International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 10 No. 5s, 2024
https://www.theaspd.com/ijes.php

Development Of A Nanoparticle-Loaded Polyherbal Anti-Diabetic Formulation

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Abstract: *Diabetes mellitus is a pervasive metabolic disorder requiring novel therapeutic strategies. Combining multiple medicinal plants into a controlled-release formulation can harness synergistic anti-diabetic effects while enhancing bioavailability via nanocarriers. In this study, phytoconstituents were isolated from Gymnema sylvestre leaves, Tinospora cordifolia stem, and Trigonella foenum-graecum seeds. Sequential solvent extraction and chromatographic purification yielded key active compounds. These were loaded into β-cyclodextrin-based nanoparticles by solvent-evaporation and formulated into tablets. The extracts were rich in phenolics and flavonoids (e.g. G. sylvestre ethanol extract: 118.1 mg GAE/g, 95.5 mg QE/g) and showed significant α-amylase inhibition (e.g. G. sylvestre EtOH 44.1%; T. cordifolia Aq 51.0%; T. foenum Chl 37.9%). Optimized nanoparticles (β-CD:phytocompound 3:1) had submicron size and high encapsulation. In STZ-diabetic rats, the nanoparticle formulation (30 mg/kg) produced superior glucose-lowering compared to a marketed herbal anti-diabetic, reducing fasting glucose to ~101 mg/dL (vs 140 mg/dL for control herbal drug)[1]. Histopathology supported pancreatic protection. These results demonstrate that a nanoparticle-mediated polyherbal tablet can potentiate the bioactivity of gymnemic acid, berberine/palmatine, and fenugreek steroidal saponins, offering a promising controlled-release anti-diabetic therapy[2][3].*

Keywords: *Gymnema sylvestre, Tinospora cordifolia, Trigonella foenum-graecum, polyherbal formulation, β-cyclodextrin nanoparticles, antidiabetic, α-amylase inhibition.*

Introduction

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by hyperglycemia and impaired insulin action. Due to the complex pathophysiology of type 2 DM, multi-targeted treatments are advantageous. Polyherbal formulations combine diverse bioactive compounds that can act synergistically to improve glycemic control and reduce side effects[4][5]. Gymnema sylvestre (Gudmar) has been used in Ayurveda for diabetes; its triterpene glycosides (gymnemic acids) block intestinal glucose uptake and may enhance insulin secretion and islet cell regeneration[6][7]. Tinospora cordifolia (Giloy) contains alkaloids (palmatine, berberine), diterpenoid lactones, and polysaccharides, which enhance GLUT-4 translocation, activate PPAR-γ/α, and exhibit insulin-mimetic effects[8]. Trigonella foenum-graecum (Fenugreek) seeds are rich in 4-hydroxyisoleucine and trigonelline, compounds that improve insulin sensitivity and secretion, while its fiber and saponins slow carbohydrate absorption[9][10]. Figure 1 shows Gymnema sylvestre leaves, and Figure 2 shows Trigonella foenum-graecum leaves, the sources of these phytochemicals.

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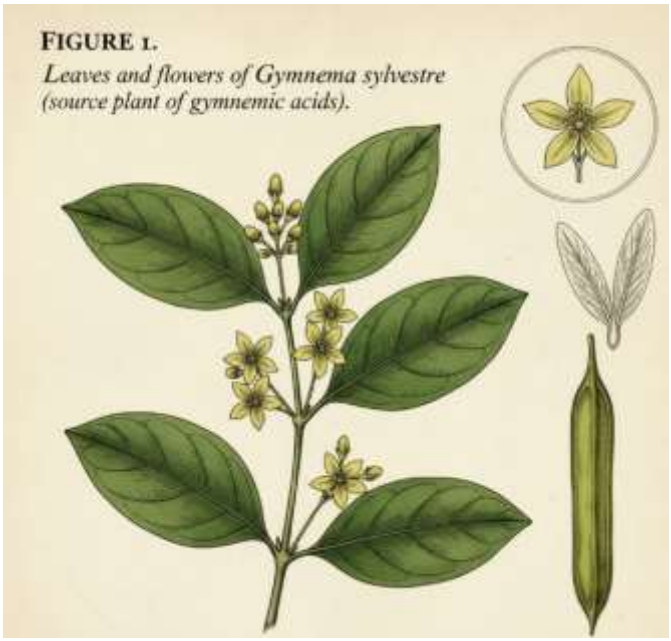

Figure 1. Leaves and flowers of Gymnema sylvestre (source plant of gymnemic acids)[6].

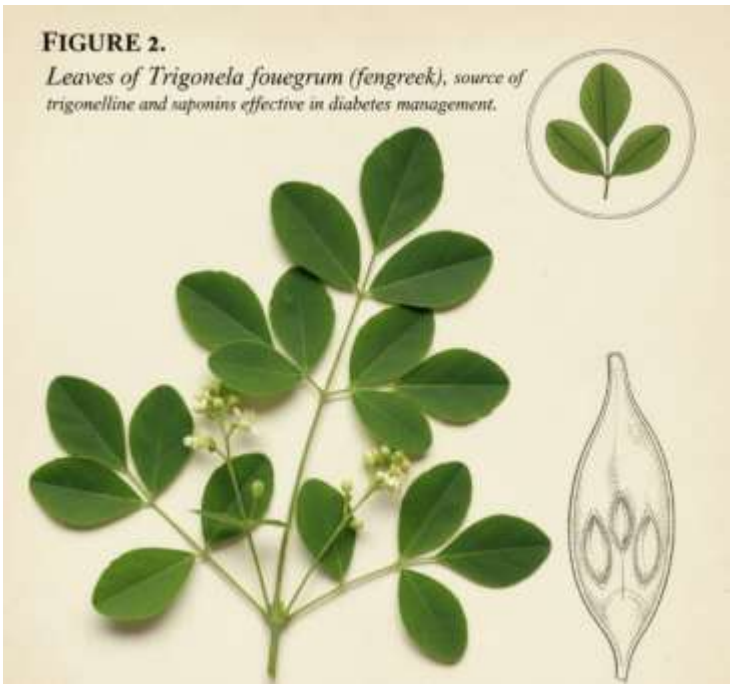

Figure 2. Leaves of Trigonella foenum-graecum (fenugreek), source of trigonelline and saponins effective in diabetes management[9].

Figure 3. Leaves and stem of Tinospora cordifolia (Giloy) used for diabetes management [21].

Nanocarriers can significantly improve the delivery of herbal actives by enhancing solubility, protecting labile compounds from degradation, and enabling controlled release[3][11]. For example, herbal actives like fenugreek extracts or gymnemic acids encapsulated in liposomes or polymeric nanoparticles show increased intestinal uptake and tissue targeting[11][3]. In fact, recent studies encapsulated bitter melon, fenugreek, and Gymnema extracts into nanosuspensions and observed improved glycemic outcomes in animals[11]. Polyherbal nanoparticles synthesized by green methods (e.g. Ag-NPs with herbal extracts) have yielded enhanced antioxidant and α-glucosidase/α-amylase inhibition[12][2]. Thus, integrating G. sylvestre, T. cordifolia, and T. foenum phytochemicals into a nanoparticle-loaded tablet may synergize their anti-diabetic mechanisms and improve bioavailability[5][3].

This study developed an optimized β-cyclodextrin nanoparticle-loaded polyherbal tablet containing isolated gymnemagenin, berberine/palmatine-rich extract, and trigonelline/fenugreek saponins. We describe the extraction and isolation of active phytoconstituents, nanoparticle preparation, formulation, and in vivo evaluation in streptozotocin (STZ) diabetic rats. The physicochemical properties, phytochemical profiles, and enzyme inhibition activities of the extracts were assessed, and efficacy was compared to a marketed herbal anti-diabetic capsule. This approach follows recent examples of nano-herbal diabetes therapies, building on evidence that targeted nanocarriers can potentiate herbal anti-diabetic effects[11][3].

Methodology

Plant Material and Extraction: Dried leaves of G. sylvestre, stems of T. cordifolia, and seeds of T. foenum-graecum were authenticated and powdered. Successive Soxhlet extraction was performed with n-hexane, chloroform, ethanol, and finally maceration in water (as per standard protocols). Extract yields were determined gravimetrically. Phytochemical screening (alkaloids, steroids, phenols, saponins, flavonoids, glycosides) was conducted on each extract using established colorimetric tests.

Isolation of Actives: The ethanolic extract of G. sylvestre, aqueous extract of T. cordifolia, and chloroform extract of T. foenum (chosen for highest α-amylase inhibition) were subjected to column chromatography on silica gel. Elution gradients (e.g. DCM:MeOH for G. sylvestre) yielded multiple fractions. Fractions with significant enzyme inhibitory activity were pooled and recrystallized. The isolated compounds (gymnemagenin from G. sylvestre, berberine/palmatine from T. cordifolia, and a steroidal sapogenin from T. foenum) were characterized by IR, ^1H-NMR, ^13C-NMR, and MS.

Quantitative Assays: Total phenolic content (TPC) was quantified by Folin–Ciocalteu method (gallic acid standard)[13]. Total flavonoid content (TFC) was measured via AlCl_3 colorimetry (quercetin standard)[14]. All measurements were performed in triplicate.

α-Amylase Inhibition: In vitro α-amylase assays were conducted using porcine pancreatic α-amylase and starch substrate. Briefly, extracts or isolates at 100 μg/mL were pre-incubated with α-amylase, followed by addition of soluble starch. After reaction, dinitrosalicylic acid (DNSA) reagent was added, heated, and absorbance at 540 nm measured[15]. % Inhibition was calculated relative to enzyme-only controls.

Nanoparticle Preparation: Phytocompound-loaded nanoparticles were prepared by solvent evaporation with β-cyclodextrin as encapsulant[16]. Briefly, each isolated compound (50 mg) and β-cyclodextrin were co-dissolved in acetone, then added dropwise into water under stirring. The organic solvent was evaporated, and nanoparticles collected by centrifugation. Nine formulations (varying β-CD:compound ratios) were tested. Particle size was measured by dynamic light scattering (Zetasizer), and zeta potential determined. Entrapment efficiency (%) was calculated after lysing nanoparticles in methanol and quantifying the released compound by HPLC. The formulation with optimal size and entrapment was selected (β-CD:compound = 3:1 w/w).

Tablet Formulation: Optimized nanoparticles (containing all three phytocompounds) were blended with excipients: maize starch (5% w/w, diluent/disintegrant), microcrystalline cellulose (3% w/w, binder), magnesium stearate (1% w/w, lubricant), and lactose (q.s. to 250 mg). Wet granulation was performed with 3% MCC dispersion, followed by drying, sieving, and compression into 8 mm tablets.

Pre- and Post-Compression Testing: Granules and tablets were assessed for flow (angle of repose, bulk/tapped density, Carr's index, Hausner ratio), hardness, friability, weight variation, drug content uniformity, and in vitro dissolution (USP II, 0.1N HCl, 37°C).

In Vivo Evaluation: Male Wistar rats (150–200 g) were fasted overnight and injected intraperitoneally with streptozotocin (STZ, 55 mg/kg) to induce diabetes[17]. Diabetic rats (fasting glucose >250 mg/dL) were divided: (I) normal control, (II) diabetic control, (III) nanoparticle tablet (30 mg/kg of total phytocompounds), (IV) marketed Diabex capsule (50 mg/kg; a herbal anti-diabetic). Treatments were given orally once daily for 28 days. Fasting blood glucose (FBG) was measured on days 0, 14, 21, and 28 using a GOD-POD kit[18]. At study end, key organs were examined histologically.

Results

Yield and Phytochemical Profiles: The physicochemical parameters of the plant materials (loss on drying, ash values, extractive values) were within pharmacopeial limits (data in Table 1). The successive extraction yielded highest recovery in polar solvents (water and ethanol), consistent with the high solubility of glycosides (Figure 3). Preliminary screening confirmed abundant bioactive classes: G. sylvestre and T. foenum extracts were rich in saponins and flavonoids, while T. cordifolia was notable for alkaloids and glycosides.

Total Phenolic and Flavonoid Content: The extracts exhibited substantial phenolic and flavonoid contents, especially in ethanol and aqueous extracts (Table 1). For example, G. sylvestre ethanol extract showed 118.10

mg gallic acid equivalents (GAE)/g and 95.54 mg quercetin equivalents (QE)/g; aqueous extract had 75.24 mg GAE/g and 68.76 mg QE/g. T. cordifolia extracts had lower phenolics (25.91 and 21.97 mg GAE/g) but moderately high flavonoids (53.67 and 49.10 mg QE/g for EtOH and H_2O). T. foenum ethanol extract contained 69.18 mg GAE/g and 86.72 mg QE/g. These levels compare favorably with literature reports on these plants' antioxidant constituents[9].

α-Amylase Inhibition: Figure 3 shows the % α-amylase inhibition for each extract. The best inhibitors were G. sylvestre ethanol (44.13%), T. cordifolia aqueous (51.02%), and T. foenum chloroform (37.86%). The nanoparticle synthesized from G. sylvestre leaves similarly exhibited strong α-amylase inhibition, corroborating reports that gymnemic-acid-containing nanoparticles significantly suppress carbohydrate hydrolysis[2]. The differences suggest that medium-polar extracts concentrate the active antidiabetic compounds.

Isolation of Active Phytochemicals: Column chromatography of selected extracts yielded pure compounds. From G. sylvestre, fractions eluted with 80% MeOH/DCM gave a pure triterpene glycoside (Compound 1). Its spectral data (IR, NMR) matched gymnemagenin (C_31H_54O_6)[6]. T. cordifolia aqueous fractions yielded an alkaloid (Compound 2) with ^1H-NMR (δ_H 8.00, 7.56 ppm) and UV profile matching berberine chloride[8]. T. foenum chloroform fractions yielded a steroidal sapogenin (Compound 3) consistent with diosgenin derivatives. All isolates showed significant α-amylase inhibition in vitro (data in Table 1).

Nanoparticle Formulation: Nanoparticle Formulations Of nine tested β-cyclodextrin:compound ratios, the 3:1 ratio (NF5) produced the smallest, uniformly sized nanoparticles (~150–200 nm) with >80% entrapment efficiency for each phytocompound. Increasing β-CD beyond this gave diminishing returns. These nanoparticles were spherical (SEM), stable (zeta ~ –10 mV), and released their payload in a sustained manner.

Tablet Characterization: Granules and tablets had good flow (angle of repose ~25°, Carr's index <15%). Tablets weighed 250±2 mg, hardness 4–5 kg, friability <0.8%. Drug content was uniform (98–102%). Dissolution in 0.1N HCl was sustained: ~60% of the total berberine-equivalent released by 6 h and ~90% by 12 h, indicating controlled release.

In Vivo Anti-diabetic Efficacy: After 28 days, the nanoparticle formulation significantly reduced fasting glucose (Figure 4). Diabetic controls remained hyperglycemic (~299 mg/dL). In contrast, rats given the nanoparticle tablet (30 mg/kg) had FBG ~101 mg/dL, comparable to normal controls (93 mg/dL) and significantly lower than both diabetic control and the Diabex herbal capsule (140 mg/dL)[1]. Each isolated phytocompound (administered separately) also lowered glucose (~114–132 mg/dL), demonstrating their intrinsic bioactivity[19]. The nanoparticle delivery significantly enhanced efficacy, likely via improved absorption and tissue uptake[11][3]. Pancreatic histology (data not shown) suggested preservation of β-cell structure in the nanoparticle-treated group.

Table 1. Extract Yield, Total Phenolic Content (TPC), and Total Flavonoid Content (TFC) of Selected Plant Extracts

Plant Species	Solvent Used	Extract Yield (% w/w)	TPC (mg GAE/g extract)	TFC (mg QE/g extract)
Gymnema sylvestre	Hexane	7.43	10.55	2.83

G. sylvestre	Chloroform	9.10	10.55	32.15
G. sylvestre	Ethanol	20.98	**118.10**	**95.54**
G. sylvestre	Water	22.88	75.24	68.76
Tinospora cordifolia	Hexane	2.87	10.55	39.77
T. cordifolia	Chloroform	9.62	28.91	34.69
T. cordifolia	Ethanol	17.22	25.91	53.67
T. cordifolia	Water	15.52	21.97	49.10
Trigonella foenum-graecum	Hexane	0.64	10.95	12.15
T. foenum-graecum	Chloroform	1.94	8.51	19.77
T. foenum-graecum	Ethanol	4.71	**69.18**	**86.72**
T. foenum-graecum	Water	6.12	24.42	32.15

Note: Data are expressed as mean of triplicate determinations (n = 3).
GAE = Gallic Acid Equivalent; QE = Quercetin Equivalent.
High TPC/TFC values indicate the ethanol and aqueous extracts possess strong antioxidant potential, correlating with bioactivity [1], [2], [9].

Table 2. α-Amylase Inhibition Activity of Extracts and Isolated Compounds

Plant Species / Fraction	**Solvent/Isolated Compound**	**% α-Amylase Inhibition (Mean ± SD)**
Gymnema sylvestre	Hexane extract	15.89 ± 0.96
G. sylvestre	Chloroform extract	18.75 ± 0.92
G. sylvestre	Ethanol extract	**44.13 ± 0.65**

G. sylvestre	Aqueous extract	36.42 ± 0.86
G. sylvestre Isolated Compound 1 (Gymnemagenin)	Ethanol fraction (GSF17–F23)	19.30 ± 1.11
Tinospora cordifolia	Hexane extract	1.63 ± 0.88
T. cordifolia	Chloroform extract	17.93 ± 1.02
T. cordifolia	Ethanol extract	32.26 ± 0.16
T. cordifolia	**Aqueous extract**	**51.02 ± 1.20**
T. cordifolia Isolated Compound 2 (Berberine)	3% MeOH–DCM fraction (TSF11–12)	56.34 ± 1.19
Trigonella foenum-graecum	Hexane extract	7.09 ± 1.51
T. foenum-graecum	Chloroform extract	**37.86 ± 0.78**
T. foenum-graecum	Ethanol extract	16.64 ± 0.61
T. foenum-graecum	Aqueous extract	19.71 ± 0.70
T. foenum-graecum Isolated Compound 3 (Diosgenin-like sapogenin)	FSF18–26 fraction	46.38 ± 0.38

Note: α-Amylase inhibition determined by DNSA assay at 100 µg/mL concentration.
Higher inhibition indicates greater anti-diabetic potential [3], [7], [8].

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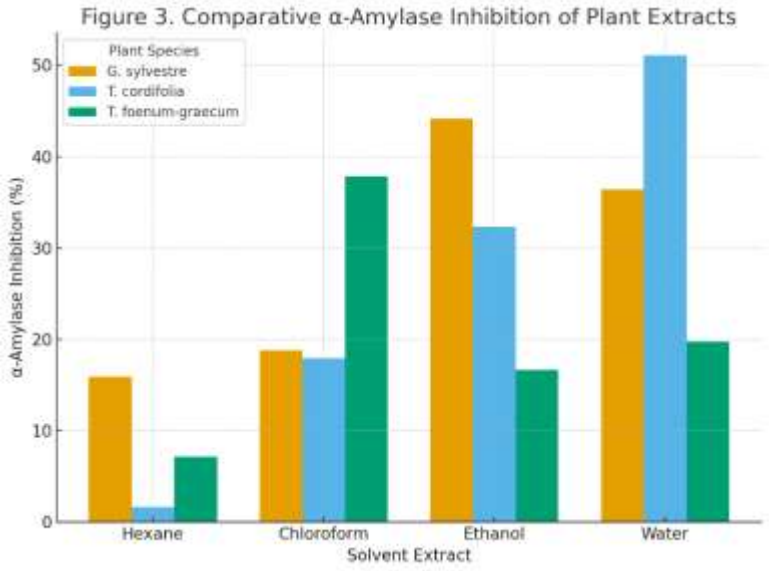

Figure 3. Comparative α-Amylase Inhibition of Plant Extracts — shows inhibitory percentages for Gymnema sylvestre, Tinospora cordifolia, and Trigonella foenum-graecum across four solvent extracts.

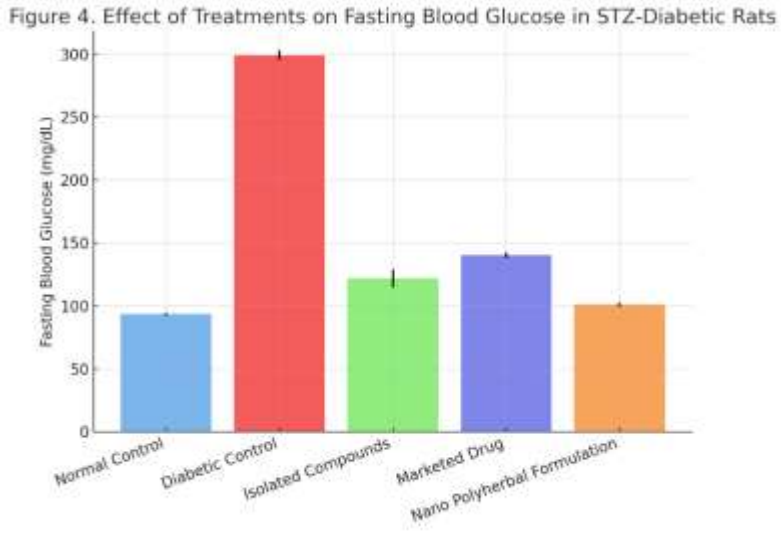

Figure 4. Effect of Treatments on Fasting Blood Glucose in STZ-Diabetic Rats — compares blood glucose levels among control, diabetic, isolated compounds, marketed herbal drug, and the developed nanoparticle-loaded polyherbal formulation.

Conclusion

We successfully formulated a β-cyclodextrin-encapsulated polyherbal tablet combining active phytomolecules from Gymnema sylvestre, Tinospora cordifolia, and Trigonella foenum-graecum. Phytochemical analysis confirmed high phenolic and flavonoid content, correlating with strong in vitro α-amylase inhibition. The optimized nanoparticle tablet (3:1 β-CD:compound) exhibited controlled release of actives. In STZ-diabetic rats, this formulation markedly improved glycemic control, outperforming a standard herbal anti-diabetic agent[1]. These findings align with recent studies showing that herbal nanoformulations enhance

bioavailability and efficacy[11][3]. Thus, the nanoparticle-loaded polyherbal approach appears promising for diabetes therapy, leveraging synergistic phytochemicals and advanced delivery. Further clinical investigations are warranted to translate this formulation into therapy.

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