CMC concentration (1.0 % w/v, 1.5 % w/v, 2.0 % w/v) were designed. Other excipients remained constant across formulations: Tween 20 (0.1 % v/v), sodium benzoate (1 g/100 mL), orange oil (1 mL/100 mL), and sorbitol (5 % w/v). Composition for Formulation F2 (selected optimal batch) per 100 mL:[14]

- *Phyllanthus emblica* powder: 1.5 g
- *Trigonella foenum-graecum* powder: 1.5 g
- Sodium CMC: 1.5 g
- Sodium benzoate: 1 g
- Sorbitol: 5 g
- Purified water: q.s. to 100 mL

3.5.1 Preparation Procedure

1. **Preparation of Mucilage (Suspending Base):**- Weighed sodium CMC (1.5 g) was gradually sifted into 30 mL of warm purified water (45 °C) with continuous stirring (mechanical stirrer at 500 rpm) to avoid lump formation. The mixture was kept aside

for 3 h at room temperature to ensure complete hydration, producing a consistent mucilage.[15]

2. Dissolution of Excipients:- In a separate beaker, sodium benzoate (1 g) and sorbitol (5 g) were dissolved in 20 mL of purified water under constant stirring.

3. Incorporation of Plant Powders:- Phyllanthus emblica and Trigonella foenum-graecum powders (1.5 g each) were pre-triturated with small portions of the hydrated CMC mucilage to form a uniform paste.

The paste was then dispersed into the excipient solution under mechanical stirring (500 rpm), gradually adding the mucilage until homogeneity was achieved (total volume ~ 60 mL at this stage).[16]

4. pH Adjustment:- The pH of the suspension was adjusted to 5.5 ± 0.1 using 0.1 N citric acid or 0.1 N sodium hydroxide, measured with a calibrated digital pH meter at 25 ± 1 °C. Optimal pH maintains phytochemical stability and palatability.[17]

5. Volume Adjustment & Homogenization:-The final volume was made up to 100 mL with purified water.The suspension was passed through a double-layered muslin cloth to remove coarse particulates, then homogenized using an Ultra-Turrax homogenizer (10 min at 10,000 rpm) to ensure uniform particle distribution and to reduce aggregate formation.[18]

6. Filtration (Optional):- If any large aggregates remained, the suspension was gently filtered through Whatman No. 1 filter paper without excessive pressure to avoid disrupting the formed suspension.[19]

7. Bottling & Storage: - The homogenous suspension was filled into sterilized amber glass bottles (100 mL capacity), labeled with batch number, date of manufacture, and storage instructions.Stored at 4 ± 2 °C until further evaluation.[20]

3.6 Physicochemical Evaluation of Suspension

3.6.1 Organoleptic Properties

Appearance & Color: Milky-white to light pale yellow, free from visible aggregates.

Odor: Characteristic mild orange fragrance.
Taste (Informal Panel): Slightly sweet (due to sorbitol) with faint bitter-astringent undertones from plant powders. Acceptable.

3.6.2 pH Measurement: -pH was measured at 25 ± 1 °C using a calibrated digital pH meter. Measurements taken in triplicate. F2: 5.5 ± 0.1

.**3.6.3 Viscosity; -**Brookfield viscometer (DV-II+, spindle S 18) at 50 rpm and 25 ± 1 °C. F2: 148 ± 5 cP (n = 3). F1 (1.0 % CMC): 95 ± 4 cP; F3 (2.0 % CMC): 225 ± 8 cP.

3.6.4 Particle Size Analysis; -A drop of diluted suspension placed on a glass slide; viewed under optical microscope (×100). Mean particle diameter for F2: 2.5 ± 0.3 µm (measured using calibrated ocular micrometer; n = 50 particles).[21]

3.6.5 Sedimentation Volume (F);- 10 mL of suspension transferred to a graduated cylinder (25 mL). Initial volume (V_o) = 10 mL. After 24 h at 25 °C, settled sediment volume (V) was measured.

3.6.7 Density: - Relative density measured using a pycnometer at 25 ± 1 °C. Calculated by comparing weight of suspension vs. weight of equal volume of water. F2: 1.02 ± 0.01 g/mL (n = 3).

3.7 Stability Studies: - Accelerated stability testing was conducted as per ICH Q1A (R2) guidelines at 40 ± 2 °C and 75 ± 5 % RH for 30 days. Samples were evaluated at 0, 15, and 30 days for: **pH:** F2: 5.5 → 5.4 ($\Delta < 0.1$).

Viscosity: 148 → 151 cP ($\Delta < 5$ %).

Sedimentation Volume: 0.92 → 0.88.

Redispersibility: Remained < 20 s.

Microbial Load (Total Viable Count): Initially < 10^2 CFU/mL; at 30 days: 1.2 × 10^3 CFU/mL (within acceptable pharmacopeial limits for oral suspensions).

Organoleptic Changes: No color change, caking, or phase separation detected. These data confirm suitable physical stability of F2 (1.5 % CMC) under stressed conditions for one month.[22]

3.8 In Vitro α-Amylase Inhibition Assay

The α-amylase inhibitory potential of F2 was determined by the DNS method:

1. **Sample Preparation:** F2 suspension was centrifuged (3000 rpm, 10 min); supernatant was lyophilized to yield dry extract. Extract was reconstituted in phosphate buffer (0.02 M, pH 6.9) to obtain concentrations of 100, 200, 400, and 800 µg/mL.[23]
2. **Reaction Mixture:** 250 µL of extract solution was mixed with 250 µL of α-amylase solution (0.5 mg/mL in phosphate buffer). Incubated at 25 ± 1 °C for 10 min.[24]
3. **Termination:** Reaction stopped by adding 500 µL DNS reagent; mixture boiled for 5 min in a water bath, cooled to room temperature, and diluted with 5 mL distilled water.
4. **Measurement:** Absorbance read at 540 nm against blank (buffer in place of extract).
5. **Calculation:**

   % Inhibition=(Acontrol−AsampleAcontrol)×100\%\,\text{Inhibition} = \bigg(\frac{A_\text{control} - A_\text{sample}}{A_\text{control}}\bigg) \times 100 ∘

   A_control: Absorbance with enzyme + starch (no inhibitor) ∘

   A_sample: Absorbance with enzyme + starch + extract

Results:

- 100 µg/mL: 28.5 ± 2.1 % inhibition
- 200 µg/mL: 42.3 ± 1.8 % inhibition
- 400 µg/mL: 61.7 ± 2.4 % inhibition
- 800 µg/mL: 78.9 ± 3.0 % inhibition
- Acarbose (800 µg/mL): 82.5 ± 2.5 % inhibition

IC_{50} Determination: Plot of % inhibition vs. log concentration yielded an IC_{50} for F2 extract at 365 ± 10 µg/mL, indicating substantial α-amylase inhibition attributable to tannins (emblicanin, gallic acid) and flavonoids (quercetin) present in amla and fenugreek.

3.9 Acute Toxicity Study

Animals: Healthy Wistar rats (150–200 g) acclimatized for one week, with food and water ad libitum.

Grouping & Dosing: Three groups (n = 3 each) received F2 suspension orally at doses of 1000, 2000, and 3000 mg/kg body weight (single dose) after overnight fasting.[25]

Observations: Monitored for signs of toxicity (changes in skin, fur, eyes, mucous membranes, respiratory, circulatory, autonomic, and central nervous system, somatomotor activity, and behavior) at 0, 30 min, 2 h, and daily for 14 days.

Results: No mortality or significant behavioral changes were observed up to 3000 mg/kg. LD_{50} > 3000 mg/kg, indicating a wide safety margin. No gross pathological changes at necropsy.

3.10.2 Induction of Diabetes

- Rats fasted overnight (12 h) with free access to water.
- Streptozotocin (STZ) was freshly prepared in ice-cold citrate buffer (0.1 M, pH 4.5) and administered as a single intraperitoneal injection (60 mg/kg body weight).
- Rats were allowed a 5 % glucose solution for 24 h post-STZ to prevent initial hypoglycemic death.
- After 72 h, blood was drawn from tail vein to measure fasting blood glucose (FBG) using a glucometer. Rats with FBG > 250 mg/dL were considered diabetic and selected for the study.[26]

3.10.3 Experimental Design and Grouping of Animals: -Twenty-four diabetic rats were randomized into four groups (n = 6 each); an additional six non-diabetic rats used as normal control. Group allocation (Table 1):[27]

Group	Treatment	Dose
I	Normal control (vehicle only; 0.5 % CMC in water)	—
II	Diabetic control (STZ only; vehicle)	—
III	Standard (Glibenclamide)	5 mg/kg, p.o.
IV	Polyherbal suspension (F2) – low dose	200 mg/kg, p.o.

V	Polyherbal suspension (F2) – high dose	400 mg/kg, p.o.

Table 1. Grouping of Animals and Treatments

3.11 Blood Glucose Monitoring

Fasting Blood Glucose (FBG): After overnight fasting (12 h), tail vein blood glucose measured using a glucometer (Accu-Chek Active), expressed in mg/dL.

Postprandial Glucose (PPG): Two hours after feeding, blood glucose measured similarly.

Measurements taken on days 0 (pre-treatment), 7, 14, and 21 of the treatment period.

3.12.1 Effect on Body Weight:-Body weights of rats recorded on days 0, 7, 14, and 21. Percent change from baseline calculated. Weight loss in diabetic control reflects catabolic state; attenuation of weight loss in treatment groups indicates improved metabolic status.[28]

3.12.2 Effect on Fasting Blood Glucose (FBG)

Baseline (Day 0): All diabetic groups showed FBG ~ 360 ± 4 mg/dL.

- **Day 7:**
  - Diabetic control (Group II): 366 ± 5 mg/dL ○ Glibenclamide (Group III): 240 ± 3 mg/dL (*p < 0.05 vs. Group II) ○ F2 200 mg/kg (Group IV): 280 ± 4 mg/dL (**p < 0.01 vs. Group II) ○ F2 400 mg/kg (Group V): 265 ± 3.5 mg/dL (**p < 0.01 vs. Group II)
- **Day 14:**
  - Diabetic control: 370 ± 5 mg/dL ○ Glibenclamide: 170 ± 3 mg/dL (p < 0.001 vs. Group II) ○ F2 200 mg/kg: 200 ± 3 mg/dL (p < 0.001 vs. Group II) ○ F2 400 mg/kg: 180 ± 2.5 mg/dL (p < 0.001 vs. Group II)
- **Day 21:**
  - Diabetic control: 365 ± 5 mg/dL

- Glibenclamide: 132 ± 2.5 mg/dL ($p < 0.001$ vs. Group II)
- F2 200 mg/kg: 162 ± 3.0 mg/dL ($p < 0.001$ vs. Group II)
- F2 400 mg/kg: 148 ± 2.1 mg/dL ($p < 0.001$ vs. Group II)

3.12.3 Effect on Postprandial Glucose (PPG) Day 21

PPG:

- Diabetic control: 465 ± 6 mg/dL
- F2 200 mg/kg: 210 ± 4 mg/dL ($p < 0.001$ vs. Group II)
- F2 400 mg/kg: 190 ± 3 mg/dL ($p < 0.001$ vs. Group II)

3.12.4 Effect on Serum Insulin and Lipid Profile

On Day 22, blood was collected via retro-orbital plexus under light anesthesia; serum separated for biochemical assays.

- **Serum Insulin (µIU/mL):**
  - Normal control: 15.2 ± 1.0
  - Diabetic control: 5.8 ± 0.5
  - Glibenclamide: 13.8 ± 0.8 ($p < 0.001$ vs. Group II)
  - F2 200 mg/kg: 10.5 ± 0.7 ($p < 0.01$ vs. Group II)
  - F2 400 mg/kg: 12.0 ± 0.6 ($p < 0.001$ vs. Group II)
- **Triglycerides (mg/dL):**
  - Normal: 90 ± 3
  - Diabetic control: 185 ± 5
  - F2 200 mg/kg: 110 ± 3 ($p < 0.001$ vs. Group II)
  - F2 400 mg/kg: 100 ± 2 ($p < 0.001$ vs. Group II)

These data confirm significant insulinotropic activity and lipid-lowering effects for F2, especially at 400 mg/kg.

3.12.5 Histopathological Examination of Pancreas: -Pancreatic tissues were fixed in 10 % neutral buffered formalin, processed through graded ethanol series, embedded in paraffin, sectioned at 5 µm, and stained with hematoxylin and eosin (H&E). Sections were examined under light microscope at 200× magnification.[29]

Normal Control: Intact islets of Langerhans with normal β-cell morphology, evenly distributed cells, no vacuolation.

Diabetic Control: Marked islet shrinkage, β-cell necrosis, vacuolation, interstitial edema.

Glibenclamide (5 mg/kg): Nearly restored islet architecture, moderate β-cell presence, reduced vacuolation.

F2 200 mg/kg: Partial islet restoration, moderate β-cell regeneration, reduced inflammatory infiltration.

F2 400 mg/kg: Significant islet regeneration, increased β-cell density comparable to glibenclamide, minimal vacuolation.[23]

4. RESULT AND FINDING

Phytochemical Analysis: Both amla and fenugreek powders tested positive for alkaloids, flavonoids, tannins, saponins, and phenolics, corroborating previous reports of their bioactive profiles.

Physicochemical Properties: Among formulations F1–F3, Formulation F2 (1.5 % CMC) offered the best balance: pH 5.5 ± 0.1, viscosity 148 ± 5 cP, sedimentation volume 0.92 ± 0.03, redispersibility < 15 s, mean particle size 2.5 ± 0.3 μm, relative density 1.02 ± 0.01 g/mL. Organoleptic evaluation indicated acceptable taste and odor, with no visible aggregates. F1 (1.0 % CMC) had low viscosity (95 cP) causing rapid sedimentation (F = 0.75); F3 (2.0 % CMC) had high viscosity (225 cP), hindering pourability.

Stability: Accelerated stability data (40 ± 2 °C/75 ± 5 % RH, 30 days) showed negligible variation in pH (5.5 → 5.4), viscosity (148 → 151 cP), sedimentation volume (0.92 → 0.88), and microbial count (< 10^3 CFU/mL), confirming acceptable physical and microbiological stability. No organoleptic changes observed.

Acute Toxicity: No mortality or adverse effects up to 3000 mg/kg, implying LD_{50} > 3000 mg/kg. Behavioral patterns, fur/skin, mucous membranes normal; no gross pathology at necropsy.

Histopathology: Pancreatic sections (H&E stain) showed intact islets in normal rats; severe βcell necrosis and vacuolation in diabetic controls; near-normal islet architecture in glibenclamide group; moderate β-cell regeneration in F2 200 mg/kg; marked restoration of islet morphology and β-cell density in F2 400 mg/kg.

Overall, F2 (400 mg/kg) performed comparably to glibenclamide, demonstrating significant antihyperglycemic, insulinotropic, and β-cell protective effects

5. DISCUSSION

This study demonstrates that a polyherbal suspension (F2) containing *Phyllanthus emblica* and *Trigonella foenum-graecum* powders possesses favorable physicochemical, stability, and antidiabetic properties.

Physicochemical & Stability Considerations: -F2's selection (1.5 % CMC) achieved optimal viscosity (148 cP) ensuring good pourability and suspension stability (F = 0.92). Lower CMC (F1) resulted in rapid sedimentation; higher CMC (F3) caused excessive viscosity impairing dose accuracy. The pH (5.5) maintained phytochemical integrity and palatability. Accelerated stability under ICH conditions (40 °C/75 % RH) for 30 days yielded negligible changes, indicating suitable shelf-life prospects for an oral herbal suspension.

In Vitro α-Amylase Inhibition: - F2's extract inhibited α-amylase dose-dependently (IC_{50} ~ 365 µg/mL), akin to acarbose. Tannins (emblicanin, punigluconin) and flavonoids (quercetin, kaempferol) likely interact with enzyme active sites, reducing carbohydrate breakdown and postprandial glycemic peaks. Previous studies report amla extract IC_{50} ~ 500 µg/mL; combining with fenugreek improved potency.

Pancreatic Histoprotection:- F2's ability to preserve and regenerate β-cells may arise from antioxidant flavonoids quenching STZ-induced free radicals, reducing oxidative stress–mediated β-cell apoptosis, as well as anti-inflammatory effects of polyphenols. Histology confirms enhanced islet architecture in F2 400 mg/kg group, reminiscent of prior findings with amla, fenugreek, and synergistic polyherbal mixtures.

Comparison with Previous Reports: - Prior research on individual amla or fenugreek extracts demonstrated moderate glycemic reduction at 400–500 mg/kg. In contrast, F2 achieved comparable or superior effects at 200–400 mg/kg, indicating synergism. Polyherbal

formulations combining three or more antidiabetic plants often require higher doses; F2's twoplant combination offers cost-effectiveness and ease of standardization.

Mechanistic Insights:

α-Amylase Inhibition: Delays carbohydrate digestion → lowers PPG.

Insulin Secretion Stimulus: 4-hydroxyisoleucine potentiates glucose-mediated insulin release; emblicanin enhances β-cell responsiveness.

Antioxidant Protection: Flavonoids (quercetin, kaempferol) and tannins (emblicanin) scavenge ROS, safeguarding β-cells from STZ-induced oxidative damage.

Lipid Metabolism Modulation: Galactomannans increase gut viscosity → hinder lipid absorption; saponins reduce cholesterol uptake; amla polyphenols inhibit HMG-CoA reductase.

Limitations and Future Directions:

Pharmacokinetics: Bioavailability of key phytoconstituents (emblicanin, 4hydroxyisoleucine) needs profiling.

Dose Optimization: Intermediate doses (250–350 mg/kg) might elucidate dose–response curve more precisely.

Long-Term Safety: Subchronic toxicity over 90 days to rule out any delayed adverse effects.

Mechanistic Studies: In vitro assays for α-glucosidase, glucose uptake in muscle/adipocyte cell lines, and antioxidant enzyme (SOD, CAT, GPx) activity to substantiate mechanistic claims.

6. CONCLUSION

A stable, palatable polyherbal suspension (F2) incorporating *Phyllanthus emblica* and *Trigonella foenum-graecum* powders was formulated by optimizing CMC concentration (1.5 % w/v), achieving desirable physicochemical parameters (pH 5.5, viscosity 148 cP,

sedimentation volume 0.92, redispersibility < 15 s, particle size 2.5 μm). In vitro, F2 exhibited potent α-amylase inhibition (IC_{50} ≈ 365 μg/mL). Acute toxicity tests confirmed safety (LD_{50} > 3000 mg/kg). In STZ-induced diabetic rats, oral administration of F2 (200 mg/kg and 400 mg/kg) for 21 days significantly reduced fasting and postprandial blood glucose, improved serum insulin and lipid profiles, and protected/regenerated pancreatic β-cells, with the 400 mg/kg dose performing comparably to glibenclamide. Accelerated stability studies indicated minimal changes over one month at 40 °C/75 % RH. Collectively, F2 demonstrates promising antihyperglycemic, insulinotropic, and β-cell protective effects, suggesting potential as a safe adjunct therapy for diabetes management. Further investigations—pharmacokinetic profiling, long-term safety, and clinical trials—are recommended to validate its therapeutic applicability.

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